UNIVERSITY OF SOUTHAMPTON

FACULTY OF FACULTY OF SOCIAL, MATHEMATICAL,

AND HUMAN SCIENCES

Psychology

INVESTIGATING THE FRONTO-LIMBIC AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS SYSTEMS IN CONDUCT DISORDER

by

Karen Denise González-Madruga

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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In this thesis, I report studies investigating the neurobiology of conduct disorder (CD) – a disorder diagnosed in children and adolescents who display a persistent pattern of disruptive and aggressive behaviour. My particular focus is on the role of frontal, limbic and hypothalamic-pituitary-adrenal (HPA) axis systems, and especially whether CD-related alterations in these systems differ between males and females. A range of different imaging techniques were applied to data from the Neurobiological and Treatment of Adolescent Female Conduct Disorder study (Fem-NAT-CD). In study 1 (Chapter 4), we employed surface-based morphometry techniques to assess frontal and limbic (cortical and subcortical) brain structures. Similar patterns of CD-related related reductions in cortical volume, thickness, and surface area in the superior frontal gyrus were seen in both sexes. The second study (Chapter 5) assessed the shape of subcortical limbic structures. Youths with CD exhibited shape deformations (i.e., inward) in the shell of the nucleus accumbens compared to controls, independent of sex. The third study (Chapter 6) used spherical deconvolution based-tractography and virtually dissected key fronto-limbic white matter tracts, namely: the uncinate fasciculus, fornix, and the subgenual, retrosplenial and parahippocampal bundles of the cingulum. We observed reduced fractional anisotropy in the retrosplenial cingulum in the CD group relative to healthy controls. However, this result was moderated by sex: males with CD showed reduced, while females with CD showed increased fractional anisotropy compared to sex-matched healthy controls. Finally, we investigated sex differences in HPA axis function (Chapter 7) by measuring cortisol response during the Trier Social Stress Test for Children. Both males and females with CD showed blunted cortisol response to stress, and such effects were not explained by low levels of self-rated fear or anxiety. In a small, proof of concept analysis we observed a positive correlation between cortical volume of the superior frontal gyrus and cortisol reactivity. I conclude that the neurobiological basis of CD is relatively similar in males and females. Thus, previous findings in males with CD may also apply for females with CD. Suggestions for future research are presented and clinical implications are discussed.

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Academic Thesis: Declaration Of Authorship

I, Karen Denise González-Madruga declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

INVESTIGATING THE FRONTO-LIMBIC AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS SYSTEMS IN CONDUCT DISORDER

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;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission
8. Signed: Karen Denise González Madruga

Date: 11/03/2018

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Definitions and Abbreviations

5-HIAA	5-Hydroxy indoleacetic acid (a serotonin metabolite)
5-HT	5-Hydroxytryptamine (serotonin)
AB	Antisocial Behaviour
ACC	Anterior Cingulate Cortex
ACTH	Adrenocorticotropic hormone
AD	Axial Diffusivity
ADHD	Attention-deficit/hyperactivity disorder
ANOVA	Analysis of Variance
AO	Adolescent Onset
ASPD	Antisocial Personality Disorder
BA	Brodmann Area
CC	Corpus Callosum
CD	Conduct Disorder
CN	Caudate Nucleus
CO	Childhood Onset
CP	Conduct Problems
CRH	Corticotrophin Releasing Hormone
CT	Cortical Thickness
CU	Callous Unemotional
CV	Cortical Volume
DA	Dopamine
DBD	Disruptive Behaviour Disorder
DG	Dentate Gyrus
DL-PFC	Dorsolateral prefrontal cortex
DMN	Default Mode Network
dMRI	diffusion Magnetic Resonance Imaging
DS	Dorsal Striatum
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion Tensor Imaging
DW	Diffusion Weighted
EF	Executive Functions

FA	Fractional Anisotropy
FDR	False Discovery Rate
FemNAT-CD	Neurobiological and Treatment of Adolescent Female Conduct Disorder
fMRI	functional Magnetic Resonance Imaging
FWE	Family Wise Error
GAD	Generalised Anxiety Disorder
GLM	General Linear Model
GMV	Grey Matter Volume
GR	Glucocorticoid Receptor
HARDI	High Angular Resolution Diffusion Imaging
HC	Healthy Control
HMOA	Hindrance Modulated Orientational Anisotropy
HPA	Hypothalamic Pituitary Adrenal
IA	Instrumental Aggression
IFG	Inferior Frontal Gyrus
IRR	Inter Rater Reliability
K-SADS-PL	Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime version
MANOVA	Multivariate Analysis of Variance
MD	Mean Diffusivity
MDD	Major Depression Disorder
mPFC	medial Prefrontal Cortex
MR	Mineralocorticoid Receptor
MRI	Magnetic Resonance Imaging
MTL	Medial Temporal Lobe
NAcc	Nucleus Accumbens
ODD	Oppositional Defiant Disorder
OFC	Orbitofrontal Cortex
PCC	Posterior Cingulate Cortex
PDS	Pubertal Developmental Scale
PFC	Prefrontal Cortex
PHC	Parahippocampal Cingulum
PRU	Pupil Referral Unit

PTSD	Post-traumatic Stress Disorder
RA	Reactive Aggression
RD	Radial Diffusivity
ROI	Region of Interest
RPQ	Reactive Proactive aggression Questionnaire
RSC	Retrosplenial Cingulum
SA	Surface Area
SBM	Surface Based Morphometry
SD	Spherical Deconvolution
SES	Socio-economic Status
SFG	Superior Frontal Gyrus
SLF	Superior Longitudinal Fasciculus
SGC	Subgenual Cingulum
sMRI	structural Magnetic Resonance Imaging
TBSS	Tract Based Spatial Statistics
TSST-C	Trier Social Stress Test for Children
UF	Uncinate Fasciculus
VAS	Visual Analogue Scale
VBM	Voxel Based Morphometry
vmPFC	ventromedial Prefrontal Cortex
VS	Ventral Striatum
WM	White Matter
YPI	Youth Psychopathic traits Inventory

Chapter 1 An Introduction to Conduct Disorder

This chapter introduces the topic of antisocial behaviour in young people and defines the characteristics of disruptive behaviour disorders (e.g. oppositional defiant disorder and conduct disorder). It focuses specifically on conduct disorder (CD), as this is the focus of the present thesis.

1.1 Delimiting the characteristics of Antisocial Behaviour

The term 'antisocial behaviour' (AB) covers a range of different behaviours, including theft, burglary, physical violence, and substance abuse. The way AB is defined depends strongly on the field of study (e.g. legal, or psychiatric/psychological). Although there are some similarities in definitions of AB across the fields (e.g. violating societal norms, aggressive behaviour, rule breaking, theft and vandalism), there are also different concepts that are important to consider (B B. Lahey, Waldman, & McBurnett, 1999).

The Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 2003), considers a diagnosis of CD for children and adolescents with persistent and severe AB. As such, in psychological and psychiatric contexts discussion of AB tends to focus on its severe consequences to the individual and their families, such as, peer rejection, school dropout, and police arrest. A person convicted of antisocial behaviour that violates laws may not meet the criteria for a diagnosis of CD. Legal sources tend to focus on the effect of AB on society. Here AB is often considered an act of harming, lacking consideration or negatively affecting the well-being of others which may result in arrest (Coie & Dodge, 1998).

Antisocial behaviour is a major public health concern in young people. For instance, CD is the most common reason for referral to Child and Adolescent Mental Health Services amongst young people (Murphy & Fonagy, 2012). In addition, the costs of CD on society are particularly high, with some estimating that each affected individual costs around ten times more to raise to adulthood than a child without the disorder (Murphy & Fonagy, 2012; Scott, Knapp, Henderson, & Maughan, 2001). They are more likely to develop antisocial personality disorder (a personality disorder characterised by impulsive, irresponsible, and criminal behaviour) (Robins, 1978). Moreover, children and adolescents with AB have a poor prognosis with negative adult outcomes, such as criminality, alcohol abuse, unemployment, and poor mental and physical health (Odgers et al., 2008). Although AB is less common in girls than in boys, it often has more severe consequences in girls including teenage pregnancy, prostitution, financial problems and school dropout (Odgers et al., 2008; Pajer, 1998). Therefore, AB has a profoundly negative impact on the affected individual as well as on their family, teachers and fellow students, and society.

Many different terms have been used to describe variants of AB. Here we focus especially on Disruptive Behaviour Disorders (DBDs), such as Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD).

1.2 Disruptive behaviour disorders

DBD is a generic term for AB and covers a range of different disorders. It is characterised by three components: defiance, anger and aggression. Although children at every developmental stage may refuse to obey parental or, teacher demands at times, compliance increases with age in typically-developing children (Brumfield, Roberts, & Mark, 1998). However, it is important to note that in children and adolescents with conduct problems, the frequency, intensity, forms and effects of these behaviours represent abnormal expressions of defiance.

Similarly, anger is a natural emotional response to one´s psychological interpretation of having been threatened or frustrated. However, in a similar way to defiant behaviour, anger becomes abnormal when it is expressed frequently and severely, to the extent that the individual´s quality of life is affected. It is expected that with increasing age children learn how to communicate and manage their anger in appropriate ways such as, verbalising their feelings, understanding facial expressions and social cues, rather than showing a hostile attitude or fighting (Ridgeway & Waters, 1985).

Severe forms of anger usually lead to violent and aggressive behaviour (Hortensius, Schutter, & Harmon-Jones, 2012). Therefore, severe anger acts as a precursor to aggressive behaviour in CD. Particularly, anger is a key feature of reactive aggression, which is a product of emotional arousal (Dodge, Lochman, Bates, Harnish, & Pettit, 1997). Aggressive behaviour encompasses actions, which cause physical harm or injury to others. Rates of aggression are also expected to decline from childhood to adolescence. Although boys tend to show more overt types of aggression during childhood, declining trajectories of aggression apply equally to both genders (Bongers, Koot, van der Ende, & Verhulst, 2003). Moreover, males and females are indistinguishable in terms of rates of aggressive behaviour in late adolescence (Bongers et al., 2003).

1.2.1 Oppositional Defiant Disorder

Oppositional Defiant Disorder is a diagnostic category defined in the DSM-IV and DSM-5. It is a variant of DBD and may predict later CD. However, there are important differences between these two diagnoses (Rowe, Costello, Angold, Copeland, & Maughan, 2010). Although the focus of this thesis is CD, it is important to understand the way in which ODD differs from CD, as numerous

studies that have focused on CD patients have treated ODD and CD as different manifestations of the same condition (Burke, Loeber, & Birmaher, 2002). ODD is defined as a recurrent disobedient, negativistic, defiant, and hostile behaviour towards authorities (American Psychiatric Association, 1994). According to the DSM-IV, individuals with ODD need to display four out of eight of the following symptoms: loses temper, argues with adults, actively defies or refuses to comply with adults' rules, blames others for his or her mistakes, deliberately annoys people, is touchy or easily annoyed by others, is angry and resentful, and is spiteful or vindictive (American Psychiatric Association, 1994).

Although many children or adolescents with CD will have had a history of ODD at earlier ages, follow-forward studies have shown that this is not always the case. For instance, a study assessed children (aged 7-11) with ODD and CD symptoms, and ODD alone, a subset of the cohort was further assessed four years later, when the participants entered mid-adolescence. It was reported that while children with ODD and CD symptoms manifested more severe conduct problems and had an increased risk for having a CD diagnosis several years later, children with ODD only (without CD symptoms) largely did not progress to CD by mid-adolescence (Biederman et al., 1996). In line with this evidence that ODD and CD are partly distinguishable, the DSM-5 treats CD as a different disorder from ODD, meaning that young children who manifest ODD and CD symptoms in parallel are not treated as individuals with ODD but rather they are considered as having the childhood-onset form of CD.

1.2.2 Conduct Disorder

CD is defined as a repetitive and persistent pattern of behaviour in which the basic rights of others or major age-appropriate societal norms or rules are violated (American Psychiatric Association, 1994). Four clusters of CD symptoms have been differentiated through factor analysis (Frick et al., 1993; Frick & White, 2008):

1) Aggression to people and animals (e.g. bullying, initiating physical fights, stealing with confrontation)
2) Destruction of property (e.g. fire setting, vandalism)
3) Deceitfulness or theft (e.g. breaking into someone's car, stealing without confrontation)
4) Serious violations of rules (e.g. truancy, staying out all night, running away from home)

1.3 Characteristics and prevalence of Conduct Disorder

1.3.1 Diagnostic Criteria

According to the DSM-IV classification, in order to be diagnosed with CD, an individual has to exhibit three behavioural symptoms during a twelve-month period and one behavioural symptom during a six-month period prior to the age of eighteen years (see **Table 1.1**).

There is also a sub-classification according to the age of onset of CD symptoms: the childhood onset subtype of CD is diagnosed if at least one symptom emerged prior to age 10, and adolescent onset CD is diagnosed if no symptoms emerged prior to age 10, but the young person meets full criteria for CD after this point (American Psychiatric Association, 1994).

Table 1.1 - Conduct Disorder (CD) symptoms

Aggressive CD		
Aggression towards animals and people		
Initiates physical fights		
Threatens, Bullies, or intimidates others		
Uses a weapon		
Forces someone into sexual activity		
Physically cruel towards people		
Physically cruel towards animals		
Steals while confronting a victim		
Non-Aggressive CD		
Deceitfulness or theft	Runs away from home	Destruction of property
Lies to obtain good or favours	Serious violations of rules	Destroys others' property
Breaks into someone's house or car	Stays out at night	Set Fires
Steals without confrontation	Truant from school	

The DSM-IV identifies whether the symptoms are mild (e.g. lying, truancy, staying out, rule breaking), moderate (stealing without confrontation, vandalism), or severe (forced sex, physical cruelty, use of a weapon, stealing with confrontation, as well as breaking and entering). It also

specifies that the frequency of the symptom should be far greater than would be expected in a typically developing peer (e.g., very frequent versus occasional lying), and the symptoms should significantly impair the individual's social, academic, or work life.

1.3.2 Criticisms of the Conduct Disorder Diagnostic Criteria

The timeframe of the diagnostic criteria can make diagnoses complicated. In addition, since CD can be diagnosed when just three symptoms out of a list of fifteen are met, a child or adolescent manifesting three symptoms and another displaying all fifteen will receive the same clinical diagnosis. This has been argued to make CD too broad as a diagnostic category, and overall a highly heterogeneous disorder. As an example of this heterogeneity, it has been estimated that there are 32,647 distinct symptom combinations for a diagnosis of CD (Nock, Kazdin, Hirpi, & Kessler, 2006).

There is also a high probability that youths without CD may occasionally display some of the symptoms categorized in the CD criteria, such as staying out all night (32.8%), and truancy (26.6%), compared to other symptoms of CD such as forced sexual activity (0.3%) (Nock et al., 2006). Therefore, some researchers have suggested that treating CD as a dimension, using a continuous scale, would be more accurate than identifying different groups (Krueger, Markon, Patrick, & Iacono, 2005). However, categorical models (i.e., using different groups) allow researchers and clinicians to have clearer cut-offs to identify the individual's condition and allocate treatment to those who most need it. Additionally, it improves the communication between clinicians and researchers, and aids the development of better treatments and research protocols for the investigation of CD.

1.3.3 Prevalence

The prevalence of CD increases substantially in the transition from childhood to adolescence. However, this rise seems to be stronger for nonaggressive (rule-breaking) forms of CD (Maughan, Rowe, Messer, Goodman, & Meltzer, 2004). The aggressive form of CD appears to reduce in prevalence in adolescence.

1.3.3.1 Sex Differences in Conduct Disorder

There are sex differences in the prevalence of CD, with males being more frequently diagnosed than females (Maughan et al., 2004). Overall, a male to female sex ratio of 2:1 was reported in the Great British national survey of children and adolescents aged 5 to 15 (Meltzer, Gatward &

Goodman, 2000). Although antisocial behaviours committed by males are usually more serious and more violent than those committed by females, the sex ratios for antisocial behaviours such as rule breaking, drug and alcohol use offences are very similar, particularly in mid-adolescence (Moffitt et al., 2001).

In relation to childhood-onset (CO) CD, the sex ratio is lower for girls, appearing to be about 10:1 (Moffitt, Caspi, Rutter, & Silva, 2001). Although there are more males than females with CD at every stage of lifespan, the sex ratio drops to as low as 1.5:1 in favour of males by mid-adolescence (Wasserman, McReynolds, Ko, Katz, & Carpenter, 2005). Although the increased prevalence is particularly dramatic in girls, they tend to have a lower symptom count, and lower levels of aggression than their male counterparts (Maughan et al., 2004). Instead, females seem to display an increase in rule-breaking forms of CD (Maughan et al., 2004). In late adolescence, the sex ratio increases again to become 2:1 with males being more likely to retain a CD diagnosis compared to females (Moffitt & Caspi, 2001).

At younger ages, females with CD tend to display more overt aggressive behaviour, while they display more covert aggressive behaviour during adolescence (Wolke, Woods, Bloomfield, & Karstadt, 2001). However, females with CD generally exhibit lower levels of aggression than males, with less severe consequences (e.g., damage and physical harm to others) (Lahey et al., 1998). In addition, females with CD tend to display more relational or social aggression than their male counterparts (Crick & Grotpeter, 1995). Consequently, some researchers have suggested integrating relational forms of aggression into the criteria for CD (Hartung, Milich, Lynam, & Martin, 2002).

Most antisocial behaviour in females occurs during adolescence (Fontaine, Carbonneau, Vitaro, Barker, & Tremblay, 2009). Thus, it has been suggested that females typically show an adolescent-onset (AO) presentation of CD (Silverthorn & Frick, 1999). However, although females are more prone to develop AO-CD than CO-CD, they also display conduct problems in early childhood. However, they exhibit other kinds of conduct problems relative to symptoms of the CD criteria (e.g., relational aggression; Odgers et al., 2008). Furthermore, it has been shown that when females are diagnosed with CD - either with a CO or AO subtype - there is a higher prevalence of comorbid disorders (especially anxiety) (Von Polier, Vloet, Herpertz-Dahlmann, Laurens, & Hodgins, 2012). Therefore, there is a higher risk that females with CD will develop other psychopathologies in adulthood (e.g. borderline personality disorder, major depressive disorder). Whereas in men there seems to be more continuity in antisocial behaviour as they are more likely to develop antisocial personality disorder as adults (Odgers et al., 2008).

1.3.3.2 Possible causes of prevalence sex differences in CD

The underlying causes of sex differences in the prevalence of CD are not well understood. It has been argued that the observed differences in prevalence may be due to cultural biases towards the sexes, aggressive behaviours are more tolerated in boys than in girls (Robins, 1991). Also, the diagnostic criteria for CD are thought to capture the more overt forms of antisocial behaviour that are typically displayed by boys (e.g. aggression and vandalism) as compared to the more covert forms of antisocial behaviour (e.g. deceit, manipulation, lying, staying out late), the latter being more frequently displayed by antisocial females (Leadbeater et al., 1999). This may be the result of the fact that the empirical validation of the diagnostic criteria of CD in the DSM-III-R and DSM-IV field trials did not examine sufficient numbers of girls (Zoccolillo & Tremblay, 1996).

1.3.3.3 Implications of sex differences

Research on females with CD has gained a lot of attention in recent years. This is partly because they are at higher risk of negative outcomes than CD males (Keenan, Loeber, & Green, 1999). For instance, females with CD tend to drop out of school more frequently than CD males. In addition, it is more likely that females with CD would find partners exhibiting antisocial behaviours, and raise antisocial offspring, compared to males with CD (Hill, 2002; Keenan et al., 1999), thereby leading to the intergenerational transmission of antisocial behaviour. They are also more susceptible to becoming victims of grooming, participating in prostitution, and becoming pregnant at a younger age when compared to typically developing females (Bardone et al., 1998; Hill, 2002).

Sex differences in antisocial behaviour are important to understand because although the number of females displaying severe aggressive behaviour has increased over the last two decades (Youth Justice Board, 2008), the majority of studies examining genetic, environmental and neurobiological risk factors for CD have primarily focused on male individuals. This is despite preliminary evidence that the aetiology and neurobiology of CD may differ in important ways across the sexes (Meier, Slutske, Heath, & Martin, 2011).

1.3.4 Subtypes of CD

As mentioned above, CD is a clinically heterogeneous disorder. Many approaches to subtyping have been proposed and some of these are used in the current DSM-5. These include aggressive versus nonaggressive, childhood onset versus adolescence onset, and CD with high versus low levels of callous unemotional traits (also known as ‘limited prosocial emotions’ in the DSM-5).

1.3.4.1 Aggressive and non-aggressive forms of CD

The symptoms comprising the aggressive type of CD are those including physical aggression, such as physical fighting, bullying, cruelty to people, and animals. On the other hand, symptoms comprising the non-aggressive subtype include lying, truancy, and those related to rule breaking (Burt, 2009; Frick & Ellis, 1999). The distinction between the aggressive and non-aggressive types of CD was included in the DSM-III, however, DSM-IV gave priority to classifying the disorder in terms of age of onset. Nevertheless, is important to consider the former classification, as aggressive types of CD are the most socially disruptive and often have the most severe consequences.

In addition, it has been suggested that these two forms of CD (aggressive vs. nonaggressive) have different aetiologies (e.g. genetic factors vs. environmental factors) and therefore different developmental pathways (Burt, 2009). For instance, research has shown that aggressive behaviours are more heritable than non-aggressive ones. Conversely, the role of environmental influences - and particularly shared environmental influences - seems to be higher for non-aggressive symptoms (e.g. rule-breaking behaviours). Also, aggressive behaviour has shown to be more stable over time compared to nonaggressive forms of antisocial behaviour (Burt, 2009). It has also been reported that aggressive behaviour in childhood predicts negative outcomes in adolescence such as alcohol abuse, drug abuse and truancy. Therefore, children with aggressive behaviour are more likely to escalate to show more serious antisocial behaviours later on in adolescence and adulthood (Loeber et al., 1993), whereas non-aggressive forms of CD are more likely to worsen in early adolescence, but decline in the transition to adulthood (Burt, 2009; Loeber et al., 1993).

1.3.4.2 Reactive, instrumental and relational aggression

As well as the distinction between aggressive and non-aggressive behaviour, some authors think it is important to subtype CD according to the type of aggression displayed (Frick & Ellis, 1999; Gao, Zhang, & Fung, 2015). These subtypes mostly focus on reactive aggression, which is elicited in response to frustration or threat, instrumental aggression (IA), which is an unprovoked and goal-orientated type of aggression used for personal gain (Hubbard, McAuliffe, Morrow, & Romano, 2010). Relational aggression involves efforts to cause harm by damaging others' relationships or threatening to do so, for example, spreading rumours and excluding others from friendship groups (Crick & Grotpeter, 1995). Instrumental aggression has been associated with delinquency and psychopathic traits whereas reactive aggression has been associated with impulsivity and a lack of peer relationships in adolescence (Frick & Ellis, 1999). Relational aggression is associated with peer rejection and signs of depression (Crick & Grotpeter, 1995), this latter form of aggression has shown to be higher in girls than in boys (Bongers et al., 2003).

1.3.4.3 Childhood-Onset versus Adolescent-Onset CD

The age of onset distinction originated in 1993 when Terrie Moffitt proposed the developmental taxonomic theory. This model builds on our understanding of the relationship between age and antisocial behaviour. The theory argues that the childhood-onset (CO) form of CD is a neurodevelopmental condition, whereas the adolescent onset (AO) variant of CD is caused by a maturity gap in which individuals with AO-CD mimic the antisocial behaviours of their deviant peers. Thus, it has been argued that the two subtypes arise from different aetiological factors (Moffitt, 1993). Childhood onset CD develops during the early stages of life, and it has been regarded to emerge from a combination of individual characteristics, such as, irritability, hyperactivity, and impulsivity (Moffitt, 1993), and psychosocial and biological factors, such as, prenatal or perinatal problems, and genetic risk factors (Moffitt, 2015; Silverthorn & Frick, 1999).

Adolescent onset CD, on the other hand, is considered to be a less pathological condition. It usually manifests in individuals without a previous history of antisocial behaviour, and it is thought that the AO-CD will remit as the individuals enter adulthood (Moffitt, 1993). Also, it has been argued that those with CO-CD tend to manifest more aggressive symptoms, whereas those with AO-CD tend to be more involved in rule-breaking behaviour (Frick & White, 2008). Furthermore, those who show CO-CD are predicted to have more criminal convictions (Loeber, 1982), and are more prone to develop antisocial personality disorder, than those with an AO-CD type (American Psychiatric Association, 2003). However, some authors have shown that the AO form of CD also predicts later antisocial outcomes and other difficulties in adulthood (Matthys & Lochman, 2009; Moffitt et al., 2008; Odgers et al., 2008).

Finally, individuals with CO-CD appear to manifest more neuropsychological deficits, compared to individuals with AO-CD – perhaps linked to structural and functional alterations in the brain (Moffitt, 1993). However, further research comparing the CO-CD and AO-CD subtypes in terms of brain structure and function found no significant differences between the CD subgroups, although both groups significantly differed from controls (Fairchild et al., 2011; Passamonti et al., 2010). This suggests that, contrary to the developmental taxonomic model, the aetiology of the CO-CD and AO-CD subtypes may have a similar neurodevelopmental basis (Fairchild et al., 2013).

1.3.4.4 Callous-Unemotional Traits

The DSM-5 added the 'limited prosocial emotions' specifier as a descriptive feature and subtyping index of CD (American Psychiatric Association, 2013). This characteristic identifies individuals with CD and callous-unemotional (CU) traits. Lack of remorse or guilt and general lack of concern about the negative consequences of his/her actions must have been displayed for at least 12 months (American Psychiatric Association, 2013). In addition, this information should be provided by both

the youth and another informant such as the parent/teacher/carer. However, it is not yet very clear how to assess for these criteria. Commonly, researchers investigating this domain in CD use questionnaires assessing CU traits, such as the Inventory of Callous Unemotional traits and the Youth Psychopathic traits Inventory (Andershed, Hodgins, & Tengström, 2007; Kimonis et al., 2008). However, researchers have not agreed on consistent cut offs or norms for these measures. Thus, to date, there is no formal or widely agreed definition of what is a low versus a high level of CU traits and research studies on this topic have adopted various cut-offs.

Callous-unemotional (CU) traits are personality traits that reflect a disregard of others' feelings and lack of empathy for others. Individuals that manifest CD and high levels of CU traits exhibit a more severe, aggressive, and persistent pattern of antisocial behaviour (Frick & Dickens, 2006; Frick & White, 2008). Therefore, individuals with CD and CU traits are argued to be a different group from those with CD alone (Fanti, 2013). In addition, it has been proposed that CD with high levels of CU traits designates individuals who are at higher risk of becoming psychopaths as adults (Fergusson, Horwood, & Ridder, 2005). Individuals classified according to this subtype manifest preferences for novel and dangerous activities. Furthermore, individuals with CD and CU traits may show both RA and IA, whereas individuals with CD alone only show higher levels of RA (Frick & Ellis, 1999; Loney, Frick, Clements, Ellis, & Kerlin, 2003).

1.4 Conclusion

In summary, CD is a clinical diagnosis that is given to individuals who display a persistent and pervasive pattern of antisocial behaviour. It is included within the broader category of 'disruptive behaviour disorders'. It is important to understand the relationship between ODD and CD as several studies have treated ODD and CD as manifestations of the same condition. CD has a major impact on the affected individual and their family, as well as on society. The prevalence of CD in males is higher than in females. Males show much more continuity and stability of CD than females. However, the sex ratio for CD is narrowest in mid-adolescence compared to the other phases of the lifespan, probably due to dramatic increases in non-aggressive (e.g. rule breaking) forms of antisocial behaviour amongst adolescent females. Finally, CD is a heterogeneous disorder that includes both aggressive and nonaggressive behaviours, childhood-onset versus adolescence onset subtypes, and individuals who vary in the level of CU traits (also known as 'limited prosocial emotions').

It is important to mention that CD is not always associated with a negative trajectory or poor adult outcomes. Many antisocial adults have a history of CD in childhood. However, not all children with CD become antisocial adults (Moffitt, 1993). Therefore, the development of CD is complex in

terms of continuity and change. More specifically, CD is a stable condition for some individuals, while in others the symptoms either escalate or remit (Rowe et al., 2010).

Chapter 2 Biological correlates of Conduct Disorder

This chapter describes the biological systems associated with conduct disorder, namely, the frontal, limbic and hypothalamic pituitary (HPA) axis systems. The chapter provides a brief overview of risk factors such as environmental stress that influence the development of CD. It will further describe the relationship between the frontal and limbic systems and the HPA axis, followed by discussing neuroimaging and neuroendocrinological studies that have been conducted in CD populations.

2.1 Risk Factors for Conduct Disorder

The causes of conduct disorder (CD) have long been a subject of debate. Specifically, authors have discussed whether CD predominantly reflects the influences of nature or nurture, or an interaction between biological and environmental factors. It has been challenging for scientists to agree on a simple developmental pathway for this disorder as its aetiology originates from biological (e.g., genetic) and environmental factors, as well as mediating processes. These processes include cognitive and neurobiological factors, which alter the risk of developing the psychiatric disorder (Morton & Frith, 1995; Rutter, Michael, 2000). For instance, CD may emerge as a result of the effects of environmental adversity upon a genetic predisposition to CD (Foley et al., 2004). Neurobiological factors contribute to the development of CD and mediate the joint effect of genes and environmental stress (Morton & Frith, 1995). Further, neurobiological factors that play a role in initiating disruptive behaviour disorder symptoms may also go on to play a role in the maintenance and development of CD (Matthys & Lochman, 2010; see **Figure 2.1**).

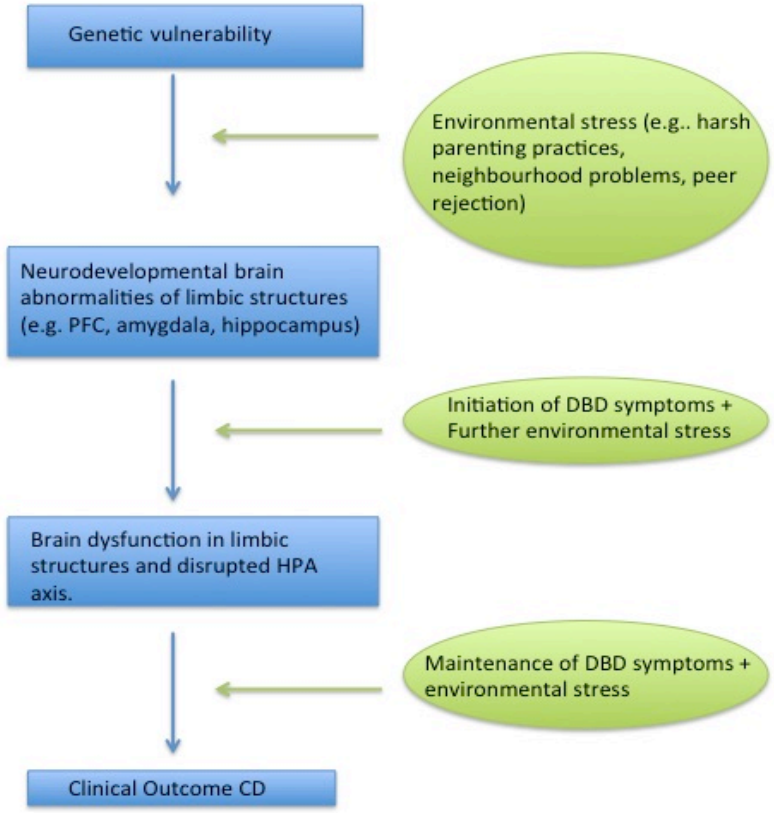


Figure 2.1 - A hypothetical causal model of CD

It has been demonstrated, that the origins and maintenance of CD in childhood derive from environmental characteristics such as, family factors, parenting practices, peer factors, community and school factors (Burke et al., 2002; Frick et al., 1992). A large number of factors, especially those that are considered to be stressful, affect childhood aggression and CD. For instance, children exposed to financial stress in families with a low socioeconomic status (SES) or living in poverty have been shown to have higher rates of antisocial behaviour than those from other SES groups (Macmillan, Mcmorris, & Kruttschnitt, 2004). Moreover, in a longitudinal study, it was revealed that an increase in family income decreased the children's conduct problems (Macmillan et al., 2004), suggesting, that family income is related to the antisocial behaviour of these individuals. However, it has been argued that rather than poverty being a direct causative factor itself, poverty negatively affects other family processes like parenting or quality of supervision.

On the other hand, it has been proposed that those children who are at high risk of developing childhood-onset or adolescence-onset conduct disorder may be due to individual characteristics, such as, genetic influences, neuropsychological deficits, temperament and perceptual processes (Tremblay, 2010). Moreover, several researchers have suggested that risk factors operate differently between males and females. The section below, will consider potential sex differences in risk factors.

2.1.1 Sex differences in risk factors

Given that antisocial behaviour (AB) is less common in females compared to males, it has been suggested that in order to develop AB, females may require a greater load of genetic, neuro-biological, or environmental risk factors (Cloninger, 1978). On one hand, this model has been supported by studies showing higher heritability of AB in females (Gelhorn et al., 2006), but on the contrary, other studies have not found significant differences between the sexes in the level of genetic and environmental influences on AB (Burt, 2009).

With regards to the relationship between risk factors and the development of AB, a large-scale longitudinal study reported that there are no sex differences between these factors (Moffitt et al., 2001). However, the level of exposure to the risk factor may differ among the sexes. For instance, child abuse appears to be more strongly related to the development of AB in females compared to males (Burnette, Oshri, Lax, Richards, & Ragbeer, 2012). In contrast, males are more frequently punished for bad behaviour than females (Meier et al., 2011), and harsh parenting appears to be more strongly associated with antisocial behaviour in males than in females (Leve, Kim, & Pears, 2005). However, antisocial females appear to be more strongly related antisocial behaviour in parents, especially when the mother is the one exhibiting the behaviour (Pajer et al., 2008), suggesting that females with CD are more vulnerable to difficult parental relationships than their male counterparts (Ehrensaft, 2005).

To conclude, it has been argued that the development of AB is a product of genetic, neurobiological, and environmental risk factors. However, we cannot be certain of which risk factors is more important or influential. The study of brain development and the impact of environmental influences are crucial to understanding the underpinnings of CD. The relevance of understanding normal brain development lies in the fact that childhood and adolescence are critical periods for brain development. Therefore, understanding changes in cortical structures and rearrangements of brain connections during this period are crucial to characterising the neurobiological mechanisms in individuals with a high risk of developing conduct disorder. In addition, as environmental stress has shown to be a major factor in the development of CD, it is important to understand how stress alters brain function and the way in which this may lead to the development of CD. It is thought that a complex interaction between the frontal-limbic and the hypothalamic-pituitary-adrenal (HPA) axis systems mediates the body's response to stress. Next, we will review the mechanisms of these systems and how they have been associated with CD. It will firstly describe the HPA axis and its functions, followed by a description of the frontal and limbic neuroanatomical and functional networks.

2.2 The Hypothalamic-Pituitary-Adrenal Axis

The role of the HPA axis is to coordinate and regulate neurophysiological responses to physical and psychological stressors (Gunnar & Quevedo, 2007). The way we adapt to stress is a major priority for all organisms, as it determines the response to the demands of the environment. Stress may involve a real, or an anticipated, disruption of homeostasis or threat to our well-being (Ulrich-Lai & Herman, 2009). The stressor could be either physical (e.g. blood loss, infection, and body pain), which requires a systemic reaction, or it could be a psychogenic (i.e. psychological) stressor, which is based on prior experience or innate reactions. These responses can occur in anticipation of, or as an instant reaction to, stressful events. Neural and endocrine systems work together to obtain a successful response to stress (Ulrich-Lai & Herman, 2009). The system also helps organisms to cope with a volatile environment (Kudielka & Kirschbaum, 2005).

A stressor triggers signals to the hypothalamus to secrete corticotrophin releasing hormone (CRH), followed by the activation of the anterior pituitary to secrete adrenocorticotropic hormone (ACTH). ACTH activates the release of cortisol through its action at the adrenal glands (Kudielka & Kirschbaum, 2005). This process is controlled by regulatory negative feedback mechanisms in which cortisol acts to downregulate the secretion of ACTH and CRH, thereby inhibiting its own production. The responsiveness to this feedback is due to the affinity of cortisol to inhibit ACTH and CRH release. This is achieved by cortisol binding to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR; **Figure 2.2**).

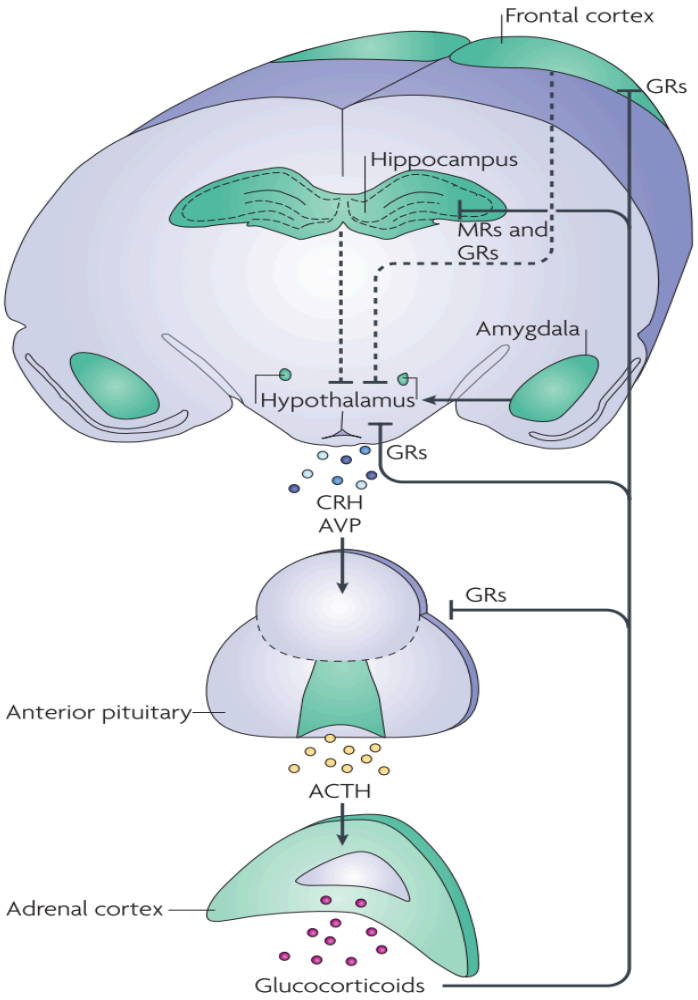


Figure 2.2 - The stress system

Figure 2.2. The hypothalamic pituitary adrenal axis, the body's main stress system. When the brain encounters a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH). CRH stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, this in turn stimulates the adrenal cortex to produce glucocorticoids (including cortisol). Glucocortocoids act on the hypothalamus and other limbic regions in a negative feedback fashion to reduce the secretion of CRH. Once the stressor has ended, the axis returns to its homeostatic norm.

Reprinted from: Effects of stress throughout the lifespan on the brain, behaviour and cognition. By S.Lupien et al., 2009, Nature Reviews. Neuroscience, 10 (6), p. 435.

2.2.1 Associations between the HPA axis and antisocial behaviour

Maternal substance use and chronic exposure to stress have also shown to be risk factors for developing AB (Gao, Huang, & Li, 2017; Silberg et al., 2003). This environmental factor may be associated with the development of the HPA axis. The regulation of the HPA-axis is initially developed and programmed in the prenatal period. During pregnancy, CRH is not only produced by the hypothalamus but also by the placenta (Lupien et al., 2009). This production can alter the length of the gestational period. It has been revealed that women with higher levels of cortisol, which could be an indicator that they are either under stress or suffering from depression or anxiety, were more likely to give birth prematurely (Lupien et al., 2009). High levels of cortisol during pregnancy may have an impact on the baby's brain development. The baby that develops under these conditions may become more vulnerable to neurodevelopmental disorders such as, depression, attention defecit hyperactivity disorder (ADHD) and conduct problems (O'Donnell, O'Connor, & Glover, 2009). Therefore, stressed or depressed mothers are not only affecting their own HPA axis but also the development of the unborn child's (O'Donnell et al., 2009).

Environmental stress such as, adverse childhood family environment, exposure to sexual and physical abuse, and deviant peer affiliation, are all thought to be risk factors for the development of oppositional defiant disorder (ODD) and CD. The stress-regulating system is highly dynamic; as such, it is important to understand how the environmental stress influences its development. The long-term consequences of the stress response may be influenced by how the individual copes with stress, and is proportional to the duration and intensity of the stressor. (Van Goozen, Fairchild, Snoek, & Harold, 2007). It is thought that dysfunction of stress regulating mechanisms has a role in the development of antisocial behaviour. However, the HPA axis does not function in isolation; rather the fronto-limbic system also plays a major role in stress regulation.

2.3 An Introduction to the Limbic System

Given that the limbic system is a concept based on functionally and anatomically interconnected structures, this section will firstly provide a brief overview of the limbic system.

The concept of the limbic system originates with Paul Broca, who was a pioneer in studies of brain function localisation, and who discovered the neural network that plays a critical role in emotion (MacLean, 1949). In 1878, he described a ring, or limbus, of temporal cortex surrounding the underlying thalamus, naming it 'le grand lobe limbique' - the limbic lobe (**Figure 2.3a**). The areas compromised the limbic lobe included, curved ridges of the parahippocampal gyrus, the cingulate gyrus, and the subcallosal gyrus, as well as the hippocampus. In 1937, James Papez described a set of brain structures that constituted the anatomic basis of emotions. The Papez circuit included the

thalamus and hypothalamus (**Figure 2.3b**; MacLean, 1949). Further, Paul MacLean in 1952 extended Papez's circuit to include the amygdala, and frontal cortex (**Figure 2.3c**). This circuit was referred as the limbic system (Newman & Harris, 2009).

Since then, the limbic system is a way of describing functionally and anatomically interconnected cortical and subcortical brain structures (Catani, Dell, & Thiebaut De Schotten, 2013). Cortical regions that are involved in the limbic system include the orbitofrontal cortex, cingulate gyrus, subcallosal gyrus, and the parahippocampal gyrus. Subcortical regions include the hippocampus, amygdala, hypothalamus, and thalamus (Newman & Harris, 2009; Rolls, 2013).

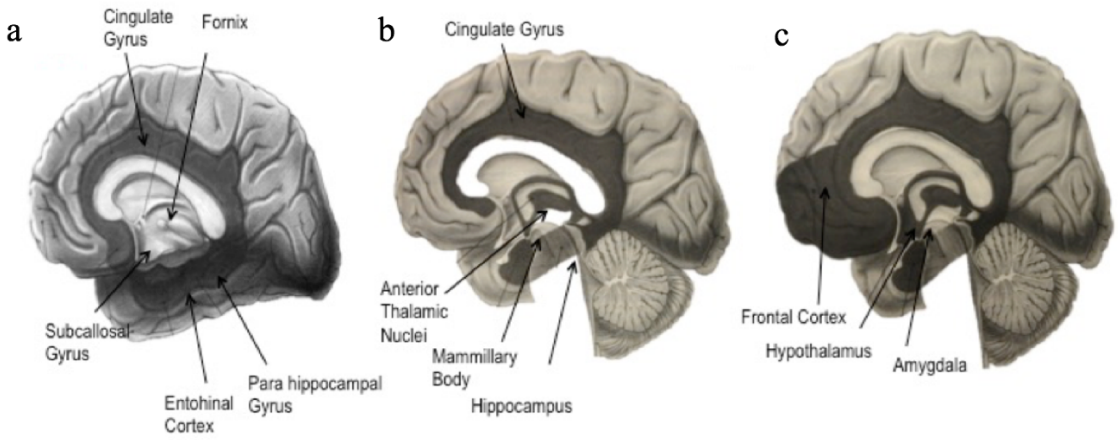


Figure 2.3 - The limbic system: Le Grand Lobe Limbique (shaded area), as defined by Broca (a). The emotional circuit of Papez (b).The limbic system of MacLean (c). Adapted from *The Biology Of Violence* (p.73), by Niehoff, 1999, The Free Press, Copyright 1999 by Debra Niehoff.

However, given that the insula and striatum are strongly associated with the aforementioned limbic regions, further studies have also included these structures (i.e., striatum and insula) as part of the limbic concept – which is sometimes also referred as the para-limbic system (Kiehl, 2006). In the next section, the neuroanatomical and functional features of the frontal and limbic structures will be delineated.

2.4 The neuroanatomy and functions of the fronto-limbic system

2.4.1 Cortical Brain Structures

2.4.1.1 Insular cortex

The insular cortex is located beneath the temporal, frontal and parietal lobes (Fernandez-Baca Vaca, Maciunas, Koubeissi, & Lüders, 2011). The most anterior part is a component of the limbic system. The anterior insula is divided in three short gyri – the anterior, middle, and posterior (Stephani et al., 2011). It has been proposed that the anterior insula is involved in the perception and processing of subjective feelings, emotional processing, self-awareness (Craig, 2009), and fear conditioning (Birbaumer et al., 2005).

2.4.1.2 Orbitofrontal cortex

The orbitofrontal cortex (OFC) is located at the front and sides of the brain, towards the bottom of the prefrontal cortex (PFC). The OFC includes Brodmann areas (BA): 10, 11, and 47 (Kringelbach, 2005). The ventromedial prefrontal cortex (vmPFC) overlaps with these BA areas. Hence both terms are commonly used to describe the same set of brain regions. Occasionally, the vmPFC is used to describe a wider area in the lower, and central region of the prefrontal cortex, which includes BA regions: 25, 12, and 32 (Öngür & Price, 2000). The OFC and vmPFC have been associated with learning and reversal of stimulus reinforcement associations (Rolls, 2004).

2.4.1.3 Dorsolateral and medial prefrontal cortex

The Dorsolateral prefrontal cortex (DL-PFC) is composed of Brodmann areas 8, 9, 10 and 46 and the medial prefrontal cortex (mPFC) includes BA: 8, 9, 11, 12. These BA are mainly formed by the superior and middle frontal gyrus. The DL-PFC is involved in cognitive and self-regulatory processes such as attention, cognitive flexibility, and impulse control. Deficits in the DL-PFC are related to problems in goal-directed behaviour. The mPFC is associated with introspection and social function. Deficits in this area have been related to deficient judgments of affective morality (Raine & Yang, 2006).

2.4.1.4 Anterior and posterior cingulate cortex and parahippocampal gyrus

The anterior cingulate cortex (ACC) is situated superiorly and anteriorly to the splenium of the corpus callosum (BA 24,32,33), towards the front of the cingulate cortex, a region that is situated

directly above the corpus callosum (Ramachandran, 2002). The functions of the ACC have been subdivided into emotional and cognitive processes along the dorsal-ventral axis (Bush, Luu, & Posner, 2000). The dorsal ACC seems to be more responsible for processing cognitive information, whereas the ventral ACC is more responsible for affective processing. The ACC has been implicated in error and conflict detection, as well as socio-emotional processes (Luu, Flaisch, & Tucker, 2000).

The posterior cingulate cortex (PCC) is situated superiorly and posteriorly to the splenium of the corpus callosum (BA 23 and 31; Ramachandran, 2002). It is a central node of the default mode network, which is a distributed network of brain regions which are more active during rest than during performance of many cognitive tasks (Uddin, Clare Kelly, Biswal, Castellanos, & Milham, 2009). The PCC has been suggested to mediate interactions between emotion and memory (Maddock, Garrett, & Buonocore, 2001).

The parahippocampal gyrus covers a large portion of the temporal lobe. It is adjacent to brain regions with an important role in memory (e.g. hippocampus) and visual processes (e.g. fusiform cortex, Aminoff, Kveraga, & Bar, 2016). The parahippocampal gyrus has been implicated in episodic memory, and in the identification of social context (Rankin et al., 2009).

2.4.2 Subcortical Brain Structures

2.4.2.1 Hippocampus

The hippocampus is located within the medial temporal lobe, adjacent to the centre of the brain. The hippocampus has a sea horse shape, and it is divided into cytoarchitecturally different subfields, namely the dentate gyrus and cornu ammonis (Andersen, Morris, Amaral, Bliss, 2006). The first of these is the dentate gyrus (DG), followed by a series of Cornu Ammonis areas (Andersen, et al., 2006). The hippocampus is involved in memory processes (Moscovitch et al., 2005), in novelty processing (Chong et al., 2007), self-referential processing during recall and prospection (Muscatell, Addis, & Kensinger, 2010), formation of emotional memories in social contexts (Eisenberger, Gable, & Lieberman, 2007) , processing of social and emotional contexts (Immordino-Yang & Singh, 2013) and stress-regulation (Herman, Ostrander, Mueller, & Figueiredo, 2005).

