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Investigating sex differences in the relationship between conduct disorder and brain structure and neural activity during emotion processing

by

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ABSTRACT

INVESTIGATING SEX DIFFERENCES IN THE RELATIONSHIP BETWEEN CONDUCT DISORDER AND BRAIN STRUCTURE AND NEURAL ACTIVITY DURING EMOTION PROCESSING

Irini Areti Eugenia Smaragdi

Conduct disorder (CD) is a psychiatric disorder characterised by aggressive and rule-breaking behaviour. It is a debilitating disorder that brings substantial costs for affected individuals, their families and society more generally. It can lead to educational failure, unemployment, mental health issues, and in some cases, criminality in adulthood. The prevalence of CD is over two times higher in males compared to females, but it is still one of the most common reasons for referral to child and adolescent mental health services in the UK for both sexes. Although there is evidence that males and females with CD differ in terms of risk factors, clinical presentation, and adult outcomes of the disorder, little research has been devoted to studying these potential sex differences, and the vast majority of research has thus far focused on males.

There is increasing evidence to suggest that alterations in brain structure and function may contribute to the risk of developing CD. Despite this, few studies have investigated whether such effects are observed in females with CD. This study is part of the Female Neurobiology and Treatment of Conduct Disorder (FemNAT-CD) study, which is a large-scale European collaboration between 11 universities and psychiatric clinics

aiming to investigate the neurobiology of female CD and sex differences in CD. The current study selected 96 adolescents with CD (48 females) and 102 sex-, age-, and puberty-matched healthy controls (14-18 years old) that had undergone magnetic resonance imaging (MRI) at four of the sites. We investigated common and sex-dependent associations between CD and brain structure using two independent approaches: voxel-based morphometry and surface-based morphometry. In addition, we tested for shared and sex-dependent effects on brain activity during emotion processing using functional MRI, in a sub-set of the CD and healthy control participants (n=103). We also tested the validity of collapsing across childhood-onset and adolescent-onset forms of CD, and repeated each analysis to assess the influence of ADHD comorbidity.

Across the three studies, males and females showed common and distinct abnormalities in brain structure and function. Structurally, males and females with CD both showed lower cortical thickness and grey matter volume, and increased gyrification in prefrontal cortex relative to controls, while there were sex-by-diagnosis interactions in some areas, such as the insula and amygdala. These appeared to be driven by structural alterations in males but not females with CD. Furthermore, CD-related associations with brain structure were sometimes in the opposite directions in males and females: relative to controls, males with CD showed higher, and females with CD showed lower, superior frontal gyrus surface area and gyrification. Sex-by-diagnosis interactions were also seen for amygdala activity during processing of angry facial expressions (i.e., males with CD showed higher, and females with CD lower, activity relative to their respective control groups). These results were largely unrelated to CD age-of-onset, IQ differences, and ADHD comorbidity.

This study provides the first robust evidence for sex differences in the relationship between CD and brain structure and neural activity during emotion processing. Overall, the findings from the three studies suggest that there may be important sex differences in the neurobiological basis of CD. This highlights the importance of studying males and females

with CD separately in future neuroimaging studies, as combining the sexes might obscure or bias results. Furthermore, if the neurobiological basis of CD differs between the sexes, males and females with CD may require different treatments in clinical settings.

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DECLARATION OF AUTHORSHIP

I, Areti Smaragdi, declare that this thesis and the work presented in it are my own and have been generated by me as the result of my own original research.

[Investigating sex differences in the relationship between conduct disorder and brain structure and neural activity during emotion processing]

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
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Abbreviations

ACC =Anterior cingulate cortex

ACR = American College of Radiology

ADHD = Attention-deficit/hyperactivity disorder

AO-CD = Adolescent-onset conduct disorder

ASPD = Anti-Social Personality Disorder

CAMHS = Child and Adolescent Mental Health Services

CD = Conduct disorder

CO-CD = Childhood-onset conduct disorder

CP = Conduct problems

CU = Callous-unemotional traits

DARTEL = Diffeomorphic Anatomical Registration Through Exponential Lie Algebra

DBD = Disruptive behaviour disorders

DSM = Diagnostic and Statistical Manual of Mental Disorders

EHI = Edinburgh Handedness Inventory

F = Female

FBIRN = Functional Biomedical Informatics Research Network

FDR = False Discovery Rate (correction for multiple comparisons)

FemNAT-CD = Female Neurobiology and Treatment of Conduct Disorder

FEW = Family Wise Error (correction for multiple comparisons)

GMV = Grey matter volume

HC = Healthy controls

ICU = Inventory of Callous-Unemotional traits

IES = Integrated Emotion Systems

IFG = Inferior frontal gyrus

IQ = Intelligence Quotient

K-SADS-PL = Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version

*l*GI = Local gyrification index

M = Male

MNI = Montreal Neurological Institute

MP-RAGE = Magnetization-prepared rapid-acquisition gradient-echo sequence

MRI = Magnetic Resonance Imaging

ODD = Oppositional defiant disorder

OFC = Orbitofrontal cortex

PDS = Pubertal Developmental Scale

PFC = Prefrontal cortex

PTSD = Post-traumatic Stress disorder

ROI = Region of interest

SBM = Surface based morphometry

SFG = Superior frontal gyrus

SIP = Social Information Processing

SPM = Statistical Parametric Mapping

STG = Superior temporal gyrus

SVC = Small volume correction

TPM = Tissue probability maps

VBM = Voxel-based morphometry

VlPFC = Ventrolateral prefrontal cortex

VmPFC = Ventromedial prefrontal cortex

WASI = Wechsler Abbreviated Scale of Intelligence

WISC = Wechsler Intelligence Scale for Children

WM = White matter

YOS = Youth offending services

YPI = Youth Psychopathic traits Inventory

Chapter 1: What is conduct disorder

The overall aim of this thesis is to investigate sex differences in the relationship between conduct disorder (CD) and neural activity during emotion processing and brain structure. The motivation for this thesis comes from the overall underrepresentation of females in the neuroimaging literature on CD and our consequent lack of understanding of the neurobiological underpinnings of the disorder in females. This chapter will introduce CD by providing an overview of the clinical characteristics and diagnostic criteria of the disorder, and continue by introducing sex differences in the presentation, risk factors, and adult outcomes of the disorder. We will also discuss the important considerations of these potential sex differences, which have been largely overlooked in the literature thus far. Furthermore, the costs and consequences of the disorder for the individual, the families, and society will briefly be reviewed, and theories of CD will be discussed in relation to empirical findings of the neuropsychological and neurophysiological deficits associated with the disorder.

1.1 Definition and assessment of CD

Conduct disorder (CD) is a psychiatric condition that emerges in childhood or adolescence and is characterised by persistent and repetitive patterns of aggressive and antisocial behaviour (American Psychiatric Association, 2013). Diagnoses of CD are categorical in nature, and based on the presence of a minimum of three out of fifteen symptoms listed in the DSM-5 (APA, 2013). These symptoms are purely behavioural and have been divided into four clusters (aggression to people and animals, destruction of property, deceitfulness or theft, and serious rule violations; **Table 1.1**). To obtain a diagnosis, these symptoms must have been present in the last year, with at least one symptom displayed in the last 6 months. In addition, there must be evidence of significant impairment in the individual's family or social relationships, or in academic functioning,

such as poor peer-, parent-, or teacher relationships, or suspension from school (APA, 2013).

Table 1.1. DSM-5 criteria for conduct disorder categorised under four headings. To meet the criteria, at least three symptoms must be present in the previous year, with at least one present in the last 6 months

Aggression to people and animals
Bullying
Fighting
Use of weapon
Physical cruelty to people
Physical cruelty to animals
Stealing with confrontation of the victim
Forced sexual activity
Property destruction
Fire setting
Vandalism
Deceitfulness or theft
Breaking and entering
Lying for personal gain
Stealing without confrontation with victim
Serious rule violation
Staying out late
Run away from home over night
Truancy

1.2 The consequences of CD

Conduct disorder incurs major costs, both for the individual, their family, and society, as it is the main reason for referral of children and adolescents to Child and Adolescent Mental Health Services (CAMHS) in the UK, accounting for 30-40% of all referrals (Baker, 2013). It is thought that around 6% of males and 3% of females in the UK will develop CD, with the risk being highest in adolescence (Maughan, Rowe, Messer, Goodman, & Meltzer, 2004). Although males are about twice as likely to develop CD compared to females, the prevalence of female CD has steadily increased over the last decades, making it one of the most commonly diagnosed disorders in both sexes (Collishaw, Maughan, Goodman, & Pickles, 2004; Moffitt, Caspi, Rutter, & Silva, 2001).

The cost for the families with a child diagnosed with CD is often high, sometimes leading to loss of employment for the parents, and a financial strain due to the damage to property that the young person may cause as a consequence of their disorder. There are

several agencies in the UK that are likely to be involved in treatment of individuals with CD, including Youth Offending Services (YOS), CAMHS, and social services. Altogether, it has been estimated that it costs society at least £100,000 more to raise a child with CD to adulthood compared to a child without conduct problems (Baker, 2013) – these costs are principally due to special educational provision, legal costs, and additional benefits. In addition, individuals with CD often show lower estimated IQ, with particular impairments in verbal skills and reading, and it is not uncommon for these young people to drop out or be excluded from mainstream schools (Baker, 2013; Moffitt et al., 2001). This group is also at an increased risk of psychopathology in adult life, leading to greater unemployment, higher rates of alcohol and drug abuse, and increased risk of suicide (Baker, 2013; Odgers et al., 2007). Furthermore, around half of all youths with CD will go on to develop antisocial personality disorders in adulthood, with a high proportion continuing their criminal careers into adulthood (Robins, 1966; Robins, 1978).

1.3 Introduction to sex differences in CD

As a result of the higher prevalence of CD amongst males, the majority of research into the aetiology, clinical characteristics, and treatment of CD has focused on males. As a consequence, we know far less about the disorder in females, leading many researchers and clinicians to treat them in the same way. However, differences and similarities between the sexes across several domains of antisocial behaviour have been noted (e.g., Moffitt et al., 2001).

A large-scale prospective longitudinal study investigated sex differences in the relationship between early risk factors and the development of antisocial behaviour (Moffitt et al., 2001). The researchers found that although risk factors were generally similar in males and females, the sexes differed in their levels of exposure to these risk factors. For example, while family environment such as low socioeconomic status (SES), harsh discipline, and inconsistent parenting were strongly associated with later antisocial

behaviour in both sexes, individual risk factors, such as neurological abnormalities, poorer reading ability, and hyperactivity were more common in males prior to developing antisocial behaviour. Low IQ was a risk factor that was equally present in both sexes (Moffitt et al., 2001).

In contrast, child abuse seems to be more strongly linked with the development of antisocial behaviour and other forms of psychopathology in females compared with males (Burnette, Oshri, Lax, Richards, & Ragbeer, 2012; Herrera & McCloskey, 2001; Siegel & Williams, 2003). An estimated 35-40 % of antisocial females had been victims of sexual abuse compared to 8% of antisocial males (Chesney-Lind & Shelodon, 2013), which is far higher than rates of sexual abuse in the general population (7% versus 2.6% of 11-17 year old females and males, respectively; Radford et al., 2011).

The expression of CD also differs to some extent between the sexes. Relative to females, males show higher levels of aggression and antisocial behaviour across the life span. These differences can be seen as early as the toddler years, where males are more than twice as likely to show elevated levels of aggression compared to females (Baillargeon et al., 2007). Differences in aggression are reflected not only in higher rates of CD in childhood and adolescence, but in the substantial gap between males and females in the prison system; the Ministry of Justice reported a sex ratio in incarceration of 20.5:1 in favour of males across England and Wales (Ministry of Justice, 2015).

However, while forms of physical aggression such as fighting, physical bullying, and weapon use tend to be higher in males (Gorman-Smith & Loeber, 2005), relational forms of aggression such as manipulation, ostracism of peers, and non-physical bullying do not differ between the sexes in adolescence (or are higher in females; Underwood, 2003). Furthermore, while aggressions remains fairly stable (or increases) throughout the lifetime in males, aggression levels in females decrease at around age four (Keenan & Shaw, 1997) and then drastically increase during adolescence, where rates of non-aggressive antisocial behaviour become almost indistinguishable between the sexes

(Archer, 2004; McGee, Feehan, Williams, & Anderson, 1992). Thus, the higher prevalence of CD and incarcerations in males may be due to the fact that physical aggression is more likely to lead to arrest or punishment in the educational system compared to non-physical aggression.

Furthermore, adult consequences of having CD differ between the sexes. Males are more likely than females to both retain their diagnosis of CD through adolescence, and develop antisocial personality disorder (ASPD) in adulthood (48 % versus 16 % develop ASPD respectively; Moffitt et al., 2001). Females are more likely than males to switch from CD to other psychopathologies in late adolescence/early adulthood, such as borderline personality disorder, depression, or substance dependence (Pajer, 1998). However, although there does not seem to be any differences between the sexes in the severity of adult outcomes; adolescent CD increases risk of suicide, poverty, and unemployment for both sexes (Odgers et al., 2008), males display more homotypic continuity of their disorder in adulthood (i.e., ASPD) whereas females show more heterotypic continuity. The negative consequences for females might therefore be mediated by the development of additional psychiatric disorder (Samuelson, Hodgins, Larsson, Larm, & Tengström, 2010).

1.4 Considerations when studying sex differences

The evidence for somewhat different risk factors, trajectories, and adult outcomes of CD for males and females raises issues regarding sex differences in the assessment of CD. Since CD is more prevalent in males and the majority of research on CD has focused on males, several researchers have argued that the diagnostic criteria for CD is biased by the behavioural patterns typically displayed by males (i.e., physical aggression). As we have seen, the type, but not necessarily the rate, of aggression differs by sex, thus many symptoms of antisocial behaviour considered in the DSM criteria for CD might not be very suitable for assessing CD in females (Zoccolillo, 1993). It is possible that the sexes display

different symptoms that nevertheless reflect the same underlying impairment (Rutter, Caspi, & Moffitt, 2003). It has also been suggested that the number of symptoms required to reach a diagnostic threshold differ between males and females. Zoccolillo and colleagues (1996) assessed behavioural problems in over 1400 females and found that many six year old girls did not reach the DSM threshold of the disorder, despite displaying persistent and disruptive antisocial behaviour in kindergarten. If sex specific diagnostic thresholds were used, more females might be diagnosed early on in life, which may reveal a different picture of the prevalence of CD for females than has previously been thought (Zoccolillo et al., 1996).

Potential sex differences in the prevalence and expression of CD could partly be explained by the *polygenic threshold model* (Cloninger, Christiansen, Reich, & Gottesman, 1978). This model suggests that as antisocial behaviour is less common amongst females compared to males, females may require a higher loading of genetic, neurobiological, or environmental risk factors in order to develop antisocial behaviour. From this perspective, the females that do reach the threshold for a CD diagnosis may be more impaired compared to their male counterparts. Although this model has received some support from genetic studies, suggesting a higher heritability of antisocial behaviour in females (Gelhorn et al., 2005), other studies have found no significant difference between the sexes in the magnitude of genetic and environmental influences on antisocial behaviour (Burt, 2009; Rhee & Waldman, 2002).

1.5 Comorbidity

Rates of psychiatric comorbidity within CD samples are high, with externalising disorders such as attention-deficit/hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD) being the most common (Turgay, 2005). Although comorbid ADHD is more common in males with CD compared to females with CD - simply because CD is far more common in males – females are more likely to show CD+ADHD compared to CD or

ADHD alone, whereas males are more likely to have CD or ADHD alone compared to CD+ADHD (Waschbusch, 2002).

Furthermore, a young person with CD is more than twice as likely to have comorbid internalising disorders, such as depression and anxiety, compared to their healthy peers (Polier, Vloet, Herpertz-Dahlmann, Laurens, & Hodgins, 2012), and females with CD are twice as likely to have these comorbidities than males with CD (Rosenfield & Mouzon, 2013). These are important considerations when studying sex differences in CD, as comorbidity may alter the course of CD differently for males and females. For example, ADHD typically precedes CD, and while the presence of ADHD is associated with a fourfold increase in risk of developing CD as well as often resulting in more severe symptomology, the presence of CD does not appear to worsen the clinical symptoms of ADHD (Gaub & Carlson, 1997). Furthermore, having ADHD at a young age increases the risk of developing CD for females more than it does for males (Loeber & Keenan, 1994).

In summary, although males and females share many of the same risk factors, behaviours, comorbidities, and negative adult outcomes associated with CD, there is evidence for sex differences in the level of exposure to risk factors required to develop the disorder, types of antisocial behaviour (i.e., rule-breaking versus aggressive behaviour), the adult consequences of having the disorder, and types and rates of comorbidity. Next, we will discuss the challenge of heterogeneity within the disorder, and how that has influenced diagnostic strategies and research in this area.

1.6 Heterogeneity of CD

Regardless of the added complexity of comorbidity, heterogeneity of CD poses difficulties in assessment of the disorder. The behavioural approach to assessing CD is helpful as information relevant to diagnosis can be obtained from secondary sources, such as parents and teachers, or school and police records. However, compared to other disorders such as depression or anxiety, the criteria for CD do not require assessment of

internal processes that lead to the 'symptoms', i.e., the emotional or cognitive patterns that may underlie the behaviours in question (Blair, Peschardt, Budhani, Mitchell, & Pine, 2006).

This issue is exacerbated by the fact that there are over 32,000 possible combinations of symptoms that would yield a CD diagnosis (Nock, Kazdin, Hiripi, & Kessler, 2006), with the possibility that these have shared or distinct aetiologies. The broad classification of the disorder brings additional difficulties in terms of studying CD and developing treatment strategies. Thus, a considerable amount of research has been devoted to finding methods of subtyping the disorder that may help differentiate groups of CD individuals with distinct symptom profiles, developmental trajectories, and responsiveness to treatment.

1.6.1 Age of onset: The developmental taxonomic theory

The most influential of these subtyping approaches has been the developmental taxonomic theory of antisocial behaviour, put forward by Moffitt (1993). She proposed the age of onset of CD symptoms as a basis for sub-typing individuals in a way that helps distinguish between the different forms of antisocial behaviour with distinct causes. This model distinguishes between 'life-course persistent' antisocial behaviour, which includes individuals who display symptoms of CD in childhood (specified in the DSM-5 as appearing prior to the age of 10, and referred to as 'childhood-onset'), and 'adolescence-limited' antisocial behaviour, which includes individuals whose antisocial behaviours are restricted to the teenage years (referred to in the DSM-5 as 'adolescent-onset'). Using data from a longitudinal study, Moffitt's research group found strong support for this distinction on the basis of these subgroups having distinct childhood predictors and trajectories. Individuals with life-course persistent CD had a more severe manifestation of antisocial behaviour and were identified as being exposed to more childhood risk factors (such as abuse) and more likely to have neurodevelopmental impairments. Individuals with

adolescent-limited CD, on the other hand, did not show the same pathological backgrounds or severe manifestation as the life-course persistent individuals (Moffitt et al., 2001).

This classification of CD has received some empirical support and influenced the method used to diagnose and classify individuals with CD in the DSM-IV (APA, 1994) and DSM-5 (APA, 2013). However, there are issues with basing a classification of a disorder on retrospective accounts of symptom onset as this risks underestimating the age of onset of the first symptoms (Henry, Moffitt, Caspi, Langley, & Silva, 1994). In addition, the usefulness of the classification has been challenged by research showing no differences between childhood-onset and adolescent-onset CD on neuropsychological tasks (Fairchild, Van Goozen, Calder, Stollery, & Goodyer, 2009), neuroendocrinological measures of cortisol secretion (Haltigan, Roisman, Susman, Barnett-Walker, & Monahan, 2011), neuroanatomical measures of grey matter volume (Fairchild et al., 2011) and neural activity (Passamonti et al., 2010). Furthermore, there seem to be many individuals who only show antisocial behaviour in childhood, a so-called 'childhood-limited' antisocial group, and many individuals classified in the adolescence-limited group who continue to show serious antisocial behaviour and functional impairment in adulthood (Odgers et al., 2007, 2008). In addition, the majority of research supporting this approach has been based on male samples, and large-scale studies such as the Dunedin study suggest that childhood-onset CD is relatively rare in females (Moffitt et al., 2001). Indeed, most of the research on female CD has been conducted on adolescent samples. Thus, a critical issue concerns whether the age-of-onset classification is appropriate to females with CD. Silverthorn and Frick (1999) proposed that females might show a 'delayed-onset' form of CD, which, although it develops in adolescence, shares many similarities in terms of risk factors with childhood-onset CD in males (White & Piquero, 2004).

1.6.2 Behavioural subtypes: Aggressive and non-aggressive CD

CD can also be divided on the basis of whether aggressive behaviour is present. Violent antisocial behaviour is defined as acts of physical aggression such as fighting,

sexual assault, cruelty to humans and animals, and weapons use. Non-violent antisocial behaviours, on the other hand, refer to rule-breaking behaviours such as theft, vandalism, persistent lying, and truancy. Considerable support for the distinction between aggressive and non-aggressive classifications of CD comes from neuropsychological and family-based genetic research. It has been shown that while individuals displaying aggressive behaviour seem to be impaired on executive function tasks and perform less well on tasks measuring verbal IQ, non-aggressive antisocial individuals display intact, or sometimes superior, performance on these measures (Barker, Séguin, White, & et al, 2007). The same study also identified distinct developmental trajectories for the two groups. The study followed ~700 males from approximately age 12 until 31, with assessments of aggression and theft made every three years. Two trajectories were identified with regard to violence- those individuals on a high violence trajectory (13%) used violence by the age of 12 continuing into adulthood, while those on a low violence trajectory (87%) rarely engaged in physical aggression. The research group further identified four trajectories for theft; low theft through adolescence and adulthood, medium theft, a group that increased linearly in rates of theft with age, and a high theft group that showed an inverted U-shape in their theft trajectory, with the highest rates of theft seen at around 21 years old (Barker et al, 2007). These asymmetrical developmental trajectories for violence and theft imply that they have distinct aetiologies.

Furthermore, violent antisocial behaviour is more strongly related to genetic factors, while nonviolent antisocial behaviour is more related to environmental factors (Burt, 2009; Eley, Lichtenstein, & Moffitt, 2003). Monuteaux and colleagues (2004) looked at how these two constructs relate inter-generationally, and found that aggressive behaviour in fathers predicted aggressive, but not rule-breaking, behaviour in their biological children. The same relationship was found for fathers displaying mainly rule-breaking behaviour (Monuteaux, Fitzmaurice, Blacker, Buka, & Biederman, 2004). Individuals displaying aggressive behaviour in childhood and adolescence are also more

likely to continue showing aggressive behaviour, while rule-breaking behaviours are more likely to desist in adulthood (Broidy et al., 2003; Eley et al., 2003; Verhulst & Der Ende, 1995). In this respect, there is an overlap between this distinction and the age-of-onset classification - individuals with childhood-onset CD also tend to show more aggression compared to adolescent-onset CD individuals (Moffitt et al., 2001). This is complicated further by the fact that females with CD are more likely to show non-aggressive antisocial behaviour, or non-violent aggressive behaviour.

1.6.3 Personality traits: Callous-Unemotional traits

A third way of subtyping CD is according to the presence or absence of callous-unemotional (CU) traits. CU traits refer to personality features such as diminished empathy or guilt (Barry et al., 2000; Frick & White, 2008). This distinction also partly overlaps with the two previous subdivisions, as CD individuals with high CU traits have higher levels of aggression and tend to display more severe antisocial behaviour (Frick, Cornell, Bodin, et al., 2003; Frick, Ray, Thornton, & Kahn, 2014; Frick & White, 2008; Viding, Frick, & Plomin, 2007). Several studies have provided support for the idea that individuals with both CD and high levels of CU traits fare worse than those with CD alone (or low CU traits), that is, they tend to have more, and more severe, symptoms (e.g., violent crimes; Frick, Cornell, Bodin, et al., 2003). In addition, they have an impaired capacity for empathy (Blair, 2005b), are more likely to show both instrumental and reactive aggression (Fanti, Frick, & Georgiou, 2008), and are more impaired in recognising emotions (Barry et al., 2000; Loney, Frick, Clements, Ellis, & Kerlin, 2003). Due to this possible distinction between high and low CU traits, a 'limited pro-social emotions' specifier has been added to the diagnostic criteria for CD in the DSM-5 (APA, 2013). However, there are no formal definitions or guidelines of what constitutes 'high' and 'low' levels of CU traits. This causes issues both for diagnosis and research, as there is no standardised way of subdividing these groups, which runs the risk of adding to the issues of heterogeneity in the disorder and replicability between studies.

In addition, there is no evidence for specific impairment in those with high compared to low CU traits (Sully, Sonuga-Barke, & Fairchild, 2015; Sully, Sonuga-Barke, Savage, & Fairchild, 2016). Additional impairments seen in those with high CU traits may therefore simply be a consequence of a higher number of CD symptoms, as there is a high correlation between CU traits and CD symptom severity (Fairchild et al., 2011; Passamonti et al., 2010).

1.6.4 Alternative approaches

It should be noted that all of the approaches of subtyping CD mentioned above have received mixed empirical support, suggesting that more complex and multi-faceted approaches, which incorporate more aspects of the CD phenotype may be required to effectively sub-categorise the disorder. In an attempt to achieve this, some researchers have used data-driven approaches to subtyping rather than the previously mentioned hypothesis-driven approaches. These approaches have the power to identify clusters of symptoms based on the probability of certain symptoms occurring together (Vermunt & Magidson, 2004). Using this approach, Nock, Kazdin, Hiripi, and Kessler (2006) identified five subgroups of CD individuals from a population-based sample of over 3000 individuals: these were termed 'rule violation', 'deceit/theft', 'aggressive', 'severe covert behaviour', and 'pervasive CD symptoms'. Similar classes of CD individuals were found in an epidemiological study including over 20,000 individuals (Breslau, Saito, Tancredi, Nock, & Gilman, 2012). However, apart from the separation of aggressive and non-aggressive CD discussed previously, these classes have not been tested using neuropsychological or neuroimaging measures. Without some evidence that individuals in these classes perform differently in neuropsychological tasks or show distinct neurobiological underpinnings, the usefulness of these classifications remains uncertain, and replication of these findings is needed.

Other researchers have argued that applying a categorical approach in order to classify psychopathology is flawed altogether. Several studies have shown that

dimensional models (i.e., using a continuous scale of CD severity rather than identifying distinct groups), better explain externalising behaviour (Krueger, Markon, Patrick, & Iacono, 2005). In a sample of over 40,000 individuals in the USA, Marcon and Krueger (2005) found that categorical (latent class) models were less effective than continuous (latent trait) models in classifying individuals with externalising disorders, including antisocial personality disorder and alcohol-and substance abuse. Although this study was on adults, the continuous model was supported for both current and past symptoms, and across males and females, indicating that the results were robust (Markon & Krueger, 2005). These results have were later replicated in a mixed sex community sample of adolescents with antisocial behaviour (Walton, Ormel, & Krueger, 2011). Although there is compelling evidence that antisocial behaviour is better viewed as a continuous construct, it is difficult to use dimensional approaches in a clinical setting. The well-defined and clear criteria for all mental disorders in the DSM allows clinicians to base their diagnosis on the same symptom and standardised cut-offs. This facilitates understanding between clinicians and standardised treatment planning across clinics, as well as the facilitation of researchers in terms of systematically studying and replicating others' work. Thus, clinicians need, to some degree, a categorical classification system that increases the validity, reliability, and consistency of diagnosis and treatment (Coghill & Sonuga-Barke, 2012). This on-going debate has no straightforward solution, but it is clear that researchers and clinicians must work together and agree on the best way forward in classifying psychopathology that will be beneficial for both clinical practice and research.

In summary, CD is one of the most heterogeneous childhood disorders, and although considerable effort has been devoted to developing subtyping approaches, none of the existing approaches fully incorporate or are able to explain all of the clinical features associated with the disorder. In the next section, we will review theories of CD, which aim to explain the origin of the underlying mechanisms that may lead to antisocial behaviours.

1.7 Theories of CD

Neuropsychological studies of CD have found that these individuals tend to engage in risky decision-making, have lower levels of empathy, and show abnormal reinforcement learning and processing of negative stimuli/emotions (e.g., Lovett & Sheffield, 2007; Moffitt et al., 2001; Sonuga-Barke, Cortese, Fairchild, & Stringaris, 2016). Theories of psychiatric disorders emphasise the interplay between biology, cognition, and behaviour; with each domain also interacting with environmental factors (e.g., Morton & Frith, 1995). Although there are several models and theories regarding the development and manifestation of CD, no theory has been able to effectively integrate cognitive, behavioural, and biological aspects of the disorder to fully explain the deficit in cognitive and emotional processes seen by individuals with CD. Although some theories of CD have already briefly been discussed, such as the developmental taxonomic theory (Moffitt, 1993), this theory aimed to explain differences in severity and developmental trajectories of the disorder, rather than explaining impairments related to specific cognitive or emotional processes that might lead to CD. These processes, and the theories that attempt to explain them, will briefly be reviewed below.

1.7.1 Risky decision-making

Individuals with CD tend to make risky decisions, which are thought to arise from increased sensation seeking, and decreased ability to learn from punishment and negative consequences (Byrd, Loeber, & Pardini, 2013; Fairchild, van Goozen, et al., 2009; Sully et al., 2016; Syngelaki, Moore, Savage, Fairchild, & Goozen, 2009). It has been suggested that individuals with CD suffer from chronic physiological under-arousal. According to the *sensation-seeking theory*, this under-arousal drives them to engage in thrill-seeking or risky behaviour in order to increase their physiological arousal (Raine, Venables, & Mednick, 1997; Zuckerman, 1979). Studies investigating the relationship between physiological state and antisocial behaviour have found extensive support for lower resting

heart rate (Farrington, 1997; Portnoy et al., 2014; Adrian Raine, 2002a; Rogeness, Cepeda, Macedo, Fischer, & Harris, 1990) and skin conductance levels (Gatzke-Kopp, Raine, Loeber, Stouthamer-Loeber, & Steinhauer, 2002; Lorber, 2004) in youth with antisocial behaviour compared to controls. Such physiological markers predispose towards delinquent behaviour (Raine et al., 1997). In addition, a meta-analysis of heart rate responses in antisocial populations have found that both males and females with antisocial behaviour exhibit lower heart rate compared to their control groups (Ortiz & Raine, 2004). These studies provide some evidence for the sensation seeking theory to explain risky-decision making, but do not explain why the individuals chose to engage in antisocial behaviour rather than only thrill-seeking activities that are not against laws and rules of society.

1.7.2 Reduced fear and its effect on processing reward and punishment

In addition to increasing risk-seeking behaviour, Raine (1993) suggested that the low resting heart rate and skin conductance levels seen in antisocial individuals with high levels of psychopathic traits reflect abnormal emotion processing and reduced levels of fear. He proposed the *fearlessness theory*. According to this theory, healthy individuals experience levels of anxiety and fear when anticipating punishment (such as when engaging in rule or legal violations), while the under-active autonomic system in antisocial individuals decreases their levels of fear, thus decreasing feelings of anxiety associated with punishments and risky decisions, and hinders their ability to learn from punishment (Raine, 1993). Several studies have showed that high levels of CU traits are associated with reduced fear and emotional distress (Frick, Lilienfeld, Ellis, Loney, & Silverthorn, 1999; Pardini, Lochman, & Frick, 2003), as well as reduced response to punishment (Fisher & Blair, 1998; O'Brien & Frick, 1996). However, although these studies help to explain the behaviour of individuals with both CD and high CU traits, its applicability to antisocial behaviour more broadly has not been tested, and remains debatable. Furthermore, although the vast majority of research into CU traits has been conducted

using males (Frick & White, 2008), there is some evidence to suggest that the insensitivity to punishment is not present in females (Frick, Cornell, Barry, Bodin, & Dane, 2003).

1.7.3 Reduced empathy

The ability to understand others' emotional states, or as it is termed, empathy, is an important determinant of pro-social behaviour, and success in interpersonal relationships (Lovett & Sheffield, 2007), and normally acts to inhibit aggressive and antisocial behaviour (Eisenberg, Eggum, & Di Giunta, 2010). Reduced empathy is a key characteristic of CD with high psychopathy/CU-traits (Blair, 2005a; Carrasco, Barker, Tremblay, & Vitaro, 2006; Cohen & Strayer, 1996; Dadds et al., 2009) in both males and females (Dadds et al., 2009), and forms the basis of several theories of psychopathy (Blair, 2005b; Soderstrom, 2003). These theories posit that structural and functional abnormalities in brain regions such as the insula, anterior cingulate cortex, and the limbic system are at the core of these empathy deficits (Shirtcliff, Dahl, & Pollak, 2009). Similar to the theories of fearlessness and risk-taking, deficits in empathy have been linked to lower activity in the autonomic nervous system (Hastings, Zahn-Waxler, & McShane, 2006; Raine, 2002). Interestingly, aggressive behaviour shows a different relationship with empathy relative to high CU/psychopathic traits. While some have noted a positive correlation between empathy and aggression (Gill & Calkins, 2003) these results are highly inconsistent, and there is no clear link between aggression and empathy (Lovett & Sheffield, 2007). Although theories of reduced empathy focus on CD with high levels of psychopathic/CU traits, it would be beneficial to investigate whether this impairment is specific to high psychopathic/CU individuals or whether it extends to CD populations without CU traits.

1.7.4 Emotion regulation and distress cues

Related to impairments in empathy in antisocial individuals are deficits in recognising others' emotional expressions. It has been suggested that the recognition of others emotional states is the first crucial step in empathic processing (Marshall &

Marshall, 2011); impairments in recognising emotions and distress cues are therefore one of the most widely studied processes in antisocial populations. Individuals with CD are impaired in recognising negative facial expressions of fear (Fairchild, Van Goozen, et al., 2009; Stevens, Charman, & Blair, 2001), anger (Best, Williams, & Coccaro, 2002; Fairchild, Stobbe, van Goozen, Calder, & Goodyer, 2010; Fairchild, Van Goozen, et al., 2009), sadness (Fairchild, Van Goozen, et al., 2009; Sully et al., 2015), and disgust (Best et al., 2002; Fairchild et al., 2010; Fairchild, Van Goozen, et al., 2009). They also have decreased ability to recognise sad vocal tones in others (Stevens et al., 2001). Individuals with high CU/psychopathic traits further display lower accuracy and slower reaction times when responding to emotional faces (Barry et al., 2000) and words (Loney et al., 2003) compared to those with low CU/psychopathic traits. These deficits are thought largely to be a consequence of dysfunction of the brain circuits involved in emotion processing and reinforcement learning (Blair, 2007). According to the *integrated emotion systems (IES)* model (Blair, 2005a), aggressive behaviour can arise from a failure to identify distress cues in others. This is based on the idea that identifying such distress cues, such as sad or scared facial expressions, elicits compassion and motivates pro-social behaviour (Blair et al., 2006; Marsh, Kozak, & Ambady, 2007). Thus, deficits in identifying these distress cues can lead to a failure to inhibit aggression towards others (Blair, 2005a). This is thought to be particularly important in the development of instrumental aggression, i.e., goal-directed aggression. Although similar deficits in emotion recognition have been observed in females with CD (Fairchild et al., 2010; Schwenck et al., 2014), the neural mechanisms underlying emotion processing are not well understood. It is also unclear whether females with CD share the same deficits as males in this regard.

1.7.5 Hostile attribution bias

The *social information processing (SIP) theory* (Crick & Dodge, 1994) postulates that individuals with CD tend to interpret neutral or ambiguous stimuli as negative or threatening, a so-called 'hostile attributional bias'. Studies testing this theory have

commonly used vignettes of ambiguous social situations, and ask participants to state what their reaction would be if they were the person in the situation. Individuals with aggressive tendencies tend to show more hostile reactions in these ambiguous situations. Males also tend to act in a more hostile way than females (Fontaine, Burks, & Dodge, 2002). Furthermore, the cognitive pattern of this type of information processing, i.e., interpreting ambiguous stimuli or situations as threatening, predicted children's aggressive behaviour as early as one (Dodge, Laird, Lochman, & Zelli, 2002) and three years of age (Lansford et al., 2006). Support for this theory has also come from studies of violent offenders, who tended to label ambiguous emotional faces (Schönenberg & Jusyte, 2014) and neutral facial expressions (Hoaken, Allaby, & Earle, 2007) as negative or hostile. This attribution bias is also associated with self-reported aggressive tendencies in community samples (Burt, Mikolajewski, & Larson, 2009). In addition, the tendency of these individuals to show biased attention towards threatening stimuli extends to ambiguous and threatening words, both among offenders and within a healthy population with elevated aggressive tendencies (Smith & Waterman, 2003). Finally, there is some evidence to suggest that this bias is displayed by males, but not females, with antisocial behaviour (Frick, Cornell, Bodin, et al., 2003).

1.7.6 Conclusion

Many of the studies discussed, and indeed the theories proposed to explain the results, have been based on CD groups with high levels of CU traits. Although CU traits are important characteristics that frequently co-occur with CD, it is important to test these theories by contrasting individuals with CD and either high or low levels of CU traits to be able to conclude what characteristics are specific to those with high CU traits, and which applicable to those with CD more generally (irrespective of CU traits). Furthermore, irrespective of the possible distinction between high and low CU traits, none of the theories incorporate biological, cognitive, and behavioural elements in their models. For example, the IES model (Blair, 2005a; Marsh & Blair, 2008a), the fearlessness theory (Raine, 1993;

2013), and the sensation-seeking theory (Raine et al., 1997) focus almost exclusively on neurophysiological deficits. Furthermore, while Crick and Dodge's (1994) SIP model focuses exclusively on the cognitive impairments seen in antisocial youths, the developmental taxonomic theory (Moffitt, 1993) argues for a substantial role of biological and environmental causes but has been criticised for not clearly specifying the nature of the cognitive impairments associated with CD (Krol, Morton, & De Bruyn, 2004). Thus, none of the existing theories have successfully incorporated biological, cognitive, behavioural, and environmental aspects of CD, and it is questionable whether one model will succeed in doing so, given the complexity of the interactions between these domains and the heterogeneity of the disorder (Krol et al., 2004). Furthermore, it is not clear if and how any of the theories discussed above extend to females with CD. Although some similarities and differences have been found between males and females with CD in emotion recognition and empathy, there have been no studies to date on risky decision-making or sensation-seeking in females with CD to inform theories of these processes that extend to both sexes.

The next chapter will focus on neurobiological differences between males and females and the effects of age and puberty on brain development. Neuroimaging studies of CD will be discussed in relation to the clinical characteristics discussed previously.

Chapter 2: Sex differences in brain development and an overview of neuroimaging studies of conduct disorder

There are substantial sex differences in the development and organisation of the brain and the cortex more specifically (Cahill, 2014). It is critical to understand these sex differences in brain development in order to understand sex-specific expressions of neuropsychiatric disorders, such as differences in the age of onset, prevalence, and symptomatology (Cosgrove, Mazure, & Staley, 2007; Giedd, Raznahan, Mills, & Lenroot, 2012). Normative sex differences may therefore go some way in explaining the differences between males and females with CD discussed in Chapter 1. This chapter will lay the foundation for the case-control neuroimaging studies in the chapters that will follow, by first considering sex differences in the organisation and development of the brain in normative populations, as well as the issues that need to be considered when including both of the sexes in neuroimaging studies. Following this overview, the chapter will briefly review structural and functional findings from previous neuroimaging studies of CD and document the striking differences in numbers of males and females with CD included in previous studies in this area - and why this matters.

2.1 Sex differences in the development of the brain

On average, males have ~10 % larger overall brain size compared to females, a robust relationship that holds true from childhood to adulthood (Bellis et al., 2001; Giedd, Castellanos, Rajapakse, Vaituzis, & Rapoport, 1997; Goldstein et al., 2001; Gur & Gur, 2016). The difference in total brain size is partly due to the overall larger body size in male and thus reflects a relative difference. Interestingly, when overall brain size is taken into account, it has been found that there are few differences between the sexes (Bellis et al., 2001), and if anything, females have more grey matter volume compared to males (Lenroot

et al., 2007), as well as thicker cortex overall (Luders et al., 2006). These differences seem to be most pronounced in frontal regions of the brain, and dorsal striatum (Bellis et al., 2001). Males, on the other hand, seem to have comparatively greater volumes in limbic and paralimbic structures such as the amygdala (Giedd et al., 1996, 2012; Ruigrok et al., 2014).

There are also sex differences in the relationship between age and white- and grey matter development. White matter volume increases linearly up to adulthood for both sexes, and across brain regions, but the increase is slower in females; a ~17% increase in females versus ~45% increases in males between the ages of 6 and 18 years (Bellis et al., 2001). Furthermore, although the trajectory of grey matter volume follows an inverted U-shaped curve for both males and females, the sexes differ in the timings of these curves, peaking between 8.5 – 11 years in females, compared to between 10.5 - 12 years in males (Figure 2.1); Giedd et al., 1999, 2012; Lenroot et al., 2007).

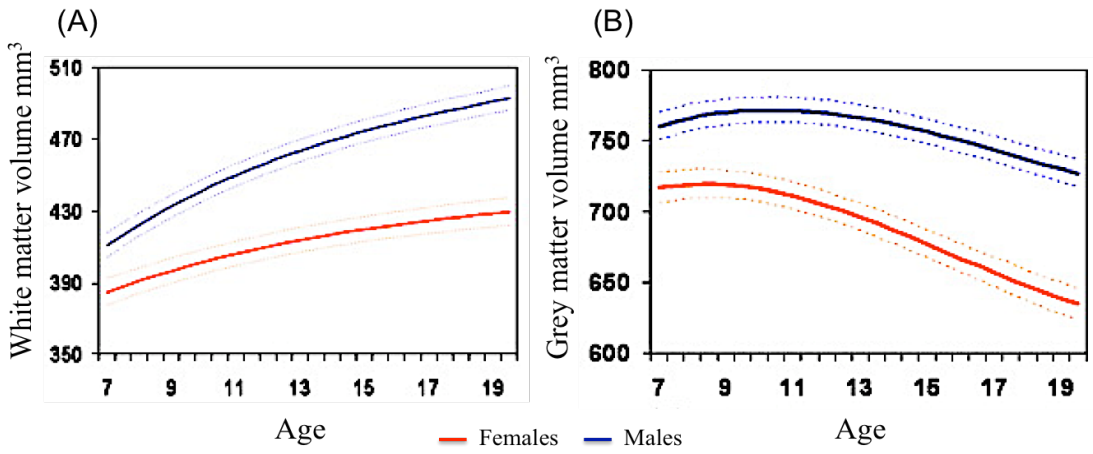

Figure 2.1. The relationship between age and (A) white matter volume and (B) grey matter volume for males (blue line) and females (red line). *Adapted from* "Sexual dimorphism of brain developmental trajectories during childhood and adolescence" by R. K Lenroot *et al.,* 2007, *NeuroImage, 36*(4), p 1068. Copyright 2016 by Elsevier B.V.

In addition, grey matter volume is a composite measure that is made up of different cortical properties which have distinct aetiologies and developmental trajectories (Panizzon et al., 2009). These properties include the thickness of the cortex (CT; measured in mm as the distance between the grey-white matter boundary and the grey-cerebrospinal

fluid boundary), the surface area (SA) of the cortical sheet, and gyrification (the degree of cortical folding within a sulcus compared to that outside the sulcus; (Dale, Fischl, & Sereno, 1999; Fischl & Dale, 2000; Winkler et al., 2012)). While CT and SA follow an inverted-U trajectory, peaking at 8.5 years and 9 years respectively, gyrification peaks at around 1.5 years and decreases steadily until adulthood (Raznahan et al., 2011). In addition, while SA and gyrification have different developmental trajectories for males and females, such that females peak earlier than males, there is no difference between the sexes in the development of CT (Raznahan et al., 2011). Of note, the developmental trajectories and sex differences of these cortical properties also vary substantially across the brain (Vijayakumar et al., 2016)

There are also sex differences in the connections across brain regions. Ingalhalikar et al., (2014) investigated the microstructural properties of white matter tracts in 949 healthy children and adolescents and found greater intra-hemispheric connections for males, while females had greater inter-hemispheric connections. The authors interpreted these findings as support for suggested differences in functions between the sexes; intra-hemispheric connections facilitate connectivity between perception and coordinated actions, while inter-hemispheric connections facilitate connections between analytical and intuitive processing networks (Ingalhalikar et al., 2014). Other studies that have investigated sex differences in the functions of the brain have proposed that females have a higher overall blood flow to the brain compared to males, both during resting and cognitive activities (Cosgrove et al., 2007). Finally, it has been suggested that males and females differ in brain activation during emotion regulation (McRae, Ochsner, Mauss, Gabrieli, & Gross, 2008) and processing (Killgore & Yurgelun-Todd, 2001), and empathy (Derntl et al., 2010). The differences observed in healthy males and females in terms of both structure and function are important to take into account when investigating the neural basis of psychiatric disorders. If groups are not matched on sex - which is often the case in neuroimaging literature - it is possible that effects that are interpreted as diagnostic effects

could in fact reflect sex differences. This point is important to consider because there are sex differences in the prevalence of many disorders – for example, as we saw in Chapter 1, externalising disorders such as CD are far more common in males than females.

2.2 The effect of puberty on the brain

Increases in grey matter during early childhood are thought to reflect a second wave of synaptic production (Giedd et al., 1999), whereas decreases are likely to reflect dendritic pruning processes (as opposed to cell loss; Obonai, Mizuguchi, & Takashima, 1998). However, it is not well established what causes these increases and decreases, and why they seem to differ between males and females. The timing of the grey matter peaks correspond roughly to the onset of puberty, suggesting a strong association between pubertal maturation and brain maturation, and that the onset of puberty may trigger dendritic pruning processes (Lenroot et al., 2007). Support for this notion came from Campbell, Grimm, Bie, & Feinberg (2012) who found that pubertal timing strongly predicted the steep decline in delta waves seen from early to late adolescence, which in turn is thought to be driven by synaptic pruning. The relationship between synaptic pruning, decline in delta waves, and pubertal timing holds irrespective of the onset of puberty - that is, individuals who started puberty early started their decline of delta waves earlier compared to late starters (Campbell et al., 2012).

However, it is not known whether the sex hormones that determine onset of puberty are able to influence brain structure differences only in utero and early childhood, or if they also have a direct effect on the developing brain throughout puberty. However, some research has shown that gonadal steroid (pubertal) hormones do seem to have a direct influence on brain development in adolescence, specifically the increase in amygdala volume in males and hippocampal volume in females (Neufang et al., 2009).

Although the impact of pubertal onset on brain development is not well understood, it is interesting to note that the timing of puberty seems to affect males and females

differently. A large-scale longitudinal study that followed girls from pre-puberty to adulthood found that early maturation is associated with a wide range of negative outcomes in adolescence, including higher rates of antisocial behaviour and substance-related problems (Copeland et al., 2010). Furthermore, the problems experienced by early-maturing girls seem to be limited to the adolescent period, and there is little evidence of persistent, long-term effects for the majority of the early-starters (Copeland et al., 2010). In contrast, pubertal timing seems to have mixed effects on males, with some suggesting early starters having higher rates of externalising problems (Kaltiala-Heino, Marttunen, Rantanen, & Rimpelä, 2003), while others suggest pro-social benefits (Stattin & Magnusson, 1990).

The drastic increase in antisocial behaviour seen in females going into puberty, together with the fact that females are more likely to show remission from CD than males, may therefore be partly explained by an early onset of puberty in a subset of females.

Taken together, we have seen that there are substantial sex differences in the development of the brain, as well as the structural and functional organisation of the brain. These differences have implications not only in terms of ensuring that samples are matched for sex, as discussed above, but also in the ages of the sample selected. Age is closely linked to the developmental curves seen in grey matter, thus the magnitude of sex differences may depend on the age of the samples being compared (Giedd et al., 2012; Ruigrok et al., 2014). Furthermore, it is likely that the pubertal development is closely linked to brain development (Lenroot et al., 2007). Thus, it is important that samples not only are matched on age, but to avoid averaging across different developmental stages, a narrow age-range should be selected. Furthermore, since females tend to go through puberty earlier than males (Marshall & Tanner, 1986), it is beneficial to ensure that males and females are at the same pubertal stage in studies of sex differences. This is especially an issue if, as will be discuss below, studies use samples with an age-range of up to 6-7 years (potentially averaging across several different stages of pubertal development).

In summary, sex differences in the size and organisation of the brain are highly robust findings. Studies of brain development point to the onset and course of puberty as factors that influence the development of the brain, and may offer an explanation as to the different timing of increases and decreases of grey matter seen in males and females. Sex differences in brain development in the normative population may offer important insights into sex differences in the neurobiology of different forms of psychopathology. In the next section, we will review findings of structural and functional brain abnormalities in the CD population, and highlight further methodological issues facing neuroimaging research in this area.

2.3 Functional and structural brain abnormalities in the CD population

Studies that have investigated structural and functional brain differences in children and adolescents with CD have found some overlap in terms of the areas identified as showing differences compared to healthy control groups. The most common structures implicated in CD are the amygdala, striatum, ventromedial- and orbitofrontal cortex, anterior cingulate cortex, and insula (Fairchild et al., 2011; Huebner et al., 2008; Sterzer, Stadler, Krebs, Kleinschmidt, & Poustka, 2005; Stevens & Haney-Caron, 2012). In the typically developing population, these brain regions have been associated with emotional regulation and recognition, empathy, and reward processing (Bush et al., 2002; Knutson, Fong, Adams, Varner, & Hommer, 2001; Sergerie, Chochol, & Armony, 2008; Singer, Critchley, & Preuschoff, 2009).

2.3.1 Reward and punishment processing in the brain

Studies in normative populations investigating reward and punishment have identified brain networks including orbitofrontal cortex, amygdala, striatum (O'Doherty, 2004), and anterior cingulate cortex (Bush et al., 2002). As discussed in Chapter 1, CD is associated with increased risk-taking and abnormal processing of reward and punishment

(Byrd et al., 2013; Syngelaki et al., 2009). fMRI studies have shown lower activity during processing of reward in orbitofrontal cortex (Rubia et al., 2009) and striatum (Cohn et al., 2015; Finger et al., 2011), and higher activity during processing of punishment in ventromedial prefrontal cortex (Finger et al., 2008), striatum (White et al., 2013), and amygdala (Cohn et al., 2015) in individuals with CD compared with controls. Of note, these studies included relatively small sample sizes (n<20), and none had the power to look for any sex differences. Furthermore, the samples included individuals with CD and high levels of psychopathic traits, making it difficult to separate out the effects of CD and psychopathic traits.

2.3.2 Empathy

Decreased activation of the insula, inferior frontal gyrus, and anterior cingulate cortex have been found in individuals with CD and high levels of CU traits while viewing images of people in pain (Lockwood et al., 2013; Marsh et al., 2013). This lower activity is thought to reflect reduced ability to experience empathy in this population. However, in a similar study involving viewing images of others in pain. Decety, Michalska, Akitsuki, and Lahey (2009) found *higher* activation in medial and orbitofrontal cortex in a small group of CD males compared to healthy controls. The authors proposed that the higher activation observed when viewing others in pain might reflect an increase in arousal in a subset of CD males. Indeed, Lockwood et al (2013) included individuals with varying levels of CU-traits, which are associated with lower physiological responses to distressing stimuli (Hastings et al., 2006; Raine, 2002), whereas Decety et al. (2009) specifically tested individuals with aggressive CD, showing yet another example of the challenges posed by the heterogeneity in samples between studies of CD.

2.3.3 Emotion processing

Emotion processing in CD populations has received a great deal of attention in the last decade. This is not surprising, considering several theories of CD and

CU/psychopathic traits build upon the deficits in recognising and understanding others emotions and intentions seen in these individuals (Blair, 2005a). Studies of emotion processing have commonly found abnormal activation in CD groups in several of the cerebral regions associated with emotional processing. These include the amygdala (Phelps & LeDoux, 2005), insula (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003), orbitofrontal- and ventromedial prefrontal cortex (OFC, vmPFC; Davidson & Irwin, 1999), and anterior cingulate cortex (Etkin, Egner, & Kalisch, 2011) - providing strong support for the argument that CD is associated with deficits in emotion processing.

As with the fMRI studies of empathy, findings of emotion processing are inconsistent. Most commonly, CD has been associated with lower activity in the amygdala during fear processing (Jones, Laurens, Herba, Barker, & Viding, 2009; Marsh et al., 2008; White, Marsh, et al., 2012), and in the amygdala, vmPFC, OFC, and insula during anger processing (Passamonti et al., 2010). However, while it remains unclear whether CD subtypes are related to these effects; CD and high CU/psychopathic traits have been associated with hypo activity, whereas CD and low CU/psychopathic traits have been associated with hyper activity in the limbic system (Sebastian et al., 2013), it is clear that CD in general is liked to abnormal emotion processing. The hypo activity seen in CD individuals with high CU/psychopathic traits fits well with Blair's (2005) IES model, which suggests that increased aggression may arise from a deficit in recognising distress cues- this deficit in recognising distress cues may be mediated by lower neural activity in response to these cues. In contrast, the hyper activity seen in individuals with CD and low CU/psychopathic traits could be explained through the SIP theory (Crick & Dodge, 1994) discussed in Chapter 1, and may thus be a consequence of their increased attention to distress stimuli compared to controls, and their tendency for hostile attribution bias.

2.3.4 Structural MRI studies of CD

In addition, the areas identified as showing abnormal activation in CD groups overlap with those highlighted as altered in structural MRI studies of these populations.

These have found lower GMV in CD groups compared to controls in the insula (Fahim et al., 2011; Sterzer, Stadler, Poustka, & Kleinschmidt, 2007), amygdala (Fairchild et al., 2011; Huebner et al., 2008; Sterzer et al., 2007), orbitofrontal- (Fairchild et al., 2011; Huebner et al., 2008; Sebastian et al., 2015), medial/ ventromedial prefrontal cortex (PFC; Fahim et al., 2011; Olvera et al., 2014; Rogers & De Brito, 2016), and anterior cingulate cortex (ACC; Olvera et al., 2014; Sebastian et al., 2015; Sterzer et al., 2005). Interestingly, higher GMV has also been found in male children with high levels of CU traits (De Brito et al., 2009).

2.3.5 Evaluation of structural and functional MRI studies

As discussed in section 2.2, age and pubertal timing are important factors to consider when comparing groups in a cross-sectional neuroimaging study. The published functional and structural MRI studies of CD vary greatly in the ages of the samples. Some include children as young as eight or nine years of age (Fahim et al., 2011; Michalska, Decety, Zeffiro, & Lahey, 2015; Michalska, Zeffiro, & Decety, 2016; Stadler et al., 2007; Sterzer et al., 2005), while others have participants over 20 years of age (Fairchild et al., 2011; Fairchild, Hagan, et al., 2013; Passamonti et al., 2010). Although it is important to understand what happens at different developmental stages, this variation in ages between studies increase difficulty in replication. Even within a single study, the age ranges often span up to seven years (Huebner et al., 2008; Marsh et al., 2013; Passamonti et al., 2010; Rubia et al., 2008), more or less covering the entire pubertal period. This issue is exacerbated when combining both sexes in one study without testing for sex differences, since males and females show different brain development trajectories (Giedd et al., 1997; Lenroot et al., 2007). Further, although several studies have included a sample that, as a consequence of their age, would likely decrease the risk of averaging across pubertal stages (i.e., ages 16-21; Passamonti et al., 2010), none of the studies have systematically matched their groups on puberty or included stage of pubertal development as a covariate.

Considering the evidence that pubertal status influences brain development, this is potentially a considerable limitation of previous neuroimaging studies.

Furthermore, there is substantial heterogeneity between samples (Table 2.1 and Table 2.2), with some studies using a pure CD sample, some only included CD with high levels of CU or psychopathic traits, CD with comorbid substance dependence, or used disruptive behaviour disorder samples with a mixture of CD and ODD diagnoses.

Finally, there has been a gross underrepresentation of females in the studies of CD performed to date. The majority of studies have either not included females in the sample, or have only included a small number, and have therefore been unable to investigate whether there were any sex differences in the relationship between CD and brain structure, or whether CD in females is associated with any structural or functional abnormalities. Across all structural MRI studies of adolescent CD, 557 males with CD compared to only 170 females with CD have been studied (Table 2.1). The difference in sample sizes between the sexes is even greater in functional MRI studies, with a total of 541 CD males compared to 106 CD females included in these studies (Table 2.2). This striking underrepresentation of females with CD is likely a reflection of the sex ratio in diagnosis; however, this is a major limitation in the literature, as potentially overlooking important differences between the sexes runs the risk of distributing intervention programs, therapy, or pharmacological treatment to CD that have largely been based on studies of males with the disorder. As we have seen in Chapter 1, the risk factors, clinical presentation, and to some extent, adult outcomes differ between males and females with CD. Furthermore, as we have seen in sections 2.1 and 2.2 of this chapter, there are substantial normative differences between the sexes in the organisation and development of the brain. The fact that the majority of the previous studies mentioned in Table 2.1 and Table 2.2 have combined males and females in their groups, often with grossly unequal numbers in favour of males, means that results are likely influenced by both the presence of sex differences in the CD groups, and normative sex differences in brain development. The effects of sex and

CD diagnosis cannot be teased apart when groups are averaged across sexes and when samples are predominantly male.

Sex differences have only recently gained attention, with neuroimaging studies from just two research groups investigating the relationship between CD/DBD and brain structure (Fairchild, Hagan, et al., 2013a; Michalska et al., 2015), and function (Fairchild et al., 2014; Michalska et al., 2016) in females. These studies have found preliminary evidence for both similarities and differences between the sexes. However, the aforementioned studies still included relatively modest samples sizes (n~20; Table 2.1 and 2.2), and were not comparable in terms of method used for the structural analysis (i.e., factorial versus correlational analysis), nor did they use the same functional task (i.e., emotion processing versus empathic processing).

In summary, functional and structural neuroimaging studies largely overlap in terms of CD-related alterations. Abnormal activity and structure in the amygdala, striatum, insula, and areas in the prefrontal cortex are consistently seen in individuals with CD. The findings from these studies support the association between CD and altered emotion processing, empathy, reward learning, and decision making discussed in Chapter 1. However, there is considerable variability between studies in age, comorbidity, and characteristics of the CD samples, making interpretation and replication difficult. In addition, females with CD have been severely underrepresented across both functional and structural neuroimaging studies, with no studies including a large enough sample to reliably test for sex differences in the relationship between CD and brain structure and function.

Table 2.1. Summary of structural MRI studies of conduct disorder and related disorders

Study	Sample description	M : F	Age	Method
Sterzer et al (2007)	CD	12:0	12*	VBM
Huebner et al (2008)	CD	23:0	12-17	VBM
De Brito et al (2009)	CP + CU	23:0	10-13	VBM
Fairchild et al (2011)	CO-CD and AO-CD	63:0	16-21	VBM
Fahim et al (2011)	DBD	12:10	8*	SBM
Stevens et al (2012)	CD	12:12	15-16	VBM
Hyatt et al (2012)	CD	10:9	12-18	SBM
Wallace et al (2012)	CD	16:6	15*	SBM
Fairchild et al (2013)	CD	0:22	14-20	VBM
Ermer et al (2013)	CD + psychopathy	191:0	17*	VBM
Cope et al (2013)	CD + psychopathy	0:39	15-19	VBM
Sarkar et al (2014)	CD	21:0	12-19	SBM
Olvera et al (2014)	CD, CD+BPD	12:12	13-17	VBM
Fairchild et al (2015)	CD	36:0	16-21	SBM
Hummer et al (2015)	DBD	21:12	13-17	VBM
Sebastian et al (2015)	CP + CU	60:0	10-16	VBM
Michalska et al (2015)	DBD	23:20	9-11	VBM
Dalwani et al (2015)	CD + substance use	0:22	14-18	VBM
Jiang et al (2015)	AO-CD	22:6	14-16	SBM

*Note: AO-CD = adolescent onset conduct disorder, BPD = bipolar disorder, CD = conduct disorder, CO-CD = childhood onset conduct disorder, CP = conduct problems, CU = callous unemotional traits, DBD = disruptive behaviour disorders, F = females, M = males SBM = surface-based morphometry, VBM = voxel-based morphometry. * Indicates mean age when age range was not available.*

Table 2.2. Summary of fMRI studies of conduct disorder and related disorders

Study	Sample description	M : F	Age	Task used
Sterzer et al. (2005)	CO-CD	13:0	9-15	Passive viewing of IAPS pictures
Stadler et al. (2007)	CO-CD	13:0	9-14	Passive viewing of IAPS pictures
Banich et al. (2007)	CP + substance use	12:0	14-18	Stroop task
Marsh et al. (2008)	DBD + CD	7:5	10-17	Emotion processing
Herpertz et al. (2008)	CO-CD	22:0	12-17	Passive viewing of IAPS pictures
Finger et al. (2008)	DBD + CD	9:5	10-17	Probabilistic reversal learning
Jones et al. (2009)	CP + CD	17:0	10-12	Emotion processing
Decety et al. (2009)	CO-CD	8:0	16-18	Empathy for pain task
Rubia et al. (2008)	CO-CD	13:0	9-16	Stop task
Rubia et al. (2009a)	CO-CD	14:0	9-16	Rewarded performance
Rubia et al. (2009b)	CO-CD	13:0	9-16	Simon interference task
Gatzke-Kopp et al. (2009)	Externalizing disorder	19:0	12-16	Monetary incentive delay
Bjork et al. (2010)	Externalizing disorder	9:3	13-17	Monetary incentive delay
Passamonti et al. (2010)	CO-CD and AO-CD	40:0	16-21	Emotion processing
Rubia et al. (2010)	CO-CD	14:0	9-17	Switch task
Crowley et al. (2010)	CD + substance use	20:0	14-18	Risk taking task
Finger et al. (2011)	DBD + CU	9:6	14*	Passive avoidance learning
Marsh et al. (2011)	DBD + CU	8:6	10-16	Implicit association with
White et al. (2012a)	DBD + CU	13:4	10-17	Spatial attention with eye
White et al. (2012b)	DBD + CU	12:3	10-17	Face processing
Sebastian et al. (2012)	CP +/- CU	31:0	10-16	Affective theory of mind task
Viding et al. (2012)	CP +/- CU	31:0	10-16	Subliminal face
Cogn et al (2013)	DBD +/- psychopathy	38:12	17*	Fear conditioning
White et al. (2013)	DBD + CU	17:3	10-18	Passive avoidance task
Marsh et al. (2013)	DBD + CU	8:6	10-17	Empathy for pain task
Sebastian et al. (2013)	CP +/- CU	34:0	10-16	Empathy for pain task
Lockwood et al. (2013)	CP +/- CU	37:0	10-16	Empathy for pain task
Fairchild et al (2014)	CP +/- CU	0:20	17*	Emotion processing
Cohn et al (2015)	DBD +/- psychopathy	34:11	17*	Reward processing
Michalska et al	CP +CU/aggression	26:22	9-11	Empathy for pain task

*Note: AO-CD = adolescent onset conduct disorder, CD = conduct disorder, CO-CD = childhood onset conduct disorder, CP = conduct problems, CU = callous unemotional traits, DBD = disruptive behaviour disorders, F = females, M = males. * indicates mean age when age range was not available.*

2.4 Aim of the thesis and outline of remaining chapters

The aim of this thesis is to investigate sex differences in the relationship between adolescent CD and neuroanatomical and functional brain abnormalities. Firstly, Chapter 3 will give an overview of the FemNAT-CD study, which this thesis is based on, and

describe the methods used to obtain the data. Chapter 4 will describe the use of voxel-based morphometry (VBM) to explore similarities and differences in the relationship between CD and grey matter volume alterations. Chapter 5 will extend the analysis on brain structure by using surface-based morphometry to investigate sex differences in the relationship between CD and cortical structure (cortical thickness, surface area, and gyrification). In Chapter 6, we will examine sex differences in CD-related differences in emotion processing between males and females with CD compared to their respective control group, in a subset of the total sample. Finally, in Chapter 7, findings from the three chapters will be summarised, integrated, and discussed in relation to previous studies in the area. Here, clinical and scientific implications of the results will be described.

Chapter 3: General methods

This chapter will outline the methods and analytical approaches used in the FemNAT-CD study in general and those used in this thesis.

3.1 Sample recruitment

The Female Neurobiology and Treatment of Conduct Disorder (FemNAT-CD) study is a large-scale multi-disciplinary European study investigating the neurobiology of female conduct disorder (CD), and the extent to which males and females with CD share similar clinical, neuropsychological, and neurobiological profiles. The project set out to recruit 1,840 children and adolescents aged between 9 to 18 years from 11 universities and psychiatric clinics across Europe (Southampton and Birmingham, UK; Frankfurt, Aachen, and Heidelberg, Germany; Basel, Switzerland; Amsterdam, Netherlands; Bilbao and Barcelona, Spain; Athens, Greece; Szeged, Hungary). A sub-sample of 600 participants will then to be re-assessed 18 months after their first assessment in a longitudinal study at each site.

Briefly, the project has 6 main work-packages: 1) *Genetics and Epigenetics*, which will investigate genetic and environmental risk factors and gene-environment interactions; 2) *Neurocognitive Characteristics*, including behavioural measures of emotion recognition, emotion regulation, and learning, and questionnaire measures (e.g., psychopathy, aggression, empathy); 3) *Psychophysiology and Neuroendocrinology*, involving the investigation of the autonomic nervous system (heart rate and skin conductance) during emotional and stressful tasks, and collection of cortisol from saliva samples; 4) *Neuroimaging,* involving the use of magnetic resonance imaging (MRI) techniques to study brain structure and function; 5) *A randomized control trial*, evaluating group-based psychological treatment using Dialectic behavioural therapy and; 6) *Oxytonergic and serotonergic transmission*, including implementing animal models of aggression, and

investigating the effect of manipulating brain levels of serotonin and oxytocin on aggression and emotion processing.

The neuroimaging assessments were performed on a subsample of the total recruited sample, and were undertaken at five of the 11 sites (Southampton, Birmingham, Aachen, Frankfurt, and Basel). The sample used in Chapters 4 and 5 in the current thesis was selected from participants that were tested between April 2014 and April 2016 at four of these data collection sites (Frankfurt, Aachen, Southampton, and Birmingham; see Table 3.1 for distribution across sites). At the time of data analysis (April 2016), the longitudinal part of the study had not yet begun, thus all data included in this thesis are from the cross-sectional part of the FemNAT-CD study. Within this time period, the fifth neuroimaging site (Basel, Switzerland) was focused on the recruitment of female participants. Given the focus of the current thesis on sex differences, the sample was selected from the four remaining sites that had recruited both male and female participants in order to ensure approximately equal numbers in each group from each site. Participants were selected if they were between 14 and 18 years old, had a late- or post-pubertal status (as measured with the Pubertal Developmental Scale (Petersen, Crockett, Richards, & Boxer, 1988), see section below), and had a good quality T1-weighted structural scan. The age and pubertal status restriction in the current study was put in place to ensure comparability of the developmental stage reached by individuals in the different groups (Lenroot et al., 2007).

Table 3.1. Number of participants in the overall sample contributed by each of the four sites

Groups	Site 1 Frankfurt (*n*=32)	Site 2 Aachen (*n*=42)	Site 3 Southampton (*n*=64)	Site 4 Birmingham (*n*=62)	Chi Square (p value)
No. F-CD	7	11	16	14	X^2=2.24 (.99)
No. M-CD	5	11	16	16	
No. F-HC	10	10	16	16	
No. M-HC	10	10	16	16	

Note: F-CD = females with conduct disorder; F-HC = female healthy controls; M-CD = males with conduct disorder; M-HC = male healthy controls. Differences between sites were tested using a Chi Square test.

Based on these criteria, 96 adolescents (48 females) with CD and 104 adolescents (52 females) without psychiatric disorders were included in the structural MRI studies (Chapters 3 and 4). The sample reported on in Chapter 5 comprised a subsample of these

groups from the Southampton and Birmingham sites. Due to fMRI data sharing protocols between countries in the FemNAT-CD study, the data from three sites (Aachen, Frankfurt, Basel) were not available to researchers at the University of Southampton by April 2016. Hence, the subsample was selected from the available data, and represent only those recruited from the UK sites. Participants were recruited from mainstream schools and colleges, and CD participants were additionally recruited from pupil referral units, clinics, and youth offending services. Clinical diagnoses of CD and comorbid disorders for research purposes were made using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL), which is a semi-structured interview based on DSM-IV criteria that can be used with young people and their parents (Kaufman et al., 1997).

3.2 Exclusion criteria

Study exclusion criteria were an estimated full-scale IQ of < 70, the presence of any neurological disorders (e.g., epilepsy), any serious psychiatric conditions or pervasive developmental disorders (e.g., schizophrenia, autism spectrum disorders, bipolar disorder- as assessed by the K-SADS-PL), and a history of head trauma. In addition, we also applied standard MRI exclusion criteria (e.g., whether the subjects were claustrophobic or had ferro-magnetic metal in their bodies). Questions relating to these criteria were asked at the time of the interview or over the phone prior to the MRI scanning sessions, using the MRI safety screening (Appendix 1). We also asked female participants whether there was any possibility that they could be pregnant – this was a further exclusion criterion. All participants had to be fluent in English in order to understand the safety aspects of the MRI procedures and to complete the questionnaires. The healthy control groups were free of current DSM-IV Axis I disorders, as assessed using the K-SADS-PL.

3.3 Diagnostic assessment

The K-SADS-PL is a semi-structured diagnostic interview, based on DSM-IV criteria (APA, 2013), which assesses current and lifetime history of psychiatric disorders in children and adolescents. The interview covers five overall domains: Affective, psychotic, anxiety, and behavioural disorders, substance abuse and other disorders. The screening used in the current study assessed for the presence of 23 disorders (Appendix 2). The interviews were conducted separately with the participants and their parents by trained masters- or doctoral-level staff. At the start of the interview, basic demographic information regarding participants' sex and date of birth were collected, as well as information about whether the participants were in contact with child and adolescent mental health services, or were taking any medication for mood or behavioural problems at the time of the interview or had done so in the past. This section had the potential to yield useful initial information regarding potential psychopathology prior to the screening section of the interview. Following this, participants and their parents were asked screening questions related to key symptoms of each of the 23 disorders (e.g., persistent low mood in the major depressive disorder screen). For diagnoses not central to the study design if the participant endorsed any of the symptoms of the psychiatric diagnoses on the initial screen, the interviewer then went on to complete the full K-SADS-PL supplement for that specific disorder. However, full supplements for CD, ODD, and ADHD were completed for every participant in order to obtain dimensional information on these disorders for the entire sample (See appendices 3-5, respectively). After the interview was completed, the two interviewers computed a combined score from the participant and their parent. If either the participant or their parent endorsed a symptom to threshold, it was considered present (Kaufman et al., 1997).

A current research diagnosis of CD was given if parent or participant reported three or more of 15 symptoms as occurring over the previous 12 months in addition to at least one symptom being present in the last six months (APA, 2013). For this study, participants

were only included in the CD group if they met the criteria for current CD, however, symptoms reported prior to the last year were considered when assessing the severity of the disorder (i.e., number of lifetime CD symptoms) and to classify the age of onset of the disorder. Both of these measures were used in all subsequent analyses. Childhood-onset CD was defined by the presence of at least one CD symptom prior to the age of 10 years, and a full CD diagnosis thereafter, whereas adolescence-onset CD was diagnosed if the individual only developed CD symptoms after age 10 (APA, 2013).

3.3.1 Medication

Information about current medication use was obtained at the start of the K-SADS-PL interview. We also obtained information regarding the reason for the prescribed medication, the amount taken, and the period in which it was taken. For the purpose of the current study, only the presence of current medication use was of interest.

3.4 Demographic measures

3.4.1 Estimated IQ

IQ was measured at the time of the interview and estimated using two-subscales (vocabulary and matrix) of the Wechsler Abbreviated Scale of Intelligence (Southampton, Birmingham; WASI; Wechsler, 1999), or the same two-subscales of Wechsler Intelligence Scale for Children (Frankfurt, Aachen; WISC-IV; Wechsler, 2003).

The vocabulary subtest included 37 words, which became progressively more difficult as the test went on. Participants were required to provide definitions of these words, which were presented both visually in a stimuli booklet and verbally by the interviewer. The matrix-reasoning subtest required participants to correctly complete a logical pattern of stimuli by identifying the correct options from a set of 5 possibilities. There were 35 patterns in total, which became progressively more difficult as the test went on. There was no time limit for either of the subtests. The scores from the two subtests

were converted into standardised z-scores (in order to improve comparability across sites), and then combined to form the estimated full-scale IQ.

3.4.2 Socioeconomic status and ethnicity

Socioeconomic status and ethnicity of the participant were recorded using the social information section in the Medical History interview (Appendix 6). This is a structured interview that was conducted with parents after the K-SADS-PL interview. It contains questions regarding the mother's pregnancy (e.g., illness, use of smoking, alcohol or drugs), incidence of birth complications, developmental milestones (e.g., age of first words and first steps), nursery and school experiences (e.g., did the child ever change school due to academic or behaviour problems?), chronic medical problems, social information (e.g., living situation, education and occupation of the parents, ethnicity), and history of psychiatric disorders in the family.

3.4.3 Puberty measure

Puberty status was assessed using the Pubertal Developmental Scale (PDS; Appendix 7-8 for males and females, respectively; Petersen et al., 1988). This self-report measure requires participants to answer five questions regarding their physical development. The first three questions apply to both sexes (e.g., growth in height) and the last two questions are sex specific (e.g. growth of breasts or facial hair). The participants are asked to choose between five options for each question that applies to them ("Has not yet started", "Has barely started", "Is definitely underway", "seems complete", and "I don't know"). In this study, we removed the option of "I don't know" to reduce missing responses as it was crucial to obtain a more complete measure of puberty. Measuring puberty is important when investigating sex differences, as girls tend to pass through puberty earlier than boys (Marshall & Tanner, 1986). Furthermore, and as discussed in Chapter 2, cortical development is associated with the onset and the progression of puberty

(Campbell et al., 2012; Lenroot et al., 2007). Thus, in order to reliably compare cortical structure and function between the sexes it was crucial that the groups were matched on this measure.

Scores were computed based on the categorical criteria developed by Petersen and colleagues (Petersen et al., 1988), with the exception of the cut-off point for boys. In the original calculation, across the five questions, a sum total of nine would classify boys in the late pubertal phase and a total sum of 12 would categorise boys to post-puberty. However, two studies which set out to validate the PDS against other pubertal development measures (sexual maturation scale and clinical evaluation) found that while the reliability across measures was good for girls, boys tended to underestimate their pubertal development using the PDS (Bond et al., 2006; Shirtcliff et al., 2009). Thus, we modified the original cut-off points of 9 and 12 (for late- and post puberty, respectively), and instead used a cut-off point of 8 for late puberty, and 11 for post-puberty in the male sample.

3.4.4 Edinburgh Handedness Inventory

Handedness was measured using the self-report Edinburgh Handedness Inventory (Oldfield, 1971; Appendix 9). The questionnaire includes ten activities (e.g., writing, using scissors) and participants indicated whether they predominately used their left or right hand during these activities. These could be classified as either predominantly left or right handed or ambidextrous. Assessing handedness of participants is important in (f)MRI studies, as left and right handed individuals differ to some degree in the asymmetries of cortical volume (Snyder, Bilder, Wu, Bogerts, & Lieberman, 1995) and activation across the cortex (Pool, Rehme, Eickhoff, Fink, & Grefkes, 2015).

3.5 Personality measures

3.5.1 Youth Psychopathic traits Inventory

Psychopathic traits were measured using the Youth Psychopathic traits Inventory (YPI; Appendix 10; Andershed, Kerr, Stattin, & Levander, 2002). This self-report measure contains 50 items relating to psychopathic tendencies, scored in a 1-4 point scale ("does not apply at all", "does not apply well", "applies fairly well", and "applies very well"). The total score ranges from 50 to 200, with higher scores indicating higher levels of psychopathic tendencies. There are three dimensions of psychopathy that make up the total psychopathy score: Grandiose-manipulative, callous-unemotional, and impulsive-irresponsible. In addition to the total psychopathic traits score, we used the summed scores of the callous-unemotional subscale as a measure of callous-unemotional traits, as these traits have been argued to be particularly elevated in CD (Frick et al., 2014). For the purpose of the current thesis, the YPI (and the CU sub-scale of the YPI) were used to assess the clinical characteristics of the sample.

3.5.2 Inventory of Callous-Unemotional traits

Callous-unemotional traits were also assessed using the Inventory of Callous-Unemotional traits (ICU; Appendix 11; Barry et al., 2000). The ICU is administered as a self-report or a parent-report questionnaire, which contains three sub-scales (uncaring, callous, and unemotional) that measures the affective personality features of psychopathy over 24 items scored on a 0-4 point scale ("not at all true", "somewhat true", "very true", and "definitely true"). Total scores range from 0-72, with higher scores indicating higher levels of CU-traits. At the start of the FemNAT-CD study, ICU data were collected from parents at all sites, and from participants only in Southampton. The remaining sites adopted the self-report measure only after about a third of data collection had been completed. Due to the relatively high number of young people from Birmingham and

Southampton participating in the study without parental involvement, a high proportion of parent-report ICU data were missing, and a high proportion of self-report ICU data were missing from participants tested in Frankfurt, Aachen, and Birmingham. For these reasons, this measure was not used in the current study.

3.6 Site qualification procedures

To assure comparability of clinical assessment between sites, each site completed inter-rater reliability (IRR) assessments on eight K-SADS-PL interviews (five CD subjects and three healthy controls). This involved either filming the diagnostic interviews and asking other members of the research team to judge whether a symptom had been endorsed and whether the participant met criteria for particular diagnoses (Aachen, Frankfurt) or to ask a second rater to attend the interviews and make diagnoses separately to look at inter-rater agreement (Birmingham, Southampton). For the sites used in the current study (Southampton, Birmingham, Frankfurt, Aachen), the Cohen's kappa coefficients for each of the main disorders were: CD=.90, ODD=.94, ADHD=.79, and MDD=.88., which indicates strong to almost perfect agreement between raters (McHugh, 2012).

To ensure validity of questionnaire items across all sites involved in FemNAT-CD, the study used validated translations of the PDS, EHI, and YPI where available. When no validated translation was available, the documents were translated and back-translated by two independent researchers fluent in both languages of interest. For the purpose of the current study, German translations of the PDS (Watzlawik, 2009) and YPI (Stadlin, Pérez, Schmeck, Gallo, & Schmid, 2015) were already available and have been widely used in research settings. The questions asked in the EHI are clear and simple enough not to require a formal translation.

Magnetic resonance imaging (MRI) is a widely used method of imaging the human brain. However, there may be significant variability between sites in terms of the performance of the equipment and the quality of MRI images - even though they may be

using scanners produced by the same manufacturer. Functional and structural MRI scans were acquired using Siemens 3T (Frankfurt and Southampton: Tim Trio; Aachen: Prisma) or Philips 3T (Birmingham: Achieva) scanners, in all cases using a 32-channel head coil. To ensure comparability of functional and structural MRI data between the five sites, each site adopted similar scanning parameters and image acquisition sequences, and underwent site qualification procedures to ensure that sequences were comparable. These included scanning: (i) an American College of Radiology (ACR; Chen, Wan, Wai, & Liu, 2004) phantom; (ii) a functional Biomedical Informatics Research Network (FBIRN; Glover, 2005) phantom; and (iii) a human volunteer. The ACR phantom is designed to assess structural scan sequences, and the FBIRN is designed to verify and measure scanning stability during functional MRI sequences, and provide information concerning scanner drift, percent fluctuation in signal, signal-to-noise ratio, and signal-to-fluctuation-noise ratio. Once collected, the three sets of data were reviewed by an MRI physicist and each site adjusted the scanning parameters according to the physicist's recommendations until the scanning procedures produced comparable results. The sites were only able to start collecting data once they had successfully passed this site qualification procedure and had been given permission to start acquiring data.

3.7 Procedure

Prior to the interview, the participants and/or their parents were asked several screening questions related to the exclusion criteria (e.g., metal in their body, history of head trauma). If the participant was not affected by any of these exclusion criteria, they were invited to take part in the first session, which took place either at the participant's home or at the university/clinic.

At the UK sites, informed consent was obtained from participants aged 16 years or above, whereas informed assent and parental consent were obtained from participants and their parent or legal guardian if the participants were under the age of 16 years. Parental

consent was obtained at the German sites for participants of all ages. The interviews were conducted separately for the participants and their parents using the KSADS-PL (Kaufman et al., 1997) in order to assess for CD and other common comorbid disorders. The WASI (Wechsler, 1999) or the WISC-IV (Wechsler, 2003) was used at the UK or German sites, respectively, in order to obtain estimates of full-scale IQ for the participants. If the participants met all inclusion criteria, the MRI safety screening form (Appendix 1) was completed, either at the time of the interview, or over the phone prior to the MRI scan.

Participants were invited to the study site to complete a number of questionnaires (including the YPI, ICU, PDS, and EHI), neuropsychological tests, psychophysiological measures, as well as providing saliva samples for DNA and hormone level measurement. The testing session lasted between 3-3.5 hours, however; only three questionnaires from this battery are of direct relevance to the current study; the Edinburgh handedness inventory (EHI), the Youth Psychopathic traits Inventory (YPI) and the Pubertal Developmental Scale (PDS). In this session, the medical history interview was conducted with the participant if a parent was not willing to be involved in the study.

Subsequently, participants were invited to the respective imaging centre at each site for a session lasting approximately 1.5-2 hours. Each site had slightly different fMRI tasks and scanning order, however, each site collected a T1-weighted structural scan, and an emotion processing functional MRI task. The scanning at the Southampton site took place at the Centre for Neuroscience and Neurodynamics (CINN), Reading University. We first collected a T1 scan, followed by two fMRI tasks: an emotion-processing task and a reward-learning task. After the second fMRI task, we collected a Diffusion Tensor Imaging (DTI) scan, and finally, the last functional MRI task: empathy for pain. Prior to the scan, participants were screened using two additional detailed safety questionnaires (Appendices 12-13) to ensure safety during the MRI scans. Following this, the participants were given detailed instructions and information about the scanning session (e.g., the order of the scans, not to cross their arms or legs) and completed short off-line practice sessions

of each of the three fMRI tasks to familiarise themselves with the tasks. Between each scan or fMRI task, the MRI operator or the assistant operator talked to the participant via the intercom to ensure they were awake and comfortable, as well as to remind them about the instructions for the upcoming scan or task. However, for the purpose of this thesis, only the structural data and the facial emotion processing task will be considered.

3.8 Ethical approval

The study was conducted according to legal regulations of the European Union, national legislation, and the Declaration of Helsinki. The study protocols were approved by the relevant ethical committees at each site prior to the start of data collection; the Ethics Committee of the RWTH Aachen University Hospital (EK027/14) for the Aachen site, the medical faculty of Goethe University Frankfurt for the Frankfurt site, and in the UK sites, the NHS Research Ethics Committee (NRES Committee West Midlands, Edgbaston; REC reference 13/WM/0483).

3.9 Publishing agreement

Due to the rich and extensive data collected in the FemNAT-CD consortium, author- and publication agreements were put in place by the steering committee. Biannually, each site could present an abstract to propose consortium publications. These suggestions would then be discussed and determined in a meeting with the primary investigators from each site. Each PhD student and post doc fellow further presented a project proposal at the start of the study. Chapter four in this thesis was accepted as a consortium publication at the start of the study, and has subsequently been written as a paper for publication, whereas the topics and general methods of Chapter three and five will be published with the final, larger data set by other members of the consortium with the PhD candidate and her supervisors in the authorship. Furthermore, although Southampton has collected data for neurophysiological and neurocognitive tasks, Southampton was primarily a neuroimaging

site, thus neuropsychological and neurophysiological data was not readily available to use in this thesis.

Chapter 4: Sex differences in the relationship between conduct disorder and grey matter volume in adolescence: A voxel-based morphometry analysis.

Background

Conduct disorder (CD) is characterised by marked impairments in emotion recognition, empathy, and decision-making - to name just a few. Neurodevelopmental theories of CD propose that these impairments may be rooted in abnormalities in brain development. Indeed, CD has been associated with structural abnormalities in brain regions that are thought to be involved in these processes. However, it is currently unclear whether the structural abnormalities seen in males with CD are also present in females with CD. The aim of this study is therefore to investigate potential similarities and differences between the sexes in the relationship between CD and changes in grey matter volume at a whole-brain level and in specific regions of interest.

Method

Structural magnetic resonance imaging data were collected from 96 adolescents with CD (48 females) and 104 sex-, age- and pubertal-status matched typically developing controls (52 females; all aged 14-18 years). We used voxel-based morphometry to investigate differences in grey matter volume, testing for main effects of diagnosis, sex, and sex-by-diagnosis interactions, while statistically controlling for age, IQ, and scan site. In addition, we tested for differences between childhood- and adolescent-onset CD subgroups in order to ensure that it was valid to combine them in the main analyses, and re-ran analyses including ADHD symptoms as an additional covariate.

Results

In line with our predictions and previous research, we observed lower grey matter volume in the CD relative to the control group in the amygdala, anterior cingulate cortex, ventromedial prefrontal cortex, and fusiform gyrus - areas involved in the processing of emotions, reward processing, and behavioural inhibition. Importantly, we also observed

sex-specific effects in the amygdala and insula, showing lower volume in these areas in males with CD only. In addition, we observed opposite effects in anterior cingulate cortex, where males with CD showed lower, and females with CD higher, grey matter volume relative to their respective control groups. Finally, sex differences were observed in the effects of disorder severity; males showed a negative, and females a positive, association between CD severity and fusiform gyrus grey matter volume. There were no differences between childhood- and adolescent-onset CD subgroups, and the results remained largely significant when controlling for ADHD symptoms.

Conclusion

This study shows that although males and females with CD have shared brain abnormalities in several areas relative to healthy controls, there are also several areas that seem to be structurally abnormal only in males with CD or show changes in opposite directions in males and females with CD. This suggests that it is not appropriate to combine the sexes in structural neuroimaging analyses as this might obscure or bias results.

4.1 Introduction

The most commonly used neuroimaging data analysis technique for measuring brain structural differences in individuals with psychopathology is voxel-based morphometry (VBM). This analysis technique uses the approach of statistical parametric mapping to calculate grey matter volume (GMV) in voxels across the whole brain to allow for comparisons between groups in terms of brain structure (Ashburner & Friston, 2000). Studies using VBM have found lower GMV in CD groups compared to typically-developing controls in the insula (Fahim et al., 2011; Sterzer et al., 2007), amygdala (Fairchild et al., 2011; Huebner et al., 2008; Sterzer et al., 2007), orbitofrontal cortex (Fairchild et al., 2011; Huebner et al., 2008; Sebastian et al., 2015), medial/ ventromedial prefrontal (Fahim et al., 2011; Olvera et al., 2014), and anterior cingulate cortex (Olvera et al., 2014; Sebastian et al., 2015; Sterzer et al., 2005). Although less commonly reported,

effects of CD have also been found in the hippocampus (Huebner et al., 2008), temporal cortex (Olvera et al., 2014), and superior frontal gyrus (SFG; Olvera et al., 2014). In the typically developing population, these brain regions are implicated in emotion regulation and recognition, affective empathy, and reward processing (Bush et al., 2002; Knutson et al., 2001; Sergerie et al., 2008; Singer et al., 2009). Furthermore, fMRI studies have shown atypical activation in CD groups in some of these areas during empathy (Lockwood et al., 2013; Marsh et al., 2013), emotion processing (Fairchild et al., 2014; Fairchild, Van Goozen, et al., 2009; Passamonti et al., 2010), and reinforcement learning/reward processing (Finger et al., 2011; Rubia et al., 2008) tasks.

The majority of VBM studies on CD have included male-only (or predominantly male) samples, and very few studies have included sufficient numbers of males and females to test for common and sex-specific effects of CD on brain structure. However, there are some indications from previous research that we should expect both common and different patterns of brain alteration in males and females. For example, a study of females with CD and severe substance dependence reported lower volume in ventro- and dorsolateral PFC, OFC, and ACC (Dalwani et al., 2015). Although substance dependence was a confound in this study, which limits its comparability with other CD studies, the findings are largely overlapping with findings from male-only CD samples.

In addition, a study that included males and females with CD found that there was both a main effect of diagnosis on right amygdala volume (CD < controls), and a sex-by-diagnosis interaction in anterior insula, where females with CD showed lower, and males with CD higher, volume in this region compared to their respective control groups (Fairchild, Hagan, et al., 2013). Associations between CD severity (i.e., number of CD symptoms) and GMV may also be different in males and females – in female only samples, CD severity has been found to negatively correlate with GMV in motor cortex, supramarginal gyrus (Dalwani et al., 2015), and dorsolateral PFC (Fairchild, Hagan, et al., 2013). Furthermore, a recent study that included a large sample of both males and females

reported a strong significant negative association between the CD severity and GMV in the superior temporal cortex in females, but not males with CD (Michalska et al., 2015). In contrast, a negative association between CD symptoms and GMV has been reported in the anterior insula in males with CD (Fairchild et al., 2011).

Although lower GMV in CD groups has been the most consistent finding, higher GMV has also been noted in some regions. For example, a recent meta-analysis reported higher GMV in CD subjects in the inferior parietal lobule, superior parietal lobule, and fusiform gyrus (Aoki, Inokuchi, Nakao, & Yamasue, 2014). In addition, a study of pre-adolescent males (~11 years) with more general conduct problems and high levels of callous-unemotional traits (CU; personality features encompassing diminished empathy or guilt; Frick & White, 2008), found higher grey matter concentration (GMC) in OFC and ACC, and higher GMC and GMV in the temporal lobes in the conduct problems group compared to controls (De Brito et al., 2009). Taken together, it is evident that the results from previous VBM studies are mixed, although most have reported GMV reductions in adolescents with CD.

Although VBM has increasingly been criticised for combining several independent features of the cortex (surface area and cortical thickness) that contribute to grey matter variability (Panizzon et al., 2009; Winkler et al., 2012), there are several benefits to this approach. These include the fact that VBM provides whole-brain coverage rather than testing for group differences purely at the surface of the cortex. Surface-based morphometry (SBM) methods gives estimates of the surface area, thickness, and gyrification of the cortex, but are suboptimal for studying subcortical areas - such as the amygdala and hippocampus, which are thought to be affected in CD (Fahim et al., 2011; Fairchild et al., 2011; Huebner et al., 2008; Rubia et al., 2009; Sterzer et al., 2007). VBM is also the most commonly used, and most well established, method for assessing brain structure; thus taking a VBM approach would facilitate replication of previous studies and integration of findings with the rest of the literature. Furthermore, this method requires less

preparation and pre-processing of the T1-weighted structural images compared to SBM methods, providing efficiency in processing, which is an advantage when dealing with large datasets such as the one generated by the FemNAT-CD study.

Leaving to one side the above-mentioned limitations of VBM, the inconsistencies in findings reported (both in males and females) may have arisen from the inconsistencies in the characteristics of CD samples included in previous studies. Firstly, the majority of previous studies have included a small sample size (n = ~20 in the CD group), which increases the risk of both false positives and negatives (Button et al., 2013). This is also a concern considering that many of these studies have included both males and females with CD without providing sufficient power to test for sex-by-diagnosis interactions. The findings from these studies are therefore subject to issues relating to statistical power as well as the possibility that results obtained are products of averaging across the sexes. The two largest VBM studies of CD/conduct problems to date (n= ~ 60 in the CD/conduct problems groups; Fairchild et al., 2011; Sebastian et al., 2016), had increased power compared to previous studies, partly due to their larger sample sizes and partly because they only included males. Although these studies are valuable in understanding brain structure changes in males with CD, there are no comparable studies including a large sample of females with CD to be able to examine whether males and females with CD show similar or distinct GMV changes relative to sex-matched control groups.

In the current study, we attempt to bridge this gap in the literature by including a large and equal number of males and females (with and without CD) and reporting diagnostic effects that are common to both sexes as well as those that are sex-dependent (i.e., both main effects of diagnosis and sex-by-diagnosis interactions). Secondly, there are significant differences between prior studies in the age of the samples, with some studies recruiting participants as young as 8 or 9 years of age (Fahim et al., 2011; Michalska et al., 2015; Sterzer et al., 2007) and other with participants as old as 19 or 21 years (Cope, Ermer, Nyalakanti, Calhoun, & Kiehl, 2013; Fairchild et al., 2011; 2013), with the

majority of samples spanning a developmental period of 6 or 7 years, i.e., covering childhood and adolescence. As has been discussed in Chapter 2, age (and pubertal changes broadly associated with age) influences on the structural development of the brain, and averaging across ages may dilute or average out important age-specific results. Thus, in the present study, we selected a narrow age-range (14-18 years) and matched our groups on pubertal development. Thirdly, there have been discrepancies between studies in terms of the threshold of statistical significance chosen by researchers. Some studies report results that are whole-brain corrected (the most stringent level of corrections for multiple comparisons; Michalska et al., 2015; Olvera et al., 2014), while others reported results that only survive small-volume correction (Fairchild et al., 2011; Fairchild, Hagan, et al., 2013; Huebner et al., 2008), uncorrected thresholds (Huebner et al., 2008), or both corrected and uncorrected thresholds (De Brito et al., 2009; Fairchild et al., 2013; Sebastian et al., 2015). While all of the above statistical thresholds are widely used in the literature, whole-brain corrected thresholds are the most stringent and least susceptible to type I error (Bennett, Wolford, & Miller, 2009). However, applying thresholds of this type increases the risk of false negatives, particularly when using relatively small samples and studying heterogeneous disorders. Hence, comparisons between studies in terms of results and interpretation of findings need to be made cautiously. In the current study, we report both whole-brain and small-volume corrected findings, and for the sake of completeness, also report uncorrected findings in the results tables.

Finally, it is not clear how the presence of ADHD comorbidity affects the results, since some studies report results only when controlling for ADHD comorbidity (De Brito et al., 2009) while some present results only without controlling for ADHD (Fahim et al., 2011; Olvera et al., 2014; Sebastian et al., 2015; Sterzer et al., 2007). Other report results both with and without controlling for ADHD (Dalwani et al., 2015; Fairchild et al., 2011; Fairchild et al., 2013; Michalska et al., 2015). To report results that are both representative of clinical reality as well as presenting results that may be specific to CD, here we first

report results that were significant without controlling for comorbid ADHD symptoms, and then note which results remained significant when the number of ADHD symptoms was included as a covariate of no interest.

In summary, the aim of this study was to investigate whether CD is associated with similar or distinct abnormalities in GMV in males and females compared to their respective control groups. For this purpose, we included a larger than previously studied sample (N=200), with groups that were deliberately matched in terms of sex, age, and pubertal development. Based on previous studies, we predicted that the CD group would mainly show lower GMV compared with the healthy control group, specifically in the amygdala, prefrontal areas of the cortex, anterior cingulate cortex, and insula. Furthermore, based on the fact that some sex differences in the relationship between CD and GMV have been noted, we predicted that there would also be some sex-dependent abnormalities, such that lower GMV in CD may be driven more by one sex than the other, or that males and females with CD would show opposite relationships, as would be evident from sex-by-diagnosis interactions. However, due to the limited nature of prior research on females with CD, it was difficult to make predictions regarding the direction of these findings. Finally, based on previous research showing negative associations between CD severity and GMV, we predicted negative correlations between CD severity and GMV in the areas investigated in the main factorial analysis. As with the main analysis, we expected to observe both common and sex-dependent associations between CD severity and brain structure.

4.2 Method

4.2.1 Participants

The sample was selected from the participants tested at the Southampton, Birmingham, Frankfurt, and Aachen data collection sites participating in the Female Neurobiology and Treatment of Conduct Disorder (FemNAT-CD) study, which is

described in detail in Chapter 3. A total of 96 adolescents (48 females) with CD and 104 adolescents (52 females) without psychiatric disorders were selected. All participants were aged between 14-18 years (M_{age}=16.02). Diagnoses were made using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL; Kaufman et al., 1997; see Chapter 3 for a detailed description).

4.2.2 MRI data acquisition and pre-processing

Structural MRI data were acquired using Siemens 3T (Frankfurt and Southampton: Tim Trio; Aachen: Prisma) or Philips 3T (Birmingham: Achieva) scanners. Each site underwent a site qualification procedure prior to commencing data collection (see Chapter 3 for a detailed description). T1-weighted scans were collected using a magnetization-prepared rapid-acquisition gradient-echo sequence (MP RAGE), TE(Philips) = 3.7, TE(Siemens) =3.4, TR=1.9, flip angle = 9, FHxAP FoV = 256, RL FoV = 192, matrix = 256, voxel size =1×1×1 mm, sagittal slices = 192, bandwidth(Philips) = 174 hz/pix, bandwidth(Siemens) = 180 Hz/pix, total scan time = 4 min 26 sec (Siemens) or 6 min 5 sec (Philips).

The quality of the data was assessed immediately after the scan by the MRI operator, and repeated until a high quality image was acquired. Data were pre-processed and analysed using Statistical Parametric Mapping software (SPM 12; http://www.fil.ion.ucl.ac.uk/spm/). Prior to statistical analysis, several pre-processing steps and quality control procedures were performed (see Figure 4.1). Firstly, all images were inspected for gross neuroanatomical abnormalities and excessive movement. Secondly, all T1-weighted images were re-oriented according to the anterior and posterior commissure line in order to aid the normalisation process. Thirdly, to ensure comparability between participants' brain areas, i.e., so that voxels fall within the same brain structure for each participant, we then proceeded with an automated normalisation process, registering images from all participants using the same coordinates. Due to the paediatric nature of the sample in this study, it was likely that the data would deviate from the standard Montreal

Neurological Institute (MNI) template provided with the SPM12 toolbox. Thus, to minimise problems with normalisation, we adopted an additional pre-processing step of creating customized Tissue Probability Maps (TPMs). Consequently, we used the Template-O-Matic Toolbox for SPM8 (Wilke, Holland, Altaye, & Gaser, 2008) with the age and sex of all participants as defining variables to create the TPMs. The T1-weighted images were segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using VBM8 (Gaser, 2009), and the individual native-space GM and WM segments were normalised to the TPM using an affine registration. Fourthly, a Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL; Ashburner, 2007) template was created in SPM12 and normalised to MNI space using the GM and WM segments from all participants. From the segmented grey matter, we calculated estimated total grey matter volume for each participant to account for inter-individual variability in this measure. Finally, the images for each participant were warped to the template, and the data were smoothed with a Guassian kernel of 6 mm full-width/half-maximum. This step was included to render the data closer to a normal distribution, by averaging the intensity in each voxel to the weighted average of the surrounding voxels (Ashburner & Friston, 2000).