2.4.2.2 Amygdala

The amygdala is a structure located within the medial temporal lobe (Kim et al., 2011). It is extensively interconnected with the hypothalamus and prefrontal cortex (Kim et al., 2011). The

amygdala is well known to have a central role in emotion processing (Pessoa, 2010), and fear recognition (Adolphs & Tranel, 1995). In fact, the amygdala is one of the most important brain regions involved in processing of affective stimuli (Sergerie, Chochol, & Armony, 2008). In normal populations, neuronal activation within the amygdala has been reported in tasks using facial expressions (Williams, 2004), moral reasoning (Luo et al., 2006), and during tasks of empathy (Baron-Cohen et al., 1999).

2.4.2.3 Thalamus and Hypothalamus

The thalamus is oval shaped, and communicates impulses to and from the brain stem, spinal cord, cerebrum, and cerebellum. It functions to relay sensory input to the cerebral cortex, and therefore all sensory (i.e. the eyes, ears, and body) input transverses the thalamus (Ramachandran, 2002).

The hypothalamus is located below the thalamus. The hypothalamus is an important structure for functions such as monitoring of water and hormone concentrations, and body temperature. The hypothalamus plays an important role mediating the autonomic nervous system and the peripheral neural network, orchestrating the flight or fight body response, and the endocrine system (Ulrich-Lai & Herman, 2009). The hypothalamus is connected to the pituitary, and is also involved in the regulation and production of hormones (Ulrich-Lai & Herman, 2009).

2.4.2.4 Striatum

The striatum is mainly divided in two segments: the dorsal striatum (DS) and the ventral striatum (VS). The DS includes the caudate nucleus (CN) and putamen, while the VS mainly consists of the nucleus accumbens (NAcc, (Glenn & Yang, 2012)), however the olfactory bulb, ventral caudate and ventral putamen are sometimes also included in the VS component (Neto, Oliveira, Correia, & Ferreira, 2008). It has been suggested that deficits in VS function are associated with impulsivity, and heightened sensitivity to reward while the DS is more associated with learning from positive outcomes (Glenn & Yang, 2012).

2.5 The Associations between the Fronto-Limbic and HPA axis Systems

Brain regions of the limbic system, such as the hippocampus, amygdala, and mPFC are highly integrated with the regulation of the HPA axis, and the maintenance of basal cortisol levels during the diurnal circadian rhythm (Herman et al., 2003). This regulation is itself mediated by the HPA axis through the expression of glucocorticoid receptors (GR), and mineralocorticoid (MR) receptors (Bittencourt & Sawchenko, 2000; de Kloet, Joels, & Holsboer, 2005) which differ in the affinity and localisation within the brain.

Mineralocorticoid receptors are high-affinity receptors to cortisol and corticosterone. Consequently, during basal conditions, at the trough of the circadian rhythm, it is primarily the MRs that are occupied. However, during stress or the circadian peak, the MRs become saturated, and the GRs appear to "take over", acting in coordination to ensure the return to homeostasis (Herman & Cullinan, 1997). In the rodent brain, MRs are most densely located in the hippocampus, whereas GRs are widely distributed including hippocampus, hypothalamus, and prefrontal cortex (Herman & Cullinan, 1997). In the amygdala, expression of MRs is weaker than GRs. Interestingly, unlike in rodents, there is significant expression of MR in the human prefrontal cortex (de Kloet, Vreugdenhil, Oitzl, & Joëls, 1998). This suggests that MR may have a role in modulating higher brain functions (e.g. social behaviour and cognitive processing) in humans.

In addition, the hippocampus also plays a role in terminating HPA axis responses to stressors (Herman et al., 2005). For instance, lesions to the hippocampus, extend GC or ACTH release following exposure to stress (Herman et al., 2003). Furthermore, GR over-activation has been found to decrease hippocampal output creating HPA axis dysregulation (Makino, Hashimoto, & Gold, 2002), and prolonged exposure to glucocorticoids alters the morphology and the functional integrity of the hippocampus(McEwen, 2001).

2.6 The Effects of Stress on Fronto-Limbic Structures and the HPA Axis

It is well established that brain regions of the limbic system are involved in the stress response. Studies using gene expression as a marker of neuronal activation, have revealed widespread induction of c-fos mRNA (i.e. neuronal marker) in the brain following restraint, swim, or audiogenic stress in rodents (López, Akil, & Watson, 1999). The brain regions that were activated by these acute stressors included the hippocampus, amygdala, anterior cingulum, and vmPFC (López et al., 1999). However, the degree of activation depends on the type of stressor. For example, swim stress causes higher expression of c-fos in the hippocampus than does restraint, or audiogenic stress in rats. Thus, there seems to be a general and stimuli-specific response to stress.

While the HPA axis seems to regulate the stress response, the limbic system plays an important role in processing threatening stimuli, initiating and modifying the stress response. In humans, an association between HPA function and morphology of the hippocampus has been reported (Bremner et al., 2000). It has been suggested that the hippocampus may be involved in the interpretation of stressor intensity. The hippocampus down regulates certain types of stressors, but not other stressors, for example, exposure to restraint stress caused elevated glucocorticoid secretion, however, this was not seen in stressors that involved ether inhalation or hypoxia (Herman & Watson, 1995; Mueller, Dolgas, & Herman, 2004). In addition, corticosteroid responses to mild stress, such as, cage relocation or following open field exposure, was greater in rats with hippocampal lesions compared to typical rats (Herman, Dolgas, & Carlson, 1998), and chronic stress is associated with decreases in the MR/GR ratio in the hippocampus (López, Chalmers, Little, & Watson, 1998).

The medial prefrontal cortex (mPFC) plays a specific role in stress regulation. It mainly acts as a negative feedback mechanism in the HPA axis by attenuating its responses (Herman et al., 2005). The anterior cingulate cortex of the mPFC projects to regions such as the stria medularis, and hypothalamic nuclei (Herman et al., 2003). Therefore, these regions are thought to regulate the anticipatory response to a stressor.

As mPFC neurons are rich in glucocorticoid receptors and are stimulated by stressors, mPFC lesions enhance ACTH and corticosterone responses in rats (Herman et al., 2005). It has been reported that lesions in the anterior cingulate and vmPFC increase ACTH and corticosterone secretion following some forms of stress, such as restraint, but not to other stressors such as ether inhalation (Diorio, Viau, & Meaney, 1993; Figueiredo, Bodie, Tauchi, Dolgas, & Herman, 2003).

Thus, it has been suggested that the mPFC has a stressor-specific role in HPA inhibition. In contrast, the amygdala is mainly involved in activating the HPA axis and has a feed-forward role in stress regulation (Herman et al., 2005). Lesions in the amygdala have been shown to decrease HPA

axis responses to stress (Dayas, Buller, & Day, 1999), whereas amygdala stimulation increases HPA axis output (Dunn & Whitener, 1986).

Stress also seems to alter plasticity in the limbic system. Studies have demonstrated adverse alterations to neuronal microstructure, such as interruption of neurogenesis and retraction of dendritic processes in rats in response to stress (Sapolsky, 2003). However, other types of plasticity are considered to be beneficial. For example, the vmPFC is associated with reversal learning and with resilience after stress. This means that the vmPFC activates in the presence of one stressor and further activates or modifies its function in the presence of a new stressor (Maier & Watkins, 2010).

In addition, animal models have shown that stress modifies the expression of the limbic system´s neurotransmitters. Serotonin (5-HT) functioning in the limbic system appears to be altered after stress exposure. For example, rats exposed to physical stress showed increased levels of 5-HT in the hypothalamus, nucleus accumbens, amygdala and mPFC (Inoue, Tsuchiya, & Koyama, 1994). In addition, psychosocial stress appears to reduce the number of 5HT binding sites in the hippocampus and cingulate cortex (Flugge, 1995). Interestingly, decrease of serotonin function relates to increased levels of aggression. A study comparing CD/ODD children with controls found significantly reduced plasma 5-HIAA in the CD/ODD group relative to controls, and increased aggression in CD/ODD children was correlated with lower levels of plasma 5-HIAA (Van Goozen, Matthys, Cohen-Kettenis, Westenberg, & van Engeland, 1999).

Stressful experiences also appear to alter dopamine metabolism and release in the limbic system. A study investigating the effects of repeated exposures to restraint stress found that levels of dopamine in limbic areas of the rat increased during and following the stressor (Imperato, Angelucci, Casolini, Zocchi, & Puglisi-Allegra, 1992). These alterations appear to remain even after the stress exposure. For instance, exposure to social defeat stress during adolescence changes dopamine (DA) levels in the medial prefrontal cortex not only after the stress exposure but also as rodents enter into adulthood (Novick, Forster, Tejani-Butt, & Watt, 2011). Dopamine plays a role in reward mechanisms, behavioural activation, and goal directed behaviours (Gregor et al., 2009). Interestingly, increased DA functioning is usually associated with increased aggressive behaviour. However, studies have shown that plasma homovanillic acid (HVA), a dopamine metabolite, is reduced in young boys with CD (Van Goozen et al., 1999). In addition, a recent study reported that low levels of dopamine in the anterior cingulate cortex are associated with impairments in reversal learning. In this context, difficulties in learning through reinforcement, a characteristic of individuals with CD, may be a product of low dopaminergic levels in the mPFC.

Exposure to chronic stress has shown to cause changes in limbic architecture. For instance, a study in rats showed that after exposure to chronic stress, the anterior cingulate and medial PFC manifested alterations in dendritic length and density (Radley et al., 2006). Morphological changes

of the hippocampus (McLaughlin, Gomez, Baran, & Conrad, 2007) and amygdala (Ding, Han, & Shi, 2010) have also been shown after acute and chronic stress exposure in rodents.

Human studies looking at individuals that have experienced traumatic and stressful events, such as the loss of a child, have reported lower hippocampal volumes compared to a control group (Luo et al., 2016). Young individuals that have witnessed domestic violence, or suffered from physical or sexual abuse have shown smaller volumes in dorsolateral, and orbitofrontal cortex (Hanson et al., 2010), and increased amygdala and hippocampal volumes compared to a control group (McCrory, De Brito, & Viding, 2010). However, interestingly, children who suffered similar traumatic events and develop posttraumatic stress disorder (PTSD) show decreased vmPFC compared to controls and to those who did not develop PTSD (Morey, Haswell, Hooper, & De Bellis, 2015). This may suggest that further abnormalities in the vmPFC, affect the fear extinction which is associated with the traumatic experience.

Similarly, a fMRI study revealed that children that experienced early stress in life (e.g. neglect, verbal abuse, physical abuse) showed increased amygdala response to angry faces (McCrory, De Brito, & Viding, 2013). However, it is not known whether adversity in early life is associated with amygdala dysfunction in individuals with CD. Abnormalities in the limbic white matter of the uncinate fasciculus, fornix and cingulum have also been reported in subjects with a history of childhood maltreatment and in adults with PTSD (Choi, Jeong, Rohan, Polcari, & Teicher, 2009; Eluvathingal et al., 2006; Fani et al., 2012), suggesting that these white matter tracts are susceptible to stress.

Stressful experiences are usually interpreted with an emotional response. Activation of the HPA axis and sympathetic nervous system (SNS) generates physiological changes that heighten the experience of an emotion in response to a stressor (Heilman, 1994). Thus, as mentioned above, the stress response is highly integrated with limbic components that are involved in the evaluation and regulation of emotions, which are necessary for behavioural changes that would allow an organism to adapt to the environment. Learning from previous experiences also plays an important role in the mediation of the stress response. Given that individuals with CD are more likely to experience environmental stress (e.g., witness domestic violence) relative to typically developing peers, and display deficits in emotional processing as well as in reinforcement learning, the fronto-limbic system and HPA axis may play an important role in the neurobiology of CD.

Fronto-limbic system dysfunction plays a major role in CD. While CD research to date has focused on the role of this system in emotion regulation and decision-making, this system is also highly sensitive to stress. This is important because as discussed above, stress plays an important role in the emergence of CD. The ability to regulate emotions is particularly important when it comes to stressful situations. Inappropriate processing or interpretations of stressful information or threat may lead to psychiatric conditions, including, antisocial personality. It has been shown that

exposure to traumatic events exacerbates symptoms of conduct disorder (Greenwald, 2002). Thus, it is important to look the role that psychological stress plays in brain regions of the PFC and limbic system – so we can understand how stress may influence the neurobiology of CD. It is important to note, that the majority of information looking at the direct effects of stress on the brain comes from animal studies. However, there are indicators from functional imaging studies of individuals undergoing stressful events.

2.7 Prefrontal and Limbic Brain Areas implicated in Conduct Disorder

It has been largely argued that antisocial behaviour is caused by deficits in the frontal lobe, in particular, the vmPFC (Raine et al., 2005; Raine & Yang, 2006; Séguin, 2009). However, more recent studies have suggested that brain abnormalities in antisocial behaviour extend beyond the PFC, and involve more limbic brain areas, such as, anterior and posterior cingulate, amygdala, and hippocampus which are commonly activated during the performance of decision-making, emotion processing, and reward tasks (Bechara, 2000; Rolls, 2000; Rushworth, Behrens, Rudebeck, & Walton, 2007; Sergerie et al., 2008).

Numerous neuroimaging studies have been devoted to the investigation of anatomical abnormalities in adults with antisocial personality disorder and psychopathic traits (Craig et al., 2009; Kiehl et al., 2001; Raine, 2002; Sethi et al., 2014; Sundram et al., 2012). Relative to this large body of evidence, the number of studies devoted to the investigation of brain abnormalities in children and adolescents with CD is much smaller, especially in females. However, evidence has revealed similar abnormalities in brain areas among adults with antisocial personality disorder and individuals with CD (Sebastian et al., 2016; Yang & Raine, 2009).

2.7.1 Cortical Brain Structures

2.7.1.1 Insula cortex

Structural neuroimaging studies have revealed similar brain abnormalities in both adolescent onset (AO) and childhood onset (CO) forms of CD, revealing reduced bilateral amygdala volumes and reduced volumes of the anterior insula relative to healthy comparison subjects (Fairchild et al., 2011; Sterzer, Stadler, Poustka, & Kleinschmidt, 2007). Interestingly, reductions in anterior insula volume have also been observed in female teenagers with CD (Fairchild et al., 2013). Furthermore, reductions in anterior insula volume have also been revealed in adult populations with

psychopathic traits (de Oliveira-Souza et al., 2008). Studies using surface-based morphometry have also reported insula abnormalities (Hyatt, Haney-Caron, & Stevens, 2012). However this is not always observed (Wallace et al., 2014). The findings in anterior insula cortex are interesting, as they have been associated with empathy levels (Sterzer et al., 2007). However, a study conducted in teenagers with CD and high callous unemotional traits did not find volumetric differences in anterior insular, rather, increases in volume were found in posterior insula (De Brito et al., 2009).

In line with neuroanatomical abnormalities, functional neuroimaging studies showed reduced responses in individuals with early and adolescent onset of CD compared to healthy peers during tasks of empathy and emotion recognition (Passamonti et al., 2010). However, the opposite response (i.e. increased insula activity) was observed in females with CD (Fairchild et al., 2014).

2.7.1.2 Prefrontal cortex

The PFC is of particular interest in CD, as they play a crucial role in executive functions, and decision-making (Sonuga-Barke et al., 2016). Impaired decision making has been shown to increase the risk for antisocial behaviour (White et al., 2013). For instance, these impairments are seen in paradigms such as, reversal learning (Blair, Colledge, Murray, & Mitchell, 2001), risk-taking (Fairchild, van Goozen, et al., 2009), and passive avoidance (Finger et al., 2011) in neuropsychological tasks.

Structural neuroanatomical evidence has revealed reduced dorsolateral PFC (Dalwani et al., 2015) and orbitofrontal cortex when comparing adolescents with AB and healthy controls (Huebner et al., 2008). However, one study showed OFC reductions, only in the adolescent onset of CD subgroup (Fairchild et al., 2011), and not in the early onset CD type. Although, it has been suggested that high levels of callous unemotional (CU) traits might mediate OFC reductions (Sebastian et al., 2016), the opposite pattern has been reported in boys (i.e. increased grey matter; De Brito et al., 2009) with elevated CU traits, and in female adolescents with CD (Cope, Ermer, Nyalakanti, Calhoun, & Kiehl, 2014).

Functional MRI studies have reported reduced OFC response to reward in CD relative to controls (Crowley et al., 2010;Finger et al., 2011; Rubia et al., 2009). Reduced sensitivity to expected value of information (White et al., 2013), and increase responses to unexpected punishment (Finger et al., 2008) has also been revealed in this brain area in individuals with disruptive behaviour disorders, youths with conduct problems and substance abuse (Crowley et al., 2010). Finally, increased activations in OFC have also been reported during emotional processing tasks in youths with conduct problems (Sebastian et al., 2014). However, the opposite pattern has been described in adolescent females, showing reduced activations within the OFC relative to controls (Fairchild et al., 2014).

Reduced functional connectivity has also been revealed between the amygdala, OFC, and rostral anterior cingulate cortex in adolescents with CD compared to a healthy control group (Finger et al., 2012). In line with this, structural connectivity studies using tractography methods have revealed abnormal structural connectivity of the uncinate fasciculus (UF), which is a ventro-temporo-amygdala tract connecting the anterior temporal cortex, amygdala and the OFC.

Interestingly, male adults with antisocial personality disorder and psychopathic traits have reported a decrease in fractional anisotropy (FA; Craig et al., 2009), whereas male youths with CD have shown an increased in FA (Passamonti et al., 2012; Sarkar et al., 2013; Zhang, Gao, et al., 2014). However, diverging from findings in males, females with CD showed a preserved structural connectivity of the UF (Zhang et al., 2014).

2.7.1.3 Anterior and posterior cingulate cortex and parahippocampal gyrus

Structural MRI studies have reported grey and white matter volumetric abnormalities in the anterior cingulate cortex (De Brito et al., 2009), in the entire cingulate (Fahim et al., 2011) and parahippocampal gyrus (Wu, Zhao, Liao, Yin, & Wang, 2011) in males with CD compared to controls. Functional neuroimaging studies have mainly reported abnormal activations in the anterior cingulate during emotion processing (Lockwood et al., 2013; Sebastian et al., 2012; Sterzer, Stadler, Krebs, Kleinschmidt, & Poustka, 2005), empathy (Lockwood et al., 2013; Marsh et al., 2013), and response inhibition (Völlm et al., 2004). However, decreased sensitivity has been reported in the posterior cingulate during sustained attention tasks in CD subjects (Rubia, Smith, et al., 2009). Moreover, individuals with antisocial personality or psychopathic tendencies display reduced activation during tasks of emotion processing and conflict resolution within the anterior cingulate, posterior cingulate, and parahippocampal gyrus (Kiehl et al., 2001). Studies of functional connectivity in CD have revealed reduced connectivity between the anterior and posterior cingulate cortices under resting state conditions (Broulidakis et al., 2016). Similarly, diffusion tensor imaging studies have also revealed abnormal structural connectivity in the cingulum tract that connects medial PFC and posterior cingulate cortex in antisocial individuals with psychopathic tendencies compared to their healthy counterparts (Sethi et al., 2014).

2.7.2 Subcortical Brain Structures

2.7.2.1 Hippocampus

Reduced hippocampal volume has been reported in individuals with antisocial personality (Laakso et al., 2001), in individuals with CD (Huebner et al., 2008), however, increased hippocampal

volumes have been reported in youths with CD and psychopathic tendencies (De Brito et al., 2009). The hippocampus has also been associated with antisocial and aggressive behaviour (Soderstrom et al., 2002). An early functional imaging study of violent offenders observed unusual asymmetry in hippocampal functioning (Raine et al., 2004; Raine, Buchsbaum, & Lacasse, 1997). Functional MRI studies have also reported lower hippocampal activity during risk taking (Crowley et al., 2010), sustained attention (Rubia, Smith, et al., 2009) and emotion processing tasks (Sterzer et al., 2005) in individuals with CD, and in individuals with antisocial personality and psychopathic tendencies (Kiehl et al., 2001) compared to healthy controls.

2.7.2.2 Amygdala

Due to its involvement in aversive conditioning, emotion processing, and fear processing, the amygdala is a crucial region of interest in studies of antisocial populations. Fearlessness is hypothesised to be a key characteristic of antisocial individuals (Raine, 1997), and has been investigated using aversive conditioning tasks (Schneider et al., 2000). Increased activations in the dorsolateral PFC and amygdala have been reported during the acquisition phase of aversive conditioning in individuals with antisocial personality disorder (Schneider et al., 2000). Reduced function of the amygdala to distress cues such as fearful (Jones et al., 2009; Marsh et al., 2008) and angry and sad (Passamonti et al., 2010) facial expressions have also been reported in adolescents with CD or disruptive behaviour disorders (DBD). In addition, reduced amygdala activity has been reported during emotion processing tasks in youths with CD (Herpers, Scheepers, Bons, Buitelaar, & Rommelse, 2014; Jones et al., 2009a; Marsh et al., 2008; Passamonti et al., 2010; Sebastian et al., 2012; Sterzer et al., 2005).

Structurally, the amygdala has been shown to be reduced in volume in youths with CD (Sterzer et al., 2007), and in individuals with antisocial personality (Boccardi et al., 2011), compared to healthy controls. Structural connectivity between the amygdala and vmPFC is altered in adolescents and adults with antisocial behaviour (Passamonti et al., 2012; Sarkar et al., 2013).

However, the involvement of the amygdala may be dependent on the presence of callous unemotional traits. A study examining neural responses to subliminal facial expressions showed that high or low rates of callous unemotional traits influenced the extent of amygdala activation in response to fearful faces (Viding et al., 2012). Moreover, a study measuring empathy for pain of others, showed that differences in CU traits were highly correlated with differences in amygdala responses (Marsh et al., 2013).

2.7.2.3 Thalamus and Hypothalamus

Reactive aggression is a core component of the aggressive type of CD. The hypothalamus was one of the first brain structures to be associated with rage and aggressive behaviour (Brazier, 1988). Some of the earliest experiments on aggression discovered that sham rage in cats was eliminated after ablating the hypothalamus (Bard, 1928). However, reactive aggression is thought to be mediated not only by the hypothalamus but also by a neural system including the hypothalamus, amygdala and the periaqueductal grey mater (Blair, 2004; Gregg & Siegel, 2001).The thalamus and amygdala interact to retrieve an emotional pattern of images or circumstances to initiate a similar response (e.g. muscular, hormonal, and endocrine) in a subsequent situation. However, when the emotional pattern or stimuli changes and become unfamiliar, the vmPFC plays a critical role in initiating or moderating the new response (LeDoux, 2003).

Thus, it was suggested that reactive aggression is not triggered by a basic threat response in antisocial individuals, but instead is due to vmPFC dysfunction, as antisocial individuals may struggle more than healthy controls to cope with the new or unfamiliar stimuli (Blair, 2010). This is because, a key part of the basic threat circuitry, the amygdala, appears to be under-responsive, rather than over-reactive, and the other core elements, hypothalamus and periaqueductal grey mater, do not appear to be hyper-responsive in antisocial individuals (Blair, 2010). For instance, it was concluded that reactive aggression in antisocial adults with psychopathic tendencies, is due to their increased susceptibility for experiencing frustration (Blair, 2010). However, the literature regarding these brain structures in antisocial individuals is very scarce.

2.7.2.4 Striatum

Deficits in reinforcement learning is a key characteristic of CD. For instance, studies investigating youths with CD have demonstrated increased activation in the ventral striatum in paradigms assessing reward sensitivity (Bjork, Smith, Chen, Hommer, & Makris, 2010). However, increased activation of the VS has also been shown in tasks assessing aversive stimuli in youths with CD relative to controls (Decety, Michalska, Akitsuki, & Lahey, 2009).These findings suggest that deficits in reinforcement learning are associated with striatal dysfunction. Moreover, structural studies have also reported reduced volume in dorsal and ventral striatum in adolescents with CD compares to healthy peers (Fairchild et al., 2013).

In conclusion, brain regions of the fronto-limbic system have been shown to be abnormal in CD. However, it appears that their contribution to the disorder varies depending on the CD subtype, and the gender of the subject. For example, the insula seems to play a role in empathy and reduced volume in this structure is not moderated by the age of onset nor the sex factor. However, the neural mechanisms (or function) of the insula in CD seem to differ across the sexes. The vmPFC

seems to influence deficits on reward based decision-making. However, abnormalities seem to differ depending on the age of onset of CD, and on levels of CU traits. The anterior cingulate seems to play a critical role in emotion regulation, and the posterior cingulate in sustained attention. The amygdala is involved in processing affective stimuli, and the presence of CU traits may modulate its function, with amygdala hyporeactivity observed in those with elevated CU traits and hyperreactivity in those with low or normative levels of CU traits. The hippocampus appears to be involved storing and retrieving emotional memories. The striatum appears to have a vital role in reinforcement learning. Thus, each of these structures contributes to the aetiology of CD, and problems in emotion regulation or attention might explain why individuals develop CD. Finally, the thalamus and hypothalamus have been associated with reactive aggression. However, the evidence for an association between structural or functional changes in this brain region and antisocial behaviour in humans is very limited.

2.8 Hypothalamic-Pituitary-Adrenal Axis Alterations in Conduct Disorder

Two main theories are used to explain reduced cortisol levels and attenuated reactivity in CD. The risk-seeking theory proposes that the association between low cortisol and DBDs is as a result of antisocial individuals having elevated thresholds for stress and being physiologically under aroused. As a consequence, these individuals might seek out stimulation (including increased cortisol levels) by engaging in sensation-seeking behaviours, such as breaking into houses or robbery (Zuckerman, 1979). Although this theory explains why these individuals engage in risk taking behaviour, it does not explain their lack of engagement in thrill seeking behaviours. (e.g. extreme sports), nor their involvement in repetitive behaviours with negative consequences (e.g. school expulsion).

The fearlessness theory states that low hypothalamic- pituitary-adrenal axis activity indicates that antisocial individuals have a lack of fear, or are less anxious. Therefore, reduced levels of arousal lead to low fear and anxiety arousal, which may lead to behavioural disinhibition in which youths with CD appear to be unconcerned with negative outcomes related to their behaviours (Burke et al., 2002; Raine, 1993), and are not afraid of receiving any type of punishment (Raine, 1993). For instance, it has been reported that children with DBD display reduced cortisol reactivity to stress in tasks assessing frustration and anger (Van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & van Engeland, 2000) which has been associated with punishment processing deficits (Matthys, Vanderschuren, & Schutter, 2013).

Previous research on the HPA axis has shown low cortisol levels in antisocial individuals (Van Goozen et al., 2007). It has been suggested that these individuals may be under-aroused, or that

their HPA axis is over-regulated (Kruesi, Schmidt, Donnelly, Hibbs, & Hamburger, 1989). Although few of these studies have been carried out in children and adolescents with antisocial behaviour, there have been varying findings in studies comparing levels of cortisol young people with AB and their typically developing peers.

2.8.1 Basal cortisol levels in Conduct Disorder

A large number of studies looking at HPA axis activity in individuals with CD (Pajer, Gardner, Rubin, Perel, & Neal, 2001; Popma, Doreleijers, et al., 2007; Vanyukov et al., 1993) and severe ODD (Van Goozen et al., 1998) have demonstrated reduced basal cortisol levels. This also appears true for children with aggressive tendencies (Tennes, Kreye, Avitable, & Wells, 1986), and those who show hostile behaviour towards their teachers (Tennes & Kreye, 1985), as well as in adolescents with CD and CU traits (Von Polier et al., 2013). The low basal cortisol levels of the latter study appeared to be influenced by individuals with CD and high levels of CU traits when compared to those with CD and low levels of CU traits. Although comorbid internalising disorders (e.g. anxiety and depression) are more common in girls with CD than their male counterparts (Moffitt et al., 2001), low cortisol levels have also been observed in adolescent girls with CD (Pajer et al., 2001). This is interesting, as internalising disorders have generally been associated with higher, rather than lower, cortisol levels (Nader & Weems, 2011).

On the other hand, the opposite direction of findings have also been reported, whereby individuals with CD show increased basal cortisol levels, however, these findings are only seen at certain times of the day (e.g., the evening) (Fairchild, van Goozen, et al., 2008; Van Bokhoven et al., 2005). Finally, other studies have found no significant relationship between basal cortisol levels and disruptive behaviour disorders in children (Van Goozen et al., 2000).

2.8.2 Cortisol response to stress in Conduct Disorder

Research has also shown an attenuated HPA axis response to stress in adolescents with CD (Fairchild, van Goozen, et al., 2008; Howard B Moss, Vanyukov, & Martin, 1995; Van Goozen et al., 1998, 2000). In addition, studies of maltreated children have also reported reduced cortisol responses to stress (Hart, Gunnar, & Cicchetti, 1995). Therefore, findings of lower stress reactivity in CD may be explained by increased exposure to early adversity. In fact, it is thought that the difficulties with behavioural and emotional regulation experienced by individuals with CD may be related to exposure to adversity early in their lives (van Goozen, Fairchild, & Harold, 2008).

However, the associated between cortisol reactivity and cognitive-affective processing is not yet understood in youths with CD. Studies have also demonstrated contrary findings whereby increased cortisol responses to stress are associated with conduct disorder severity (measured by number of symptoms), although this was not seen in the whole CD sample (McBurnett et al., 2005). Also, higher cortisol levels in a non-clinical population with rule breaking behaviour have also been reported (Halpern, Campbell, Agnew, Thompson, & Udry, 2002).

2.8.3 Possible explanations of Inconsistent findings

The inconsistencies in the results of neuroendocrine studies could be a result of several methodological variations. For instance, cortisol assessments have with different methodologies, e.g. single measurements versus multiple daily measurements, plasma samples vs saliva samples – (where the procedure of obtaining blood could be stressful per se), and under stressful versus basal (naturalistic) conditions. The range of ages has also varied widely, and studies have included pre- and post-pubertal individuals. The types of participants included in the studies (clinical versus population-based samples) have also differed. Finally, it is important to note that conflicting findings might also be attributed to the heterogeneity that characterises CD.

2.8.3.1 Heterogeneity of Conduct Disorder and findings in cortisol levels

It has been considered several domains influence the pattern of high or low cortisol levels, such as, the type of antisocial behaviour, internalising comorbidity, and early adversity (Hawes, Brennan, & Dadds, 2009).

It seems that the attenuated cortisol reactivity during stress is more predominant in adolescents with a CO-CD compared to those with AO-CD (Fairchild, van Goozen, et al., 2008). In addition, the aggressive and non-aggressive sub-types of CD are thought to have different aetiologies, and some studies have shown that individuals with CD and high aggression show high cortisol levels, whereas those with CD and non-aggressive behaviour show low cortisol levels (Van Bokhoven et al., 2005; Von Polier et al., 2013). In addition, studies have demonstrated a negative relationship between cortisol levels with aggressive tendencies (Alink et al., 2008) and antisocial behaviour (AB; Popma, Vermeiren, et al., 2007). However, it has been argued that this relationship may not necessarily be attributed to AB in general, but rather it may be explained by the presence of impulsivity and CU traits (Von Polier et al., 2012).

Patterns of high cortisol levels are observed in individuals displaying reactive aggressive forms of CD (Van Bokhoven et al., 2005). As reactive aggression is related to internalising disorders such as anxiety (Frick & Ellis, 1999) and greater cortisol levels have been reported in individuals with anxiety (Kallen et al., 2008), it is possible that anxiety may be influencing the observed higher cortisol levels in some CD groups. In fact, a study which compared a group with pure CD and CD with comorbid anxiety, observed that higher cortisol levels were only present in those with comorbid anxiety. (McBurnett et al., 1991). This highlights the importance to consider that a large number of children and adolescents with CD will have comorbid internalising psychiatric disorders (e.g. anxiety and depression) rather than CD alone.

As mentioned above, early adversity is highly associated with the development of CD. However, several studies examining the relationship between salivary cortisol and CD did not control for environmental stress (Popma, Doreleijers, et al., 2007). This may be important, a study which looked at the HPA –axis pattern in patients with major depression disorder (MDD), found that the impact of maltreatment has been shown to significantly increase cortisol levels in children with MDD, but only in those currently undergoing environmental stress. Thus HPA –axis activity in maltreated children may be dependent on whether they are currently being exposed to a threatening environment (Kaufman et al., 1997). Therefore, comorbid internalising disorders (e.g. depression, anxiety) and exposure early environmental adversity should be considered when studying CD.

In conclusion, although the relationship between cortisol levels and AB has been inconsistent, there is strong evidence that supports the linkage between this neurobiological marker and CD. In particular, attenuated cortisol in response to stress is the most frequently reported finding in studies of individuals with CD. However, this relationship seems to be dependent on different factors such as, internalising comorbidity, and CD subtypes (e.g. aggressive vs non-aggressive, high CU vs low CU traits.

2.9 Aims and Thesis overview

As reviewed above, CD is accompanied by alterations in brain regions of the frontal and limbic system as well as alterations in cortisol secretion under basal conditions and in response to stress. However, there are some inconsistencies in the literature. A possible reason for these discrepancies may be due to the use of small samples. In addition, there is a clear gap in the literature regarding sex differences in CD, as most of the research in CD has been conducted in male-only samples. Therefore, the broad aim of this thesis is to investigate fronto-limbic and hypothalamic-pituitary-adrenal axis systems in a larger sample of youths with CD. A second, but no less important, broad aim is to examine sex differences in the relationship between CD and these brain systems.

In **Chapters 1 and 2** we reviewed the literature that provides a basis for this thesis. Having established the general aims of this thesis, I will now further outline the work that will form this PhD. Firstly, **Chapter 3** will provide an overview of the principal data source of this thesis: the FemNAT-CD project. It will further describe the general methods used to obtain the data that was used in all of the studies described in this thesis. **Chapter 4** will investigate group and sex differences in the cortical structure of the key prefrontal and limbic brain regions that have previously been implicated in the pathophysiology of CD. **Chapter 5** will comprehensively study differences in the shape of subcortical limbic structures in youths with CD relative to healthy controls. **Chapter 6** will further investigate whether there are significant differences between CD and control groups in key white-matter pathways of the fronto-limbic system (i.e., the cingulum, fornix and uncinate fasciculus) and examine whether these effects are similar in males and females. **Chapter 7** aims to look at differences between participants with CD and healthy controls in salivary cortisol responses to a laboratory-based psychosocial stress procedure: the Trier Social Stress Test for Children (TSST-C). We will further investigate whether there are main effects of sex and sex-by-diagnosis interactions on these measures. Within this chapter, a proof of concept analysis will examine the relationship between cortisol reactivity and brain structure. Finally**,** **Chapter 8** will provide a general discussion of the findings and themes raised by this thesis. It will cover the key findings across the chapters, they will further be synthesised and discussed with regards to previous studies in the field. Strengths and limitations of the thesis as a whole will also be described, and the clinical and scientific implications of the work will be discussed.

Chapter 3 General Methods

This chapter will review the general methods that were used in the Neurobiological and Treatment of Adolescent Female Conduct Disorder (FemNAT-CD) study, as a general methodological introduction to the field.

3.1 The FemNAT-CD project

3.1.1 Overview

The FemNAT-CD study is a large-scale, multi-disciplinary cross-European study that aims to investigate the neurobiology of female CD, and the extent to which males and females with CD share similar clinical, neuropsychological, and neurobiological profiles.

The original aim of the project was to recruit 1,840 children and adolescents aged between 9 to 18 years old. Eleven universities and psychiatric clinics across Europe (Southampton and Birmingham, UK; Frankfurt, Aachen, and Heidelberg, Germany; Basel, Switzerland; Amsterdam, the Netherlands; Bilbao and Barcelona, Spain; Athens, Greece; Szeged, Hungary) contributed to this project. The project also aims to conduct a longitudinal study by re-assessing a sub-sample of 240 participants 18 months after their first assessment. However, this thesis will focus on a case-control study design.

The project mainly consists of six work-packages:

1) Genetic and Epigenetics, which will investigate genetic and environmental risk factors for CD and gene-environment interactions. 2) Neurocognitive Characteristics, which includes 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) and cortisol response during emotional and stressful tasks, as well as during basal conditions

4) Neuroimaging; involving the use of magnetic resonance imaging (MRI) techniques to study brain structure and function.

5) A randomised controlled trial, evaluating group-based psychological treatment using dialectical behavioural therapy for girls with CD living in welfare institutions.

6) Oxytocinergic and serotonergic transmission, including animal models of aggression, and testing the effect of manipulating brain levels of serotonin and oxytocin on aggression and emotion processing.

Members of the Fem-NAT CD consortium study have been involved in the design of the study as a whole, as well as collecting and sharing data across sites in a standardised way. Each site has been allocated specific research questions, and it is agreed that data would be shared upon mutual consent of the principal investigators of each site.

While I was involved in collecting data for all of the aforementioned work packages, this thesis mainly contributes towards work packages 3 and 4. However, it also used data from work package 2 (i.e., questionnaires). The specific aims and contribution of this thesis to the Fem-NAT CD project were described in the 'aims of this thesis' section above (Chapter 2.9). Only measures, methods and procedures relevant to this thesis will be described below.

Design

This thesis conducted a case-control study design.

3.1.2 Ethical issues and approval

Local ethics committees approved the study at each site. In Southampton, this project received ethical approval from the University of Southampton's Ethics Committee, the Research Governance Office of the University of Southampton (ERGO Ref No. 8215), the Southampton City Council Children's Services Directorate, the Hampshire County Council Research and Evaluation Unit, and the Edgbaston NHS Research Ethics Committee (REC ref 13/WM/0483).

We subsequently obtained approval from the Research and Ethics Committee of the University of Reading for the neuroimaging component of the study which was carried out at the University of Reading (see below for details).

3.1.3 Recruitment

Participants were recruited from different sources such as mainstream schools and further education colleges in Southampton and Reading (via mail-out information sheets and contact details forms, invited presentations in school assemblies and the distribution of flyers, information packs, or e-mails to school distribution lists, Appendix A.1), and Facebook by using the platform available at: www.callforparticipants.com.

Individuals with CD were also recruited through pupil referral units (PRUs) or education centres. The PRUs are facilities that provide education to children who cannot attend mainstream schools due to a range of different reasons (e.g. school exclusion, emotional and behavioural difficulties). Participants were also recruited through the Southampton, Hampshire and Reading Youth Offending Services, and Child and Adolescent Mental Health Services (CAMHS) in the NHS in the local area.

Before booking an appointment, we conducted a brief telephone screening to ensure that participants were not affected by some of the exclusion criteria such as traumatic brain injury, genetic disorders (e.g. Down syndrome), neurological disorder (e.g., cerebral palsy) and autism or psychosis.

3.1.4 Interview

During initial contact with potential participants, we provided information regarding the study, including the rationale, the duration, and the financial reimbursement for their time. This meeting was usually held at the participant's home, however, if they preferred, it was held at the University. They were fully informed of the study requirements and potential risks. They were given the opportunity to ask any questions before giving informed consent. The young people themselves gave consent if they were aged ≥16 years, however, for younger participants, at least one parent/carer gave written informed consent on their behalf, and they provided written assent after being given a full description of the study (see Appendix A.2). Participants were told that they could withdraw from the study at any point.

Following this, participants and their parent /carers were interviewed in separate rooms for confidentiality purposes. We used the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL; Kaufman et al., 19997) to test for any psychiatric condition. The K-SADS-PL is a semi structured interview assessing symptoms of psychiatric disorders covering five main domains: Affective, psychotic, anxiety, and behavioural disorders, substance abuse and other disorders. This study evaluated for the presence of 23 disorders based on DSM-IV criteria (see Appendix B). Trained post-graduate staff, conducted the K-SADS- PL at each site.

After the interview was completed, the 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. A current research diagnosis of CD was given if the parent or participant reported three or more of the 15 symptoms which had to be occurring over the previous 12 months as well as at least one symptom in the last six months (APA,2013). Finally, the

researchers decided whether the participant fully met the inclusion criteria for the study and ensured that they were not affected by the exclusion criteria (see **Table 3.1**).

Table 3.1 - Inclusion and exclusion criteria

Inclusion Criteria	Exclusion criteria
Age 9 to 18 years old	Age <9 years old and >18 years old
IQ >=70	Any chronic or acute neurological disorder (eg. Cerebral palsy, current treatment for epilepsy, history of moderate to severe traumatic brain injury
Good level of spoken English	Current clinical diagnosis of Bipolar Disorder or Mania according to ICD-10, DSM-IV- or DSM-5
Inclusion criteria for controls: Not meeting current criteria for any psychiatric disorder in the K-SADS-PL and no past ODD or CD diagnosis	Known monogenetic disorder (eg. Fragile-X-syndrome, Down's syndrome, Prader-Willi-Syndrome)
Inclusion criteria for CD group: Meeting a current diagnosis for CD on the basis of the K-SADS-PL OR 9-12 years: meeting full criteria for ODD plus at least 1 Conduct Disorder symptom in the K-SADS-PL OR >=13 years: meeting full criteria for ODD + at least 2 CD symptoms in the K-SADS-PL	Clinical diagnosis of autism spectrum disorder according to ICD-10, DSM-IV or DSM-5 (current or lifetime)
	Clinical diagnosis of schizophrenia according to ICD-10, DSM-IV or DSM-5 (current or lifetime)
	Additional exclusion criteria for controls: -Any known current psychiatric diagnosis (besides learning disorder) -Any history of ODD, CD, ADHD, Bipolar Disorder or Mania.

Key: K-SADS-PL, Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version; CD, conduct disorder; ODD, oppositional defiant disorder; ADHD, Attention-deficit/hyperactivity disorder

3.1.5 Assessing inter-reliability of the K-SADS-PL diagnostic interview

To assure agreement of clinical assessments between sites, each site performed an inter-rater reliability (IRR) procedure. This was done on a diagnosis level, rather than per symptom/item level. The IRR assessment started only after successful training with K-SADS-PL (training in University of Southampton was supervised by Dr. Graeme Fairchild- who has experience in using this diagnostic tool from previous studies). Each site rated 5 patients with CD and 3 healthy controls by two interviewers. Aachen, Basel, and Frankfurt filmed the diagnostic interviews and asked another member of the research team to rate the interview (without any exchange between the raters). At the UK sites (Birmingham and Southampton), two members of the research team (one interviewer and one rater) attended to the same interview and coded each diagnosis separately. Once, this procedure was finalised for each site, data from a total of 75 patients were added to the IRR-database. Percent agreement between raters and Cohen's kappa (κ) with the respective 95%-

confidence interval was calculated (in SAS 9.4) for several diagnoses such as ADHD, CD, ODD, Major Depressive Disorder, and Post-traumatic stress disorder (PTSD) (the assessment included scores for past and current diagnosis). The Cohen's kappa coefficients for each current diagnosis were: ADHD=0.83, CD=0.90, Major depressive disorder=0.86, ODD=0.94, PTSD=0.58, and agreements across raters was >= 92% in all instances, which indicates strong to almost perfect agreement between raters (Landis & Koch, 1977).

3.1.6 Additional assessments relevant to the thesis

3.1.6.1 Estimated IQ

Intelligence quotient (IQ) was assessed on the interview day. In the UK the Wechsler Abbreviated Scale of Intelligence- I (WASI; Wechsler, 1999) was used. We used the two-subtest versions as it provides a rapid estimate of full-scale IQ. In Germany (Frankfurt, Aachen) and Switzerland (Basel), IQ was calculated using two-subscales of Wechsler Intelligence Scale for Children (WISC-IV; Wechsler, 2003). The scores from the five sites were transformed into z-scores and then combined to form the estimated full-scale IQ.

3.1.6.2 Neuropsychological assessment

On a different day, participants were invited to complete several questionnaires and neuropsychological tasks, and their physiological activity (heart rate and skin conductance) was also assessed. Saliva samples were also collected to provide DNA data and measures levels of hormones such as, cortisol, DHEA-S, testosterone, oestrogen, progesterone, oxytocin and vasopressin under resting (basal) conditions. This testing session lasted between 3 and 3.5 hours. Three questionnaires from this battery were used in this thesis to assess clinical characteristics: the Youth Psychopathic traits Inventory (YPI, Appendix C.1), Reactive-Proactive aggression Questionnaire (RPQ, Appendix C.2), and Pubertal Developmental Scale (PDS, Appendix C.3 and C.4). Finally, some additional questions were asked as part of a checklist for data collection in each site. The relevant sections of this checklist for this thesis were the questions regarding handedness (relevant to Chapter 4-6) and contraceptive use (relevant to Chapter 7).

3.1.6.3. Questionnaires

The Youth Psychopathic traits Inventory (Andershed, Kerr, Stattin, & Levander, 2002) measures self-reported total psychopathic and callous-unemotional traits. This self-report measure contains 50 items relating to psychopathic tendencies (e.g., I like to do exciting and dangerous things, even if it is forbidden or illegal), scored on a 1-4 point scale (does not apply at all, does not apply well,

applies fairly well, or applies very well. Total scores range from 50 to 200, with higher scores indicating higher levels of psychopathic tendencies. This inventory includes three dimensions that make up the total score of psychopathic traits: Callous-unemotional, grandiose-manipulative and impulsive-irresponsible dimensions.

The Reactive-Proactive aggression Questionnaire (RPQ; Raine et al., 2006), distinguishes between reactive (e.g., yelled at other when they have annoyed you) and proactive/instrumental forms of aggression (e.g., vandalized something for fun). Each item was rated based on the frequency of occurrence (i.e., 0 = never, 1 = sometimes, 2 = often). The questionnaire contains 23 items, 12 items for proactive and 11 for reactive aggression.

The Puberty Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988), assesses pubertal stage using words, rather than pictures, in those participants aged 12 and above. This self-report measure contains 5 questions regarding the respondent's physical development (e.g., growth in pubic hair). The participants are instructed to choose between five options that best describes them ('Has not yet started', 'Has barely started', 'Is definitely underway', 'Seems complete', and 'I don't know').

3.1.7 Site qualification procedure for MRI data

Acquiring magnetic resonance imaging (MRI) data across multiple centres can introduce significant variability in the data and analysis (e.g., different image quality). Functional, structural and diffusion MRI scans were acquired using a Phillips 3T (Birmingham: Achieva) and Siemens 3T (Frankfurt and Southampton: Tim Trio; Basel, and Aachen: PRISMA) scanners with actively shielded magnetic field gradients (maximum amplitude 40 mT/m), using a 32 (Frankfurt, Southampton and Birmingham) or 20 (Aachen and Basel) channel head coils. Therefore, to ensure comparability of structural and diffusion MRI data between the five sites, each site standardised their protocol for image acquisition and went through a site qualification procedure. The procedure included the scanning of two phantoms: an American College of Radiology phantom (to assess structural MRI sequences), and a functional Biomedical Informatics Research Network phantom (to assess functional MRI sequences), as well as scanning a human volunteer. Once collected, the images were reviewed by the same, experienced MRI physicist (based in Birmingham), and each site adjusted the scanning parameters according to the physicist's recommendations until the protocols were equivalent and if necessary, scanning artefacts had been corrected. The sites were not allowed to start data acquisition until they had successfully passed this site qualification.

3.1.8 MRI testing procedure

On their third visit, participants were invited to their respective imaging centre for an MRI session lasting between 1.5-2 hours. Due to problems in accessing the MRI scanner at the Southampton General Hospital, the research team a Southampton used a scanner based at the Centre for Neuroscience and Neurodynamics (CINN) at Reading University On the day of testing, participants travelled to the CINN neuroimaging centre at Reading University. All travel expenses were reimbursed.

3.1.9 MRI safety

Participants were screened before, and again, on the day of, the brain scan, for eligibility for MRI scanning (this was done with their parents if participants were under 16 years of age). They were asked whether they had any metallic objects in their body (e.g. as a result of an accident), or if they had undergone surgery involving insertion of metal objects (e.g. cardiac stents or metal pins). Female participants were also asked if there was any possibility that they could be pregnant or whether they have had an intrauterine device or coil implanted (see Appendices C.5 and C.6 for MRI safety screening questions). In case where participants had undergone surgery or had had intrauterine coil inserted, contact was made with their GP to check that the coil did not contain ferromagnetic elements.

3.1.10 MRI scanning procedure

Each site had different research questions. Therefore, not all the sites followed the same scanning order, nor they acquired the same sequences. For example, the research team at Frankfurt acquired T1-weighted structural MRI images but did not obtain diffusion MRI (dMRI) data. In Southampton, we first acquired a T1-weighted image, followed by two functional MRI (fMRI) tasks (an emotion processing task and a reward learning task). We additionally ran a diffusion MRI scan, followed by the last fMRI task (empathy for pain). The T1-weighted image was reviewed during the scanning session and repeated as needed until high-quality image was available.