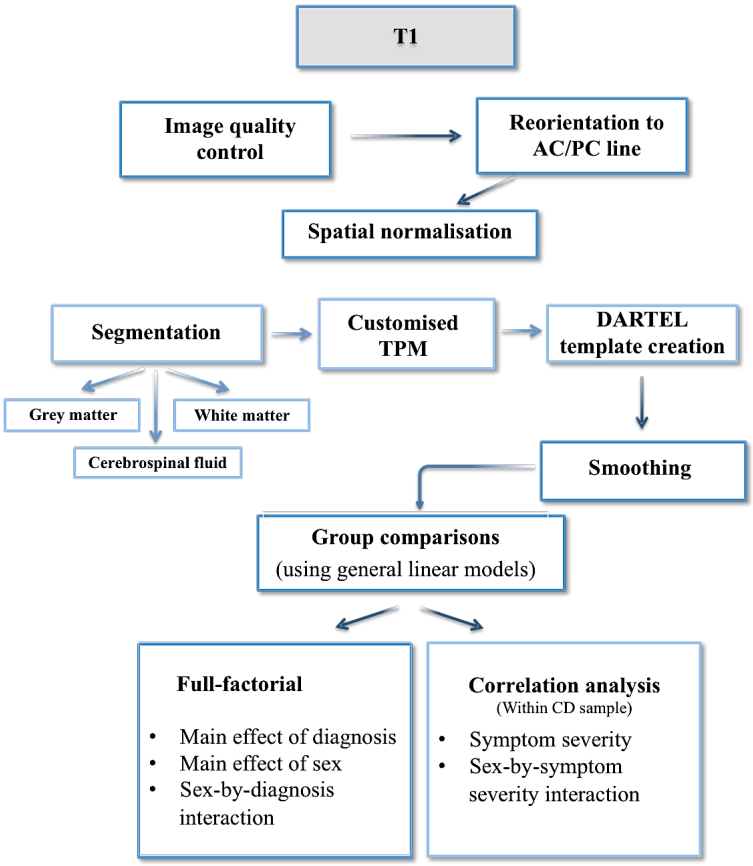

Figure 4.1. Schematic representation of the voxel-based morphometry pre-processing and analysis steps. *Key*: AC/PC = anterior commissure/posterior commissure, CD = conduct disorder, DARTEL = Diffeomorphic Anatomical Registration Through Exponential Lie Algebra, TPM = Tissue Probability Map.

4.2.3 VBM analysis

Following the pre-processing steps, statistical analyses were performed using General Linear Models (GLM) for group comparisons. We conducted two main analyses: (i) a full-factorial model assessing main effects of diagnosis and sex, and sex-by-diagnosis interactions; and (ii) correlational analyses within the CD sample assessing the correlations between grey matter volume and CD severity (i.e., number of lifetime CD symptoms) as well as sex-by CD severity interactions (i.e., where the correlation between number of CD symptoms and grey matter volume significantly differs by sex). In addition, given previous evidence suggesting quantitative brain structural differences between childhood-onset (CO) and adolescent-onset (AO) CD (Fairchild, Hagan, et al., 2013; Fairchild et al., 2015), we first ran a set of GLM-based factorial analyses comparing these subgroups in order to assess the validity of collapsing across them in our main analyses. In addition, to confirm independence of findings from ADHD comorbidity, each analysis was repeated including

lifetime ADHD symptoms (from the K-SADS-PL) as a covariate of no interest. Age, IQ, total GMV, and scan site were included in both models as covariates of no interest.

We used two main thresholding approaches when analysing the data. First, we took a region of interest (ROI) approach based on an anatomical definition of brain regions. This commonly employed statistical procedure ensures a robust protection against both type I and type II errors, especially in those ROIs for which there is a strong *a priori* hypothesis (Poldrack, 2007). We created seven regions of interest (ROIs) using the atlas for automated anatomical labelling (aal.02; Tzourio-Mazoyer et al., 2002). The regions were chosen based on results from previous MRI studies showing differences between CD and HC groups (Fairchild et al., 2011; Huebner et al., 2008; Sterzer et al., 2007) and included the amygdala, ventrolateral/medial PFC, OFC, insula, ACC, fusiform gyrus, and superior temporal gyrus (STG). Results from the ROIs are reported at $p \leq .05$, family-wise error (FWE) correction for multiple comparisons in small volumes (i.e., small volume correction; SVC; Friston, 1997).

Second, we report any findings in other brain regions that met a threshold of $p \leq .05$, FWE, whole-brain correction – the most stringent threshold available in SPM12. Finally, for the sake of completeness, we also report the results that met a "borderline" threshold of $p \leq .001$ uncorrected and a cluster size (k) more than 10 voxels in the Tables.

4.3 Results

4.3.1 Sample and demographics

Table 4.1 summarises the demographic characteristics of the sample. The groups did not differ in age, pubertal status, handedness, or ethnicity. Within the male and female samples, both CD groups had lower total IQs and reported more CD and ADHD symptoms than HCs. Males with CD displayed more ADHD symptoms than the other three groups. Furthermore, the CD group had significantly higher levels of comorbidity and medication use. However, the male and female CD groups did not differ in terms of comorbidity or medication use. Total GMV were higher in males compared to females (p<.001), as

expected (Giedd et al., 1997), but there were no effects of diagnosis on volume in males (p=.81) or females (p=.92) considered separately.

Table 4.1. Demographic and clinical characteristics of the sample used in the VBM study

	Females		Males				
Variable	CD (n=48)	HC (n=52)	CD (n=48)	HC (n=52)	Tgroup (p)	Tsex (p)	FgroupXsex (p)
	M (SD)						
Age (years)	15.83 (1.29)	16.13 (1.07)	15.92 (1.32)	16.21 (1.14)	T=1.75 (.08)	T=.46 (.64)	F= 1.09 (.35)
Estimated IQ	92.51 (12.26)	99.67 (12.01)	93.01 (11.95)	101.33 (11.42)	T=4.58 (<.001)	T=.66 (.51)	F= 7.14 (<.001)
Lifetime CD symptoms	6.06 (2.78)	.37 (1.12)	7.50 (2.84)	.42 (.67)	T=21.42 (<.001)	T=1.33 (.18)	F=164.30 (<.001)
ADHD symptoms	4.98 (6.53)	.13 (.60)	8.75 (6.62)	.06 (.42)	T=10.11 (<.001)	T=2.17 (.03)	F= 41.98 (<.001)
	N (%)				X^2group (p)	X^2sex (p)	X^2groupXsex (p)
No. Lifetime *DSM-IV* diagnoses							
ODD	31(66)	1(2)	32(70)	0(0)	X^2=99.83 (<.001)	X^2=.002 (1.00)	X^2=100.01 (<.001)
ADHD	10(21)	0(0)	24(52)	0(0)	X^2=42.95 (<.001)	X^2=7.14 (=.01)	X^2=61.49 (<.001)
MDD	14(29)	0(0)	8(17)	0(0)	X^2=31.30 (<.001)	X^2=1.77 (.26)	X^2=27.70 (<.001)
Alcohol abuse	3(6)	1(2)	4(9)	0(0)	X^2=5.43 (.02)	X^2=.00 (1.00)	X^2=6.00 (.11)
Drug abuse (cannabis)	7(15)	0(0)	10(22)	2(4)	X^2=15.07 (<.001)	X^2=1.51 (.24)	X^2=47.38 (<.001)
Medication	4(8)	1(2)	8(17)	0(0)	X^2=10.94 (.001)	X^2=.74 (.57)	X^2=13.84 (.003)
Puberty							
Late	34(71)	34(65)	34(71)	40(77)	X^2=.02 (.96)	X^2=.87 (.35)	X^2=1.68 (.64)
Post	14(29)	18(35)	14(29)	12(23)			
Age of Onset							
Childhood	19 (40)	-	26 (54)	-		X^2=1.58 (.45)	
Adolescent	27 (56)	-	22 (46)	-			
Missing	2 (4)	-	0 (0)	-			
Handedness							
Right	41 (86)	48 (92)	38 (79)	48 (92)			
Left	3 (6)	4 (8)	10 (21)	2 (4)	X^2=4.92 (.09)	X^2=2.37 (.31)	X^2=15.93 (.14)
Ambidextrous	3 (6)	0 (0)	0 (0)	1 (2)			
Missing	1 (2)	0 (0)	0 (0)	1 (2)			
Ethnicity							
Caucasian	45 (93)	51 (98)	46 (96)	49 (94)	X^2=3.78 (.29)	X^2=5.67 (.13)	X^2=12.75 (.17)
Non-white	3 (6)	1 (2)	2 (4)	3 (6)			

Note: ADHD = attention-deficit/hyperactivity disorder; CD = conduct disorder; HC = healthy control; IQ = intelligence quotient (measured using the WASI or WISC-IV); MDD = major depressive disorder ODD = oppositional defiant disorder. Group and sex differences were computed using independent sample t-tests or Chi square tests, and sex-by-diagnosis interactions were computed using ANOVAs and Chi square tests.

4.3.2 VBM results

4.3.2.1 Preliminary analysis

4.3.2.1.1 Conduct disorder age-of-onset effects

There were no significant differences at $p < .05$, SVC between the childhood-onset and adolescence-onset CD subgroups in any of the ROIs, either in the full factorial model or in the correlational analyses (age-of-onset-by-CD severity interaction). Thus, in all analyses, the childhood-onset and adolescence-onset CD subgroups were combined when performing comparisons with the HC groups using a factorial design or when testing for correlations with CD severity.

4.3.2.2 Full factorial analysis

Main effects of diagnosis: The CD group showed lower GMV compared to the HC group in several of the ROIs, including bilateral fusiform gyrus (Figure 4.2), bilateral amygdala, bilateral anterior cingulate, bilateral insula, right superior temporal gyrus, and left ventrolateral/ventromedial PFC (Figure 4.3; all $p < .05$, SVC). The CD group also showed lower GMV in several areas outside the ROIs, most notably the right superior frontal gyrus, left inferior gyrus, and middle temporal gyrus ($p < .001$, uncorrected; Table 4.2). There were no regions where the CD group had significantly higher volume compared to the HC group.

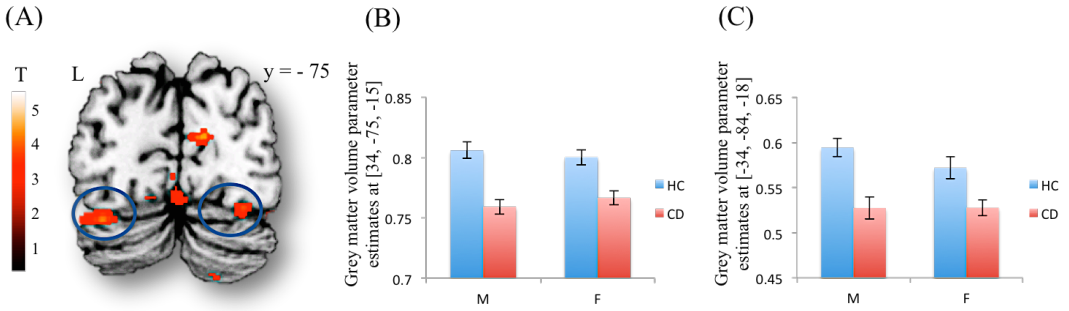

Figure 4.2. Lower grey matter volume in the conduct disorder (CD) group compared to the healthy control (HC) group in bilateral fusiform gyrus (blue ovals in Panel A). The image is thresholded at $p < .005$, uncorrected, for display purposes. The colour bar represents T statistics. Plots of the data extracted from left and right fusiform gyrus are displayed in panels B and C, respectively. *Key:* F= females, L = left, M = males.

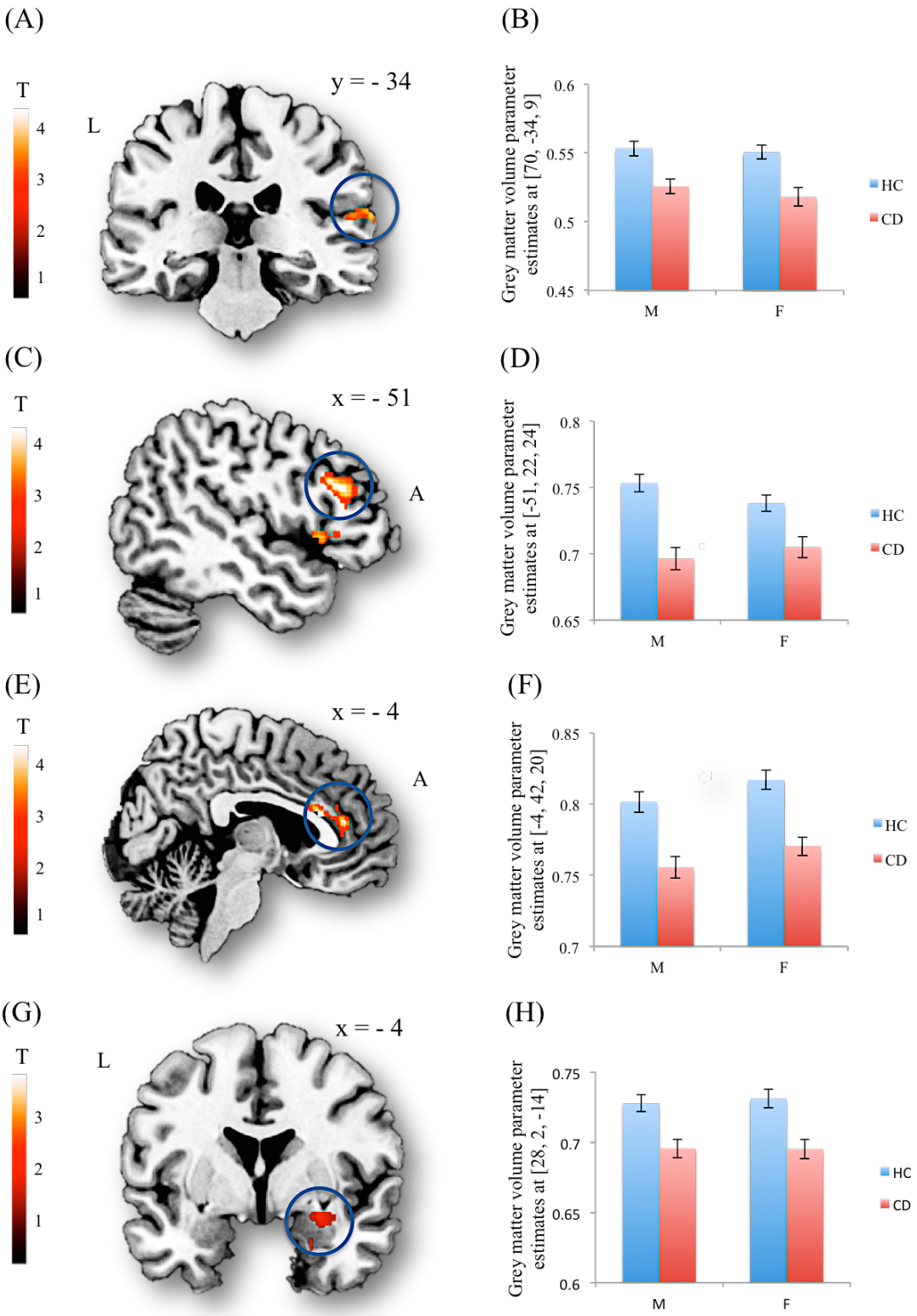

Figure 4.3. Lower grey matter volume in the conduct disorder (CD) group compared to healthy controls (HC) in right superior temporal gyrus (Panel A), left ventrolateral prefrontal cortex (Panel C), left anterior cingulate cortex (Panel E), and right amygdala (Panel G). The images are thresholded at $p < .005$, uncorrected, for display purposes. The colour bars represent T statistics. Plots of the data extracted from right STG, left vlPFC, left ACC, and right amygdala are displayed in panels B, D, F, and H, respectively. *Key:* A = anterior, ACC = anterior cingulate cortex, F= females, L = left, M = males, STG = superior temporal gyrus, vlPFC = ventrolateral prefrontal cortex.

Main effect of sex: Main effects of sex were observed in widespread areas of the brain; females showed higher volume compared to males in a large cluster including right anterior insula, OFC, and ACC ($p < .05$, FWE whole-brain corrected). Additional clusters were seen in bilateral STG, right SFG, bilateral caudate ($p < .05$, FWE whole-brain corrected), as well as bilateral amygdala ($p < .05$, SVC) and right fusiform gyrus ($p < .05$, SVC; Table 4.2).

Sex-by-diagnosis interactions: We observed sex-by-diagnosis interactions in bilateral insula (Figure 4.4), left ACC (Figure 4.5A-B), and left amygdala Figure 4.5C-D) at $p < .05$, SVC. In left ACC, males with CD showed lower, and females with CD higher, GMV relative to their respective control groups. Left amygdala volume was lower for both males and females with CD relative to their respective controls, but this effect was stronger in males with CD. In bilateral insula, the sex-by-diagnosis interactions were driven by the males with CD showing lower GMV compared to control males (all significant at $p < .05$, SVC).

In addition, there were several sex-by-diagnosis interactions in areas outside the ROIs; females with CD had higher, and males with CD lower, volume compared to their respective control groups, most notably in bilateral posterior insula and bilateral SFG ($p < .001$, uncorrected; see Table 4.2).

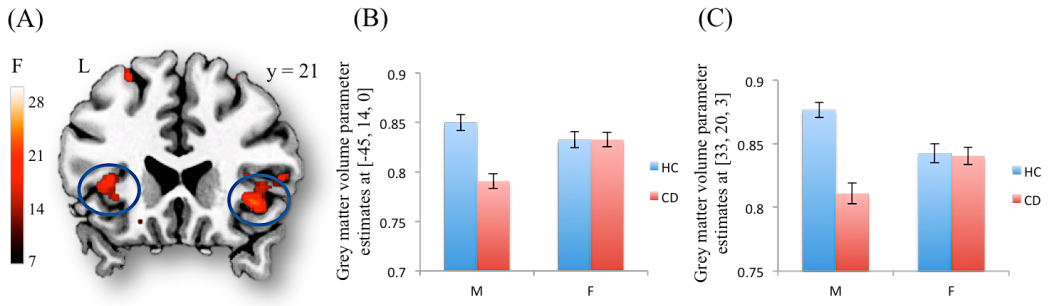

Figure 4.4. Sex-by-diagnosis interaction in bilateral anterior insula (Panel A); males with conduct disorder (CD) showed lower volume compared the male healthy control (HC) group, whereas there was no difference between females with CD and female HC. The colour bar represents F statistics. The image is thresholded at $p < .005$, uncorrected, for display purposes. Plots of the data extracted from the left and right anterior insula are displayed in panels B and C, respectively. *Key:* F= females, L = left, M = males.

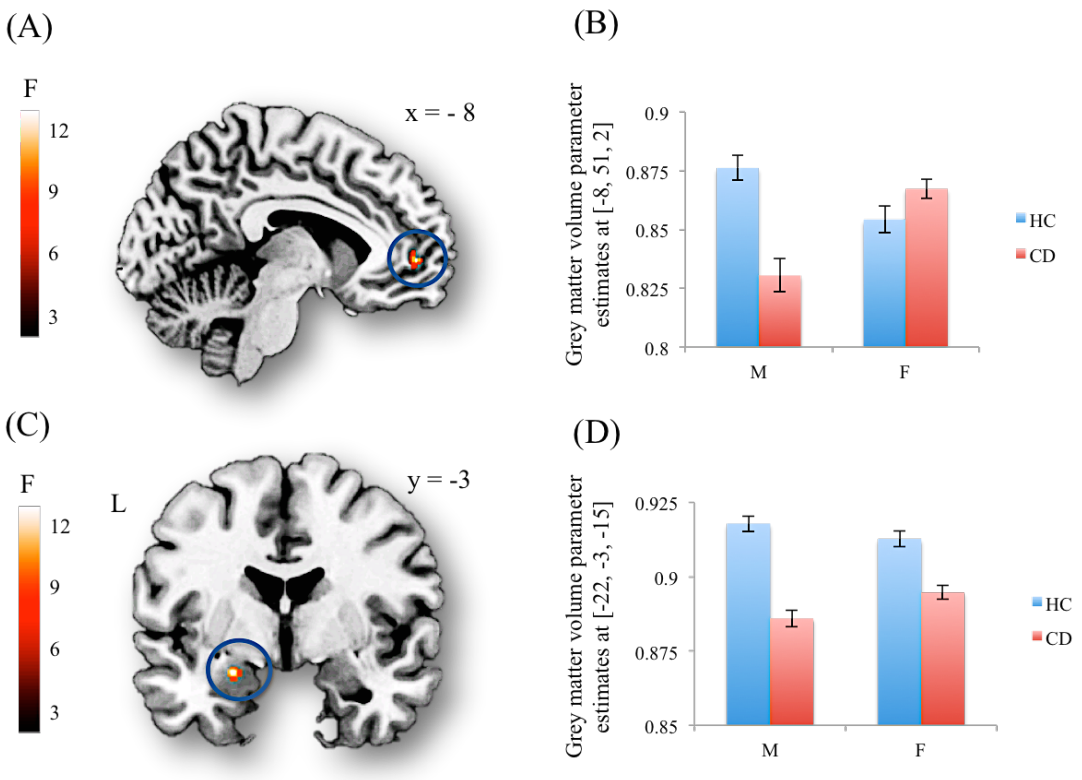

Figure 4.5. Sex-by-diagnosis interactions in left ACC and left amygdala grey matter volume. Males with conduct disorder (CD) showed lower, and females with CD higher, volume relative to male and female healthy controls (HC) in left ACC (Panel A). Left amygdala volume was lower for both males and females with CD relative to their respective controls, but this effect was stronger in males with CD (Panel C). The data extracted from the left ACC and the left amygdala are displayed in panel B and D, respectively. The colour bars represent F statistics. The images are thresholded at $p < .005$, uncorrected, for display purposes. *Key:* ACC = anterior cingulate cortex, F = females, L = left, M = males.

Table 4.2. Coordinates and cluster sizes for main effects of diagnosis, sex and sex-by-diagnosis interactions

Contrast		Local	No. of significant	MNI Coordinates		
Cerebral regions	Hemisphere	maxima z	Voxels in Cluster	*x*	*y*	*z*
Main effects of Diagnosis						
All CD < All HC						
Amygdala	L*	4.11 [a]	38	-28	-2	-16
	R	3.43 [a]	13	28	2	-14
Anterior cingulate cortex	L*	4.46 [a]	82	-4	42	20
	R	3.89 [a]	135	4	24	20
Fusiform gyrus	L	4.33 [a]	21	-34	-84	-18
	R	3.93 [a]	55	34	-75	-15
Anterior insula	L*	4.00 [a]	38	-45	14	0
	R	3.86 [a]	39	33	20	3
Superior temporal gyrus	R	3.89 [a]	29	70	-34	9
Ventrolateral PFC	L*	4.16 [a]	173	-51	22	24
Ventromedial PFC	L	5.58	116	-28	60	-6
Inferior temporal	L	4.55	190	-50	-54	-21
Temporal pole	R	4.43	78	56	10	-32
Postcentral gyrus	R	4.35	79	66	-18	28
Precentral gyrus	R	4.11	143	14	-20	74
Middle temporal gyrus	L	4.04	77	-66	-8	-15
Superior frontal gyrus	R*	3.87	128	6	57	16
All HC < all CD						
Supplementary motor area	R	3.71	24	4	-3	70
Main effects of Sex						
Females > Males						
Insula (anterior)/OFC/ anterior cingulate	R	6.51 [a]	490	39	16	-14
	L	6.35 [a]	473	-38	14	-10
Posterior cingulate	R	6.18 [a]	51	-3	-30	42
Medal OFC (anterior)	L	6.09 [a]	207	-4	62	-12
Superior temporal cortex	R	5.91 [a]	25	46	-21	21
	L	5.71 [a]	61	-50	-26	18
Cerebellum crus 2	R	5.87 [a]	145	42	-46	-44
Caudate nucleus	R	5.87 [a]	39	10	12	6
	L	5.51 [a]	16	-10	12	6
Lingual gyrus	R	5.52 [a]	19	4	-78	-2
Anterior cingulate	Medial	5.47 [a]	34	0	33	-4
Middle temporal pole	R	5.45 [a]	18	24	15	-36
Superior frontal gyrus	R	5.44 [a]	22	4	18	58
Angular gyrus	L	5.34 [a]	11	-45	-56	57
Cerebellum crus 1	R	5.21 [a]	11	48	-66	-28
	L	5.14 [a]	10	-46	-70	-32
Amygdala	L	5.44 [b]	126	-20	-3	-22
	R	4.29 [b]	63	21	0	-21
Fusiform gyrus	R	4.25 [b]	87	36	-24	-28
Males > Females						
Precentral gyrus	R	4.54	75	30	-4	45
Cerebellum 6	R	4.10	61	21	-63	-33
Inferior temporal gyrus	R	3.79	36	46	-21	-28
Middle/superior frontal gyrus	R	3.76	48	33	10	45

Sex-by-diagnosis interactions						
FCD > FHC, MCD < MHC						
Amygdala	L	3.35 [a]	7	-22	-3	-15
Anterior cingulate	L	5.12 [a]	47	-8	51	2
Anterior insula	L	4.06 [a]	94	-36	21	6
	R	4.62 [a]	118	36	24	-3
Posterior insula	R	3.91	137	38	-20	20
	L	3.81	34	-39	-16	14
Superior frontal gyrus	L	3.79	14	-26	22	57
	R	3.59	15	24	16	56
Parahippocampal gyrus	L	3.70	27	-10	-40	-9
Post-precentral gyrus	R*	3.63	17	57	-6	36
Cerebellum crus 1	R	3.57	22	21	-84	-24
MCD > MHC, FCD < FHC						
Angular gyrus	R	3.74	20	57	-56	14
Inferior temporal gyrus	R	4.22	39	64	-36	-20
Thalamus	L*	3.65	60	-8	-4	4
Middle frontal gyrus	L*	3.56	14	-39	14	50
Caudate	L	3.55	46	-15	4	10
Lateral occipital cortex	L	3.52	22	-40	-69	6

*Note: Unless otherwise indicated, the regions reported are significant at p< .001, uncorrected, for > 10 continuous voxels. * Indicates regions that were rendered non-significant when including ADHD symptoms as a covariate of no interest. Key: CD = conduct disorder, FCD = females with conduct disorder, FHC = female healthy controls, HC = healthy controls, L = left, MCD = males with conduct disorder, MHC = male healthy controls MNI = Montreal Neurological Institute, R = right. PFC = prefrontal cortex.*
[a] *p<0.05, family-wise error whole brain corrected*
[b] *p<0.05, family-wise error small volume corrected*

4.3.2.3 Correlations with CD severity and sex-by-CD severity interactions within the CD group

Correlations with CD symptoms: There were no positive or negative correlations between CD severity and GMV in any of the ROIs, but several correlations were observed outside the ROIs at an uncorrected level, most notably in medial frontal cortex, and ventromedial PFC ($p < .001$, uncorrected; see Table 4.3)

Sex-by-CD severity interactions: There was a sex-by-CD severity interaction in left fusiform gyrus ($p < .05$, SVC), whereby females showed a positive, and males a negative, correlation between CD severity and GMV (Figure 4.6). There were no significant sex-by-CD severity interactions in any of the other ROIs, but several interactions were detected outside the ROIs, most notably in right superior frontal gyrus, angular gyrus, and frontal pole ($p < .001$, uncorrected; see Table 4.3).

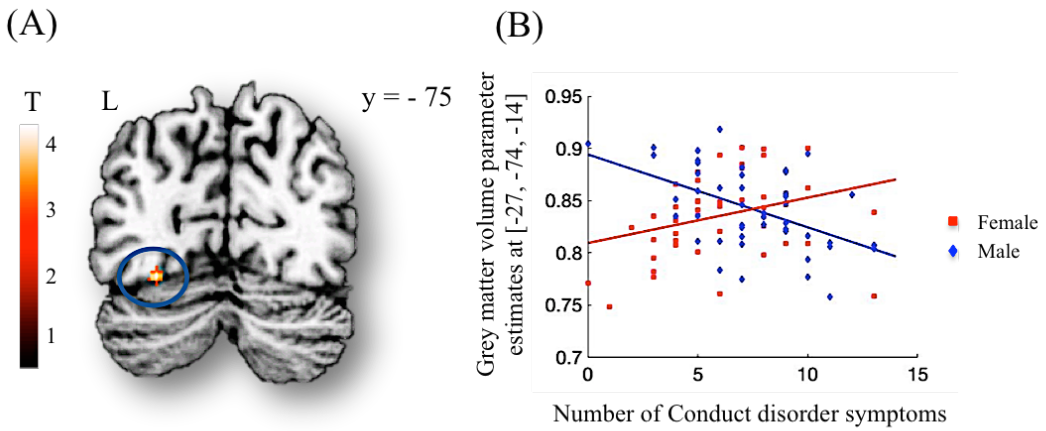

Figure 4.6. Sex-by-CD severity interaction in left fusiform gyrus. Females (red squares and line) showed a positive, and males (blue diamonds and line) showed a negative correlation between lifetime conduct disorder symptoms and grey matter volume (Panel A). The colour bar represents T statistics. The image is thresholded at $p < .005$, uncorrected, for display purposes. A scatterplot of the sex-by-CD severity interaction in left fusiform gyrus is shown in panel B. Key: L = left.

Table 4.3. Coordinates and cluster sizes for the correlations and interactions between lifetime CD severity and grey matter volume within the CD group

Contrast Cerebral region	Hemisphere	Local maxima z	No. of significant Voxels in Cluster	MNI Coordinates *x*	*y*	*z*
Positive correlations						
Postcentral gyrus	L	4.12	43	-30	-42	70
Frontal pole	L	3.93	16	-4	66	-21
Inferior temporal gyrus	R	3.88	15	51	-2	-33
Negative correlations						
Cerebellum crus 2	R	4.13	408	39	-63	-45
Medial frontal cortex	R	4.11	129	2	22	-27
Supplementary motor area	R	4.06	33	8	-15	62
Ventromedial PFC	L	3.90	30	-26	60	6
Lateral occipital cortex		3.83	59	40	-69	26
Parahippocampal gyrus	R	3.63	29	24	-28	-20
Precuneus cortex	L	3.53	19	-4	-58	21
	R	3.51	14	9	-62	57
Middle frontal gyrus	L	3.52	12	-38	21	56
Sex-by-CD severity interactions						
Females positive, males negative						
Fusiform gyrus	L	4.01[b]	15	-27	-74	-14
Superior frontal gyrus	R	3.70	15	14	16	48
Supplementary motor area	R	3.70	47	2	-9	56
Frontal pole	R	3.64	28	27	40	28
Middle frontal gyrus	L	3.48	25	-32	36	30
Males positive, females negative						
Angular gyrus	L*	3.91	20	-58	-57	36
Lateral occipital cortex	L*	3.85	44	-40	-75	-10
	R*	3.48	22	45	-64	30
Middle frontal gyrus	R*	3.65	15	50	20	36

*Note: * Indicates regions that were rendered non-significant when including ADHD symptoms as a covariate of no interest. Key: ADHD = Attention-deficit/hyperactive disorder, CD = conduct disorder, FCD = females with conduct disorder, L = left, MCD = males with conduct disorder, MNI = Montreal Neurological Institute, R = right, PFC = prefrontal cortex.*

[b]*p<0.05, family-wise error small volume corrected*

4.3.2.4 ADHD symptoms as an additional covariate of no interest

Factorial analysis: When the factorial analyses were run with ADHD symptoms included as an additional covariate of no interest, all sex-by-diagnosis interactions in the ROIs remained significant. The effects of diagnosis in right amygdala, right anterior insula, and right superior temporal gyrus remained significant ($p < .05$, SVC) and the effects of diagnosis in bilateral fusiform gyrus became stronger (i.e., higher F value and larger cluster size). The effects of diagnosis in left amygdala and left anterior cingulate cortex were now only significant at an uncorrected level ($p < .001$, uncorrected), while the diagnosis effects in left anterior insula, and left ventrolateral PFC were rendered non-significant when controlling for ADHD symptoms.

Correlational analyses: When the correlational analyses were run with ADHD symptoms included as an additional covariate, the sex-by-CD severity interaction in left fusiform gyrus remained significant ($p < .05$, SVC; Table 1.3).

4.3.2.5 Testing for potential confounding effects of scanning site and IQ

The majority of the effects of diagnosis and sex-by-diagnosis interactions remained significant when excluding IQ as a covariate. When excluding site as a covariate, the effects of diagnosis in bilateral ACC, bilateral fusiform gyrus, bilateral anterior insula, right superior temporal gyrus, left vmPFC, and left amygdala remained significant, while the effect of diagnosis in right amygdala was rendered non-significant. Similarly, the sex-by-diagnosis interactions in bilateral anterior insula and anterior cingulate cortex remained significant, while the sex-by-diagnosis interaction in left amygdala was rendered non-significant. When running separate analyses excluding IQ and site as covariates, all correlations with CD severity and sex-by-CD severity interactions remained significant.

4.4 Discussion

The aim of this study was to investigate potential sex differences in the relationship between CD and grey matter volume and to examine whether findings from previous research on brain structure abnormalities in males with CD extend to females with CD.

In line with our predictions and previous research (Fahim et al., 2011; Fairchild et al., 2011; Huebner et al., 2008; Olvera et al., 2014; Rogers & De Brito, 2016; Sebastian et al., 2015; Sterzer & Stadler, 2009; Sterzer et al., 2007), we found lower grey matter volume (GMV) in the CD group compared to the HC group in bilateral amygdala, bilateral anterior cingulate cortex (ACC), bilateral insula, bilateral fusiform gyrus, right superior temporal gyrus (STG), and left ventrolateral/ventromedial prefrontal cortex (PFC). Structural deficits in these regions could lead to impairments in emotion recognition and processing, empathy, and decision-making – which are often seen in individuals with CD (Sonuga-Barke et al., 2016). For example, the amygdala is strongly associated with emotion processing and recognition (Derntl et al., 2009; Phelps & LeDoux, 2005; Stanton, Wirth, Waugh, & Schultheiss, 2009; Whalen et al., 2001).Ventrolateral/ventromedial PFC and ACC play a key role in regulating amygdala activity during these processes (Buhle et al., 2014; Bush et al., 2002; Davis, Taylor, Crawley, Wood, & Mikulis, 1997; Lane, Fink, Chau, & Dolan, 1997). Lower GMV in these areas may therefore lead to the characteristic problems in regulating emotions that are observed in males with CD (Blair, 2007; Davidson, Jackson, & Kalin, 2000; Rubia, 2011). Similarly, STG is thought to be involved in inhibition (Horn, Dolan, Elliott, Deakin, & Woodruff, 2003) and social perception (Pelphrey, Morris, & McCarthy, 2004), and lower GMV in this area has previously been found in CD groups (Rogers & De Brito, 2016) and incarcerated male adults (Müller et al., 2008). Furthermore, the reduced empathy and altered processing of aversive stimuli often associated with CD (Cohen & Strayer, 1996) has been linked to abnormal activity (Decety et al., 2009) and volume (Fahim et al., 2011; Sterzer et al., 2007) in the insula. This structure, which plays a key role in empathic processing (Craig et al., 2009; Singer et al.,

2009), has also shown to be associated with the severity of disorder in males (Fairchild et al., 2011). The fact that GMV changes in this area were more evident in males with CD may reflect the fact that males often display a more severe form of the disorder than females.

Importantly, however, we also observed sex-by-diagnosis interactions in similar regions that showed diagnostic effects: namely, left amygdala, left ACC, and bilateral anterior insula. In left amygdala and bilateral insula, the sex-by-diagnosis interactions were driven by males with CD showing lower GMV relative to male controls. However, the sex-by-diagnosis interaction in ACC showed that there were opposite changes in males and females with CD relative to their respective control groups: while males with CD displayed *lower*, females with CD showed *higher,* GMV in this area relative to sex-matched controls.

As mentioned above, lower GMV in males with CD relative to controls has previously been reported in the amygdala (Fairchild et al., 2011; Huebner et al., 2008; Sterzer et al., 2007), ACC (Olvera et al., 2014; Sebastian et al., 2015; Sterzer et al., 2005), and insula (Fahim et al., 2011; Sterzer et al., 2007). Although one previous study found lower ACC volume in females with CD relative to controls (Dalwani et al., 2015), substance dependence was a confound in that study, and may explain the discrepancy in findings with the current study. Similarly, lower insula GMV has been noted in a group of females with CD (Fairchild et al., 2013). However, the current study included twice as many individuals with CD, and findings of the Fairchild et al. (2013) study may be a consequence of a relatively small sample size. In addition, it is worth noting that the anterior insula clusters identified as showing sex-by-diagnosis interactions do not appear to overlap between the two studies; the current study seems to have identified a more anterior part of the insula[1].

[1] y= 21 vs. y=3 in the left hemisphere; y=24 vs. y=3 in the right hemisphere in the current study and Fairchild et al.'s (2013) study, respectively.
[2] Fairchild et al.'s (2013) study was an all-female study, but also included data from a previously published

We also observed lower bilateral fusiform gyrus GMV in the CD groups compared to the HC groups and a sex-by-CD severity interaction in the left fusiform gyrus. Lower cortical thickness in fusiform gyrus has been found in previous mixed-sex (but predominantly male) CD samples (Hyatt, Haney-Caron, & Stevens, 2012; Jiang et al., 2015). Males with CD further showed a negative correlation between CD severity and fusiform gyrus GMV, whereas a positive correlation was found for females with CD. The fusiform gyrus is functionally connected to the amygdala (Pujol et al., 2009) and has been suggested to be involved in emotion processing (Geday, Gjedde, Boldsen, & Kupers, 2003; Pujol et al., 2009).

Considering the high co-occurrence of ADHD and CD in this sample, and in clinical settings more generally (Waschbusch, 2002), it is also important to consider the influence of comorbid ADHD symptoms on the results. Previous VBM studies have varied in terms of how they dealt with this comorbidity issue. Here, we were able to show that while ADHD comorbidity influenced some of the main effects of diagnosis (especially in the anterior insula), all of the sex-by-diagnosis interactions, as well as the sex-by-CD severity interactions, remained unaffected.

Taken together, the results of the sex-by-diagnosis interactions appear to suggest that the relationship between CD and alterations in GMV is stronger in males than females - some structural differences (e.g., anterior insula) were absent in females with CD. On the face of it, while males and females with CD show abnormalities in overlapping areas, there are also several areas where only males with CD show lower GMV or the structural deficits are greater in magnitude. This strongly indicates that males and females have abnormalities in similar areas, but that there are differences in the strength, and to some extent the direction, of these abnormalities. This has implications for future neuroimaging studies of CD, as combining the sexes to investigate diagnosis effects might obscure differences that might only be present in males or females with CD. It might also explain why previous findings in this area have been so mixed. Furthermore, the fact that we see

lower GMV in anterior insula in males only and significantly greater reductions in left amygdala GMV in males relative to females suggests that deficits in empathy and emotion processing seen in CD groups might be more characteristic of males with CD.

Similarly, sex-dependent effects on brain structure have previously been found in autism and PTSD. Males, but not females, with autism showed lower GMV compared to their healthy controls in parietal brain regions (Beacher et al., 2012). Males with PTSD further showed more widespread structural alterations compared to females with PTSD; for example, lower frontal lobe volume and smaller ventricles and corpus callosum (De Bellis & Keshavan, 2003).

The current study had several strengths, including a larger than previously studied sample, and equal numbers of males and females with CD, which increased our statistical power to detect common and sex-specific effects of CD on GMV. In addition, our groups were matched on pubertal status and handedness, and our CD groups were matched on age-of-onset, medication use, and, apart from ADHD, which was more common in males, rates of comorbid disorders. In addition, running the analyses both with and without including ADHD symptoms as a covariate of no interest allowed us to demonstrate unique contributions of CD to GMV alterations, as well as the changes seen in a representative sample of CD adolescents. We also ran an analysis contrasting childhood-onset CD and adolescent-onset CD subgroups to ensure that it was valid to combine them in the main analyses.

However, the study should be viewed in the light of a number of limitations. Firstly, we were unable to match our CD and control groups on IQ. However, differences in IQ between CD and control groups are difficult to avoid, and excluding CD participants with low IQ would likely reduce the generalisability of the findings while disregarding important aspects of the CD phenotype. Furthermore, there were no differences in IQ between the male and female CD groups or between the male and female HC groups. Therefore group differences in this measure seem unlikely to explain the sex-by-diagnosis

interactions observed here. Secondly, being a multi-site study, it is possible that differences in hardware and software between sites could have introduced noise into the data. In order to minimise these potential issues, data acquisition protocols were matched across sites, and each site underwent site qualification procedures (see Chapter 3). In addition, site was included as a covariate of no interest in all analyses. Thirdly, we intentionally selected participants with either late- or post-pubertal status, and matched our groups on this metric to limit the variability in developmental stages between groups. However, by restricting our sample by age and puberty, we are unable to make any inferences regarding sex differences in the relationship between CD and brain structure in younger ages. Finally, VBM as a method has been criticized on a number of points, including averaging across different properties of the cortex (Panizzon et al., 2009; Winkler et al., 2012). Cortical thickness, surface area, and folding are three separate properties of the cortex. Although these properties are somewhat dependent on each other, such that, the amount of cortical folding is proportional to the surface area within the intracranial volume, the three cortical measures assess different aspects of cortical structure and there are strong arguments that these properties should be investigated independently rather than being averaged together (Panizzon et al., 2009; Wallace et al., 2014; Winkler et al., 2012). However, as mentioned in the introduction, VBM allows analysis of both cortical and subcortical structures using the same methodological approach. While it is possible to obtain volumetric data on subcortical volumes using the same pipeline as was used to obtain the cortical measures, this method is inherently different from the SBM methods used to analyse cortical structures, and thus it is more difficult to reconcile these findings. Thus, using VBM facilitates replication of, and comparison with, previous research in a way that is not yet possible with SBM (due to the fact that considerably less research on CD has used SBM). In addition, VBM uses the same statistical parametric mapping approach as is used in fMRI analyses, and the same

anatomical ROIs and statistical thresholds can be used, which creates the opportunity to relate volumetric changes and neural activity.

4.5 Conclusion

We observed main effects of CD diagnosis in frontal, temporal, and limbic areas, where the CD group showed lower GMV relative to controls. We also found areas, such as the insula, where only males with CD showed lower GMV relative to male controls. In rostral areas of the anterior cingulate cortex, males and females with CD showed opposite structural changes in relation to their respective control groups. Additional sex differences were observed in the effects of the severity of the disorder; males showed a negative, and females a positive, association between CD severity and fusiform gyrus volume. Furthermore, although controlling for ADHD symptoms rendered some of the main effects of diagnosis non-significant, the sex-by-diagnosis interactions and sex-by-CD severity interactions remained unchanged. These results indicate that there are both similarities and differences between the sexes in the relationship between CD and structural abnormalities, suggesting that it is not appropriate to combine the sexes in neuroimaging studies of CD as this might obscure or bias results – especially in predominantly male samples that have been used in the majority of previous research.

Chapter 5: Sex differences in the relationship between conduct disorder and cortical structure in adolescence: A surface-based morphometry approach.

Background:

Previous studies have reported lower cortical thickness and surface area, and altered gyrification in frontal and temporal regions in adolescents with conduct disorder (CD). While there is evidence that the clinical phenotype of CD differs between males and females, no imaging studies have included a sample appropriate for examining whether such sex differences extend to cortical structure.

Method:

As part of a European multi-site study (FemNAT-CD), structural magnetic resonance imaging data were collected from 48 female and 48 male adolescents with CD and 104 sex-, age- and pubertal-status matched typically-developing controls (aged 14-18 years). Data were analysed using surface-based morphometry implemented in FreeSurfer, testing for effects of sex and diagnosis and their interaction, while controlling for age, IQ, scan site, and total grey matter volume.

Results:

CD was associated with cortical thinning and higher gyrification in ventromedial prefrontal cortex in both males and females. Several sex-by-diagnosis interactions were also observed: males with CD showed lower, and females with CD showed higher, supramarginal gyrus cortical thickness, relative to their respective control groups. Males with CD showed higher gyrification and surface area in superior frontal gyrus relative to male controls, whereas the opposite pattern was seen in females. Within the CD group, CD severity was positively associated with posterior cingulate cortex gyrification, and several sex-by-CD severity interactions were observed: males showed a positive, and females a

negative, correlation between CD severity and both surface area and gyrification in superior frontal gyrus. These results were not influenced by CD age-of-onset differences between the sexes or ADHD comorbidity.

Conclusion:

This study provides evidence for sex differences in the relationship between CD and brain structure across three different properties of the cortex, suggesting that the pathophysiological basis of the disorder may be sex-specific. These results highlight the need to consider males and females separately in future neuroimaging studies and the possibility that males and females may require different treatments.

5.1 Introduction

It is increasingly thought that brain abnormalities may contribute to the risk of developing conduct disorder (CD; Frick & Viding, 2009). As discussed in Chapter 4, recent meta-analyses confirmed that individuals with CD show altered brain structure, such as lower volume in amygdala, insula, prefrontal cortex, and superior temporal gyrus (Rogers & De Brito, 2016), and higher volume in fusiform gyrus and parietal cortex (Aoki et al., 2014).

While the lifetime prevalence of CD is between two to 10 times higher in males than females (Fontaine, Carbonneau, Vitaro, Barker, & Tremblay, 2009; Moffitt et al., 2001; Nock et al., 2006), it is nevertheless one of the most common disorders in adolescent females (Merikangas et al., 2010), and one of the main reasons for referral to mental health services (Baker, 2013; Pajer, 1998). As was discussed in Chapter 1, CD also presents in different ways in males and females; males with CD display higher levels of aggression (Gorman-Smith & Loeber, 2005), but lower levels of comorbid disorders, such as depression (Rosenfield & Mouzon, 2013), and are more likely to develop antisocial personality disorder in adulthood (Moffitt et al., 2001).

Although very few imaging studies have investigated sex differences in CD, there is preliminary evidence that CD is associated with lower amygdala (Fairchild, et al., 2013) and orbitofrontal/ventromedial prefrontal cortex (OFC/vmPFC) volume (Dalwani et al., 2015) in both males and females. In contrast, one study found lower anterior insula volume in females with CD relative to female controls, but the reverse effect in males (Fairchild et al., 2013). Furthermore, a negative association between CD severity and superior temporal cortex grey matter volume (GMV) was reported in females, but not males, with CD (Michalska et al., 2015).

The majority of previous neuroimaging studies of CD have used voxel-based morphometry (VBM, see Chapter 4), which measures differences in grey matter volume (GMV) across the whole brain (Ashburner & Friston, 2000). However, an alternative, and increasingly popular, method of assessing for differences in brain structure between individuals with psychopathology and healthy individuals is to use surface-based morphometry (SBM) methods. Compared to the more commonly-used method of VBM, SBM is capable of measuring different properties of the cortex, which contribute to volume: cortical thickness (CT), surface area (SA), and gyrification (i.e., the degree of cortical folding within a sulcus compared to outside the sulcus). It is important to distinguish between these cortical properties because they have distinct aetiologies and different developmental trajectories: while CT and SA follow an inverted-U trajectory across childhood and adolescence, peaking at 8.5 years and 9 years respectively, gyrification peaks at around 1.5 years and decreases steadily during childhood and adolescence (Raznahan et al., 2011). In addition, these metrics show different brain development trajectories for males and females, such that females show an earlier peak than males (Raznahan et al., 2011). Despite the fact that males and females show different brain development trajectories, previous SBM studies of CD have frequently combined data from both sexes.

These studies have demonstrated lower CT in prefrontal cortex (PFC; Fahim et al., 2011; Fairchild et al., 2015; Hyatt et al., 2012; Jiang et al., 2015; Wallace et al., 2014), superior temporal cortex (Fahim et al., 2011; Fairchild et al., 2015; Hyatt et al., 2012; Jiang et al., 2015; Wallace et al., 2014), supramarginal/angular gyrus (Hyatt et al., 2012; Jiang et al., 2015), precuneus (Fahim et al., 2011; Hyatt et al., 2012; Jiang et al., 2015; Wallace et al., 2014), and fusiform gyrus (Hyatt et al., 2012; Jiang et al., 2015), and lower SA in PFC (Fairchild et al., 2015; Sarkar et al., 2015) in CD participants compared to controls. Furthermore, lower gyrification in vmPFC and orbitofrontal OFC (Jiang et al., 2015), and higher gyrification in superior frontal gyrus, insula, fusiform gyrus (Fairchild et al., 2015), and precentral gyrus (Jiang et al., 2015) have been reported in individuals with CD versus typically-developing controls.

Given previous evidence suggesting both common and sex-dependent structural differences, it is surprising that only four out of the six SBM studies of CD have included any females in their sample (Fahim et al., 2011; Hyatt et al., 2012; Jiang et al., 2015; Wallace et al., 2014). In fact, and as described in Chapter 2, only 31 females with CD have been included across all SBM studies of CD (compared to 117 males with CD). The findings reported from these papers are consequently those obtained when collapsing across the sexes and have been interpreted as suggesting that there are no sex differences in the relationship between CD and SBM measures. The current study aimed to improve upon the methods used, and extend on the results found, in Chapter 4, by using SBM measures to address the lack of reliable evidence relating to sex differences in CD-related cortical abnormalities. To this, end, we included a large, balanced sample of male (n=48) and female (n=48) adolescents with CD and similar-sized typically-developing control groups.

Accordingly, we predicted that CD would be associated with: lower CT in ventromedial prefrontal/orbitofrontal cortex, and superior temporal cortex, gyrification abnormalities in insula and PFC, and lower SA in PFC. We predicted that the SBM abnormalities would be most evident in the most severely-disordered individuals, i.e.,

those with more CD symptoms (Fairchild et al., 2011). Given that this is the first study to include a large enough sample to investigate whether sex differences exist in the relationship between CD and cortical structure, we could not make clear predictions regarding sex differences, as would be demonstrated by sex-by-diagnosis interactions.

5.2 Method

5.2.1 Participants

The sample was selected from the participants tested at Southampton, Birmingham, Frankfurt, and Aachen data collection sites participating in the Female Neurobiology and Treatment of Conduct Disorder (FemNAT-CD) study, which is described in detail in Chapter 3. A total of 96 adolescents (48 females) with CD and 104 adolescents (52 females) without psychiatric disorders were selected. All participants were aged between 14-18 years (M_{age}=16.02). Diagnoses were made using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL; Kaufman et al., 1997; see Chapter 3 for a detailed description).

5.2.2 MRI data acquisition

Structural MRI data were acquired using Siemens 3T (Frankfurt and Southampton: Tim Trio; Aachen: Prisma) or Philips 3T (Birmingham: Achieva) scanners. Each site underwent a site qualification procedure prior to commencing data collection (see Chapter 3). T1-weighted scans were collected using a magnetization-prepared rapid-acquisition gradient-echo sequence (MP RAGE) (TE(Philips) = 3.7, TE(Siemens) =3.4, TR=1.9, flip angle = 9, FHxAP FoV = 256, RL FoV = 192, matrix = 256, voxel size =1×1×1 mm, sagittal slices = 192, bandwidth(Philips) = 174 hz/pix, bandwidth(Siemens) = 180 Hz/pix, total scan time = 4 min 26 sec (Siemens) or 6 min 5 sec (Philips). Data were collected at the start of the scanning sequence, and the quality of the data was assessed immediately after the scan by the MRI operator, and repeated until a high quality image was acquired.

5.2.3 Image processing

CT, SA, and gyrification were estimated at each vertex using Freesurfer v5.3.0 (http://surfer.nmr.mgh.harvard.edu; Dale et al., 1999; Fischl, Sereno, Tootell, & Dale, 1999; Winkler et al., 2012). FreeSurfer surface-based cortical reconstruction and analysis have been described (Dale et al., 1999; Fischl et al., 1999) and validated (Han et al., 2006) in previous studies. Briefly, the reconstruction process involves segmentation of the white matter and identification of the white-grey matter interface followed by the identification of the grey matter-cerebrospinal fluid interface to create the pial surface; a two-dimensional mesh of triangular elements consisting of more than 100,000 vertices per hemisphere.

All surfaces were visually inspected (blind to participant group status) and segmentation errors or topological defects were manually corrected in Freeview. The corrections included manual edits to the white- and grey matter boundaries, and adding control points where needed. CT was calculated at each vertex as the shortest distance (mm) between the white and pial surfaces (Han et al., 2006). SA was estimated by mapping each vertex into a spherical atlas space and calculating the transformation change (expressed in mm^2; Winkler et al., 2012). Gyrification (termed 'local gyrification index' or *l*GI) was calculated using the method developed by Schaer at al. (Schaer et al., 2008). Each surface was resampled into a common anatomical space and smoothed using Gaussian kernels of 10 mm full-width/half-maximum for CT and SA, whereas no smoothing was applied for *l*GI. Finally, total GMV was estimated for each participant and included to control for inter-individual variability in global brain size.

5.2.4 Statistical analysis

First, for each hemisphere, a full-factorial general linear model (GLM) was fitted separately for CT, SA, and *l*GI, which tested for effects of diagnosis, sex, and sex-by-diagnosis interactions. Second, separate GLM correlation analyses were conducted within the CD group to test for correlations and sex-by-CD severity interactions between CT, SA,

and *l*GI, and number of lifetime CD symptoms, i.e., CD severity (from the K-SADS-PL). Third, given previous evidence suggesting quantitative brain structural differences between childhood-onset (CO) and adolescent-onset (AO) CD (Fairchild et al., 2013; Fairchild et al., 2015), we compared these subgroups in order to assess the validity of collapsing across them in our main analyses. In addition, each analysis was repeated including lifetime ADHD symptoms (from the K-SADS-PL) as a covariate of no interest. All models included age, IQ, total GMV, and scan site as covariates of no interest (coded as a binary fixed-effect). In line with previous research (Fairchild et al., 2015; Hyatt et al., 2012), results were corrected for multiple comparisons using a Monte Carlo z-field simulation, and clusters were reported if they met a whole-brain corrected threshold of $P<0.05$.

5.3 Results

5.3.1 Sample and demographics

Table 5.1 summarises the demographic characteristics of the sample. The four groups did not significantly differ in age, pubertal status, or handedness. Within the male and female samples, the CD groups had lower total IQs and reported more CD and ADHD symptoms than HCs. Males with CD further displayed more ADHD symptoms than the other three groups. Furthermore, by design, our control groups were free of current psychiatric disorders; thus the CD group had significantly higher levels of comorbidity and medication use. However, apart from ADHD comorbidity, the male and female CD groups did not differ in terms of comorbidity or medication use. Total GMV values were higher in males overall compared to females ($p<.001$), as expected (Giedd et al., 1999), but there were no differences in total GMV between males with CD and male controls ($p=.81$) or females with CD and female controls ($p=.92$).

Table 5.1. Demographic and clinical characteristics of the sample in the SBM study

	Females		Males				
Variable	CD (*n*=48)	HC (*n*=52)	CD (*n*=48)	HC (*n*=52)	*T*group (*p*)	*T*sex (*p*)	*F*groupXsex (*p*)
	M (SD)						
Age (years)	15.83 (1.29)	16.13 (1.07)	15.92 (1.32)	16.21 (1.14)	T=1.75 (.08)	T=.46 (.64)	F= 1.09 (.35)
Estimated IQ	92.51 (12.26)	99.67 (12.01)	93.01 (11.95)	101.33 (11.42)	T=4.58 (<.001)	T=.66 (.51)	F= 7.14 (<.001)
Lifetime CD symptoms	6.06 (2.78)	.37 (1.12)	7.50 (2.84)	.42 (.67)	T=21.42 (<.001)	T=1.33 (.18)	F=164.30 (<.001)
ADHD symptoms	4.98 (6.53)	.13 (.60)	8.75 (6.62)	.06 (.42)	T=10.11 (<.001)	T=2.17 (.03)	F= 41.98 (<.001)
	N (%)				X^2group (*p*)	X^2sex (*p*)	X^2 groupXsex (*p*)
No. Lifetime *DSM-IV* diagnoses							
ODD	31(66)	1(2)	32(70)	0(0)	X^2=99.83 (<.001)	X^2=.002 (1.00)	X^2=100.01 (<.001)
ADHD	10(21)	0(0)	24(52)	0(0)	X^2=42.95 (<.001)	X^2=7.14 (=.01)	X^2=61.49 (<.001)
MDD	14(29)	0(0)	8(17)	0(0)	X^2=31.30 (<.001)	X^2=1.77 (.26)	X^2=27.70 (<.001)
Alcohol abuse	3(6)	1(2)	4(9)	0(0)	X^2=5.43 (.02)	X^2=.00 (1.00)	X^2=6.00 (.11)
Drug abuse (cannabis)	7(15)	0(0)	10(22)	2(4)	X^2=15.07 (<.001)	X^2=1.51 (.24)	X^2=47.38 (<.001)
Medication	4(8)	1(2)	8(17)	0(0)	X^2=10.94 (.001)	X^2=.74 (.57)	X^2=13.84 (.003)
Puberty							
Late	34(71)	34(65)	34(71)	40(77)	X^2=.02 (.96)	X^2=.87 (.35)	X^2=1.68 (.64)
Post	14(29)	18(35)	14(29)	12(23)			
Age of Onset							
Childhood	19 (40)		26 (54)			X^2=1.58 (.45)	
Adolescent	27 (56)		22 (46)				
Missing	2 (4)		0 (0)				
Handedness							
Right	41 (86)	48 (92)	38 (79)	48 (92)			
Left	3 (6)	4 (8)	10 (21)	2 (4)	X^2=4.92 (.09)	X^2=2.37 (.31)	X^2=15.93 (.14)
Ambidextrous	3 (6)	0 (0)	0 (0)	1 (2)			
Missing	1 (2)	0 (0)	0 (0)	1 (2)			
Ethnicity							
Caucasian	45 (93)	51 (98)	46 (96)	49 (94)	X^2=3.78 (.29)	X^2=5.67 (.13)	X^2=12.75 (.17)
Other	3 (6)	1 (2)	2 (4)	3 (6)			

Note: ADHD = attention-deficit/hyperactivity disorder; CD = conduct disorder; HC = healthy control; IQ = intelligence quotient (measured using the WASI or WISC-IV); MDD = Major depressive disorder ODD = Oppositional defiance disorder. Group and sex differences were computed using independent sample t-tests or Chi square test, and sex-by-diagnosis interactions were computed using between-group ANOVAS and Chi square tests.

5.3.2 SBM results

5.3.2.1 Preliminary analysis

5.3.2.1.1 Conduct disorder age-of-onset effects

There were no differences between the CO-CD and AO-CD subtypes in CT or SA. The CD subgroups differed from each other in *l*GI in some regions including the anterior insula and temporal pole; however, these regions were not altered as a function of CD, sex,

or their interaction, thus we did not distinguish between these subgroups in the main *l*GI analyses.

5.3.2.2 Full factorial analysis

Effects of diagnosis: CD was associated with lower CT and, contrary to our predictions, higher SA. The CD group showed lower CT in bilateral ventromedial prefrontal cortex (vmPFC), left rostral middle frontal gyrus, and left precentral gyrus (Figure 5.1A; Table 5.2). Higher SA was observed in left precentral extending to postcentral gyrus, left middle temporal gyrus/fusiform gyrus and right lateral occipital cortex (see Table 5.3). The CD group also showed lower *l*GI in left superior temporal gyrus/posterior insula, vmPFC/lateral orbitofrontal cortex (OFC), and postcentral/precentral gyrus (Figure 5.3A) and lower *l*GI in right inferior frontal gyrus (IFG) and supramarginal gyrus (Table 5.4).

Effects of sex: Consistent with previous data showing sex differences in SBM measures during adolescence and young adulthood (Im et al., 2006; Eileen Luders et al., 2004; Sowell et al., 2007), females overall showed higher cortical thickness in precentral and postcentral gyrus, greater surface area in temporal and frontal areas, and higher folding in frontal, temporal and occipital regions relative to males (Tables 2-4).

Sex-by-diagnosis interactions: Sex-by-diagnosis interactions were observed for each of the SBM measures; males with CD showed lower CT in left superior parietal lobule and right supramarginal gyrus, whereas these effects were reversed in females (see Figure 5.1B). In superior frontal gyrus (SFG), males with CD displayed higher, while their female counterparts displayed lower, SA and *l*GI (see Figure 5.2 and Figure 5.3C, respectively). In left parahippocampal cortex, males with CD displayed higher, whereas females with CD showed lower, *l*GI (Table 5.4).

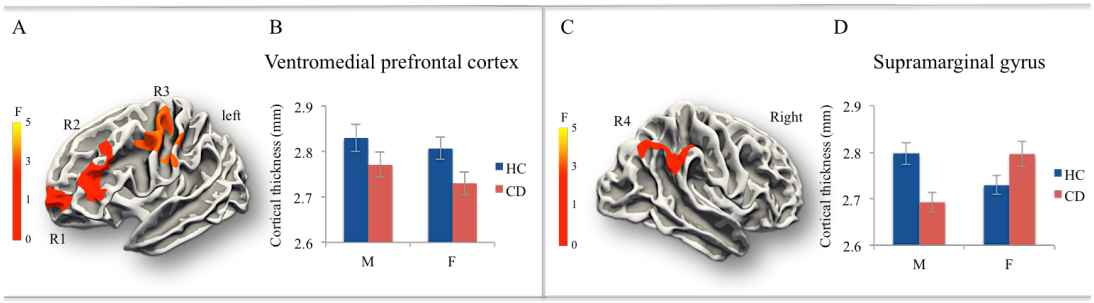

Figure 5.1. Effect of diagnosis and sex-by-diagnosis interaction in cortical thickness. Panel A displays lower cortical thickness in the conduct disorder (CD) group compared to healthy controls (HCs) in left ventromedial prefrontal cortex (R1), rostral middle frontal gyrus (R2), and precentral gyrus (R3). Panel B displays the group differences in ventromedial prefrontal cortex (R1); these results remained significant when controlling for attention-deficit/hyperactivity disorder symptoms. Panel C displays a sex-by-diagnosis interaction in right supramarginal gyrus (R4); females with CD showed higher thickness compared to female HCs, whereas males with CD showed lower thickness in this region compared to male HCs. Panel D displays the sex-by-diagnosis interaction, which remained significant after controlling for attention-deficit/hyperactivity disorder symptoms. Error bars show ± 1 standard error.

Table 5.2. Main effects of diagnosis, sex, and sex-by-diagnosis interactions on cortical thickness that were obtained without controlling for ADHD symptoms.

Contrast and Cerebral Region	Hemisphere	Size (mm^2)	NVtxs	MNI coordinates *x*	*y*	*z*	CWP	*Max*
Main effect of diagnosis								
All CD < All HC								
Precentral gyrus	L*	2621	4411	-52	0	38	0.0002	3.09
Rostral middle frontal	L*	1846	1922	-41	36	17	0.0062	3.00
Ventromedial PFC	L	2048	2219	-7	50	8	0.0024	2.68
	R*	2373	2565	7	65	6	0.0006	3.08
Cuneus	R*	1618	1768	3	-81	27	0.0106	2.59
Main effect of Sex								
All females > All males								
Postcentral	L	1350	2392	-58	-9	34	0.0284	8.66
	R	2509	4431	62	-8	34	0.0005	5.92
Precentral	L	5745	10766	-49	0	50	0.0001	5.57
	R	2035	4141	18	-16	71	0.0024	5.28
Sex-by-diagnosis interaction								
FCD > FHC, MCD < MHC								
Superior parietal lobule	L*	1287	2195	-19	-65	44	0.0367	4.38
Supramarginal gyrus	R	2008	2895	57	-48	30	0.0027	3.24

*Note: ADHD = attention-deficit/hyperactivity disorder; CD = Conduct disorder; CWP = cluster-wise-P value; FCD = Females with conduct disorder; HC = Healthy control; Max = maximum –log10(P value); MCD = Males with conduct disorder; MNI = Montreal Neurological Institute; NVtxs = number of vertices in the cluster. * Indicates regions that were rendered non-significant when including ADHD symptoms as a covariate of no interest.*

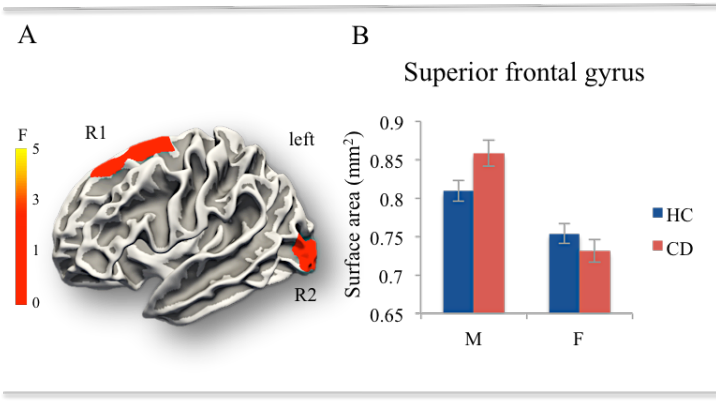

Figure 5.1. Sex-by-diagnosis interaction in surface area. Panel A displays sex-by-diagnosis interactions in superior frontal gyrus (R1) and lateral occipital cortex (R2) surface area; males with conduct disorder (CD) showed higher surface area compared to male healthy controls (HCs), whereas females with CD showed lower surface area in these regions compared to female HCs. Panel B displays the sex-by-diagnosis interaction in superior frontal gyrus (R1), a result that remained significant after controlling for attention-deficit/hyperactivity disorder symptoms. Error bars show ± 1 standard error.

Table 5.3. Main effects of diagnosis, sex, and sex-by-diagnosis interactions in surface area that were obtained without controlling for ADHD symptoms.

Contrast and Cerebral Region	Hemisphere	Size (mm^2)	NVtxs	MNI coordinates *x*	*y*	*z*	CWP	*Max*
Main effect of diagnosis								
All CD > All HC								
Fusiform/inferior temporal gyrus	L*	2312	2557	-42	-43	-25	0.0112	3.01
Postcentral/superior frontal gyrus	L*	3044	5840	-50	-27	41	0.0013	2.78
Lateral occipital	R*	2953	3311	12	-95	15	0.0021	1.83
Main effect of Sex								
All females > All males								
Caudal middle frontal	R	2318	3864	35	14	28	0.0102	4.17
Middle/superior temporal gyrus/insula	R	4589	7072	49	-25	-6	0.0001	3.44
Middle/inferior temporal gyrus	L	2011	2221	-61	-39	-17	0.0246	2.92
All males > All females								
Lateral occipital	L	2695	2689	-19	-98	-18	0.0039	4.28
Rostral middle frontal	R*	2008	1980	43	48	-3	0.0272	3.23
Cuneus/ occipital cortex	R	2618	2610	11	-91	16	0.0042	2.73
Sex-by-diagnosis interaction								
MCD > MHC, FCD < FHC								
Lateral occipital	L*	2229	2357	-28	-98	-1	0.0130	2.29
Superior frontal gyrus	L	1936	2183	-17	2	72	0.0298	1.93

*Note: ADHD = attention-deficit/hyperactivity disorder; CD = Conduct disorder; CWP = cluster-wise-P value; FCD = Females with conduct disorder; HC = Healthy control; MCD = Males with conduct disorder; Max = maximum –log10(P value); MNI = Montreal Neurological Institute; NVtxs = number of vertices in the cluster. * Indicates regions that were rendered non-significant when including ADHD symptoms as a covariate of no interest.*

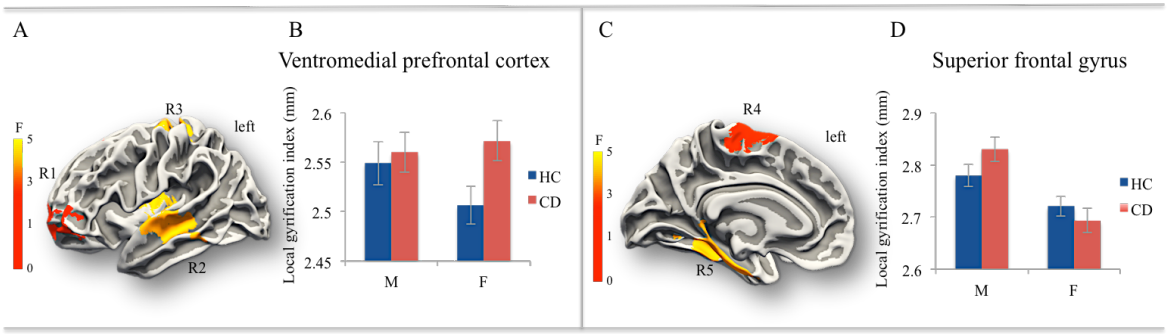

Figure 5.2 Effect of diagnosis in local gyrification index. Panel A display higher gyrification in the conduct disorder (CD) group compared to healthy controls (HCs) in left ventromedial prefrontal cortex (vmPFC; R1), insula/superior temporal gyrus (R2), and pre/postcentral gyrus (R3). Panel B displays the group difference in vmPFC (R1), a result that remained significant after controlling for attention-deficit/hyperactivity disorder symptoms. Panel C display sex-by-diagnosis interactions in left superior frontal gyrus (R4) and parahippocampal cortex (R5); males with CD showed higher gyrification compared to male HCs, whereas females with CD showed lower gyrification in these regions compared to female HCs. Panel D displays the sex-by-diagnosis interaction in superior frontal gyrus (R4), which remained significant after controlling for attention-deficit/hyperactivity disorder symptoms. Error bars display ± 1 standard error.

Table 5.4. Main effects of diagnosis, sex, and sex-by-diagnosis interactions on cortical folding, as quantified using local gyrification index, that were obtained without controlling for ADHD symptoms.

Contrast and Cerebral Region	Hemisphere	Size (mm^2)	NVtxs	MNI coordinates *x*	*y*	*z*	CWP	*Max*
Main effect of diagnosis								
All CD > All HC								
Superior temporal/ insula	L*	2554	4830	-64	-19	-4	0.0001	3.10
Ventromedial PFC	L	988	846	-27	46	-13	0.0068	2.86
Post/precentral gyrus	L*	1689	3571	-28	-29	53	0.0001	2.41
Lateral orbitofrontal	L*	892	726	-13	60	-10	0.0122	1.86
All CD < All HC								
Inferior frontal gyrus	R*	1647	2577	42	17	22	0.0001	2.99
Supramarginal	R*	872	1495	54	-36	22	0.0171	2.74
Main effect of Sex								
All females > All males								
Precentral gyrus	R	1575	2880	38	5	28	0.0001	3.96
Middle/inferior frontal gyrus	L	1928	3393	-40	15	34.2	0.0001	3.34
Orbitofrontal cortex	L	1029	1389	-38	21	-17	0.0053	3.34
Postcentral gyrus	R	1008	1917	53	-9	26	0.0072	3.33
Lateral occipital cortex	L	2486	3115	-22	-97	18	0.0001	2.78
Middle temporal gyrus	L*	3069	4197	-65	-23	-11	0.0001	2.43
Rostral anterior cingulate	R*	897	1334	4	33	-10	0.0145	2.23
All males > All females								
Fusiform/lingual gyrus	L	1761	2249	-15	-67	-12	0.0001	3.16
	R	3718	6043	20	-63	-11	0.0001	2.97
Sex-by-diagnosis interaction								
MCD > MHC, FCD < FHC								
Fusiform /parahippocampal gyrus	L*	2463	4023	-17	-17	-26	0.0001	2.54
Superior frontal gyrus	L	1251	2129	-8	-9	70	0.0009	2.18

*Note: ADHD = attention-deficit/hyperactivity disorder; CD = Conduct disorder; CWP = cluster-wise-P value; FCD = Females with conduct disorder; HC = Healthy control; MCD = Males with conduct disorder; Max = maximum –log10(P value); MNI = Montreal Neurological Institute; NVtxs = number of vertices in the cluster; PFC = prefrontal cortex. * Indicates regions that were rendered non-significant when including ADHD symptoms as a covariate of no interest.*

5.3.2.3 Correlations with CD severity and sex-by-CD severity interaction in the CD sample

Correlations with CD severity: There were no significant correlations between CD severity and CT within the CD sample. However, CD severity were positively correlated with *l*GI in caudal middle frontal, and negatively correlated with *l*GI in isthmus cingulate (Table 5.5). In addition, CD severity was positively correlated with SA in right posterior cingulate cortex/precuneus (see Figure 5.4A, Table 5.5).

Sex-by-CD severity interactions: There were no sex-by-CD severity interactions for CT. However, there was a significant sex-by-CD severity interaction for SA: males showed a positive, whereas females showed a negative, correlation between CD severity and right superior frontal/precentral gyrus SA (Figure 5.4C). Finally, several sex-by-CD severity interactions were observed for *l*GI: females showed a positive, and males a negative, correlation between CD symptoms and left fusiform gyrus *l*GI (Figure 5.4E). In contrast, females showed a negative correlation between CD severity and left SFG/paracingulate cortex *l*GI, whereas no such correlation was observed in males (Figure 5.4G, Table 5.5).

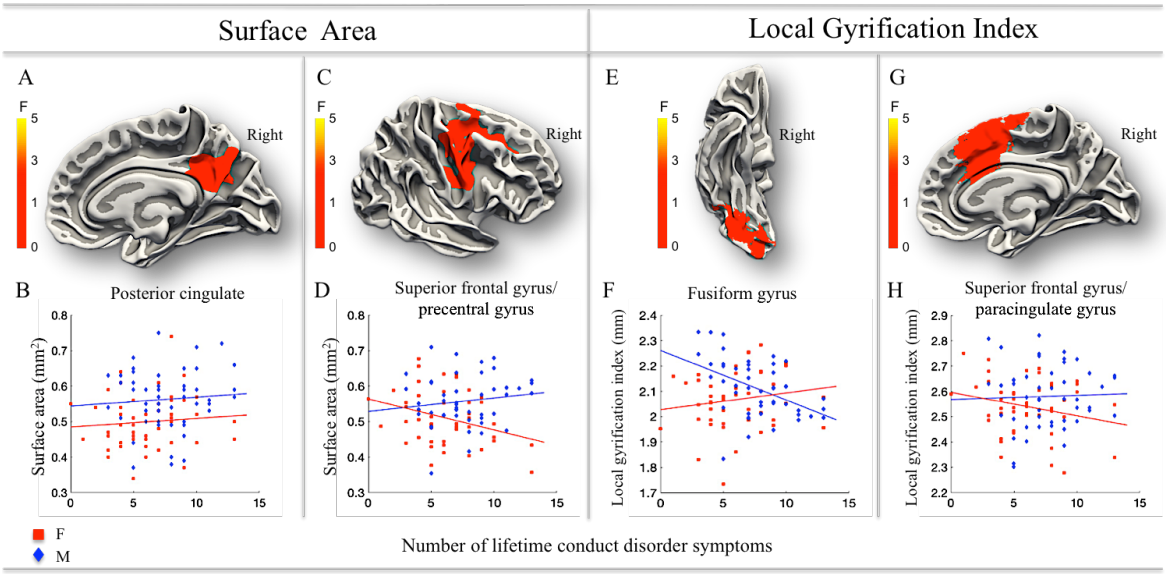

Figure 5.3. Correlations between conduct disorder (CD) severity and surface area and gyrification within the CD group. Panel A displays a positive correlation between CD severity and surface area in posterior cingulate cortex/precuneus. Panel B shows the correlation in both males (blue symbols and least square lines) and females (red symbols and least square lines). Panel C displays a sex-by-CD severity interaction in superior frontal/precentral gyrus surface area. Panel D shows that females with CD showed a negative, and males showed a positive correlation in this region. Panel E displays a sex-by-CD severity interaction in fusiform gyrus local gyrification index (*l*GI). Panel F shows that females with CD had a positive, and males with CD a negative correlation in this region. Panel G displays a sex-by-CD severity interaction in superior frontal gyrus/paracingulate cortex *l*GI. Panel H shows that females with CD had a negative correlation in this region, whereas there was no significant correlation for males.

Table 5.5. Correlations between cortical thickness, surface area, and gyrification and CD severity, and sex-by-CD severity interactions within the CD group. Results are presented without controlling for ADHD symptoms*.

Contrast and Cerebral Region	Hemisphere	Size (mm^2)	MNI coordinates NVtxs	*x*	*y*	*z*	CWP	*Max*
Surface area								
CD severity Correlations								
Overall positive correlation								
Posterior cingulate cortex/precuneus	R	2045	3715	8	-54	26	0.0240	3.39
Sex-by-CD severity interaction								
MCD > FCD								
Precentral gyrus /Superior frontal gyrus	R	4298	8054	53	3	44	0.0001	2.89
Local gyrification index								
CD severity Correlations								
Overall positive correlation								
Caudal middle frontal	L	848	1373	-30	22	45	0.0166	3.06
Overall negative correlation								
Isthmus cingulate	R	1051	1663	17	-50	-0	0.0052	2.06
Sex-by-CD severity interaction								
MCD > FCD								
Superior frontal gyrus	R	1105	1835	24	-0	52	0.0037	3.07
FCD > MCD								
Temporal pole	L	899	1032	-33	3	-21	0.0119	3.37
Fusiform gyrus	R	2560	2661	30	-76	-13	0.0001	2.52
Middle temporal	L	1424	1991	-65	-45	-9	0.0001	2.10
Supramarginal gyrus	L	1050	1785	-58	-55	20	0.0047	1.75

*Note: ADHD = attention-deficit/hyperactivity disorder; CD = conduct disorder, CWP = cluster-wise-P value; FCD = Females with conduct disorder; MCD = Males with conduct disorder; Max = maximum –log10(P value); MNI = Montreal Neurological Institute; NVtxs = number of vertices in the cluster. * Re-running the analysis with ADDH symptom as a covariate did not alter the results.*

5.3.3 ADHD as an additional covariate of no interest

The main effects of CD on vmPFC CT and *l*GI, and the sex-by-diagnosis interactions for supramarginal CT and superior frontal gyrus *l*GI and SA remained significant after controlling for ADHD symptoms. In addition, all CD severity correlations and sex-by-CD severity interactions reported above remained significant.

5.3.4 Confounding effects of site and IQ

We also tested the impact of excluding site and IQ from the statistical models. For CT, the effects of diagnosis and sex-by-diagnosis interactions remained significant when excluding IQ or site as a covariate. For *l*GI, excluding IQ or site did not affect the effect of diagnosis or the sex-by-diagnosis interaction, whereas the effects of diagnosis and sex-by-diagnosis interaction in SA were rendered non-significant.

5.4 Discussion

In Chapter 4, we used voxel-based morphometry (VBM) to investigate CD-related alteration, and sex-by-diagnosis interactions, in grey matter volume. Because VBM has been criticised for averaging across different properties of the cortex, the current chapter adopted surface-based morphometry measures (SBM) to extend on the methods used in the previous chapter. To our knowledge, this is the first SBM study specifically designed and with a large enough sample to test for sex differences in the relationship between CD and cortical structure. Our results confirmed previous associations between CD and alterations in CT, SA and *l*GI. As hypothesised, and as previously found in predominantly male samples (Fahim et al., 2011; Fairchild et al., 2015; Hyatt et al., 2012; Jiang et al., 2015; Wallace et al., 2014), CD was associated with lower CT in the ventromedial prefrontal cortex (vmPFC). This was accompanied by higher gyrification in overlapping regions of the vmPFC, as well as in the posterior insula, in the CD group. The vmPFC is implicated in reward processing (Knutson et al., 2001), emotion regulation (Goldin, McRae, Ramel, & Gross, 2008), and empathic processing (Shamay-Tsoory, Tomer, Berger, & Aharon-Peretz,

2003). Neuropsychological studies have consistently provided evidence for deficits in these processes in CD (Fairchild, Van Goozen, et al., 2009; Puzzo, Smaragdi, Gonzalez, Martin-Key, & Fairchild, 2016; Sonuga-Barke et al., 2016). Of note, although we did not perform statistical tests of the difference in strength between males and females with CD relative to their respective control groups, the main effect of diagnosis in vmPFC appears somewhat stronger in the female sample. We may have been underpowered to detect a sex-by-diagnosis interaction. Although CD-related alterations were observed in a more posterior location in the present study, higher insula gyrification has been reported previously in CD populations (Fairchild et al., 2015). The insula plays a key role in empathy and the processing of aversive stimuli (Craig et al., 2009; Singer et al., 2009) - processes that are reported to be abnormal in CD (Schwenck et al., 2014). Against expectation and previous findings (Fairchild et al., 2015; Sarkar et al., 2015), we found greater SA in the CD group relative to controls – although the regions affected were different from those reported previously. These observations of greater SA and *l*GI in males with CD may reflect delayed brain development in CD individuals. Longitudinal imaging data suggest that children with ADHD show delays in cortical maturation (as measured by SBM methods) relative to their healthy peers (Shaw et al., 2007). Although similar studies have not been performed in children with CD, it is plausible that CD is also associated with delayed cortical maturation.

Of interest, sex-by-diagnosis interactions were detected in several brain regions– for example, males with CD showed lower, and females with CD higher, supramarginal gyrus CT relative to their sex-matched control groups. Lower supramarginal gyrus CT has been reported in two SBM studies of CD (Hyatt et al., 2012; Jiang et al., 2015), both of which used mixed-sex (but predominantly male) samples. Lower supramarginal CT therefore appears specific to males with CD. Interestingly, the supramarginal gyrus is implicated in decision-making (Silani, Lamm, Ruff, & Singer, 2013) and emotional processing (Herpertz et al., 2008). Therefore, structural alterations in this region may be

related to the behavioural deficits reported in male CD populations during decision-making and emotional processing tasks (Fairchild, van Goozen, et al., 2009; Sonuga-Barke et al., 2016).

Sex-by-diagnosis interactions were also observed in the superior frontal gyrus, an area involved in higher cognitive functions and working memory (Boisgueheneuc et al., 2006). In this region, males with CD showed higher, and females with CD lower, *l*GI and SA relative to their respective control groups. Higher superior frontal gyrus *l*GI and SA in males with CD is consistent with findings from a predominantly male sample (Jiang et al., 2015). However, this is the first study to show that the relationship between CD and superior frontal gyrus *l*GI and SA is reversed in females. Furthermore, males and females showed different relationships between CD severity and *l*GI and SA in several regions, including the fusiform gyrus. Again, this suggests that the relationship between CD and cortical structure partly differs by sex. The fusiform gyrus is functionally connected to the amygdala (Pujol et al., 2009) and CD-related changes in the activity of this region have been reported in previous fMRI studies of emotion processing (Geday et al., 2003; Pujol et al., 2009). Furthermore, it is interesting to note that we observed lower GMV in the fusiform gyrus in both males and females with CD, as well as a sex-by-CD severity interaction in GMV in Chapter 4.

We note that some of our findings were influenced by ADHD comorbidity. This was most apparent for the effects of diagnosis on CT and SA, whereas the sex-by-diagnosis interactions and CD severity correlations remained largely unaffected. This reflects the strong overlap between the two disorders, and supports the idea that CD-related findings should be interpreted both with and without considering ADHD comorbidity.

The fact that several of the present findings were sex-dependent– i.e., there were significant sex-by-diagnosis interactions for all three SBM measures - could be interpreted as evidence that the neurobiological basis of CD differs in males and females. Conversely, these findings may partly be explained by complex effects of sex and diagnosis on brain

development – that, in general, CD may be associated with delayed cortical maturation, but that this is most pronounced in males in late adolescence. However, to investigate this hypothesis, longitudinal neuroimaging studies of males and females with CD are needed.

On the basis of the findings presented here, we recommend that researchers avoid collapsing across the sexes in neuroimaging studies of CD, since combining males and females runs the risk of cancelling out diagnosis effects. Accordingly, future cross-sectional studies of CD might opt to recruit single-sex samples if they are only able to test relatively small samples, or deliberately recruit large numbers of males and females and contrast these groups with sex-matched control groups.

The current study had several strengths – we included large, sex-balanced groups matched for age and pubertal status, examined three separate SBM measures and subcortical volumes, and accounted statistically for group differences in IQ and ADHD comorbidity. However, several limitations should be noted, similarly to Chapter 4. First, although data acquisition protocols were matched across sites, it is possible, as with any multi-site study, that scanner hardware and software differences between sites could introduce error/noise into the data. However, the results remained similar when site was excluded as a covariate. Second, we were unable to match the CD and control groups on IQ. However, we note that our results remained largely the same when dropping IQ as a covariate, and since both CD groups in this study had lower IQs compared to controls, IQ differences cannot explain the observed sex-by-diagnosis interactions. This was also true for comorbidity- although comorbidity was higher in the CD groups, males and females with CD did not differ in rates of comorbid disorders (aside from ADHD). Although we cannot exclude the possibility that psychiatric comorbidity influenced some results, controlling for all comorbidity would have reduced generalisability of the findings. Third, by design, we matched our groups on pubertal development to reduce the possibility of differences in brain developmental stages (Marshall & Tanner, 1986). However, this was an approximate estimation, as the relationship between pubertal stage and brain

development may differ by sex. Future analysis of data from younger children, as well as the use of longitudinal designs, is needed to investigate whether the results presented here are stable across development.

5.5 Conclusion

As with the findings reported in Chapter 4, there were both similarities and differences between males and females in the relationship between CD and cortical structure, implying that the neurobiological basis of CD may partly differ between males and females. These results were largely unrelated to ADHD comorbidity, differences in IQ, or CD age-of-onset. The findings reinforce that it is important to study males and females with CD separately and potentially treating them differently in clinical settings.

Chapter 6: Sex differences in the relationship between conduct disorder and neural activation during the processing of negative facial expressions

Background:

An increasing number of studies have investigated emotion-processing deficits in conduct disorder (CD); however, the vast majority of these have focused on males. Thus, it is currently unclear whether females with CD display similar deficits in the neural underpinnings of emotion processing as their male counterparts. The aim of this study was to address this gap in the literature by investigating differences in brain activity in male and female adolescents with CD compared with typically developing males and females during the processing of negative facial expressions. We predicted lower activity in the CD group, relative to controls, in areas known to be involved in emotion processing (i.e., amygdala, insula, and prefrontal cortex). We further hypothesised that these effects would be less pronounced in females with CD. We had similar predictions in terms of the correlations between CD severity and neural activity in these brain areas – that males would show stronger correlations than females. However, we were agnostic to the direction of these correlations.

Method

Functional magnetic resonance imaging data were obtained from 63 adolescents (30 females) with CD and 66 healthy controls (33 females; 14-18 years old). A gender discrimination paradigm was employed, in which participants viewed negative facial expressions (angry and fearful) and neutral expressions. We tested for main effects of diagnosis, sex, and sex-by-diagnosis interactions on brain activity for the contrasts angry > neutral, fearful > neutral, and all facial expressions (angry, fearful, and neutral) > low-level baseline, i.e., a fixation cross. In addition, we tested for correlations between neural

activity and CD severity (i.e., number of CD symptoms), and sex-by-CD severity interactions across all contrasts. In all analyses, we statistically controlled for age and IQ, tested for differences between childhood and adolescent-onset CD subgroups, and conducted further analyses including ADHD symptoms as an additional covariate.

Results

There was no main effect of diagnosis on activation in the regions of interest specifically during the processing of angry or fearful expressions compared to neutral expressions. However there was a sex-by-diagnosis interaction in the amygdala during the processing of angry expressions. This was driven by females with CD showing lower activity, and males with CD showing higher activity, compared to their respective control groups. There were no correlations between CD severity and brain activity or sex-by-CD severity interactions. Faces in general, compared with a fixation cross, elicited less activity in the fusiform gyrus in individuals with CD compared to controls. For this contrast, there was also a sex-by-diagnosis interaction in bilateral amygdala – females with CD showed lower, whereas males with CD showed higher, activation relative to their respective control groups.

Conclusion

This is the first study to show sex differences in the relationship between CD and amygdala activity during processing of emotional expressions; males and females with CD both showed changes in amygdala activity, but in opposite directions, relative to sex-matched controls. These results suggest that CD might affect key regions of emotion and face processing network differently in males and females. These findings suggest that males and females should be considered separately in future functional neuroimaging studies of CD, as combining the sexes might average out effects of diagnosis.

6.1 Introduction

Emotional facial expressions play a central role in emotional and social behaviour, and the ability to process and recognise such expressions is important for effective

communication between people (Blair, 2003). It has been suggested that difficulties in identifying and understanding the emotions of others may contribute to the development of conduct disorder (CD) in particular, and antisocial behaviour more generally (van Goozen, Fairchild, Snoek, & Harold, 2007). Consequently, emotion recognition and processing in CD has received considerable attention in recent years. Neuropsychological studies of emotion recognition have suggested that CD is associated with a global impairment in recognition across all emotional expressions (Short, Sonuga-Barke, Adams, & Fairchild, 2016; Sully et al., 2015). Several studies have also highlighted that such impairments may be especially prominent in the recognition of negatively valenced emotions. These include fear (Fairchild et al., 2009; Stevens et al., 2001), anger (Best et al., 2002; Fairchild et al., 2010; Fairchild, Van Goozen, et al., 2009), sadness (Fairchild et al., 2009; Sully et al., 2015), and disgust (Best et al., 2002; Fairchild et al., 2010; Fairchild et al., 2009). Similarly, individuals with elevated levels of psychopathic traits, that frequently co-occur with CD (Salekin & Frick, 2005), show a deficit in recognising 'distress cues', such as fearful and sad facial expressions (Blair, Colledge, Murray, & Mitchell, 2001; Blair et al., 2004; Dadds, El Masry, Wimalaweera, & Guastella, 2008; Marsh & Blair, 2008; Stevens et al., 2001).

Brain imaging studies of emotion processing in CD typically employ gender-discrimination paradigms, whereby participants view photographs of male and female facial emotion expressions and are asked to determine the sex of the face in the photographs. This paradigm allows measures of brain activity during processing of emotions, while the participant is engaged in this secondary task that does not interfere with the measured processes. Two early studies using this paradigm found that the combination of CD and high callous-unemotional traits (CU; personality features encompassing diminished empathy or guilt; Frick & White, 2008) was associated with lower activity in the amygdala during the processing of fearful, compared to neutral, facial expressions (Jones et al., 2009; Marsh et al., 2008). Lower activity in this brain region,

together with lower inferior frontal gyrus and posterior cingulate cortex activity, has also been found in youth with disruptive behaviour disorders (DBDs; including CD and oppositional defiant disorder) and high levels of psychopathic traits, during processing of fearful faces (White, Marsh, et al., 2012). Furthermore, the relationship between CD and emotion processing appears to vary as a function of CU traits. Two studies that used a pre-attentive masking paradigm (i.e., facial expressions were presented for 17 ms, below the level of conscious processing), found that individuals with low levels of CU traits had higher activity in the amygdala compared to individuals high in CU traits when processing fearful versus calm facial expressions (Sebastian et al., 2013; Viding et al., 2012). Another study reported that although individuals with CD had higher amygdala activity compared to controls, they had lower activity in orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), and insula compared to controls during the viewing of negatively-valenced stimuli (Herpertz et al., 2008). Reduced activity in amygdala and ventromedial prefrontal cortex (vmPFC) has also been found in a CD group with comparably lower levels of CU traits during processing of sad faces, and similarly, in amygdala, vmPFC, OFC, and insula, when processing angry faces (Passamonti et al., 2010), and in ACC when processing negative emotional stimuli (Stadler et al., 2007). Furthermore, studies that have investigated associations between CD severity and neural activity have found negative correlations between CD symptoms and activity in several of the aforementioned areas during anger and sadness processing (Passamonti et al., 2010). Aggression levels have also been found to negatively correlate with ACC activity during the processing of negative stimuli (Sterzer et al., 2005).

fMRI studies of typically-developing individuals have provided extensive support for the involvement of amygdala in fear (Adolphs et al., 2005; Adolphs, Tranel, Damasio, & Damasio, 1995; Fanselow & Gale, 2003; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; LeDoux, 2000; Phelps & LeDoux, 2005; Phillips et al., 1997), anger (Derntl et al., 2009; Stanton et al., 2009; Whalen et al., 2001), and sadness processing (Blair, Morris,

Frith, Perrett, & Dolan, 1999). The prefrontal cortex is known to be implicated in cognitive control processes (Duncan & Owen, 2000) and it has been suggested that the vmPFC and the ACC act to modulate amygdala activity during emotion processing (Buhle et al., 2014; Bush et al., 2002; Davis et al., 1997; Lane et al., 1997). In addition, the insula has consistently been associated with empathic processing (Bernhardt & Singer, 2012; Singer et al., 2004, 2009) and is thought to play a specific role in the recognition and processing of negative emotions, such as disgust (Phillips et al., 1997; Schienle et al., 2002) and fear (Schienle et al., 2002).

Existing neuroimaging studies of emotion processing in CD have almost exclusively focused on males (see Chapter 2), therefore we know little about the neural correlates of emotion processing in females with CD. This is because the majority of the studies have recruited male only samples (Passamonti et al., 2010), or have only recruited a small number of females and combined the sexes in their analyses (Marsh et al., 2008; White, Marsh, et al., 2012; White, Williams, et al., 2012), which could obscure findings if the relationship between CD and abnormalities in emotion processing differs by sex. The lack of research into sex differences in emotion processing in the CD population is especially surprising considering prior findings from normative studies showing that females are more accurate than males in emotion recognition (McClure, 2000). Females also display higher emotional expressivity than males (Deng, Chang, Yang, Huo, & Zhou, 2016), and may have enhanced emotion regulation and reappraisal abilities compared to males (McRae et al., 2008). Data on whether the altered neural activity during emotion processing seen in males with CD extends to females with CD (while taking sex into account) are therefore urgently needed.

Behaviourally, there is evidence that females with CD, like their male counterparts, show impaired recognition of anger and disgust (Fairchild et al., 2010; Fairchild, Van Goozen, et al., 2009). High levels of psychopathic traits in females with CD have also been associated with deficits in recognising sadness (Fairchild et al., 2010) - similar to deficits

observed in male groups (Dawel, O'Kearney, McKone, & Palermo, 2012; Eisenbarth, Alpers, Segrè, Calogero, & Angrilli, 2008; Fairchild, Van Goozen, et al., 2009). However, one study found that there was no significant difference between females with CD and female controls in the recognition of emotional expressions (Pajer, Leininger, & Gardner, 2010). It therefore remains unclear whether these deficits are consistently present in females. Although no neuroimaging study has included a sufficient number of males and females to directly test for sex differences in the relationship between CD and functional abnormalities during emotion processing, one study investigated emotion processing in females with CD compared to female controls only (Fairchild et al., 2014). This study found no differences between groups during anger or sadness processing (relative to processing neutral faces), but reported lower activity in medial OFC and higher activity in anterior insula during face processing in general (i.e., when neural responses to angry, sad, and neutral expressions were compared to a low level baseline condition (fixation cross)). In addition, a negative correlation was found between CD severity and neural activity in amygdala, superior temporal gyrus, fusiform gyrus, and dorsolateral prefrontal cortex during face processing in general (Fairchild et al., 2014).

The aim of the current study was to directly investigate sex differences in the relationship between CD and neural activity during emotion processing. To this end, we selected a large sex-balanced sample of adolescents with CD (30 females, 33 males) and a group of healthy adolescents (29 females, 33 males), matched in age and pubertal development, from the Southampton and Birmingham samples described in Chapter 3. Angry, fearful, and neutral emotional expressions were selected since CD and CU traits have been linked to recognition and processing problems that are particularly marked for anger and fear, respectively (Fairchild et al., 2010; Fairchild, Van Goozen, et al., 2009; Jones et al., 2009; Passamonti et al., 2010; Stevens et al., 2001; White, Marsh, et al., 2012, 2012; White, Williams, et al., 2012)

Our main analysis focused on the contrast of emotionally valenced and neutral faces (angry versus neutral, and fearful versus neutral). However, we also included an analysis aimed at testing for CD-related difficulties in face processing per se by comparing all faces with a low-level baseline (fixation cross). This contrast was important to test for main effects of condition in order to ensure that the task reliably elicited activity in the face processing network. That is, if we observed activation in brain areas known to be associated with processing faces, such as the fusiform gyrus and occipital cortex, we could be confident that we had the ability to detect activity in these regions (and therefore potentially find group differences in these regions as well). Previous research has used either the same contrast or a similar contrast involving a low-level baseline. For example, Fairchild et al., (2014) used a similar task and contrasted angry and sad facial expressions with neutral facial expressions, as well as all three facial expressions with fixation cross. Whilst they did not find a main effect of diagnosis when contrasting emotional expressions with neutral faces, they found reduced activity in the CD group relative to controls during the contrast all faces versus fixation. Similarly, Passamonti et al. (2010) contrasted angry, sad, and neutral facial expressions with fixation cross trials in order to assess which expression was driving the main effects of group for the emotion versus neutral contrasts. In all cases, they found similar effects of diagnosis when processing facial expressions relative to fixation crosses. Including this contrast is therefore important in terms of replication, as well as the fact that the only emotion processing study of females with CD (Fairchild et al., 2014) found significant effects of diagnosis only when contrasting all facial expressions with fixation cross.