Prior to the scanning session, participants were familiarised with the scanner and the tasks they would be doing during the scanning session (e.g., they completed a few trials of each fMRI task). They were further instructed not to cross their arms and legs and were told they could stop at any time if they felt uncomfortable by pressing a panic ball. The assistant operator would ensure that the participant was comfortable throughout the session, and reminded them of the instructions for each of the following tasks or sequences. For the purposes of this thesis, only the structural and

diffusion MRI data will be considered, and the specifics of the methodology that was used to address each aim will be described in its corresponding chapter.

Chapter 4 Fronto-Limbic, Cortical and Subcortical Structures in Youths with Conduct Disorder

4.1 Abstract

Conduct Disorder (CD) has been associated with structural abnormalities in a range of frontal and limbic brain regions. However, there have been some discrepancies between studies in terms of the location or direction of the structural changes, which may partly be due to the use of small samples. Importantly, few studies have examined the influence of sex on the findings, with most studies restricted to males only. To address these gaps in the literature, this study tested the largest sample of males and females with CD recruited to date. We acquired data from 156 (79 females) youths with CD and 159 (80 females) typically developing youths. Structural MRI data were collected, and surface-based morphometry analysis was used to assess different properties of the cortex such as cortical thickness, surface area and volume. Key brain regions of the frontal and limbic regions, such as, dorso-lateral, medial, and ventromedial prefrontal cortex, insula and anterior cingulate cortex, as well as subcortical (amygdala, hippocampus, and striatum) structures, were selected as regions of interest. Furthermore, we assessed psychopathic, impulsive, narcissistic and callous-unemotional traits using the Youth Psychopathic traits Inventory. Our findings revealed that adolescents with CD showed reduced thickness, surface area, and cortical volume in the right superior frontal area (including the gyri and sulci) compared with typically-developing youths. Sex did not moderate these CD-related effects. Furthermore, we observed an inverse correlation between the cortical thickness of the superior frontal area and psychopathic and impulsive traits in the CD sample. Cortical thinning in the superior frontal gyrus may contribute to the impulsive and reckless decision-making displayed by youths with CD. Our results are not consistent with previous findings of structural abnormalities in the insula, amygdala or ventromedial prefrontal cortex. The present findings indicate that sex does not moderate the relationship between CD and alterations in the structure of frontal and limbic areas. Future studies investigating the pathophysiology of CD would benefit from using whole-brain approaches in which posterior brain regions can also be investigated.

4.2 Introduction

In recent years, it has been debated whether conduct disorder (CD) manifests similarly among young males and females (Berkout, Young, & Gross, 2011). It is estimated that CD is more prevalent among males than females, with a lifetime sex ratio CD of 2.4:1 in favour of males

(Moffitt, Caspi, Rutter, & Silva, 2001). It has been claimed that this fact could be attributed to the approach that was taken with the generation and validation of the diagnostic criteria, as it mainly relied on male samples (Moffitt et al., 2008). For instance, the diagnostic criteria are more likely to diagnose the more overt antisocial behaviours of males (e.g., theft, physical fights) rather than more covert forms of antisocial behaviour (e.g., manipulation, lying), which are more frequently displayed by antisocial females (Leadbeater et al., 1999).

It has been proposed that the genetic, environmental factors and neurobiological influences underpinning CD may differ across the sexes (Meier et al., 2011; Smaragdi et al., 2017). It has been proposed that biological correlates of antisocial behaviour would be stronger in females than in males (Cloninger, 1978). However, it has also been argued that biological mechanisms underlying antisocial behaviour in males and females do not differ from each other (Moffitt et al., 2008). Research in neuropsychological measures of executive functioning (i.e. assessing the abilities of inhibition/attention, and decision making), have shown divergent results in males and females with CD (Hartung et al., 2002; Sidlauskaite et al., 2017), however few neuroimaging studies have investigated the differences in brain structure and function between boys and girls with CD. Although, recent studies have supported that CD has different clinical characteristics (e.g., sex differences in risky behaviour), (Holliday, Ewing, Storholm, Parast, & D'Amico, 2017), as well as sex-specific neural alterations (Fairchild et al., 2014; Menks et al., 2017., Smaragdi et al., 2017), to date few studies have directly compared males and females, limiting our understanding of whether the neurobiological bases of CD are similar or different among males and females (McCarthy, Arnold, Ball, Blaustein, & De Vries, 2012).

Understanding neurobiological differences in CD would contribute to the debate of whether the causes of antisocial behaviour are similar or different across the sexes. Moreover, if CD is sex-specific, as it has been suggested, and given that CD is less frequently diagnosed in females than males, it may be thought that females are protected from developing CD relative to males. Therefore, understanding sex-specific protective agents may lead to better potential therapies, or identification of new drug targets (McCarthy et al., 2012).

A key characteristic of individuals with CD is that they tend to reoffend or commit antisocial acts despite threats of punishment, such as, arrest by the police, expulsion from school or parental discipline at family home. Thus, a well-established model that explains the neurobiological factors involved in understanding CD consists of three interconnected psychological domains: punishment processing, reward processing, and cognitive control. These domains are regulated by interconnected neurobiological systems (e.g., limbic system and the hypothalamic-pituitary-adrenal axis) (Matthys, Vanderschuren, Schutter, & Lochman, 2012).

When considering brain structure and CD, the prefrontal cortex and limbic systems are key brain regions associated with decision-making, autonomic and stress/threat response (Hänsel & von

Känel, 2008). In fact, neurodevelopmental theories have attributed the emergence of CD to dysfunctions of frontal and limbic brain regions (Blair, 2007; Blair, 2013), these include the amygdala, striatum, ventromedial prefrontal cortex (vmPFC), dorsolateral prefrontal cortex (DL-PFC), insula, and posterior cingulate cortex (Ameis et al., 2013; Anderson & Kiehl, 2012; R. J. R. Blair, 2007; Oostermeijer et al., 2016; Raine, Lee, Yang, & Colletti, 2010). Neurodevelopmental models propose that dysfunction in the aforementioned brain structures leads to impaired stimulus-reinforcement learning and reduced responsiveness to distress cues (Blair, 2013; White et al., 2013). This suggests that there might be brain alterations in areas associated with fear conditioning, which if impaired may lead to deficits in punishment processing and cognitive control. Interestingly, these brain regions also show high expression of glucocorticoid receptors suggesting they are stress/threat -sensitive (Lupien et al., 2009).

4.2.1 Previous imaging studies of CD

In line with cognitive and neurodevelopmental models, functional MRI (fMRI) studies have shown abnormal activation in the amygdala in individuals with CD during emotion processing tasks, as well as in the striatum, the nucleus accumbens (NAcc) and prefrontal cortex (PFC) during decision-making tasks (Crowley et al., 2010; Finger et al., 2008, 2011; Marsh & Blair, 2008; Marsh et al., 2008; White et al., 2008, 2012). However, a recent fMRI meta-analysis in youths with conduct disorder did not observe abnormal activations in the amygdala under any of the tasks assessing executive functions (i.e., both cool and hot) and emotional processing. The most prominent abnormal activity was seen as lower activations in the anterior cingulate cortex (ACC), medial-prefrontal cortex, and ventral striatum in youths with CD relative to their comparison group. However, when the sample was further explored by selecting a subsample of youths with conduct problems and psychopathic traits, it was reported that relative to controls, youths with conduct problem and psychopathic traits showed decreased activation in the ventromedial prefrontal cortex (vm-PFC), hypothalamus, thalamus, ventral striatum and increased activation in the DL-PFC (Alegria, Radua, & Rubia, 2016).

Furthermore, similar to fMRI studies, structural MRI studies of young people with CD have revealed brain anomalies in structures associated with decision–making and fear conditioning such as, the amygdala, ACC, DL-PFC, vmPFC, and insula (Rogers & De Brito, 2016).

4.2.2 Studies using voxel-based morphometry

A recent structural MRI (sMRI) meta-analysis looking at studies using voxel based morphometry (VBM) techniques in youths with conduct problems reported that the most consistent brain

abnormalities are found in frontal and limbic lobe areas (Rogers & De Brito, 2016). However, to date, VBM studies have shown some inconsistencies. For instance, lower grey matter volume (GMV) has been reported in CD groups compared to typically developing controls in orbitofrontal cortex ((OFC);Fairchild et al., 2011; Huebner et al., 2008; Sebastian et al., 2015), ACC (Sebastian et al., 2015; Sterzer, Stadler, Poustka, & Kleinschmidt, 2007) and vmPFC (Olvera et al., 2014). GMV reductions in temporal regions, such as the amygdala, insula and hippocampus have also been detected (Fahim et al., 2011; Fairchild et al., 2011, 2013; Huebner et al., 2008; Sterzer et al., 2007; Stevens & Haney-Caron, 2012). However, the opposite effect (i.e. increased volume) in the aforementioned regions has also been reported (De Brito et al., 2009). In addition, it is important to note that effects in the aforementioned brain regions are not always detected across the studies. For instance, changes in the vmPFC, a region that is well-documented to show deficits in CD, are not always observed in either males (Dalwani et al., 2011; Sterzer et al., 2007) or in females with CD (Fairchild et al., 2013). Furthermore, no differences in brain morphology were reported between a CD and a healthy control group (Michalska, Decety, Zeffiro, & Lahey, 2015; Olvera et al., 2014). However, reduced GMV were instead identified in the right medial and superior frontal gyrus (SFG), the ACC and temporal gyrus in a group of adolescents with CD and comorbid bipolar disorder compared with healthy peers (Olvera et al., 2014).

Although the majority of brain imaging studies have focused on male subjects only, there is some evidence that suggests that females with CD show overlapping brain alterations as those reported in male-only CD samples, such as: lower grey matter volume in amygdala, ventro- and dorsolateral PFC, OFC, and ACC (Dalwani et al., 2015; Fairchild et al., 2013). To date, just two VBM studies have tested whether there are sex-specific alterations in brain structure. Fairchild et al. (2013) reported that males showed a higher, whereas females showed a lower volume compared to their healthy counterparts in anterior insula, while Michalska et al. (2015) reported no group by gender interactions in grey matter volume, however there were stronger negative correlations in the number of CD symptoms with GMV along the superior temporal sulcus in girls relative to boys (Fairchild et al., 2013; Michalska et al., 2015)

However, the above studies used VBM techniques, and this is problematic as this method combines both thickness and surface features to calculate the overall GMV (Winkler et al., 2010). Thus, the extent to which each metric (i.e., surface or thickness) contributes to the observed volumetric differences in unclear. Also, it has been shown that these features have different genetic and cellular mechanisms in the brain (Panizzon et al., 2009). A more recent technique, known as surface-based morphometry (SBM), overcomes this issue and estimates the measurement of the different cortical features, such as cortical thickness, surface area and folding (Dale, Fischl, & Sereno, 1999). The distinct metrics provided by this method potentially offer a better understanding of the underlying causes of brain structural alterations as well as understanding the cognitive processes that may be affected by these abnormalities (Jiang et al., 2015). Moreover, SBM methods

provide a more accurate form of volume-based registration, and they are also more sensitive to grey matter changes than VBM techniques (Ghosh et al., 2010; Palaniyappan & Liddle, 2012).

4.2.3 Studies using surface-based morphometry

SBM studies investigating brain abnormalities in CD have yielded similar results to VBM studies. However, they have also identified alterations in additional brain regions that have not previously been reported using VBM techniques. For instance, individuals with CD have shown thinner cortex or folding irregularities in the OFC, ACC and insula (Fahim et al., 2011; Fairchild et al., 2015; Hyatt, Haney-Caron, & Stevens, 2012; Jiang et al., 2015; Smaragdi et al., 2017; Wallace et al., 2014). Cortical thinning has also been observed in superior temporal cortex and in more posterior brain regions, including the supramarginal/angular gyrus, precuneus and fusiform gyrus (Hyatt et al., 2012; Jiang et al., 2015). Lower gyrification in vmPFC, and OFC (Jiang et al., 2015) and increased gyrification in SFG, insula, and fusiform gyrus (Fairchild et al., 2015), and precentral gyrus (Jiang et al., 2015) have also been detected in individuals with CD compared to controls.

Surface area (SA) abnormalities are less frequently reported, however two studies demonstrated SA reductions in vmPFC (Fairchild et al., 2015; Sarkar,Daly,Feng,Ecker, Craig et al., 2015), and in dorsolateral PFC (Sarkar et al., 2015), in CD individuals compared to healthy controls. Finally, both, cortical and subcortical volume estimated with SBM techniques, have also revealed reduced volume of the DL-PFC, striatum and amygdala in subjects with CD compared to healthy peers (Sarkar et al., 2015; Wallace et al., 2014).

Similar to VBM studies, the majority of SBM studies have looked at samples that are predominantly male - only two studies have investigated whether alterations in cortical structure are similar or distinct in males and females with CD. Wallace et al. (2014) did not find any group by gender interaction. However, the number of female participants included in this sample was very small (n = 6), and therefore the study was not well powered to adequately test for sex differences (Wallace et al., 2014). However, a recent well-powered sample from our lab demonstrated sex-specific alterations in brain structure in CD for all three cortical (i.e. folding, thickness, surface area) measures in multiple brain regions (Smaragdi et al., 2017).

4.2.4 Limitations of previous studies

Inconsistencies in the results of structural imaging studies might be due to the methodology that was used (SBM vs VBM). In addition, the samples that were used in previous studies are considerably heterogeneous in terms of the phenotype of the CD sample, e.g. clinical (Sterzer et al.,

2007) vs population (Fairchild et al., 2013) vs incarcerated (L M Cope et al., 2014). Also, the mean age varied significantly across the studies (e.g. M age= 8 vs 18, (Fahim et al., 2011; Fairchild et al., 2011)). Furthermore, some studies included participants with CD and high comorbidity with other disorders (e.g. severe drug use or bipolar disorder (Dalwani, Sakai, Mikulich-Gilbertson, et al., 2011)) while other studies have failed to consider heterogeneity within CD, in terms of age of onset (childhood- versus adolescence-onset) and levels of callous-unemotional (CU) traits - which designates individuals that lack remorse or guilt and show a general lack of concern about the negative consequences of his/her actions (American Psychiatric Association, 2013).

Given that SBM provides further insight into the nature of the neuroanatomical changes previously found in individuals with CD, and since it is the methodology that will be used for the current study, it is important to note that with the exception of two studies (Fairchild et al., 2015; Smaragdi et al., 2017), most of the previous SBM studies included small samples (i.e.<=25), and this may have resulted in both false positive or false negative results (Ioannidis, 2011). Moreover, although the results of these previous studies are somewhat consistent regardless of the methodological approach used, and these studies largely implicate brain regions associated with the hypothalamic pituitary adrenal axis (i.e., physiological systems that have shown anomalies in CD), it should be noted that to date structural imaging studies of CD are frequently lacking in female participants. Of all the participants included in SBM studies published to date, only 20% were female, and the majority, but not all (Smaragdi et al., 2017; Wallace et al., 2014), of the findings reported from the studies which did include female participants, reported overall group differences (CD vs healthy controls -HC), assuming a lack of sex differences. This may have obscured important case-control and sex specific brain differences.

4.3 Rationale and Aims

Adolescence is a crucial period for brain development, especially in regions linked with self-control and decision making (Casey, Jones, & Hare, 2008). However, adolescent males and females undergo neurodevelopmental changes at different rates (Giedd et al., 1999), displaying sexually dimorphic brain development (Lenroot & Giedd, 2006). Further, it has been shown that there are sex differences in terms of the emergence of CD, as most antisocial behaviour occurs during adolescence (Fontaine, Carbonneau, Vitaro, Barker, & Tremblay, 2009), and some researchers argue that age of onset in females is restricted to adolescents onset (AO) of CD (Silverthorn & Frick, 1999). Phenotypic sex differences in CD may be driven by separate biological or genetic risks in females and males (Cloninger, 1978), emphasising the investigation of females and males separately.

Although Smaragdi et al. (2017) had adequate power to test whether brain abnormalities are similar or distinct in males and females, the relative lack of studies, and especially those using novel SBM techniques, still leaves this question unresolved. The current study will differ from Smaragdi et al. (2017) in that it will employ a stronger *a priori* hypothesis-driven approach by using a slightly different SBM method (i.e., region of interest (ROI)-based analyses with or without a priori hypotheses versus hypothesis-free whole brain vertex-based). These approaches differ in the technique to estimate spatial averaging on measurement error. For instance, the sum and average of ROI-based measures vary considerably from region to region (increases in larger regions), whereas in vertex-based measures, the estimate of spatial averaging is settled across all vertices on the surface (Eyler et al., 2012).

This study will address limitations of previous studies by using a large sample with adequate numbers of males and females to examine sex differences. We will use an ROI approach to examine key brain structures (i.e. areas of the PFC and limbic system) which are associated with physiological systems involved in fear conditioning (e.g. HPA axis). We also attempt to replicate previous sMRI findings in CD in brain regions associated with the physiopathology of the disease (e.g. insula and striatum). Based on previous GMV and cortical thickness studies, we hypothesised that youths with CD would show reductions in these morphometric measures in PFC and limbic areas compared with healthy volunteers. Based on the work of Smaragdi et al. (2017), we hypothesise that males and females will show opposite effects in cortical structure measures in the SFG.

In addition, it has been claimed that individuals with CD and CU traits have a different neurological profile relative to those with CD and low levels of CU traits (Moffitt et al., 2008). Thus, similar to previous studies (Fairchild et al., 2015; Jiang et al., 2015), we will investigate whether the degree of brain alterations are associated with the following psychopathic dimensions: callous/unemotional traits, narcissism and impulsivity. Furthermore, similar to previous studies, we will investigate the relationship between CD symptoms and grey matter volume to explore severity effects (Michalska et al., 2015). Further, as attention deficit hyperactivity disorder (ADHD) has shown to be an important confounding factor in studies of CD, we will investigate the influence of ADHD symptoms on the SBM findings. Finally, to assess whether neural alterations exist in the different subtypes of CD, we will also compare the childhood- onset and adolescent-onset subtypes of CD in cortical structure.

4.4 Methods:

4.4.1 Participants

Participants for this study were recruited from five different sites involved in the Female Neurobiology and Treatment of Conduct Disorder (FemNAT-CD) study (for more details see chapter 3). The sites that were included in this study were as follows: University of Southampton (UOS), University of Birmingham (UOB), Goethe University Frankfurt (GU), University Hospital Aachen (UKAACHEN), and University of Basel (UNIBAS). The total sample of participants of the FemNAT-CD that underwent a structural MRI scan was 562 (GU=94, UKAACHEN=131, UOS=140, UNIBAS=65, UOB=132) aged 9-18. However, due to previous findings showing brain anatomical changes during childhood and adolescence (Lenroot & Giedd, 2006), and especially in fronto-limbic regions (Casey et al., 2008), this study excluded children aged 9 to 11 (n=87), leaving a sample of N=475. The structural images were then inspected for image quality, seven participants' data were removed due to poor image quality.

Match is a computer program that facilitates the process of matching large groups of items and participants in large data sets. Match is a fully automated command-line program that operates through an algorithm (van Casteren & Davis, 2007). Thus, we used Match to exclude potential outliers from each group (CD and HC) and to further select an IQ-, age- and gender-matched sample. After this procedure, we ended up with a final sample of N=315 adolescents, 156 with conduct disorder (79 females) and 159 healthy controls (80 females).

As mentioned in Chapter 3, a research diagnosis was made using the Schedule for Affective Disorders and Schizophrenia for School-Age-Children –Present and Lifetime version (KSADS-PL; Kaufman et al., 1997).

4.4.2 Image acquisition

Structural MRI data were acquired using Siemens 3T (GU, and UOS: Tim Trio; UNIBAS, and UKAACHEN:PRISMA) or Philips 3T (UOB) scanners. All sites went through a site qualification procedure before collecting imaging data (See Chapter 3 for more details).

The FemNAT-CD T1-weighted images were acquired using a magnetization-prepared rapid-acquisition gradient-echo sequence (MP-RAGE) were acquired with a matrix size of 256 mm and a voxel size of 1x1x1 mm isotropic, a flip angle of 9 degrees, 192 sagittal slices, a bandwidth of 174 HZ/pix (Phillips), and of 180 HZ/pix (Siemens). An echo time of 3.7 ms (Phillips), 3.4 ms (Siemens), a field of view of 256x192 and a repetition time of 8 ms (Phillips) and 8.3 ms (Siemens). The total scan time was 4 min 26 seconds (Siemens) and 6 min 5 sec (Philips). The T1-weighted image was the first sequence to be collected, followed by fMRI and diffusion MRI data.

Once the scan was collected, the quality of the data was immediately evaluated by the MRI operator to ensure that there were not significant head movements or unwanted noise. In case it was needed, the scan was repeated to ensure that a high quality T1-weighted image was available.

4.4.3 Image analysis

Cortical reconstruction and volumetric segmentation were conducted using FreeSurfer image analysis suite version 5.3, which is documented and available for download online (http://surfer.nmr.mgh.harvard.edu/). A detailed reconstruction process has previously been described (Dale et al., 1999; Fischl et al., 2002). Briefly, the pipelines in FreeSurfer are used to carry out cortical reconstructions and subcortical volumetric segmentations (See **Figure 4.1** for a processing stream overview). This process corrects for motion and averages several T1-weighted images (Reuter, Rosas, & Fischl, 2010). It further corrects and normalizes intensity of T1-weighted images. Next, it removes non-brain tissue such as the skull, eyeballs, and images using a hybrid watershed/surface deformation procedure (Ségonne et al., 2004). This algorithm creates a boundary between the brain and the skull to accurately deform the surface models (Ségonne et al., 2004). An automated Talairach transformation is applied, and it further conducts a segmentation procedure based on signal intensity and geometric structure of the grey-white matter interface. Next, it generates representations of each hemisphere, and segments subcortical deep grey matter (including, amygdala, hippocampus, putamen, caudate, ventricles) and white matter structures (Fischl et al., 2002, 2004). The image intensity is further normalised (Sled, Zijdenbos, & Evans, 1998) and the resulting image/volume is tessellated with triangular meshes of the white matter, grey matter, and cerebrospinal fluid (CSF) boundary. Further, an automated topology correction is performed followed by a surface deformation, which works by following intensity gradients to accurately localize the grey/white and grey/cerebrospinal fluid boundary (the greatest shift in intensity demarks the change to the other tissue) (Dale et al., 1999; Fischl & Dale, 2000).

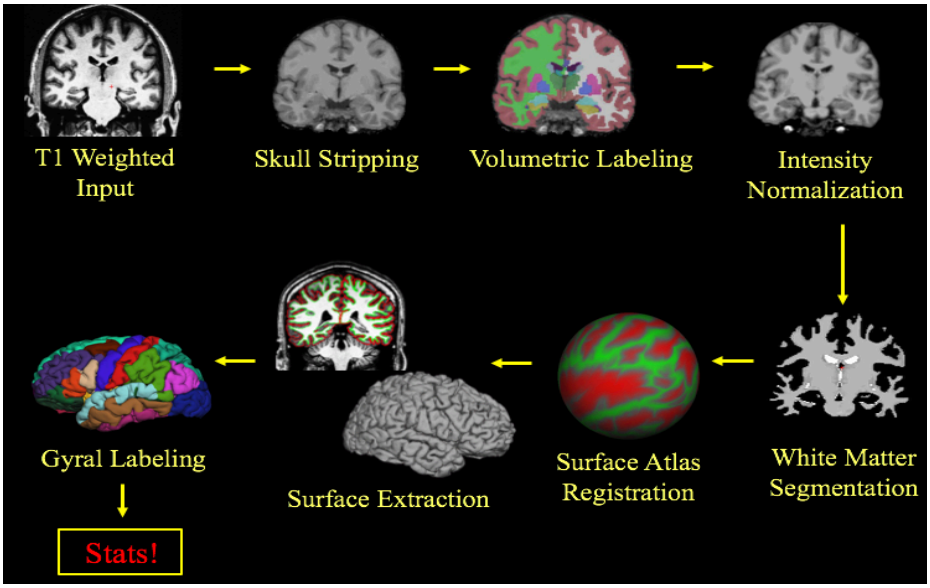


Figure 4.1 - Processing Stream Overview: *Reprinted from FreeSurfer Tutorials, (2017), http://surfer.nmr.mgh.harvard.edu/fswiki/Tutorial*

Once the cortical model is completed, FreeSurfer automatically performs a deformable procedure for processing data and analysis which includes surface inflation (Fischl, Sereno, & Dale, 1999), individual cortical folding patterns are mapped to a spherical atlas based on cortical geometry across subjects (Fischl, Sereno, Tootell, & Dale, 1999) which are then registered to a spherical atlas. The gyral and sulcal structure of each hemisphere of the cerebral cortex is further parcellated into units. It further calculates total cortical surface area of the pial (in mm^2), total cortical GMV (in mm^3), and Cortical thickness (in mm) which is calculated at each location of the cortex as the shortest distance between the pial and white surface (**Figure 4.2**) (Han et al., 2006). Histological analysis has been used to validate the procedures for measurement of cortical thickness, (Rosas et al., 2002) as well as through manual measurement (Kuperberg et al., 2003). Freesurfer procedures have been shown to have reliability across a range of scanners strengths (Han et al., 2006).

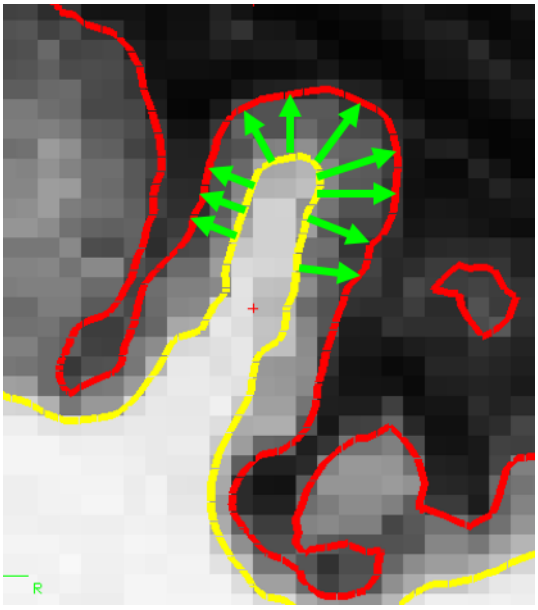


Figure 4.2 - Estimate of Cortical Thickness: Green arrows indicate the distance between white (yellow) and pial (red) surfaces. *Reprinted from Free Surfer Tutorials, (2017), http://surfer.nmr.mgh.harvard.edu/fswiki/Tutorial*

4.4.4 Volume-based subcortical structures

The volume based stream designed to estimate MRI subcortical tissue classes has previously been described in detail (Fischl et al., 2002, 2004). Briefly, the first stage involves affine registration with MNI305 space specifically designed to be insensitive to pathology and maximise the accuracy

of the final segmentation. Each voxel is then normalised, and volumetric labelling is further assigned on a probabilistic atlas obtained from a manually labelled training set (Fischl et al., 2002).

Estimated total intracranial volume was estimated for each participant and included for interindividual variability in global brain size. Surfaces were inspected to control for potential outliers and extreme inaccuracy on the segmentations.

4.4.5 Anatomically-defined cortical and sub-cortical regions of interest

FreeSurfer can automatically label the cortex in a way that attempts to replicate the labelling of a trained anatomist. The labels are adapted to each participant based on curvature statistics stored in the ROI atlas.

The study used an automated atlas-based Bayesian parcellation procedure (Desikan et al., 2006; Destrieux, Fischl, Dale, & Halgren, 2010) to extract quantitative estimates of brain structure and to label cortical and subcortical tissue classes. We selected fear conditioning and CD-related ROIs, which included: ten regions of interest located in the prefrontal cortex (**Figure 4.3**), five insula regions (**Figure 4.4**), and seven cingulate gyrus regions (**Figure 4.5**). The estimate for the vmPFC followed an approach proposed by Morey at al (Morey et al., 2015). The total volume was composed by two regions defined by Freesurfer, the sum of the lateral orbitofrontal and right and left medial orbitofrontal cortex's (Desikan et al., 2006). The orbitofrontal sulcus laterally, the orbitofrontal cortex medial to the straight gyrus, and the ventral sector of the medial PFC, are included in the vmPFC estimate (**Figure 4.6**). Finally, seven subcortical volumetric regions including: the amygdala, hippocampus, putamen, pallidum, thalamus, accumbens and caudate, were also estimated. All ROIs were examined in both hemispheres. Details of these freesurfer parcellations have been previously described (Desikan et al., 2006; Destrieux et al., 2010).

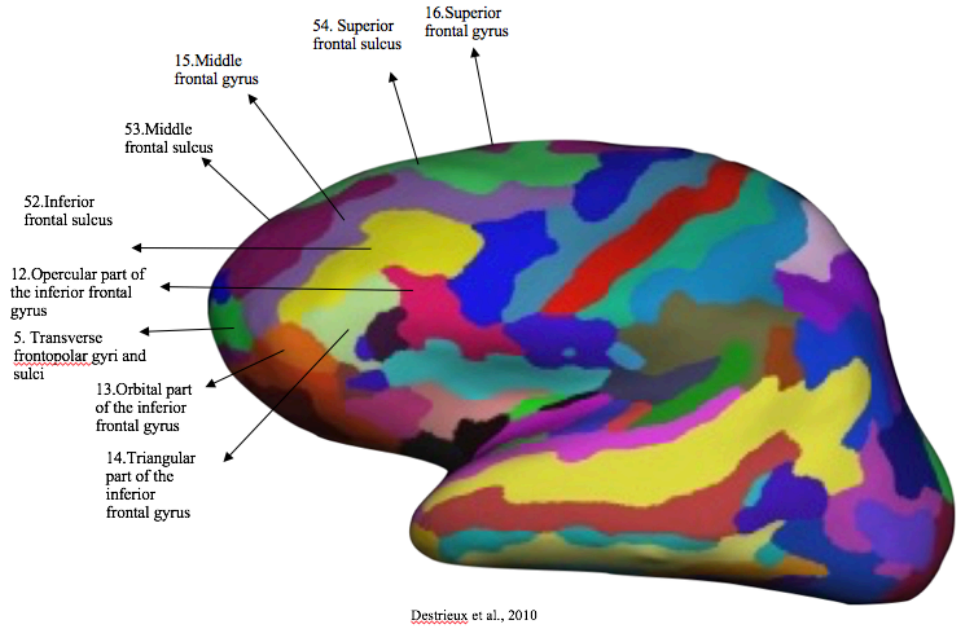


Figure 4.3 - Cortical Frontal areas: the cortical parcellations are generated with Free surfer based on a parcellation system of Destrieux et al. (2010). This figure displays all of the parcellation units. The black arrows indicate the labels of the nine prefrontal regions examined in this study.

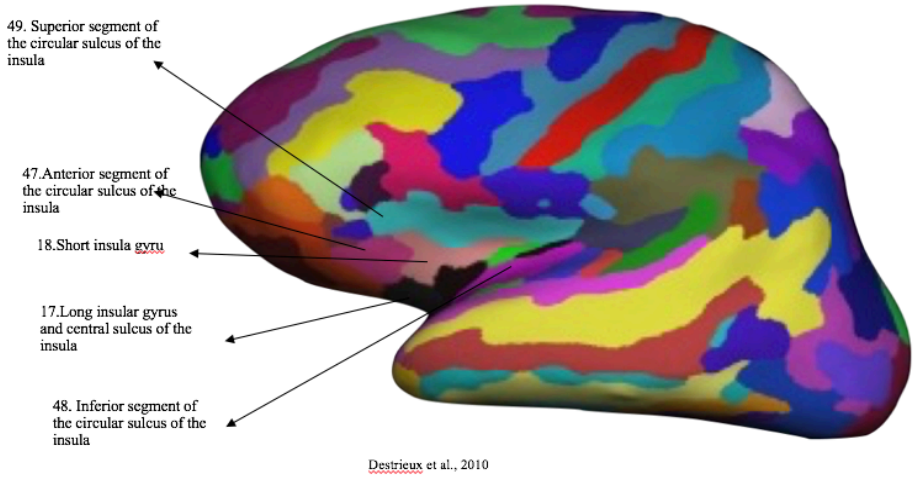


Figure 4.4 – Insular Cortex: the cortical parcellations are generated with Free surfer based on a parcellation system of Destrieux et al. (2010). This figure displays all of the parcellation units. The black arrows indicate the labels of five insular cortex regions (including gyri and sulci) that were examined in this

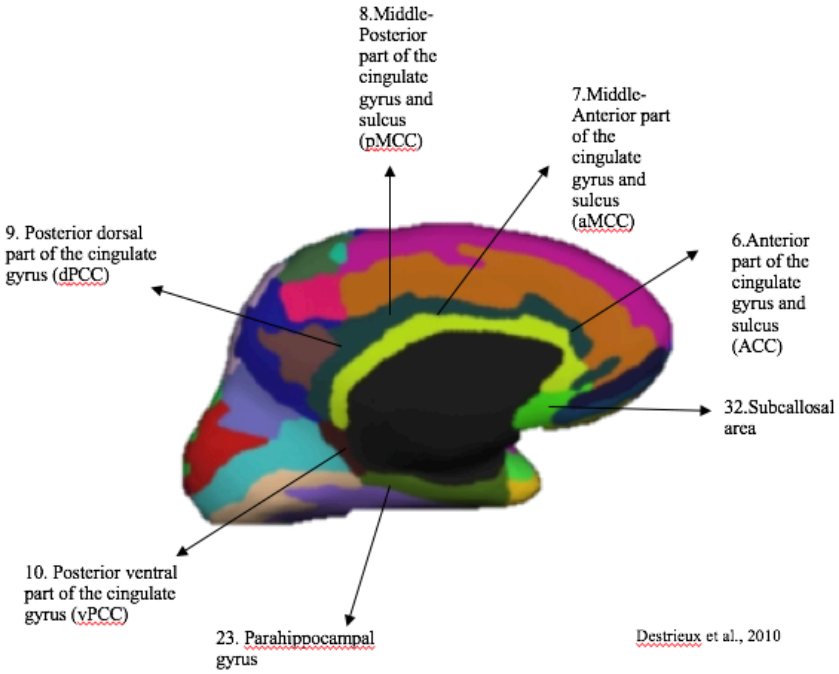


Figure 4.5 - Cingulate Cortex: the cortical parcellations are generated with FreeSurfer based on the parcellation system of Destrieux et al. (2010). This figure displays the parcellation units. The black arrows indicate the labels of the seven cingulate regions examined in this study.

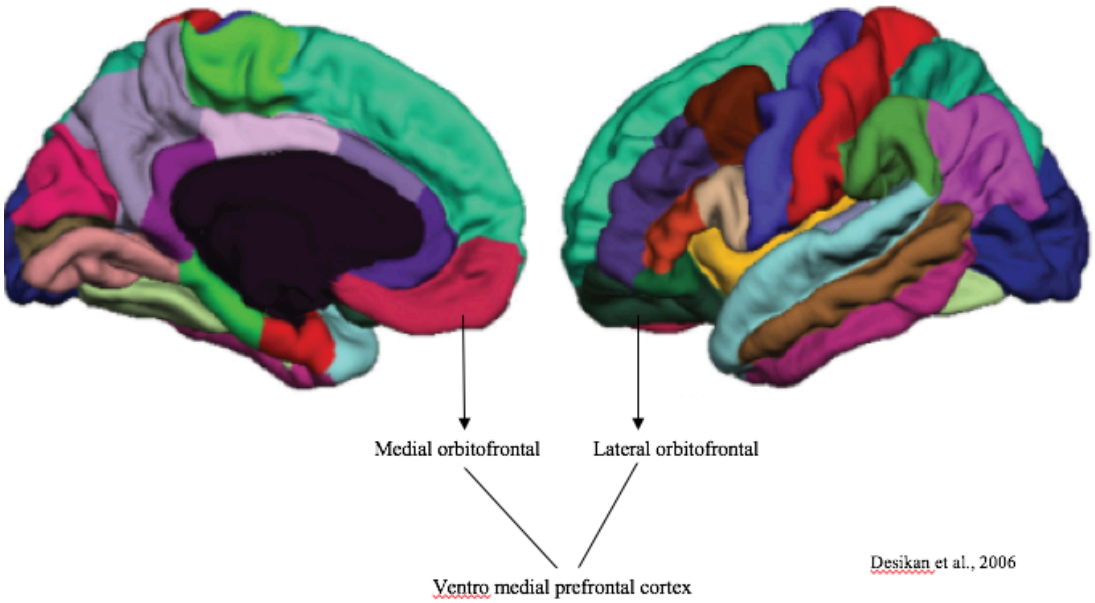


Figure 4.6 – Ventro-medial Prefrontal Cortex: the cortical parcellations are generated with FreeSurfer based on Desikan et al.'s (2006) parcellation system. This figure displays all of the parcellation units. The black arrows indicate the labels that formed the ventromedial prefrontal cortex.

FreeSurfer segmentation has been validated in young individuals (Ostby et al., 2009). In addition, automated segmentation of the hippocampus and amygdala compared with manual tracing with FreeSurfer has also been validated (Morey et al., 2009). The current study selected regions of interest on cortical thickness (CT), surface area, and cortical volume (CV) – measures that have recently been found to show abnormalities in individuals with CD (Fairchild et al., 2015; Sarkar et al., 2015; Wallace et al., 2014). These regions were chosen based on previous studies showing the involvements of these brain regions and the relation with CD, and the considerable link of these brain regions with stress/threat adaptation.

4.5 Statistical analysis

Matlab_R2016B was used to carry out the statistical analysis. We used a general linear model (GLM) to examine diagnosis effects and gender effects as well as test for gender by diagnosis interactions. The general linear model included the following covariates which have been shown to be associated with brain structural volumes, particularly in adolescents: age (Giedd & Rapoport, 2010), IQ (Lange, Froimowitz, Bigler, Lainhart, & Brain Development Cooperative Group, 2010), total intracranial volume (Trivedi et al., 2011), and site (coded as a binary fixed effect). We further ran the GLM adding the current symptoms of ADHD as a covariate (i.e., symptoms had been displayed in the last year). Finally, as there is some evidence that has suggested brain structural differences between the childhood–onset (CO) and adolescent-onset (AO) in conduct disorder (Fairchild et al., 2011), we used the same model, to compare these subgroups in order to assess the validity of combining these subgroups in our main analysis. The significant threshold was adjusted using the Benjamin –Hochberg false discovery rate (FDR: $q<0.05$) correction for multiple comparisons. Corrections were applied in each hemisphere. Chi squares test were performed using the Social Science Statistic web site (http://www.socscistatistics.com/Default.aspx) to observe data distribution. The effect size for diagnosis and sex effects were calculated using Cohen's d extracted from effects size calculators (LeeBecker; http://www.uccs.edu/~lbecker/) and effect size for sex by diagnosis interactions were estimated using partial eta squared (ηp^2) displayed by SPSS (v24).

Previous studies using a vertex-wise approach, had suggested that to detect a 10% difference between groups in any of the cortical measures it is required to test effects with sufficient power. Some cortical properties are more sensitive to others, therefore the N of participants varies between the cortical measures (Liem et al., 2015). For instance, it is recommended to use a sample of 39 individuals for cortical thickness, 21 for surface area, 80 for cortical volume and between 16-76 for different subcortical regions. I did not conduct power analysis myself as the approach used in this study is the first one using an ROI-based approach in CD, thus I could not compute effect sizes. In addition, as there are several potential interactions and variables (ROIs and cortical measures), an a

priori analysis would have been time consuming, unreliable and inaccurate. However, it is important to note that the present study is to some extent the largest of its type in the CD literature.

Consistent with previous studies (Sarkar et al., 2013), in order to elucidate what could be driving significant group differences in any of the selected ROIs within the CD sample, a GLM was run to test for associations between the three different subsets of the Youth Psychopathic traits Inventory scores: irresponsibility and impulsivity, narcissism and callous and unemotional traits, as well as with total psychopathic traits and CD symptoms. To reduce the probability of finding spurious statistically significant associations, correlations were only made in structures in which significant group effects were observed. Finally, to obtain more information about our sample, the CD sample was subdivided into higher (Females, n=28; Males; n=40) and lower (Females, n=51; Males, n=37) callous-unemotional traits subgroups using a median split procedure based on CU dimension scores of the YPI. Participants scoring >33 were classified as CD/CU+ while those scoring <33 were classified as CD/CU-.

4.6 Results

4.6.1 Sample demographics:

Demographic characteristics along with group comparisons and their corresponding p values are detailed in **Table 4.1**

The four groups did not significantly differ in age, IQ or handedness. They were also gender matched (**Table 4.1**). As expected, the conduct disorder group showed significantly more CD, ODD and ADHD symptoms, as well as significantly more traumatic experiences than their control counterparts. Significantly more males had a childhood onset of CD, whereas more females displayed an adolescent onset CD. Moreover, as expected, the CD group showed significantly higher scores in reactive and proactive aggression, as well as in the three different components of psychopathic traits: impulsive and irresponsible, narcissism and callous and unemotional traits, and the total score of psychopathic traits.

Table 4.1 - Clinical and demographic characteristics of the sample included in the surface-based morphometry analysis

Variable	Females (Mean ± SD)		Males (Mean ± SD)		Statistics		
	CD (n=79)	HC (n=80)	CD (n=77)	HC (n=79)	Group F(*p*)	F gender F(*p*)	F GxG F(*p*)
Age (years)	15.35±1.58	15.41±1.68	14.87±2.02	15.09±1.90	0.76 (0.38)	4.81 (0.03)	0.01 (0.90)
Estimated IQ	98.21±12.10	99.06±12.27	96.70±9.54	99.40±10.84	2.6 (0.10)	0.023 (0.89)	0.54 (0.46)
CD symptoms (K-SADS-PL)	5.25±2.78	0.14±0.38	5.46±2.65	0.13±0.38	567 (0.001)	0.49(0.48)	0.211 (0.64)
ODD symptoms (K-SADS-PL)	5.57±3.08	0.03±0.24	5.24±2.75	0.12±0.43	530 (0.001)	0.23 (0.63)	0.651 (0.42)
ADHD symptoms (K-SADS-PL)	5.25±6.28	0.05±0.35	7.55±6.53	0.02±0.16	156 (0.001)	5.66 (0.02)	5.30 (0.02)
PTSD (No. traumatic events)	2.89±2.18	1.15±1.18	2.53±2.0	1.28±1.12	63.5 (0.001)	0.48 (0.49)	1.71 (0.19)
CD age of onset, No (%)							
Childhood onset	32 (40.5)		48 (62.3)			X^2=7.44 (0.006)	
Adolescent onset	47 (59)		29 (37.6)				
Handedness No (%)							
Right	71 (90)	67 (84)	63 (82)	70 (89)	X^2=5.6 (.13)	X^2=1.01 (0.79)	X^2=8.12 (0.52)
Left	3 (4)	10 (13)	9 (12)	7 (9)			
Ambidextrous	4 (5)	1 (1)	3 (4)	1 (1)			
Missing	1 (1)	2 (3)	2 (3)	1 (1)			
Psychological measurements (Mean ± SD)							
Reactive aggression (RPQ)	12.03±5.36	5.62±3.51	11.58±5.06	5.87±3.59	151.60 (0.001)	0.001 (0.98)	0.24 (0.62)
Proactive aggression (RPQ)	4.51±4.48	0.75±1.45	5.18±5.02	1.35±2.22	92.80 (0.001)	2.84 (0.10)	0.05 (0.83)
Total RPQ	16.54±8.84	6.37±4.38	16.77±9.39	7.21±5.03	152.85 (0.001)	0.709 (0.40)	0.037 (0.84)
Grandiose manipulative (YPI)	38.40±10.62	32.45±9.01	39.55±13.91	35.41±9.34	17.37 (0.001)	3.18 (0.08)	0.42 (0.52)
Callous/Unemotional (YPI)	29.70±7.76	25.83±6.49	34.65±9.69	30.60±5.21	24 (0.001)	32.22 (0.001)	0.03 (0.88)
Impulsive/Irresponsible (YPI)	40.84±9.40	31.88±6.83	38.98±10.26	33.78±6.13	56.9 (0.001)	0.007 (0.94)	3.87 (.05)
Total YPI	108.94±24.19	90.16±18.73	113.16±29.41	99.8±16.68	40 (0.001)	7.6 (0.006)	0.99 (0.32)
Current Psychiatric comorbidity - No. with K-SADS-PL diagnoses (%)							
ADHD	25 (32)		39 (50.7)			X^2=7.2(.03)	
ODD	49 (63)		54 (70)			X^2=2.4(.29)	
PTSD	9 (11.4)		3 (4)			X^2=4.0 (.13)	
MDD	17 (21.5)		7 (9)			X^2=4.6 (.03)	
Alcohol abuse	7 (8.9)		7 (9)			X^2=.003(.96)	
Alcohol dependence	3 (4)		1 (1)			X^2=.97(.32)	
Substance abuse	7 (8.9)		9 (11.7)			X^2=.34(.56)	
Substance dependence	4 (5)		10 (13)			X^2=2.9(.08)	
Anxiety	5 (6.4)		3 (4)			X^2=.52 (.47)	

Key: +CU, high callous unemotional traits, -CU, low callous unemotional traits, ADHD, attention-deficit/hyperactivity disorder; ODD, Oppositional defiant disorder; PTSD, post-traumatic stress disorder; MDD, major depressive disorder; YPI, youth psychopathic traits inventory; RPQ, reactive proactive aggression questionnaire. *Number of traumatic events were estimated

In terms of psychiatric comorbidity in individuals with CD, males and females showed similar rates of ODD, PTSD, alcohol/substance abuse and dependence. However, the sexes differed in rates of ADHD (males>females) and MDD (females>males). Finally, the sample distribution across the sites showed that there was an adequate observed distribution (**Table 4.2**).

Table 4.2 - Sample distribution across the sites

	GU (n=50)	UKAACHEN (n=58)	UOS (n=87)	UNIBAS (n=54)	UOB (n=66)	Total (n=315)	
Males CD	11	15	24	6	21	77	X^2= 19.28 (.08)
Males HC	11	15	24	8	21	79	
Females CD	14	14	19	20	12	79	
Females HC	14	14	20	20	12	80	

Note: CD; Conduct disorder, HC; healthy controls, UOS; University of Southampton, UOB; University of Birmingham, GU; Goethe University Frankfurt, UKAACHEN; University Hospital Aachen, and UNIBAS, University of Basel. Differences between sites were tested using a Chi Square test.

4.6.2 Age of CD onset

To confirm our findings were not affected by grouping childhood and adolescent onset forms of CD together, we first conducted a GLM comparing these subgroups. There were no significant differences between the CO-CD and AO-CD subtypes in any of the investigated metrics at $p < 0.05$.

4.6.3 Main effects of CD

4.6.3.1 Cortical Volume

In order to test for group differences in cortical volume, cortical thickness and surface area, a series of GLMs was conducted. We found strong effects of CD on cortical volume of the left ACC (t (304) = -1.93, p=0.05, d=0.09), right superior frontal gyrus (t (304) = -3.47,p=0.001,d=0.26), right superior frontal sulcus (t(304) = -1.93,p=0.05,d=0.21), posterior medial cingulate cortex (t (304) = -2.65,p=0.008,d=0.26), and anterior medial cingulate cortex (t (304) = -2.01,p=0.04,d=0.21), indicating reduced volume of these regions in the CD group relative to the control group. However, after correcting for multiple comparisons, only the effect of diagnosis in the right superior frontal gyrus remained significant (pFDR=0.01; **Figure 4.7a**).

Means and standard deviations for main effects of diagnosis on cortical volume are displayed in **Table 4.3.** For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.3 (Appendix D.1)

Table 4.3 - Main effects of diagnosis on Cortical Volume

Hemisphere	Brain Region	Healthy Controls N=159	Conduct disorder N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	*Cohen's d*
Left	Anterior part of the cingulate gyrus and sulcus (ACC)	5387.42±883.96	5309.04±782.00	-1.93	0.05	0.63	0.09
Right	Middle-anterior part of the cingulate gyrus and sulcus (aMCC)	3546.95±619.83	3420.02±568.90	-2.01	0.04	0.24	0.21
	Middle-posterior part of the cingulate gyrus and sulcus (pMCC)	3169.81±532.83	3034.25±512.03	-2.65	0.01	0.09	0.26
	Superior frontal gyrus	19628.70±2552.53	18965.26±2483.80	-3.47	0.01	0.01	0.26
	Superior frontal sulcus	5025.96±971.46	4836.49±864.19	-2.3	0.02	0.16	0.21

4.6.3.2 Surface Area

Finally, there were also significant effects of diagnosis on surface area of the right superior frontal gyrus (t(304)=-3.04, p=0.003, d=0.26), middle posterior part of the cingulate gyrus and sulcus (t (304)=-2.05, p=0.04, d=0.19) with the CD group showing lower surface area in these regions. When correcting for multiple comparisons the main effect of diagnosis on superior frontal gyrus surface area remained significant (pFDR=0.05; **Figure 4.7c**).

Means and standard deviations for effects of diagnosis on the surface area are displayed in **Table 4.4**. For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.4 (Appendix D.2)

Table 4.4 - Main effects of diagnosis on Surface Area

Hemisphere	Brain Region	Healthy Controls N=159	Conduct disorder N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	*Cohen's d*
Right	Middle-posterior part of the cingulate gyrus and sulcus (pMCC)	1060.58±165.64	1030.13±157.99	-2.05	0.04	0.39	0.19
	Superior frontal gyrus	4889.98±616.93	4726.86±614.79	-3.04	0.003	0.05	0.26

4.6.3.3 Cortical Thickness

The CD group showed higher cortical thickness in the left inferior segment of the insula sulcus (t(304)=-=-1.96, p=0.05, d=-0.1), whereas they showed lower cortical thickness in the right superior frontal sulcus (t (304)=-=-3.10, p=0.002, d=0.24), middle posterior part of the cingulate gyrus and sulcus (t(304)=-2.14, p=0.03, d=0.18), and middle frontal sulcus (t(304)=-1.95, p=0.05, d=0.24). However, when correcting for multiple comparisons, only the effect of diagnosis in the right superior frontal sulcus remained significant (pFDR=0.04; **Figure 4.7d**).

Means and standard deviations for effects of diagnosis on cortical thickness are displayed in **Table 4.5**. For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.5 (Appendix D.3).

Table 4.5 - Main effects of diagnosis on Cortical Thickness

Hemisphere	Brain Region	Healthy Controls N=159	Conduct disorder N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	*Cohen's d*
Left	Inferior segment of the circular sulcus of the insula	2.96±0.24	3.00±0.23	1.96	0.05	0.56	-0.16
Right	Middle-posterior part of the cingulate gyrus and sulcus (pMCC)	2.80±0.14	2.78±0.16	-2.14	0.03	0.28	0.18
	Opercular part of the inferior frontal gyrus	3.06±0.19	3.03±0.17	-1.93	0.05	0.28	0.17
	Middle frontal sulcus	2.37±0.18	2.33±0.17	-1.95	0.05	0.28	0.24
	Superior frontal sulcus	2.62±0.15	2.59±0.17	-3.10	0.002	0.04	0.24

4.6.3.4 Subcortical Volume

We also found a significantly larger effect on the volume of the left nucleus accumbens (t=1.99, p=0.05, d=-0.08) on the CD group compared with the control group. However, this effect did not survive when correcting for multiple comparisons.

Means and standard deviations for effects of diagnosis on subcortical volume are displayed in **Table 4.6**. For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.6 (Appendix D.4)

Table 4.6 - Main effects of diagnosis on Subcortical Cortical Volume

Hemisphere	Brain Region	Healthy Controls N=159	Conduct disorder N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	Cohen's *d*
Left	Accumbens	561.54±132.48	572.69±144.96	1.99	0.05	0.39	-0.08

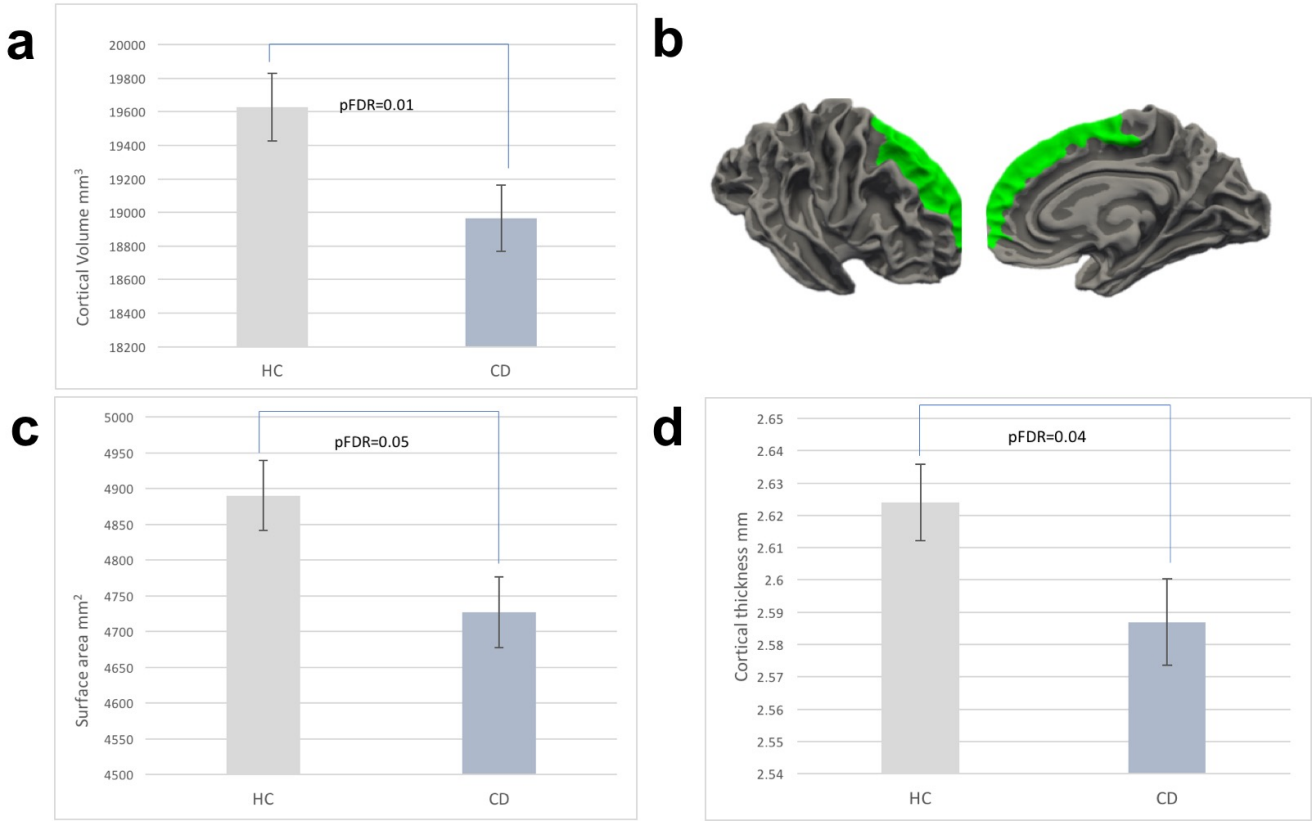


Figure 4.7 – Effects of Diagnosis in the Superior Frontal Area

a) Main effects of diagnosis on superior frontal gyrus cortical volume.
b) The green area in the brain map is showing both; superior frontal gyrus and superior frontal sulcus.
c) Main effects of diagnosis on superior frontal gyrus surface area.
d) Main effects of diagnosis on superior frontal sulcus cortical thickness. Group differences in cortical volume, cortical thickness and surface area are significant at $p<.05$, false discovery rate correction. Error bars show standard errors.

4.6.4 Main effects of sex

4.6.4.1 Cortical Volume

We found main effects of sex in the left ACC (t(304)=-2.17, p=0.03,d=0.64), ventral posterior part of the cingulate cortex (t (304)=-2.52, p=0.01,d=0.73), anterior segment of the circular sulcus of the insula, and in the right vmPFC (t(304)=-3.31, p=0.001,d=0.62), ACC (t(304)=-3.09, p=0.002,d=0.85), transverse frontopolar (t(304)=-2.69, p=0.04,d=0.42), triangular part of the IFG (t(304)=-1.98, p=0.05,d=0.56),superior frontal gyrus (t(304)=-2.59, p=0.01,d=0.71) and in superior segment of the circular sulcus of the insula (t(304)=-2.68, p=0.008,d=0.79), and in bilateral short insular gyri (left: t(304)=-2.02, p=0.04,d=0.6; right: t(304)=-2.12, p=0.03,d=0.6), and anterior segment of the circular sulcus of the insula (left: t (304)=-1.96, p=0.05,d=0.63; right: t(304)=-2.07, p=0.04,d=0.5) . When correcting for multiple comparisons only the main effects in the right vmPFC (Males: M=14846.45, SD=2095.56; Females: M= 13678.16, SD=1614.19; pFDR=0.02), and ACC (Males: M=6444.04, SD=951.66; Females: M= 5726.761, SD=718.2391; pFDR=0.02), transverse frontopolar gyri (Males: M=3123.39, SD=567.38; Females: M= 2887.27, SD=557.72; pFDR=0.05), and in the triangular part of the IFG (Males: M=3415.92, SD=812.65; Females: M=3013.58, SD=617.30; pFDR=0.05) remained significant – in each case, males showed significantly higher volumes than females.

Means and standard deviations for effects of sex on cortical volume are displayed in **Table 4.7**. For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.7 (Appendix D.5)

Table 4.7 - Main effects of sex on Cortical Volume

Hemisphere	Brain Region	Males N=159	Females N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	*Cohen's d*
Left	Anterior part of the cingulate gyrus and sulcus (ACC)	5605.94±896.18	5096.13±683.33	-2.17	0.03	0.23	0.64
	Posterior-ventral part of the cingulate gyrus (vPCC)	714.97±200.03	577.80±176.69	-2.52	0.01	0.23	0.73
	Short insular gyri	2456.32±461.74	2228.09±330.80	-2.02	0.04	0.23	0.57
	Anterior segment of the circular sulcus of the insula	1078.24±220.50	951.23±181.97	-1.96	0.05	0.23	0.63

Table 4.7 Continued - Main effects of sex on Cortical Volume

Hemisphere	Brain Region	Males N=159	Females N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	*Cohen's d*
Right	Transverse frontopolar gyri and sulci	3123.39±567.38	2887.27±557.73	-2.069	0.04	0.11	0.42
	Anterior part of the cingulate gyrus and sulcus (ACC)	6444.04±951.67	5726.76±718.24	-3.086	0.002	0.02	0.85
	Triangular part of the inferior frontal gyrus	3415.92±812.66	3013.58±617.31	-1.98	0.05	0.11	0.56
	Superior frontal gyrus	20157.39±2642.59	18459.07±2121.04	-2.597	0.01	0.05	0.71
	Short insular gyri	2237.72±420.78	2003.56±307.93	-1.975	0.05	0.11	0.64
	Anterior segment of the circular sulcus of the insula	1225.12±232.80	1112.67±200.75	-2.118	0.03	0.11	0.52
	Superior segment of the circular sulcus of the insula	2526.44±400.93	2224.70±361.94	-2.686	0.008	0.05	0.79
	Inferior frontal sulcus	3860.37±845.87	3483.08±597.86	-2.425	0.02	0.07	0.52
	Middle frontal sulcus	3915.99±759.27	3582.57±738.23	-1.732	0.08	0.16	0.45
	Ventromedial PFC	14846.45±2095.57	13678.16±1614.20	-3.308	0.001	0.02	0.62

4.6.4.2 Cortical Thickness

We also found significantly higher cortical thickness in males in the left inferior frontal sulcus (t=-3.21, p=0.001,d=0.5), posterior ventral cingulate cortex (t=-2.69, p=0.007,d=0.3), ACC (t=-2.28, p=0.02,d=0.2), anterior insula (t=-2.24, p=0.02,d=0.2), vmPFC (t=-1.95, p=0.05,d=0.3), and in the right ACC (t=-3.47, p=0.001,d=0.3), inferior frontal sulcus (t=-2.35, p=0.01,d=0.3), superior frontal sulcus (t=-2.23, p=0.02,d=0.3), middle frontal sulcus (t=-2.22, p=0.02,d=0.4), middle posterior part of the cingulate gyrus and sulcus (t=-2.19, p=0.02,d=0.3), and in the vmPFC (t=-2.18, p=0.02,d=0.2). However, the areas that survived correction for multiple testing were the left inferior frontal sulcus (Males: M=2.48, SD=0.17; Females: M=2.40, SD=0.13; pFDR=0.03), and the right ACC (Males: M=2.89, SD=0.21; Females: M=2.82, SD=0.18; pFDR=0.01) – in both cases, males showed higher cortical thickness than females.

Means and standard deviations for effects of sex on cortical thickness are displayed in **Table 4.8.** For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.8 (Appendix D.6)

Table 4.8 - Main effects of sex on Cortical Thickness

Hemisphere	Brain Region	Males N=159	Females N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	*Cohen's d*
Left	Anterior part of the cingulate gyrus and sulcus (ACC)	2.96±0.20	2.93±0.19	-2.28	0.02	0.13	0.17
	Posterior-ventral part of the cingulate gyrus (vPCC)	2.62±0.33	2.54±0.27	-2.69	0.007	0.08	0.26
	Anterior segment of the circular sulcus of the insula	3.06±0.25	3.01±0.21	-2.25	0.03	0.13	0.23
	Inferior frontal sulcus	2.48±0.18	2.40±0.14	-3.22	0.001	0.03	0.52
	Ventromedial PFC	5.55±0.34	5.45±0.30	-1.96	0.05	0.21	0.31

Table 4.8 continued - Main effects of sex on Cortical Thickness

Hemisphere	Brain Region	Males N=159	Females N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	*Cohen's d*
Right	Anterior part of the cingulate gyrus and sulcus (ACC)	2.89±0.22	2.82±0.19	-3.478	0.0006	0.01	0.35
	Middle-posterior part of the cingulate gyrus and sulcus (pMCC)	2.82±0.16	2.76±0.14	-2.19	0.03	0.1	0.35
	Inferior frontal sulcus	2.44±0.16	2.39±0.15	-2.35	0.02	0.1	0.31
	Middle frontal sulcus	2.38±0.18	2.32±0.17	-2.22	0.03	0.1	0.37
	Superior frontal sulcus	2.63±0.16	2.58±0.15	-2.23	0.03	0.1	0.27
	Ventromedial PFC	5.55±0.36	5.47±0.33	-2.19	0.03	0.1	0.24

4.6.4.3 Surface Area

Finally, we also found higher surface area in the right SFG (t=-2.56, p=0.01, d=0.7), superior insula (t=-2.51, p=0.01,d=0.7), vmPFC (t=-1.97, p=0.05,d=0.6) in males relative to females. However, none of these regions survived correction for multiple comparisons.

Means and standard deviations for effects of sex on surface area are displayed in **Table 4.9.** For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.9 (Appendix D.7).

Table 4.9 - Main effects of sex on Surface Area

Hemisphere	Brain Region	Males N=159	Females N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	*Cohen's d*
Right	Superior frontal gyrus	5030.31±618.09	4592.25±542.44	-2.56	0.01	0.13	0.75
	Superior segment of the circular sulcus of the insula	1038.00±140.72	937.66±131.72	-2.51	0.01	0.13	0.69
	Ventromedial PFC	4651.92±653.11	4317.05±525.47	-1.97	0.05	0.21	0.38

4.6.4.4 Subcortical Volume

Significant effects of sex were observed in the left caudate (t=2.48, p=0.01, d=-0.3), right amygdala (t=-2.16, p=0.03, d=0.7), caudate (t=1.99, p=0.05, d=-0.2), putamen (t=-2.03, p=0.04, d=-0.7), and thalamus (t=-2.28, p=0.02, d=-0.3); in each case, in the direction of males showing greater volumes than females. However, none of these results in the ROIs survived when correcting for multiple comparisons.

Means and standard deviations for effects of sex on subcortical volume are displayed in **Table 4.10.** For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.10 (Appendix D.8).

Table 4.10 - Main effects of sex on Subcortical Cortical Volume

Hemisphere	Brain Region	Males N=159	Females N=156	Statistics			
		Mean ± SD	Mean ± SD	T	P	Corrected p value	Cohen's *d*
Left	Caudate	4098.07±722.51	3930.15±567.37	2.48	0.01	0.39	0.3
Right	Amygdala	1658.83±276.70	1491.74±196.47	-2.16	0.03	0.39	0.7
	Caudate	4107.91±670.88	3955.63±570.65	1.99	0.05	0.58	0.2
	Putamen	6030.19±783.50	5509.52±619.08	-2.03	0.04	0.58	0.7
	Thalamus	8243.44±1558.99	7865.11±1249.04	-2.28	0.02	0.39	0.3

4.6.5 Sex by diagnosis interactions

We did not find any reliable sex by diagnosis interactions, although we found several sex by diagnosis interactions that were present at an uncorrected level, meaning that none of the ROIs survived correction for multiple comparisons. The observed uncorrected effects are listed below:

4.6.5.1 Cortical Volume

We found changes in opposite directions (M-HC>MCD; F-HC<FCD) in the cortical volume of the left ACC (t=2.19, p=0.03,n^2=0.014). In addition, we found a significant diagnosis effect in males (M-HC>MCD) but not in females in the right SFG (t=2.31, p=0.02, n^2=0.015) and right vmPFC (t=1.95, p=0.05, n^2=0.012).

Means and standard deviations for sex by diagnosis interactions in cortical volume are displayed in **Table 4.11.** For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.11 (Appendix D.9).

Table 4.11 - Sex by diagnosis interactions on Cortical Volume

Hemisphere	Brain Region	Healthy Controls		Conduct Disorder		Statistics			
		Males (n=79)	Females (n =80)	Males (n =77)	Females (n=79)	T	P	Corrected p value	np^2
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD				
Left	Anterior part of the cingulate gyrus and sulcus (ACC)	5737.28±976.47	5041.94±614.87	5471.19±789.34	5151.00±746.24	2.19	0.03	0.61	0.016
Right	Superior frontal gyrus	20769.54±2649.08	18502.13±1874.91	19529.34±2500.14	18415.47±2355.58	2.31	0.02	0.44	0.017
	Ventromedial PFC	15117.87±2113.24	13577.81±1548.99	14567.97±2053.70	13779.77±1681.42	1.95	0.05	0.44	0.012

4.6.5.2 Cortical Thickness

We also found an effect of diagnosis in females (F-HC>F-CD) and not in males, on the cortical thickness of the left parahippocampal gyrus (t=-2.13, p=0.03, n^2=0.015) and an effect of diagnosis in males (M-HC>MCD) but not in females in the right superior frontal sulcus (t=2.36, p=0.01, $n^{2=}$0.019) was also observed.

Means and standard deviations for sex by diagnosis interactions on cortical thickness are displayed in **Table 4.12.** For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.12 (Appendix D.10).

Table 4.12 - Sex by diagnosis interactions on Cortical Thickness

Hemisphere	Brain Region	Healthy Controls		Conduct Disorder		Statistics			
		Males (n=79)	Females (n =80)	Males (n =77)	Females (n=79)	T	P	Corrected p value	np^2
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD				
Left	Parahippocampal gyrus	3.13±0.29	3.23±0.33	3.18±0.31	3.14±0.26	-2.13	0.03	0.71	0.001
Right	Superior frontal sulcus	2.67±0.14	2.58±0.14	2.59±0.17	2.59±0.16	2.37	0.02	0.39	0.003

4.6.5.3 Surface Area

Finally, we also observed changes in opposite directions (M-HC>M-CD; F-HC<F-CD) on the surface area of the left parahippocampal gyrus (t=2.5, p=0.01, n^2=0.020).

Means and standard deviations for sex by diagnosis interactions on the surface area are displayed in **Table 4.13.** For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.13 (Appendix D.11).

Table 4.13 - Sex by diagnosis interactions on Surface Area

Hemisphere	Brain Region	Healthy Controls		Conduct Disorder		Statistics			
		Males (n=79)	Females (n =80)	Males (n =77)	Females (n=79)	T	P	Corrected p value	np^2
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD				
Left	Parahippocampal gyrus	876.61±162.83	794.33±109.47	824.88±151.51	830.85±188.29	2.5	0.01	0.27	0.02

4.6.5.4 Subcortical Volume

Significant diagnosis effect was seen in females (F-HC>F-CD), but not in males in the subcortical volume of the left caudate (t=-2.09, p=0.04, n^2=-0.007).

Means and standard deviations for sex by diagnosis interactions on subcortical volume are displayed in **Table 4.14.** For further results from other brain regions, including non-significant values at an uncorrected level see supplementary table S4.14 (Appendix D.12).

Table 4.14 - Main effects of sex by diagnosis interaction on Subcortical Cortical Volume

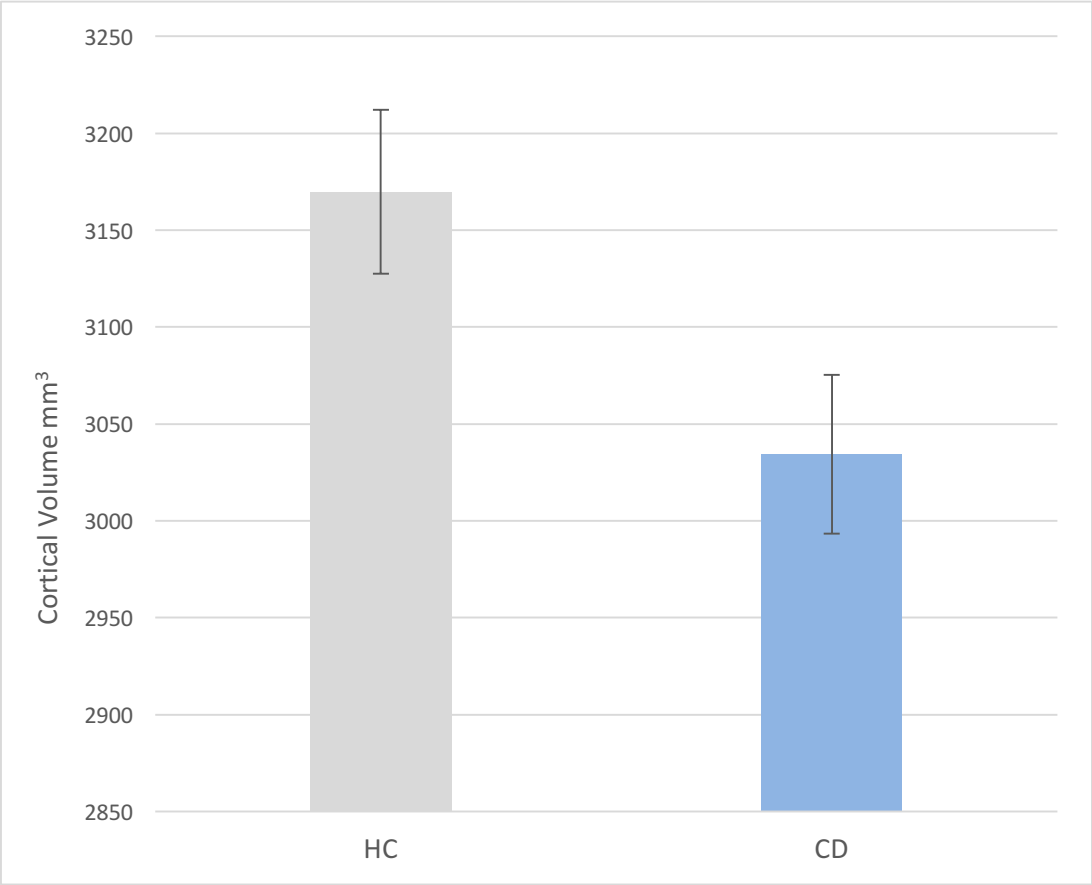
Hemisphere	Brain Region	Healthy Controls		Conduct Disorder		Statistics			
		Males (n=79)	Females (n =80)	Males (n =77)	Females (n=79)	T	P	Corrected p value	np^2
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD				
Left	Caudate	4102.47±733.56	4019.56±533.53	4093.56±715.78	3839.62±589.29	-2.09	0.04	0.3	<0.001

4.6.6 Testing of the potential confounding effects of ADHD comorbidity

The main effects of CD diagnosis observed on the cortical volume (t(303)=-3.34, p=0.0009) and surface area (t(303)=-2.57, p=0.01) of the superior frontal gyrus and the cortical thickness of the superior frontal sulcus (t(303)=-2.50, p=0.01) remained significant after controlling for current ADHD symptoms. However, after correcting for multiple comparisons, only the right cortical volume of the SFG (pFDR=0.02) survived. In addition, a significant main effect of diagnosis emerged in the middle part of the anterior cingulate (t(304)=-3.00, pFDR=0.03, d=0.3; **Figure 4.8**) when controlling for ADHD symptoms. Similarly, to the GLM analysis performed without controlling for ADHD symptoms, there were no sex by diagnosis interactions that survived correction for multiple comparisons.

4.6.7 Correlations between surface-based morphometry measures and CD symptoms or psychopathic traits

There were no significant correlations between SA or cortical volume of the right superior frontal gyrus and CD symptoms, nor total psychopathic traits, or the three sub facets of psychopathic traits (impulsive/irresponsible, callous/unemotional, narcissistic/manipulative) within the CD sample. However, total scores of psychopathic traits and impulsivity/irresponsibility were negatively correlated with the CT of the right superior frontal sulcus (**Figure 4.9**). There was no sex by CD nor sex-by-psychopathic traits interactions for any of the measures.



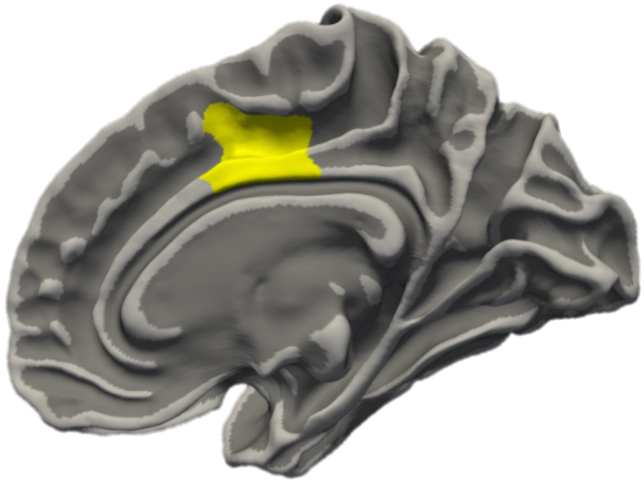


Figure 4.8 - Main effects of Diagnosis on cortical volume of the middle posterior part of the cingulate gyrus: Main effect after controlling for co-occurring ADHD symptoms. Group differences in cortical volume are significant at $p<.05$, false discovery rate correction. Corresponding brain area highlighted in yellow.

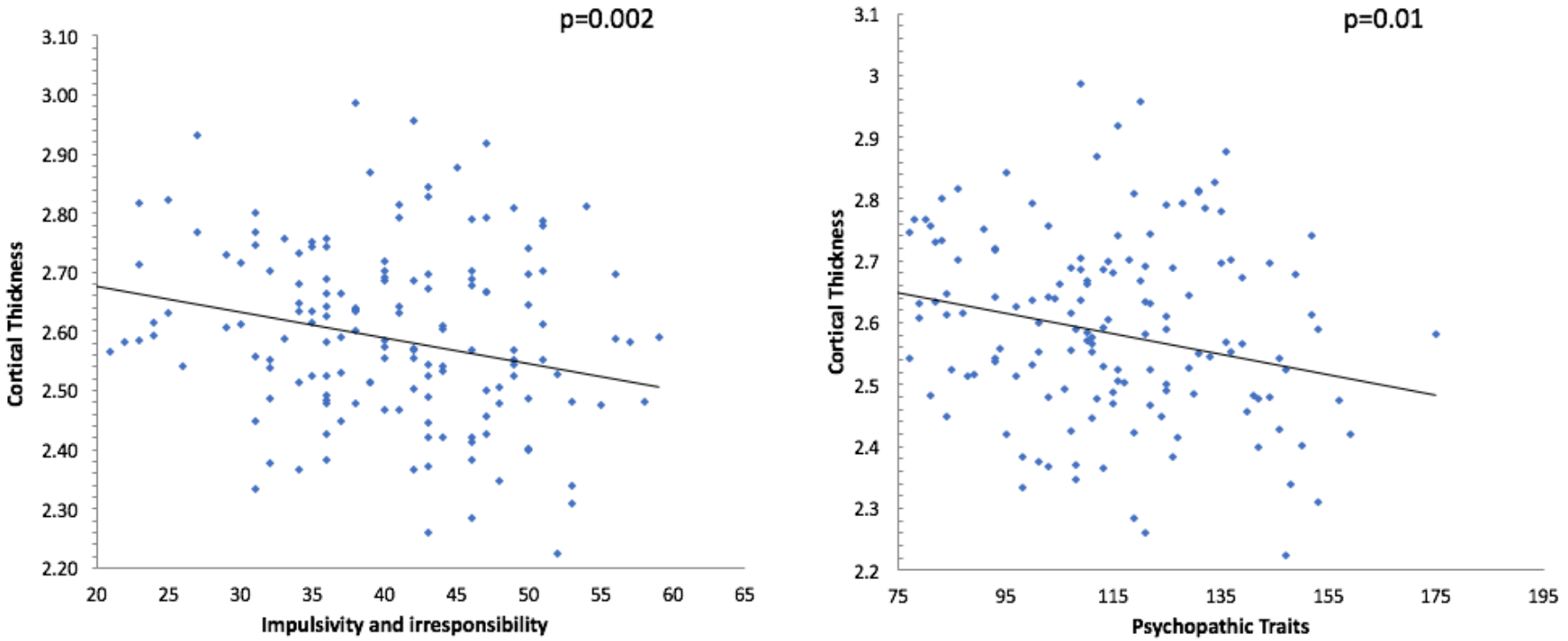


Figure 4.9 – Negative associations between Impulsive and Irresponsible traits and Cortical thickness, and total psychopathic traits and cortical thickness in the right superior frontal sulcus in the conduct disorder group only

4.7 Discussion

The goal of this study was to examine sex differences in CD in cortical thickness, surface area and volume as well as subcortical volumes, in limbic and prefrontal brain regions that have previously been implicated in antisocial behaviour (e.g. striatum and insula). We have extended the previous literature by focusing on these key brain regions of interest, in a much larger sample of male and female participants than has been studied to date (N = 315), which allowed us to adequately test whether female and male adolescents with CD show common or distinct alterations in brain structure.

Taking all our findings together, it appears that many of the findings reported in earlier studies of CD were not confirmed in this larger, better-matched sample. In addition, there were no significant sex-by-diagnosis interactions in the current study. There are no significant sex differences the key brain areas that have previously been associated with CD. However, irrespective of sex, we observed an overall effect of CD diagnosis in the superior frontal gyrus and sulcus – which is one of the key brain areas that constitutes the dorsolateral prefrontal cortex (DL-PFC).

4.7.1 Conduct Disorder Effects

Consistent with previous SBM findings, we observed lower cortical thickness, surface area and cortical volume in the CD group compared to their comparison peers. The main effect observed in this study was reduced CV, CT and SA in the right superior frontal area (i.e. superior frontal gyrus and sulcus). This finding is in line with earlier SBM findings conducted in male only samples which reported lower SA and CV in the right DL-PFC (Sarkar et al., 2015). In addition, a study investigating a group of youths with disruptive behaviour disorders, reported that when dividing the sample in those with oppositional defiant disorder and those with CD, the CD group showed reduced CT of the superior frontal area in a combined group of males and females with CD compared to healthy controls (Fahim et al., 2011), however the statistical power of the latter subsample was relatively small (n=11). Although the present study did not assess cortical folding, as expressed as local gyrification index, folding irregularities have also been detected in the SFG in male-only samples, as well as in a combined group of males and females (Fairchild et al., 2015; Hyatt, Haney-Caron, & Stevens, 2012b; Jiang et al., 2015). Furthermore, although a recent sMRI meta-analysis included studies using VBM methods, one of the key findings revealed that compared with HC, those with conduct problems showed reduced GMV in the superior frontal gyrus. However, this finding was observed in the left hemisphere (Rogers & De Brito, 2016). Finally, our results are also in line with a recent fMRI meta-analysis which reported more prominent reduced activations in the right DL-PFC under tasks

assessing emotion processing, and increased activations in a sub sample of youths with CD and psychopathic traits (Alegria et al., 2016).

The SFG is part of the DL-PFC, and in particular the right DL-PFC has been associated with cognitive control, decision-making, and delaying gratification (Baumgartner, Knoch, Hotz, Eisenegger, & Fehr, 2011; Knoch et al., 2006.; Shackman, Mcmenamin, Maxwell, Greischar, & Davidson, 2009). This is partly due to the contribution of the right SFG to response inhibition. The SFG is implicated in the inhibition of a motor response, which differs from more cognitive forms of inhibitory control such as interference control (Rubia et al., 2001). Although the finding in the SFG is nonspecific, as it has also been largely associated with other psychiatric disorders, such as ADHD (Rubia, 2011), it is important to note that the effects remained when controlling for ADHD.

Interestingly, the right DL-PFC is also linked with responses to threat and stress (Shackman, Mcmenamin, Maxwell, Greischar, & Davidson, 2009). The DL-PFC regulates our state of arousal by assisting in reducing the stress response (Amat, Paul, Zarza, Watkins, & Maier, 2006). Abnormalities in this region may lead to difficulties in learning to discriminate periods of threat from safety, thus getting involved in sensation-seeking situations. Also, neurodevelopmental aberrations in the DL-PFC may affect stress response regulation leading to thrill seeking behaviour in adolescents with CD.

Contrary to previous SBM study findings, we did not find group differences in other frontal areas (e.g. vmPFC) nor did we find diagnosis effects in subcortical - limbic brain areas (e.g. amygdala, striatum). The finding of no differences between the CD and control groups in subcortical regions is in line with a recent study of CD that adopted similar methods (Smaragdi et al., 2017).

Previous SBM studies in this area have had varying results which may be the result of different methodologies. For example, one study was conducted in children rather than adolescents, and as adolescence is an active period of brain development, it may be that brain alterations emerge at different stages of development. In addition, the heterogeneous nature that characterises CD might have affected earlier results. For instance, Fahim et al (2011) included children with oppositional defiant disorder as well as CD within their case group, and they were diagnosed using a self-report method (Fahim et al., 2011). This might have affected the reliability of information about the disorder and obscured whether the observed anomalies were associated with disorder-specific neuroanatomical deficits. In fact, the study demonstrated that when the sample was divided in those with ODD and those with CD, the anomalies were observed in different brain regions. Similarly, another study did not find thinning differences in any of the frontal or limbic areas in CD (Hyatt et al., 2012). However, the latter study included a rather pure CD sample without comorbid ADHD symptoms, and this sample may represent a different clinical population compared to the other studies that included samples of CD participants with a higher number of ADHD symptoms. Jiang et al. (2015) included a

sample of participants with adolescent-onset CD. Thus it is unclear whether their findings are specific to this form of CD (Jiang et al., 2015).

In addition, some of the previous studies did not match their samples in terms of IQ (Hyatt et al., 2012; Sarkar et al., 2015; Smaragdi et al., 2017), and it has been suggested that IQ has an important effect on brain alterations (de Zeeuw et al., 2012). In fact, a study of CD in which analyses were run in an unmatched IQ sample - yet co-varying for IQ, and with a matched IQ sample, found that their prior diagnostic effects in the vmPFC were no longer observed in the matched IQ subsample (Wallace et al., 2014). Interestingly, a recent longitudinal design conducted in adolescents with distinct trajectories of conduct disorder reported that the neither the vmPFC, nor the amygdala have a particular role in the neural pathophysiology of CD. However reduced CT in the DL-PFC appeared to have a considerable effect on the developmental pathway of CD (Oostermeijer et al., 2016). The authors suggested that delayed brain maturation may explain this effect.

It is surprising that very few robust CD-related alterations were seen in areas that are commonly associated with CD, such as, the amygdala, insula and ACC. However, this is in line with a VBM study in a large sample of adolescents with conduct problems which did not find any brain alterations between the CD and the healthy control group (Michalska, Decety, Zeffiro, & Lahey, 2015). Perhaps the most important issue to take into consideration here is that the majority of previous studies did not estimate effect-sizes of CD brain correlates. Small sample size could reach statistical significance, given the nature of the sample. However, it reduces the possibility that the result reflects a true effect (Ioannidis, 2011). Here, in a larger sample, we report small effect sizes in areas that have shown a significant effect. This may not mean that brain alterations with small effect sizes are unpractical – considering the complexity of the human brain. However, it may reduce the likelihood of replicability. Furthermore, a recent mega-analysis suggest that brain alterations associated with childhood disorder have previously been over-estimated in small samples (Schmaal et al., 2017). Therefore, future studies should be cautious when interpreting results and may want to report effect sizes.

4.7.2 Effects of Sex

We also found, reduced CT and CV in frontal ROIs, such as the vmPFC, ACC, Frontopolar, and IFG in females compared to males. However, no SA differences between the sexes were observed. Previous studies have shown thicker cortex in parietal and temporal areas in females compared to males (Sowell et al., 2007), but these ROIs were not included in the current study. Cortical thinning has been shown to occur with age as part of normal brain maturation (Sowell et al., 2007), and since

brain development occurs slightly earlier in females than males, this may be a normal developmental effect. Moreover, thinning of frontal areas have been associated with improvement in cognitive tasks. Since adolescence is a period where there are several developmental changes in myelination, cortical thinning may assist to improve cognitive performance. Thus, the reduced CV and CT in frontal regions seen in females may be related to normative sex differences in brain development.

4.7.3 Sex by diagnosis interactions

Contrary to our hypothesis, and prior studies investigating sex differences in CD (Fairchild et al., 2013) we did not find any reliable evidence of a sex by diagnosis interaction in any of our ROIs or for any of the SBM measures. This is in line with an earlier VBM study (Michalska et al., 2015), however a recent study from our lab demonstrated folding and SA irregularities in the left SFG, in which males with CD showed an increased gyrification and SA, whereas the opposite pattern was seen in females with CD (Smaragdi et al., 2017). Here, we did not observe sex by diagnosis interactions (e.g. increased SA in males with CD but decreased SA in females). Instead, the nominal results on this issue (i.e. sex by diagnosis interaction of SA in SFG), demonstrate that males with CD display lower SA values compared to the other research groups, thus effects in males with CD are more pronounced than those seen in females.

In addition, the discrepancy between the study by Smaragdi et al. (2017) and the current one, may be due to methodological issues. For instance, here we employed an a priori, ROI based approach instead of vertex-wise analysis (i.e. hypothesis free). The former method compromises stricter corrections for multiple comparisons (i.e. number of multiple comparisons is fixed) than vertex-wise analysis - which corrects across the clusters that are observed, therefore it may facilitate obtaining significant effects. Relevant to this issue, a study using both approaches within the same sample reported vertex – wise effects which were not found using the ROI approach (Greve et al., 2013). The authors suggested that perhaps clusters from the vertex-wise analysis did not fit precisely into the anatomically defined regions of the ROI approach. Moreover, the data processing done in the study by Smaragdi et al. (2017) was more comprehensive, in that segmentation errors were manually corrected. Although this improves the quality of the outputted data, this method is a time-consuming process and is not convenient for larger samples sizes, such as the one used in the present study. It is also worth noting that the present study only included frontal and limbic brain regions. Thus, findings in posterior and temporal regions would not have been detected.

4.7.4 Associations between cortical thickness and psychopathic traits and impulsivity

Interestingly, we found negative correlations between the right cortical thickness of the SFG and impulsive and psychopathic traits in the CD group. This domain reflects impulsiveness, thrill seeking and irresponsibility - which are important characteristics of CD. This is in line with previous studies that observed abnormal activations in the superior frontal areas during tasks assessing risky-decision-making in adolescents with CD (Crowley et al., 2010). Impulsivity relies on the regulation of executive functions involved in attention, learning and inhibition. For instance, impulsivity is a product of an inability to use available information to reflect on the consequences of actions. Secondly, it is an inability to sacrifice a small reward for a later reward. Finally, it is the inability to suppress a motor response (Torregrossa, Quinn, & Taylor, 2008). This response requires distinct cortical nodes but highly interconnected, which includes both, affect and inhibition. Given that response inhibition is linked in specific with the right SFG (Rubia, Smith, et al., 2009), the association found in this study may underlie the impulsive and risk-taking behaviour displayed by adolescents with CD. Future fMRI studies might consider including the SFG as a region of interest in tasks assessing this type of functions. However, it is important to note that this association was not reported in an earlier study that also looked at brain structure associations with impulsive traits (Jiang et al., 2015)

4.7.5 Confounding effects of comorbid ADHD symptoms

The most common co-occurring disorder in CD is ADHD (Klein, 1997). Both disorders overlap behaviourally, clinically, and cognitively. It has been claimed that ADHD is most prominently associated with the abnormal structure of brain areas involved in cognition and attention (i.e., DL-PFC), whereas CD is associated with motivation and affect (i.e. ventromedial PFC and amygdala) (Rubia, 2011). Our results contradict this hypothesis and demonstrate that the main brain abnormality seen in adolescents with CD was in the DL-PFC. Although our study included adolescents with CD with significant ADHD comorbidity (over 40% had ADHD), the present results survived even after factoring out ADHD symptoms. Thus, the current study challenges previous findings and reports a possible emerging area of interest involved in the pathophysiology of CD, and which could be involved in the poor decision-making of youths with CD.

Interestingly, when ADHD symptoms were controlled for, a main effect of diagnosis was observed in a new area – the posterior cingulate cortex (PCC). The PCC is a key component of the default mode network (DMN). Similarly to the present study, a study looking at the DMN in youths with CD reported that only after adjusting for co-occurring ADHD, the CD group showed hypo connectivity

between core DMN regions compared to the healthy comparison group (Broulidakis et al., 2016). In addition, the PCC has also been implicated in response inhibition. However, it has been claimed that it contributes differently to the SFG. For instance, two processes of response inhibition have been described, intentional response inhibition (the absence of an external command) and external response inhibition (triggered by an external stimulus). External response inhibition has commonly been associated predominantly but not exclusively with the right SFG, in contrast, the decision of intentional response inhibition has been associated with the PCC (Filevich, Kühn, & Haggard, 2012). Intentional inhibition predicts the longer-term consequences of the current action. The fact that this area was significant after controlling for ADHD symptoms suggest that ADHD can have an important effect on reported results.

4.7.6 Strengths and Limitations

The major strength of this study was the large sample size which means it is one of the first with an adequate number of males and females to address the question of sex differences among youths with CD. To date, this is the largest group of youths with CD studied using structural MRI. Also, our research groups were well-matched in terms of IQ and age. We also assessed the impact of ADHD comorbidity by treating it as a potential confounding factor in further analysis. We also tested for differences in brain structure between the CD subtypes (i.e. AO-CD and EO-CD), and we further evaluated the effects of individual differences in psychopathic traits within the CD group. A standardized approach was taken to obtain a current diagnosis of CD based on DSM-IV criteria and semi-structured interviews with the young people and their parents. Further, the method employed in this study of using SBM rather than VBM enabled the examination of separate cortical structure measures (i.e. CV, CT, SA) within our ROIs.

However, our study had a number of limitations. First, although collecting data across multiple sites offers the possibility of increasing statistical power, it also has some limitations. For instance, although we obtained a large study cohort by including data from multiple facilities, and although a cautious protocol to match data acquisition was created, combining data from multiple centres and scanners produced by different manufacturers (Siemens and Phillips) may have introduced unwanted noise and variations into the data. However, in an attempt to reduce this, all analyses included site as a covariate of no interest. Further, although surfaces were inspected to ensure that were not significant outliers, we did not correct for segmentation errors or topological defects with manual corrections. In addition, the methodology employed in this study was an ROI approach, reducing our understanding of a whole-brain analysis level. Although, ROI analysis provides a means through which the Freesurfer ROI results can be compared with those of VBM studies as well as with vertex-wise

analysis (Greve et al., 2013), an advantage of vertex-wise analysis is that it finds clusters that are not included in standard anatomical definitions, and it is also more similar to the unbiased approach taken in VBM methods.

In summary, in this is the first ROI-based SBM study with a large enough sample to adequately test for sex differences in the relationship between CD and cortical structure in frontal and limbic brain regions. We found that irrespective of sex, adolescents with CD have significant reduced CT, SA and CV in the superior frontal areas compared to their healthy peers. When adding ADHD symptoms as a covariate, an additional diagnosis effect was observed in the middle posterior cingulate cortex. Both CD age of onset subtypes appeared to show the same morphological anomalies. Further, negative correlations were seen with impulsive and psychopathic traits and CT of the superior frontal region. This finding highlights that superior frontal areas might be involved in the impulsive-poor decision making of CD. However, future studies investigating CD neural pathophysiology could progress from the well-stablished CD correlates of frontal and limbic brain regions to more posterior brain areas.

In addition, our study does not support that the suggestion that sex moderates the relationship between CD and cortical structure in frontal and limbic areas. Although future SBM and VBM studies looking at these ROIs should consider gender as a potentially important variable, our findings do not support the idea of treating males and females with CD separately, at least when looking at frontal and limbic areas. Finally, since this study had a cross-sectional design, we cannot infer that the observed structural anomalies (e.g. in the superior frontal area) play a causative role in the development of CD or impulsive/irresponsible traits.

Chapter 5 Morphology of the Amygdala, Hippocampus and Striatum in Youths with Conduct Disorder

5.1 Abstract

Volumetric studies of Conduct Disorder (CD) have reported structural abnormalities in subcortical regions such as the amygdala, hippocampus and striatum. However, there have been no studies of CD-related variation in the morphology of these regions. This could potentially tell us more about their functional contributions to the pathophysiology of CD. Thus, this study aimed to investigate shape differences in subcortical regions such as the amygdala, hippocampus, caudate nucleus, and nucleus accumbens between youths with CD and typically developing youths. A total of 156 (79 females) individuals with CD and 159 (80 females) typically developing youths were included in this study. Structural MRI data were collected and shape analysis of subcortical structures was conducted using FSL/FIRST vertex analysis to examine localised measures of shape differences between the CD and typically developing groups. Our findings demonstrated that youths with CD exhibited inwards shape deformations in the shell of the nucleus accumbens (NAcc). Sex did not moderate this effect. This is the first study to investigate shape differences in adolescents with CD relative to healthy controls. The Nacc is of great relevance to CD because it is linked to reward and risk-taking. Thus, our findings suggest that abnormalities in the shape of the NAcc may underpin alterations in reward-based decision making in CD. However, future studies should investigate the functional consequences of the shape deformations in the NAcc observed here.

5.2 Introduction

Youths with conduct disorder (CD) tend to reoffend or commit antisocial acts despite threats of punishment, such as arrest by the police, expulsion from schools, and parental discipline at family home (Burke et al., 2002). CD has great costs for society, affecting not only the individual but also their families and society in general (Murphy & Fonagy, 2012). Psychological features of CD are impulsivity, reward seeking, novelty seeking, and poor decision making (Glenn & Yang, 2012). Consequently, studies examining neural correlates in CD have focused on brain regions associated with inhibition, behavioural control and decision making.

The brain circuits controlling these above mentioned processes include frontal and limbic areas, such as, the amygdala, insula, anterior cingulate cortex (ACC), ventromedial frontal cortex (vmPFC),

orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DL-PFC), and posterior cingulate cortex (Ameis et al., 2013; Anderson & Kiehl, 2012; Blair, 2007; Oostermeijer et al., 2016; Raine, Lee, Yang, & Colletti, 2010). In fact, neurodevelopmental models of CD have attributed the emergence of CD to dysfunctions of the aforementioned structures (Blair, 2013). This model suggests that aberrations in these structures would lead to impaired stimulus-reinforcement learning, reduced responsiveness to distress cues and poor cognitive control (Blair, 2013; White et al., 2013).

The limbic system is a set of functionally and anatomically interconnected subcortical and cortical brain structures, which appear to be highly involved in emotion, motivation, learning and memory formations (Rajmohan & Mohandas, 2007). However, other structures strongly associated with the limbic system such as the striatum, have been less extensively investigated and may be of equal importance in terms of understanding deficits in reinforcement learning and reward-based decision making in CD (Glenn & Yang, 2012; Kiehl et al., 2001).

The striatum is divided into two components: the dorsal striatum (DS) and the ventral striatum (VS). The DS includes the caudate nucleus (CN) and putamen, while the VS mainly consists of the nucleus accumbens (NAcc; (Glenn & Yang, 2012)), however the olfactory bulb, ventral caudate and ventral putamen are sometimes also included in the VS component (Neto et al., 2008). It has been suggested that the VS is associated with impulsivity, and heightened sensitivity to reward while the DS is more associated with learning from positive outcomes (Glenn & Yang, 2012). Another subcortical structure that is relevant to the limbic system is the thalamus. It receives input from the amygdala and striatum, and projects to cortical limbic areas (e.g., OFC, insula).Thus, the subcortical regions of the limbic system (e.g. amygdala and hippocampus), as well as related subcortical structures of the limbic system (e.g., striatum) are of considerable importance in reward processing, learning and altering behaviour (Boccardi et al., 2010; Cope, Ermer, Nyalakanti, Calhoun, & Kiehl, 2012; Sergerie, Chochol, & Armony, 2008).