For all contrasts, we ran a full-factorial model assessing for effects of condition, diagnosis, sex, and sex-by-diagnosis interactions. We also assessed the effects of CD severity within the CD group across the sexes, and sex-by-CD severity interactions (which would indicate that the relationship between CD severity and brain activity differs between males and females). In all analyses, age, IQ, and scan site were included as covariates of

no interest. In addition, we conducted further analyses to investigate the robustness of the findings. First, in order to present findings that are representative of clinical reality, given the considerable overlap between CD and ADHD (Waschbusch, 2002), we report the group effects and correlations observed when not controlling for comorbid ADHD symptoms, and then test for effects specifically related to CD (i.e., after controlling for comorbid ADHD symptoms). Secondly, previous studies have noted subtle differences in emotion processing between childhood-onset (CO) and adolescence-onset (AO) CD, such that males with CO-CD might show more widespread abnormalities compared to males with AO-CD (Passamonti et al., 2010). For this reason, it may not be valid to consider CO-CD and AO-CD as one homogeneous group, when comparing CD groups with controls. Thus we tested the validity of collapsing across these CD subtypes by first comparing them in terms of neural activity, before combining the subtypes when running the main analyses investigating effects of diagnosis, sex, and sex-by-diagnosis/symptom severity interactions. Third, since several studies of emotion processing have found differences between individuals with CD and high or low CU traits, such that those with high levels of CU traits have shown higher activity relative to individuals with low CU traits (Sebastian et al., 2013; Viding et al., 2012), we performed a similar analysis to that employed to test for differences between the age-of-onset subtypes, to test the validity of collapsing across the high and low CU traits groups.

Due to the limited research on emotion processing in females with CD, it was difficult to make specific predictions about sex differences in this regard. There is evidence that males and females with CD show similar behavioural impairments in emotion recognition (Fairchild et al., 2010; Fairchild, Van Goozen, et al., 2009), and lower neural activity when processing faces in general (Fairchild et al., 2014). However, there are also indications that the neural abnormalities in females with CD may not be as widespread or robust as those seen in their male counterparts, and may not be emotion-specific (Fairchild et al., 2014). Based on these observations, we first hypothesised that the CD group would

in general show lower neural activity in the amygdala, insula, ventromedial/ventrolateral PFC, ACC, and OFC relative to healthy controls. Second, we hypothesised that these effects would be less pronounced in females with CD, especially in relation to the contrasts between anger or fearful versus neutral facial expressions (i.e., emotion-specific rather than face-general contrasts). We also had similar predictions in relation to the correlations between CD severity and neural activity in these areas, that is, we expected to see some common effects of CD severity across males and females, as well as sex-by-CD severity interactions, in areas overlapping with the ones mentioned above.

6.2 Methods

6.2.1 Participants

The sample was selected from the participants tested at Southampton and Birmingham sites participating in the Female Neurobiology and Treatment of Conduct Disorder (FemNAT-CD) study, described in Chapter 3. A total of 63 adolescents (30 females) with CD and 66 adolescents (33 females) without psychiatric disorders had completed the fMRI task. All participants were aged between 14-18 years (M_{age}=16.02). Diagnoses were made using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL; Kaufman et al., 1997; see Chapter 3 for a detailed description).

6.2.2 The conscious face processing task

Participants viewed photographs of 30 individuals (15 females) from the NimStim Face Stimulus Set (Tottenham et al., 2009) portraying angry, fearful, or neutral expressions (see Figure 6.1). They were asked to press either the left or right button on a button box if the person in the photograph was female or male, respectively. Stimuli were presented in 17.5-second epochs, which contained five faces from the same emotion category (i.e., angry, fearful, or neutral) intermixed with five null events (fixation cross). Each

experimental trial consisted of the presentation of a face (1000 ms). This was followed by a fixation cross (750 ms). In contrast, control trials involved presenting a second fixation cross for 1750 ms in place of the face. To enhance design efficiency, while preserving the unpredictability of stimulus onset for participants, the stimuli within each epoch were pseudorandomised with respect to trial type and sex of the individual in the photograph. Images depicting each emotion category (angry, fearful, neutral) were presented 60 times across 12 epochs, with 180 facial expressions and 180 null events presented in total. The task had a total duration of 10 minutes 30 seconds.

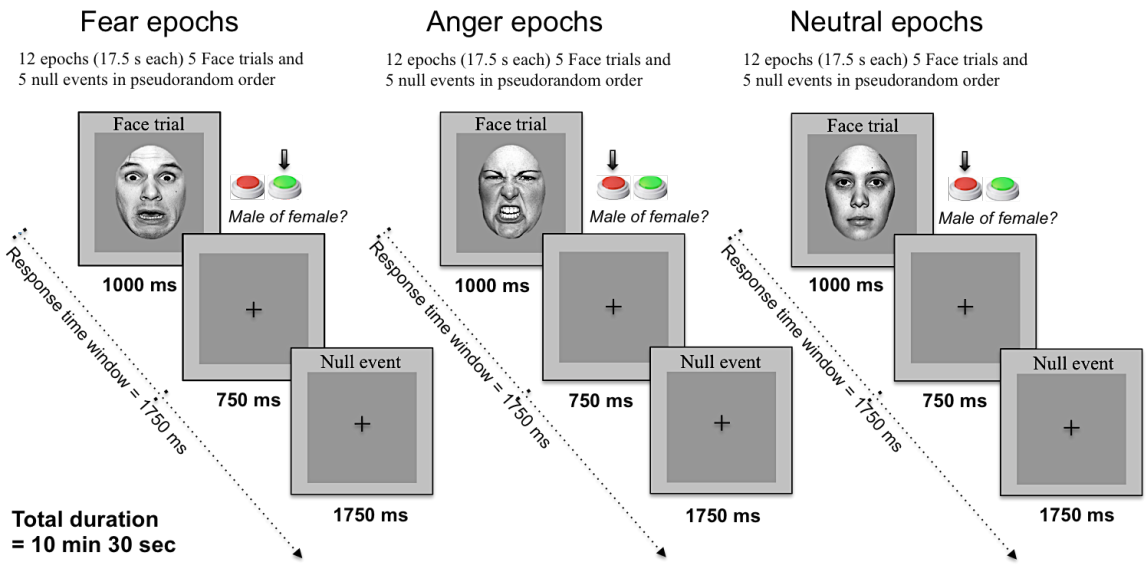

Figure 6.1. Schematic representation of the fMRI paradigm (gender discrimination of emotional and neutral faces) and examples of stimuli used.

6.2.3 fMRI data acquisition and pre-processing

Functional and structural MRI scans were acquired using a Siemens Tim Trio 3T scanner in Southampton, or a Philips Achieva 3T scanner in Birmingham, in both cases using a 32-channel head coil. Each site underwent a site qualification procedure to ensure that data collected between sites were comparable (see Chapter 3 for a detailed description). T1-weighted scans were collected using a magnetization prepared, rapid acquisition gradient-echo sequence (Echo time (Philips)=3.7, Echo time (Siemens)=3.4, Repetition time=1.9, flip angle=9 degrees, Foot to Head and Anterior to Posterior field of view= 256, Right to Left field of view=192, matrix=256, voxel size=1×1×1 mm, sagittal

slices=192, bandwidth(Philips)=174 hz/pix, bandwidth(Siemens)=180 Hz/pix, total scan time=4 min 26 sec (Siemens) or 6 min 5 sec (Philips). Head movements were minimized by using foam pads placed around the participants' heads. Structural MRI data were collected at the start of the scanning process, and if required, the scan was repeated until a good quality structural image was acquired.

Functional MRI data were collected using echo-planar T2-weighted imaging (EPI) sensitive to the blood oxygenation level-dependent (BOLD) signal contrast covering the whole brain (axial slices=41, Echo time=30 ms, Repetition time=2500 ms, voxel size= 3×3×3 mm, flip angle=83 degrees, slice thickness= 2 mm, Right to Left and Anterior to Posterior field of view = 192, Foot to Head Field of view= 122).

Data were pre-processed and analysed using statistical parametric mapping software (SPM 12; http://www.fil.ion.ucl.ac.uk/spm/). Prior to statistical analysis, several pre-processing steps and quality control procedures were performed, following standard procedures in fMRI analysis (see Figure 6.2 for an overview of the analysis process). These pre-processing steps had the purpose of increasing the validity and sensitivity of the analysis by reducing the influence of data acquisition (such as scanner drift and thermal noise) and physiological artefacts (such as signals related to respiration, heart beat, and movement), as well as to standardise the locations of brain regions across participants.

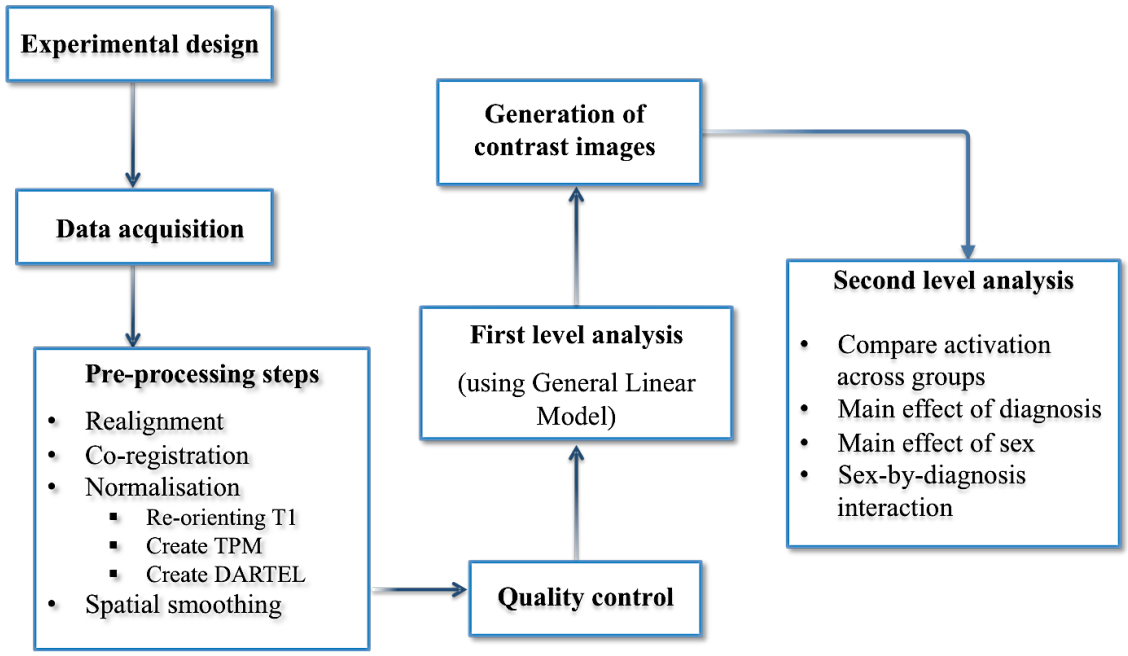

Figure 6.2. Steps included in the fMRI analysis procedure. *Note:* T1=T1-weighted structural MR image, TPM= tissue probability map, DARTEL= Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (an advanced method of normalisation that involves creating a single template using the brains of all subjects in the study, and warping all subjects' brains back to this template).

First, participant movement inside the scanner is common and results in a mismatch in location between the images in a time-series, hence adding to the noise and reducing sensitivity of the measurements. To reduce this artefact, all functional scans from a participant were realigned to the first scan (reference scan) in the time-series using rigid body transformation. The mean EPI was computed for each participant. Second, the realigned EPI images were co-registered to the individual T1-weighted image to allow for the final pre-processing step - normalisation. Third, to ensure comparability between participants' brain areas, i.e., that voxels fall within the same brain region for each participant, a normalisation process registered images from all participants into the same coordinates. This involved several steps: the T1-weighted images were first re-oriented according to the anterior and posterior commissure line. Given that this was a multi-site study with a paediatric sample with a mean age of 16 years, it was necessary to create our own Tissue Probability Maps (TPMs), as it is more likely that children's and adolescents' data deviate from the standard Montreal Neurological Institute (MNI) template provided with the SPM12 toolbox. Despite this known issue, most previous studies of CD using adolescent samples have not adopted this approach to minimise problems with

normalisation (Fairchild et al., 2014.; Jones et al., 2009; Marsh et al., 2008; Passamonti et al., 2010). Hence, we calculated customized TPMs in MNI space using the average Template approach of the Template-O-Matic Toolbox for SPM8 (Wilke et al., 2008) with the age and sex of all participants included as defining variables. The T1-weighted images were segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using VBM8 (Gaser, 2009), and the individual native-space GM and WM segments were normalised to the TPM using an affine registration. A Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL; Ashburner, 2007) template was then created in SPM12 and normalised to MNI space using the GM and WM segments from all participants. The EPI images were then normalised to our sample-specific DARTEL template on an individual basis. Fourth, smoothing was applied to increase the signal-to-noise ratio (SNR). To maintain a balance between the resolution of the image and SNR, we used the most commonly recommended dimensions of twice the voxel size (3x3x3), hence a smoothing kernel of 6 mm full-width/half-maximum was used. Finally, to ensure a high image quality after pre-processing, we visually inspected the EPI data. The Art toolbox in SPM (http://www.nitrc.org/projects/artifact_detect/) was used to detect and create a list of realigned images that were affected by excessive movements in terms of translation and rotation (> 1.5 mm and 2^{o} respectively). These outliers, as well as the mean image created using the Art toolbox, were visually inspected in SPM for artefacts (using the 'Check Reg' function). Participants that had more than 20 scans (10%) considered outliers were removed without further visual inspection. The artifactual images were interpolated by using the mean of the images before and after the affected image.

6.2.4 fMRI analysis

The subject-specific general linear models (GLMs; Friston et al., 1994) included four experimental factors (neutral-, angry-, and fearful faces, and null events). Six realignment parameters were added as variables of no interest to account for residual motion-related variance. Low-frequency signal drift was removed using a high-pass filter

(cut-off 128 s), and an autoregressive modelling [AR(1)] of temporal autocorrelations was applied. At the first GLM level, contrast images assessing the effect of the condition 'angry>neutral', 'fearful>neutral', 'all faces>null' were generated for each participant (see Figure 6.3). The angry and neutral facial expressions were selected based on previous research showing the processing of these emotions to be most impaired in individuals with CD (Fairchild et al., 2014; Jones et al., 2009; Marsh et al., 2008; Passamonti et al., 2010). The neutral facial expressions acted as a comparison baseline for the two emotions, while the null events were included for two reasons; firstly, to ensure that participants were showing activation in areas involved in processing faces in general, in order to validate the task before conducting group analyses. Secondly, a previous study on female CD only found differences between CD and controls in this contrast.

Second level analysis tested for sex differences in brain response between controls and adolescents with CD. Here, we ran a full factorial model for each of the conditions including diagnosis and sex as main factors (see Figure 6.3). The contrasts of interest related to the main effect of condition, main effect of diagnosis, main effect of sex, and sex-by-diagnosis interaction. Age, IQ, and scan site were included into the model as covariates of no interest. Furthermore, we ran additional analyses in order to assess the validity of collapsing across age-of-onset CD subgroups and those with CD and high versus low CU traits in our analyses. Childhood-onset and adolescent-onset CD were classified from the K-SADS-PL. CU traits were calculated from the CU-subscale of the Youth Psychopathic traits Inventory (Andershed et al., 2002), and a median split of the total CU-subscale scores in the CD group were used to classify CD individuals into high or low CU traits subgroups. Moreover, each analysis was repeated with lifetime ADHD symptoms included as a covariate of no interest in order to present findings that are representative of clinical reality, as well as effects specifically related to CD.

As with Chapter 4, two main approaches were employed to threshold second-level maps. The first was based on a region of interest (ROI) approach using anatomical

definition of brain regions. We created seven regions of interest (ROI) using the atlas for automated anatomical labelling (aal.02; Tzourio-Mazoyer et al., 2002). ROIs were chosen on the basis of results from previous fMRI studies showing differences between CD and HC groups (Fairchild et al., 2014; Marsh et al., 2008; Passamonti et al., 2010; Rubia et al., 2008; Sebastian, McCrory, Cecil, & et al, 2012; White, Williams, et al., 2012), and included the amygdala, ventromedial prefrontal cortex, orbitofrontal cortex, insula, anterior cingulate cortex, superior temporal gyrus, and fusiform gyrus. Results from the ROI analyses are reported at $p \leq .05$, family-wise error correction for multiple comparisons in small volumes (i.e., small volume correction; SVC; Friston, 1997).

Second, we report any findings in other brain regions that met a threshold of $p \leq .05$, FWE, whole-brain correction – the most stringent threshold available in SPM12. Finally, for sake of completeness, we also report the results that met a "borderline" threshold of $p \leq .001$ uncorrected and a cluster size (k) of more than 10 voxels.

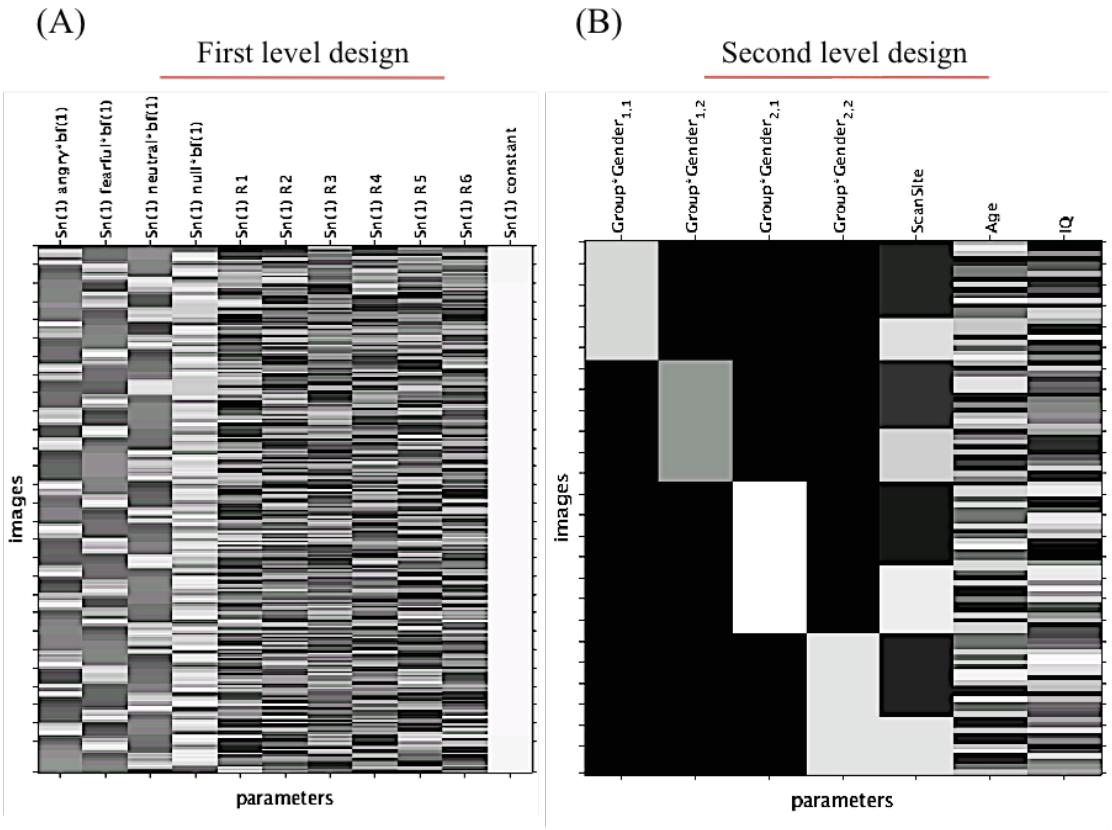

Figure 6.3. Design matrix used for first and second level fMRI analysis. A) The first three columns show angry, fearful, neutral, and null trials, respectively. The following six columns show the realignment parameters. B) The first four columns show females with conduct disorder (CD), males with CD, female healthy controls (HCs), and male HCs, respectively. The last three columns show the covariates of no interest: scan site, age, and IQ.

6.3 Results

6.3.1 Sample and demographics

We excluded data from 7 females with CD, 4 female controls, 10 males with CD, and 5 male controls because they either did not complete the task or displayed excessive head movement in the scanner. Table 6.1 summarises the demographic and clinical characteristics of the participants included in the fMRI analysis. The four groups did not differ in age, stage of pubertal development, handedness, or ethnicity, and males and females with CD did not differ significantly in age-of-onset or CU-subtype. Compared to the control groups, the CD groups had significantly lower estimated full-scale IQ scores, and higher levels of lifetime CD and ADHD symptoms, as well as higher scores of psychopathic and callous-unemotional traits (as measured by the total score, and CU-

subscale, of the Youth Psychopathic Inventory (YPI; Andershed et al., 2002). Furthermore, by design, our control groups were free of psychiatric disorders at the time of testing; thus the CD group had significantly higher levels of comorbidity and medication use. However, apart from ADHD, the male and female CD groups did not differ in terms of comorbidity or medication use.

Table 6.1. Demographic and clinical characteristics of the sample used in the emotion processing study

Variable	Females		Males				
	CD (*n*=23)	HC (*n*=29)	CD (*n*=23)	HC (*n*=27)	*T*group (*p*)	*T*sex (*p*)	*F* sex-by-diagnosis (*p*)
	M (SD)						
Age (years)	16.26 (1.18)	16.14 (.95)	16.09 (1.35)	16.26 (1.16)	*T*=.10 (.92)	*T*=.05 (.96)	*F*=.14 (.94)
Estimated IQ	89.61 (12.07)	96.90 (11.36)	90.30 (10.32)	97.19 (10.25)	*T*=.3.26 (.002)	*T=.15 (.88)*	*F*=.3.50 (.02)
Lifetime CD symptoms	6.74 (3.08)	.34 (.77)	8.26 (3.14)	.59 (.75)	*T=16.08* (<.001)	*T=1.16 (.25)*	*F*=.91.54 (<.001)
ADHD symptoms	2.61 (3.68)	.07 (.26)	6.43 (6.25)	0 (0)	*T=6.19* (<.001)	*T=2.13 (.04)*	*F*=.9.07 (<.001)
Total YPI	113.83 *(18.14)*	90.55 *(16.61)*	117.85 *(22.14)*	97.92 *(16.54)*	*T*=5.71 (<.001)	*T*=1.42 (.16)	*F*=12.33 (<.001)
CU subscale of YPI	21.65 *(4.41)*	18.59 *(4.73)*	26.35 *(6.85)*	22.35 *(3.27)*	*T*=3.17 (.002)	*T*=4.08 (<.001)	*F*=10.22 (<.001)
	N (%)				X^2group (*p*)	X^2sex (*p*)	X^2 sex-by-diagnosis (*p*)
No. Lifetime *DSM-IV* diagnoses							
ODD	13(57)	0(0)	15(65)	0(0)	X^2=49.50 (<.001)	X^2=.20 (.82)	X^2=49.56 (<.001)
ADHD	2(9)	0(0)	7(30)	0(0)	X^2=12.59 (<.001)	X^2=3.05 (.16)	X^2=18.45 (<.001)
MDD	6(26)	0(0)	4(17)	0(0)	X^2=14.14 (<.001)	X^2=.44 (.074)	X^2=16.67 (.001)
Alcohol abuse	2(9)	0(0)	1(4)	0(0)	X^2=3.94 (.082)	X^2=.34 (1.00)	X^2=4.95 (.18)
Drug abuse (cannabis)	3(13)	0(0)	6(26)	0(0)	X^2=12.59 (<.001)	X^2=1.10 (.49)	X^2=14.45 (.002)
Medication use	4(8)	1(2)	8(17)	0(0)	X^2=5.07 (.04)	X^2=.002 (1.00)	X^2=5.07 (.04)
Puberty							
Late	12(52)	18(62)	14(67)	20(74)	X^2=0.82	X^2=1.87	X^2=2.70
Post	11(48)	11(38)	7(33)	7(26)	(.37)	(.17)	(.44)
Age of Onset							
Childhood	11 (48)		15 (65)			X^2=2.09 (.35)	
Adolescent	11 (48)		8 (35)				
Missing	1 (4)		0 (0)				
CU subgroups							
High	8 (35)		15 (65)			X^2=4.26 (.08)	
Low	15 (65)		8 (35)				
Handedness							
Right	19 (82)	27 (93)	19 (83)	26 (96)			
Left	2 (9)	2 (7)	4 (17)	0 (0)	X^2=5.72	X^2=1.92	X^2=12.10 (.60)
Ambidextrous	2 (9)	0 (0)	0 (0)	0 (0)	(.06)	(.38)	
Missing	0 (0)	0 (0)	0 (0)	1 (4)			
Ethnicity							
Caucasian							
Other	23 (100)	28 (97)	22 (96)	25 (93)	X^2=0.68 (.41)	X^2=1.12 (.29)	X^2=1.84 (.61)

Note: ADHD = attention-deficit/hyperactivity disorder; CD = conduct disorder; CU = callous-unemotional traits; HC = healthy control; IQ = intelligence quotient (measured using the WASI or WISC-IV); MDD = major depressive disorder ODD = oppositional defiance disorder. Group and sex differences were computed using independent sample t-tests or Chi square test, and sex-by-diagnosis interactions were computed using ANOVAs and Chi square tests.

6.3.2 Behavioural results

Accuracy and reaction times (RTs) for correct responses were entered into a 2 x 2 x 3 mixed-measures ANOVA (assessing effects of diagnosis, sex, and emotion). Due to an error in the design of the task, responses after 750 ms were not recorded for the majority of the Southampton participants. This resulted in a higher number of total missing trials ($T(90) = 6.10$, $p < .001$), a lower total RT ($T(44.15) = 13.18$, $p < .001$) and higher accuracy ($T(90) = 2.23$, $p = .03$) across the three emotions compared to the Birmingham sample. Despite this error, no participants missed more than 30% of responses. Importantly, the two sites showed a similar relationship between RT and accuracy across the three emotions and the four group, thus the data from the two sites were combined prior to analysis. Due to the higher number of missing responses in the Southampton data, accuracy and RTs for both sites was reported as the proportion of correct responses from the total of correct and incorrect responses.

Accuracy: There was a main effect of emotion ($F(2,176) = 21.04$, $p < .001$), whereby, for all groups, gender-discrimination accuracy for angry faces was significantly lower than for fearful and neutral faces ($p < .001$). There was also a significant main effect of diagnosis ($F(1,88) = 12.14$, $p < .001$; see Figure 6.4A), where the healthy control group were more accurate than the CD group across all emotions. There was also a main effect of sex ($F(1,88) = 5.32$, $p = .023$), where females in general were more accurate at the gender discrimination task compared to males ($p = .023$). Diagnosis did not significantly interact with sex or emotion, and there was no interaction between sex and emotion or three-way interaction between these factors (all $p > 0.11$).

RT: There was a main effect of emotion ($F(1.39, 122.05) = 10.74$, $p < .001$; see Figure 6.4B), RTs to neutral faces were significantly shorter than to angry ($p = .003$) and fearful faces ($p = .003$). There were no two-way or three-way interactions (all $p > .31$).

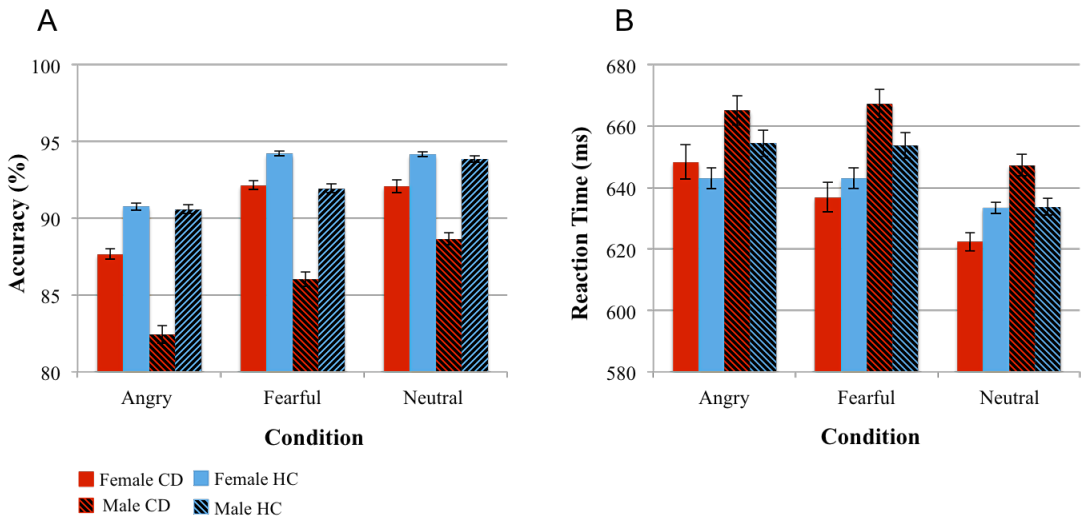

Figure 6.4. A) Accuracy (%) and B) reaction times (ms) for the four groups across the three conditions in the gender discrimination task. CD = conduct disorder; HC = healthy control.

6.3.3 fMRI results

6.3.3.1 Preliminary analysis

6.3.3.1.1 Conduct disorder age-of-onset effects

There were no significant differences between the childhood- and adolescence-onset CD subgroups in any of the ROIs in the full factorial model or in the correlational analyses for the contrasts "All faces versus fixation", "Angry versus neutral" and "Fearful versus neutral". Thus, in all subsequent analyses, the CD subgroups were combined together.

6.3.3.1.2 Effects of callous-unemotional traits

As with the age-of-onset analysis, there were no significant differences between the CD groups with high versus low levels of CU traits in any of the ROIs in the full factorial model or in the correlational analyses (i.e., no age-of-onset-by-CD severity interaction) for the contrasts "All faces versus fixation", "Angry versus neutral" and "Fearful versus neutral". Consequently, in the main analyses, these subgroups were collapsed together.

6.3.3.2 Full factorial analysis

6.3.3.2.1 Activation during facial emotion processing

6.3.3.2.1.1 Angry versus Neutral faces

We observed a main effect of condition in the right anterior insula ROI, where participants overall showed greater activation to angry compared to neutral faces ($p < .05$, SVC; see Table 6.2). There were no main effects of diagnosis in any of the ROIs. There were, however, main effects of sex in left insula and superior temporal gyrus, where males displayed higher activity compared to females ($p < .05$, SVC; Table 6.2).

A sex-by-diagnosis interaction was observed in right amygdala ($p < .05$, SVC), where females with CD displayed lower, and males with CD higher, activity compared to their respective control groups (see Table 6.2; Figure 6.5). This relationship was also seen in several regions outside the ROIs - most notably, in bilateral precentral and postcentral gyrus, and right superior temporal gyrus ($p < .001$, uncorrected; see Table 6.2).

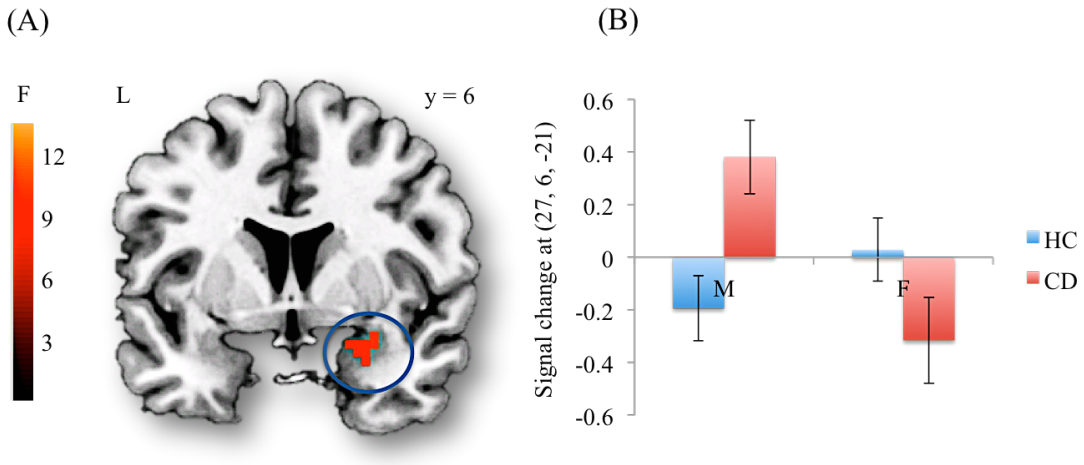

Figure 6.5. Sex-by-diagnosis interaction for the contrast "Angry versus neutral". Females with conduct disorder (CD) showed lower activity compared with the female healthy control (HC) group, whereas males with CD showed higher activation compared with the male HC group in right amygdala (panel A). The colour bar represents F statistics. The image is thresholded at $p<.005$, uncorrected, for display purposes. Plots of the data extracted from the right amygdala are displayed in panel B.

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Table 6.2. Coordinates and cluster sizes for the main effects of condition and sex, and sex-by-diagnosis interaction in the contrast "Angry versus neutral"

Contrast				MNI Coordinates		
Cerebral regions	Hemisphere	Local maxima z	No. of significant Voxels in Cluster	*x*	*y*	*z*
Main effect of Condition						
Anterior insula	R*	3.55[b]	4	48	-3	-6
Inferior occipital	L	3.80	36	-36	-84	-6
Fusiform gyrus	L	3.65	12	-42	-36	-15
Main effect of Diagnosis						
CD > HC, HC > CD						
No significant clusters						
Main effect of Sex						
Females > Males						
No significant clusters						
Males > Females						
Insula	L*	3.90[b]	16	-33	-6	12
Superior temporal gyrus	R*	4.07[b]	69	69	-30	9
Precentral gyrus	L	3.95	64	-9	-33	69
	R	3.91	42	18	-36	69
Parietal operculum cortex	R	3.91	84	45	-21	18
	L	3.78	39	-45	-42	24
Rolandic operculum	R	4.68	33	57	3	3
Hippocampus	R	3.67	28	27	-15	-18
Cerebellum 6	R	3.62	18	21	-57	-27
Postcentral gyrus	R	3.61	18	15	-48	69
Lateral occipital cortex	L	3.50	30	-18	-66	42
Superior frontal gyrus	R	3.43	19	6	54	21
Sex-by-diagnosis Interaction						
FCD > FHC, MCD < MHC						
No significant clusters						
MCD > MHC, FCD < FHC						
Amygdala	R	3.33[b]	2	27	6	-21
Postcentral gyrus	R	3.97	56	18	-33	69
	L	3.46	18	-45	-12	45
Superior temporal gyrus	R	3.54	10	39	-57	21
Precentral gyrus	L	3.62	22	-54	9	36
	R	3.47	20	30	-21	48
Inferior parietal	R	3.42	11	36	-51	42

*Note: * indicates regions that were rendered non-significant when including ADHD symptoms as a covariate. Unless otherwise indicated, the regions reported are significant at p < .001, uncorrected, for > 10 continuous voxels. CD= conduct disorder, FCD= Females with conduct disorder, HC = Healthy control, L = left, MCD= Males with conduct disorder, MNI = Montreal Neurological Institute, R = right.*

[b] p < .05, family-wise error small volume corrected

6.3.3.2.1.2 Fearful versus Neutral faces

There was a main effect of condition in the right superior temporal gyrus ($p < .05$, SVC), where participants overall showed greater activation to fearful compared to neutral faces. There were no main effects of diagnosis in any of the ROIs; however, outside the

ROIs, individuals with CD showed higher activation in left precentral gyrus and right cuneus relative to controls ($p < .001$, uncorrected; see Table 6.3. There were no main effects of sex in any of the ROIs (Table 6.3).

There were no sex-by-diagnosis interactions in any of the ROIs. However, sex-by-diagnosis interactions were observed in left caudate and right precentral gyrus at an uncorrected threshold ($p < .001$, uncorrected; see Table 6.3); in these cases, males with CD showed higher, and females with CD lower, activity compared to their respective control groups, following a similar pattern to the results found in the ROIs.

Table 6.3. Coordinates and cluster sizes for the main effects of condition and sex, and sex-by-diagnosis interactions for the contrast "Fearful versus neutral"

Contrast				MNI Coordinates		
Cerebral regions	Hemisphere	Local maxima z	No. of significant Voxels in Cluster	*x*	*y*	*z*
Main effect of Condition						
Superior temporal gyrus	R	3.85[b]	5	69	-6	0
Middle frontal gyrus	L	3.36	29	-27	45	6
Main effect of Diagnosis						
CD > HC						
Precentral gyrus	L*	3.60	15	-33	-24	54
Cuneus	R*	3.32	10	18	-66	27
HC > CD						
No significant clusters						
Main effect of Sex						
Females > Males						
No significant clusters						
Males > Females						
Rolandic operulum	R	3.93	70	42	-21	21
	L	3.61	18	-42	-15	21
Putamen	L	3.82	27	-27	6	-9
Paracingulate gyrus	R	3.67	44	3	54	15
Inferior frontal gyrus	L	3.65	22	-48	30	3
Precentral gyrus	R	3.59	10	63	0	12
Sex-by-diagnosis interaction						
FCD > FHC, MCD < MHC						
No significant clusters						
MCD > MHC, FCD < FHC						
Caudate	L	3.67	12	-18	0	24
Precentral gyrus	R	3.45	22	36	-18	45

*Note: * indicates regions that were rendered non-significant when including ADHD symptoms as a covariate. Unless otherwise indicated, the regions reported are significant at p ≤ .001, uncorrected, for > 10 continuous voxels. CD= conduct disorder, FCD= Females with conduct disorder, HC = Healthy control, L = left, MCD= Males with conduct disorder, MNI = Montreal Neurological Institute, R = right.*
[b]p ≤ .05, family-wise error small volume corrected

6.3.3.2.2 Activation during face processing in general

There was a main effect of condition whereby participants showed greater activation for faces considered together compared to fixation in widespread areas of the brain, including inferior- and middle occipital cortex and fusiform gyrus (all significant at $p < .05$, family-wise error [FWE] whole-brain correction; see Figure 6.6). In addition, activation was seen in all of our a priori ROIs, and the effects were present bilaterally (all significant at $p < .05$, small volume correction [SVC]; see Table 6.4).

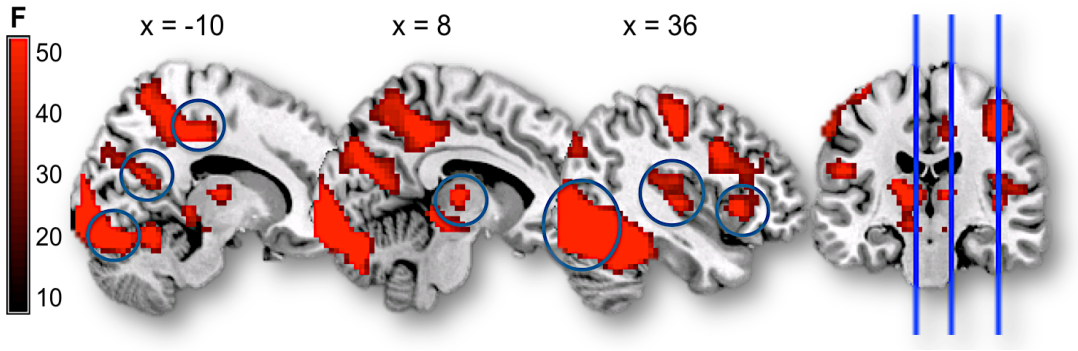

Figure 6.6. Main effect of condition in the contrast "All faces versus fixation". The figure shows from left to right as highlighted using blue circles: inferior occipital cortex/fusiform, cuneus, middle cingulate cortex, thalamus, middle occipital cortex, rolandic operculum, and anterior insula. The coronal view illustrates the x-coordinates of the three sagittal slices. The colour bar represents F statistics. The image is thresholded at $p <$ .05 Family-Wise-Error, whole-brain correction.

For this contrast, there was a main effect of diagnosis in the left occipital fusiform gyrus, where the CD group displayed less activation compared to the control group ($p <$ .05, SVC; see Table 6.4, Figure 6.7). Of note, the peak activation is located in the occipital fusiform gyrus, which is at posterior end of the fusiform ROI.

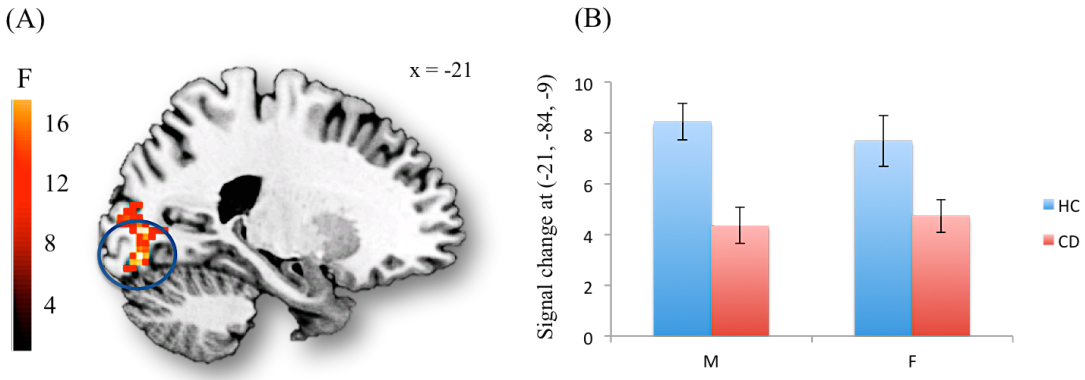

Figure 6.7. Main effect of diagnosis for the contrast "All faces versus fixation". The conduct disorder (CD) groups showed significantly lower activity in the left occipital fusiform gyrus compared to the healthy control groups (HC). The colour bar represents F statistics. Panel A shows the location of the effect in left fusiform gyrus; the image is thresholded at $p <$ .005, uncorrected, for display purposes. Plots of the data extracted from the fusiform gyrus are displayed in panel B.

There were no significant main effects of sex in any of the ROIs (Table 6.4), but there was a significant sex-by-diagnosis interaction in bilateral amygdala. Females with CD showed lower, and males with CD higher, activity compared to their respective control groups when processing faces in general ($p <$ .05, SVC; see Table 6.4, Figure 6.8).

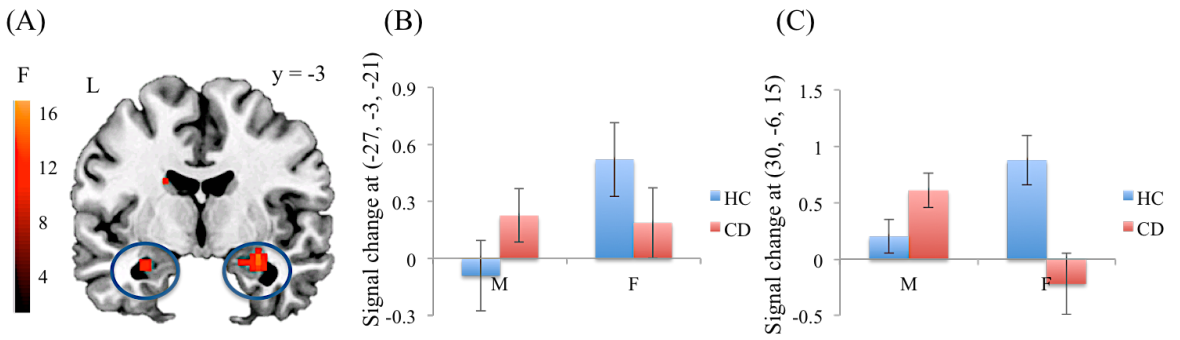

Figure 6.8. Sex-by-diagnosis interaction for the contrast "All faces versus fixation". Males with conduct disorder (CD) showed higher activation compared with the male healthy control (HC) group, whereas females with CD showed lower activation compared to female HCs in both left and right amygdala (blue circles). The colour bar represents F statistics. Panel A shows the activation in left and right amygdala; the image is thresholded at $p < .005$, uncorrected, for display purposes. Plots of the data extracted from the left and right amygdala are displayed in panels B and C, respectively.

Table 6.4. Coordinates and cluster size for the main effect of condition, diagnosis, sex, and sex-by-diagnosis interaction for the contrast "All faces versus fixation"

Contrast				MNI Coordinates		
Cerebral regions	Hemisphere	Local maxima z	No. of significant Voxels in Cluster	*x*	*y*	*z*
Main effect of Condition						
Inferior Occipital /Fusiform gyrus	R	>8.00[a]	5882	42	-81	-9
Thalamus	R	>8.00[a]	949	21	-30	0
Middle occipital cortex	L	>8.00[a]	389	-39	-78	33
Middle cingulate cortex/ precentral gyrus	L	>8.00[a]	1268	-9	-42	45
Cuneus	L	>8.00[a]	250	-18	-63	21
Rolandic opercularis	R	7.73[a]	81	-42	-3	12
Calcarine/cuneus	R	7.48[a]	192	18	-60	18
Precentral gyrus/ Insula	R	7.46[a]	638	42	6	33
Parahippocampal cortex	L	7.22[a]	35	-30	-42	-9
Middle temporal gyrus	L	7.19[a]	211	-66	-51	-6
Amygdala	L	6.41[a]	41	-21	-3	-15
	R	6.42[a]	36	-18	-3	-15
Fusiform gyrus	L	>8.00[a]	457	-39	69	-15
	R	>8.00[a]	553	36	-54	-18
Insula	L	7.16[a]	281	-39	3	15
Anterior Insula	R	7.38[a]	136	39	21	0
Orbitofrontal cortex	L	4.86[a]	26	-30	27	-6
	R	6.11[a]	73	36	27	-6
Superior temporal gyrus	L*	4.22[a]	36	-57	-3	-15
	R	6.47[a]	436	48	-27	15
Ventrolateral PFC	L	5.65[a]	195	-33	24	12
	R	6.97[a]	489	45	12	24
Anterior Cingulate cortex	L	5.37[a]	289	-3	39	-6
	R	5.27[a]	225	3	39	-6
Main effect of Diagnosis						
CD < HC						
Fusiform gyrus	L*	3.74[b]	7	-21	-84	-9
Main effect of Sex						
Females > Males						
Cerebellum crus 1	R	4.23	37	39	-51	-27
Sex-by-diagnosis interaction						
Amygdala	L	2.98[b]	4	-27	-3	-21
	R	3.30[b]	22	30	-6	-15

*Note: * indicates regions that were rendered non-significant when including ADHD symptoms as a covariate. CD= conduct disorder, HC = Healthy control, L = left, MNI = Montreal Neurological Institute, R = right, PFC = prefrontal cortex.*

[a]p < .05, family-wise error whole brain corrected

[b]p < .05, family-wise error small volume corrected

6.3.3.3 Correlation with CD severity and sex-by-CD severity interactions within the CD group

Correlations with CD severity: There were no significant correlations between CD severity and neural activity in any of the ROIs for the contrasts "Angry versus neutral", "Fearful versus neutral", or "All faces versus fixation". However, there were several correlations in areas outside the ROIs ($p < .001$, uncorrected; see Table 6.5. Most notably, there was a negative correlation between CD severity and right fusiform gyrus activity for the contrast "All faces versus fixation", and positive correlations between symptom severity and signal change in superior frontal gyrus during "Angry versus neutral" and right superior temporal gyrus during "Fearful versus neutral".

Sex-by-severity interactions: There were no significant sex-by-CD severity interactions in any of the ROIs for any contrast. However, we did observe some interactions that were present at an uncorrected threshold ($p < .001$) - females with CD showed a negative, and males with CD a positive, correlation between CD severity and signal change in left superior frontal gyrus for the contrast "Angry versus neutral". The reverse relationship (negative correlation in males and positive correlation in females) was observed in left supramarginal gyrus during "Fearful versus neutral" and right superior parietal cortex during "All faces versus fixation" (see Table 6.5).

Table 6.5. Coordinates and cluster sizes for the correlations between CD severity and neural activity, and sex-by-severity interactions for the contrasts "All faces versus fixation", "Angry versus neutral", and "Fearful versus neutral".

Contrast Cerebral region	Hemisphere	Local maxima *z*	No. of significant Voxels in Cluster	MNI Coordinates *x*	*y*	*z*
All faces versus fixation						
Positive correlation						
No significant results						
Negative correlation						
Fusiform gyrus	R	3.29	47	33	-54	-21
Lateral occipital cortex	L	3.43	10	-24	-78	27
Sex-by-symptom severity interactions:						
Females positive, males negative						
Superior parietal cortex	R	4.76	57	24	-51	57
Males positive, females negative						
No significant results						
Angry versus Neutral						
Positive correlation						
Superior frontal gyrus	R	3.62	60	18	30	45
Middle frontal gyrus	R	3.34	32	36	45	3
	L	3.25	18	-21	48	30
Middle cingulate cortex	R	3.16	20	6	33	36
Frontal inferior operculum	R	3.01	29	57	18	15
Negative correlation						
No significant results						
Sex-by-symptom severity interactions:						
Females positive, males negative						
No significant results						
Males positive, females negative						
Superior frontal gyrus	L	3.47	28	-12	0	72
Superior occipital cortex	R	3.23	39	12	-90	33
Fearful versus Neutral						
Positive correlation						
Superior temporal gyrus	R	3.40	10	54	-33	-9
Negative correlation						
No significant results						
Sex-by-symptom severity interactions:						
Females positive, males negative						
Supramarginal gyrus	L	3.64	22	-54	-48	57
Middle frontal gyrus	R	3.08	14	45	12	51
Males positive, females negative						
No significant results						

Note: Unless otherwise indicated, the regions reported are significant at $p \leq .001$, uncorrected, for > 10 continuous voxels. FCD= Females with conduct disorder; L = left; MCD= Males with conduct disorder; MNI = Montreal Neurological Institute; R = right.