Brain abnormalities in the aforementioned regions may contribute to the development, and maintenance of CD. For instance, it has been suggested that deficits in the amygdala may have an effect on social dysfunction and impaired moral decision-making in individuals with antisocial behaviour (AB; Raine & Yang, 2006). The hippocampus has mainly been associated with the acquisition of fear conditioning (Boccardi et al., 2010). The DS and VS have both been associated with reinforcement learning and reward prediction errors. However, the VS is more strongly associated with reward processing whereas the DS is more associated with executive functions (e.g., cognitive control) (Pauli, O 'reilly, Yarkoni, & Wager, 2016). Both sub-regions of the striatum are components of the mesolimbic fronto-striatal dopamine pathway regulating reward-based decision making, and motivation control (Shenhav, Botvinick, & Cohen, 2013). The thalamus is implicated in sensory information processing, and it has been suggested that thalamic deficits may contribute to an

inability of suppressing intrusive memories and thoughts leading to poor behavioural control in individuals with AB (Kumari et al., 2013).

5.2.1 Structural MRI studies

Structural magnetic resonance imaging (sMRI) data analysis methods, can be used to extract volumetric values of subcortical structures. Using these sMRI-based volumetric techniques, numerous studies have investigated subcortical limbic and associated areas (e.g., striatum) in adults with antisocial personality and psychopathic traits (Boccardi et al., 2010; Kumari et al., 2013; Raine et al., 2004; Yang, Raine, Narr, Colletti, & Toga, 2009). Although there is preliminary evidence for similar neural abnormalities in adolescents with CD, the number of studies targeting these brain regions in young people with CD remains small.

Moreover, previous studies examining volumetric alterations in subcortical areas in CD have been inconsistent. For instance, several sMRI studies investigating the neural correlates of CD have used the amygdala as a primary region of interest (ROI) (for review see; Rogers & Brito, 2016). Studies have variously reported reduced grey matter volume (GMV) in the amygdala in adolescents with CD compared to typically developing peers (Fairchild et al., 2011; Huebner et al., 2008; Sterzer et al., 2007; Wallace et al., 2014). However, the opposite effect (i.e., increased GMV in amygdala), or no volumetric changes in the amygdala have also been reported in participants with CD (De Brito et al., 2009; Fairchild et al., 2013). Increased, and reduced GMV of the hippocampus(Cope, Ermer, Nyalakanti, Calhoun, & Kiehl, 2014; De Brito et al., 2009; Huebner et al., 2008b), pallidum, putamen, caudate and ventral striatum have also been reported in youths with CD (Fairchild et al., 2011b, 2013; Wallace et al., 2014).

The discrepancy between the studies in terms of findings might be a consequence of limitations in imaging methods. For instance, although volumetric sMRI approaches can provide information about the atypical development of brain regions, it cannot identify particular cellular differences (i.e. missing localised abnormalities). More specifically, volumetric approaches involve averaging volume measurements over a given area. Previous studies that have reported volumetric differences in subcortical regions have frequently treated different brain regions as a single structure (e.g. striatum). This is problematic, as it is well established that subcortical structures are composed of sub-regions, and each subregion is responsible for a range of specific functions. In addition, each region has heterogeneous developmental trajectories, and may be differentially sensitive to brain insult, especially during adolescence (Andersen & Teicher, 2008; Raznahan et al., 2013).

For example, thirteen different nuclei have been distinguished in the amygdala. Consequently, while several nuclei are implicated in emotional processing and fear conditioning, the central nucleus of the amygdala has been associated with processing motivational and affective stimuli. Thus, previous authors have hypothesised that deficits within amygdala may contribute to features of antisocial personality disorder (ASPD) and psychopathy (Yang & Raine, 2009). The hippocampus has six distinct subdivisions (i.e., dentate gyrus, cornu ammonis (CA4, CA3, CA2, CA1, and subiculum)) which are implicated in distinct specialised functions (Gilbert, Kesner, & Lee, 2001). The caudate is formed by a head, body and tail. The putamen is divided in dorsal, caudal and ventral regions (Kumar et al., 2014). The globus pallidus contains two parts, globus pallidus pars externa and globus pallidus interna. The NAcc links the basal ganglia and the limbic system and is considered to be a motor-limbic interface. It has shown to be involved in several emotional, motor, and motivational processes (Neto et al., 2008). The medial, ventral and lateral portion of the NAcc is referred to be the shell of the NAcc (which has stronger limbic connections), while the central and dorsal portion is usually referred as the core of the NAcc (Neto et al., 2008).Both sub regions have shown to have distinct contributions to reinforcement learning (Mannella, Gurney, & Baldassarre, 2013). Finally, the thalamus is comprised of approximately 60 structurally and functionally distinct nuclei, and each has a different pattern of anatomical connections to cortical and subcortical areas (Fama & Sullivan, 2015).

In addition, segmentations of subcortical structures are frequently subject to errors. This is due to the considerable overlap among subcortical structures, challenging the signal intensity to distinguish between each structure's limits. For example, the overlap of the hippocampus and the adjacent amygdala is almost total (Fischl et al., 2002). Similarly, the boundaries of the NAcc and caudate nucleus are challenging to differentiate (Neto et al., 2008). Thus, previous volumetric studies might have risked obscuring group differences by performing analysis that focuses on the gross volume of the structure (e.g., whole hippocampal volume) rather than on its subregions.

Adolescence is a critical period for brain development and neural reorganisation. Volumetric changes might not detect modest age-related changes in a particular area, yet vital changes that happen in this dynamic stage (G. E. Alexander, DeLong, & Strick, 1986). Thus, a method to overcome the afore-mentioned limitations is by incorporating the use of statistical models of intensity, shape and appearance model. In fact, novel techniques in neuroimaging have become increasingly sophisticated, and automated segmentation packages offer the possibility of investigating structural changes in subcortical regions by using surface and mesh modelling methods (Patenaude, Smith, Kennedy, & Jenkinson, 2011). Surface modelling of subcortical structures allows us to precisely localise differences within subcortical grey matter regions, as well as to detect the direction of the shape deformation (e.g., concave/inwards or convex/outwards).

This approach has previously been used in adults with ASPD and psychopathy. The subcortical regional deformations that researchers were interested in investigating were in the amygdala and hippocampus. It was reported that localised inward deformations were most prominent in regions corresponding to the basolateral and superficial nuclei of the amygdala (Yang & Raine, 2009; Yang et al., 2010). This is of interest because this specialised subregion is implicated in establishing stimulus-reinforcement associations in fear conditioning, which may lead to deficits in the ability to learn from punishment. However, outwards deformations have also been detected in the lateral and central nucleus of the amygdala (Boccardi et al., 2011). These subregions are crucial in terms of mediating rapid fight or flight responses.

Similarly, localised inward deformations in the dentate gyrus of the hippocampus have been observed in violent adults with psychopathy. This sub-region contains CA4 neurons which are responsible for visceral sensory and automatic response (Boccardi et al., 2010). Interestingly, the latter study demonstrated that while there was specific abnormal hippocampal morphology, no grey matter volume changes were observed. Localised alterations of striatal structures have also been investigated in adults with ASPD. It was reported that psychopathy is associated with abnormal striatal morphology, however, with more local and global differences in the NAcc compared to the putamen and caudate (Boccardi et al., 2013).

Finally, sMRI volumetric approaches investigated total GMV of the putamen and globus pallidus combined (i.e., lenticular nuclei) and distinct caudal sub regions. It was reported a total GMV increase in the overall striatum, which was driven by the GMV of the lenticular nuclei. Interestingly, it was also reported that the head of the caudate was associated with impulsive traits, whereas the caudate body was associated with affective and interpersonal features of psychopathy (Glenn, Raine, Yaralian, & Yang, 2010). These findings highlight the importance of investigating localised deformations in an ASPD developmental precursor - CD.

Another factor to consider is that in recent years it has been debated whether CD manifests similarly among young males and females (Berkout et al., 2011). Indeed, CD is more prevalent in males than females, with a lifetime sex ratio of CD of 2.4:1 in favour of males (Moffitt et al., 2001). It has been proposed that the aetiology and biology of CD may differ across the sexes (Meier et al., 2011). As CD is less prevalent in females than in males, it has been suggested that biological correlates of antisocial behaviour would be stronger in females than in males (Cloninger, 1978). On the other hand, it has been argued that biological mechanisms underlying antisocial behaviour in males and females do not differ from each other (Moffitt et al., 2008). Therefore, given that female and male display different characteristic of CD and aetiology, the inconsistencies in previous volumetric sMRI studies, may reflect the possibility that previous studies obscured obtaining sex-specific effects. In fact, shape changes in sub-cortical brain regions have reported to follow sexually dimorphic trajectories over

adolescence (Raznahan et al., 2013), it is possible that these changes may contribute to sex-specific CD symptoms.

Although there are insufficient numbers of sMRI studies investigating sex-specific neural alterations in subcortical grey matter, there are some indications that females with CD showed lower GMV in the putamen and VS (Fairchild et al., 2013), while males with CD show reduced GMV in the caudate nucleus relative to typically developing peers (Fairchild et al., 2011). However, the lack of evidence or replications in these areas may also reflect the fact that few studies have focused on these regions. However, it is important to note that a recent sMRI meta-analysis in youths with conduct problems (CP) reported decreased GMV in only one subcortical limbic region, i.e. amygdala, in youths with CP relative to their healthy comparison group.

5.3 Aims of the Study

The aim of this study is to examine whether CD is associated with alterations in the shape of subcortical brain structures such as the amygdala, hippocampus, putamen, caudate nucleus, nucleus accumbens, thalamus and pallidum. In addition, the present study will further investigate sex differences in the shape of these subcortical structures in adolescence. To address these questions, this study will use a relatively large sample of male and female youths with CD, and shape changes will be analysed by using an automated model-based segmentation tool (Patenaude et al., 2011).

As this is the first study to use shape analysis to investigate brain structure in CD, it is difficult to formulate clear hypotheses regarding localised deformations that may be observed in subcortical structures. However, based on the most consistent findings from previous studies investigating subcortical regions in young people with CD and adults with antisocial personality disorder, we hypothesised that we would observe deformations in amygdala shape in CD. In addition, as deficits in reward- and punishment processing appear to be particularly important in the aetiology of CD, we further hypothesised that we would observe morphological differences between youths with CD and healthy controls, in areas associated with reward processing (e.g., the ventral caudate, ventral putamen and nucleus accumbens).

Similar approaches have previously been used and validated in young people with other neuropsychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD; (Qui et al., 2009; Shaw et al., 2014; Sobel et al., 2010)), schizophrenia (Chakravarty et al., 2015; Dean et al., 2016), and autism (Schuetze et al., 2016), and it has proven to be effective in revealing specific regional shape changes associated with these disorders. However, to date, this relatively novel technique has not been used in a sample of adolescents with CD.

5.4 Methods

5.4.1 Participants

Similar to Chapter 4, participants in this study were recruited from five different sites involved in the Female Neurobiology and Treatment of conduct Disorder (FemNAT-CD) study (for more details see Chapter 3). The sites that were included in this study were as follows: University of Southampton (UOS), University of Birmingham (UOB), Goethe University Frankfurt (GU), University Hospital Aachen (UKAACHEN), and University of Basel (UNIBAS). Similar to the previous chapter, the total sample of participants of the FemNAT-CD that underwent a structural MRI scan was 562 children and adolescents (GU=94, UKAACHEN=131, UOS=140, UNIBAS=65, UOB=132) aged 9-18 years. However, due to previous findings showing brain anatomical changes during childhood and adolescence (Lenroot, 2006) especially in fronto-limbic regions (Casey et al., 2008), this study excluded children aged 9 to 11 (n=87), leaving a sample of 475. Data was then inspected for image quality, and fourteen participants were removed due to poor quality or head movement.

Match is a computer program that facilitates the process of matching large groups of items and participants in large data sets. Match is a fully automated command-line program that operates through an algorithm (van Casteren & Davis, 2007). Thus, we used Match to exclude potential outliers from each group (CD and HC) and to further select an IQ-, age- and gender-matched sample. After this procedure, we ended up with a sample of 315 adolescents, 156 with conduct disorder (79 females) and 159 healthy controls (80 females).

Inspection of individual data revealed that subcortical segmentations for nine participants (FHC-2, MHC-2, MCD-4) were unsuccessful and therefore these participants were excluded. We therefore added two more participants that were similar in IQ scores (1 female with CD, 1 male with CD), and ended up with a final sample of 309 youths.

As mentioned in Chapter 3, a research diagnosis was made using the Schedule for Affective Disorders and Schizophrenia for School-Age-Children –Present and Lifetime version (KSADS-PL;Kaufman et al., 1997).

5.4.2 Image acquisition

Image acquisition has been described in Chapter 4 Section 4.4.2

5.4.3 Subcortical Shape analysis

Shape analyses were carried out using FMRIB's Integrated Registration and Segmentation Tool (FIRST/FSL, FMRIB Software Library). FIRST is a model-based segmentation and registration tool created by Patenaude et al., (2011). The shape and appearance models of the subcortical structures were constructed from manually segmented images from the "Center for Morphometric Analysis", Boston. This training data set and procedure has previously been described (Patenaude et al., 2011). Briefly, the training set was composed of 15 subcortical structures, right/left amygdala, globus pallidum, hippocampus, caudate nucleus, putamen, nucleus accumbens, and thalamus. The structures were traced by trained operators on T1 weighted MR images from 336 subjects. The data set contained a wide age range **(**4 to 72 years), including both typically developing brains as well as pathological brains (e.g. Schizophrenia, Alzheimer's disease, ADHD, Bipolar, and cocaine exposure).

FIRST uses a two-stage process to register each brain image onto an MNI 152 template. The first process involves a whole brain registration. While, the second one applies a subcortical mask followed by an additional affine registration to obtain optimal registration of the subcortical structures. The manual labels are further parameterised and modelled as a point distribution model. Next, it produced deformable surface 3D mesh for each subcortical structure, composed of a set of triangles, the interface of these triangles is called vertex. This procedure automatically quantifies volumetric measures in terms of meshes. The deformable surfaces conserve the vertex correspondence across the training data, and local changes in subcortical structures can be directly investigated by examining vertex locations (Patenaude et al., 2011). It deforms the shape accordingly to the vertices, however, it maintains the point (vertex) correspondence across subjects. Furthermore, normalised intensities along the surface normal are samples and modelled. It further creates a model average shape (determined by the vertex locations), it averages the shape and the modes of variation using a Bayesian formulation. It then fits the model by finding the best shape by searching along modes of variation. The modes describe the how the structure's shape varies most typically over a population, uses intensity match to judge fitting success. FIRST models all structures by meshes, this permits to create images with interior and boundary voxels. The boundary voxels (BV) are used to perform a boundary correction; this is important to decide whether the BV should belong to the structure or not, ensuring that neighbouring structures do not overlap.

5.4.4 Shape analysis

In order to investigate localised shape differences, a vertex-wise analysis was implemented.

The new FIRST version, allowed us to use FSL's Randomise tool, which is a permutation-based inference approach. Once coordinates are transformed, effects of diagnosis, sex, and sex by diagnosis interactions were adjusted for age, IQ, site (using a binary fix effect) and total intracranial volume – which was generated using FreeSurfer (v 5.0). The null distribution was built up over 5000 random permutation-based across the image. The resulting statistical maps were corrected using family-wise error correction threshold of $p < 0.05$. Given that ADHD is a neurodevelopmental disorder that frequently co-occurs with CD, we wanted to inspect the impact of this variable in our findings, therefore we repeated the analysis by adding current symptoms of ADHD (i.e., symptoms displayed in the last year) as an additional covariate.

A previous study calculated power calculations by computing the standard deviation for manual tracings. It was suggested that to detect a 10%- 12% difference between groups in subcortical volume, researcher would require a per group sample size of 24 individuals (Morey et al., 2009).

To obtain more information about our sample, the CD sample was subdivided into higher (Females, n=25; Males; n=38) and lower (Females, n=55; Males, n=36) callous-unemotional traits subgroups using a median split procedure based on CU dimension scores of the YPI. Participants scoring >33 were classified as CD/CU+ while those scoring <33 were classified as CD/CU-.

The following six contrasts were used in for this analysis:

1) Diagnostic effects: *HC>CD*, and *HC<CD*;
2) Sex effects: *M>F*, and *M<F*;
3) Sex by diagnosis interactions: *[(MHC-MCD)>(FHC-FCD)]*, and *[(MHC-MCD)<(FHC-FCD)]*.

The analysis pipeline is shown in **Figure 5.1.**

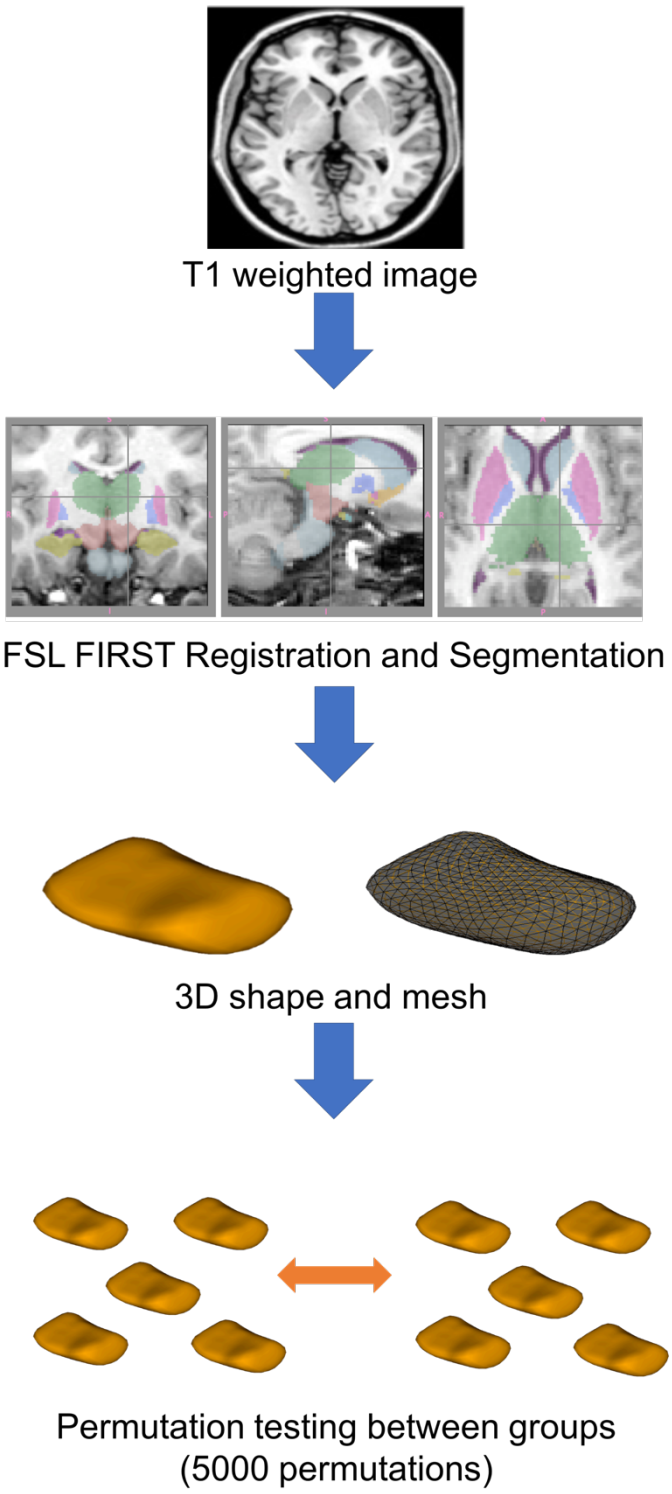


Figure 5.1 - Processing pipeline for shape analysis: The figures were adapted from Patenaude et al. (201
The displayed 3D shape corresponds to the nucleus accumbens

5.5 Results

5.5.1 Sample demographics

Demographic characteristics, along with group comparisons and their corresponding p values, are reported in **Table 5.1**.

The four groups did not significantly differ in IQ, age, or handedness. As expected, the conduct disorder group had significantly more CD, oppositional defiant disorder (ODD) and ADHD symptoms, as well as significantly more traumatic experiences than their control counterparts. Significantly more males than females displayed the childhood onset form of CD, whereas females were more likely to display the adolescent-onset subtype of CD compared to males. Moreover, as expected, the CD group showed significantly higher levels of reactive and proactive aggression, as well as in the three different components of psychopathic traits: impulsive and irresponsible, narcissism, and callous and unemotional traits, and the total score of psychopathic traits.

Regarding psychiatric comorbidity in individuals with CD, males and females showed similar rates on ODD, post-traumatic stress disorder (PTSD), alcohol/substance abuse and dependence. However, the sexes differed for ADHD (males>females) and major depressive disorder (MDD) (females>males). The sample distribution across the sites showed that there was an adequate observed distribution (**Table 5.2**).

Table 5.1 - Clinical and demographic characteristics of the participants included in the shape analyses

Variable	Healthy Controls N=155 (Mean±SD)		CD N=154 (Mean±SD)		Statistics		
	Males N=77	Females N=78	Males (n=74)	Females (n=80)	Group F(p)	F gender F(p)	F GxG F(p)
Age (years)	15.18±1.84	15.56±1.47	15.05±1.96	15.45±1.53	0.38 (0.53)	4.00 (0.05)	0.001 (0.97)
Estimated IQ	99.86±9.60	99.27±12.33	97.28±9.45	97.74±12.85	2.57 (0.10)	0.003 (0.95)	0.17 (0.68)
CD symptoms (K-SADS-PL)	0.19±0.67	0.13±0.37	5.45±2.63	5.24±2.71	558.30 (0.001)	0.393 (0.53)	0.10 (0.75)
ODD symptoms (K-SADS-PL)	0.12±0.43	0.04±0.25	5.15±2.83	5.56±3.01	497.62 (0.001)	0.502 (0.48)	1.08 (0.29)
ADHD symptoms (K-SADS-PL)	0.09±0.59	0.05±0.36	7.43±6.53	5.15±6.15	148.24 (0.001)	5.16 (0.02)	4.8 (0.03)
PTSD (No. traumatic events)	1.26±1.10	1.13±1.19	2.54±2.01	2.88±2.15	62.39 (0.001)	0.28 (0.59)	1.47 (0.22)
CD age of onset, No (%)							
Childhood onset			45 (61)	32 (40)		X^2= 6.65 (0.009)	
Adolescent onset			29 (39)	48 (60)			
Handedness No (%)							
Right	70 (91)	63 (81)	59 (80)	72 (90)	X^2= 5.29 (0.15)	X^2= 1.97 (0.57)	X^2= 9.55 (0.38)
Left	7 (9)	12 (15)	9 (12)	4 (5)			
Ambidextrous	0	2 (3)	3 (4)	4 (5)			
Missing	0	1 (1)	3 (4)	0			
Psychological measurements							
Reactive aggression (RPQ)	5.94±3.65	5.50±3.58	11.47±5.04	11.29±5.80	115.91 (0.001)	0.35 (0.56)	0.06 (0.81)
Proactive aggression (RPQ)	1.51±2.26	0.73±1.41	5.22±4.93	4.23±4.39	79.52 (0.001)	4.7 (0.03)	0.07 (0.79)
Total RPQ	7.40±5.00	6.23±4.34	16.68±9.35	15.48±9.06	124.48 (0.001)	2.04 (0.15)	0.001 (0.99)
Grandiose manipulative (YPI)	35.48±9.21	32.50±9.05	39.85±13.21	38.54±9.68	19.38 (0.001)	3.30 (0.07)	0.50 (0.48)
Callous/Unemotional (YPI)	30.61±5.08	25.54±6.31	34.55±8.97	30.10±6.82	29.21(0.001)	36.64 (0.001)	0.15 (0.69)
Impulsive/Irresponsible (YPI)	33.84±6.09	31.54±6.44	39.16±9.39	41.24±7.97	75.96(0.001)	0.018(0.894)	6.46 (0.01)
Total YPI	100.04±16.43	89.58±18.43	113.58±26.54	109.88±20.29	51.62(0.001)	9.03 (0.003)	2.0 (.15)
Current Psychiatric comorbidity - No. with K-SADS-PL diagnoses (%)							
ADHD			34 (46)	24 (30)		X^2= 4.16 (0.04)	
ODD			51 (69)	50 (63)		X^2= 0.70(0.40)	
PTSD			3 (4)	9 (12)		X^2= 2.77 (0.09)	
MDD			7 (9)	18 (22)		X^2= 4.80(0.03)	
Alcohol abuse			7 (9)	7 (9)		X^2= 0.02 (0.87)	
Alcohol dependence			1 (1)	3 (4)		X^2= 0.87 (0.34)	
Substance abuse			10 (13)	8 (10)		X^2= 0.45 (0.49)	
Substance dependence			10 (13)	5 (6)		X^2= 2.30 (0.12)	
Anxiety			3 (1)	5 (4)		X^2=.37 (0.53)	

Key: +CU, high callous unemotional traits, -CU, low callous unemotional traits, ADHD, attention-deficit/hyperactivity disorder; ODD, Oppositional defiant disorder; PTSD, post-traumatic stress disorder; MDD, major depressive disorder; YPI, youth psychopathic traits inventory; RPQ, reactive proactive aggression questionnaire. *Number of traumatic events were estimated

Table 5.2 - Sample distribution across the sites

	GU (n=50)	UKAACHEN (n=57)	UOS (n=88)	UNIBAS (n=55)	UOB (n=59)	Total (n=309)	
Males CD	11	15	24	7	17	74	X2= 15.85 (.19)
Males HC	11	15	24	8	19	77	
Females CD	14	14	20	20	12	80	
Females HC	14	13	20	20	11	78	

Note: CD; Conduct disorder, HC; healthy controls, UOS; University of Southampton, UOB; University of Birmingham, GU; Goethe University Frankfurt, UKAACHEN; University Hospital Aachen, and UNIBAS, University of Basel. Differences between sites were tested using a Chi Square test.

5.5.2 Main effects of Diagnosis

There was a main effect of CD diagnosis on the shape of the right nucleus accumbens (see **Figure 5.2**). Diagnosis effects were more pronounced in the shell (ventral, medial and dorsal) of the NAcc. In this region, individuals with CD were found to have more inward deformations than HC individuals. CD-related effects in the NAcc remained significant after controlling for ADHD symptoms. The displacements shown in the following figures are significant at the 95% significance level (FWE $p<0.05$), with the colour coding indicating the amount of displacements in millimetres.

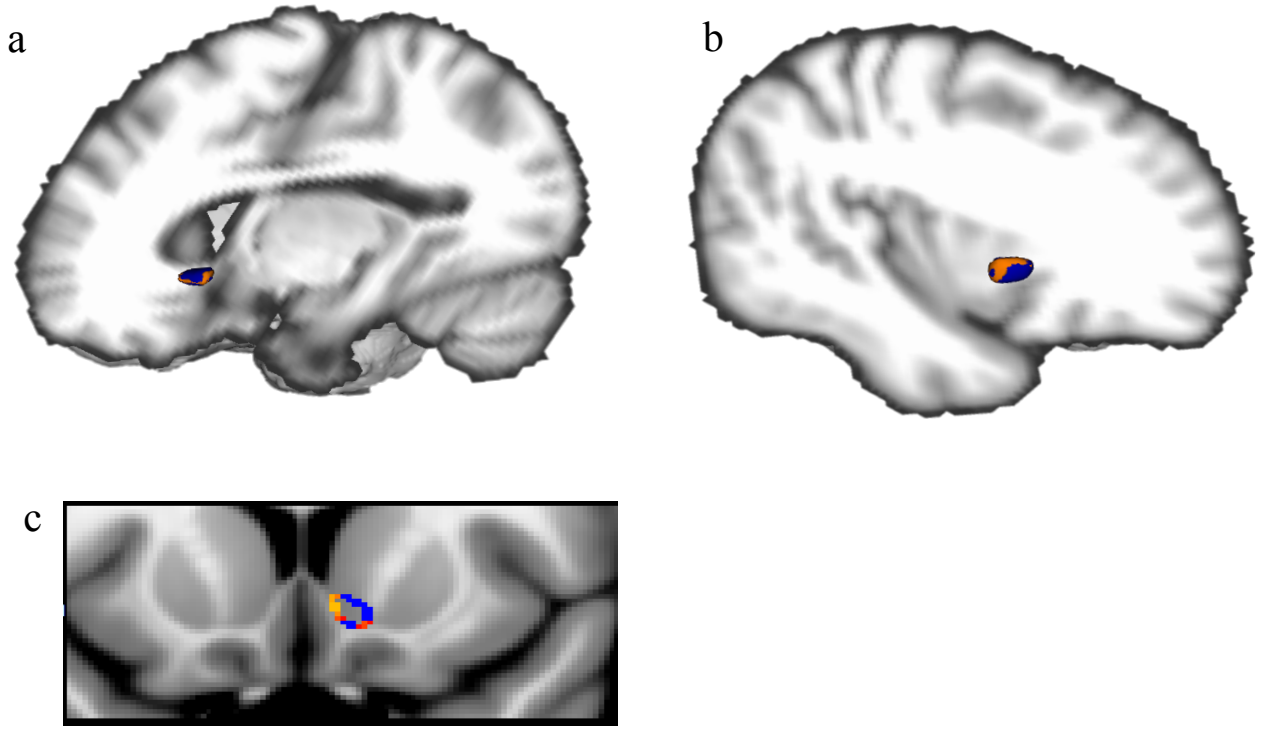


Figure 5.2 - Effects of diagnosis on right nucleus accumbens shape: a) Sagittal section of right hemisphere medial view; b) Sagittal section of right hemisphere lateral view; c) Coronal view showing nucleus accumbens. Areas in red-yellow were localized regions which were significantly displaced inwards in adolescents with Conduct Disorder relative to healthy controls ($p=0.01$). This area mainly corresponds to the shell of the nucleus accumbens. Standard mask of the nucleus accumbens is shown in blue.

5.5.3 Main effect of Sex

There were main effects of sex on bilateral caudate and right putamen shape (see **Figure 5.3**). Sex differences were detected in several striatal structures such as, bilateral caudate, right putamen and NAcc. The morphological deformations in the right caudate were seen in the anterior dorsal part of the body of the caudate, whereas in the left caudate deformations were found in the ventral anterior part of the body of the caudate. The right putamen showed aberrations in the posterior part of the putamen. The right NAcc demonstrated morphological deformation in the core and the shell (ventral-anterior) part of the NAcc. All of these structures demonstrated inward deformations in males compared to females.

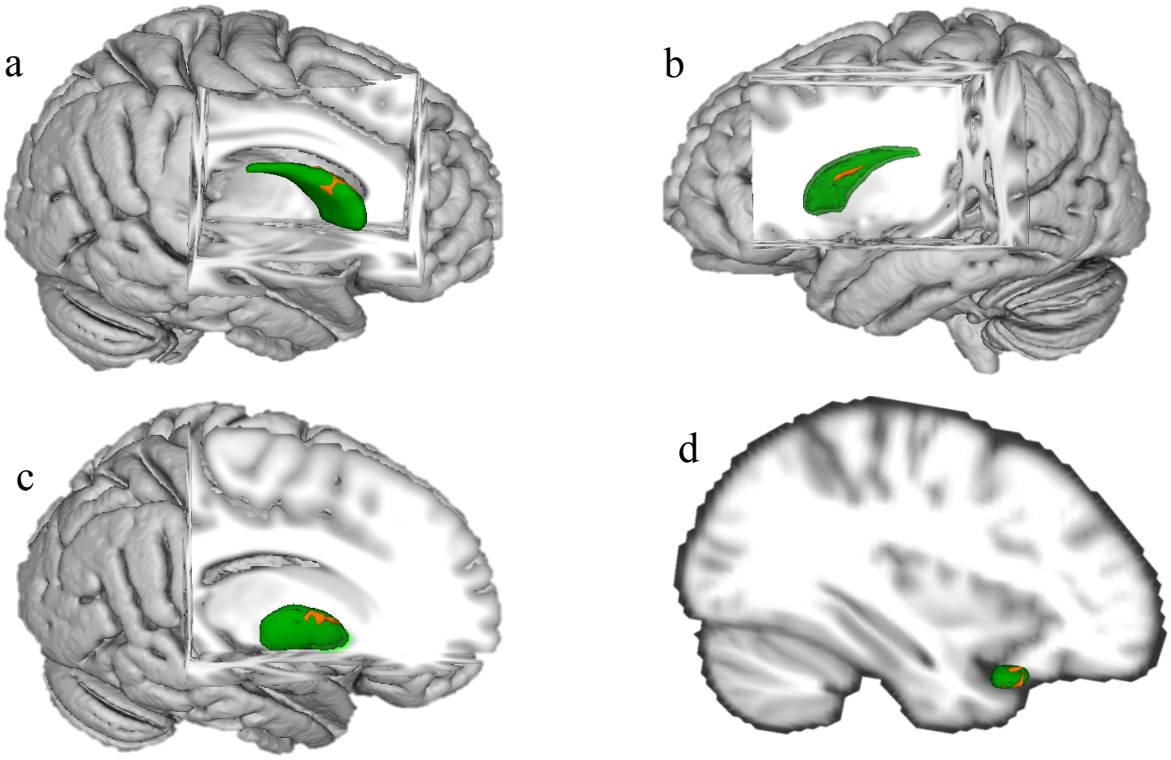


Figure 5.3 - Main effects of sex on shape of striatal structures: Green indicates the standard masks of the: a) Right Caudate (Sagittal view) p=0.02; b) Left Caudate (Sagittal view) p=0.05; c) Right Putamen (Sagittal view) p=0.04; d) Right Nucleus Accumbens (Sagittal view) p=0.01. Areas in red-yellow were localised regions which were significantly displaced inwards in males relative to females.

5.5.4 Sex by diagnosis interactions

No significant sex-by-diagnosis interactions were found at $p<0.05$, FWE. However, there was a trend towards a significant sex-by-diagnosis interaction ($p=0.06$) in the right hippocampus. The difference between the HC and CD groups in hippocampal shape was less pronounced in males than females. The localised area of such interaction was seen in the subiculum of the right hippocampus (see **Figure 5.4).**

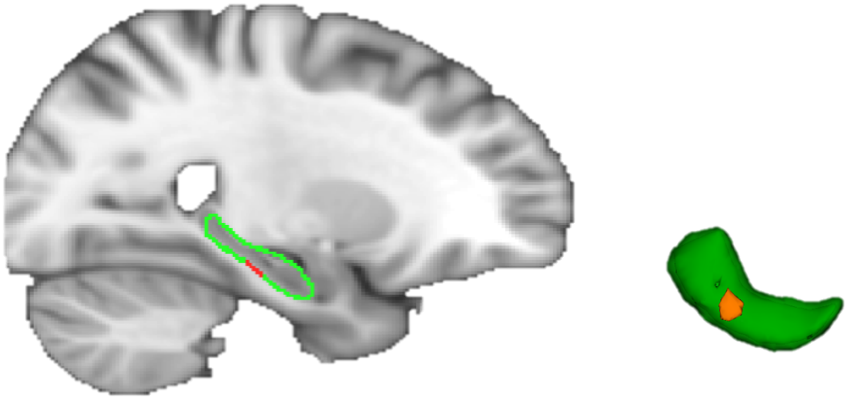


Figure 5.4 - Sex by diagnosis interaction on right hippocampal shape: Sagittal and 3D views of the right hippocampus. Areas in red-yellow were localised regions which were more significantly displaced inwards in females with conduct disorder compared to females without conduct disorder.

5.6 Discussion

This is, to our knowledge, the first study to comprehensively investigate morphological differences in striatal and limbic subcortical structures (i.e., hippocampus, amygdala, nucleus accumbens, putamen, caudate, globus pallidum and thalamus) in youths with CD. Furthermore, we also tested for sex differences and sex-by-diagnosis interactions in a large sample of males and females, which allowed us to investigate whether female and male adolescents with CD show common or distinct deformations in subcortical structures. Interestingly, our findings revealed that subtle differences in the morphology of the nucleus accumbens might play an important role in the pathophysiology of CD. Contrary to our expectations, we did not observe shape abnormalities in the amygdala, which is a region that has frequently been implicated in CD. In addition, we did not observe any significant sex-by-diagnosis interactions in these subcortical structures, although there was a trend towards such an interaction in the right hippocampus.

5.6.1 Group differences in the nucleus accumbens

Findings in the shell of the NAcc, is of considerable importance in the context of connectional arrangements. The shell is distinguished from the rest of the striatum in that it has strong associations with the limbic system. The caudo-medial part of the shell has a close relationship with the extended amygdala, but also both structures share common cellular characteristics and functions (Heimer et al., 1997). The core, on the other hand, is better linked with the extrapyramidal motor system. Interestingly, our findings demonstrated a more significant concave shape in the shell (i.e. medial, ventral and lateral part) of the right NAcc. Therefore, connections of the NAcc with the limbic system and in particular with the amygdala may be impaired in individuals with CD.

The NAcc plays an important role in motivational and affective processes, and reward and reinforcement learning (Galvan et al., 2006). Thus, the diagnostic effect observed in the NAcc is consistent with typical deficits displayed by patients with CD, specifically in terms of the impaired reward processing. Individuals with CD tend to display deficits in learning from aversive outcomes, resulting in poor and reckless decision making (Sonuga-Barke et al., 2016). In addition, studies using functional magnetic resonance imaging (fMRI) in individuals with CD have frequently reported impaired decision-making related to punishment or reward which have been measured in tasks of temporal discounting, gambling,

and reward reversal (Alegria et al., 2016). The NAcc is innervated by mesostriatal dopamine and strongly interconnected with anterior cingulate, ventro medial prefrontal cortex, insula and thalamus (Plichta & Scheres, 2014). Interestingly, studies in youths with conduct problems have shown that deactivations in the vmPFC and ventral striatum (i.e., ventral caudate) are the most consistent findings in tasks of reward-sensitivity, in which individuals with CD tend to demonstrate a preference for immediate over delayed rewards (Alegria et al., 2016; Crowley et al., 2010; White et al., 2012). Given that decision making is influenced by neuropsychological processes (e.g., self-referential, and learning) which affect the subjective value assigned to stimuli or choices (Sonuga-Barke & Fairchild, 2012), shape alteration in the shell of the NAcc may lead to deficits in reward-based decision making.

Furthermore, connections between the vmPFC, NAcc and ventral caudate, generate cues that predict positive or negative outcomes in goal-directed behaviour. Dopaminergic processes may be acting in the NAcc, attributing motivational significance to the stimuli associated with the reward (Yau et al., 2012). The final outcome or decision-making may be based on positive or negative emotional memories (Crowley et al., 2010). Further, neurodevelopmental theories attribute the emergence of CD to the interconnections between the striatum , amygdala, and vmPFC (Crowe & Blair, 2008; Blair, 2013). However, among all the striatal structures, the NAcc is more strongly connected to the vmPFC and anterior cingulate cortex relative to the other striatal structures (Heimer et al., 1997; Salgado & Kaplitt, 2015). In fact, a specific trait that differentiates this fronto-striatal loop (e.g., NAcc-vmPFC), is that in particular the NAcc has been linked to apathy and lack of motivation (Bonelli & Cummings, 2007). In addition, the NAcc is the only striatal structure that receives further input from the amygdala and the hippocampus (Haber, 2016). Perhaps, the strong association of the NAcc with the amygdala and hippocampus mediates the effect of emotional memories and arousal on reward-based decision making.

5.6.2 The role of the nucleus accumbens in previous CD studies

Although the importance of studying the reward-related, motivational and affective functions of the NAcc in CD has been noted by several authors, few fMRI and sMRI studies of CD have specifically targeted this area. However, the lack of studies including the NAcc as a ROI may be as a result of varying methodologies for measuring the striatum. For instance, in some studies, the NAcc is included as part of the caudate (Qui et al., 2009). In addition, the ventral striatum has a more complex organisation when compared to the dorsal striatum, and sometimes its exact location is more challenging to identify (Prensa, Richard, & Parent,

2003). This might be due to the overlapping limits of the NAcc with the caudate nucleus and putamen (Neto et al., 2008).

However, considering all striatal areas as a single structure is problematic. The development of striatal structures have shown to be protracted and heterochronous, with regional specific patterns of expansion and contraction (Raznahan et al., 2013). Thus, while previous studies used volume measures and treated some of these structures as a single component, these are limited to the functional differentiation within these structures (Schuetze et al., 2016). In addition, more recent studies have shown clear distinctions and limits among striatal structures (i.e., including the NAcc), which are detectable with MRI approaches (Neto et al., 2008). In fact, although, some atlases that are provided for MRI-volume based studies do not identify the NAcc per se, and which have incorporated it within adjacent striatal structures (e.g., Tzourio-Mazoyer et al., 2002), some studies interested on explicitly studying the NAcc have used alternative and more targeted approaches, such as manual tracing or directly localizing limits that have been provided by previous neuroanatomists (Boccardi et al., 2013; Crowley et al., 2010; Neto et al., 2008).

Indeed, a study measured reward-punishment sensitivity in youths with antisocial substance disorder, and specifically aimed to investigate the NAcc. However, no abnormal activations were observed. The authors suggested that this could be attributed to opposing biological mechanisms of impulsive traits and the addictive behaviour displayed by their participants (Crowley et al., 2010). However, although, another study included a relatively small sample, it has been revealed that relative to healthy controls, adolescents with CD showed greater NAcc activation by notification of rewards (relative to cues for no incentive), and greater NAcc deactivation by missed rewards (Bjork, Chen, Smith, & Hommer, 2010). Also, a further study measuring response empathic responses to viewing others in pain in youths with CD reported strong neural activations in the ventral striatum (i.e., including the NAcc). Although, it is tempting to suggest that since the NAcc is associated with reward and pleasure, youths with CD found viewing others in pain as 'exciting'. However, the NAcc also plays an important role in fear (Glenn & Yang, 2012). Thus, the NAcc has shown to be activated with not only reward, but also with aversive, novel or intense stimuli (Hikida et al., 2013). A crucial property for these stimuli (i.e., reward and aversive behaviour) is saliency; consequently attending to the pain of others may lead to either approach or avoidance (Groenewegen, 2007). Perhaps, connections of the NAcc shell may be implicated in the functional pathophysiology of CD.

In fact, a recent fMRI meta-analysis in youths with conduct problems reported that the most consistent functional deficits in CD individuals included the ventral striatum (Alegria et al.,

2016). This was observed when considering all tasks together (e.g. executive functions and emotion processing). Nevertheless, this finding seems to be driven by individuals with high levels of psychopathic traits. Similarly, a study assessing adults with psychopathic traits reported that impulsive-antisocial traits were associated with NAcc release of dopamine (Buckholtz et al., 2010). Further, a follow-up study using fMRI showed that impulsive traits were correlated with hyperactivity in the VS during the anticipation of a monetary reward. However there was no activation during the receipt of the monetary reward. This may suggest that abnormalities in this region also leads to disrupted prediction error signalling (e.g. a mismatch between expectancy and outcome), leading to poor-decision making and risk-taking behaviour (Buckholtz et al., 2010). This is in line with an fMRI study conducted on youths with CD, which demonstrated reduced activity in the caudate when measuring prediction error of responses to reward (White et al., 2013). However, similar to the above-mentioned, the atlas that was used in this study did not include the NAcc. Perhaps some of its clusters overlapped with those labelled in the caudate.

Finally, our results are in line with the previous literature in adults with ASPD and psychopathy. A study using manual tracing to detect localised alterations of striatal structures found that the NAcc was the most severely affected by shape changes. Although both inward and outward deformations were reported along several sub-regions of the NAcc, a stronger inward alteration was observed in the anterior region of the NAcc – which also belongs to the shell of the NAcc (Boccardi et al., 2013). However, we did not find amygdala or hippocampal deformations as previous studies in adults with psychopathy have reported. However, it must be noted that these previous findings were obtained in a population of adults with high levels of psychopathic traits. In fact, it has been suggested that amygdala dysfunction has a stronger contribution to the manifestation of the affective-interpersonal impairments (e.g., shallow affect, superficial charm, lack of empathy), than to the manifestation of antisocial behaviour (e.g., criminality versatility, impulsiveness) (Glenn, Raine, & Schug, 2009; Yang et al., 2009). Callous-unemotional traits are a recognised developmental pathway to psychopathy. This is important because not all subjects with CD necessarily display high levels of callous unemotional traits. In fact, the sample used in the present study does not show high levels of CU traits, as the scale ranges from 15 to 60 and the group with the highest levels in this study (males with CD) had a mean score of 35. Thus, future studies investigating morphological differences in the amygdala must be cautious to interpret results and consider this heterogeneity within CD samples. Similarly, hippocampal deformations appeared to be more pronounced in the subgroup of adults with ASPD and greater severity of psychopathy. The hippocampus is a fundamental element contributing to the regulation of fear conditioning,

which is a key characteristic of psychopathy. In contrast, the NAcc is associated with common phenotypes displayed by antisocial individuals more generally.

5.6.3 Commonalities with attention-deficit/hyperactivity disorder

Our results were not affected by the presence of ADHD symptoms. It has been claimed that individuals with CD are differentiated with those with ADHD in that those with CD display low motivation control, whereas individuals with ADHD are mainly associated with inhibition and attention deficits (Hobson, Scott, & Rubia, 2011; Rubia, 2011). In line with this, three studies have previously investigated localized morphological changes of the striatum in ADHD participants, and reported inward deformations in the dorsal striatum (i.e., putamen, caudate, globus pallidus) (Qui et al., 2009; Shaw et al., 2014; Sobel et al., 2010). The DS is more strongly associated with executive functioning and motor planning (Shaw et al., 2014). Although, a longitudinal study in youths with ADHD demonstrated gradual atypical contractions of the ventral striatum, whereas the DS showed a fixed, non-gradual contraction. The segmentation for the VS in this study included the NAcc and sub regions of the caudate and putamen. In addition, no significant group differences were observed in the VS at baseline variables, while they were seen in DS (Shaw et al., 2014).

Moreover, a study using volumetric based techniques did not find any significant volumetric alteration in striatal structures in adolescents with ADHD (Noordermeer et al., 2017). However, although, it is important to consider, that alterations in the NAcc in individuals with ADHD appear to have a stronger effect in childhood than in adolescence, as suggested in a previous meta-analysis (Hoogman et al., 2017). It has been suggested that abnormalities observed in VS in ADHD participants may be linked with comorbidity with CD, which is usually not controlled for in ADHD studies (Alegria et al., 2016; Noordermeer et al., 2017).

5.6.4 Sex effects

Regarding sex effects, we found more concave shapes in bilateral caudate, right putamen and right NAcc in males compared to females. Striatal morphology appears to follow a curvilinear trajectory of shape change in which several structures contract (e.g., ventral regions of the striatum), and others expand (e.g., caudate tail), with a relative delay in males (Raznahan et al., 2013). Thus, our results are consistent with previous reports of sexual dimorphism on striatal structures (Goddings et al., 2014).

In addition, our results are consistent with prior results in which no sex differences were detected in the amygdala's morphology (Satterthwaite et al., 2014). However, we did not replicate previous reports of sex differences in the hippocampus (Satterthwaite et al., 2014). A previous meta-analysis investigating sex-differences on regional brain volume found that males had larger volumes in several limbic brain regions, such as, amygdala, hippocampus, insula, and bilateral putamen relative to females (Ruigrok et al., 2014).These structures follow an inverted U-shaped developmental pattern in grey matter volume (i.e., males>females) and they peak around age 17-19 (Wierenga et al., 2014). Thus, this complex trajectory might have obscured potential regional changes in our data.

It is of interest to note that our findings were mainly observed in the right hemispheres. Right striatal structures have been more strongly associated with the processing of positive emotional stimuli and outcomes (i.e., reward sensitivity) in tasks assessing reinforcement learning (Diener et al., 2012). The delay seen is in males on striatal structures, may explain why males engage in more risky behaviour than do females. For instance, women are more likely to perceive negative outcomes and lesser expectation of enjoyment from risky activities, such as, gambling, substance use, and rule breaking (Harris & Jenkins, 2006). Delayed development of striatal structures combined with delays in the development of the prefrontal regions (Lenroot et al., 2007), may impair some executive functions and impulse control leading to the expression of more risky choices in males than do in females.

5.6.5 Sex by diagnosis interaction

Finally, we found a trend to significance in sex by diagnosis interaction in the right hippocampus. The difference in hippocampal shape was especially stronger for females than in males. Interestingly, the localized change was detected in the subiculum. It has been suggested that connections of the hippocampal subiculum are strongly associated with depression (Posener et al., 2003). However, more specifically, the subiculum is strongly interconnected with the NAcc, and both structures form part of the learned helplessness depression circuit (Grace, 2016). Helplessness is considered to an aetiological factor as the antecedent cause for the onset or maintenance of depression (Bussfeld, Hegerl, Moller, & Henkel, 2002). Therefore, the fact that this specific region is more strongly impaired in females with CD may explain why there are more females with comorbid CD and depression than males.

5.6.6 Strenghts and Limitations

Our study includes several strengths, such as sample size sample size and adequate number of males and females to address the question of sex differences among youths with CD. Our research groups were well-matched in terms of IQ and age. Additionally, we assessed the impact of ADHD comorbidity by treating it as a potential confounding factor in further analysis. Finally, the use of shape analysis allowed us to localise shape changes that volumetric techniques are unable to detect.

A limitation of the present study is that we used a relatively new software package to perform the segmentation. However, this method uses an individual basis structure segmentation and can therefore be used to compare groups. In addition, the advantage of using FIRST-segmentation vs manual segmentation is that while the automated segmentation uses voxel intensity, the manual segmentation relies on visual contrast differences. In addition, the automated segmentation tool allows researchers to analyse large data sets like the one used in the current study. Although it is striking that we observed brain abnormalities in the NAcc in CD, as mentioned before, the NAcc is a difficult area to segment even with user-defined or semi-automated methods. However, other studies using similar methods have also included the NAcc to study its shape deformations (Shaw et al., 2014).

Our study did not exclude the possibility that variance of callous-unemotional, or psychopathic traits could have influenced our findings. However, as this is the first study of this kind, we attempted to first understand and this preliminary result before conducting further analysis.

5.7 Conclusion

In conclusion, this study used shape analysis to further investigate the morphology of subcortical structures in CD. This technique has the potential to provide new insights into localised deformations in subcortical structures such as the amygdala and striatum. This is of relative importance as each of these regions have specific connections and functional roles. In addition, these regions are strongly implicated in CD and behavioural studies have shown that neurocognitive functions linked to these structures are impaired in CD. This study measured these localised differences and revealed that, contrary to our expectations, there were no significant shape changes in key areas that have been associated with the pathophysiology of CD, such as the amygdala and caudate. However, we found abnormal shape deformations in the right nucleus accumbens in adolescents with CD relative to healthy controls. The NAcc is

a structure of high relevance with regards to CD-related characteristics (e.g., increased sensitivity to rewards and risk-taking). However, at the moment the amount of existing literature in this field is relatively small and structural findings in the NAcc of individuals with CD have never been reported. However, given its specific role in reward processing, we hope that this study motivates future research to integrate empirical evidence and initiate new studies in this field.

Chapter 6 White Matter Microstructure of the Extended Limbic System in Youths with Conduct Disorder

6.1 Abstract

Recent work on conduct disorder (CD) has suggested that there are alterations in the connections between the prefrontal cortex and limbic regions. However, studies have yielded inconsistent results, and the role of white-matter abnormalities within limbic areas is not completely understood. The uncinate fasciculus (UF) is a white-matter tract connecting prefrontal cortical and limbic structures, and has been implicated in previous studies of CD. However, to date, other major limbic white-matter tracts including the cingulum bundles (subgenual, retrosplenial and parahippocampal) and the fornix, have not been investigated. Critically, only a few studies have examined the influence of sex and none have been adequately powered to test whether the relationship between CD and altered structural connectivity differs by sex. Thus, this study examined whether adolescent males and females with CD exhibit differences in the structural connectivity of these limbic white-matter tracts compared to healthy controls. We collected diffusion Magnetic Resonance Imaging (dMRI) data from 101 (52 female) adolescents with CD and 99 (50 female) healthy controls. Data were processed for deterministic spherical deconvolution tractography using StarTrack. Five diffusion measures were estimated and exported to TrackVis, namely Fractional Anisotropy, Hindrance Modulated Orientational Anisotropy, Mean Diffusivity, Axial Diffusivity and Radial Diffusivity. Virtual in-vivo dissections of the UF, the three subdivisions of the cingulum (retrosplenial, parahippocampal and subgenual cingulum), and the fornix were performed. Our results extend previous findings and report CD-correlates with the right RSC tract. Importantly, these effects were moderated by sex - males with CD displayed reduced, while CD females showed increased, FA relative to sex-matched healthy control groups. Abnormalities in the RSC white-matter microstructure may contribute to deficits in self-referential processes which in turn influence the poor decision-making exhibited by youths with CD. Our results demonstrate that sex is an important factor when studying WM in youths with CD. Suggestions for future research and clinical implications are discussed.

6.2 Introduction

Conduct Disorder (CD) is diagnosed in children and adolescents that display a repetitive and persistent pattern of behaviour in which societal rules and rights of others are violated (American Psychiatric Association, 2003). Although the lifetime prevalence of CD is higher amongst males than females (a sex ratio of around 2.4:1) (Moffitt et al., 2001), it is nevertheless one of the most common disorders in adolescent females (Baker, 2013). In addition, individuals with CD of both genders have a poor prognosis with negative adult outcomes that include criminality, alcohol abuse, unemployment, poor and physical health (Odgers et al., 2008). CD is one of the main reasons for referral to child and adolescent mental health services (Baker, 2013) generating a high cost to the affected individuals, families and society in general (Scott et al., 2001). Therefore, CD is a major mental health problem, and achieving a better understanding of the neurodevelopmental underpinnings of CD is of great importance.

6.2.1 The Limbic System And Conduct Disorder

It has been proposed that limbic system disruptions may cause antisocial behaviour (Finger et al., 2012; Kiehl, 2006; Raine, Lee, Yang, & Colletti, 2010; Rubia, Smith, et al., 2009). Although the exact make-up of the structures comprising the limbic system is still subject to debate (Rolls, 2013), the most widely-accepted and well-established model states that the limbic system is a set of functionally integrated sub-systems, connecting cortical and subcortical brain structures (Catani, Dell'acqua & Thiebaut De Schotten, 2013; see **Figure 6.1**).

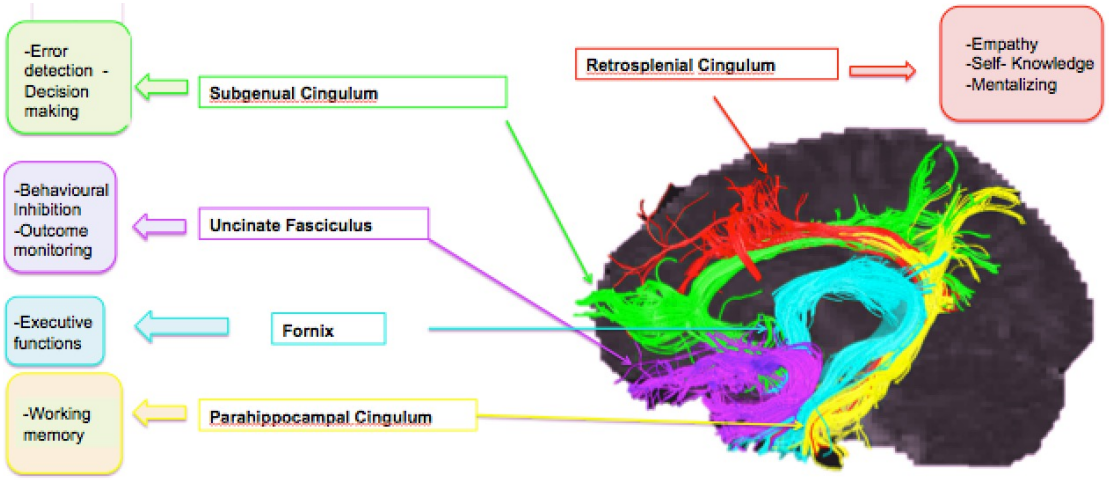


Figure 6.1 - The major limbic white matter tracts of the brain, together with their putative functions

According to this model, the brain regions that form the limbic system include: the anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC), orbitofrontal cortex (OFC), ventromedial prefrontal cortex (vmPFC), hippocampus, hypothalamus, amygdala, insula, and medial temporal lobe (MTL; Catani et al., 2013; Rubia, 2011). The limbic system is involved in emotion processing and regulation, reward-related decision-making and a range of other cognitive functions (Catani et al., 2013; Rolls, 2004, 2013). Evidence implicating limbic brain structures in antisocial behaviour comes from a number of sources. Structural neuroimaging studies in adolescents with CD have frequently identified limbic brain abnormalities, including in the amygdala (Fairchild et al., 2011; Huebner et al., 2008; Sterzer et al., 2007), insula (Fahim et al., 2011; Sterzer et al., 2007), hippocampus (Huebner et al., 2008), ACC (Sterzer et al., 2007), OFC and vmPFC (Fairchild et al., 2011; Huebner et al., 2008). In fact, two recent structural magnetic resonance imaging (sMRI) meta-analyses, concluded that the most robust brain abnormalities observed in this population are in limbic brain structures, such as the amygdala, insula and ACC (Aoki, Inokuchi, Nakao, & Yamasue, 2014; Rogers & De Brito, 2016).

In line with sMRI studies, functional MRI (fMRI) studies have also reported altered patterns of activity in CD individuals in the dorsolateral prefrontal cortex (DL-PFC), ACC, vmPFC, insula, amygdala and OFC during tasks involving emotion processing, and hot executive functions (eg. punishment/reward related decision making tasks, (Finger et al., 2011; Rubia, Halari, et al., 2009; Sterzer et al., 2005), and in dlPFC (Rubia, 2009), dorsal ACC, thalamus and hippocampus (Rubia et al., 2008) during cool executive tasks.

Similar to sMRI metanalyses in CD, a recent meta-analysis of fMRI studies reported that individuals with CD displayed the most consistent underactivations in the ACC and vmPFC

(Alegria et al., 2016). However, most of the fMRI studies conducted in teenagers with CD have used hot executive function tasks, thus due to the nature of the tasks employed in these studies, there may be a bias towards detecting functional abnormalities in these brain regions (Alegria et al., 2016). In line with these findings, a voxel-based morphometry-diffeomorphic study, which is a technique to study structural characteristics of white matter (WM), found increased WM volume in ACC, bilateral vmPFC, right insula, and parahippocampal gyrus in adolescents with CD compared to controls (Wu et al., 2011). Finally, neuroendocrine evidence has revealed that children with disruptive behaviour disorders exhibit disturbances in the hypothalamic-pituitary-adrenal (HPA) axis (Van Goozen et al., 1998). This is interesting as the HPA axis is highly regulated by brain regions of the limbic system, in particular, the amygdala, hippocampus and PFC (de Kloet et al., 2005).

6.2.2 Limbic System Pathways

The brain is formed of grey and white matter and cerebrospinal fluid. The white matter is further composed of myelinated bundles of axons, connecting different grey matter areas of the brain, these connections are usually referred as 'pathways'. Furthermore, axons within the brain (also known as fibres), can be categorized depending on the basis of their path and connection into either association fibres (axons that connect cortical areas within the same cerebral hemisphere), projection fibres (efferent and afferent fibres connecting the cerebral cortex with other centres in the brain or spinal cord), or commissural fibres (axons crossing the midline which connect the two hemispheres of the brain, Catani & Schotten, 2012).

Investigating structural connectivity is now possible by using diffusion-weighted (DW) magnetic resonance imaging, which is a technique frequently used to investigate the microstructural properties of brain tissue (specifically, WM tracts, Leemans et al., 2011). As the cerebral white matter comprises axons, it is possible to measure the diffusivity of water molecules along different directions to estimate the WM fibre orientation (Basser, Mattiello, & LeBihan, 1994). The quantification for water molecule diffusivity is achieved by generating diffusion maps of deep tissue organisation (Basser & Pierpaoli, 1996). The mostly commonly reported diffusion tensor imaging (DTI) measures include: mean diffusivity (MD; average diffusion in all directions), fractional anisotropy (FA; an index of white matter coherence and diffusion directionality), radial diffusivity (RD; perpendicular diffusivity; modulated by myelin in WM) and axial diffusivity (AD; diffusivity along the fibers; an indicator of axonal integrity; Leemans, Lee, Lazar, & Field, 2007).

The major limbic system´s pathways are formed of association and projection fibres and include the fornix, the mammillo-thalamic tract, the anterior thalamic projections, the cingulum, and the uncinate fasciculus (UF; Catani et al., 2013). The fornix is a tract connecting the hippocampus and the hypothalamus. The mammillo-thalamic pathway connects the mammillary bodies and thalamus. The anterior thalamic projection connects the fornix and mammillo-thalamic tract to the orbitofrontal and anterior cingulate cortex. The UF connects the anterior part of the temporal lobe and the OFC. The cingulum contains fibres of different lengths, and it can be divided into an anterior-dorsal component and a posterior-ventral component (Catani et al., 2013). In fact, recent studies have shown that the cingulum subcomponents have different anatomical features (Jones, Christiansen, Chapman, & Aggleton, 2013). In conduct disorder, researchers have mainly focused on investigating group differences of the microstructural properties of the UF (Passamonti et al., 2012; Sarkar et al., 2013a; Zhang, Gao, et al., 2014), perhaps due to earlier findings in adult psychopaths showing white-matter microstructural abnormalities in this tract (M. C. Craig et al., 2009; Motzkin, Newman, Kiehl, & Koenigs, 2011; Sundram et al., 2012). Although these prior studies were important first steps in understanding the developmental trajectory of CD, additional limbic tracts have lacked research interest in youths. This is despite the evidence of abnormalities observed in the structural connectivity in other limbic WM tracts (e.g., cingulum) in adults with ASPD (Sethi et al., 2014).

6.2.3 Techniques Used To Investigate White Matter Characteristics in the Brain

The two most commonly used approaches to analysing diffusion MRI data are voxel-based global search techniques (e.g., tract-based spatial statistics; TBSS), and diffusion MRI-based tractography. Tract-based spatial statistics involves a voxel-wise, fully automated, hypothesis-free approach that investigates changes in DTI parameters across the whole brain (Smith et al., 2006). Although this method facilitates the identification of WM differences in new regions that may not have been previously considered to be of importance in a particular population (Smith et al., 2006), it does not allow the investigation of specific anatomical pathways of interest (Jones, 2008). In addition, the method involves correcting for multiple comparisons across the entire white matter skeleton, which tends to inflate Type II errors (false-negatives), especially in small samples.

On the other hand, diffusion MRI based tractography approaches allow the reconstruction of three-dimensional trajectories of specific white matter tracts such as the UF. This facilitates the integration of diffusion properties along the entire length of these anatomically defined

WM pathways (Jones, 2008). Diffusion tensor (DT) algorithms are the most frequently-used method used to characterise water diffusivity in WM (Basser, Pajevic, Pierpaoli, Duda, & Aldroubi, 2000). However, this method has limitations. For example, it does not solve the problems associated with complex fibre configurations such as fibre crossing, branching problems or a mix of different tissues (e.g. white matter fibres and grey matter). Thus, volume effects corrupt the indices, and they are no longer fibre- or tissue-specific (Dell'Acqua, Simmons, Williams, & Catani, 2013). However, novel tractography approaches based on constrained spherical deconvolution (SD) algorithms (e.g. Damped Richardson Lucy; Dell'Acqua et al., 2010) potentially overcome these limitations and provide a better estimate of the underlying architecture of specific WM tracts.

6.2.4 Diffusion Tensor Imaging Studies of Conduct Disorder

To date, 12 studies have investigated, and directly compared the white matter microstructure of youths with CD and related phenotypes such as conduct problems and callous unemotional traits with typically developing peers (Breeden, Cardinale, Lozier, VanMeter, & Marsh, 2015; Decety, Yoder, & Lahey, 2015; Finger et al., 2012; Haney-Caron, Caprihan, & Stevens, 2014; Hummer, Wang, Kronenberger, Dunn, & Mathews, 2015; Li, Mathews, Wang, Dunn, & Kronenberger, 2005; Passamonti et al., 2012; Sarkar et al., 2013b, 2017; Wang et al., 2012a; Zhang, Gao, et al., 2014; Zhang, Zhu, et al., 2014; see **Table 6.1** for details). Four of these have grouped participants with different disruptive behaviour disorders, including participants with CD and oppositional defiant disorder (Finger et al., 2012; Hummer et al., 2015; T.-Q. Li et al., 2005; Yang Wang et al., 2012a). One study investigated individuals with conduct problems (CP) and varying levels of callous unemotional traits - without requiring subjects to have a research or clinical diagnosis of any disruptive behaviour disorder (Breeden et al., 2015).

Diffusion MRI studies of CD have mainly focused on investigating structural abnormalities of the UF (Passamonti et al., 2012; Sarkar et al., 2013; Zhang, Gao, et al., 2014). The UF is thought to be involved in reward processing and memory retrieval (Olson, Heide, Alm, & Vyas, 2015). Therefore, altered structural connectivity in the UF may explain why individuals with CD show deficits in reversal learning abilities (Finger et al., 2008). The UF is a limbic white matter tract, connecting the OFC to the anterior temporal lobe (Von Der Heide, Skipper, Klobusicky, & Olson, 2013). Abnormal structural connectivity in this tract has been reported in both adolescents with CD and adults with antisocial personality disorder (for review see; Waller, Dotterer, Murray, Maxwell, & Hyde, 2017). Although one study

conducted in youths with CP reported lower FA values in the UF relative to healthy controls (Breeden et al., 2015), a greater number of studies using more specific –WM tract approaches (e.g., deterministic tractography) have reported that adolescents with CD show higher FA values in the UF relative to their healthy counterparts (Passamonti et al., 2012; Sarkar et al., 2013b; Zhang, Gao, et al., 2014). Interestingly, a large number of studies in adults with antisocial personality disorder show the opposite pattern, that is, lower FA values in the UF compared to their healthy counterparts (Craig et al., 2009; Motzkin, Newman, Kiehl, & Koenigs, 2011; Sundram et al., 2011). Several authors have suggested that the higher FA values observed in the UF might indicate an abnormally accelerated maturation of WM in individuals with CD (Passamonti et al., 2012; Sarkar et al., 2013b; Zhang, Gao, et al., 2014).

Table 6.1 - Studies investigating white-matter microstructure in Conduct Disorder and related conditions using diffusion tensor imaging methods.

Study	Participants: mean age years (SD); sex	Match IQ	CD type	Assessed for sex differences	Method	Diffusion Indices	Main Findings	Correlation Findings
Li et al., 2005	25 CD/ODD: 14 ;(M) 21 HC: 14;(M) 11 CD/ODD: 14;(F) 19 HC: 14; (F)	N/A	CD/ODD	NO	Whole brain: (VBA)	FA	↓FA and AD in CD in frontal lobe and left temporal lobe	No correlations
Finger et al 2012	15 CD/ODD: 14.8; (M) 16 HC: 13.8;(M)	Yes	CD/ODD+ psychopathic traits	-	Whole brain TBSS and ROI-A (DT)	FA	No significant findings	No correlations
Wang et al., 2012	8 CD/ODD: 15; (M) 6 CD/ODD: 15; (F) 36 HC: 15; (M) 10 HC: 15; (M)	Yes	CD/ODD	NO	Whole brain TBSS	FA, RD, MD	No significant findings	No correlations
Passamonti 2012	13 CD: 18.4; (M) 13 HC: 18.6; (M)	Yes	Childhood onset	-	Whole brain VBA & ROI-M (DT)	FA, (λ1) (λ2) (λ3).	↑ FA in CD in the right external capsule (shown by VB-DTI) and ↑ FA in bilateral UF (Shown by tractography)	No correlations
Sarkar et al., 2013	27 CD: 16 16 C: 16	Yes	CD	-	ROI-M: (DT)	FA, RD, MD, AD	↑ FA and ↓ RD in CD in the left UF	Trend for a positive correlation in the Left UF and antisocial behaviour.
Haney – Caron et al., 2014	10 CD: 15.9;(M) 7 CD: 15.9; (F) 15 HC: 15.6;(M) 9 HC: 15.9; (F)	Yes	Non-comorbid CD	NO	Whole brain: TBSS	FA, RD, AD	↓ FA and AD in CD in frontal lobe and temporal lobe regions, including corona radiata, IFL, and IFOF	Negative correlations between FA and CD symptoms.
Zhang, Zhu et al., 2014	36 CD: 14.97; (M) 33 HC: 15.93; (M)	Yes	CD	-	TBSS & ROI –A (DT)	FA, RD, MD, AD	↑ FA in CD: Right genu of CC Left body of CC Right body of CC	Positive correlation between impulsivity and FA of the CC
Zhang, Gao et al., 2014	14 CD: 14.1 (M) 13 CD: 14.1 (F) 16 HC: 14.4 (M) 13 HC: 14.4 (F)	Yes	Adolescent onset	YES	ROI-A: (VBA & DT)	FA, RD, MD, AD	↑ FA in male with CD in bilateral UF compared to CD female. ↓ RD in male with CD in left UF ↑ FA in in bilateral UF compared to HC males. No group differences were found in the	Negative Correlation: between males CD with RD and MD and callous-unemotional traits in the right UF. No correlations were found in the female subgroup

Study	Participants: mean age years (SD); sex	Match IQ	CD type	Assessed for sex differences	Method	Diffusion Indices	Main Findings	Correlation Findings
							female sample.	
Breedan et al., 2015	13 CP:15.05 (M) 13 CP:15.05 (F) 12 HC:13.55 (M) 9 HC:13.55 (F)	NO	DBD	NO	ROI-A:(TBSS)	FA	↓ FA right UF, and fornix in the DBD+CU- vs HC ↓ FA in bilateral UF, and fornix in the DBD+CU+ vs HC	Negative correlation between CU traits with FA in bilateral UF and fornix
Decetey et al., 2015	16 ST-CD:10 (M) 20 ST-CD:10 (F) 11 CD:10 (M) 10 CD:10 (F) 26 HC:10 (M) 27 HC:10 (F)	N/A	Children with varying symptoms of CD	YES	Whole brain (TBSS)	FA, RD,AD	No significant differences between CD and HC	CD symptoms associated with higher AD and RD in SLF, r-SCR,r-FMIN – particularly pronounced for females
Hummer et al., 2015	24 CD:10 (M) 9 CD:10 (F) 24 HC:10 (M) 9 HC:10 (F)	YES	DBD	NO	ROI-A:(TBSS)	FA	No significant differences between CD and HC.	↑ FA with increasing age is associated only in HC but not the DBD group
Sarkar et al., 2016	27 CD: (M) 21 HC: (M)	NO	CD	-	Whole brain TBSS		↑ FA in male with CD in bilateral inferior and superior cerebellar peduncles, corticopontocereblar tract, posterior limb of internal capsule and corticospinal tract; and in right SLF and left cerebellar WM	

Key: N/A, not available.
Group acronyms: conduct disorder (CD); healthy controls (HC); callous-unemotional traits (CU); disruptive behaviour disorder (DBD); sub-threshold (ST)
DTI parameter and tract acronyms: fractional anisotropy (FA); radial diffusivity (RD), axial diffusivity (AD); mean diffusivity (MD); superior longitudinal fasciculus (SLF); superior corona radiata (SCR); forceps minor (FMIN); uncinate fasciculus (UF), white matter (WM)
DTI methods acronyms: tract-based spatial statistics (TBSS); region of interest automatic generation (ROI-A); region of interest, manually drawn (ROI-M); voxel based analysis (VBA); deterministic tractography (DT).
Papers are listed in chronological order

However, a deterministic tractography study looking at sex differences in the UF of youths with CD, found significantly higher FA and lower RD values in males with CD, while this was not seen in females with CD (Zhang, Gao, et al., 2014). These preliminary findings may suggest that alterations in temporo-frontal WM microstructure might be unique to the neuropathology of CD/antisocial personality disorder in males. However, in contrast to the latter study, a further TBSS study looked at sex differences in children with CD (aged 9-11 years). It reported that CD symptoms were associated with greater AD and RD in several WM tracts such as the right corticospinal tract, forceps minor, superior longitudinal fasciculus, and superior corona radiata. This effect appeared to be particularly pronounced for females (Decety et al., 2015). Finally, a relatively small and likely underpowered sample for assessing sex differences reported no differences between males and females with CD (Haney-Caron et al., 2014) . These studies highlight that although there have been some attempts to study sex differences in the WM tracts of individuals with CD, the relative lack of evidence still leaves this question unresolved.

In addition, voxel-based studies also included females, however, contrary to the designs used in the aforementioned studies, females and males were combined in the same CD groups. Thus sex differences were not assessed (Li et al., 2005; Wang et al., 2012). This is a surprising oversight given that it has been reported that WM tract development differs in males and females (Clayden et al., 2012). These studies with combined-sex CD groups have shown reduced FA in the fornix (Breeden et al., 2015) and frontal and temporal white-matter (Haney-Caron et al., 2014). Increased FA in the superior longitudinal fasciculus (SLF; (Sarkar et al., 2016)) and reduced RD in the corpus callosum (CC) has also been reported in males with CD (Zhang, Zhu, et al., 2014). However, findings in the SLF and CC are difficult to detect as the SLF and CC are adjacent to fibres of the cingulum, and TBSS is unable to discriminate between these tracts (Kamali, Flanders, Brody, Hunter, & Hasan, 2014).Three TBSS studies, however, did not observe any significant differences between the DBD group and their healthy counterparts in any of the DTI parameters assessed (Finger et al., 2012; Hummer et al., 2015; Yang Wang et al., 2012b).

Interestingly, apart from three studies (Breeden et al., 2015; Haney-Caron et al., 2014; T.-Q. Li et al., 2005) most of the aforementioned studies reported higher, rather than lower, FA values in CD subjects relative to healthy controls. This is important, as FA values increase with age (Asato, Terwilliger, Woo, & Luna, 2010; Schmithorst & Yuan, 2010). In addition, evidence has shown that age is positively associated with FA values in several WM tracts such as the corpus callosum and SLF (Hummer et al., 2015; Peper, de Reus, van den Heuvel, & Schutter, 2015) and the UF (Sarkar et al., 2013b), in typically developing participants, but

not in individuals with CD. Thus, these findings support that higher FA values in individuals with CD may indicate an abnormally accelerated maturation of WM.

It should be noted that the aforementioned DTI studies had several limitations. First, most had relatively small samples – some had less than 15 participants in each group (Finger et al., 2012; Haney-Caron et al., 2014; Passamonti et al., 2012; Zhang, Gao, et al., 2014). Second, all studies using tractography methods applied conventional fibre reconstruction based on DTI algorithms, thus failing to get a better orientation estimate of WM tracts. On the other hand, TBSS methods potentially misassign voxels to their corresponding WM tracts. This is particularly true for the corpus callosum, SLF and cingulum bundle (Bach et al., 2014). Third, besides one study, the possibility of sex differences in the microstructural integrity of limbic system-related tracts has seldom been investigated. This is important as neurobiological factors involved in the aetiology of CD might differ between male and female adolescents (Fairchild et al., 2013; Smaragdi et al., 2017). Finally, the majority of studies have focused narrowly on the UF, with other key limbic white matter tracts being largely ignored.

6.3 Aims of the Study

The present study will address these limitations using a large sample with similar numbers of males and females to examine sex differences. It will extend previous findings by examining two other key limbic WM tracts – the fornix and the cingulum bundle - as plausible WM tracts involved in the neural underpinnings of CD. The fornix links the hippocampus to the hypothalamus (Catani et al., 2012). The fornix is also involved in memory and executive functions (Douet & Chang, 2015). In addition, it links brain areas involved in the regulation of the HPA axis (de Kloet et al., 2005). The cingulum bundle is anatomically and functionally subdivided in three different bundles or tracts – the retrosplenial cingulum (RSC); parahippocampal cingulum (PHC) and the subgenual cingulum (SGC; Jones, Christiansen, Chapman, & Aggleton, 2013). The RSC connects the dorso-lateral prefrontal cortex (Vann, Aggleton, & Maguire, 2009), anterior cingulate cortex, and posterior cingulate cortex (Jones et al., 2013). Regions interconnected by the RSC have been shown to be involved in affective processing (Kiehl et al., 2001), social information processing (Buckner, Andrews-Hanna, & Schacter, 2008) and empathy (Decety et al., 2009). The PHC links posterior cingulate cortex, medial temporal lobe, and visual areas in the occipital lobe (Jones et al., 2013). The PHC is involved in memory recognition, retrieval and interference (Talamini, Meeter, Elvevåg & Murre, 2005). Finally, the SGC connects ventral and rostral ACC with the amygdala (Heilbronner & Haber, 2014). These regions are of specific interest due to previous findings

showing structural and functional abnormalities in the amygdala and the ACC of individuals with CD relative to healthy controls (R. J. R. Blair, 2008; Marsh et al., 2013).

The study will employ a targeted search strategy by using deterministic tractography based on high-angular-resolution diffusion imaging (HARDI) acquisition parameters. We will employ constrained spherical deconvolution techniques for reconstructing WM tracts. Multiple DTI measures (e.g., FA, MD, RD and AD) will be extracted. In addition, hindrance-modulated orientational anisotropy (HMOA) - a novel index of WM microstructural organisation defined as the absolute amplitude of the fibre orientation distribution derived by constrained spherical deconvolution algorithms (Dell'Acqua et al., 2013) - will also be computed. We will also enhance statistical power by increasing the sample size relative to previous studies in this area.

We will attempt to replicate previous results showing higher FA in limbic WM tracts in male individuals with CD relative to healthy controls. In addition, we will extend research in this area by investigating whether other limbic WM tracts also differ in participants with CD compared to controls. We hypothesise that differences between CD and control groups will be most evident in limbic WM tracts involved in socio-emotional processes (i.e. subgenual cingulum, retrosplenial cingulum, and uncinate fasciculus) in comparison with posterior and lateral limbic WM tracts (e.g., fornix and parahippocampal cingulum). We will also compare the childhood- onset and adolescent-onset subtypes of CD in terms of microstructural properties of white matter pathways, given that the former subtype is thought to have a neurodevelopmental basis whereas the latter is considered to emerge as a result of social and peer processes (Fairchild et al., 2013; Moffitt, 1993). In addition, similar to previous studies (Sarkar et al., 2013; Zhang, Gao, et al., 2014), we will investigate whether the degree of white-matter abnormality in CD is related to different dimensions of psychopathic traits (i.e., grandiose-manipulative, callous-unemotional, and impulsive-irresponsible traits) (Breeden et al., 2015; Pape et al., 2015).

6.4 Methods

6.4.1 Participants

Participants for this study were recruited from four different sites involved in the Female Neurobiology and Treatment of Conduct Disorder (FemNAT-CD) study. All participants and several parents went through a research diagnostic interview based in DSM-IV criteria by using the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL) (for more details, see Chapter 3).

Only four sites collected diffusion MRI data, and these were: University of Southampton (UOS), University of Birmingham (UOB), University Hospital Aachen (UKAACHEN), Germany, and University of Basel (UNIBAS), Switzerland.

The total sample of participants of the FemNAT-CD study that underwent a diffusion MRI scan were 325 children and adolescents (UOS=98, UOB=82, UKAACHEN=86, UNIBAS=59) aged 9-18 years. However, due to previous findings showing brain anatomical changes as well as changes in white matter integrity during childhood and adolescence especially in fronto-limbic regions (Casey et al., 2008) , this study excluded children age 9 to 12 (n = 57), leaving a sample of 268. Data were then inspected for image quality and to ensure that whole brain coverage was achieved. This led us to exclude a further 9 participants. Further, residuals to the tensor fit were inspected for outlying data points (described below), and those with significant artefacts (e.g., head movement) visible in their scans were excluded from the analysis (n = 19).

Match is a computer program that facilitates the process of matching large group of items and participants in large data sets. Match is a fully automated command-line program that operates through an algorithm (van Casteren & Davis, 2007). Thus, we further used Match to select an IQ-, age- and gender-matched sample by excluding participants with high and low values of IQ and age from each group (CD and HC). Following this procedure, we ended up with a final sample of 200 adolescents: 101 with conduct disorder (52 females) and 99 healthy controls (50 females).

6.4.2 Scanning

The diffusion MRI data were acquired using HARDI acquisition optimised for spherical deconvolution (for more details, see Chapter 3). The subjects undertook a diffusion weighted

MRI scan lasting eleven minutes. The following parameters were used in acquisition: voxel size 2 × 2 × 2 mm, matrix 128 x 128, field of view 256 mm, 62 slices, b-value 1500s/mm^2, 64 diffusion-weighted directions and 2 non diffusion-weighted volumes. A spin-echo single-shot echo-planar imaging (EPI) was used covering the whole brain (Phillips: TE/TR= 87ms/8000ms, Siemens=TE/TR = 92 /8000 ms). An automated analysis technique was used to assess the quality of the EPI data (Simmons, Moore, & Williams, 1999).

6.4.3 Pre-processing

The datasets were corrected for head motion and eddy current distortions using the FSL software package http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT/UserGuide. Distortions were estimated in the magnetic field using an algorithm developed by Andersson, Graham, et al. (2016). Subject-specific b-vectors were used during pre-processing of data from UOS and UNIBAS as angulation was applied during data acquisition at these sites. (Andersson & Sotiropoulos, 2016)

A Pair of b=0 volumes are used to estimate an off-resonance susceptibility-induced field which were acquired using reversed PE-polarities (Andersson, Skare, & Ashburner, 2003), of the b=0 volumes one was the first diffusion data set volume and the second was obtained afterwards. The diifusion data was used together with the field estimate to genereate a calculation of eddy current-induced distortions and movements of subjects (Andersson & Sotiropoulos, 2016). Non parametric Q-space interpolation replaced lost signal from movement-induced signal loss (Andersson, Graham, Zsoldos, & Sotiropoulos, 2016). Outlying data points in the residuals of the tensor fit were inspected and any scans with significant artefacts were excluded. Explore DTI was used to estimate of diffusion tensor maps (AD, MD, RD, and FA) and was then exported to Trackvis.

Whole brain deterministic tractography using a damped Richardson-Lucy algorithm (Dell'Acqua et al., 2010) was performed by using the software package StarTrack (http://www.natbrainlab.com). This algorithm provides a robust indication of Fibre Orientation Distribution (FOD) in voxels with white matter, grey matter and CSF evident, which therefore reduces false positives of partial volume effects and inaccurate fibre orientations (Dell'Acqua et al., 2013). An absolute (0.1%) and relative (5%) threshold was applied to exclude spurious local connections (Dell'Acqua et al., 2010). The seed point resolution was 2 x 2 x 2 mm, the step size was 0.5 mm, Lmax was 8 and an angle threshold of 45º. The HMOA index was extracted, and exported to Track Vis.

6.4.4 Delineation of Regions of Interest

TrackVis was used to reconstruct the fornix, the CB subdivisions and the UF. Reconstruction of these tracts has previously been described (fornix and UF: (Stieltjes, Brunner, Fritzsche, & Laun, 2013), and CB subdivisions: (Jones et al., 2013). The Boolean logic (AND, and NOT gates) was employed to delineate the CB´s subdivisions, the fornix and the UF. To increase consistency, we dissected the tracts in each hemisphere one at a time. In addition, the tracts were delineated according to a set order. The first tract to be dissected was the subgenual cingulum, followed by the retrosplenial cingulum, parahippocampal cingulum, the fornix, and finally the uncinate fasciculus.

Regions of interest (ROIs) were defined in each subject's native space on the directionally encoded colour map based on the principal eigenvector of the diffusion tensor. Fibres of the opposite hemisphere were excluded by placing a NOT gate in the sagittal plane, two or three slices lateral to the mid-sagittal plane in the hemisphere that was not being delineated. Spurious fibres which were inconsistent with known anatomy, were removed by collocating an exclusion ROI. The present author is experienced with this software, having attended a tractography workshop previously, and was also trained extensively by a staff member of the Neuroanatomy and Tractography Laboratory to ensure reliability and reproducibility of the tractography analyses.

6.4.4.1 Subgenual Cingulum

The first tract to be dissected was the SGC. The SGC is a subdivision of the cingulum bundle. This is the most anterior tract, and it follows the curved shape of the rostral portion of the corpus callosum. Fibres of the SGC arise from the anterior cingulate region, reaching the mPFC, insula and amygdala (Jones, 2013). Association fibres can be better identified on the sagittal plane however the spheres were collocated on the coronal plane. To define the location of the first sphere (radius= max 2-3 mm), the coronal plane was moved five slices in the anterior direction from the midpoint of the body of the corpus callosum. A target sphere (radius = max 2-3mm) was collocated in the subgenual part of the cingulum. **Figure 6.2**

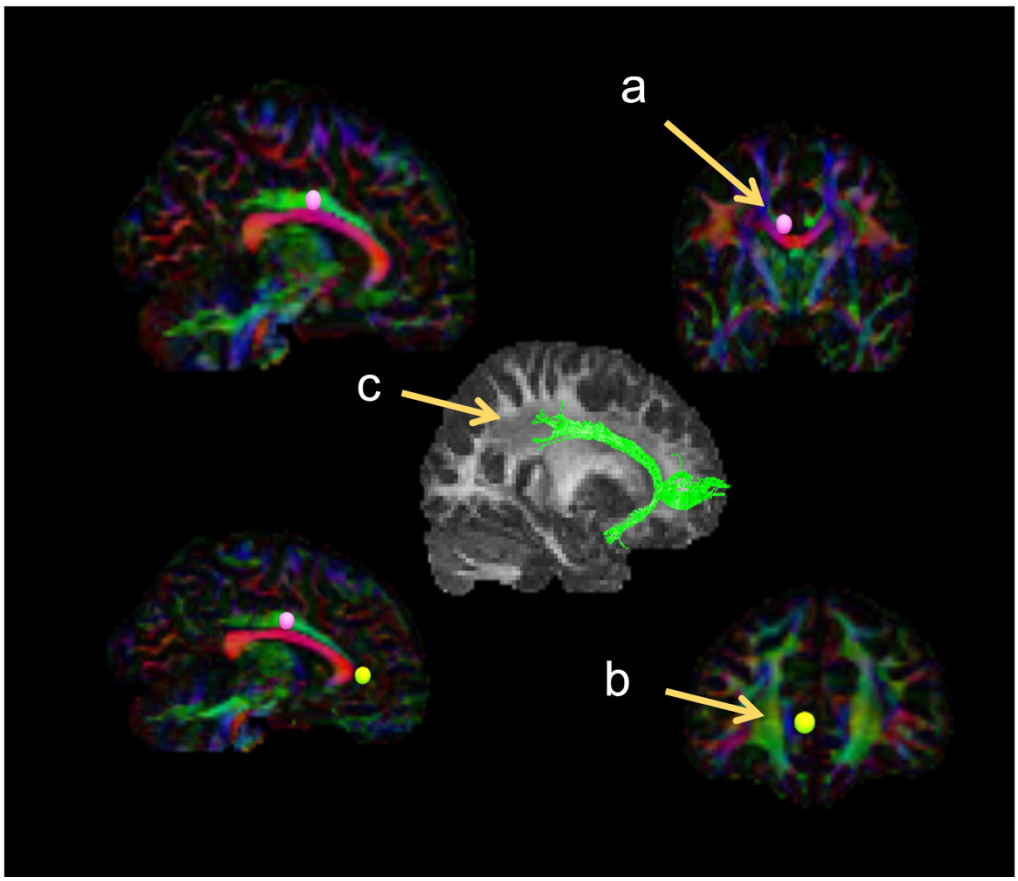


Figure 6.2 - Coronal tractography reconstruction of the subgenual cingulum: a) First sphere of interest placed 5 slices anterior to the mid-sagittal view. b) Target sphere placed 3 slices anterior the genu of the corpus callosum. c) Final reconstruction of the SGC.

6.4.4.2 Retrosplenial Cingulum

The RSC is a subdivision of the cingulum bundle. This tract follows the posterior shape of the cingulate gyrus. It contains fibres connecting the dlPFC, anterior cingulate cortex, and posterior cingulate. Similarly to the SGC, the RSC was identified in the sagittal plane, but spheres were collocated on the coronal plane (radius= 2-3 mm). The first sphere was collocated five slices posterior to the midpoint of the body of the corpus callosum. A target sphere was placed after identifying the most ventral plane of the splenium and collocating it three or four slices above the splenium. See **Figure 6.3**.

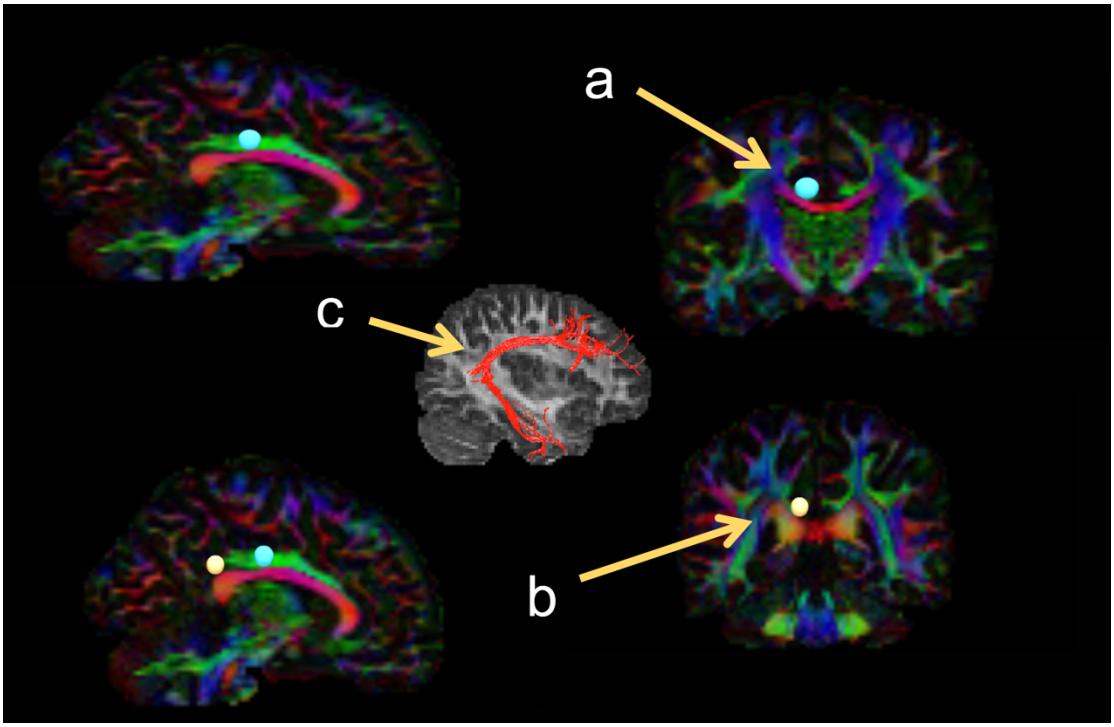


Figure 6.3 - Coronal tractography reconstruction of the retrosplenial cingulum: a) First sphere of interest placed 5 slices posterior to the mid-sagittal view. b) Target sphere placed 3-4 above the splenium. c) Final reconstruction of the RGC

6.4.4.3 Parahippocampal Cingulum

The PHC subdivision is the most lateral subdivision of the cingulum bundle. It connects the medial temporal lobe, posterior cingulate cortex and parietal areas (Jones et al., 2013). The first sphere was the same as the most posterior sphere used for the retrosplenial cingulum. The axial plane was further moved four slices below the upper sphere to identify the location for the target sphere. See **Figure 6.4**.

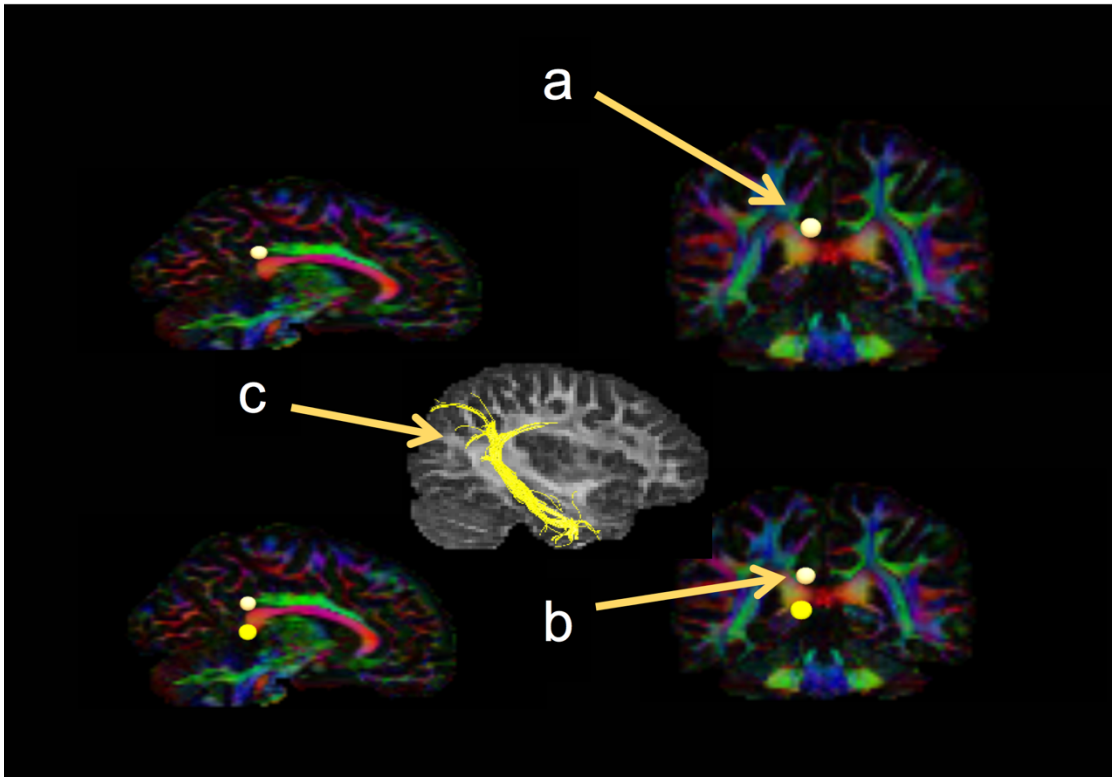


Figure 6.4 - Coronal tractography reconstruction of the parahippocampal cingulum. a) First sphere of interest placed behind the splenium. b)Target sphere placed 3-4 slices below the sphere A. c) Final reconstruction of the PHC

6.4.4.4 Fornix

The fornix is a projection tract connecting the hippocampus with the mammillary bodies, the anterior thalamic nuclei, and the hypothalamus (Catani & Schotten, 2012). To delineate the fornix, its body was identified in the mid-sagittal plane. One sphere was further co-located in the corresponding coronal slice. Spurious fibres of the corpus callosum and anterior commissure were excluded by using exclusion regions of interest (**Figure 6.5**).

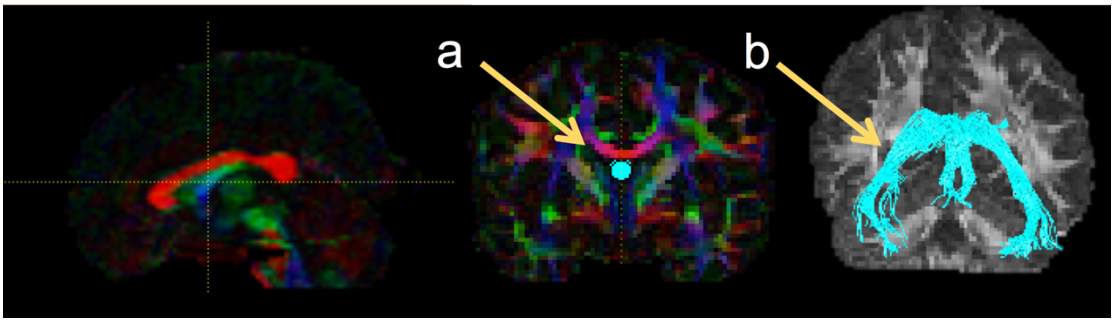


Figure 6.5 - Coronal Tractography reconstruction of the Fornix. a) One coronal sphere placed in the body of the fornix. b) Final reconstruction of the Fornix

6.4.4.5 Uncinate Fasciculus

The last tract to be dissected was the UF. This is a limbic association tract that connects the anterior temporal lobe (including the amygdala) with the OFC. Two spheres were used to dissect the UF. An initial sphere was positioned in a coronal plane in the fronto-temporal junction. A target sphere was further placed in the temporal pole of the same coronal plane (**Figure 6.6**).