6.3.3.4 ADHD symptoms as an additional covariate of no interest

When repeating the analysis controlling for ADHD symptoms, the sex-by-diagnosis interactions in the amygdala for the contrasts "Angry versus Neutral" and "All faces versus fixation" remained significant ($p < .05$, SVC), while the main effect of diagnosis in left fusiform gyrus for the contrast "All faces versus fixation" was rendered non-significant.

6.3.3.5 Confounding effects of site and IQ

When running separate analyses excluding IQ and site as covariates, the sex-by-diagnosis interaction in amygdala for the contrast "Angry versus Neutral" remained significant ($p < .05$, SVC). Similarly, dropping site and IQ as covariates did not alter the main effect of diagnosis in fusiform gyrus ($p < .05$, SVC) in the contrast "All faces versus fixation". Furthermore, while dropping IQ did not affect the sex-by-diagnosis interaction in bilateral amygdala during "All faces versus fixation", excluding *site* as a covariate rendered this interaction non-significant. Finally, the main effects of sex in insula and superior temporal gyrus remained significant when dropping both site and IQ as covariates.

6.4 Discussion

The primary aim of this study was to investigate whether males and females with CD display similar or distinct neural abnormalities when processing angry and fearful emotional expressions compared to healthy males and females.

Despite the fact that we did not observe main effects of diagnosis in our regions of interest during emotion processing, we did observe a sex-by-diagnosis interaction in right amygdala during processing of angry expressions. This is the first study that has been able

to show sex-dependent effects of CD on neural activity during emotion processing - in a structure that has consistently been associated with the processing of emotional facial expressions (Adolphs, Tranel, Damasio, & Damasio, 1995; Ralph Adolphs et al., 2005; Blair et al., 1999; Derntl et al., 2009; Fanselow & Gale, 2003; LaBar et al., 1998; LeDoux, 2000; Phelps & LeDoux, 2005; Phillips et al., 1997; Stanton et al., 2009; Whalen et al., 2001). Both lower and higher activation of the amygdala during the processing of negative emotions has previously been reported in predominantly male CD groups. However, our study differs from previous studies in a number of ways. For example, previous studies reporting reduced activity in the amygdala have included relatively few females and/or combined males and females into a single group (Marsh et al., 2008; Passamonti et al., 2010; White, Marsh, et al., 2012; White, Williams, et al., 2012) and the samples used have either been younger (10-12 years; Jones et al., 2009) or had a wide age-range (Fairchild et al., 2014.; Marsh et al., 2008; Passamonti et al., 2010; Sebastian et al., 2013; Viding et al., 2012; White, Marsh, et al., 2012). Furthermore, some studies have suggested that individual differences in CU traits are important in explaining patterns of amygdala activity, such that individuals with conduct problems and low levels of CU traits showed higher activity relative to individuals with low CU traits (Sebastian et al., 2013; Viding et al., 2012). However, in this study, there were no significant differences between the CU traits subgroups in brain activation and CU traits did not interact with sex within the CD group, across contrasts and analyses. In addition, although there were more males in the high CU traits group, the difference in sex composition between these subgroups was not statistically significant. Considering these two findings, variation in CU traits is unlikely to

be driving the sex-by-diagnosis interaction found in the amygdala (i.e., as lower CU traits have been linked to amygdala hyperactivation whereas higher CU traits have been associated with amygdala hypoactivation; Viding et al., 2012).

The fact that we did not see any main effects of diagnosis during the processing of angry or fearful expressions was surprising, considering previous research showing lower activity in amygdala during fear processing (Jones, Laurens, Herba, Barker, & Viding, 2009; Marsh et al., 2008; White et al., 2012), and in amygdala, ventromedial PFC, OFC, and insula during anger processing (Passamonti et al., 2010). However, the current study included a substantially larger number of females with CD than has been included in previous studies. The only previous study of females with CD did not report any effects of diagnosis during emotion processing (Fairchild et al., 2014), and it may be that the females in our sample masked effects that would otherwise have been seen in a predominantly male sample- especially since they showed a change in amygdala activity in the opposite direction relative to their sex-matched control group. Furthermore, our narrow age-range and restricted range in pubertal development of the sample used in this study may have influenced the findings, such that lower activation in these areas is more pronounced in younger age groups, reflecting emotion-processing difficulties that may normalise with increasing age. However, lower activation during emotion processing – but higher activation during processing of faces – have been found in an older age group of males with CD compared with controls (Passamonti et al., 2010), and other factors such as comorbidity likely played a part.

It is also noteworthy, in this regard, that we did not observe any correlations between CD severity and brain activity or sex-by-CD severity interactions in any of the ROIs. However, we observed an uncorrected positive correlation between right superior frontal gyrus activity and CD severity for both males and females, and a sex-by-CD severity interaction in left superior frontal gyrus during the processing of angry expressions: males showed a positive, and females a negative, correlation between CD severity and neural activity in this region. This is particularly interesting, as we observed sex-by-CD severity interactions in cortical structure and grey matter volume in this area in Chapters 4 and 5 (albeit in the right, rather than the left, hemisphere), showing positive correlations for males, and negative correlations for females. Consequently, alterations in structure and function in the superior frontal gyrus might be a sex-dependent marker for severity of the disorder.

Given the lack of main effects of CD on activation during emotion processing, it is interesting to note that we did find effects related to face processing in general. That is, we found a main effect of diagnosis in occipital fusiform gyrus, at the posterior end of our ROI, where the CD group showed lower activity relative to the control group. There was also a sex-by-diagnosis interaction in bilateral amygdala, where, similar to the interaction observed during processing of angry expressions, males with CD showed higher, and females with CD showed lower, activity relative to their respective control groups. Several behavioural studies have found that CD is associated with a global impairment in recognising facial expressions, rather than specific emotions (Fairchild, Van Goozen, et al., 2009; Short et al., 2016; Sully et al., 2015). The lower fusiform gyrus activity in the CD

group relative to controls found in this study may help to explain these findings, as this brain area is known to be involved in the processing of faces in general (Kanwisher, McDermott, & Chun, 1997; Rossion et al., 2003). Similarly, while the sex-by-diagnosis interactions observed in this contrast may be an indication of sex differences in the relationship between CD and the processing of facial expressions more generally, it is likely that the interaction was driven by the sex-by-diagnosis interaction seen in the contrast angry versus neutral, i.e., the presence of angry facial expressions.

Importantly, all interactions remained significant when including ADHD symptoms as a covariate of no interest, which indicates that these results are specific to CD, and that there are, at least to some extent, sex differences in the relationship between CD and emotion processing. Sex-differentiated effects during emotion processing have been noted in other forms of psychopathology, such as schizophrenia; negative images activated temporal and posterior regions of the brain in males, but not in females with schizophrenia, compared to their respective control groups (Mendrek, Mancini-Marië, Fahim, & Stip, 2007). These observations are potentially important for interventions aimed at improving emotion processing and empathy (Bowen, Morgan, Moore, & van Goozen, 2014), as it is possible that the same intervention may not be applicable for both sexes. However, before making these claims, we need to know more about the potential sex differences in both emotion recognition and emotion processing in CD. Investigating the relationship between behavioural performance in emotion recognition tasks and neural activity during emotion processing in males and females with CD may be of value for future research.

The current study had several strengths that meant that it improved upon the methodology of previous studies of this kind. We included an equal number of males and females with CD, with a relatively large total sample size, which meant that we were able to detect both similarities and differences between males and females with CD compared to their respective sex-matched control groups. Furthermore, we contrasted participants with CO-CD and AO-CD subtypes, and high versus low CU-traits subgroups to ensure that they could be validly combined in one CD group before conducting further analyses, and presented results both with and without controlling for ADHD to report results that were representative of the CD population more generally and those that were specific to CD, respectively. In addition, our groups were matched on age and pubertal development to control for potential differences in developmental stages, and age, site, and IQ were included as covariates of no interest in all analyses.

However, there were also several limitations that should be noted. First, the problem with collecting behavioural data on gender discrimination accuracy in Southampton meant that the results were statistically biased towards shorter reaction times and correct responses. The main reason for asking participants to judge the sex of the faces (as opposed to passive viewing) was to make sure that the participants were paying attention to the task. Despite the problem with recording the data, we can confidently say that this was the case, as each participant's responses were visually noted in the control room by the MRI operator or assistant operator (usually a member of the research team). In addition, the problem with non-recorded responses was not an issue in the two other fMRI tasks used in Southampton, in which responses were made in >90% of the trials, giving us

reason to believe that the participants were complying with the instructions in the current task as well.

Second, the majority of imaging studies, including the current study, have focused on negative emotions, particularly anger or fear (Fairchild et al., 2014; Marsh et al., 2008; Passamonti et al., 2010; Sebastian et al., 2013; Viding et al., 2012; White, Williams, et al., 2012). This means that the findings from the literature may be presenting a biased picture of deficits in the CD group in these specific emotions, while other emotions, such as happiness, disgust, or sadness have not been studied to the same extent. However, due to the design of this type of study, i.e., the need to have both a baseline (fixation cross) and neutral facial expressions to use as comparisons, in addition to avoiding boredom or disengagement in the participants, it was not feasible to increase the length of the experiment to include additional emotional expressions, such as sadness. This was particularly evident from the number of participants in the CD group that did not complete the task (17 individuals with CD compared to 9 HCs). Importantly, we included three facial expressions (angry, fearful, neutral), while many previous studies have only focused on two (Herpertz et al., 2008; Jones et al., 2009; Sebastian et al., 2013; Viding et al., 2012; White, Williams, et al., 2012).

Third, the use of fixation cross as a baseline condition to measure activations during face processing per se, is suboptimal, as there are a number of differences between the face and non-face contrasts other than the presence or absence of a face - such as differences in low level visual features, luminance, spatial frequency, and contrast (Eger, Schweinberger, Dolan, & Henson, 2005; Kanwisher et al., 1997). Thus, comparing facial

expressions with a fixation cross is likely to activate brain regions involved in several aspects of visual processing in addition to regions specialised for face and emotion processing. Future studies of facial emotion processing in CD would benefit from using a more appropriate baseline, such as scrambled faces (Eger et al., 2005; Kanwisher et al., 1997), which would have identical low-level visual features. However, as discussed above, adding this baseline to the current experiment would have increased the length of the task and consequently increased the risk of participants becoming restless and ending the task prematurely. In addition, the previous studies of emotion processing in CD have used fixation cross as a low-level baseline, hence using a fixation cross in the current study allowed for clearer comparisons to be made between this and previous studies.

Fourth, the amount of missing data for the Inventory of Callous-Unemotional traits (ICU) meant that the analysis of CU subgroups was conducted using the Callous-Unemotional subscale of the Youth Psychopathic traits Inventory (YPI; see Chapter 3). This limited our ability to draw direct comparisons with previous studies finding differences between individuals with conduct problems and high versus low CU traits using the ICU, and could have affected the results of this analysis. On the other hand, by comparing CD subgroups with high versus low levels of CU traits (rather than children with conduct problems who would not meet diagnostic criteria for CD), our study is well-placed to investigate the validity of the 'limited prosocial emotions' specifier to CD in the DSM-5 (American Psychiatric Association, 2013). Our null findings suggest that previous studies showing differences between high and low CU traits subgroups may have been confounded by differences in levels of CD symptoms or conduct problems.

Finally, comorbid psychiatric disorders were observed in the CD groups, and it is possible that these disorders may have influenced our results. In particular, about a quarter of all females with CD had comorbid depression and one fourth of all males with CD reported cannabis abuse. However, it is important to note that although males and females with CD differed in these measures compared to their respective control groups, there were no significant differences between the sexes in the CD or HC groups. Similarly, although there were IQ differences between the CD and HC groups, there were no differences between the sexes in IQ. Therefore, differences in IQ or psychiatric comorbidity cannot explain the sex-by-diagnosis interactions described here. Excluding CD participants with low IQ and/or comorbidity would arguably reduce the representativeness of the sample, and controlling for all comorbidities might distort the results. Thus, we focused on controlling for the most common comorbid disorder - ADHD - and included IQ as a covariate of no interest in all analyses.

6.5 Conclusion

Unlike previous studies with male-only samples or small numbers of females collapsed into single sex groups, we found no differences between CD and controls during the processing of negative facial expressions of emotion. However, we did find clear sex differences in the amygdala - with males with CD being more reactive and females with CD being less reactive than their sex-matched control groups during the processing of angry faces. These sex-differentiated effects suggest that neurobiological basis of emotion processing in CD may be partly different for males and females and highlights the importance of considering sex as a moderator of CD-related effects in future studies.

In addition to the emotion interaction, there were CD related deficits in face processing more generally, where the CD group showed reduced fusiform gyrus activation relative to the control group when processing faces in general compared with fixation. In contrast to the sex-differentiated effects during processing of angry expressions, this might help to explain why global deficits in facial recognition have been observed in several studies of CD.

Chapter 7: General Discussion

The primary aim of this thesis was to investigate whether abnormalities in brain structure, and neural activity during emotion processing, previously seen in males with CD, are also seen in their female counterparts. By doing this, we aimed to explore potential sex-dependent brain abnormalities associated with CD. In this chapter, the key empirical findings from the three studies included in the thesis will be summarised and the possible interpretation of these results will be discussed. Furthermore, specific and shared themes across the studies will be discussed, as well as the potential influences of age-of-onset of CD and ADHD comorbidity. Finally, the strengths and limitation of the studies described in the thesis will be highlighted, followed by a discussion of the implications of the findings for future research and clinical practice.

7.1 Summary of key findings

7.1.1 Voxel-based morphometry (Chapter 4)

In Chapter 4, we used voxel-based morphometry (VBM) to investigate whether reductions in grey matter volume in CD seen in previous studies with mixed-sex or predominantly male samples are also found in females with CD. We replicated previous findings of a main effect of CD (independent of sex) associated with lower grey matter volume (GMV) in CD groups relative to controls in superior temporal gyrus, ventromedial prefrontal cortex (vmPFC), anterior cingulate cortex (ACC), fusiform gyrus, and amygdala (Fahim et al., 2011; Fairchild et al., 2011; Huebner et al., 2008; Olvera et al., 2014; Rogers & De Brito, 2016; Sebastian et al., 2015; Sterzer & Stadler, 2009; Sterzer et al., 2007). Our findings are therefore consistent with previous studies by implicating regions involved in the processing of emotion and reward, and well as behavioural inhibition, in CD (Buhle et al., 2014; Horn et al., 2003; Phelps & LeDoux, 2005; Pujol et al., 2009).

Importantly, however, we also observed sex-by-diagnosis interactions in some of these regions: namely, left amygdala, left ACC, and bilateral anterior insula. In the amygdala and insula, the sex-by-diagnosis interactions showed that the lower GMV seen in CD relative to controls in these areas were stronger in males with CD. In other words, this suggests that the males with CD drove the main effects of diagnosis in these areas. However, the sex-by-diagnosis interaction in ACC showed an opposite relationship between sex and diagnosis: males with CD displayed *lower*, while females with CD showed *higher,* GMV in this area. The insula is known to be associated with empathic processing (Bernhardt & Singer, 2012), and these results suggest that deficits in empathy and emotion processing may be more pronounced in males compared to females with CD. Furthermore, sex differences were observed in the effects of the severity of the disorder; males showed a negative, and females a positive, association between CD severity and fusiform gyrus GMV.

The type of sex-dependent effects reported here have been found in other forms of psychopathology where males are overrepresented in the affected population. For example, males with autism were reported to have lower GMV in parietal regions compared to male controls, whereas there was no difference between the female groups in these regions (Beacher et al., 2012). Overall, the VBM results from this study show that several core areas previously identified as structurally abnormal in CD may only be affected in males with the condition.

7.1.2 Surface-based morphometry (Chapter 5)

In Chapter 5, we extended our investigation into sex-differences in CD-related effects on brain structure by examining three separate properties of the cortex (cortical thickness, surface area, and gyrification) using surface-based morphometry (SBM). As predicted, CD was associated with lower cortical thickness in ventromedial prefrontal cortex (vmPFC; Fahim et al., 2011; Fairchild et al., 2015; Jiang et al., 2015; Wallace et al.,

2014) – a brain area associated with decision-making, empathic processing, and emotion regulation (Goldin et al., 2008; Shamay-Tsoory et al., 2003; Studer, Manes, Humphreys, Robbins, & Clark, 2015). These processes have previously been found to be impaired in individuals with CD (Puzzo et al., 2016; Sonuga-Barke et al., 2016). We also observed higher vmPFC gyrification and surface area in the CD group relative to controls. The findings of higher gyrification and surface area may be an indication that children and adolescents with CD show delayed cortical maturation, similar to what has previously been found in children with ADHD (Shaw et al., 2007).

There were also significant sex-by-diagnosis interactions involving all three structural indices. Males with CD had lower, and females with CD higher, cortical thickness in supramarginal gyrus, whereas the opposite relationship was observed with regard to surface area and gyrification of superior frontal gyrus. In addition, sex-by-CD severity interactions were observed within the CD group in superior frontal gyrus, where males showed a positive, and females a negative correlation, between CD severity and these two measures. The supramarginal gyrus is implicated in decision-making (Silani et al., 2013), and emotional processing (Herpertz et al., 2008), while the superior frontal gyrus is thought to be associated with higher cognitive functions (Boisgueheneuc et al., 2006). Lower supramarginal gyrus thickness, and higher gyrification and surface area values in superior frontal gyrus have previously been found in male only (or predominantly male) samples (Hyatt et al., 2012; Jiang et al., 2015). However, this is the first study to show that females with CD display an opposite pattern to males with CD, relative to their respective sex-matched control groups. These results suggest that there are both common and sex-dependent effects of CD on these three cortical properties, which has implications for future research studying both males and females.

7.1.3 Brain activity during emotion processing (Chapter 6)

In Chapter 6, we investigated sex differences in brain function during the processing of anger and fearful facial expressions, and face processing in general. Against predictions and previous findings (Jones et al., 2009; Marsh et al., 2008; Passamonti et al., 2010; White, Marsh, et al., 2012), we did not observe any main effects of diagnosis, nor any significant correlations between symptom severity within the CD group and neural activity in the *a priori* defined brain areas during processing of fear or anger.

Once again, however, there was a sex-by-diagnosis interaction. In this case, in amygdala activity, where females with CD showed lower, and males with CD higher, activity compared with their respective control groups during the processing of angry versus neutral expressions, as well as faces in general. The amygdala is the structure that has most consistently been associated with emotion processing (Phelps & LeDoux, 2005); however, this is the first time that a sex-dependent effect has been found in this, or any, brain structure in CD during an emotion processing task. Similar sex-by-diagnosis interactions have previously been found in schizophrenia, where males showed lower activity in temporal and posterior parts of the brain during the processing of negative images, but there were no differences in females (Mendrek et al., 2007). In addition, we observed lower activity in the CD group compared to controls in fusiform gyrus during the processing of faces in general. The fusiform gyrus is involved in both emotion processing (Pujol et al., 2009) and the processing of faces (Rossion et al., 2003), and lower activity in this region could reflect a more general impairment in the ability of CD individuals to process facial expressions, rather than specific emotions. Similar results – abnormal activity to faces in general rather than specific classes of facial expression, were previously obtained in females with CD (Fairchild et al., 2014). These results suggest that males and females with CD may have different impairments in emotion processing. This would have

implications for intervention programs designed to improve emotion recognition and processing in CD individuals.

7.2 Identifying common brain regions emerging across studies

Table 7.1 compares the brain regions associated with CD across the different studies. A number of regions showed effects of diagnosis and sex-by-diagnosis interactions across all chapters, most notably, the amygdala, fusiform gyrus, and superior frontal gyrus.

7.2.1 Amygdala

As seen in sections 7.1.1 and 7.1.3, effects of diagnosis and sex-by-diagnosis interactions were identified in the amygdala. Structurally, the CD group displayed lower volume compared to controls, but this was driven by the males with CD. During processing of angry facial expressions, females with CD had lower, and males with CD higher, activity in the amygdala relative to their respective control groups. Changes in amygdala structure or function have been frequently observed in neuroimaging studies of CD, with a heavy focus on the involvement of this structure in emotion processing. The fact that we see sex-by-diagnosis interactions in both structure and function in this area may have important implications for sex differences in CD-related deficits in emotion-processing.

7.2.2 Fusiform gyrus

Another structure that has been noted consistently throughout the three studies is the fusiform gyrus (Table 7.1). For the effects of diagnosis (irrespective of sex), we noted lower GMV, but greater surface area in fusiform gyrus in CD subjects compared to controls, and lower fusiform activity in CD subjects compared to controls when processing faces in general. There were also sex-differentiated effects in the fusiform gyrus; males with CD showed higher, and females with CD lower, gyrification in this region, relative to their respective control groups. In contrast, symptom severity correlated positively with

both fusiform volume and gyrification in females, but negatively in males. These changes may have functional implications given what is known about the role of the fusiform gyrus in the processing of both faces in general and facial expressions of emotion specifically (Kanwisher et al., 1997; Pujol et al., 2009; Rossion et al., 2003). Furthermore, it is interesting to note that in fusiform gyrus, as with the SFG, lower grey matter volume was accompanied by higher surface area. However, more work is needed to explore the functional significance of increases versus decreases in fusiform volume, surface area, and activity to understand the implications of the opposite pattern of structural and functional changes in fusiform gyrus in males and females.

7.2.3 Superior frontal gyrus

Superior frontal gyrus (SFG), a structure thought to be associated with higher cognitive functions and working memory (Boisgueheneuc et al., 2006), was differently associated with CD in males and females relative to controls across all measures. In Chapter 4, we found that females with CD had higher, and males with CD lower, volume relative to their respective control groups in this area. Within the CD group, females further showed a positive, and males a negative correlation between CD symptoms and volume in the SFG. In Chapter 5, we found that males with CD had higher, and females with CD lower, surface area and gyrification in this area relative to their respective control groups. In addition, males displayed a positive, and females a negative, correlation between CD symptoms and both surface area and gyrification. Finally, in Chapter 6, we found a positive correlation between CD symptoms and neural activity in the SFG for males, and a negative relationship for females, during the processing of angry facial expressions.

The fact that we observed lower GMV, but greater gyrification, in males with CD relative to male controls (and the opposite relationship in the female groups) is consistent with previous VBM (Olvera et al., 2014) and SBM studies (Fairchild et al., 2015), and

could reflect the cruder approach of VBM in terms of averaging across aspects of cortical structure to obtain volumetric measures. In addition, grey matter volume, as measured by VBM, is not simply an average of the three SBM metrics, but could include other unwanted properties of the cortex (Winkler et al., 2010).

Despite the fact that we did not have *a priori* hypotheses regarding the SFG, and thus did not have a predefined ROI to use in analyses in Chapters 4 and 6, the T-values for the mentioned contrasts were comparable (or higher) in this area compared to the values observed in the ROIs, where significant results were found.

Related to this issue is the potential bias in the literature that requires researchers to have a strong *a priori* hypothesis regarding a brain region in order to include it as a ROI. This is important in order to avoid researchers choosing areas of interest based on their preliminary results, or on a whim; however, it also creates a literature where new studies base their predictions only on prior research and their well-defined areas of interest, leaving little room to convincingly identify new important areas. In VBM and fMRI analysis, it would be difficult to persuade researchers of the importance of an area without obtaining whole-brain corrected results, which, as discussed previously, is relatively rare in case-control studies with comparatively small sample sizes. Alternatively, meta analyses are capable of detecting areas that have consistently appeared in the literature but that may not have enough power in each individual study to become significant. One recent meta-analysis of VBM studies in CD identified an area that included medial SFG (Rogers & De Brito, 2016) as showing lower GMV in CD relative to controls. However, sex-by-diagnosis interactions were not tested in this analysis. Consequently, there is increasing evidence for the role of SFG in CD, and future neuroimaging studies should consider this area as an important structure in the disorder, but also pay close attention to the sex composition of their sample.

Clearly, combining structural and functional neuroimaging data is potentially of great value in terms of identifying brain areas where both structure and function is

abnormal in CD, but due to time constraints, this type of multi-modal analysis is beyond the scope of this thesis and without such formal tests we can only highlight the areas that appear to overlap, and speculate regarding the importance of these structures across different measures in terms of the aetiology of CD. Another interesting and more informative analysis would be to relate neuroimaging and neuropsychological data in the same individuals to better understand what the functional implications of altered brain structure or function mean for the participants' cognitive or emotional abilities.

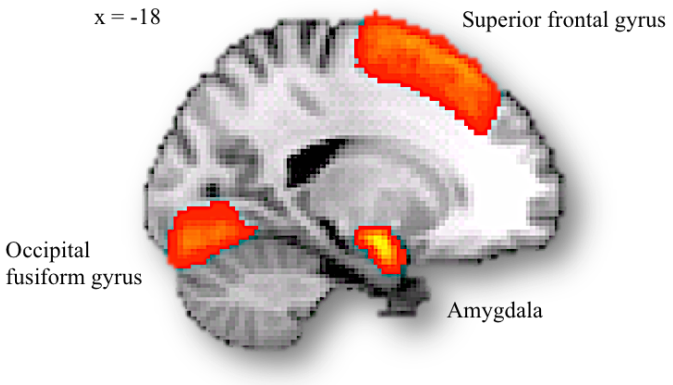

Figure 7.1. Location of the brain regions identified across the three chapters as showing effects of diagnosis, sex-by-diagnosis interactions, or sex-by-CD severity interactions. The image displays the left occipital fusiform gyrus, superior frontal gyrus, and amygdala from the Harvard-Oxford cortical and subcortical structural atlas, for display purposes.

Table 7.1. Brain regions identified across the three chapters, separated by main effect of diagnosis, sex-by-diagnosis interactions, correlations with CD symptoms, and sex-by-CD severity interactions

	Measure				
	VBM		SBM		fMRI
Cerebral structure Contrast	Volume	CT	SA	*l*GI	Neural activity
Superior frontal gyrus					
Main effect of diagnosis	X				
Sex-by-diagnosis interaction	X		X	X	
Symptom severity					
Sex-by- severity interaction	X		X	X	X
Fusiform gyrus					
Main effect of diagnosis			X		X
Sex-by-diagnosis interaction				X	
Symptom severity					
Sex-by- severity interaction	X			X	
Amygdala					
Main effect of diagnosis	X				
Sex-by-diagnosis interaction	X				X
Symptom severity					
Sex-by- severity interaction					

Key: CT = cortical thickness, *l*GI = local gyrification index, SA= surface area, X = significant effect.

7.3 Is female CD neurobiologically distinct from male CD?

Sex differences in CD have previously been investigated with regards to risk factors, clinical presentation, neuropsychological and cognitive processes, and adult outcomes - and commonalities and differences have been noted in each of these domains. However, these differences are not always clear-cut. For example, although childhood risk factors seem to be largely similar for males and females, the sexes seem to be exposed to these risk factors in different ways and to different extents (e.g., child abuse is linked to developing antisocial behaviour in both sexes, but the rates are higher in females (Chesney-Lind & Shelodon, 2013; Moffitt et al., 2001). Similarly, although the severity of adult outcomes/consequences of CD seem to be comparable between the sexes, males and females differ in the *type* of consequences, such that males are more likely to continue on an antisocial path, leading to a greater chance of arrest or imprisonment, whereas females are likely to show a more heterotypic continuity of the disorder, whereby they might desist from showing CD in early adulthood, but other psychopathologies such as depression might emerge in its place (Odgers et al., 2008; Samuelson et al., 2010). Therefore, the sex differences that are present in CD may be fairly complex, and researchers are still relatively far away from understanding the implications of these sex differences for clinical practice. Thus, a greater understanding as to whether the neurobiology of CD is similar or differs between the sexes may help answer questions regarding differences in diagnosis and clinical presentation, and ultimately, whether males and females should be studied together and treated in the same way.

The results presented in this thesis suggest that males and females with CD share some of the same brain abnormalities (such as lower thickness and higher gyrification in vmPFC and reduced fusiform gyrus activity), indicating that there is a shared neurobiological basis for the disorder. However, across the three studies, we also observed several sex differences in the relationship between CD and brain structure and function - and in several of the same brain regions across the studies (e.g., superior frontal gyrus

surface area and gyrification). These differences could be an indication of a partly different neurobiological basis of CD in males and females. However, before making this claim, we must consider that the results may have been partly influenced by other unknown or unmeasured factors.

7.3.1 The potential influence of psychiatric comorbidity and life events

The ability to distinguish between the variables of interest (in this instance main effects of CD and interactions between sex and CD) and other potential factors that could influence the brain is an issue common to all case-control imaging studies (Peterson, 2003). Karatsoreos & McEwen (2013) argued that this issue might lead researchers to disregard the possibility that observed effects might arise from compensatory or adaptive changes in the brain, rather than reflecting a diagnostic effect. The changes observed in brain structure or function may therefore partly be a result of differences in co-occurring disorders, early adversity, brain injury, or other life events that may directly or indirectly cause brain alterations. In addition, the cross-sectional design of the study casts uncertainty on whether the brain abnormalities noted in the CD groups contribute to developing CD, or whether acquiring the disorder causes the brain to change. If the former is true, the sex differences we have observed may be related to different neurobiological causes of CD in males and females, whereas the latter may indicate that the clinical presentation of CD differs between the sexes, and this *leads* to differences in the brain. In the current study, the males and females with CD did not differ in CD severity, rates or type of comorbidity (except for ADHD), IQ, or medication use, and thus these types of behavioural and treatment characteristics seem unlikely to have influenced the sex-by-diagnosis interactions - at least not in a direct way.

7.3.2 Could differences in the diagnostic criteria underlie sex differences in the relationship between CD and brain structure or function?

Another factor that warrants consideration relates to the diagnostic criteria for CD, as discussed in Chapter 1. If the criteria used to determine whether someone has CD are indeed biased towards behaviours typically displayed by males, they may not apply as well to females. This poses a fundamental question when studying sex differences in CD: what meaning could we infer to sex-by-diagnosis interactions in the brain if the criteria for including participants in the CD group are only applicable to one of the sexes? For example, is some other (or additional) criteria are needed for females with CD, we may not have included a sample that is representative of clinical reality, or have missed some individuals that could have been included in the female CD sample. This may in turn influence the effects of diagnosis and the sex-by-diagnosis interactions.

It is clear the a better understanding of the presentation and behaviour of females with CD in general is needed to be certain that our results were not biased by the diagnostic criteria of the disorder. Additional classifiers of CD, which include cognitive and emotional deficits and findings at a biological levels (Wakefield et al., 2002), may help to create reliable criteria for diagnosing CD, which would apply to both sexes. For example, the study in Chapter 6 suggests that deficits in emotion processing may be different for males and females with CD, whereas low IQ has previously been found to be a characteristic of the disorder in both sexes. Nevertheless, it is currently not possible to estimate to what extent, if any, research on sex differences is restricted by the current classification system.

7.3.3 Sex differences in other psychopathologies: the bigger picture?

Sex differences in symptom presentation have been reported in several other disorders. In autism spectrum disorders (ASDs), males are similarly overrepresented in the population, which may be at least partly be due to their display of more 'traditional' symptoms of the disorder compared to females, much like the issue discussed regarding

CD (Mandy et al., 2012). In this disorder, sex-by-diagnosis interactions in brain structure have been noted; females with autism had higher volume in temporal–parietal regions compared to controls, whereas there were no differences between males with autism and male controls (Lai et al., 2013). Contrary to this, Beacher et al. (2012) found a sex-by-diagnosis interaction whereby males with ASD had lower GMV in inferior parietal lobe and rolandic operculum compared to male controls, whereas there was no difference between the female groups. However, the latter study had a relatively low autism group sample size (just 15 males and 13 females), and it could be that although the effect may be there for both sexes, it might be stronger in one sex (i.e., males) and thus differences with smaller effect sizes (in females) could go undetected in studies with small samples.

Sex differences in the relationship between ADHD and brain structure have also been reported. A recent study using SBM measures found that although children with ADHD of both sexes had reduced total surface area (SA) compared to controls, they differed in the area which was most affected. SA was found to be lower in the prefrontal cortex for females with ADHD compared to female controls, whereas males with ADHD did not differ from male controls in this region. In addition, while lower primary motor cortex SA was found in males with ADHD compared to male controls, there was no difference between females with ADHD and female controls in this region (Dirlikov et al., 2015). Again, the evidence suggests that ADHD presents differently for the two sexes; females tend to have greater intellectual impairment, and lower levels of hyperactivity compared to males (Gaub & Carlson, 1997). These findings are of particular relevance to the current studies considering the overlap between CD and ADHD; if males and females differ in the behavioural presentation of both CD and ADHD, as well as in the prevalence of both disorders, this adds to the complexity of interpreting the sex-by-diagnosis interactions. In addition, it shows that it is important to investigate effects of CD with and without taking ADHD comorbidity into account, in an attempt to separate out the effects related to the two disorders.

Furthermore, males with post-traumatic stress disorder (PTSD) have been found to have more widespread brain abnormalities compared to females with the disorder (De Bellis & Keshavan, 2003), despite the fact that females in general are at higher risk, and twice as likely to develop the disorder compared to males (Breslau, Peterson, Poisson, Schultz, & Lucia, 2004; Christiansen & Hansen, 2015).

Finally, differences between neural activity of males and females with schizophrenia have been noted - a disorder where the prevalence is similar between the sexes (Saha, Chant, Welham, & McGrath, 2005) - and males, but not females with schizophrenia, showed lower activity in temporal and posterior parts of the brain during the processing of negative images (Mendrek et al., 2007). Taken together, sex differences in brain structure and function are seen across several forms of psychopathology, and are often coupled with differences in symptomology and clinical presentation in males and females. This implies that there is a growing need to investigate potential sex differences across psychopathologies, and to focus resources on finding out what these sex differences mean for treatment and management across different disorders.

7.3.4 Delays in brain development

Yet another feasible explanation for the sex-by-diagnosis interactions presented here is the possibility that CD is associated with a general delay in brain development, and that this may be specific to, or more pronounced in, males with CD- or at least more evident in the late- or post-pubertal stages of development that were the focus of the present studies. As discussed in Chapter 2, and subsequently in Chapter 5, individuals with ADHD show delayed cortical maturation compared to their healthy peers (Rubia, 2007; Shaw et al., 2007). Specifically, in prefrontal regions, children with ADHD reached their peak of cortical thickness around three to five years later compared to controls (Rubia, 2007; Shaw et al., 2007). Although longitudinal imaging studies such as the one mentioned in children with ADHD have not been conducted to measure cortical development in CD, it is feasible that CD might be associated with similar delays in cortical maturation. In a

recent VBM study, Hummer et al (2015) noted an atypical relationship between age and GMV: they found that while older typically-developing adolescents had lower GMV compared to their younger peers, there were no differences in GMV between younger and older adolescents with disruptive behaviour disorders (including CD and ODD; Hummer, Wang, Kronenberger, Dunn, & Mathews, 2015). Furthermore, in normative populations, males and females show brain developmental trajectories that follow an inverted U-shaped curve in GMV and surface area (peaking in late childhood), and a decrease in gyrification (from early childhood; (Giedd et al., 1997). The lower surface area and gyrification seen in controls compared to the CD group could be an indication that males and females with CD do not follow the same curve (or the same time-course of the curves) in these cortical properties as their normative peers. For example, the fact that females with CD show higher supramarginal CT relative to female controls whereas males with CD show lower CT relative to male controls might reflect the fact that the females are 'ahead' of the males in the brain maturation process, while simultaneously reflecting a relative delay in thickness and surface area maturation in the CD groups relative to controls. Similarly, the higher gyrification values seen in the CD groups could be an indication of this delay, since gyrification typically declines linearly with increasing age (and we see lower gyrification values in control females relative to control males; Raznahan et al., 2011).

Despite these issues and other potential explanations for the findings discussed here, we cannot disregard the possibility that the different relationships between CD and brain structure and function seen in males and females reflect fundamental sex differences in how CD is expressed at the level of the brain. However, to reduce the risk that the effects are due to differences in symptomology, comorbidity, or early adversity, it is important to be aware of the potential limitations of the current classification system, as well as the complexity that comorbidity and variability in demographic characteristics add to the analysis. Furthermore, to understand whether CD is associated with a delay in brain

development, and whether this delay is superimposed on sex differences in brain development, longitudinal research designs would have to be adopted.

7.4 Confounding and complicating factors

7.4.1 Age-of-onset of CD

The developmental taxonomic theory has been one of the most influential theories of the development and subcategorisation of CD. The distinction based on age-of-onset has received mixed empirical support, with earlier studies reporting differences in risk factors, severity, and adult outcome (Moffitt & Caspi, 2001; Moffitt et al., 2001), but subsequent studies finding no difference between the groups in types of biological risk factors (Roisman et al., 2010) or in adult outcomes (Odgers et al., 2008). Results from neuroimaging studies have also been varied. While no differences between the subtypes have been found in grey matter volume (Fairchild et al., 2011), a recent SBM study found that males with childhood-onset (CO) CD exhibited greater gyrification in SFG, fusiform, and temporal gyrus relative to those with adolescence-onset (AO) CD, but these groups did not differ in cortical thickness or surface area (Fairchild et al., 2015). Furthermore, a previous study reported lower activity in CO-CD males compared to AO-CD males when processing sad facial expression. However, there were no differences between the two sub-groups when processing angry facial expressions (Passamonti et al., 2010),

The results of the three analyses in this thesis are consistent - there were no significant differences between CO-CD and AO-CD subgroups in GMV or neural activity during processing of angry or fearful facial expressions, or faces in general. Furthermore, we did not observe any differences in GMV, cortical thickness or surface area. However, similarly to Fairchild et al., (2015), we found that CO-CD individuals had greater gyrification compared to AO-CD individuals, albeit in separate areas from those identified as showing main effects of diagnosis in the main analyses: i.e., anterior insula. Because these areas did not overlap with any of the areas identified in the main analysis, we

combined the two CD age-of-onset groups. There were furthermore no differences between males and females with CO-CD and AO-CD across the measures, as would be evident from a sex-by-age-of-onset interaction. The results from these three experiments, and the findings published previously raise questions regarding the validity of the developmental taxonomic theory. This theory has had a major impact not only on research but also on the diagnosis of CD and clinical treatment of this condition. Specifically, the theory emphasises the greater importance of neurobiological and genetic influences on CO-CD, whereas AO-CD is likened to an extreme form of normative teenage rebellion (Moffitt, 1993). Practically, this means that the two groups should receive different intervention strategies and therapies (Searight, Rottnek, & Abby, 2001), such that individuals with CO-CD may be more likely to receive pharmacological treatment and those with AO-CD being more likely to receive family-based therapy (or no treatment at all). However, if the two subgroups are in fact largely the same in terms of their neurobiological underpinnings, the DSM-5 distinction based on age-of-onset may act to hinder clinicians to formulate appropriate treatment strategies. In a review article published in 2013, Fairchild and colleagues suggested a reformulation of the theory to suggest quantitative, rather than qualitative, differences between the two subtypes of CD, whereby they may differ in the degree, but not necessarily the type, of risk- and protective factors that may lead to the disorder (Fairchild, van Goozen, et al., 2013). Exposure to abuse, neglect, or other childhood adversities may therefore play a greater part in influencing the type and course of antisocial behaviour, and may be a mediating factor in the timing of antisocial behaviour onset.

However, it is worth noting that since the sample used in the present studies were late adolescents, questions regarding age of first symptom onset would be more likely to be recalled incorrectly than if the sample were younger - thus potentially reducing reliability in the age-of-onset classifications made here. Nevertheless, there was an approximately similar number of CO-CD and AO-CD cases in the male and female CD groups, and in the

direction that we would expect, i.e., a slightly higher proportion of male CO-CD compared to male AO-CD, and a slightly higher proportion of female AO-CD compared to female CO-CD (albeit not significantly different). It is clear that, in the absence of clear neurobiological evidence to support the age-of-onset classification, more research is needed to determine the validity and clinical benefits of making this distinction.

In contrast, and as discussed in Chapter 1, research have shown that the distinction between aggressive versus non-aggressive antisocial behaviour is more predictive of trajectories and adult outcome (Burt, Donnellan, Iacono, & McGue, 2011). Although investigating neurobiological differences between these behavioural subtypes were beyond the scope of this thesis, it would be of great value for future research to compare the different subtyping approaches in terms of neurobiological measures to potentially bridge the different approaches.

7.4.2 Comorbidity

Rates of comorbidity are high in individuals with CD, with rates of depression and anxiety reported to be around 35% in community samples (Polier et al., 2012). Alcohol and drug abuse comorbidity is also high, estimated to be present in around 20% of those with CD (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003). The rates of comorbidity vary greatly depending on the setting - with increasing rates of different psychopathologies in clinical samples (Polier et al., 2012), and the type typically differs between the sexes, such that females are more likely to have internalising comorbidity and males more likely to have externalising comorbidity. The rates of comorbidity within the current CD sample were similar to those expected from a (predominantly) community sample (23% depression, 17% drug abuse), with numerically higher rates of depression in the female sample, and numerically higher rates of drug abuse in the male sample. Although several psychopathologies are commonly comorbid with CD, including the ones mentioned, ADHD is by far the most common comorbidity- particularly in males (Turgay, 2005). This

was the case in our sample as well, with half of our male CD group and a quarter of our female CD group having comorbid ADHD. The high prevalence of co-occurring disorders, and the complex relationship between them, present several challenges in cross-sectional (f)MRI designs as it is difficult to interpret to what extent the effects seen in the brain are related to CD, and which could be effects that reflect the presence of comorbid disorders.

Furthermore, disorders such as ADHD, depression, or alcohol and substance dependence may present themselves more or less independently from one another but may also be affecting CD on one or more levels in regards to the development, course, or severity of the disorder (Loeber & Keenan, 1994). For example, the presence of ADHD has been suggested to worsen the severity of CD, but the presence of CD does not appear to affect ADHD in the same way, creating an asymmetrical relationship between the two disorders (Loeber & Keenan, 1994). In the neuroimaging literature on CD, approaches to dealing with ADHD comorbidity have varied. A straightforward solution would be to only recruit individuals with pure CD, i.e., those with no comorbid disorders. However, this would greatly reduce the generalisability of the findings and the resulting samples would generally not be representative of CD populations. Another option is to re-run analyses excluding all participants with comorbidity and compare the two sets of results. This option is also suboptimal, as excluding a large part of the sample essentially creates two different samples- one larger group with comorbidity and one much smaller group with pure CD. The fact that one result was statistically significant in one group, but not another, could therefore reflect issues related to statistical power rather than true differences between pure and comorbid forms of CD. In this thesis, we adopted another approach- where we re-ran the analysis including ADHD symptoms as a covariate of no interest to examine the impact of controlling for this factor. Although this approach has limitations concerning assumptions of covariance, i.e., the significant difference in number of ADHD symptoms between CD and controls, this method was used as it does not affect the sample

size, yet is able to present results that were both affected and unaffected by ADHD comorbidity.

Overall, controlling for ADHD symptoms predominantly influenced the main effects of CD diagnosis, whereas the sex-by-diagnosis interactions and sex-by-CD severity interactions were largely unaffected across the three studies. More specifically, when including ADHD as an additional covariate, the findings that were originally present bilaterally in five out of the seven ROIs, that is, lower GMV in the CD group relative to controls, remained significant only in the right hemisphere (amygdala, anterior cingulate cortex, fusiform gyrus, anterior insula, and superior temporal gyrus). Of note, the main effect of diagnosis on fusiform gyrus volume became even stronger when including ADHD symptoms as a covariate. Furthermore, although the main effects of CD diagnosis on vmPFC thickness and gyrification remained significant, the remaining main effects of diagnosis across the three SBM measures were rendered non-significant. The only main effect of diagnosis in the emotion processing task- i.e., lower fusiform gyrus activity in CD subjects relative to controls during face processing in general, was also rendered non-significant. In contrast, all sex-by-diagnosis interactions in GMV remained significant when controlling for ADHD symptoms, and the sex-by-diagnosis interactions in superior frontal gyrus gyrification and surface area and supramarginal thickness also remained significant. In the emotion processing study, the sex-by-diagnosis interaction in the amygdala during anger processing and face processing in general also remained significant. Finally, none of the CD symptom correlations or sex-by-CD severity interactions were affected by the inclusion of ADHD symptoms as a covariate. This is not surprising, considering the fact that CD and ADHD symptoms were highly correlated within the whole sample, but less so within the CD sample considered alone, such as in the correlation analyses. The fact that some effects of diagnosis and sex-by-diagnosis interactions became stronger and some weaker (rather than only weaker) when including ADHD as a covariate suggests that these results represent effects that are related to the

presence of CD in the absence of ADHD, rather than simply reflecting reduced significance levels as a result of decreased statistical power.

7.5 Strengths and weaknesses of the studies described in this thesis

7.5.1 Strengths

The studies included in the thesis had a number of strengths. Firstly, we were able to investigate, for the first time, interactions between CD and sex in terms of brain structure and activity during emotion processing. Previous studies have achieved this in other psychopathologies, such as autism (Lai et al., 2013), ADHD (Dirlikov et al., 2015), PTSD (De Bellis & Keshavan, 2003), and schizophrenia (Mendrek et al., 2007). However, this is, to our knowledge, the first set of studies to include a large enough CD sample (n=96) with equal number of males and females to be able to assess sex differences in brain structure, and to directly investigate sex differences in the neural substrates of emotion processing. In Chapter 2 (Tables 2.2 and 2.3), we noted that the total number of females studied across all previous SBM studies of CD was 31 (compared to 117 males with CD). Our female sample is therefore greater than the combined number of females included in all previous studies of this kind. Across the 13 previous VBM studies, the numbers of females included in previous studies are somewhat higher, with 139 females with CD in total (compared to 440 males with CD). However, three of the four studies that included a female sample of n>20, did not include males with CD and thus were unable to investigate sex differences directly[2] (Cope et al., 2013; Dalwani et al., 2015; Fairchild, Hagan, et al., 2013). Furthermore, apart from Fairchild et al. (2013), the primary focus of the studies were CD together with psychopathy (Cope et al., 2013), substance abuse (Dalwani et al., 2015), and disruptive behaviour disorders more generally (Michalska et al., 2015).

[2] Fairchild et al.'s (2013) study was an all-female study, but also included data from a previously published study of males (Fairchild et al., 2011) to investigate sex differences in the relationship between CD and brain structure.

Secondly, across all fMRI studies in the literature, only 106 females with CD have been studied compared to 541 males with CD. However, the relative number of females is even smaller when focusing on studies of emotion processing. Despite that fact that there has been a substantial focus on emotion processing deficits in behavioural studies and theories of CD (van Goozen et al., 2007), only one previous fMRI study have investigated these processes in females with CD specifically (Fairchild et al., 2014), giving a total of 28 females with CD (compared to 166 males with CD) across all studies. Thus none of the previous studies of emotion processing included a sufficiently large sample of males and females with CD to investigate sex differences in the relationship between CD and neural activity during emotion processing.

Thirdly, we have improved on previous studies by including a narrower age range and matching our participants in terms of pubertal status, as well as age. No previous studies have systematically matched their groups in pubertal status. In addition, several of these studies (both structural and functional) have included samples with age ranges spanning up to seven years, and consequently may have averaged over different developmental stages (Huebner et al., 2008; Marsh et al., 2013; Passamonti et al., 2010; Rubia et al., 2008). Although this type of matching for pubertal status does not guarantee that our groups were at the same stage of brain development, that is, the effect of pubertal stage on brain development may not be the same in males and females, pubertal development is nevertheless associated with brain development (Lenroot et al., 2007), and thus it is less likely that pubertal status directly influenced our results.

Fourthly, we separated our CD sample into CO-CD and AO-CD subgroups and directly compared these subgroups in terms of brain structure and function. Since males and females with CD did not differ in their ratio of CO-CD and AO-CD subjects, and the age-of-onset groups did not significantly differ in most of the measures studied (except *l*GI), we can be more confident that the results were not driven solely by the presence of childhood-onset CD individuals in the sample. Finally, to reduce the risk that IQ, site, or

differences in total GMV influenced our results, we re-ran all analyses individually, excluding these covariates from the analyses. This was to ensure that none of these factors significantly impacted the results, and in each case the findings remained largely similar to those obtained when including each of these factors as covariates of no interest.

Finally, the diagnoses of CD and comorbid disorders were made using a semi-structured interview- the K-SADS-PL (Kaufman et al., 1997; see Chapter 3), rather than self- or parent-reported questionnaires assessing psychopathology such as the Child Behaviour Check List. This means that we obtained rich and detailed information from both parents and participants, and the semi-structured format allowed the interviewers to tailor their strategies of asking questions to the informant, while having a script to go back to if needed. The assessor can use information provided earlier in the interview to probe answers and ask about significant developmental milestones (e.g., transition from primary to secondary school) or holidays (e.g., Christmas, birthdays) to enhance recall accuracy, which is a particularly important consideration in relation to the age-of-onset of CD symptoms.

7.5.2 Limitations

The limitations of each individual study have been discussed in detail in each of the three chapters; thus, these issues will not be revisited in full here; rather, we will draw out limitations that apply across the three studies.