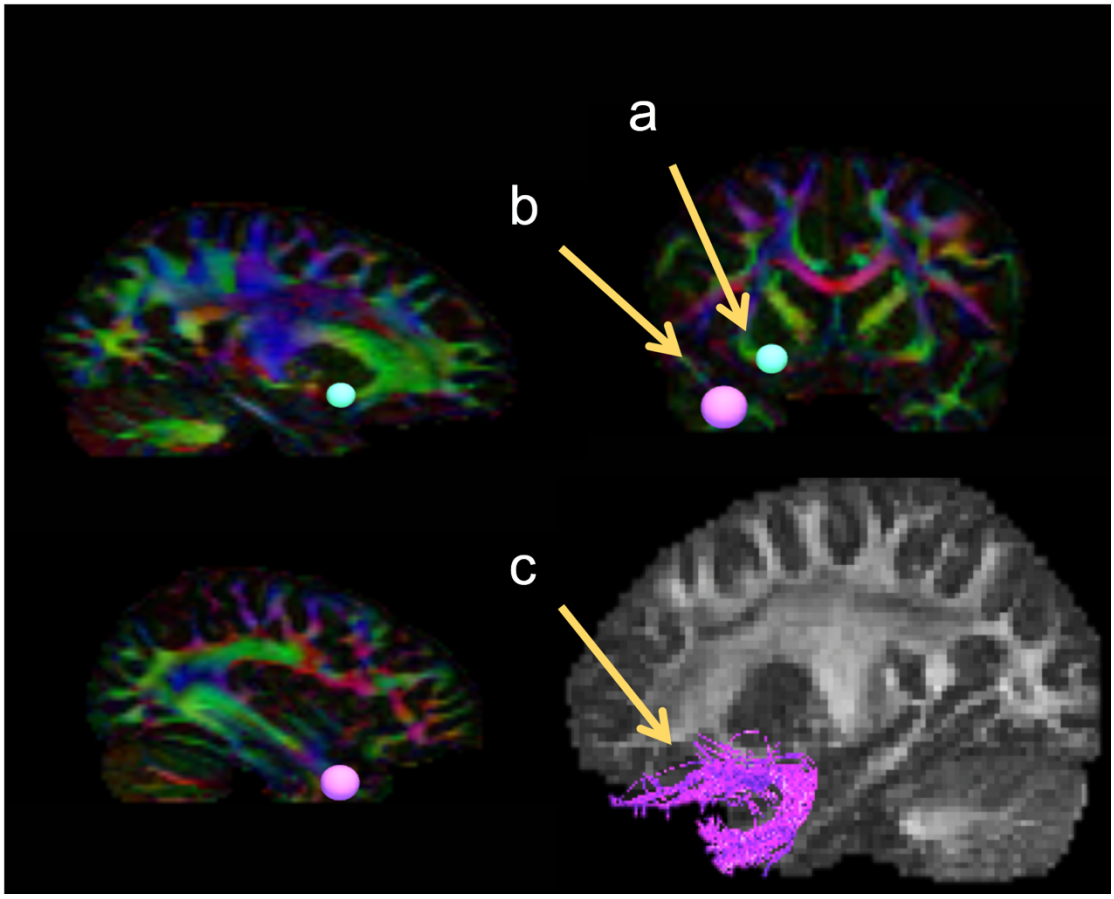


Figure 6.6 -Coronal tractography reconstruction of the uncinate fasciculus: a) First sphere of interest placed in the fronto-temporal junction. b) Target sphere placed at the temporal pole. c) Final reconstruction of the UF

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6.5 Statistical Analysis

Shapiro-Wilk tests were used to verify normal distribution of the quantitative indices using SPSS version 24 (SPSS Inc., Chicago, IL). The values for RD and MD in the fornix were deviated significantly from normality in the healthy control group. Therefore, values of the detected outliers (n=2) were changed to the next highest score plus one (Field, 2009). All measures were normally distributed after this procedure.

Matlab_R2016 B was further used to carry out statistical analysis. Tract measures of FA, RD, MD, AD and HMOA, using a general linear model to examine for diagnosis and gender effects, as well as to test for gender by diagnosis interactions. The general linear model included the following covariates which have been shown to be associated with white matter microstructural integrity in studies of adolescents: age (Asato et al., 2010), IQ (Dunst, Benedek, Koschutnig, Jauk, & Neubauer, 2014), and site (coded as binary fixed effect). ADHD is a neuro-developmental disorder that frequently co-occurs with CD, and previous DTI studies have shown that comorbid ADHD strongly modulates WM effects (Yang Wang et al., 2012b). Thus, we repeated the GLM adding current symptoms of ADHD (i.e., symptoms displayed in the last year) as an additional covariate. In addition, there is some evidence that there are brain structural differences between the childhood – onset (CO) and adolescent-onset (AO) variants of CD (Fairchild et al., 2011). Accordingly, we used the same model to compare these subgroups to assess the validity of combining these subgroups in our main analysis.

The significance threshold was adjusted using the Benjamin – Hochberg false discovery rate (FDR: $q<0.05$) correction for multiple comparisons. Corrections were applied for each parameter independently. Chi square tests were performed using the Social Science Statistic web site (http://www.socscistatistics.com/default.aspx) to examine the distribution of categorical variables. Effect sizes for diagnosis and sex effects were calculated using Cohen's d extracted from effect size calculators (LeeBecker; http://www.uccs.edu/~lbecker/) and effect size for sex by diagnosis interactions were estimated using partial eta squared (ηp^2) displayed by SPSS.

A priori power calculations were estimated based on effect sizes from the study by Sarkar et al. (2013). The results of these calculations indicate that we need 17 subjects in each group, power = 0.8, and $\alpha = 0.05$ to measure the effect.

In cases where significant group differences were observed, we followed these up by running a GLM to test for associations between CD symptoms, dimensions of psychopathic traits (i.e., grandiose-manipulative, callous-unemotional, and impulsive-irresponsible traits), and DTI measures. The alpha level was set as $p < 0.05$. To reduce the probability of finding spurious statistically significant

associations, correlations were only made in structures in which significant group effects were observed. Finally, to obtain more information about our sample, the CD sample was subdivided into higher (Females, n=17; Males; n=26) and lower (Females, n=35; Males, n=23) callous-unemotional traits subgroups using a median split procedure based on CU dimension scores of the YPI. Participants scoring >33 were classified as CD/CU+ while those scoring <33 were classified as CD/CU-.

6.6 Results

6.6.1 Demographic Variables

The four groups did not significantly differ in IQ, age, or handedness. As expected, the CD group had significantly more CD, ODD, and ADHD symptoms, as well as significantly more traumatic experiences than their control counterparts. The CD group also scored higher in terms of psychopathic traits, as well as for the three sub-dimensions of psychopathy: callous-unemotional, grandiose and manipulative, and impulsive and irresponsible (**Table 6.2)**. There were no significant differences between males and females in the age-of-onset of CD (i.e., childhood-onset vs. adolescent-onset).

In terms of psychiatric comorbidity in individuals with CD, males and females differed only in rates of substance abuse (M>F), while similar rates of ADHD, ODD, PTSD, MDD, alcohol abuse, substance dependence and anxiety disorders were observed. Finally, the sample distribution across the sites showed that there was an unequal sample distribution (**Table 6.3**). In an attempt to address this issue we used site as a covariate of no interest.

6.6.2 Tractography results

To test for effects of diagnosis, sex, and sex by diagnosis interactions for the HMOA, FA, RD, MD and AD values within each white matter tract of interest, general linear models were conducted.

6.6.3 Age of CD onset

There were no significant differences between the CO-CD and AO-CD subtypes in any of the diffusion MRI parameters at a significance level of $p<0.05$ - uncorrected.

Table 6.2 - Sample demographic and clinical characteristics of SD Tractography Study

Variable	Females (Mean±SD)		Males (Mean±SD)		Statistics		
	CD (n = 52)	HC n = 50	CD (n = 49)	HC n = 49	Diagnosis F(*p*)	Sex F(*p*)	Sex x Diagnosis F(*p*)
Age (years)	15.44±1.64	15.38±1.66	15.38±1.765	15.34±1.80	0.045 (0.83)	0.032 (0.85)	0.002(0.96)
Estimated IQ	99.48±11.58	100.24±12.20	96.16±9.49	97.43±11.58	0.403 (0.53)	3.69 (0.06)	0.025 (0.87)
CD symptoms (K-SADS-PL)	5.00±2.89	0.06±0.23	5.53±2.73	0.14±0.40	328.50 (0.001)	1.15 (0.28)	0.617 (0.43)
ODD symptoms (K-SADS-PL)	5.61±2.94	0.02±0.14	5.12±2.92	0.08±0.40	321.26 (0.001)	0.53 (0.47)	0.873 (0.35)
ADHD symptoms (K-SADS-PL)	5.37±5.91	0.08±0.44	7.06±6.58	0.02±0.14	96.04 (0.001)	1.69 (0.19)	1.94 (0.16)
*PTSD (No. traumatic events)	2.85±1.98	1.06±1.17	2.59±2.02	1.41±1.19	41.03 (0.001)	0.013 (0.91)	14.22 (0.001)
CD age of onset - No (%)							
Childhood onset	24(46)		28(58)			X^2=1.22 (0.26)	
Adolescent onset	28(54)		21(43)				
Handedness No (%)							
Right	45(87)	40(80)	40(82)	46(94)	X^2= 2.04(0.56)	X^2= 1.54(0.67)	X^2= 9.70(0.37)
Left	2(4)	8(16)	7(15)	2 (4)			
Ambidextrous	3(6)	0	1(2)	0			
Missing	2(4)	2(4)	1(2)	1 (2)			
Psychological measurements							
Reactive aggression (RPQ)	11.67±4.81	5.80±3.33	11.59±5.15	6.02±3.49	89.37 (0.001)	0.013 (0.90)	0.062 (0.803)
Proactive aggression (RPQ)	3.80±3.68	0.60±1.19	5.02±5.06	1.44±2.23	50.38 (0.001)	4.66 (0.03)	0.145 (0.70)
Total RPQ	15.48±7.42	6.40±3.83	16.61±9.60	7.46±5.01	88.64 (0.001)	1.29 (0.25)	0.001 (0.97)
Grandiose manipulative (YPI)	38.13±9.34	31.92±9.09	38.18±13.07	34.93±9.09	10.59 (0.001)	1.11 (0.29)	1.044 (0.30)
Callous/Unemotional (YPI)	29.73±7.69	26.44±6.03	35.57±10.43	30.36±5.29	15.53 (0.001)	20.54 (0.001)	0.788 (0.37)
Impulsive/Irresponsible (YPI)	41.19±8.12	32.16±6.74	39.55±10.01	32.85±5.90	50.12 (0.001)	0.18 (0.67)	1.108 (0.29)
Total YPI	109.07±20.86	90.52±17.89	113.30±28.43	98.18±15.93	31.24 (0.001)	3.89 (0.05)	0.324 (0.57)
Current Psychiatric comorbidity - No. with K-SADS-PL diagnoses (%)							
ADHD	16(31)		21(43)			X^2=1.58(.20)	
ODD	34(65)		30(61)			X^2=0.18 (0.66)	
PTSD	7(14)		2(4)			X^2= 2.73 (0.09)	
MDD	11(21)		4(8)			X^2= 3.36 (0.06)	
Alcohol abuse	1(2)		4(8)			X^2=2.08 (0.14)	
Alcohol dependence	0		0				
Substance abuse	1(2)		7(14)			X^2=5.28 (0.02)	
Substance dependence	1(2)		5(10)			X^2=3.09 (0.07)	
Generalized Anxiety Disorder	7(14)		2(4)			X^2= 2.73 (0.09)	

Key: -CU, low callous unemotional traits, +CU, high callous unemotional traits, ADHD, attention-deficit/hyperactivity disorder; ODD, Oppositional defiant disorder; PTSD, post-traumatic stress disorder; MDD, major depressive disorder; YPI, youth psychopathic traits inventory; RPQ, reactive proactive aggression questionnaire. *Number of traumatic events were estimated

Table 6.3 - Distribution of participants and numbers in each group across the four sites

	UKAACHEN (n=54)	UOS (n=64)	UNIBAS (n=46)	UOB (n=36)	Total (n=200)	
CD males	14	18	4	13	49	X2=27.80 (0.001)
HC males	14	18	4	13	49	
CD females	14	14	19	5	52	
HC females	12	24	19	5	50	

Note: CD; Conduct disorder, HC; healthy controls, UOS; University of Southampton, UOB; University of Birmingham, GU; Goethe University Frankfurt, UKAACHEN; University Hospital Aachen, and UNIBAS, University of Basel. Differences between sites were tested using a Chi Square test.

6.6.4 Effects of Diagnosis

Relative to controls, individuals with CD had a significantly **lower** bilateral HMOA (right :t(190)=-2.22, p=0.03, d=0.10; left: t(190)=-2.27, p=0.02, d=0.16), and **lower** FA (t(190)=-2.91, p=0.004, d=0.28) in the right retrosplenial cingulum. In addition, the CD group also showed significantly **lower** MD (t(190)=-2.03, p=0.04,d=.14) and AD (t(190)=-1.93, p=0.05,d=.14) values in the left uncinate fasciculus compared to healthy controls. However, after correcting for multiple comparisons, only the effect of diagnosis in FA of the right retrosplenial cingulum remained significant (pFDR=.03; **Figure 6.7**). Means and standard deviations for effects of diagnosis on all dMRI parameters are shown in **Table 6.4**. No other significant differences were found in FA, RD, MD, AD and HMOA in any of the other limbic WM tracts.

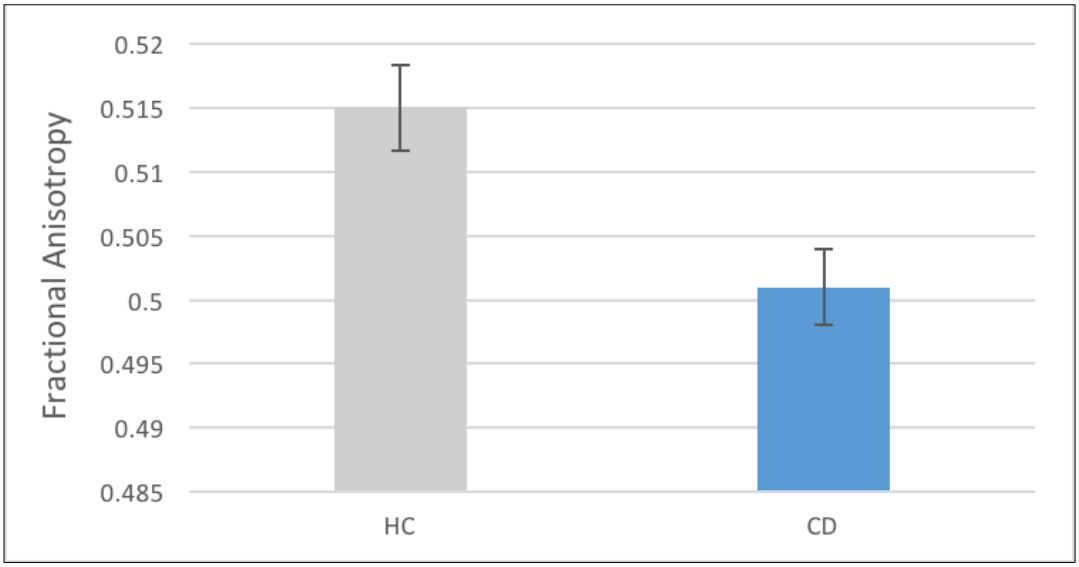


Figure 6.7 - Main effects of diagnosis on fractional anisotropy values in the right retrosplenial cingulum tract. Group differences are significant at p<.05, false discovery rate correction. Error bars show standard errors. HC, healthy controls; CD, conduct disorder.

Table 6.4 - Main effect of diagnosis on measurements of white matter structural connectivity

Tract	Parameter	Hemisphere	Healthy controls (Mean ± SD) n=99	Conduct Disorder (Mean ± SD) n=101	T	P	pFDR	Cohen´s D
SGC	FA	Right	0.427±0.0421	0.424±0.038	-0.001	0.999	1.00	0.07
	HMOA		0.101±0.018	0.099±0.016	-0.08	0.936	0.94	0.11
	AD		1.15±0.05	1.13±0.05	-1.29	0.199	0.26	0.24
	MD		0.75±0.02	0.74±0.02	-1.542	0.125	0.26	0.23
	RD		0.55±0.03	0.55±0.03	-0.728	0.468	0.70	0.06
	FA	Left	0.406±0.042	0.410±0.044	-0.006	0.995	1.00	0.05
	HMOA		0.089±0.018	0.090±0.017	0.107	0.915	0.94	0.08
	AD		1.10±0.04	1.11±0.05	-1.36	0.176	0.26	0.06
	MD		0.74±0.02	0.74±0.03	-1.464	0.145	0.26	0.05
	RD		0.56±0.03	0.56±0.04	-0.428	0.669	0.86	0.09
RSC	FA	Right	0.510±0.0329	0.506±0.029	-2.913	0.004	0.04	0.12
	HMOA		0.127±0.018	0.125±0.017	-2.22	0.027	0.12	0.10
	AD		1.18±0.04	1.18±0.04	-1.402	0.163	0.16	0.12
	MD		0.72±0.02	0.72±0.02	0.191	0.849	0.32	0.13
	RD		0.49±0.03	0.48±0.03	1.249	0.213	0.51	0.06
	FA	Left	0.488±0.034	0.485±0.026	-1.404	0.162	0.49	0.07
	HMOA		0.113±0.016	0.111±0.012	-2.271	0.024	0.12	0.16
	AD		1.16±0.04	1.15±0.04	-1.875	0.062	0.26	0.14
	MD		0.72±0.02	0.72±0.02	-1.154	0.25	0.85	0.14
	RD		0.50±0.03	0.50±0.03	0.016	0.987	0.99	0.07
PHC	FA	Right	0.421±0.035	0.420±0.033	0.635	0.526	0.71	0.06
	HMOA		0.076±0.010	0.077±0.010	0.905	0.367	0.42	0.07
	AD		1.14±0.04	1.13±0.04	-0.677	0.499	0.16	0.11
	MD		0.76±0.03	0.76±0.03	-1.068	0.287	0.32	0.03
	RD		0.57±0.03	0.57±0.03	-0.893	0.373	0.67	0.03
	FA	Left	0.422±0.036	0.418±0.035	-0.86	0.391	0.71	0.11
	HMOA		0.078±0.010	0.082±0.045	1.091	0.277	0.47	0.12
	AD		1.13±0.04	1.12±0.05	-1.815	0.071	0.50	0.19
	MD		0.75±0.03	0.75±0.03	-1.066	0.288	0.32	0.11
	RD		0.57±0.03	0.57±0.03	0.129	0.897	0.99	0.02
UF	FA	Right	0.416±0.031	0.416±0.029	1.47	0.14	0.49	0.01
	HMOA		0.076±0.010	0.076±0.0010	1.61	0.11	0.33	0.05
	AD		1.16±0.03	1.15±0.03	-0.7	0.48	0.50	0.28
	MD		0.77±0.02	0.77±0.02	-1.75	0.08	0.26	0.20

Tract	Parameter	Hemisphere	Healthy controls (Mean ± SD) n=99	Conduct Disorder (Mean ± SD) n=101	T	P	pFDR	Cohen´s D
	RD		0.58±0.03	0.59±0.03	-1.74	0.08	0.51	0.10
	FA		0.424±0.024	0.422±0.026	0.82	0.41	0.71	0.07
	HMOA		0.080±0.008	0.079±0.009	1.22	0.22	0.40	0.11
	AD	**Left**	1.15±0.07	1.14±0.06	-1.94	0.05	0.50	0.14
	MD		0.77±0.03	0.76±0.03	-2.03	0.04	0.16	0.14
	RD		0.57±0.03	0.57±0.03	-1.41	0.16	0.51	0.05
	FA		0.413±0.019	0.415±0.020	0.597	0.551	0.71	0.10
	HMOA		0.100±0.011	0.101±0.011	1.268	0.206	0.40	0.11
Fornix	**AD**	**N/A**	1.58±0.06	1.58±0.07	1.841	0.067	0.16	0.07
	MD		1.06±0.04	1.06±0.05	1.467	0.144	0.26	0.01
	RD		0.80±0.04	0.80±0.05	1.218	0.225	0.51	0.02

Key: FA, fractional anisotropy; HMAO, hindrance modulated orientational anisotropy; AD, axial diffusivity; MD, mean diffusivity;RD, radial diffusivity and SGC, subgenual cingulum; RSC, retrosplenial cingulum; PHC, parahippocampal cingulum and UF, uncinated fasciculus
MD, AD, and RD values and S.D. ×10−3mm2/s.

6.6.5 Effects of Sex

Relative to females, males showed significantly higher FA t(190) =-2.77,p=0.006,d=0.29), HMOA t(190)=-2.99,p=0.003,d=0.35), and AD values (t(190)=-2.52,p=0.006,d=0.35) in the right retrosplenial cingulum. Males also showed higher AD in bilateral SGC (left: t(190)=-2.70,p=0.007,d=0.43; right: t(190)=-2.01,p=0.04,d=0.43), and in the left UF (t(190)=2.70, p=0.02, d=0.36). Furthermore, males showed lower RD t(190) =1.89,p=0.05,d=-0.10), MD t(190)=2.04,p=0.04,d=0.13), and AD t(190)=1.93,p=0.05,d=0.12) in the fornix compared to females. All of the afore mentioned sex effects survived correction for multiple comparisons except those seen in the fornix and the left SGC. Means and standard deviations for effects of sex on all dMRI parameters are shown in **Table 6.5**. No other significant sex differences were found in FA, RD, MD, AD and HMOA in any of the other limbic WM tracts

Table 6.5 - Main effects of sex on measurements of white matter structural connectivity

Tract	Parameter	Hemisphere	Males (Mean ± SD) n=98	Females (Mean ± SD) n=102	T	P	pFDR	Cohen´s D
SGC	FA	Right	0.432±0.039	0.419±0.041	-0.762	0.447	0.66	0.34
	HMOA		0.103±0.017	0.098±0.018	-0.848	0.398	0.48	0.31
	AD		1.15±0.04	1.13±0.06	-2.016	0.045	0.06	0.44
	MD		0.75±0.03	0.74±0.02	-1.829	0.069	0.09	0.21
	RD		0.55±0.04	0.55±0.03	-0.491	0.624	0.80	0.10
	FA	Left	0.413±0.046	0.405±0.040	-0.712	0.477	0.66	0.18
	HMOA		0.092±0.018	0.088±0.015	-1.298	0.196	0.72	0.25
	AD		1.12±0.05	01.10±0.04	-2.7	0.008	0.10	0.43
	MD		0.75±0.03	0.74±0.03	-2.606	0.01	0.21	0.32
	RD		0.56±0.04	0.56±0.03	-0.224	0.823	0.93	0.00
RSC	FA	Right	0.513±0.033	0.504±0.030	-2.778	0.006	0.05	0.29
	HMOA		0.129±0.019	0.123±0.016	-2.99	0.003	0.03	0.35
	AD		1.19±0.04	1.18±0.04	-2.529	0.012	0.06	0.36
	MD		0.72±0.02	0.72±0.02	-1.013	0.312	0.31	0.14
	RD		0.48±0.03	0.49±0.03	0.714	0.476	0.72	0.09
	FA	Left	0.488±0.033	0.486±0.028	-0.508	0.612	0.66	0.06
	HMOA		0.113±0.016	0.111±0.012	-1.776	0.077	0.35	0.16
	AD		1.16±0.04	1.1532±0.04	-1.425	0.156	0.23	0.09
	MD		0.72±0.02	0.72±0.02	-1.482	0.14	0.40	0.05
	RD		0.50±0.03	0.50±0.02	-0.702	0.483	0.72	0.00
PHC	FA	Right	0.419±0.039	0.422±0.029	0.916	0.361	0.66	0.08
	HMOA		0.077±0.010	0.076±0.008	0.179	0.858	0.89	0.14
	AD		1.13±0.05	1.14±0.04	-0.131	0.896	0.67	0.06
	MD		0.76±0.03	0.76±0.03	-0.676	0.5	0.39	0.01
	RD		0.57±0.03	0.57±0.03	-0.753	0.452	0.72	0.03
	FA	Left	0.416±0.040	0.424±0.029	0.602	0.548	0.66	0.22
	HMOA		0.083±0.046	0.078±0.009	-0.133	0.895	0.89	0.17
	AD		1.12±0.06	1.13±0.04	-0.641	0.522	0.90	0.03
	MD		0.75±0.03	0.75±0.02	-1.181	0.239	0.50	0.20
	RD		0.57±0.04	0.56±0.03	-0.951	0.343	0.72	0.27
UF	FA	Right	0.418±0.029	0.414±0.030	0.69	0.49	0.66	0.12
	HMOA		0.077±0.010	0.075±0.009	0.37	0.71	0.48	0.26
	AD		1.15±0.03	1.15±0.03	-0.29	0.77	0.87	0.21

Tract	Parameter	Hemisphere	Males (Mean ± SD) n=98	Females (Mean ± SD) n=102	T	P	pFDR	Cohen´s D
	MD		0.77±0.03	0.77±0.02	-0.92	0.36	0.39	0.11
	RD		0.58±0.03	0.58±0.03	-0.92	0.36	0.72	0.02
	FA		0.427±0.025	0.419±0.024	-0.92	0.36	0.66	0.34
	HMOA		0.081±0.008	0.077±0.008	-1.24	0.22	0.89	0.49
	AD	**Left**	1.16±0.03	1.14±0.08	-2.35	0.02	0.06	0.37
	MD		0.77±0.02	0.76±0.03	-1.13	0.26	0.41	0.24
	RD		0.57±0.03	0.57±0.02	-0.09	0.93	0.93	0.06
	FA		0.416±0.023	0.413±0.016	-0.445	0.657	0.66	0.16
	HMOA		0.101±0.012	0.100±0.010	-0.485	0.628	0.89	0.12
Fornix	**AD**	**N/A**	1.58±0.06	1.59±0.06	1.93	0.055	0.10	0.12
	MD		1.06±0.05	1.06±0.04	2.044	0.042	0.19	0.13
	RD		0.80±0.05	0.80±0.04	1.896	0.059	0.54	0.10

Key: FA, fractional anisotropy; HMAO, hindrance modulated orientational anisotropy; AD, axial diffusivity; MD, mean diffusivity;RD, radial diffusivity and SGC, subgenual cingulum; RSC, retrosplenial cingulum; PHC, parahippocampal cingulum and UF, uncinated fasciculus
MD, AD, and RD values and S.D. ×10−3mm2/s.

6.6.6 Sex by diagnosis interactions

We found sex by diagnosis interactions in the right FA $t(190)=2.75, p=0.006, \eta p^2=0.04$)), and in bilateral HMOA (right: $t(190)=2.08, p=0.04, \eta p^2=0.02$: left: $t(190)=1.99, p=0.05, \eta p^2=0.02$) of the right RSC. All interactions followed the same pattern: namely that healthy control males showed higher values relative to males with CD, while healthy control females showed lower values relative to females with CD (**Figure 6.8).** However, only the sex by diagnosis interaction on FA in the right RSC survived for multiple comparisons (pFDR=.05). Means and standard deviations for interactions between sex and diagnosis on all dMRI parameters are shown in **Table 6.6.** No other significant interaction effects were found in FA, RD, MD, AD and HMOA in any of the other limbic tracts.

Table 6.6 - Sex by diagnosis interaction on measurements of white matter structural connectivity

			Healthy Controls		Conduct Disorder					
Tract	**Parameter**	**Hemisphere**	**Male (Mean ± SD) n=49**	**Female (Mean ± SD) n=50**	**Male n=49**	**Female n=52**	**T**	**P**	**pFDR**	**ηp^2**
SGC	**FA**	**Right**	0.433±0.039	0.420±0.043	0.431±0.036	0.4170±0.038	-0.176	0.861	0.88	0.0001
	HMOA		0.103±0.016	0.098±0.019	0.102±0.016	0.096±0.016	-0.215	0.83	0.90	0.0002
	E1		1.15±0.04	1.13±0.06	1.14±0.05	1.12±0.05	0.759	0.449	0.35	0.003
	MD		0.75±0.02	0.75±0.02	0.75±0.03	0.74±0.02	1.078	0.282	0.38	0.006
	Dperp		0.55±0.04	0.55±0.03	0.55±0.03	0.55±0.03	0.619	0.537	0.80	0.002
	FA	**Left**	0.412±0.048	0.404±0.04	0.414±0.045	0.407±0.043	0.157	0.875	0.88	0.001
	HMOA		0.091±0.017	0.087±0.014	0.092±0.018	0.088±0.016	0.12	0.905	0.90	0.001
	E1		1.12±0.05	1.09±0.04	1.11±0.05	1.10±0.05	1.277	0.203	0.58	0.008
	MD		0.75±0.02	0.74±0.03	0.74±0.03	0.74±0.03	1.453	0.148	0.43	0.007
	Dperp		0.56±0.04	0.56±0.03	0.56±0.04	0.56±0.04	0.218	0.828	0.92	0.001
RSC	**FA**	**Right**	0.520±0.033	0.500±0.030	0.505±0.031	0.507±0.028	2.755	0.006	0.05	0.038
	HMOA		0.132±0.018	0.121±0.015	0.125±0.018	0.124±0.016	2.7	0.038	0.21	0.022
	E1		1.19±0.04	1.17±0.04	1.18±0.04	1.18±0.04	1.194	0.234	0.35	0.007
	MD		0.72±0.02	0.72±0.02	0.72±0.02	0.71±0.02	-0.516	0.607	0.46	0.001
	Dperp		0.48±0.03	0.49±0.03	0.47±0.03	0.48±0.03	-1.464	0.145	0.41	0.011
	FA	**Left**	0.491±0.038	0.484±0.030	0.484±0.027	0.487±0.027	1.29	0.199	0.60	0.009
	HMOA		0.116±0.018	0.110±0.014	0.110±0.014	0.112±0.010	1.995	0.047	0.21	0.02
	E1		1.16±0.04	1.15±0.04	1.15±0.05	1.15±0.03	1.621	0.107	0.35	0.014
	MD		0.72±0.02	0.72±0.02	0.72±0.03	0.72±0.02	0.834	0.405	0.61	0.004
	Dperp		0.50±0.03	0.50±0.02	0.50±0.03	0.50±0.02	-0.207	0.837	0.92	0.001
PHC	**FA**	**Right**	0.418±0.041	0.425±0.028	0.420±0.037	0.419±0.029	-0.814	0.417	0.75	0.003
	HMOA		0.076±0.011	0.076±0.008	0.078±0.010	0.076±0.009	-0.793	0.429	0.55	0.003
	E1		1.14±0.04	1.14±0.04	1.13±0.05	1.13±0.04	0.469	0.639	0.35	0.001
	MD		0.76±0.03	0.76±0.03	0.75±0.03	0.76±0.03	1.066	0.288	0.43	0.006
	Dperp		0.57±0.03	0.57±0.04	0.57±0.03	0.57±0.03	1.021	0.308	0.56	0.005
	FA	**Left**	0.420±0.040	0.424±0.031	0.413±0.041	0.423±0.028	0.632	0.528	0.79	0.002
	HMOA		0.079±0.012	0.078±0.009	0.087±0.064	0.078±0.008	-0.859	0.391	0.55	0.004
	E1		1.13±0.05	1.13±0.04	1.12±0.06	1.13±0.04	1.387	0.167	0.72	0.01
	MD		0.76±0.03	0.75±0.02	0.75±0.03	0.75±0.02	0.821	0.413	0.46	0.004
	Dperp		0.57±0.04	0.56±0.03	0.57±0.04	0.56±0.03	-0.099	0.922	0.92	0.001

			Healthy Controls		Conduct Disorder					
Tract	**Parameter**	**Hemisphere**	**Male (Mean ± SD) n=49**	**Female (Mean ± SD) n=50**	**Male n=49**	**Female n=52**	**T**	**P**	**pFDR**	**ηp^2**
UF	**FA**	**Right**	0.414±0.029	0.417±0.032	0.421±0.028	0.411±0.028	-1.55	0.12	0.56	0.012
	HMOA		0.076±0.010	0.076±0.010	0.078±0.009	0.074±0.008	-1.8	0.07	0.22	0.017
	E1		1.16±0.03	1.15±0.03	1.15±0.03	1.14±0.02	0.08	0.94	0.35	0.0001
	MD		0.78±0.02	0.77±0.02	0.78±0.03	0.77±0.02	1.38	0.17	0.38	0.01
	Dperp		0.59±0.03	0.58±0.03	0.57±0.03	0.58±0.03	1.59	0.11	0.41	0.013
	FA	**Left**	0.43±0.024	0.422±0.024	0.428±0.026	0.416±0.024	-1.04	0.3	0.68	0.006
	HMOA		0.081±0.008	0.078±0.008	0.082±0.009	0.076±0.008	-1.54	0.13	0.28	0.012
	E1		1.18±0.03	1.14±0.09	1.15±0.03	1.14±0.08	1.29	0.2	0.94	0.009
	MD		0.77±0.02	0.76±0.03	0.76±0.02	0.76±0.03	1.7	0.09	0.38	0.015
	Dperp		0.57±0.03	0.57±0.02	0.57±0.03	0.57±0.03	1.37	0.17	0.41	0.01
Fornix	**FA**	**N/A**	0.414±0.022	0.413±0.016	0.417±0.023	0.413±0.015	-0.354	0.724	0.88	0.001
	HMOA		0.099±0.012	0.100±0.010	0.102±0.013	0.099±0.009	-1.066	0.288	0.52	0.006
	E1		1.57±0.06	1.59±0.06	1.59±0.06	1.58±0.07	-1.781	0.077	0.35	0.016
	MD		1.05±0.0	1.07±0.04	1.06±0.06	1.06±0.05	-1.533	0.127	0.38	0.012
	Dperp		0.80±0.04	0.81±0.04	0.80±0.06	0.80±0.04	-1.335	0.183	0.41	0.009

Key: FA, fractional anisotropy; HMAO, hindrance modulated orientational anisotropy; AD, axial diffusivity; MD, mean diffusivity;RD, radial diffusivity and SGC, subgenual cingulum; RSC, retrosplenial cingulum; PHC, parahippocampal cingulum and UF, uncinated fasciculus
MD, AD, and RD values and S.D. ×10−3mm2/s.

6.6.7 Testing for the potentially confounding effects of ADHD comorbidity

The main effects of CD diagnosis observed in the right FA (p=0.003) and bilateral HMOA (left:p=0.03: right: p=0.03) of the RSC remained significant after factoring out current ADHD symptoms (i.e., symptoms occurring in the past year). In addition, the group effects on AD (p=0.03) and MD (p=0.01) values within the left UF also remained significant after controlling for ADHD symptoms. Moreover, a significant main effect of diagnosis emerged in the RD (189)=-2.27, p=0.02), FA t(189)=2.00, p=0.05), MD t(189)=-2.06, p=0.04,d=) and HMOA t(189)=2.07, p=0.04) of the right UF. All of the aforementioned measures of structural connectivity were higher in healthy controls relative to the CD group. However, only the FA difference in the right RSC remained significant (pFDR=0.03) after correcting for multiple comparisons.

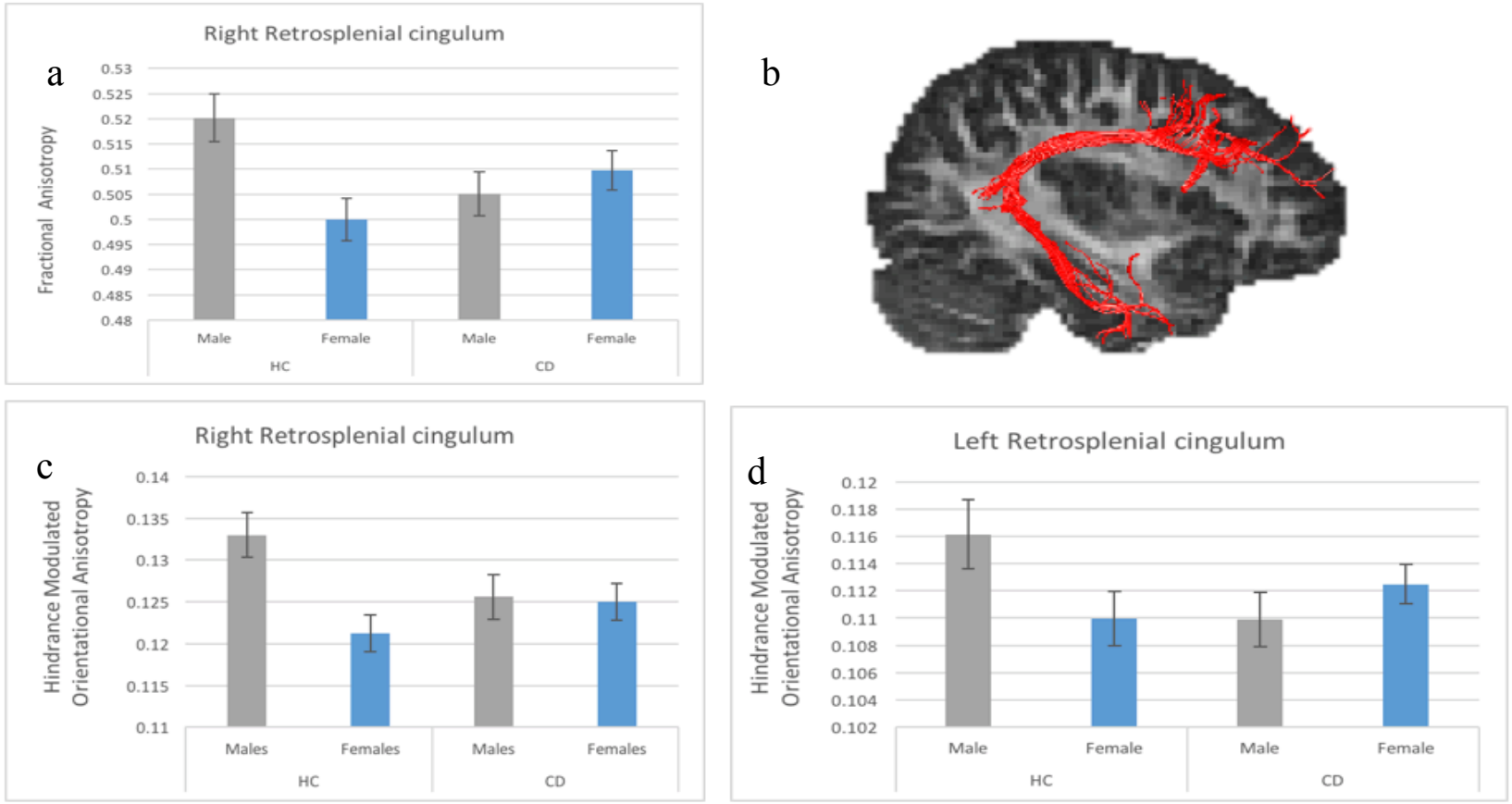


Figure 6.8 – Sex by diagnosis interactions. a) Sex by diagnosis interactions on fractional anisotropy values in the right retrosplenial cingulum. pFDR=0.05 False Discovery Rate correction. b) 3D reconstruction of the Retrosplenial cingulum tract. c) Sex by diagnosis interactions in the Hindrance Modulated Orientational Anisotropy of the right retrosplenial cingulum. d) Sex by diagnosis interactions in the Hindrance Modulated Orientational Anisotropy of the left retrosplenial cingulum.

6.6.8 Correlations between measures of structural connectivity and CD symptoms or psychopathic traits

Within the CD sample, there was a positive correlation between current CD symptoms and the HMOA of the right retrosplenial cingulum (p=0.007; **Figure 6.9**). There were no other significant correlations between any of the other measures of structural connectivity of the right RSC or bilateral UF. Moreover, there were no sex by diagnosis or sex-by-psychopathic traits (including the three distinct sub facets, impulsivity, grandiose/narcissistic, and callous-unemotional) interactions for any of the other DTI parameters.

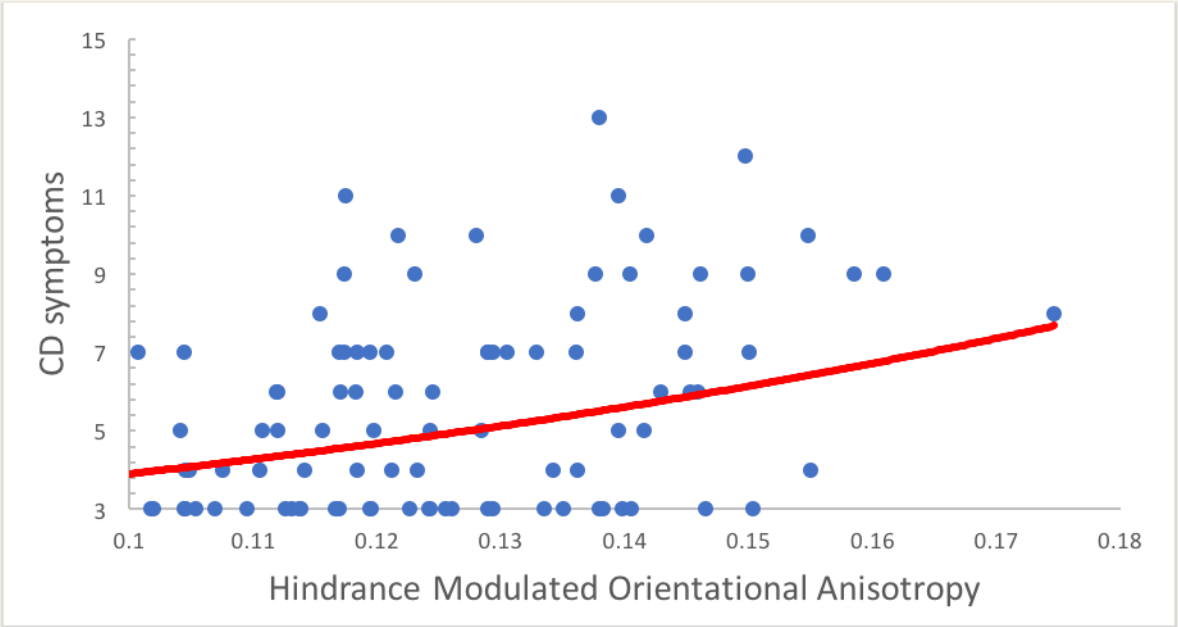


Figure 6.9 - Associations between CD symptoms and HMOA in the right retrosplenial cingulum in the CD group. There was a significant positive correlation between Conduct Disorder symptoms and hindrance modulated orientational anisotropy (HMOA) values in the right retrosplenial cingulum tract.

6.7 Discussion

Abnormalities in the limbic system have been consistently implicated in the pathophysiology of Conduct Disorder. However, no diffusion tensor imaging studies have investigated other key limbic white-matter tracts beyond the uncinate fasciculus. The current study capitalised on methodological advances in diffusion weighted imaging by applying a novel analysis approach: spherical deconvolution (SD) tractography to study limbic WM tracts in adolescents with CD.

In addition to these methodological innovations, we have extended the literature by including a much larger sample of male and female participants than has been studied to date (n=200), which allowed us to adequately test whether female and male adolescents with CD show common or distinct alterations in the limbic white-matter microstructural integrity.

Taking all our findings together, our results extend our understanding of alterations in the limbic WM tracts in CD, and support the hypothesis that fronto-limbic WM tracts such as the uncinate fasciculus (UF) and retrosplenial cingulum (RSC) bundles are implicated in CD. This study found significantly lower FA in participants with CD relative to controls in the right RSC. In addition, although the result does not survive when correcting for multiple comparisons, given its novelty, it is worth mentioning that HMOA values were lower in CD participants relative to controls in bilateral RSC. Furthermore, sex by diagnosis interactions in the RSC were also identified. Increased FA (corrected) in the right and HMOA (uncorrected) in bilateral RSC was evident in healthy control males relative to males with CD, while the opposite effect was observed in females, where increased FA and HMOA in females with CD relative to females in the HC group was observed. Below, we will consider these findings and their implications in depth.

6.7.1 Effects of diagnosis

With regards to our finding in the RSC, only one previous study investigating white-matter microstructural abnormalities in youths with CD has reported lower AD in bilateral cingulum in CD compared to HC (Haney-Caron et al., 2014). Although the latter study found main effects in a different measure of structural connectivity relative to the present study (i.e., FA), it is worth noting that it employed a whole-brain approach (i.e., TBSS). However, our findings are more consistent with findings reported in a study conducted in male adults with antisocial behaviour and psychopathy in which a more similar approach to the present study (i.e., manual ROI DTI) was used. Sethi et al., reported reduced FA in male adults with antisocial behaviour and psychopathy in the left dorsal cingulum relative to HC (Sethi et al., 2014). Furthermore, although, an only-female adult sample with a history of CD as adolescents used TBSS rather than tractography, decreased AD in CD relative to HC was reported in bilateral cingulum (Lindner et al., 2016).

The RSC is composed of fibres that connect the medial prefrontal cortex, dorsolateral prefrontal cortex, anterior cingulate cortex, posterior cingulate cortex, medial temporal lobe, and angular gyrus (Jones et al., 2013; Sethi et al., 2014). These regions have been associated with social-emotional, self-reflection, executive functions and moral processing, and are key nodes of the default mode network responsible for self-referential processing (Leech, Braga, & Sharp, 2012; Whitfield-Gabrieli & Ford, 2012). Of interest, fMRI studies have shown abnormal activations in the right ACC (Crowley et al., 2010; Marsh et al., 2011) during hot executive tasks, and right dlPFC (Passamonti et al., 2010) during emotion processing tasks, and reduced activations in the PCC during inhibition tasks in individuals with CD compared to healthy controls (Rubia et al., 2008). Further, fMRI studies conducted in adults with antisocial personality disorder have shown abnormal activation of the PCC during moral decision-making (A L Glenn et al., 2009), and affective processing tasks (Kiehl et al., 2001b). These studies are also consistent with sMRI studies that have investigated individuals with antisocial behaviour. For instance, in a study investigating cortical thickness in youths with CD and age-matched controls, Wallace et al. (2014) reported reduced cortical thickness in the right posterior cingulate cortex (PCC) in the former group. In addition, lower cortical thickness, volume and surface area in the right dlPFC has also been reported in youths with CD relative to healthy comparison groups (Fahim et al., 2011; Rogers & Brito, 2016; Sarkar et al., 2015).

Given that individuals with CD are characterised by deficits in a range of social, cognitive and affective processes, it is not surprising that functionally interconnected regions which contribute to these processes are impaired in CD. In fact, the areas interconnected by the RSC connect regions within the default mode network (DMN). Thus, the RSC is the most affine structural tract to core areas involved in the default mode network (DMN). The DMN is thought to be involved in two contrasting functions: attention to the external environment and internal mentation processes (e.g., self-reflection, mentalizing, auto-biographical memory, imagery) (Zhou et al., 2015). While both anterior and posterior areas of the DMN are related with self-referential processes, the medial prefrontal cortex hub appears to have an important role in social cognition, while the PCC is related with autobiographical memory and moral judgement making (Whitfield-Gabrieli & Ford, 2012).

Interestingly, previous studies investigating the DMN activity in youths with CD reported reduced connectivity between core DMN regions including the medial PFC, PCC, precuneus and superior temporal gyrus, relative to typically developing controls (Broulidakis et al., 2016; Zhou et al., 2016). It has been proposed that DMN dysfunction in CD may reflect an impaired neurodevelopmental trajectory related to self-awareness, regulating emotions, moral judgments and future planning in individuals with CD (Zhou et al., 2015). Such characteristics have been suggested to be key behavioural features displayed by individuals with CD.

6.7.2 Effects of Sex

In terms of sex effects, we found sex differences in MD in the right subgenual cingulum (SGC), and AD in the RSC and uncinate fasciculus. Sex differences were also observed in the right RSC (for FA and HMOA). Males showed higher MD in the right SGC. Females had higher AD in the right SGC, while males had higher AD in the right RSC, and uncinate fasciculus. Furthermore, males showed higher FA and HMOA in the right RSC compared to females. Our findings of sex differences in FA are consistent with a previous longitudinal study which used a large sample which reported higher FA values in males relative to females (Catherine Lebel & Beaulieu, 2011). However, the latter study showed higher MD values in females relative to males in the cingulum, whereas we show higher MD values in males relative to females in the subgenual cingulum. Although this previous study used a tractography approach, the cingulum was treated as a unitary tract and as mentioned above this is problematic, as the cingulum contains distinct populations of white matter fibres. Thus results may differ depending on where and how the tract is reconstructed (Jones et al., 2013).

However, it is important to note that the current study tested sex differences in samples that included both CD and HC participants. This could also have affected the gender difference seen in the RSC, as in particular this WM tract also showed a sex by diagnosis interaction. In addition, further TBSS studies investigating sex differences in WM microstructure have reported higher global AD values in females compared to males (Wang et al., 2012). This contrasts with our findings showing higher AD in males relative to females in two WM tracts (i.e., RSC, UF). However, Wang et al., used a small and likely underpowered sample. In contrast, other DTI studies which have used TBSS have not detected sex differences in FA, RD, MD or AD across the WM skeleton (Bava et al., 2010; Giorgio et al., 2010). Thus, sex differences in WM should be interpreted with caution.