Using data from a multi-site study has several benefits, including increased sample size and hence increased power to detect differences between groups, as well as potentially increased generalisability of findings. On the other hand, collecting data at multiple sites also increases the risk that scanner hardware and software differences introduce unwanted noise in the data. Although we reduced this risk by undergoing site qualification procedures at each site to ensure that scanning sequences and procedures were comparable before the study started (see detailed description in Chapter 3), it is still possible that

factors beyond our control influenced the data directly or indirectly. However, to further reduce the possibility that these potential differences influenced the results, all analyses were run both with and without including site as a covariate - and the results remained largely similar.

Similarly, including interview data from two countries in Europe increased the generalisability of the results, but also introduced the possibility that subtle differences in language and ways of interviewing across countries/sites may impacted the assessment and/or the validity of the diagnoses. To avoid this issue, each site completed inter-rater reliability assessment on the K-SADS-PL interviews, and received significant training in interviewing techniques. A related issue to cultural differences in interviewing style concerns the potential cultural differences in the manifestation of CD. As one of the criteria for assessing CD, the DSM-5 states that the behaviours shown by individuals with CD are violations of societal norms (APA, 2013). However, some societal norms, i.e., what is considered part of normative behaviour or not, likely differ to some degree between European countries.

There are furthermore some concerns regarding the design and implementation of assessing CD and comorbid disorders using the K-SADS. Firstly, by design and as discussed in section 7.4, our control groups were free from any current psychiatric conditions. This was done to improve study design and ensure that interpretations of group differences were not affected by psychopathology in the control groups. However, this meant that the control groups may not be representative of general adolescent populations (they may have been 'super-controls'), and there was no ideal way to account for the psychiatric comorbidity seen in the CD group. To improve study design, future neuroimaging studies might consider including a psychiatric control group, who similarly to the first control group are free from CD or ODD, but may have diagnoses of ADHD, depression, anxiety or other common childhood disorders to say with more confidence that the key results are explained by the presence of CD rather than common comorbid

disorders. This might also help in terms of matching the groups on important demographic characteristics such as IQ or socioeconomic status.

Secondly, due to using an adolescent sample with a mean age of 16 years, CD age-of-onset was assessed retrospectively. Although the semi-structured nature of the K-SADS-PL interview allows the interviewer to use a number of strategies to enhance accurate recall, such as asking about key milestones in development, assessing the age of onset of the first CD symptom retrospectively is not optimal. Therefore, future studies that aim to focus on similar issues would benefit from using data from longitudinal studies where the onset could be assessed closer to the age when it occurred, or datasets where clinical information is available from the participants below the age of 10. Thirdly, our sample included participants from 14 to 18 years old, many of whom did not live with their parent or under any adult supervision. This meant that in some cases, a diagnosis of CD was based on the participant's account alone. Furthermore, while it is not required to obtain consent from a legal guardian if the participant over 16 years old in the UK, this was a requirement at the German sites, who as a consequence also obtained more diagnostic information from parents. Consequently, the issue of missing parental data was much greater at the UK sites.

Thirdly, the three empirical chapters used different methods, and to some extent, different statistical approaches and thresholds. This is of particular relevance for Chapters 4 and 5, where both studies assessed brain structure alterations (in the same population). While SBM methods assess three different properties of the cortex and are restricted to the cortex (rather than the whole brain), VBM methods average over these properties and measure grey matter volume (and concentration) across the whole brain. The standard statistical thresholds for these analyses also differed - SBM analysis typically report whole-brain corrected results for the entire cortex, whereas it is common in VBM analyses to use a region-of-interest (ROI) approach within *a priori* anatomically defined regions. Although it is possible to use a similar predefined ROI approach in FreeSurfer, it is more

common to use whole-brain cluster-analysis, and thus, the latter approach was adopted in the current thesis. It should be noted that we also reported whole-brain corrected results in Chapter 4; however, it is uncommon for VBM (or fMRI) results to survive this stringent threshold. Apart from measuring different properties of the cortex (i.e., integrated measures of volume versus different SBM metrics), these constraints may help to explain why we did not find a complete overlap in terms of brain areas identified across our analyses.

Another important distinction between the two structural data analyses is the option to create customised tissue probability maps and a paediatric template based on the sample. We were able to do this in the studies described in Chapters 4 and 6 to improve normalisation and registration from native-space to the Montreal Neurological Institute (MNI) space. In contrast to MNI space, which is typically used as the 'average' brain template, FreeSurfer typically uses FSAverage, which is a template averaged over 40 adult subjects using SBM measures (Fischl et al., 1999). The different templates adopted reflect the different approaches use to calculate brain structures - that is, volumetric versus surface based methods - and thus each template is appropriate for the approach used. Consequently, although SBM and VBM methods have been used in this thesis to answer similar questions regarding alterations in brain structure, they are, inherently, quite different methods. In contrast, while fMRI measures changes in neural activity rather than structure, this method of analysis shares many similarities to the VBM approach used in Chapter 4. Both analyses used the statistical parametric mapping (SPM) approach. In addition, the same statistical approach was adopted, the same seven ROIs were used, and the same statistical thresholds applied. Furthermore, both the VBM and fMRI analyses were able to detect abnormalities in subcortical structures - which represents a valuable addition to cortical analysis. With these methodological differences in mind, comparisons between findings from the three studies have to be made cautiously, and it is critical to point out that no formal statistical comparison was made between the findings of the three

studies. Although it is possible to adopt similar thresholds, and modify the registration process (such as normalising the images to the adult MNI template in both studies and using the same corrections for multiple comparisons) to be able to compare the two methods, it was not a primary concern in these studies; rather, it was more important to adopt similar methods of analysis to those used in previous studies using the respective analysis approach to extend their findings by investigate sex differences. However, this type of comparison would be of great value in future studies. Nevertheless, it is interesting to note that several brain structures were identified in common across the three studies, showing both abnormal structure and function in CD subjects compared to controls or sex-by-diagnosis interactions.

Finally, since the findings presented here are cross-sectional, we can only speculate regarding the possibility that main effects of diagnosis and sex-by-diagnosis interactions may reflect a consequence of a delay in brain maturation in CD (particularly in males with CD). Similarly, because we restricted our sample to late- and post adolescents aged between 14 and 18 years, our findings may not generalise to younger age groups. Future studies would greatly benefit from adopting longitudinal research designs to follow the development of CD groups from pre- to post-puberty in order to investigate whether CD is associated with the same type of delay in brain maturation as has been found in ADHD samples, and whether this delay might be sex-specific, or whether sex and CD diagnosis interact to influence rates of brain development.

7.6 Implications of findings for research and clinical practice

The findings presented in this thesis fit with the literature showing sex differences in psychopathology generally, and suggest that the neurobiology of CD partly differs between males and females. The fact that we see sex-by-diagnosis interactions across all three sets of results strengthens the robustness of this inference. These findings have implications both for research and clinical practice. Firstly, and as has been discussed at

length, females with CD have been severely under-represented in neuroimaging research to date, and there has been an assumption that they do not differ from their male counterparts (and therefore data from males and females can be validly combined). We believe that the findings presented here should encourage researchers to question this assumption and devote resources to studying females with CD specifically. We therefore recommend that future neuroimaging studies of CD avoid collapsing across the sexes on the grounds that combining males and females in the same group runs the risk of cancelling out diagnosis effects (if they actually differ in different ways from their respective control groups). Accordingly, if studies are only able to recruit a relatively small sample size, where sex-by-diagnosis interaction tests would be inappropriate, we suggest that only one sex be included in the study.

Secondly, if the assumption that CD is associated with the same causes and behavioural features in males and females is inaccurate, it may hinder treatment of the disorder. Indeed, interventions aiming to improve emotion processing and empathy in CD have been based on research from males (Bowen et al., 2014). Clinicians therefore need to be alerted to the possibility that the neurobiological bases of CD may differ in males and females and that more research is needed in emotion processing (and to understand what the structural alterations mean for function) to know whether the same training and treatment strategies may not be applicable to both sexes. However, it is clear that much more research into these sex differences are needed before any concrete recommendations can be made regarding treatment programmes. Within the FemNAT-CD project, considerable focus has been put on investigating differences between males and females with CD across multiple levels of analyses and measures, including behavioural measures of empathy, risk-taking, and aggression, hormonal differences in the effect of oxytocin and serotonin, as well as gene-environment interactions (see Chapter 3.1). Thus, within the next few years, we may have a much clearer understanding of the similarities and

differences in the neurobiological basis and neuropsychological profiles associated with CD in males and females.

7.7 Future work

Apart from the recommendation of studying males and females separately or recruiting large samples of males and females with CD, there are several extensions of this and other work that would be of great value in understanding the neurobiology of CD. For example, we adopted a full factorial model in order to study the effects of sex, diagnosis, and their interaction; however, by doing so, we excluded analysis of diagnosis effects in females only. Previous studies have investigated volumetric and emotion processing abnormalities in females with CD relative to female controls (Fairchild et al., 2014; Fairchild, Hagan, et al., 2013); however, no studies have investigated cortical thickness, surface area, and gyrification in a female-only sample. A female only study of CD would be a valuable addition to the literature, as the majority of results from males with CD have been obtained in similar ways, and there may be diagnostic effects that might not be detected as significant in a full factorial model.

Furthermore, we saw in this thesis that lower volume in one brain area was often accompanied by higher surface area and gyrification, most prominently in males with CD. Although the relationship between VBM and SBM measures has been studied previously (Winkler et al., 2010), it would be of interest to further explore the relationship between these two measures of brain structure in regards to CD - especially since the vast majority of structural studies used in CD research have adopted VBM methods. Rather than aiming to replicate previous research methods of each approach, similar statistical methods (i.e., corrections for multiple comparisons) could be used in the two sets of analyses to be able to reliably compare them to each other. In addition, although we observed common alterations in both structure and functions in several brain regions, we did not perform any statistical tests of this relationship, for example in SPM12. Future studies may also wish to

investigate the relationship between altered neural activity and structure in areas such as the amygdala, fusiform gyrus, and prefrontal cortex – for example, do structural changes explain abnormal patterns of neural activity seen in CD groups?

Finally, and as mentioned throughout this chapter, there is a growing need for longitudinal neuroimaging designs to be adopted in CD research. Although the FemNAT-CD study has a longitudinal component, this will likely include participants who are already mid- or late pubertal, and is restricted to measuring brain changes at just two time points. Nevertheless, this is a promising start in understanding the neurobiological development in males and females with CD, and may help to pave the way for additional longitudinal research in this area.

7.8 General conclusion

The present study investigated sex differences in CD-related alterations in brain structure and function. We found main effects of CD in brain regions known to be implicated in emotion processing, reinforcement learning, and decision making, such as ventromedial prefrontal cortex. However, although some of these were similar for males and females, we also identified several brain regions, such as the supramarginal gyrus, amygdala, and superior frontal cortex, where males and females with effects CD showed opposite changes in structure or function compared to their respective control groups. In this chapter, we discussed a number of possible explanations for these effects. For instance, it is possible that CD is associated with a delay in brain development, and that this delay may be more pronounced in males. These effects may also imply different neurobiological bases for CD in males and females. On the basis of these findings, we recommend that future research study males and females with CD separately, rather than collapsing across sexes. Together, these studies highlight the need for increased research on females with CD, and a potential need to adopt different treatment strategies for males and females with CD.

Appendix 1 MRI Safety Checklist

MRI Safety Checklist

ID: _____ **Date completed** _____

	Subject			Parent/Carer	
	YES	**NO**		**YES**	**NO**
Do you have a cardiac pacemaker?					
Have you had any surgery within the last 6 weeks?					
Have you ever had any operations or procedures on your heart?					
Have you ever had any operations or procedures on your brain or spine?					
Have you ever had any other operations on any part of your body involving any implants?					
Have you **ever** had any accidents where metal may have entered or become lodged in your eyes or body?					
Do you have a hearing aid or a cochlear implant?					
Are you asthmatic or claustrophobic?					
Do you have any allergies?					
Do you have any renal impairment (kidney problems)?					

Female participants -

Is there any possibility that you may be pregnant? or are you breast feeding?					

The following items must be removed before you have your scan:

1. Loose metal objects, eg money, keys, pens
2. All jewellery and watches
3. Any safety pins, hair pins and slides
4. Any card with a magnetic strip
5. Clothing with metal zips or buckles (in the area to be scanned)
6. Metal dentures, hearing aids and spectacles

I am happy that data obtained from this study may later be used for research purposes YES / NO

I understand that this is not a diagnostic scan, but if something abnormal is detected, my GP will be informed YES / NO

I have read and understood the Checklist for MRI YES / NO

Date	**Subject/Parent/Guardian Signature**	**Staff Signature**
Date	**Relative / Friend / Escort**	**Staff Signature**

Name of GP	**Adress of GP**

Appendix 2 K-SADS-PL screen

K-SADS SCREEN - Preliminary interview YOUTH

I would like to ask you a few questions about how you've been feeling and behaving ***over the last 12 months****, but also previously in your life. It isn't a test of any kind. There are no right or wrong answers – all I'd like you to do is to answer my questions as honestly as you possibly can. The information you give me today is* ***confidential*** *and will go no further, so I won't tell your parents or teachers (or any authority). However, if I think that you are having problems at the moment, which could benefit from help, we will discuss the possible options with you, e.g. referring you to a doctor. If you provide information that makes me concerned about your safety or the safety of another person in your family, I may be duty bound to refer you or them on to someone who can help, e.g., your GP. We will not pass on this information to anyone outside the research team without telling you first.*

The first thing I'd like to ask is whether you have ever seen a healthcare professional (e.g., a counsellor, Educational Psychologist, a Psychiatrist or a GP) for any other reason apart from routine illness, such as to do with your mood or behaviour?

Have you ever been prescribed medication for anything to do with your mood or behaviour?

1) MAJOR DEPRESSIVE EPISODE

At least 1 from the following 3 symptoms present for more than half the time for a period of at least 2 weeks:

(I) Depressed Mood	**C**	**P**	
	0	0	No information
Everyone has good days and bad days, but in the past 6 months has there been a time when you've felt down, miserable or depressed for days on end? How long did this feeling last? Do you feel like this at the moment? Have you ever gone through a time in your life when you felt like this?	1	1	Not at all or less than once a week.
Did you feel (___) all the time, some of the time? Did it come and go? How often? Every day? How long did it last? What do you think brought it on?	2	2	Subthreshold: Often experiences dysphoric mood at least 3 times a week for more than 3 hours each time.
	3	3	Threshold: Feels "depressed" most of the day more days than not.

(II) Irritability and anger	**C**	**P**	
	0	0	No information
Has there been a time when you've felt irritable or angry for most, or all of the time, for days on end? How long did this last? What about recently? Is there a reason why you felt angry More than before? What kinds of things made you feel angry? Did you sometimes feel angry and/or irritable and/or cranky and didn't know why? Did this happen often? Did you lose your temper? With your family? Your friends? Who else? At school? What did you do? Did anybody say anything about it?	1	1	Not at all or less than once a week
	2	2	Subthreshold: Feels definitely more angry or irritable than called for by the situation, at least 3 times a week for more than 3 hours each time. Or often argumentative, quick to express annoyance.
How much of the time did you feel angry, irritable, and/or cranky? All of the time? Lots of the time? Just now and then? None of the time?	3	3	Threshold: Feels irritable/angry daily, or almost daily, at least 50% of awake time. Or often shouts, loses temper.

DMDD

C	P
0	0
1	1
2	2
3	3

NOTE 1: Must have (1) persistently and predominantly negative mood between outbursts AND (2) negative mood must be observed by others.
NOTE 2: Consider the child's subjective report of irritable, negative mood when not noticed by others. Also consider level of accommodation to prevent irritability as noted in the section temper outbursts.

If DMDD criteria for irritability is Scored with 3, please check the odd Item on page 41 and if both items are Scored with 3, complete the section on DMDD-criteria

(III) Anhedonia, Lack if interest, Apathy, Boredom

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Several activities definitely less pleasurable or interesting. Or bored or apathetic at least 3 times a week during activities.
3	3	Threshold: Most activities much less pleasurable or interesting. Or bored or apathetic daily, or almost daily, at least 50% of the time during activities.

Boredom*:*

Do you have any activities after school? What are the things you do for fun? Give example

Has there ever been a time that you felt bored a lot of the time? When? Do you feel bored a lot now? Did you feel bored when you thought about doing the things you usually like to do for fun? (Give examples mentioned above). Did this stop you from doing those things?

Anhedonia:

Did you look forward to doing the things you used to enjoy? (Give examples) Did you try to get into them? Did you have to push yourself to do your favorite activities?
Did they interest you? Did you get excited or enthusiastic about doing them? Why not? Did you have as much fun doing them as you used to before you began feeling (sad, etc.)?
If less fun, did you enjoy them a little less? Much less? Not at all?

Did you have as much fun as your friends?
How many things are less fun now than they used to be (use concrete examples provided earlier by child)?
How many were as much fun? More fun?
Did you do____ less than you used to? How much less?

(IV) Recurrent thoughts of death

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Transient thoughts of death
3	3	Threshold: Recurrent thoughts of death, "I would be better of dead" or "I wish I was dead".

Sometimes children who get upset or feel bad, they think about death, or even feel that they'd be better off dead. Have you ever had these type of thoughts? When? Do you feel that way now? Was there ever another time you felt that way?

(V) Suicidal Ideation

Sometimes children who get upset or feel bad think about dying or even killing themselves. Have you ever had such thoughts?
How would you do it?
Did you have a plan?

C	P	
		No information
0	0	Not at all
2	2	Subthreshold: Occasional thoughts of suicide but has not thought of a specific method.
3	3	Threshold: Often thinks of suicide and has thought of a specific method.

(VI) Suicidal Acts- Seriousness

Have you actually tried to kill yourself?
When? What did you do?
Any other things?
Did you really want to die
How close did you come to doing it Was anybody in the room?
In the apartment?
Did you tell them in advance? How were you found?
Did you really want to die?
Did you ask for any help after you did it?

C	P	
0	0	No information
1	1	No attempt or gesture with no intent to die (eg., held pills in hand).
2	2	Subthreshold: Present, but very ambivalent.
3	3	Threshold: Definite suicidal intent.

(VII) Suicidal Acts- Medical Lethality

How close were you to dying after your (most serious suicidal act)?
What did you do when you tried to kill yourself?
What happened to you after you tried to kill yourself?

C	P	
0	0	No information
1	1	No attempt or gesture with no intent to die (e.g., held pills in hand).
2	2	Subthreshold: e.g., took 10 aspirins, mild gastritis.
3	3	Threshold: e.g., took 10 seconal, had brief unconsciousness.

(VIII) Non-Suicidal Physical Self-Damaging Acts

Did you ever try to hurt yourself?
Have you ever burned yourself with matches/candles?
Or scratched yourself with needles/ a knife? Your nails?
Or put hot pennies on your skin?
Anything else? Why did you do it? How often?
Do you have many accidents? What kind? How often?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Infrequent (1-3 times a year). Has never caused serious injury to self.
3	3	Threshold: Frequent (4 or more times a year) or has caused serious injury to self (e.g. burn with scarring; broken bone).

2) MANIA

(I) Elation, Expansive Mood

Has there ever been a time you felt very good, really cheerful, or high? Much more than your normal self?
If unclear:
Did you feel as if there is nothing you couldn't do?
Did you feel that everything would work out just the way you wanted?
Did you get really silly? Were you more silly than most of your friends? Have your friends ever said anything to you about being too happy or too silly? If people saw you, would they think you were just in a good mood or something more than that?
Did you get as if you were drunk? Did you feel super-happy? Give me some examples?
How long did this feeling usually last?

Note: Do not score positively if elated mood due to Recreational drugs.

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Definitely elevated mood and optimistic outlook that is somewhat out of proportion to the circumstances. Mood occurs at least 3 times a week and persists for more than 3 hours each time.
3	3	Threshold: Mood and outlook are clearly out of proportion to circumstances. Noticeable to others and perceived as odd or exaggerated. Experiences elevated mood daily, or almost daily, at least 50% of awake time for at least four days

(II) Decreased Need for Sleep

How much do you usually sleep at night? Have you ever needed less sleep than usual to feel rested, like several hours less? Did you stay up because you felt especially high or energetic? Were you with friends or by yourself? Had you taken any drugs?

Note: Do not score positively if decreased need for sleep triggered by social event or drug use, or reflective of typical irregular adolescent sleep pattern.

Note if insomnia or hypersomnia reported in response to the probes for this item.

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: 1-3 hours less than usual two or more consecutive nights. Felt high or especially energetic.
3	3	Threshold: 3 or more hours less than usual for two or especially energetic--not tired the next day.

(III) Increased Goal Directed Activity

Has there ever been a time when you were more active or involved in more things than usual, or you seemed to get a lot more done than before? Were you working on any projects at home or at school? Going out more than usual?
Were you more sexually active than usual? What was your mood like at that time?
How were you feeling about yourself? More confident than usual?

Note: Only score positively if increased activity occurs during period of mood change (eg., elation, irritability) or increased self confidence.

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Mild but definite increase in general activity level involving several areas (e.g. work, school, socially, sexually).
3	3	Threshold: Moderate to severe increase in general activity level involving several areas, or marked increase in one or more areas. Activity involvement is excessive, more than what would be expected by a typical child his/her age.

(IV) Racing Thoughts

Have there ever been times when your thoughts were racing so fast it was hard for you to keep up with them? Have you ever felt like there were too many ideas jumping around in your mind? Could you stop the thoughts if you wanted to? What was your mood like at that time?

Rate based on data reported by informant or observational data. Score positively only if racing thoughts occur during mood change (eg., elation, irritability).

Note: If racing thought was the only item initially endorsed, re inquire about mood (eg., elation, irritability), sleep and activity level during periods when racing thoughts reported.

C	P	
0	0	No information
1	1	Not Present
2	2	Subthreshold: Racing thoughts cause minor distress or impairment.
3	3	Threshold: Racing thoughts cause significant distress or impairment. Thoughts cannot be stopped voluntarily.

3) PSYCHOSIS

(I) Hallucinations

Has there ever been a time you heard, saw or smelled something that other people couldn't hear, see or smell? For example, have you ever heard someone call your name when there was no one around, or see shadows or objects move? What kind of things did you hear? Did you ever hear music which other people could not?

Did this only happen at night while you were trying to sleep, or did it happen in the daytime too? Could it have been a dream?
What did you see?

Note: If hallucinations possibly present, prior to scoring this item, assess the subject's conviction of the reality of the hallucinations with the probes below.

What did you think it was?
Did you think it is your imagination or real?
What did you do when you (heard, saw, etc.) it?
Were you sick with fever when they occurred?
Have you ever been drinking alcohol or taking any drugs when it happened?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Suspected or likely
3	3	Threshold: Definitely present

(II) Delusions

Has there ever been a time your imagination played tricks on you? Did you believe in things that other people didn't believe in? Like what?
Have you ever thought that someone was following you, or listening to your conversations, when you couldn't see anyone? Or thought that someone was out to hurt you? Who? Why?

Have you ever felt that something was happening to your body? Like did you believe it was rotting from the inside, or that something was very wrong with it?
Did you ever feel convinced that the world was coming to an end? How often did you think about ___?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Suspected or likely delusional
3	3	Threshold: Definite present

4) PANIC DISORDER

(I) Panic Attacks

Do you know what a panic attack is? It is when all of a sudden you feel like you cant breath, you get pain in your chest, start sweating or get really cold and dizzy and think that you might faint or die.

The first time you had an attack like this, what did you think brought it on? Did the feeling come from out of the blue?
What was it like?
How long did it last?
After the first time this happened, did you worry a lot about it happening again?
How many times has it happened?
How did the panic attack effect you?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: At least 1 unanticipated attack, and a minimum of 4 attacks. No persistent worry about future attacks, and no effect on behavior related to the attacks.
3	3	Threshold: At least 4 attacks with persistent worry for at least one month about having another attack or significant change in behavior related to the attacks.

5) SEPERATION ANXIETY DISORDER

(I) Fears Calamitous Event That Will Cause Separation

Did you ever worry that something bad might happen to you where you would never see your parents again? Like getting lost, kidnapped, killed, or getting into an accident?
How much do you worry about this?

C	P	
0	0	No information
1	1	Not at all
2	2	Subthreshold: Occasionally worries. Worries more severely and more often than a typical child his/her age.
3	3	Threshold: Frequently worries in separation situations.

(II) Fears Harm Befalling Attachment Figure

Has there ever been a time when you worried about something bad happening to your parents? Like what? Were you afraid of them being in an accident or getting killed? Were you afraid that they would leave you and not come back?
How much did you worry about this?

C	P	
0	0	No information
1	1	Not at all
2	2	Subthreshold: Occasionally. Worries more severely and more often than a typical child
3	3	Threshold: Frequently worries in separation situations.

(III) School Reluctance/Refusal

Was there ever a time when you had to be forced to go to school? Did you have worries about going to school? Tell me about those feelings.
What were you afraid of? Had you been going to school?
How often were you out from school or did you leave school early?

C	P	
0	0	No information
1	1	Not at all
2	2	Subthreshold: Frequently somewhat resistant about going to school but usually can be persuaded to go, missed no more than 1 day in 2 weeks.
3	3	Threshold: Protests intensely about going to school, or sent home or refuses to go at least 1 day per week.

(IV) Fears Sleeping Away From Home/Sleeping Alone

Has there ever been a time after the age of four, when you were afraid of sleeping alone?
Did you get scary feelings if you had to sleep away from home without your parents being with you?

C	P	
0	0	No information
1	1	Not at all
2	2	Subthreshold: Occasionally fearful. Fears of sleeping away or alone more severe and more frequent than a typical child his/her age.
3	3	Threshold: Frequently fearful, some avoidance of sleeping alone or away from home.

(V) Fears Being Alone at Home

Was there ever a time, after the age of 4, when you used to follow your mother wherever she went? Did you get upset if she was not in the same room with you? Did you cling to your mother?
Did you check up on your mother a lot? Did you always want to know where your mother was?
How much were you afraid?

C	P	
0	0	No information
1	1	Not at all
2	2	Subthreshold: Occasionally fearful. Fears of being alone more severe and more frequent than a typical child his/her age.
3	3	Threshold: Clings to mother; fearful, some avoidance of being alone.

6) SOCIAL PHOBIA

(I) Shrinks from Contact

A lot of children are shy. Some children are beyond that, and never warm up or feel comfortable with people outside the family. Were you ever like that? Did you always feel very uncomfortable or nervous around your teacher or the other kids at school? How about the kids in your neighbourhood?

Some kids feel very shy around people they don't know. They feel as if they just can't say anything. Were you ever like that? How long would it usually take you to warm up?

Was it hard for you to talk to a person you didn't know, even if it was another kid? Did you get so scared that you couldn't say a single word? Was this true of you most of the time?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasional discomfort around nonfamily members and/or strangers. More severe and more often than a typical child his/her age, minimal, if any, impairment.
3	3	Threshold: Frequently shows significant discomfort around non-family members and/or strangers. Moderate or more severe impairment.

(II) Fear of Social Situations

Some kids really hate to answer questions in class, talk in front of the class, talk to adults or kids they don't know well, meet new kids, use the bathroom at school if there are other kids around, or eat in front of other kids....(ask about all situations listed).

Have any of these things ever really bothered you?

Much more than other kids in your class? What bothered you about__ (e.g. fear of saying something stupid, fear of looking embarrassed, fear of trembling, choking, etc.)?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasional discomfort in one or more social situations. More than a typical child his/her age. No avoidance.
3	3	Threshold: Frequently experiences significant discomfort in one or more social situations.

(III) Social Involvement with Familiar People	**Criteria**	**C**	**P**
Do you like being with your family and other people you know? How do you and your mum/dad get along? Your brothers? Sisters? *Do you have a best friend, or one or two children you like to spend time with? Do you feel scared or nervous around___? What kind of things do you like to do together?* *Some kids don't really like to be around other people, people they don't know very well, not even other kids. Are you like that? Are there any people you like to be around, or wish you could feel more comfortable around?*	Desires involvement with familiar people	0 1 2	0 1 2
(IV) Duration			
Specify dates:_____	6 months or longer	0 1 2	0 1 2

(V) Impairment	**C**	**P**
a. Socially (with peers):	0 1 2	0 1 2
b. With Family:	0 1 2	0 1 2
c. In School:	0 1 2	0 1 2
d. Severe Anxiety/Crying/Tantrums:	0 1 2	0 1 2
e. Avoidance:	0 1 2	0 1 2
(VI) Evidence of a Precipitant (Specify):	0 1 2	0 1 2

7) AGORAPHOBIA AND SPECIFIC PHOBIAS

Only rate most recent phobia

(I) Distress

Specific Phobias: *Has there ever been a time when you were really scared of something like dogs, horses, heights, needles, elevators, subways, the dark… or any other things?*

Agoraphobia: *What about being in a crowded place or going outside in public alone? Were you ever afraid to go to a shopping center or any other places were there were lots of people?*

How scared did___ make you? Did it make your stomach upset or your heart race? How long did___ last? Are you more scared of than any of___ your friends?

C	**P**	
0	0	No information
1	1	Not present
2	2	Subthreshold: Fear of stimuli or situation more severe than a typical child his/her age. Associated with only mild transient symptoms of distress.
3	3	Threshold: Fear of stimuli or situation associated with moderate to severe symptoms of distress.

(II) Avoidance	**C**	**P**	
Has there ever been a time when your fear of___ kept you from doing anything? Like go to certain places, or see your friends, or even leave the house?	0	0	No information
	1	1	Not present
Did you try to avoid___? Were there times you could___? If someone was with you, could you___?	2	2	Subthreshold: Minimal or inconsistent avoidance.
	3	3	Threshold: Feared stimuli or situation consistently avoided.

Specify most intense phobia :

Specify other phobia :

8) GENERALISED ANXIET DISORER

(I) Unrealistic Worry about Future	**C**	**P**	
Would you describe yourself as a worrier? Do you worry a lot about things that might have happened in the past or that might happen in the future? *What kind of things do you worry about?* *Do you think you worried more than other kids your age?* *Has anyone ever said you were a worrier? Do you know why they said that?* **Note: If the only worries the child brings up relates to the attachment figure or a simple phobia, do not score here. Only rate positively if the child worries about multiple things.**	0	0	No information
	1	1	Not present
	2	2	Subthreshold: Frequently worries somewhat excessively (at least 3 times per week) about anticipated events or current behavior.
	3	3	Threshold: Most days of the week is excessively worried about at least two different life circumstances or anticipated events or current behavior.

(II) Somatic Complaints	**C**	**P**	
Was there ever a time when you got sick a lot? Did you miss school, gym or other activities a lot because you didn't feel well? Was there ever a time when you got aches and pains a lot? Did you get headaches, stomachaches, aches in your legs, backaches? Any other types of problems? Everyday? Once in a while? *When did you get a___? In the morning, evening, weekends? Only on school days?* **Note: Do not count if only related to separation situations or school refusal.**	0	0	No information
	1	1	Not present
	2	2	Subthreshold: Occational symptoms/complaints that are more severe and more often than experienced by a typical child his/her age.
	3	3	Threshold: Frequent symptoms/complaints (more than 1 time per week), somewhat of a problem.

(III) Marked Self-Consciousness	**C**	**P**	
Some people worry a lot about what other people think about them. Is this true of you? Has there ever been a time when you thought about what you were going to say before you said it? Did you worry that other people thought you were stupid or that you did things funny?	0	0	No information
	1	1	Not present
	2	2	Subthreshold: Frequently (at least 3 times per week) feels self-conscious.
	3	3	Threshold: Most days of the week feels self-conscious.

(IV) Marked Feeling of Tension/Unable to Relax

Was there ever a time when you felt "up-tight" or tense a lot? Like you couldn't relax even if you tried?
Did you get so nervous that you couldn't sit still? Did you often feel jumpy or "on edge"?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Frequently nervous/anxious (more than 1 time per week), somewhat of a problem.
3	3	Threshold: Most days of the week is nervous/anxious.

9) OBSESSIVE-COMPULSIVE DISORDER

(I) Compulsions

Has there ever been a time when you found yourself having to do things over and over, or things which you could not resist repeating like touching things, or counting or washing your hands many times, or checking locks or other things?

Were there things you always felt you had to do exactly the same way or in a special way?

Did you ever have trouble making it to school on time because it takes too long to get ready in the morning?

What about when you went to sleep, did you have to check something several times before you fell asleep? Or did you have to arrange things in your room in a particular way? Have other people ever commented about these habits?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Suspected or likely.
3	3	Threshold: Definite compulsions, causes some effect on functioning or distress.

(II) Obsessions

Has there ever been a time when you were bothered by thoughts, "pictures" or words which kept coming into your head for no reason and that you couldn't stop or get rid of?

Like did you ever worry a lot about having germs on your hands, or worry that you might get ill from germs? Did you ever worry about doing things perfectly or about making things even or arranging things in a certain way? What about thoughts that something bad might happen, or that you did something terrible, even though you knew it wasn't true?

Any other types of thoughts that kept running around your mind? What about numbers that wouldn't go away?
Do these thoughts get in your way or stop you from doing things?

Note: Do not score obsessions item positively if ideas/thoughts are delusional, or relate to another Axis I disorder.

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Suspected or likely.
3	3	Threshold: Definite obsessions, causes some effect on functioning or distress.

10) ENURISIS

A lot of kids sometimes have accidents and wet their beds when they sleep at night. Has there ever been a time when this happened to you? Did you ever have accidents during the day? What about if you laughed or sneezed real hard?

	C	P	
a. Nighttime	0	0	No information
How often did this happen at night?	1	1	Not present
*Specify:*_____	2	2	One to four times in a month for three of more months
b. Daytime	0	0	No information
How often did this happen during the day?	1	1	Not present
*Specify:*_____	2	2	One to four times in a month for three of more months
c. Total	0	0	No information
Estimate frequency of combined nighttime and daytime accidents.	1	1	Not present
Specify:_____	2	2	One to four times in a month for three of more months

Distress

What did you usually do when you had an accident? Did you tell your mom? Your teacher? What did they do? Did the kids at school know you sometimes had accidents? How much did it bother you when you had an accident?

Distress ☐ **YES** ☐ **NO**

Impairment : (Home, school, peers) ☐ **YES** ☐ **NO**

Duration > 3 months ☐ **YES** ☐ **NO**

11) ENCOPRESIS

Repeated Passage of feces

Some kids have accidents and soil their beds when they sleep at night. Did this ever happen to you? Has there ever been a time when you had accidents and went to the bathroom in your pants during the day? What about when you were really scared, or for some reason couldn't get to a bathroom when you needed to? What kinds of accidents were you having? Number one or number two?

	C	P	
a. Nighttime			
How often did this happen at night? Specify:_____	0	0	No information
	1	1	Not present
	2	2	6-11 times a year
	3	3	Threshold: 1 or more times a month
b. Daytime			
How often did this happen during the day?	0	0	No information
	1	1	Not present
	2	2	6-11 times a year
	3	3	Threshold: 1 or more times a month
c. Total			
Estimate frequency of combined nighttime and	0	0	No information
	1	1	Not present
	2	2	6-11 times a year
	3	3	Threshold: 1 or more times a month

Distress

What did you usually do when you had an accident? Did you tell your mom? Your teacher? What did they do? Did the kids at school know you sometimes had accidents? How much did it bother you when you had an accident?

Distress ☐ **YES** ☐ **NO**

Impairment : (Home, school, peers) ☐ **YES** ☐ **NO**

Duration > 3 months ☐ **YES** ☐ **NO**

12) ANOREXIA NERVOSA

(I) Fear of Becoming Obese

Has there ever been a time when you were afraid of becoming really overweight? Did you believe you were fat? Did you watch what you ate and think about what you ate all the time? Were you afraid of eating certain foods because you were afraid they'd make you fat? What foods? How much time did you spend thinking about food and worrying about getting fat? If you saw that you had gained a pound or two, did you change your eating habits? Fast for a day or do anything else?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Intense and persistent fear of becoming fat, which defies prior weight history and/or present weight, reassurance, etc. Fears have only moderate impact on behavior and/or functioning (e.g., weight loss methods utilized at least once a month)
3	3	Threshold: Intense and persistent fear of becoming fat, which has severe impact on behavior and/or functioning (e.g., constantly pre-occupied with weight concerns; or use of weight loss methods)

(II) Emaciation

Have you lost a lot of weight recently? Was there ever a time when you lost a lot of weight? Has anyone ever said that you are too thin, or that you must eat more?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Weight below 90% of ideal.
3	3	Threshold: Weight below 85% of ideal.

13) BULIMIA NERVOSA

(I) Weight Loss Methods

Have you ever used diet pills to control your weight? How about laxatives, or water pills to lose weight? Did you sometimes make yourself throw up? Did you exercise a lot, more than was usual for you, in order to lose weight? How much? How many hours a day? Did you have periods of at least 1 week during which you had nothing but noncaloric fluids (tea, diet sodas, coffee, H_2O)?

Code

0 No information
1 Not present
2 Less than once time a week
3 One or more times a week

	C	P
a. using diet pills	0 1 2 3	0 1 2 3
b. taking laxatives	0 1 2 3	0 1 2 3
c. taking water pills	0 1 2 3	0 1 2 3
d. throwing up	0 1 2 3	0 1 2 3
e. exercising a lot	0 1 2 3	0 1 2 3
f. taking only non-caloric fluids for a week of more	0 1 2 3	0 1 2 3
g. combined frequency weight loss methods	0 1 2 3	0 1 2 3

(II) Eating Binges or Attacks

Has there ever been a time when you had "eating attacks" or binges that lasted for hours, and you ate so much food it hurt? What's the most you ever ate at one time? Have there ever been times you ate so much you felt sick? How often did it happen?

What triggered a binge?
What did you usually eat when you binged?
Did you ever make yourself throw up after a binge?
How did you feel after you binged?
Did you usually binge alone or with other people?
Did other people know you binged?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Eating binges that occur less than once a week.
3	3	Threshold: Eating binges once a week or more.

14) ATTENTION DEFICIT HYPERACTIVE DISORDER

(I) Difficulty Sustaining Attention on Tasks or Play Activities

Has there ever been a time when you had trouble paying attention in school? Did it affect your school work? Did you get into trouble because of this? When you were working on your homework, did your mind wander? What about when you were playing games? Did you forget to go when it was your turn?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasionally has difficulty sustaining attention on tasks or play activities. Problems have only minimum effect on functioning.
3	3	Threshold: Often has difficulty sustaining attention. Problem has moderate to severe effect on functioning.

(II) Easily Distracted

Was there ever a time when little distractions would make it very hard for you to keep your mind on what you were doing? Like if another kid in class asked the teacher a question while the class was working quietly, was it ever hard for you to keep your mind on your work? When there was an interruption, like when the phone rang, was it hard to get back to what you were doing before the interruption? Were there times when you could keep your mind on what you are doing, and little noises and things didn't bother you? How often were they a problem?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasionally forgetful. Problems has only minimal effect on functioning.
3	3	Threshold: Attention often disrupted by minor distractions other kids would be able to ignore. Problems has moderate to severe effect on functioning.

(III) Difficulty Remaining Seated

Was there ever a time when you got out of your seat a lot at school? Did you get into trouble for this? Was it hard to stay in your seat at school? What about dinnertime?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasionally has difficulty remaining seated when required to do so. Problem has only minimal effect on functioning.
3	3	Threshold: Often has difficulty remaining seated when required to do so. Problem has moderate to severe effect on functioning.

(IV) Impulsivity

Do you act before you think, or think before you act? Has there ever been a time when these kinds of behaviors got you into trouble? Give some examples.

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasionally impulsive. Problem has only minimal effect on functioning.
3	3	Threshold: Often impulsive. Problem has moderate to severe effect on functioning.

15) OPPOSITIONAL DEFIENT DISORDER

(I) Loses Temper

Has there ever been a time when you would get upset easily and lose your temper? Did it take much to get you mad? How often did you get really mad or annoyed and lose your temper? What were you like when you had a temper tantrum? What did you do?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occational temper outbursts. Outbursts more severe and more often than a typical child his/her age.
3	3	Threshold: Severe temper outbursts 2-5 times a week

(II) Argues a Lot With Adults

Was there ever a time when you would argue a lot with adults? Your parents or teachers? What kinds of things did you argue with them about? Did you argue with them a lot? How bad did the fights get? Did you get into arguments with them?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasionally argues with parents Arguments more severe and more often than a typical child his/her age.
3	3	Threshold: Often argues with parents and/or teachers. Daily or nearly daily.

(III) Disobeys Rules a Lot

Has there ever been a time when you got into trouble at home or at school for not following the rules? Did you get into trouble with the teachers at school? For what kinds of things? Did your parents get mad at you for not doing your chores or refusing to follow other household rules? How often did this happen? How often did you get away with things without getting into trouble or without getting caught?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasionally actively defies or refuses adult requests or rules (e.g., refuses to do chores at home). Disobedient more often than a typical child his/her age.
3	3	Threshold: Often actively defies or refuses adult requests or rules. Daily or nearly daily.

16) CONDUCT DISORDER

(I) Lies

Has there ever been a time when you told lies to your friends? Your teacher? Parents? Have people ever called you a liar? Why? Tell me about the types of lies you told. What's the worst lie you ever told? Did you lie to get other people to do things for you? Did you lie to get out of paying people back money or some favor you owe them? Has anyone ever called you a con? Complained that you broke promises a lot? How often did you lie?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasionally lies. Lies more often than a typical child his/her age.
3	3	Threshold: Lies often, multiple times per week or more.

(II) Truant

Has there ever been a time when you skived off school and missed a whole day? Where did you go? Did you ever go to school and leave early when you were not really supposed to? How about going in late? Did you sometimes miss a couple of classes in the morning? How often?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Truant on one isolated incident.
3	3	Threshold: Truant on numerous occasions (e.g. 2 or more times.)

(III) Initiates Physical fights

Has there ever been a time when you got into many fist fights? Who usually started the fights? What's the worst fight you ever got into? What happened? Did anyone get hurt? Who did you usually fight with? Have you ever hit a teacher? One of your parents? Another adult? How often did you fight?
Have you ever tried or wanted to kill someone?
Are you or any of your friends in a gang?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Fights with peers only. No fight has resulted in serious injury to peer
3	3	Threshold: Reports engaging in multiple fights, with one or more fights resulting in serious injury to a peer. Or physical fights involving an adult.

(IV) Bullies, Threatens, or intimidate others

Has there ever been a time when any kids really got on your nerves? Did you sometimes do things to get back at them?
Like what? Call them names? Threaten to beat them up? Push them? Trip them? Knock their books out of their hands? Come up from behind and slap them in the face? How often did you do these things?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Bullied, threatened, or intimidated another on only one or two occasions.
3	3	Threshold: Bullied, threatened, or intimidated another on three or more occations

(V) Nonaggressive stealing

In the past year, have you stolen anything? What is the most expensive thing you stole? What other things have you stolen? From whom? From which stores? Have you stolen a toy from a store? Money from your mom? Anything else? How often have you stolen things?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Has stolen without confrontation of victim on only one occasion.
3	3	Threshold: Has stolen without confrontation of victim on 2 or more occations

17) TIC DISORDER

(I) Motor Tics

Has there ever been a time when you noticed your muscles moved in a way that you did not want them to, or that you didn't expect? Like raising your eyebrows (demonstrate), blinking a whole lot (demonstrate), scrunching up your nose (demonstrate), shrugging your shoulders (demonstrate), or moving your head like this (demonstrate)? Ever blink a whole lot or real hard and not be able to stop?
About how often did this happen?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Specific tic behaviors occur infrequently, not on a daily basis. If bouts of tics occur, they are brief and uncommon.
3	3	Threshold: Specific tic behaviors are present on a daily basis.

(II) Phonic Tics

Has there ever been a time when you made noises that you didn't want to make, repeated sounds or words that you don't want to say? Like sniffing, coughing, or clearing your throat when you didn't have a cold? Making animal sounds or grunting sounds, or even repeating things that you or other people said?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Specific tic behaviors occur infrequently, not on a daily basis. If bouts of tics occur, they are brief and uncommon.
3	3	Threshold: Specific tic behaviors are present on a daily basis.

18) AUTISM SPECTRUM DISORDER

(I) Stereotyped or repetitive speech, motor movements, or use of objects

Do you like to watch your hands while you wiggle your fingers?
Does rocking back and forth calm you when you are upset?
Do people ever tell you to stay still and stop spinning?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: A few isolated incidents, rarely observed.
3	3	Threshold: Occational or more frequent occurences.

(II) Insistence on sameness, Inflexible adherence to routines, Ritualizedf patterns of verbal or nonverbal behavior

Do you get really upset when there is an unexpected change in your plans or the way you usually do things, like if there is a delay in the start of school, if dinner is a little earlier than usual, or if you have to drive home a different way than usual?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Only mildly inflexible, or inflexibility not evident in early childhood.
3	3	Threshold: Significant and persistent rigid adherence to routines and rituals that elicit distress when interrupted. Pattern of behavior evident since early childhood.

(III) Highly restricted, fixated interests that are abnormal in intensity or focus

Is there something special you are interested in that you really like to talk about, read about, or do? Tell me about it.

NOTE: RATE THIS AS POSITIVE IF IT IS INAPPROPRIATE FOR THE AGE AND CULTURE OF THE CHILD, AND IT IS EXAGGERATED. DO NOT SCORE PREOCCUPATION WITH VIDEOGAMES OR COMPUTER GAMES HERE.

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Unusual preoccupations that do not cause significant impairment or take excessive amounts of time.
3	3	Threshold: Definitely preoccupied with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus. Causes significant impairment in social functioning or limits participation in activities

(IV) Deficits in nonverbal communicative behaviors used for social interaction	C	P	
	0	0	No information
Eye to Eye Gaze: Do you frequently have to remind your child to look at you or the person s/he is talking to?	1	1	Not present
Facial Expressions: Does your child show the typical range of facial expressions? *Can you see joy on his/her face when /she is happy? Does s/he pout when s/he is sad?*	2	2	Subthreshold: Subtle problems in one or more area, which is evident to family members and professionals but not to teachers or classmates.
Does s/he show less common facial expressions like surprise, interest, and guilt? *Gestures: As a toddler or preschooler, did your child use common gestures like pointing to show interest, clapping when happy, and nodding to indicate 'yes'? Does he /she use gestures to help show how something works or while they are explaining something?* *Indicate problematic areas of non-verbal behavior: Gaze Expressions Gestures*	3	3	Threshold: Problems with one or more aspects of non-verbal behaviors cause functional impairment.

19) CIGARETTE/TOBACKO USE **(Code: 0= No information; 1=NO; 2=Yes)**

(I) Use 0 1 2

a. Ever smoked

b. Ever chewed tobacco 0 1 2

(II) Quantity of Cigarette Use ___ ___

a. Current use (cigarettes/day)

b. Greatest amount of Use (cigarettes/day) ___ ___

Age:_____

(III) Age of first regular use (1 cigarette a day or more)

(VI) Ever attempt to quit 0 1 2

(V) Ever quit 0 1 2

(VI) If Yes, code longest ___ ___

20) ALCOHOL ABUSE (0= No Information; 1= No; 2= Yes)

Do you drink alcohol? How much alcohol do you drink in a typical week? What do you drink – beer, wine or spirits? How often? Do you get drunk? Has using alcohol ever caused you to have any health problems (physical/psychological)? Have you ever had to go to a doctor or hospital because of drinking too much? Have you ever missed school/work because you've been too hungover or drunk?

Use

a. Age of first regular use: _____

b. Drank two drinks in one week four or more times 0 1 2

ABUSE

1. Quantity	C	P	
What's the most you ever drank in a single day? When was that? How about in the last six months, what's the most you drank in a day?	0	0	No information
	1	1	1 - 2 drinks
	2	2	3 or more drinks

2. Frequency	**C**	**P**	
What's the most number of days in a given week that you had something to drink? Do you usually drink Friday and Saturday night? Midweek too?	0	0	No information
	1	1	1 - 2 days
	2	2	3 or more days

3. Concern from Others about Drinking	**C**	**P**	
Has anyone ever complained about your drinking? Friends? Parents? Teachers? Have you ever been worried about it at all?	0	0	No information
	1	1	No
	2	2	Yes

21) SUBSTANCE USE (0= No Information; 1= No; 2= Yes)

Prior to beginning this section, give the subject the list of drugs included in the back of this interview packet. Remind child about the confidential nature of the interview prior to beginning probes (if appropriate).

(I). Drug Use

Let me know if you have used any of the drugs on this list before, even if you have only tried them once. Which ones have you used?

	C	**P**
a. Cannabis (Marijuana, pot, hash, THC)	0 1 2	0 1 2
b. Stimulants (Speed, uppers, amphetamines, dexedrine, diet pills, crystal meth)	0 1 2	0 1 2
c. Sedatives/Hypnotics/Anxiolytics (Barbiturates (sedatives, downers), Benzodiazepine, Quaalude (ludes), valium, librium, Xanax)	0 1 2	0 1 2
d. Cocain (Coke, crack)	0 1 2	0 1 2
e. Opioids (Heroin, morphine, codein, methadone, Demerol, percodam)	0 1 2	0 1 2
f. PCP (Angel dust)	0 1 2	0 1 2
g. Hallucinegons (Psychedelics, LSD, mescaline, peyote)	0 1 2	0 1 2
h. Solvents/Inhalants (Glue, gasoline, chloroform, ether, paint)	0 1 2	0 1 2
i. Other (Prescription drugs, nitrous oxide, ecstasy, MDA, etc.)	0 1 2	0 1 2

SUBSTANCE ABUSE

(II) Frequency

In the past six months, what is the most you have ever used___? Everyday or almost every day for at least one week? Less? More? Was there a time when you used___ more?

Code: 0 = No information 1 = Not present 2 = Less than once a month 3 = More than once a month

	C	P
a. Cannabis	0 1 2 3	0 1 2 3
b. Stimulants	0 1 2 3	0 1 2 3
c. Sedatives/Hypnotics/Anxiolytics	0 1 2 3	0 1 2 3
d. Cocain	0 1 2 3	0 1 2 3
e. Opioids	0 1 2 3	0 1 2 3
f. PCP	0 1 2 3	0 1 2 3
g. Hallucinegons	0 1 2 3	0 1 2 3
h. Solvents/Inhalants	0 1 2 3	0 1 2 3
i. Other	0 1 2 3	0 1 2 3
j. Polysubstance	0 1 2 3	0 1 2 3

22) POST-TRAUMATIC STRESS DISORDER

(I) Traumatic Event

Probe: *I am going to ask you about a number of bad things that often happen to children your age, and I want you to tell me if any of these things have ever happened to you. Be sure to tell me if any of these things have ever happened, even if they only happened one time.*

a. Car Accident	0 1 2	g. Confronted with Traumatic News	0 1 2
b. Other Accident	0 1 2	h. Witness to Domestic Violence	0 1 2
c. Fire	0 1 2	i. Physical Abuse	0 1 2
d. Witness of a Disaster	0 1 2	j. Sexual Abuse	0 1 2
e. Witness of a Violent Crime	0 1 2	k. Other	0 1 2
f. Victim of Violent Crime	0 1 2	Please Specify_____	0 1 2

(II) Screen Items

1. Recurrent Thoughts or images of the Event *Has there ever been a time when you kept seeing again and again? How often did this happen? Did what happen keep coming into your mind? Did you think about it a lot?*	0 1 2
2. Efforts to Avoid Thoughts or Feelings Associated with the Trauma *What kind of things do you do or have you done to keep from thinking about___?* *To get rid of bad thoughts, some kids, read, do things to keep busy, or go to sleep. Did you ever do any of these things or other things to get rid of those bad thoughts and/or feelings?*	0 1 2
3. Nightmares *Has there ever been a time when you had a lot of nightmares? Did you ever dream about___? How often? How did you feel when you woke up from one of your nightmares?*	0 1 2
4. Insomnia *After___ happened, did you have trouble falling or staying asleep? How long did it take you to fall asleep? Did you wake up in the middle of the night?*	0 1 2
5. Irritability or Outburst of Anger? *After___ happened, did you feel cranky or grouchy a lot? Were you having a lot of temper tantrums?*	0 1 2

Appendix 3 Conduct disorder supplement

CD SUPPLEMENT

(1) Vandalism

Do you ever break other people's things on purpose? Like breaking windows? Smashing cars? Anything else? What's the most expensive thing you ever broke, damaged, or destroyed on purpose? How about when you are feeling really angry? About how often do you break or destroy other people's things on purpose?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Minor acts of vandalism on 1 or 2 occasions (e.g., breaks another's toy on purpose).
3	3	Threshold: Three or more instances of moderate to severe vandalism

(2) Breaking and entering

In the past six months, have you or any of your friends broken into any cars? Houses? Any stores? Warehouses? Other buildings? About how many times have you broken into a house, car, store, or other building?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Has been with friends who broke into a house, car, store, or building, but did not actively participate.
3	3	Threshold: Has broken into a house, car, store, or building 1 or more times.

(3) Aggressive Stealing

In the past six months, have you or any of your friends held anyone up to try and get their money or something else? Snatched their purse or mobile? Threatened them? How often?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Has been with friends who aggressively stole, but did not actively participate.
3	3	Threshold: Mugging, purse-snatching, extortion, armed robbery, etc. on 1 or more occasions

(4) Fire Setting

Have you ever set anything on fire?
Why did you start the fire? Were you playing with matches and did you start the fire by accident, or did you start it on purpose? Were you angry? Were you trying to cause a lot of damage or to get back at someone? What's the most damage you ever caused by starting a fire? About how many fires have you set?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Match play. No intent to cause damage, and fire(s) not started out of anger.
3	3	Threshold: Set 1 or more fires with the intent to cause damage, or out of anger.

(5) Often Stays out at Night

What time are you supposed to come home at night? Do you often stay out past your curfew?
What is the latest you ever stayed out? Have you ever stayed out all night? How many times have you done that?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Stayed out all night, or almost all night, on one isolated occasion.
3	3	Threshold: Stayed out all night, or almost all nights, on several occasions (2 or more times)

(6) Run Away Overnight

Over the past six months, have you run away from home? Why? Was there something going on at home that you were trying to get away from?
How long did you stay away? How many times did you do this?

Note: Do not score positively if child ran away to avoid physical or sexual abuse.

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Ran away overnight only one time, or ran away for shorter periods of time on several occasions.
3	3	Threshold: Ran away for at least two nights or more on one or more occasions, or ran away overnight 2 or more times.

(7) Use of a Weapon

Do you carry a knife or a gun? A numb-chuck? Have you ever used a weapon against someone else, including using bricks, broken bottles, or other things you might pick up from the street? What about in self-defense?
Have you ever threatened to use a weapon to get someone to back off?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Has threatened to use a weapon, but has never used one.
3	3	Threshold: Used a weapon that can cause serious harm on 1 or more occasions (e.g. knife, brick, broken bottle, gun).

(8) Physical Cruelty to Persons

What is the worst you ever laid into someone in a fight? Have you ever beat someone up really badly for no real reason, or just because you don't like them? What happened? Did they get hurt? Have you ever put someone in hospital?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Bullies others (e.g. pushes, intimidates others), but has never bruised anyone, or caused a more serious injury.
3	3	Threshold: Bullying or physical cruelty to others has led to moderate to severe injury (e.g. Bruises, laceration).

(V) Suicidal Ideation

Sometimes children who get upset or feel bad think about dying or even killing themselves. Have you ever had such thoughts?
How would you do it?
Did you have a plan?

C	P	
		No information
0	0	Not at all
2	2	Subthreshold: Occasional thoughts of suicide but has not thought of a specific method.
3	3	Threshold: Often thinks of suicide and has thought of a specific method.

(VI) Suicidal Acts- Seriousness

Have you actually tried to kill yourself?
When? What did you do?
Any other things?
Did you really want to die
How close did you come to doing it Was anybody in the room?
In the apartment?
Did you tell them in advance? How were you found?
Did you really want to die?
Did you ask for any help after you did it?

C	P	
0	0	No information
1	1	No attempt or gesture with no intent to die (eg., held pills in hand).
2	2	Subthreshold: Present, but very ambivalent.
3	3	Threshold: Definite suicidal intent.

(VII) Suicidal Acts- Medical Lethality

How close were you to dying after your (most serious suicidal act)?
What did you do when you tried to kill yourself?
What happened to you after you tried to kill yourself?

C	P	
0	0	No information
1	1	No attempt or gesture with no intent to die (e.g., held pills in hand).
2	2	Subthreshold: e.g., took 10 aspirins, mild gastritis.
3	3	Threshold: e.g., took 10 seconal, had brief unconsciousness.

(VIII) Non-Suicidal Physical Self-Damaging Acts

Did you ever try to hurt yourself?
Have you ever burned yourself with matches/candles?
Or scratched yourself with needles/ a knife? Your nails?
Or put hot pennies on your skin?
Anything else? Why did you do it? How often?
Do you have many accidents? What kind? How often?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Infrequent (1-3 times a year). Has never caused serious injury to self.
3	3	Threshold: Frequent (4 or more times a year) or has caused serious injury to self (e.g. burn with scarring; broken bone).

(15) Undifferentiated type *Did you do some of the things we talked about with your friends and others on your own?*	0 1 2
(18) Evidence of Conduct Disorder	
DSM-IV Criteria	
A. Meets criteria for at least three of the following 15 conduct symptoms in the past 12 months, with at least one criterion present in the past 6 months: *Lies, truant, physical fights, bullies, often stays out at night, nonaggressive stealing, vandalism, breaking and entering, aggressive stealing, fire-setting, ran away overnight, use of a weapon, physical cruelty to persons, forced sexual activity, cruelty to animals.*	0 1 2
B. Behavior causes clinically significant impairment; and, C. If 18 or older, does not meet criteria for antisocial personality disorder.	0 1 2
Childhood-Onset Type Onset of at least one criterion prior to the age of 10 years.	0 1 2
Adolescent-Onset Type Absence of any criteria prior to age 10 years.	

Appendix 4 Oppositional defiance disorder supplement

ODD SUPPLEMENT

(1) Easily Annoyed or Angered

Do people annoy you and get on your nerves a lot? What kinds of things set you off? Do you get really annoyed when your parents tell you that you can't do something you want to? Like what? What other things really get on your nerves? What do you do when you are feeling annoyed or angry? How often does this happen?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Easily annoyed or angered on occasion. Annoyed more often than a typical child his/her age (1 - 3 times a week).
3	3	Threshold: Easily annoyed or angered daily or almost daily.

(2) Angry or Resentful

Do you get angry or irritable with your parents a lot? How about with your teachers? Your brothers, sisters or friends? Do other people tell you that you get angry or worked up a lot? Who says this? How often does it happen?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally angry or resentful. Angry more often than a typical child his/her age (1 - 3 times a week).
3	3	Threshold: Angry or resentful daily or almost daily.

(3*) Spiteful and Vindictive*

When someone does something unfair to you, do you try to get back at them? Give me some examples? What if your brother or a friend did something to get you into trouble or make you mad? Would you do something back to them? Has this happened before? How often? Are there times when people do something to you and you let it slide? Does this happen a lot?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Spiteful and/or vindictive on occasion. Spiteful more often than a typical child his/her age (1-3 times a week).
3	3	Threshold: Spiteful and/or vindictive daily or almost daily.

(4) Uses Bad Language

Do you use swear words a lot? Do your parents or teachers ever complain about your language? How often do you swear in front of people?

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Occasionally. Curses more often than a typical child his/her age.
3	3	Threshold: Curses excessively daily or almost daily.

(5) Annoys People on Purpose

When your mum asks you to do something, do you usually do it? Like if she asks you to put away a game, do you keep on playing and pretending you didn't hear her? Do people say you do things on purpose to annoy them or wind them up? Your parents? Teachers? Brothers? What kinds of things do they complain about? Do you think that it's true?

Do not score teasing of a sibling.

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: On one or two occasions has deliberately done things to annoy other people.
3	3	Threshold: On multiple occasions has deliberately done things to annoy other people.

(6) Blames Others for Own Mistakes

When you get into trouble, how easy is it for you to take responsibility for what you've done? Is it usually your fault or someone else? How often do you own up to what you've done? Do you think most of your troubles are caused by other people or are they your own fault?

C	P	
0	0	No information
1	1	Not present.
2	2	Subthreshold: On occasion blames others for own mistakes. Denial of responsibility more often than a typical child his/her age.

(7) Duration

How long have you had problems with your temper or following the rules (or other symptoms?)

6 months or more

0 1 2

(8) Impairment

a. Socially (With peers) 0 1 2

b. With family: 0 1 2

c. In school 0 1 2

(9) Evidence of a Precipitant (Specify)

0 1 2

_____

(10) Evidence of Oppositional Defiant Disorder

DSM-IV Criteria	
1. To obtain a diagnosis of Oppositional Defiant Disorder (ODD), children must meet criteria for four of the 8 symptoms (e.g. loses temper, argues a lot with adults, disobey rules, easily annoyed or angered, angry or resentful, spiteful or vindictive, annoys people on purpose, blame others for own mistake). In addition, there must be evidence of functional impairment. 2. Duration of symptoms is 6 months or longer 3. Does not meet criteria for CD, and oppositional symptoms do not occur exclusively during the course of a psychotic disorder, Dysthymia, MDD, Hypomania or Manic episode.	0 1 2

Appendix 5 Attention-deficit/hyperactive disorder supplement

ADHD SUPPLEMENT

(1) Makes a lot of Careless Mistakes

Do you make a lot of careless mistakes at school? Do you often get problems wrong on tests because you didn't read the instructions right? Do you often leave some questions blank by accident? Forget to do the problems on both sides of a handout? How often do these types of things happen? Has your teacher ever said you should pay more attention to detail?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occationally makes careless mistakes. Problems has only minimal effect on functioning.
3	3	Threshold: Often makes careless mistakes. Problems has moderate to severe effect on functioning.

(2) Doesn't Listen

Is it hard for you to remember what your parents and teachers say? Do your parents or teachers complain that you don't listen to them when they talk to you? Do you "tune people out"? Do you get into trouble for not listening?

Rate based on data reported by informant or observational data.

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occationally doesn't listen. Problems have minimal effect on functioning.
3	3	Threshold: Often doesn't listen. Problems has moderate effect on functioning.

(3) Difficulty Following Instructions

Do your teachers complain that you don't follow instructions? When your parents or your teacher tell you to do something, is it sometimes hard to remember what they said to do? Does it get you into trouble? Do you lose points on your assignments for not following directions or not completing the work? Do you forget to do your homework or forget to turn it in? Do you get into trouble at home for not finishing your chores or other things your parents ask you to do? How often?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally has difficulty following instructions. Problem has only minimal effect on functioning.
3	3	Threshold: Often has difficulty following instructions. Problems have moderate effect on functioning.

(4) Difficulty Organizing Tasks

Is your desk or locker at school a mess? Does it make it hard for you to find the things you need? Does your teacher complain that your assignments are messy or disorganized? When you do your worksheets, do you usually start at the beginning and do all the problems in order, or do you like to skip around? Do you often miss problems? Do you have a hard time getting ready for school in the morning?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally disorganised. Problem has only minimal effect on functioning.
3	3	Threshold: Often has disorganised. Problem has moderate effect on functioning.

(5) Dislikes/Avoids Tasks Requiring Attention

Are there some kinds of school work you hate doing more than others? Which ones? Why? Do you try to get out of doing your assignments?
Do you pretend to forget about your homework to get out of doing it? About how many times a week do you not do your homework?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally avoids tasks that require sustained attention, and/or expresses mild dislike for those tasks. Problem has only minimal effect on functioning.
3	3	Threshold: Often avoids tasks that require sustained attention and/or expresses moderate dislike for these tasks.. Problem has moderate to severe effect on functioning.

(6) Loses Things

Do you lose things a lot? Your pencils at school? Homework assignments? Things around home? About how often does this happen?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally loses things. Problem has only minimal effect on functioning.
3	3	Threshold: Often loses things (e.g. 1 a week or more). Problem has moderate to severe effect on functioning.

(7) Forgetful in Daily Activities

Do you often leave your homework at home, or your books or coats on the bus? Do you leave your things outside by accident? How often do these things happen? Has anyone ever complained that you are too forgetful?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally forgetful. Problem has only minimal effect on functioning.
3	3	Threshold: Often forgetful. Problem has moderate to severe effect on functioning.

(8) Fidget

Do people often tell you to sit still, to stop moving, or stop squirming in your seat? Your teachers? Parents? Do you sometimes get into trouble for squirming in your seat or playing with little things at your desk? Do you have a hard time keeping your arms and legs still? How often?

Rate based on data reported by informant or observational data.

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally fidgets with hands or feet or squirms in seat. Problem causes only minimal effect on functioning.
3	3	Threshold: Often fidgets with hands or feet or squirms in seat

(9) Runs or Climbs Excessively

Do you get into trouble for running down the hall in school? Does your mom often have to remind you to walk instead of run when you are out together? Do your parents or your teacher complain about you climbing things you shouldn't? What kinds of things? How often does this happen?
Adolescents: *Do you feel restless a lot? Feel like you have to move around, or that it is very hard to stay in one place?*

Rate based on data reported by informant or observational data.

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally runs about or climbs excessively. Problem has only minimal effect on functioning (In adolescents, may be limited to a subjective feeling of restlessness).
3	3	Threshold: Often runs about or climbs excessively Problem has moderate to severe effect on functioning.(In adolescents, may be limited to a subjective feeling of restlessness).

(10) On the Go/Acts Like a Driven Motor

Is it hard for you to slow down? Can you stay in one place for long, or are you always on the go? How long can you sit and watch TV or play a game? Do people tell you to slow down a lot?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally, minimal effect on functioning.
3	3	Threshold: Often acts as if "driven by a motor". Moderate to severe effect on functioning.

(11) Difficulty Playing Quietly

Do your parents or teachers often tell you to quiet down when you are playing? Do you have a hard time playing quietly?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally, has difficulty playing quietly. Problem has only minimal effect on functioning.
3	3	Threshold: Often has difficulty playing quietly. Problem has moderate to severe effect on functioning.

(12) Blurts Out Answers

At school, do you sometimes call out the answers before you are called on? Do you talk out of turn at home? Answer questions your parents ask your siblings? How often?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally, talks out of turn. Problem has only minimal effect on functioning.
3	3	Threshold: Often talks out of turn. Problem has moderate to severe effect on functioning.

(13) Difficulty Waiting Turn

Is it hard for you to wait your turn in games? What about in line in the cafeteria or at the water fountain?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally has difficulty waiting his/her turn. Problem has only minimal effect on functioning.
3	3	Threshold: Often has difficulty waiting his/her turn. Problem has moderate to severe effect on functioning.

(14) Interrupts or Intrudes

Do you get into trouble for talking out of turn in school? Do your parents, teachers, or any of the kids you know complain that you cut them off when they are talking? Do kids complain that you break in on games? Does this happen a lot?

Rate based on data reported by informant or observational data.

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally interrupts others.
3	3	Threshold: Often interrupt others.

(15) Shifts Activities

When you are playing or doing one thing, do you often stop what you are doing because you think of something else you'd rather do? Do you have trouble sticking with one activity? (Survey multiple items; e.g., setting the table, other chores, schoolwork, video games) Have other people said you do? Your teacher? Your mom?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally shifts tasks and does not finish activities.
3	3	Threshold: Often shifts tasks and does not finish activities.

(16) Talks Excessively

Do people say you talk too much? *Do you get into trouble at school for talking when you are not supposed to? Do people in your family complain that you talk too much?*

Rate based on data reported by informant or observational data.

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally talks excessively.
3	3	Threshold: Often talks excessively.

(17) Engages in Physically Dangerous Activities

Do you sometimes run out in the street without looking? Forget to check for traffic when you ride your bike? Do other things that your parents think are dangerous, like jump from tall heights? Often?
Has anyone ever said you were a dare devil? How come?

C	P	
0	0	No information.
1	1	Not present.
2	2	Subthreshold: Occasionally engages in activities that are physically dangerous.
3	3	Threshold: Often engages in activities that are physically dangerous.

(18) Duration of Disturbance

For how long have you had trouble (list symptoms that were positively endorsed)? 0 1 2

6 months or more 0 1 2

(19) Age of Onset

How old were you when you first started having trouble (list symptoms)? Did you have these problems in kindergarten? First grade? 0 1 2

Onset before age 7 0 1 2

Specify: _____

(20) Impairment

a. Socially (With peers) 0 1 2

b. With family: 0 1 2

c. In school 0 1 2

(21) Evidence of ADHD

DSM-IV Criteria A. Either I or ii: Inattention: i) Meet criteria for at least six of the following nine symptoms: 1. Makes a lot of Careless Mistakes 2) Difficulty Sustaining Attention on Tasks or Play Activities 3) Doesn't Listen 4) Difficulty Following Instructions 5) Difficulty Organizing Tasks 6) Dislikes/Avoids Tasks Requiring Attention 7) Loses Things 8) Easily Distracted 9) Forgetful in Daily Activities or OR Hyperactivity/Impulsivity ii. Meets Criteria for at least six or more of the following nine symptoms: 1) Fidget 2) Difficulty Remaining Seated 3) Runs or Climbs Excessively 4) Difficulty Playing Quietly 5) On the go/Acts as if Driven by a Motor 6) Talks Excessively 7) Blurts Out Answers 8) Difficulty Waiting Turn 9) Often interrupts or intrudes B. Suration of symptoms 6 months or longer; C. Some symptoms that caused impairment present before the age of 7; D. Some impairment from symptoms must be present in two or more situations (e.g. school and home)	0 1 2
(22) Predominantly Inattentive Type Meets criterion Ai, but not criterion Aii for past six months.	0 1 2
(23) Predominantly Hyperactive-Impulsive Type Meets criterion Aii, but not criterion Ai for past six months.	0 1 2
(24) Combined Type Both criterion Ai and Aii are met for past six months.	0 1 2
(25) Attention-Deficit Hyperactivity Disorder Not Otherwise Specified Prominent symptoms of inattention or hyperactivity - impulsivity that do not meet criteria for Attention Deficit/Hyperactivity Disorder.	0 1 2

Appendix 6 Medical history interview

Medical History

(Mandatory)

This information needs to be obtained from any individual (several sources of information may be included) who knows about the medical history of the studied child/adolescent/adult. It should be obtained by a classical medical history interview resulting in a best estimate clinical judgment. An experienced clinical psychologist or medical doctor should do the interview if possible.

Date of birth: __.__.____ Gender: ☐ female ☐ male

Study-ID: __-_____

1. PREGNANCY

Information obtained from

☐ biological mother ☐ other primary caregiver ☐ institution official ☐ studied individual

☐ other: _____ *(please, describe)*

Mother's age at birth _____(years)

Father's age at birth _____(years)

1.1 Diabetes of biological mother during pregnancy

☐ no ☐ yes ☐ not known

1.2 Hypertension of biological mother during pregnancy

☐ no ☐ yes ☐ not known

1.3 Hypothyroidism of biological mother and/or biological mother had to take thyroid hormones during pregnancy (e.g. L-Thyroxine)

☐ no ☐ yes ☐ not known

1.4 Other chronic medical condition of biological mother during pregnancy

☐ no ☐ yes: _____ *(please, describe)* ☐ not known

1.5 Chronic psychiatric condition of biological mother during pregnancy

☐ no ☐ yes: _____ *(please, describe)* ☐ not known

1.6 Regular medication of biological mother during pregnancy

☐ no ☐ yes: _____ *(please, describe)* ☐ not known

1.7 Smoking (any) of biological mother during pregnancy

☐ no ☐ yes ☐ not known

If yes, how many cigarettes per day: _____ ☐ full time ☐ only during part of pregnancy

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1.8 Alcohol use (any) of biological mother during pregnancy

☐ no ☐ yes ☐ not known

If yes, please add, approximately how often: ☐ once ☐ once a month ☐ once a week ☐ every day

1.9 Other drug abuse (any) of biological mother during pregnancy

☐ no ☐ yes ☐ not known

What kind of drug(s): _____ *(please, describe)*

If yes, please add approximately how often: ☐ once ☐ once a month ☐ once a week ☐ every day

1.10 Verbally or physically aggressive and/or violent partner of biological mother during pregnancy

☐ no ☐ yes ☐ not known

1.11 (Im-)Migration under threatening conditions by biological mother during pregnancy (severe physical and/or psychosocial stress e.g. as a political refugee. *Clinical estimate after asking for conditions***)**

☐ no ☐ yes: _____ *(please, describe)* ☐ not known

1.12 Other severe psychosocial stress factors of biological mother during pregnancy *(any subjectively stressful events which are reported by mother)*

☐ no ☐ yes: _____ *(please, describe)* ☐ not known

1.13 Mother and father were a couple for less than 6 months before becoming pregnant

☐ no ☐ yes ☐ not known

1.14 Abortion was considered by the mother or by both parents (e.g. due to social pressure)

☐ no ☐ yes ☐ not known

2. BIRTH HISTORY AND FIRST YEAR OF LIFE *(medical records / personal interview)*

Information obtained from

☐ biological mother ☐ other primary caregiver ☐ institution official ☐ studied individual

☐ other: _____ *(please, describe)*

2.1 Birth-time: ☐ term ☐ preterm (< 37th week) ☐ not known

2.2 Birth weight: _____ g or _____pounds & ounces ☐ not known

2.3 Perinatal problems: ☐ not known ☐ incubator ☐ blue light against hyperbilirubinemia

☐ postnatal infection with stay at hospital ☐ other: _____ *(please, describe)*

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2.4 Attempt to breastfeed?	☐ yes	☐ no	☐ not known
2.5 Successful breastfeeding in early life?	☐ yes	☐ no	☐ not known
2.6 Maternal* psychiatric disorder 0 - 3 months old:	☐ yes	☐ no	☐ not known
2.7 Maternal* psychiatric disorder 4 - 12 months old:	☐ yes	☐ no	☐ not known
2.8 Maternal* psychiatric disorder > 12 months old:	☐ yes	☐ no	☐ not known
2.9 Paternal* psychiatric disorder first year of life:	☐ yes	☐ no	☐ not known

* biological mother / biological father

2.10 Adoption/foster care during first year of life	☐ yes	☐ no	☐ not known
2.11 Number of changes in primary caregiver during 1st year of life (e.g. change of care from mother to grandmother)	☐0 ☐ 1	☐ >2	☐not known
2.12 Inconsistency of early childhood care personnel during 1st year of life (e.g. frequent changes of nannies or nursery personnel)	☐yes	☐no	☐not known
2.13 Single mother/father during first year of life	☐yes	☐no	☐not known
Single mother/father after first year of life	☐yes	☐no	☐not known

3. DEVELOPMENTAL MILESTONES

Information obtained from

☐ biological mother ☐ other primary caregiver ☐ institution official ☐ studied individual

☐ other: _____ *(please, describe)*

3.1 Free Walking: ☐☐ months	☐ <18 months	☐ >= 18 months	☐ not known
3.2 Physio- or psychomotor therapy ever:	☐ yes	☐ no	☐ not known
3.3 First Words: ☐☐ months	☐ <24 months	☐ >= 24 months	☐ not known
3.4 First Sentences: ☐☐ months	☐ <33 months	☐ >= 33 months	☐ not known
3.5 Speech/language therapy ever:	☐ yes	☐ no	☐ not known
3.6 Toilet training day & night completed:	☐ <60 months	☐ >=60 months	☐ not known

4. NURSERY AND KINDERGARDEN

Information obtained from

☐ biological mother ☐ other primary caregiver ☐ institution official ☐ studied individual

☐ other: _____ *(please, describe)*

4.1 Start of nursery/kindergarten: age in _____ [months]

4.2 End of nursery/kindergarten: age in _____ [months]

4.3 How many changes of nursery/kindergarten: ☐ none ☐ 1x ☐ 2x ☐ 3x ☐ >3x ☐ not known

4.4 Child ever expelled from nursery/kindergarten because of behavior? ☐ Yes ☐ no ☐not known

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5. SCHOOL

Information obtained from

☐ biological mother ☐ other primary caregiver ☐ institution official ☐ studied individual

☐ other: _____ *(please, describe)*

5.1 Start of School

☐ regular age (4-5 yr in UK) ☐ early ☐ late ☐ not known

5.2 Repeating of grades

repeated one grade	☐ yes	☐ non	☐ not known
repeated several grades	☐ yes	☐ non	☐ not known
dropped out of school	☐ yes	☐ non	☐ not known
because of	☐somatic problems		☐behavior/learning difficulties

5.3 How many changes of schools due to academic or behavioural problems:

☐ none ☐ 1x ☐ 2x ☐ 3x ☐ >3x ☐ not known

5.4 Schools

☐ primary school	☐ mainstream	☐ special education	☐ not going to school	☐ not known
☐ secondary school	☐ mainstream	☐ special education	☐ not going to school	☐ not known/NA
☐ apprenticeship	☐ regular	☐ special support	☐ no professional training	☐ not known/NA
☐ college	☐ regular	☐ special support	☐ no professional training	☐ not known/NA

5.5 Current school / college / apprenticeship attendance (proportion of required time)

☐ ~100% ☐ > 60% ☐ less than 60% ☐ < 20% ☐ not known/applicable

6. CHRONIC MEDICAL PROBLEMS

Information obtained from:

☐ biological mother ☐ other primary caregiver ☐ institution official ☐ studied individual

☐ other: _____ *(please, describe)*

Chronic medical condition: ☐ yes ☐ no ☐ not known

If yes, please, describe: _____

_____

_____

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7. SOCIAL INFORMATION

Information obtained from

☐ biological mother ☐ other primary caregiver ☐ institution official ☐ studied individual

☐ other: _____ *(please, describe)*

7.1. Child / Adolescent lives with

☐ biological mother
☐ stepmother
☐ adoptive mother
☐ foster mother
☐ grandmother
☐ relative, other responsible female carer
☐ no mother
☐ lives alone
☐ biological father
☐ stepfather
☐ adoptive father
☐ foster father
☐ grandfather
☐ relative, other responsible male carer
☐ no father
☐ no permanent residence/homeless

☐ assisted living / shared flat / care taker: since_____ _____ (month/year)

7.2 Living area: (*If child lives in a welfare institution, provide details to the last living situation*)

Number of persons in household: _____

Number of rooms (without kitchen/bathroom) _____

7.3 Siblings

Number of full siblings: _____
Number of half siblings: _____

7.4 Parents' date of birth

Biological mother's date of birth: __.__.____ biological father's date of birth: __.__.____

☐ not known ☐ not known

7.5 Chronic disharmony between parents/caregiver ☐yes ☐no ☐not known

7.6 Paternal/Maternal social isolation concerning child care (throughout childhood)
☐yes ☐no ☐not known

7.7 Mother/father lived in care/a home before the age of 18/change of care before the age of 18

☐ yes ☐ no ☐ not known

7.8. Serious or repeated minor criminal prosecution mother/father:

☐ yes ☐ no ☐ not known

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7.9 Current employment of parents / primary caregivers:

mother/female caregiver		father/male caregiver
☐	full time worker	☐
☐	regularly part time worker	☐
☐	unemployed (looking for a job)	☐
☐	other (housekeeping, pensioner)	☐

If unemployed, length of current unemployment:

Mother/female caregiver ☐less than 12 months ☐1-2 years ☐3years or longer
Father/male caregiver ☐less than 12 months ☐1-2 years ☐3years or longer

Mother's/female caregiver's profession (description of profession, if employed):

_____

Father's/male caregiver's profession (description of profession, if employed):

_____

7.10 Education of parents / primary caregivers

Mother/female caregiver		father/male caregiver
	School career	
☐	Didn't go to school at all (ISCED 0)	☐
☐	Primary school only (ISCED 1)	☐
☐	School for special education only (ISCED 1)	☐
☐	Secondary school, but no qualification <14 y (ISCED 2A)	☐
☐	Secondary school, O Levels/GCSEs (ISCED 3C)	☐
☐	Traditional apprenticeships/YTS (ISCED 3C)	☐
☐	FE college/vocational training (ISCED 3C)	☐
☐	Secondary school/college, A Levels/Highers (ISCED 3A)	☐
	Professional degree	
☐	no educational degree/qualifications (ISCED 1)	☐
☐	GNVQ or apprenticeship/NVQ Levels1-2 (ISCED 3C)	☐
☐	NVQ Level 3/specialised vocational (ISCED 3A)	☐
☐	Higher Education Diploma (incl. nursing) (ISCED 5B)	☐
☐	Higher National Diploma/Certificate (ISCED 5B)	☐
☐	university degree (BA, BSc or MSc) (ISCED 5A)	☐
☐	doctoral studies (PhD) (ISCED6)	☐
☐	any other degree	☐

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7.11 Ethnic origin

Where were the family members born? (biological parents)

	child	mother	father
Northern Europe (Denmark, Sweden, Norway, Iceland)	☐	☐	☐
North-Eastern Europe (Estonia, Latvia, Lithuania, Finland)	☐	☐	☐
Poland	☐	☐	☐
Belgium, Netherlands, Luxemburg	☐	☐	☐
Germany, Switzerland	☐	☐	☐
Austria, Czech Republic, Slovenia	☐	☐	☐
France	☐	☐	☐
Great Britain (incl. Scotland & Wales)	☐	☐	☐
Ireland	☐	☐	☐
South-Western Europe (Portugal, Spain, Italy)	☐	☐	☐
South-East Europe (Hungary, Slovenia, Croatia, Serbia, Bulgaria, Romania, Bosnia and Herzegovina, Montenegro, Greece)	☐	☐	☐
Russia, Republic of Belarus, Ukraine	☐	☐	☐
Turkey	☐	☐	☐
Israel	☐	☐	☐
Iraq, Iran, Syria, Jordan, Palestine	☐	☐	☐
Arabian Peninsula	☐	☐	☐
North Africa (Egypt, Libya, Tunisia, Algeria, Morocco)	☐	☐	☐
West Africa (Nigeria, Niger, Mali, Benin, Ivory Cost, Senegal)	☐	☐	☐
East Africa (Sudan, Eritrea, Ethiopia, Somalia, Kenya, Tanzania)	☐	☐	☐
Central Africa (Cameroon, Central African Republic, Congo, Angola, Zambia)	☐	☐	☐
South Africa (Namibia, Botswana, Zimbabwe, Mozambique, South Africa…)	☐	☐	☐
India, Sri Lanka, Pakistan, Afghanistan, Bangladesh, Nepal	☐	☐	☐
China	☐	☐	☐
Japan	☐	☐	☐
Other Asian country (e.g. Thailand, Vietnam)	☐	☐	☐
Australia, New Zealand	☐	☐	☐
USA – Caucasian	☐	☐	☐
USA – Hispanic	☐	☐	☐
USA – African-American	☐	☐	☐
USA – Indian	☐	☐	☐
Canada, Greenland	☐	☐	☐
Central America	☐	☐	☐
South America	☐	☐	☐

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7.12. Family language

Official language spoken at home? ☐yes ☐yes, together with another language ☐no

Language spoken at home / living environment (if other than official language):_____

Child's native language (language first learned):_____

If child's first language is not the same as the country's official language, at what age did the child start learning official language:

☐ before 6 years old ☐ 6 to 9 years old ☐ 10 years and older

How long has the child been living in the country of assessment?

☐ since birth ☐ 1-3 years ☐ 4-6 years ☐ 7-11 years of age

☐ longer than 12 years

8. PSYCHIATRIC DISORDERS IN THE FAMILY

Information obtained from

☐ biological mother ☐ other primary caregiver ☐ institution official ☐ studied individual

☐ other: _____ *(please, describe)*

8.1. First degree family member with a history of ADHD?	☐ yes	☐ no	☐ not known
8.2. First degree family member with a history of depression?	☐ yes	☐ no	☐ not known
8.3. First degree family member with a history of anxiety?	☐ yes	☐ no	☐ not known
8.4. First degree family member with a history of PTSD?	☐ yes	☐ no	☐ not known
8.5. First degree family member with alcohol/drug abuse history?	☐ yes	☐ no	☐ not known
8.6. First degree family member with a criminal record/in jail?	☐ yes	☐ no	☐ not known

8.7. Any other psychiatric disorders in first degree family members?

Please describe _____

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Appendix 7 Pubertal Developmental Scale (boys)

PDS– M

ID: _____ Date completed: _____

The questions below are about changes that may be happening to your body. These changes normally happen to different young people at different ages. If you do not understand a question or do not know the answer, just mark "I don't know".

Please read each of the questions carefully and answer them by circling one number for each question:

---

1. Would you say that your **growth in height**:

   1 has not yet begun to spurt (grow more than usual)
   2 has barely started
   3 is definitely underway
   4 seems completed

2. Have you noticed the growth of your **body hair** ("Body hair" means hair any place other than your head, such as under your arms)?
   Would you say that your **body hair growth**:

   1 has not yet started
   2 has barely started
   3 is definitely underway
   4 seems completed

3. Have you noticed any **skin changes**, especially **pimples**? Would you say that **changes on your skin**:

   1 have not yet started
   2 have barely started
   3 are definitely underway
   4 seem completed

4. Have you noticed that a **deepening of your voice**? Would you say that the **deepening of your voice**:

   1 has not yet started
   2 has barely started
   3 is definitely underway
   4 seems completed

5. Have you begun to **grow hair on your face**? Would you say that the **growth of your facial hair**:

   1 has not yet started
   2 has barely started
   3 is definitely underway
   4 seems completed

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Appendix 8 Pubertal Developmental Scale (girls)

PDS – F

ID : _____ Date completed: _____

The questions below are about changes that may be happening to your body. These changes normally happen to different young people at different ages. If you do not understand a question or do not know the answer, please ask.

Please read each of the questions carefully and answer them by circling one number for each question:

---

1. Would you say that your **growth in height**:

   1 has not yet begun to spurt (grow more than usual)
   2 has barely started
   3 is definitely underway
   4 seems completed

2. Have you noticed the growth of your **body hair** ("Body hair" means hair any place other than your head, such as under your arms)?
   Would you say that your **body hair growth**:

   1 has not yet started
   2 has barely started
   3 is definitely underway
   4 seems completed

3. Have you noticed any **skin changes**, especially **pimples**? Would you say that **changes on your skin**:

   1 have not yet started
   2 have barely started
   3 are definitely underway
   4 seem completed

4. Have you noticed that your **breasts have begun to grow**? Would you say that the **growth of your breasts**:

   1 has not yet started
   2 has barely started
   3 is definitely underway
   4 seems completed

5. Have you begun to **menstruate** (started to have your period)?

   1 no
   2 yes **If YES**, how old were you in years and months when you first had a period?
   Years: _____ Months: _____ □ Don't know

Appendix 9 Edinburgh Handedness Inventory

EHI

ID : _____ Date completed: _____

Please indicate your preferences in the use of hands in the following activities by *putting + in the appropriate column*. Where the preference is so strong that you would never try to use the other hand unless absolutely forces to, *put ++*. If any case you are really indifferent put + in both columns. Some of the activities require both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.

Please try to answer all the questions, and only leave the item blank if you have no experience at all of the object or task.

	Left	Right
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking Match (match)		
10. Opening box (lid)		
TOTAL		
i. Which foot do you prefer to kick with?		
ii. Which eye do you use when using only one?		

Appendix 10 Youth Psychopathic Inventory

YPI

Version 3.0

Instructions

This sheet consists of a number of statements that deal with what you think and feel about different things. Read each statement carefully and decide how well the particular statement applies to you. You can choose between four different alternatives on each statement.

Answer each statement as you most often feel and think, not only how you feel right now.

Example:

I like reading books.

Does not apply at all	Does not apply well	Applies fairly well	Applies very well
❑	❑	❑	❑

- Put a mark in the box that corresponds to how you feel.
- Do not think too long on each statement.

REMEMBER:

- **Answer ALL statements.**
- **Do not put a mark between the alternatives.**
- **Only one answer per statement.**

IMPORTANT!!! There are no answers that are ”Right” or ”Wrong”. You cannot score worse or better than anyone else. We are interested in what you think and feel, not in what is ”Right” or ”Wrong”.

	Does not apply at all	Does not apply well	Applies fairly well	Applies very well
1. I like to be where exciting things happen.	❑	❑	❑	❑
2. I usually feel calm when other people are scared.	❑	❑	❑	❑
3. I prefer to spend my money right away rather than save it.	❑	❑	❑	❑
4. I get bored quickly when there is too little change.	❑	❑	❑	❑
5. I have probably skipped school or work more than most other people.	❑	❑	❑	❑
6. It's easy for me to charm and seduce others to get what I want from them.	❑	❑	❑	❑
7. It's fun to make up stories and try to get people to believe them.	❑	❑	❑	❑
8. I have the ability not to feel guilt and regret about things that I think other people would feel guilty about.	❑	❑	❑	❑
9. I consider myself as a pretty impulsive person.	❑	❑	❑	❑
10. I'm better than everyone on almost everything.	❑	❑	❑	❑
11. I can make people believe almost anything.	❑	❑	❑	❑
12. I think that crying is a sign of weakness, even if no one sees you.	❑	❑	❑	❑
13. If I won a lot of money in the lottery I would quit school or work and just do things that are fun.	❑	❑	❑	❑
14. I have the ability to con people by using my charm and smile.	❑	❑	❑	❑
15. I am good at getting people to believe in me when I make something up.	❑	❑	❑	❑
16. I have often been late to work or classes in school.	❑	❑	❑	❑
17. When other people have problems, it is often their own fault, therefore, one should not help them.	❑	❑	❑	❑
18. It often happens that I talk first and think later.	❑	❑	❑	❑
19. I have talents that go far beyond other people's.	❑	❑	❑	❑

	Does not apply at all	Does not apply well	Applies fairly well	Applies very well
20. It's easy for me to manipulate people.	❑	❑	❑	❑
21. I seldom regret things I do, even if other people feel that they are wrong.	❑	❑	❑	❑
22. I like to do things just for the thrill of it.	❑	❑	❑	❑
23. It's important to me not to hurt other people's feelings.	❑	❑	❑	❑
24. Sometimes I lie for no reason, other than because it's fun.	❑	❑	❑	❑
25. To be nervous and worried is a sign of weakness.	❑	❑	❑	❑
26. If I get the chance to do something fun, I do it no matter what I had been doing before.	❑	❑	❑	❑
27. When someone asks me something, I usually have a quick answer that sounds believable, even if I've just made it up.	❑	❑	❑	❑
28. When someone finds out about something that I've done wrong, I feel more angry than guilty.	❑	❑	❑	❑
29. I get bored quickly by doing the same thing over and over.	❑	❑	❑	❑
30. The world would be a better place if I were in charge.	❑	❑	❑	❑
31. To get people to do what I want, I often find it efficient to con them.	❑	❑	❑	❑
32. It often happens that I do things without thinking ahead.	❑	❑	❑	❑
33. Pretty often I act charming and nice, even with people I don't like, in order to get what I want.	❑	❑	❑	❑
34. It has happened several times that I've borrowed something and then lost it.	❑	❑	❑	❑
35. I often become sad or moved by watching sad things on TV or film.	❑	❑	❑	❑
36. What scares others usually doesn't scare me.	❑	❑	❑	❑

	Does not apply at all	Does not apply well	Applies fairly well	Applies very well
37. I'm more important and valuable than other people.	❑	❑	❑	❑
38. When I need to, I use my smile and my charm to use others.	❑	❑	❑	❑
39. I don't understand how people can be touched enough to cry by looking at things on TV or movie.	❑	❑	❑	❑
40. I often don't/didn't have my school or work assignments done on time.	❑	❑	❑	❑
41. I am destined to become a well-known, important and influential person.	❑	❑	❑	❑
42. I like to do exciting and dangerous things, even if it is forbidden or illegal.	❑	❑	❑	❑
43. Sometimes I find myself lying without any particular reason.	❑	❑	❑	❑
44. To feel guilty and remorseful about things you have done that have hurt other people is a sign of weakness.	❑	❑	❑	❑
45. I don't let my feelings affect me as much as other people's feelings seem to affect them.	❑	❑	❑	❑
46. It has happened that I've taken advantage of (used) someone in order to get what I want.	❑	❑	❑	❑
47. I like to spice up and exaggerate when I tell about something.	❑	❑	❑	❑
48. To feel guilt and regret when you have done something wrong is a waste of time.	❑	❑	❑	❑
49. I usually become sad when I see other people crying or being sad.	❑	❑	❑	❑
50. I've often gotten into trouble because I've lied too much.	❑	❑	❑	❑

Appendix 11 Inventory of Callous-Unemotional traits

ICU (YV)

ID: _____ Date completed: _____

Please read each statement and decide how well it describes you. Mark your answer by circling the appropriate number (0-3) for each statement. Do not leave any statement unrated.

		Not at all true	Somewhat true	Very true	Definitely true
1	I express my feelings openly	0	1	2	3
2	What I think is "right" and "wrong" is different from what other people think	0	1	2	3
3	I care about how well I do at school or work	0	1	2	3
4	I do not care who I hurt to get what I want	0	1	2	3
5	I feel bad or guilty when I do something wrong	0	1	2	3
6	I do not show my emotions to others	0	1	2	3
7	I do not care about being on time	0	1	2	3
8	I am concerned about the feelings of others	0	1	2	3
9	I do not care if I get into trouble	0	1	2	3
10	I do not let my feelings control me	0	1	2	3
11	I do not care about doing things well	0	1	2	3
12	I seem very cold and uncaring to others	0	1	2	3
13	I easily admit to being wrong	0	1	2	3
14	It is easy for others to tell how I am feeling	0	1	2	3
15	I always try my best	0	1	2	3
16	I apologise ("say I am sorry") to persons I hurt	0	1	2	3
17	I try not to hurt others' feelings	0	1	2	3

		Not at all true	Somewhat true	Very true	Definitely true
18	I do not feel guilty or remorseful when I do something wrong	0	1	2	3
19	I am very expressive and emotional	0	1	2	3
20	I do not like to put the time into doing things well	0	1	2	3
21	The feelings of others are unimportant to me	0	1	2	3
22	I hide my feelings from others	0	1	2	3
23	I work hard on everything I do	0	1	2	3
24	I do things to make others feel good	0	1	2	3

Appendix 12 Initial MRI screening form

UNIVERSITY OF READING – CENTRE FOR INTEGRATIVE NEUROSCIENCE AND NEURODYNAMICS

INITIAL SCREENING FORM

NAME OF PARTICIPANT …… Sex: M / F

Date of birth…… Approximate weight in kg…… (one stone is about 6.3 kg)

Please read the following questions CAREFULLY and provide answers. For a very small number of individuals, being scanned can endanger comfort, health or even life. The purpose of these questions is to make sure that you are not such a person.

You have the right to withdraw from the screening and subsequent scanning if you find the questions unacceptably intrusive. The information you provide will be treated as strictly confidential and will be held in secure conditions.

	Delete as appropriate
1. Have you been fitted with a pacemaker or artificial heart valve?	YES/NO
2. Have you any aneurysm clips, shunts or stents in your body or a cochlear implant?	YES/NO
3. Have you ever had any metal fragments in your eyes?	YES/NO
4. Have you ever had any metal fragments, e.g. shrapnel in any other part of your body?	YES/NO
5. Do you wear a hearing aid?	YES/NO
6. Have you ever suffered from any heart disease?	YES/NO
7. Do you have any body piercings that you cannot, or are unwilling to, remove?	YES/NO
8. Is there any possibility that you might be pregnant?	YES/NO
9. Have you been sterilised using clips?	YES/NO
10. Have you any surgically implanted metal in any part of your body, other than dental fillings and crowns (e.g. joint replacement or bone reconstruction)	YES/NO
11. Have you ever had any surgery that might have involved metal implants of which you are not aware?	YES/NO
12. Do you wear a denture plate or brace with metal in it?	YES/NO
13. Do you wear transdermal patches that contain metal?	YES/NO
14. Do you have any tattoos or permanent make-up?	YES/NO
15. Have you ever suffered from epilepsy or thermoregulatory problems?	YES/NO
16. Do you have a contraceptive coil (IUD) installed?	YES/NO

I have read and understood the questions above and have answered them correctly.

SIGNED…… DATE……

In the presence of …….. (name) ……..(signature)

Please enter below the name and address of your doctor (general practitioner).
(Not required for persons entering the controlled area but not being scanned.)

(UREC 08/74: MRI rules of operation for research, 12 May 2010).

Appendix 13 Second MRI screening form

UNIVERSITY OF READING – CENTRE FOR INTEGRATIVE NEUROSCIENCE AND NEURODYNAMICS

SECOND SCREENING FORM

This form should be completed and signed immediately before your scan, after removal of any jewellery or other metal objects and (if required by the operator) changing your clothes.

NAME OF PARTICIPANT ……

Date of birth……. Sex: M / F

Please read the following questions CAREFULLY and provide answers. For a very small number of individuals, being scanned can endanger comfort, health or even life. The purpose of these questions is to make sure that you are not such a person.

You have the right to withdraw from the screening and subsequent scanning if you find the questions unacceptably intrusive. The information you provide will be treated as strictly confidential and will he held in secure conditions.

BEFORE YOU ARE TAKEN THROUGH FOR YOUR SCAN IT IS ESSENTIAL THAT YOU REMOVE **ALL METAL OBJECTS** INCLUDING: WATCHES, PENS, LOOSE CHANGE, KEYS, HAIR CLIPS, ALL JEWELLERY, METALLIC COSMETICS, TRANSDERMAL PATCHES, CHEQUE/CASH POINT CARDS.

	Delete as appropriate
1. Are you wearing or carrying any metal items such as those listed above?	YES/NO
2. Have your answers to any of the questions in the initial screening form changed? (The initial screening form must be shown to you before you answer this question.)	YES/NO
Specifically, please confirm:	
3. Have you been fitted with a pacemaker, artificial heart valve or cochlear implant?	YES/NO
4. Is there any possibility that you might be pregnant?	YES/NO

I have read and understood the questions above and have answered them correctly.

SIGNATURE…… DATE…….

FOR STAFF USE:

I certify that the initial screening form and the consent form have been completed by the person named above and I have attached them to this form. The volunteer has been given the standard information sheet about MRI experiments, together with any necessary study-specific information, and has been given an opportunity to ask questions. I am satisfied that the volunteer is adequately informed and understands the content of the consent form. I have taken adequate steps to ensure that the volunteer has no ferro-magnetic metal in or on his/her person and I am satisfied that the scan can proceed.

SIGNATURE…… NAME (print) ……..

(UREC 08/74: MRI rules of operation for research, 12 May 2010)

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