6.7.3 Sex by diagnosis interaction

Contrary to Zhang et al., 2014, which investigated sex differences in the UF of individuals with CD, we did not observe any sex by diagnosis interaction in this WM tract. Both male and females appeared to be equally affected in measures of structural connectivity of the UF. However, this is the first study demonstrating that sex differences in the WM microstructure of the RSC is present in youths with CD. Males with CD showed reduced FA and females with CD showed increased FA relative to sex-matched healthy control groups. Interestingly, a previous study looking at sex differences in individuals with ADHD, found a similar pattern in FA, where females with ADHD showed increased and males showed reduced FA relative to controls in the superior longitudinal fasciculus, corticospinal tract, and inferior longitudinal fasciculus (King, Yurgelun-Todd, Stoeckel,

DiMuzio, & Lopez-Larson, 2015). This is of importance due to the considerable overlap among ADHD and CD, and symptom dimensions such as impulsivity and hyperactivity have been associated with the development of antisocial behaviour in childhood (Barkley, Fischer, Smallish, & Fletcher, 2004).

FA is a microstructural parameter which quantifies directional differences in the diffusion of water molecules inside tissues. Thus changes in FA are thought to provide information about the organisational integrity of white matter architecture within and between fibres, axonal density and diameter, pathway geometry and myelination (Beaulieu, 2009; Paus, 2010). The relationship between white matter and age is nonlinear. FA values increases from childhood to adolescence, and there is a less steep progression from adolescence to adulthood (Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008). Previous DTI studies of CD have found increased FA in male-only samples, suggesting accelerated maturation in individuals with CD. Here, however, we observe increased FA only in females and not in males. However, it has been proposed that females reach mature levels (i.e., increased FA) earlier than males (Wang et al., 2012). Thus, contrary to what was previously suggested (i.e., accelerated maturation), it may be that WM maturation is delayed in both sexes with CD, and it is manifested in opposite directions.

In addition, the opposite effect may reflect different clinical characteristics observed in individuals with CD. In fact, higher values of FA have shown to be related to anxiety disorders, such as post-traumatic stress disorder and panic disorder (Abe et al., 2006; Ayling, Aghajani, Fouche, & van der Wee, 2012; D. H. Han et al., 2008). Perhaps, higher FA values in females with CD may account for the risk of developing comorbid internalising disorders. In addition, given the role of the RSC in mentalising processes and moral decision-making, it may be that impairments in this WM tract mediate this function differently for each sex. In fact, a recent resting state study investigating sex-specific effects of narcissistic traits, showed opposite directions of brain activation (F>M) (W. Yang et al., 2015). The authors proposed that internal and external processes may interplay differently in males and females. Perhaps, higher FA values in girls with CD may contribute to impaired processing of external stimuli (e.g., seeking validation), while in males lower values of FA may contribute to impaired processing of internal stimuli (e.g., blaming their mistakes on external stimuli). Disruptions in either function (i.e., external and internal), however, may contribute to impaired decision making in both sexes. In line with this, previous studies in male-only or female-only samples suggest that females are relativly better than males in cognitive empathy (the process of understanding another's emotional state), which interestingly the neural-correlates for this trait lie on the posterior cingulum cortex - key node of the RSC (Fairchild, Stobbe, van Goozen, Calder, & Goodyer, 2010; Fairchild, Van Goozen, Calder, Stollery, & Goodyer, 2009; Martin-Key, Brown, & Fairchild, 2016). Perhaps changes in FA in opposite directions may explain different empathic processes displayed by male and females with CD.

6.7.4 Associations between measures of structural connectivity of the retrosplenial cingulum and uncinate fasciculus

Contrary to expectations, the current study found positive correlations between CD symptoms and HMOA values in the right RSC. This contradictory effect may have been due to the clinical heterogeneity of CD. Thus, this association must be moderated by other behavioural or biological mechanisms. Our results are in line with a previous sMRI study reporting associations between CD symptoms and the surface area on the right posterior cingulate cortex (Smaragdi et al., 2017).

Previous evidence from adults with psychopathy and antisocial personality disorder (an adult condition analogous to CD), which had frequently associated the behavioural phenotype of emotional detachment in psychopathy with DMN dysfunction (Hoppenbrouwers et al., 2013; Sethi et al., 2014). In line with this, sMRI studies in incarcerated male and female adolescents have also shown negative associations between psychopathic traits and the PCC (Lora M Cope et al., 2014; Ermer, Cope, Nyalakanti, Calhoun, & Kiehl, 2013). However, a recent resting state fMRI study, with a similar sample to the one recruited in the present study, did not observe associations between the DMN and psychopathic traits. In addition, studies examining associations with psychopathic traits in key nodes of the DMN did not consider the effect of antisocial personality. Thus the effects may be explained by the presence of antisocial behaviour in general rather than specific to psychopathy. In fact, it has also been reported that externalising behaviour is associated with the cortical thickness in the right retrosplenial cingulate cortex (Ameis et al., 2013).

6.7.5 Why are findings of the uncinate fasciculus inconsistent?

Three previous studies in CD have used a similar approach to the present study that is using deterministic tractography and manually drawing the ROI or an automatic generation of the ROI (Passamonti et al., 2012; Sarkar et al., 2013; Zhang, Gao, et al., 2014). These studies reported contrasting results to the present study. Firstly, our diagnostic effects showed that CD was associated with reduced AD whereas Passamonti et al. (2012) reported associations with higher AD values. When we further controlled for ADHD symptoms, we found more pronounced effects on several dMRI measures (i.e., RD, FA, HMOA). Although none of these survived for multiple testing corrections, it is important to note that while the present work observed lower values in FA, the most consistent finding in the UF of youths with CD is reporting of higher FA. However, although other studies used a different dMRI approach (eg., TBSS), lower FA (Breeden et al., 2015) and AD (Haney-Caron et al., 2014) have also been found in the UF of youths with CD.

The inconsistency seen in the UF may be due to the complex microstructure that encompasses the UF. For instance, the UF is one of the regions where multiple fibre bundles of different orientations cross (Von Der Heide et al., 2013). In addition, an alternative explanation may be that although we

estimated parameters of diffusion tractography (e.g., FA, RD, MD, AD), we did not employ a standard DTI-based tract reconstruction method. Thus, the tract was virtually dissected on models of SD-based tract reconstruction, providing a more accurate estimation of white matter diffusivity. Finally, the current study included a larger sample compared to previous studies. Thus, findings in previous studies may also be attributed to their relatively small sample sizes.

6.7.6 Strenghts and Limitations

The strengths of this study include the investigation of additional limbic WM tracts by using a more comprehensive approach – spherical deconvolution tractography. The main benefit of this approach is to resolve fibre-crossing or fibre-kissing issues. In addition, SD techniques improves the accuracy of fibre tracking compared to models based on tensor alone (Dell'Acqua et al., 2010). Moreover, the reliable correction of eddy current induced fields and participant movement enhanced robustness in the estimates of diffusion parameters (Andersson & Sotiropoulos, 2016). Secondly, the large sample size in the present study, included an adequate number of males and females with and without CD, which allowed us, for the first time, to address the question of sex differences among measurements of structural connectivity. In addition, another strength is the fact that the CD group was assessed with a standardised approach for a current diagnosis of CD based on DSM-IV criteria.

However, our study also has several limitations. Firstly, the sample ranged in age from 12-18 years. The CD and control groups did not differ in age, yet age is known to have an important effect on white matter development. Thus, we included age as a covariate of no interest in all analyses. Secondly, our sample distribution across sites was inadequate and although precautions were taken prior to starting DTI data acquisition (e.g., matching data acquisition parameters and going through a site qualification process), combining data from different sites and manufacturers (Phillips and Siemens) may introduce unwanted noise and variations in the data. However, in an attempt to reduce the impact of this variability, all analysis included site as a covariate of no interest. Third, although the author was intensively trained on carrying out virtual dissections, this study did not assess the reliability of the dissections.

6.8 Conclusion

In summary, we found that adolescents with CD show significant differences from controls in the white-matter microstructural properties of the right retrosplenial cingulum. We also report that this

difference might differ between males and females with CD, and microstructural alterations were observed in several other tracts at an uncorrected level. These differences may contribute to the neurobiological mechanisms underpinning CD, and more specifically they may account for sex differences in clinical presentation and rates of comorbidity. Given the overlap of the retrosplenial cingulum tract with brain regions that make up the default mode network, future studies may wish to investigate sex differences in the functional connectivity of this network in CD. This would improve our understanding of the pathophysiology of CD and could lead to improved diagnosis and treatment for both sexes.

Chapter 7 Cortisol Reactivity to Stress in Youths with Conduct Disorder

7.1 Abstract

Previous studies have shown that males with Conduct Disorder (CD) show blunted cortisol responses to stress. However, to date, no studies have investigated whether this effect is moderated by sex. In this study we compared male and female adolescents with CD in terms of cortisol responses to stress, as well as subjective reactivity to stress, (feelings of stress, anxiety and insecurity). We recruited a sample of 36 (21 females) adolescents with CD and 38 (21 females) healthy controls. We used the Trier Social Stress for Children (TSST-C), a paradigm that involves giving a public speech in front of a panel of judges, to introduce psychological stress. Furthermore, in a proof-of-concept analysis, we examined the relationship between cortisol reactivity and brain structure (derived as detailed in previous chapters) in a sub-sample of individuals that had also undergone a structural MRI scan. Our findings extend the literature and show that similar to males, females with CD exhibit a blunted cortisol response to stress or anxiety in the CD group – both groups reported increases in feelings of stress and anxiety during the TSST-C procedure. We found a positive association between cortisol reactivity and superior frontal gyrus volume. Our results support previous suggestions that low cortisol reactivity may be a neurobiological marker for individuals with CD, and show that this may apply to individuals with CD of both sexes. Future studies may address whether this is specifically related to social stressors or whether it would also be observed for non-psychological stressors (e.g., exercise or cold pressor).

7.2 Introduction

Conduct disorder (CD) is one of the most common reasons for referral to mental health services in the USA and UK (Kazdin, Whitley, & Marciano, 2006; Scott, Knapp, Henderson, & Maughan, 2001). The sex ratio for CD is higher for males than females with most studies finding a 2:1 or 3:1 male: female ratio (Moffit & Caspi, 2001). However, the prevalence of CD in mid-adolescence is more similar between males and females with a ratio of 1.5:1 (Moffit & Caspi, 2001). Although CD is less common in girls than in boys throughout the lifespan, the rates of females diagnosed with CD has increased over the past two decades (Ilomäki, Hakko, Ilomäki, Räsänen, 2012). Moreover, females with CD are at greater risk than their male counterparts with respect to negative outcomes, such as, teenage pregnancy, teenage prostitution, school dropout, financial problems,

delinquency, substance abuse, antisocial personality/disorder and mental and physical health problems in adulthood (Loeber & Keenan, 1994; Odgers et al., 2008; Pajer, 1998).

Females differ from males in their clinical presentation of CD. For instance, females tend to display higher rates of comorbid internalising problems (Berkout et al., 2011; Keenan et al., 1999; R Loeber & Keenan, 1994), whereas males manifest higher rates of comorbid externalising problems such as attention-deficit/hyperactivity disorder (ADHD). Moreover, females with CD tend to display more covert types of antisocial behaviour (e.g., deceit, manipulation, lying, staying out late) rather than overt forms of antisocial behaviour (e.g., physical aggression, vandalism). The underlying neurobiological mechanisms associated with sex differences in CD remain to be determined.

Over the past two decades, there has been increasing evidence that deficits in neurobiological mechanisms play a key role in aggressive and antisocial behaviour in children and adolescents (Hyde, Shaw, & Hariri, 2013). There is relatively little evidence for sex specific correlates of CD regarding both environmental (e.g. delinquent peers; Gorman-Smith & Loeber, 2005) and neurobiological risk factors (Smaragdi et al., 2017). This is in large part because few studies have been designed or adequately powered to test for sex differences. Nonetheless, it has also been argued that alterations in one neurobiological system, the hypothalamic-pituitary-adrenal (HPA) axis, may drive antisocial behaviour (AB). More specifically, it has been hypothesised that hypoactivity of the HPA axis (i.e., low cortisol levels) under both resting (basal) and stressful conditions are a risk factor for the development of conduct problems (Van Goozen, Fairchild, Snoek, & Harold, 2007). Yet, to date, no studies have investigated sex differences in HPA axis activity in a clinical group with CD. This is a significant gap in the literature, given that it has been shown that sex is an important moderating factor linking HPA axis activity to psychopathology in adolescence more generally (Klimes et al., 2001).

7.2.1 The HPA axis and conduct problems

There is growing evidence of the relationship between the activity of the HPA axis and AB in young people (Alink et al., 2008). The HPA axis is comprised of the hypothalamus, pituitary and the adrenal glands. Briefly, the hypothalamus secretes corticotropin releasing hormone (CRH) as a response to stress and/or as part of circadian rythym. CRH stimulates the production of (adrenocorticotropic hormone) ACTH from the anterior pitutary. ACTH then goes on to stimulae the production of cortisol from the adrenal glands. (Herman, Ostrander, Mueller, & Figueiredo, 2005; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). The HPA axis has an important role in the organisms response to challenging novel environments (Dickerson & Kemeny, 2004; Purves et al., 2001). Cortisol levels reflect HPA axis activity, and in research

settings, cortisol is usually assessed in saliva because this collection procedure is non-invasive and not intrinsically stressful. The relationship of cortisol reactivity to stress as well as the circadian rhythm have both been examined in CD (Alink et al., 2008).

Research on the HPA axis has been informed predominantly by the low arousal theory (Ortiz & Raine, 2004).This theory proposed that low cortisol levels are a characteristic of AB. The idea is that this leads to a particular pattern of fearlessness associated with low levels of physiological arousal (Raine, 1993). It has been suggested that individuals with CD are more prone to antisocial behaviour as they are not fearful of negative consequences (e.g., punishment) that might result from their conduct. The sensation seeking theory provides an alternative explanation. This states that individuals try to increase their low basal levels of arousal by seeking out stimulating situations (e.g. criminal activities) (Zuckerman, 1979). Thus, individuals with CD attempt to increase their levels of arousal to an optimal level by seeking out stimulation. It has also been postulated that these individuals have an increased threshold for stress (van Goozen et al., 2007).

Although the vast majority of empirical evidence gathered from antisocial populations supports the low arousal theory, contradictory results have also been reported. For instance, studies looking at *basal cortisol secretion* have largely shown lower cortisol levels in youths with CD (McBurnett, Lahey,Rathouz, Loeber, 2000; Pajer, Gardner, Rubin, Perel, & Neal, 2001; Popma et al., 2007; Vanyukov, Moss, Plail, Mezzich, & Tarter, 1993) and severe Oppositional Defiant Disorder (van Goozen et al., 1998). However, findings in the opposite direction have also been reported, whereby individuals with CD show increased basal cortisol levels - although elevated cortisol is only seen at certain times of the day (e.g., the evening) (Fairchild, van Goozen, et al., 2008; Van Bokhoven et al., 2005). Finally, other studies have found no significant relationship between basal cortisol levels and disruptive behaviour disorders (van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & van Engeland, 2000). Nonetheless, results of a meta-analysis, indicate that lower basal cortisol is significantly associated with conduct problems (CP) in children between 5 to 12 years old. However, this association was not found in adolescents (Alink et al., 2008). Thus, age may have contributed to the mixed findings of previous studies.

In addition to altered basal cortisol levels, studies of youths' *cortisol response to stress* (reaction to a laboratory-based stress induction procedure) have shown an attenuated HPA response (Fairchild et al., 2008; Van Goozen et al., 1998; van Goozen et al., 2000). However, results from these studies have been less consistent than studies of basal adrenocortical activity. In fact, the meta-analysis mentioned above found a stronger association between low basal cortisol levels and CP relative to paradigms of stress-induced cortisol reactivity (Alink et al., 2008). It is worth mentioning that the latter meta-analysis included studies of broadly defined externalising behaviours which represent a heterogeneous set of problems which does not necessarily indicate that these individuals have a clinical diagnosis of CD. In fact, out of the 72 studies that were included in the metanalysis for basal cortisol, only 13 (18%) and in the metanalysis for cortisol reactivity to a stressor, only 2 (7%)

included a sample of individuals with CD (Alink et al., 2008). This is important, as it has been shown that reduced cortisol responsivity to stress may be a neurobiological marker only for youths with a clinical diagnosis of CD (Popma et al., 2006). Furthermore, there are only a relatively small number of studies on cortisol reactivity compared to studies on basal cortisol.

In addition, and most importantly, it has been suggested that the nature of the stressor used in previous studies, may partially account for the inconsistent results (Kobak, Zajac, & Levine, 2009). In fact, Alink et al.'s meta-analysis found that when the analysis was conducted only on a subset of studies that used a stronger stressor (e.g., social evaluative threat), the relationship between cortisol reactivity and externalising behaviour was greater. Thus, studies using a standardized and more reliable laboratory stressor such as: giving a public speech with an element of social evaluation, mental arithmetic tasks or frustrating and provocative tasks, have often reported blunted cortisol response in youths with CD (Fairchild et al., 2008; Van Goozen et al., 1998; Howard B Moss, Vanyukov, & Martin, 1995; Northover, Thapar, Langley, Fairchild, & van Goozen, 2016; Popma et al., 2007; Popma, Jansen, Vermeiren, Steiner, Raine, Van Goozen, van Engeland, et al., 2006; Schoorl, Van Rijn, De Wied, Van Goozen, & Swaab, 2016; Snoek & Goozen, 2004; van Goozen et al., 2000). This effect appears to be more evident during stress than during resting periods (i.e., basal conditions; van Goozen et al., 1998; van Goozen et al., 2000). However, other studies that have used more naturalistic challenges, such as, talking about intimate aspects of their sexual behaviour or a traumatic event, have shown an increased cortisol response in youths with severe CP (McBurnett et al., 2005) and in individuals with high risk of deviant behaviour (Halpern et al., 2002). This may indicate that a pattern of low cortisol reactivity during stress in CD, may only be observed in relation to specific types of stressors (e.g., social threat). In fact, it has been suggested that hypo-responsivity may not necessarily be a general feature in CD but rather may be a state-like response to challenge (Kobak et al., 2009).

7.2.2 HPA axis correlates in females with CD

As was mentioned above, females and males with CD differ regarding comorbidity rates. It has been shown that males with CD are frequently diagnosed with comorbid externalising disorders such as ADHD, while females are more commonly diagnosed with comorbid internalising disorders such as depression and anxiety. One explanation for this relationship is that HPA axis abnormalities might operate in different ways in males and females with CD – particularly leading to more stress-related emotional problems in females. However, our knowledge regarding HPA axis function in CD mainly comes from studies of male-only samples, leaving the question of whether females with CD show HPA axis abnormalities unresolved. There is, however, some preliminary evidence suggesting that females with CD show similar reductions in basal cortisol levels (Pajer et al., 2001), which is consistent with findings reported in previous studies in male

adolescents (Alink et al., 2008). On the other hand, Shirtcliff and colleagues (2005) reported a link between externalising behaviour and low morning cortisol only for boys, while this was not observed in females, in a non-clinical sample (Shirtcliff, Granger, Booth, & Johnson, 2005). In line with this, a study looking at cortisol reactivity to a naturalistic challenge paradigm (i.e., conflict discussion), reported that females and males with CP showed lower cortisol in the pre-task measurements (i.e., basal cortisol), but girls showed an increased cortisol response to conflict stress while boys with CP showed a lower cortisol response (Kobak et al., 2009).

In summary, previous studies of youths with antisocial behaviour have provided equivocal results with regards to the association between hypocortisolism and antisocial behaviour. Yet, low cortisol levels have been the most consistent findings among this population, supporting the low arousal theory. The inconsistent results might be due to variations in the methodology that was used, (e.g., single measurements vs multiple daily measurements; plasma vs saliva samples, cortisol reactivity to stress vs basal or diurnal cortisol, and the type of stress task that was used). Another potentially important issue might be attributed to the nature of the clinical heterogeneity of CD (e.g., callous unemotional traits, comorbid internalising disorders, the age of onset). Also, some of the above-mentioned studies did not specifically include a clinical sample diagnosed with CD but merely compared those with and without conduct problems. In addition, it has recently been shown that comorbid anxiety is an important factor to consider when studying HPA axis activity in CD (Schoorl et al., 2016). However, previous studies included participants presenting with psychiatric comorbidity, yet failed to investigate the impact of comorbid internalising problems (Alink et al., 2008). With regards to cortisol response to stress, findings appear to be influenced by the nature of the stressor. Blunted cortisol response is more evident in studies that used a standardised paradigm, such as frustration and social evaluation (Fairchild et al., 2008; Snoek & van Goozen, 2004; van Goozen et al., 2000). In contrast, studies that have used more naturalistic approaches (e.g. talking about a negative experience) have reported associations between increased cortisol and severe CP (McBurnett et al., 2005).

Additionally, there is a clear gap in the literature regarding the role of HPA axis functioning in the neurobiology of sex differences in CD. Therefore, the study reported in this chapter will be the first to investigate sex differences in the cortisol response to stress amongst adolescents with CD. This will be achieved by using a standardised and effective laboratory-based stress task – the Trier Social Stress Test for Children (TSST-C; (Buske-Kirschbaum et al., 1997)). This task induces acute stress and is based on principles of social evaluative threat. We will attempt to replicate previous studies by showing blunted cortisol response to stress in boys with CD compared to their healthy peers. Given the lack of research on females with CD in this area, it is a difficult task to hypothesise the direction of the results that will be obtained in females. However, based on research investigating basal levels of cortisol in females with CD (Pajer et al., 2001), we hypothesized that females with CD will show similar effects to CD males in general.

7.2.3 A proof of concept analysis of the relationship between brain structure and HPA axis function.

In the introductory chapters (Chapter 2), we highlighted the structural and functional links between the HPA axis and brain circuits - in particular, limbic and associated prefrontal regions. We described how previous research has suggested how HPA axis dysregulation and deficits are potentially mediated by activity in brain regions such as the hippocampus, amygdala, and prefrontal cortex (Kremen et al., 2010). Given, the hypothesised role of both the HPA axis and limbic system circuitry in the aetiology of CD, we also explored the relationship between HPA axis function and volume of the amygdala, hippocampus, and given that the surface based morphometry analysis (Chapter 4) demonstrated a main effect of diagnosis in the cortical volume of the superior frontal gyrus, we selected this brain area and included it in our correlational analysis. We further assessed the relationship between HPA axis function and white matter tracts microstructure and how these variables relate to CD.

Unfortunately, because this was a multi-centre trial and data from the TSST-C were not available for all cases envisaged initially, we were unable to conduct a full, adequately powered, analysis of the relationship between cortisol stress reactivity with brain regions and white matter tracts. However, we did run a proof of concept analysis involving the correlation of the volumes of specific brain regions (e.g., hippocampus, amygdala, and superior frontal gyrus) and the measures of diffusion of white matter tracts involved in the regulation of the HPA axis and the magnitude of the cortisol stress response (DELTA cortisol) as measured during the TSST-C. The brain regions and tracts were selected partly based on CD-related effects reported in Chapters 4 and 6 and partly based on a priori hypotheses based on the review of the literature relating to the neuroanatomy of HPA axis functions. Based on the first criterion, we included the right superior frontal gyrus (SFG) in the analysis along with measures of white matter (WM) tracts of the retrosplenial cingulum and uncinate fasciculus. Based on the second criterion, we also included the volume of the amygdala and hippocampus, as well as measures of WM microstructure (e.g., fractional anisotropy (FA), radial diffusivity (RD), and hindrance modulated orientational anisotropy (HMOA)) in the fornix (WM tract connecting hippocampus and hypothalamus) and subgenual cingulum tracts (WM tract connecting amygdala and subgenual cingulate). Given the exploratory and preliminary nature of this analysis, we did make any specific predictions about the direction of associations that would be observed.

7.3 Methods

A sub-group of participants that were included in this study had previously taken part in the main Fem-NAT-CD project (i.e. the neuropsychological testing, questionnaire battery, and neuroimaging). The study received separate ethical approval from the University of Southampton Ethics Committee and the Research Governance of the University of Southampton (Ethics Ref: 12177). Also, since this study involved collecting human tissue (saliva) samples, NHS ethics approval was secured from the Edgbaston NRES Committee in June 2015 (Ethics Ref: 15/WM/0086). All participants provided written informed consent for their participation in this study (Appendix E.1).

7.3.1 Participants

To measure the physiological stress response, the Trier Social Stress Test for Children (TSST-C) was conducted. The TSST-C is based on the principle of inducing social evaluative threat, and it has previously been established and validated (Buske-Kirschbaum et al., 1997). Five of the eleven sites in the FemNAT-CD study (UOS, UK Aachen, VUA, GU, UNIBAS) contributed to the TSST sub-study. However, at the time of data analysis for this thesis (July 2017), data collection at the other sites had not been completed, therefore only data from the participants that were tested at University of Southampton (UOS) will be reported in this chapter. In light of comments made by the NRES ethic committee, it was agreed that children below 12 years old would not participate in this study, as they may find the task too upsetting. Thus, only those aged 12-18 years old were included in this study. A total sample of 74 adolescents, 38 healthy controls (21 females) and 36 adolescents with CD (21 females) were tested.

For the proof of concept study of the relationship between brain structure and HPA axis function, complete data were available for 38 participants: 21 healthy controls (11 females) and 17 with CD (11 females). While for the relationship between white matter tract microstructure and HPA axis function, data were available for 29 participants: 14 healthy controls (7 females), 15 CD (9 females).

7.3.2 General Procedure

Subjects were invited to the University of Southampton where they were given and told about the rationale of the study (i.e. to assess the stress response). However, the key features of the task were not revealed, as that might affect the quality of the stress response or the effectiveness of the stressor. This is a standard approach in TSST research. Upon arrival participants were asked

questions regarding aspects of their lifestyle that could affect HPA axis responsiveness to stress, such as, whether they had smoked, exercised or drank any drinks containing stimulants (e.g., coffee) " in the past hour", as well as whether they had consumed alcohol or used drugs in the past 24 hrs. If they answered affirmatively to any of these questions, the TSST assessment was postponed; by one hour for the use of caffeine or cigarettes, and one day for the use of soft or hard drugs (Appendix E.2). In addition, females were asked about the use of contraceptives or other steroid based medication, as this could also impact their HPA axis responsiveness to stress (Kirschbaum et al., 1999).

7.3.3 Trier social Stress test Procedure

Two rooms were used for the TSST-C: one relaxing/resting room, and one testing room. The testing room was plain, large and contained a table with chairs for two-panel members. A dummy video camera was also fitted in the room. For this study, three researchers were needed: a test leader and two-panel members. The HPA axis activity follows a circadian rhythm with the highest cortisol levels in the early morning which continuously decreases over the course of the day. Thus, if a stressor is applied in the morning, basal cortisol levels (i.e., pre-test) would be higher compared to cortisol levels in the afternoon or evening (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). Therefore, to maximise reliability, we conducted the TSST-C sessions (i.e., introduction to the stressor) in the afternoon, from 1 pm (earliest) to 4 pm (latest). The TSST-C was divided into three different phases and eight periods (**Figure 7.1**).

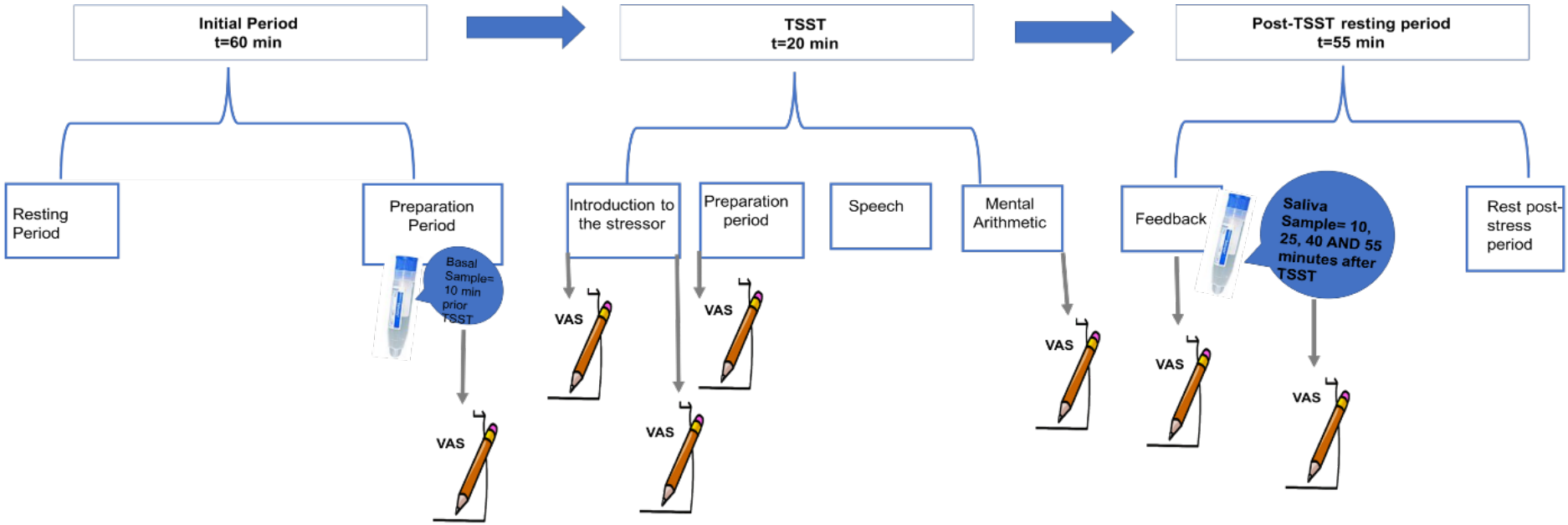


Figure 7.1 - Overview of the protocol followed for the Trier social stress Test for children in the present study

7.3.4 Phases of the experiment

Prior to the TSST-C

Period 1- Resting period (50 minutes)- The first period started when subjects arrived at the laboratory. As part of the FemNAT-CD protocol was to determine heart rates and skin conductance levels, during this period, we placed electrodes on the participant's chests and fingertips using the Vrije Universitat- Ambulatory Monitoring System (VU-AMS). Heart data was not analysed for the purposes of this thesis. In addition, during this period, participants completed a battery of questionnaires which were part of the overall Fem-NAT-CD protocol (see chapter 3 for details). To minimise anticipatory anxiety during the resting period before the social stress task, the exact content of the entire test-session was not explained beforehand, they were only told that they were taking part in a 'challenging task'. Also, as cortisol levels may be influenced by

exercise, or food intake (Lovallo, Farag, Vincent, Thomas, & Wilson, 2006), this period ensured that subjects were at least one hour free of eating, smoking, or exercising. In addition, this period provided a standardised laboratory environment for all participants (e.g. same resting room) before collecting the first basal sample (10 minutes prior the challenging tasks – 'TSST-C').

Period 2- The second period was a preparation period (10 minutes) prior to the stressful task. In this period subjects were asked to collect the first saliva sample (basal sample- time 1) and stand up for 10 minutes. The latter instruction was related to the need to record heart data.

TSST-C

Period 3 - Introduction to the stressor (5 min) – Participants were moved to the experimental room, where a table with two chairs and a video camera were mounted (**Figure 7.2**). The test leader explained the specifics of the task which is described in detail elsewhere (Buske-Kirschbaum et al., 1997). Briefly, the public speech involves giving a talk in front of a male and a female panel member, who were previously unknown to the children. In this study, the speech was about a movie or a book of the subject's choice. To make the situation more stressful, they were told that their performance was going to be video-recorded. The speech was followed by a challenging arithmetic task. Each task lasted for 5 minutes.

Period 4- Preparation period (3 min)- Participants were left alone and were provided with paper and pencils to prepare their speech. However, they were told not to use this written information during their speech.

Period 5- Free speech (5 minutes) - The panel members entered the testing room (both wearing white coats for standardisation) and asked the participant to stand in front of the camera and deliver their speech for the next 5 min. If the speech finished prior to the end of 5 min, they were asked to continue the speech until the time was up. The judges were instructed not to give positive (or negative) encouragement, but instead to maintain a neutral stance. This process was crucial to ensure standardisation and successful stress induction.

Period 6 – Mental arithmetic task (5 minutes)- Depending on the subject's age, a specific number was selected, and participants were asked to subtract it from a particular number (see Appendix E.3). They were told to be as quick and accurate as possible. If they gave an incorrect answer, the subjects were asked to restart at the original number, respectively, with one-panel member interjecting: "stop, please start again."

Post TSST-C/ resting period

Period 7- Feedback (5 min)- The principal researcher then came to the room and took the participant back to the resting room. Participants were further told that their performance was as good as that of other participants and that the stern behaviour of the committee was a pretence to induce competitive conditions. They were also told that the camera was a setup and that they had not been recorded.

Period 8- Rest post-stress period (50 minutes) - Subjects stayed in the resting room to relax after the stress response ended, during this time they filled in some non-stressful questionnaires and were asked to collect and fill in the remaining saliva samples.

Figure 7.2 - Experimental set-up for the Trier Social Stress Test for Children

7.3.5 Procedure for saliva collection

In total, five saliva samples were collected for each participant. Saliva samples were collected at period 2 (10 minutes before the TSST; -10),and during period 8: +10 (time 2) , +25 (time 3), +40 (time 4) and +55 (time 5) minutes after the TSST-C (**Figure 7.1**).

7.3.6 Recording of subjective responses to stress

Participants rated their feelings six times using a visual analogue scale (VAS, Appendix E.4). The scale contained three items (anxious, emotionally insecure, stressed). The VAS was presented as a straight horizontal line of fixed length (10 cm) which ranged from 'No, not at all' to 'Yes, very much'. Participants were told to draw a line along the scale to indicate their feelings. In total, 9 VASs were completed at different time points; at the end of period 2 (VAS 1), at the start of period 3 (VAS 2) and 4 (VAS 3), and at the ends of periods 6 (VAS 4), and 7 (VAS 5). The remaining VAS were completed during period 8 (VAS 6, 7, 8 and 9) at the time that the saliva samples were taken (Figure 1).

7.3.7 Cortisol Analysis

Each cortisol sample was analysed in duplicate using an enzyme-linked immunosorbent assay kit (Salimetrics, State College, PA). The intraassay and interassay coefficients of variation were 3.69% and 4.77% respectively. Results are reported in nmol/L.

7.3.8 Statistical Analysis

Before conducting the statistical analysis, cortisol data were screened to eliminate potential outliers. One participant (CD male) showed cortisol levels greater than three standard deviations above the mean at all time points and was excluded from the analysis. All data were analysed in SPSS v.24 with alpha set at $p < 0.05$, two-tailed. Effect sizes are reported as partial eta-squared (η_{p2} = small $\geq$.01, medium $\geq$ .06, large $\geq$ .14, (Cohen, 1988)). Demographic, clinical and questionnaire data were analysed using ANOVAs or Chi-square for categorical variables. Repeated measures ANOVAs' were used to assess effects of diagnosis and sex, and sex by diagnosis interactions, in cortisol measures, and subjective feelings of insecurity, stress and anxiety with respect to changes

over time. Sex and diagnosis were entered as between-subjects factors, and time was used as a within-subjects factor. Where assumptions of sphericity were violated, Greenhouse-Geisser corrections were employed. When there was a main effect of time, posthoc Sidak-corrected pair wise comparisons between the basal value and all other values were conducted. In case of an interaction effect of time and diagnosis, these pairwise comparisons were made separately in the healthy control and the CD groups. Furthermore, one-way ANOVAs were used to explore differences in cortisol levels among CD and healthy controls at specific time points.

To estimate the extent of stress reactivity in salivary cortisol, we calculated the DELTA measures of the stress response. This was performed by calculating the difference between cortisol concentrations at time 1 (basal levels; 10 minutes before the TSST-C), and the value at time 2 (10 minutes after the TSST-C). General linear models were further used to assess effects of diagnosis, sex and sex by diagnosis interaction on the DELTA cortisol values.

A priori power calculations were estimated based on effect sizes from Popma et al., (2006). The results of these calculations indicate that we need 19 subjects in each group, power = 0.8, and α = 0.05 to measure the effect. To obtain more information about our sample, the CD sample was subdivided into higher (Females, n=6; Males; n=10) and lower (Females, n=15; Males, n=5) callous-unemotional traits subgroups using a median split procedure based on CU dimension scores of the YPI. Participants scoring >33 were classified as CD/CU+ while those scoring <33 were classified as CD/CU-.

We further conducted bivariate Pearson's correlations within the CD group to test for the relationship between DELTA cortisol and the three different subsets of the youth psychopathic traits inventory scores: irresponsibility and impulsivity, narcissism and callous and unemotional traits, as well as with total psychopathic traits. We also explored the effects of several covariates that might affect HPA axis activity, such as generalised anxiety disorder (GAD) symptoms, ADHD, age, puberty, and smoking. Smoking was assessed by asking the participant the quantity of cigarettes they smoked on an average day. We did not examine the effect of medication use of this variable, as only two participants (1 male and 1 female with CD) were taking medication (i.e., atomoxetine) at the time of the assessment, therefore, it was felt that medication use would be unlikely to affect the results. Finally, since the use of contraceptive may affect cortisol levels, we conducted a sensitivity analysis by excluding female participants that were currently using contraceptives and repeating the main analyses.

For the proof of concept analysis of the relationship between brain structure and TSST cortisol reactivity, we ran a number of partial correlations between delta cortisol values (representing stress reactivity) and the volume of subcortical (amygdala and hippocampus) and cortical (superior frontal gyrus) volumes and key microstructural parameters of WM tracts such as, fractional anisotropy (FA), hindrance modulated orientational anisotropy (HMOA) and radial diffusivity

(RD). The volumetric values were extracted from the surface based morphometry analysis (Chapter 4), and the measures of white matter diffusivity were extracted from Chapter 6 – as discussed in the introduction.

7.4 Results

7.4.1 Characteristics of the sample

The demographic characteristics of the sample, along with group comparisons, and their corresponding p values are reported in **Table 7.1**.

The groups did not differ significantly regarding age, pubertal developmental status or IQ. There was a sex by diagnosis interaction for IQ; CD males had lower IQ scores relative to control males, whereas CD females had higher IQ scores compared to control females. Because of this subsequent analysis were repeated with IQ included as a covariate. However, as all results remained the same with and without the covariate, we decided to report the analysis without this covariate. As predicted, the CD group showed significantly more CD, ODD and ADHD symptoms, and also reported significantly more traumatic experiences and cigarette use, than their control counterparts. Females in both groups did not differ in the use of contraceptives. Moreover, as expected, the CD group showed significantly higher scores in reactive and proactive aggression, as well as in the three different components of psychopathic traits: impulsive and irresponsible, narcissism, and callous and unemotional traits, and the total score of the psychopathic traits measure. Females and males in the CD group did not differ regarding the age-of-onset distribution. They also had similar rates of comorbid disorders, and this applied to both externalising and internalising disorders.

7.4.2 Effects of CD and sex on cortisol reactivity

Basal cortisol levels did not significantly differ between the CD and control groups at the pre-test baseline (-10 minutes, time 1; p=0.83). Repeated measures ANOVA, with greenhouse-geisser correction, revealed a significant main effect of time (F=12.52, p=0.001, ηp^2=.15), and a significant time by diagnosis interaction (F=9.91, p=0.002, ηp^2=.13; **Figure 7.3**), as well as a significant main effect of diagnosis (F=6.31, P=0.014, ηp^2=.08). Stress reactivity (DELTA cortisol) was significantly lower in the CD group compared to controls (F=19.51, p=0.0001, ηp^2=.22; **Figure 7.4**). The analysis also revealed a significant effect of sex (F=7.01, p=0.010, ηp^2=.092); in which females, as a group overall, displayed reduced cortisol levels compared to males at all time points. However, both sexes showed a similar pattern of cortisol changes across time. Thus, there was not a significant sex by diagnosis by time interaction (F=0.43, p=0.51).

Table 7.1 - Sample demographics and clinical characteristics of the TSST-C

Variable	**Healthy Controls (Mean±SD)**		**Conduct Disorder (Mean±SD)**		**Statistics**		
	Females n=21	**Males n=17**	**Females n=21**	**Males n=15**	**Group F(*p*)**	**F sex F(*p*)**	**F GxG F(*p*)**
Age (years)	15.85±1.49	16.17±1.55	15.33±1.82	15.80±2.04	1.45 (0.23)	0.97 (0.33)	0.03 (0.87)
Estimated IQ	94.61±11.48	101.41±11.28	97.19±10.89	92.86±13.64	0.84 (0.36)	0.18 (0.66)	4.69 (0.03)
CD symptoms (K-SADS-PL)	0.19±0.40	0.29±0.46	3.71±2.17	5.26±2.21	80.67 (0.001)	1.34 (0.25)	0.99 (0.32)
ODD symptoms (K-SADS-PL)	0.29±0.64	0.29±0.77	4.66±2.59	4.06±3.45	48.80 (0.001)	0.24 (0.62)	1.86 (0.17)
ADHD symptoms (K-SADS-PL)	0.10±0.44	0.18±0.52	4.85±4.89	4.80±5.40	32.50 (0.001)	0.003 (0.955)	0.001 (0.97)
PTSD (No. traumatic events)	1.57±1.57	2.06±1.08	3.14±1.71	3.13±1.92	14.63 (0.001)	0.39 (0.53)	0.20 (0.65)
Cigarettes per day	0	0.12±0.48	2.05±3.50	4.07±6.85	12.67 (0.001)	1.61 (0.20)	1.27 (0.26)
Contraceptive use (%)	4 (19.04)		5 (23.80)		X^2=0.12 (0.70)		
Pubertal developmental status - No (%)							
Mid pubertal	1 (4.7)	3 (17.65)	3 (14.28)	2 (13.34)	X^2=0.19 (0.65)	X^2=0.63 (0.42)	X^2= 1.66 (0.64)
Late pubertal	11 (52)	12 (70.59)	10 (47.62)	11 (73.33)	X^2=0.04 (0.84)	X^2=3.60 (0.06)	X^2=3.72 (0.29)
Post pubertal	9 (42.86)	2 (11.76)	8 (38.10)	2 (13.34)	X^2=0.01 (0.91)	X^2= 6.99 (0.008)	X^2=7.12 (0.07)
CD age of onset - No (%)							
Childhood onset			11	10		X^2=0.73 (0.39)	
Adolescent onset			10	5			
Psychological measurements							
Total RPQ	6.42±4.65	7.29±4.95	12.52±6.15	16.60±8.08	20.67 (0.0101)	1.82 (0.18)	0.36 (0.54)
Reactive aggression (RPQ)	3.95±3.54	5.70±4.29	9.14±5.06	10.93±5.10	20.00 (0.001)	1.71 (0.19)	0.06 (0.80)
Proactive aggression (RPQ)	2.47±4.17	1.58±2.15	3.38±4.17	5.66±5.44	3.52 (0.065)	0.40 (0.52)	1.35(.24)
Total YPI	89.00±15.89	99.11±12.47	99.00±19.79	110.06±15.15	4.57 (0.04)	5.96 (0.02)	0.08 (0.77)
Grandiose manipulative (YPI)	30.47±8.49	33.70±7.48	32.61±9.65	34.06±11.74	0.001 (0.99)	0.88 (0.35)	1.18 (0.28)
Callous/Unemotional (YPI)	26.04±4.88	32.00±5.19	28.71±7.02	36.06±6.71	2.78 (0.10)	19.06 (0.001)	0.06 (0.79)
Impulsive/Irresponsible (YPI)	32.47±6.81	33.41±5.26	37.66±7.55	39.93±5.78	14.96 (0.001)	0.63 (0.42)	0.36 (0.55)

Key: -CU, low callous unemotional traits, +CU, high callous unemotional traits, ADHD; attention deficit hyperactive disorder, ODD; oppositional defiant disorder, PTSD; post traumatic stress disorder, MDD; major depressive disorder, YPI; youth psychopathic inventory, RPQ; reactive proactive questionnaire

Table 7.1 Continued – Current psychiatric comorbidity - No. with K-SADS-PL diagnoses (%)							
Variable	**Healthy Controls (Mean±SD)**		**Conduct Disorder (Mean±SD)**		**Statistics**		
	Females, n=21	**Males, n=17**	**Females, (n=21, -CU=15, +CU=21)**	**Males, (n=15, -CU=5, +CU=10)**		**F sex F(*p*)**	
ADHD			4 (19.04)	3 (20)		X^2= 0.005 (0.94)	
ODD			11 (52.38)	7 (46.66)		X^2=0.11 (0.73)	
PTSD			0	0			
MDD			3 (14.28)	0		X^2=0.51 (0.47)	
Alcohol abuse			0	0			
Alcohol dependence			0	0			
Substance abuse			0	2 (13.34)		X^2=0.92 (0.33)	
Substance dependence			0	0			
Generalised Anxiety Disorder			2	0		X^2=0.03 (0.84)	

Key: -CU, low callous unemotional traits, +CU, high callous unemotional traits, ADHD; attention deficit hyperactive disorder, ODD; oppositional defiant disorder, PTSD; post traumatic stress disorder, MDD; major depressive disorder, YPI; youth psychopathic inventory, RPQ; reactive proactive questionnaire

Cortisol levels diverged in the groups following stress onset. Pair-wise comparison for each diagnostic group independently showed that in the healthy control group cortisol levels were significantly increased at time 2 ($p>0.001$) and 3 ($p=0.002$) following stress onset, relative to time 1 (basal), while the CD group did not show a significant alteration of basal cortisol levels in any of the time points. However, both the HC and CD groups returned to similar start point cortisol levels at the last recovery sample (+55 minutes, time 5) and did not significantly differ from each other at this timepoint ($p=0.23$). Male and female healthy showed a similar pattern of cortisol reactivity (albeit starting from different pre-stress levels). Similarly, males and females with CD showed similar patterns of cortisol reactivity, but males showed a slightly greater reduction than females in cortisol response following the stress onset.

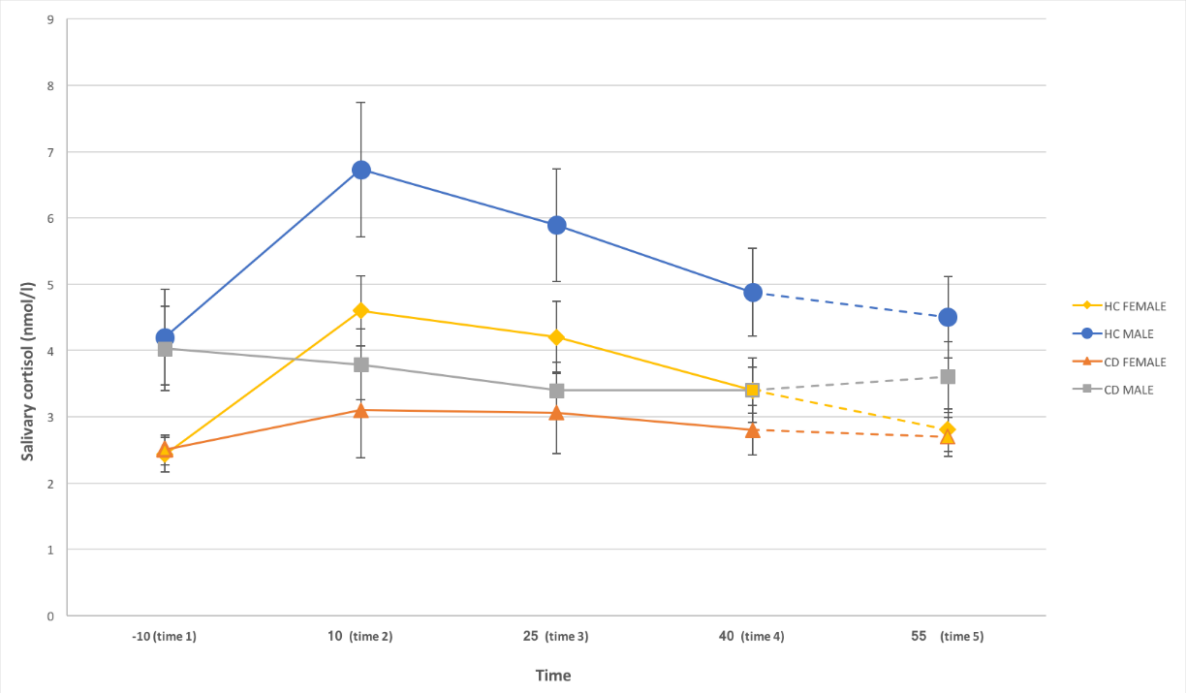


Figure 7.3 - Sex by diagnosis interactions in salivary cortisol. Results are during baseline conditions, just after social stress, and again after the stress induction. Mean and standard errors values are reported.

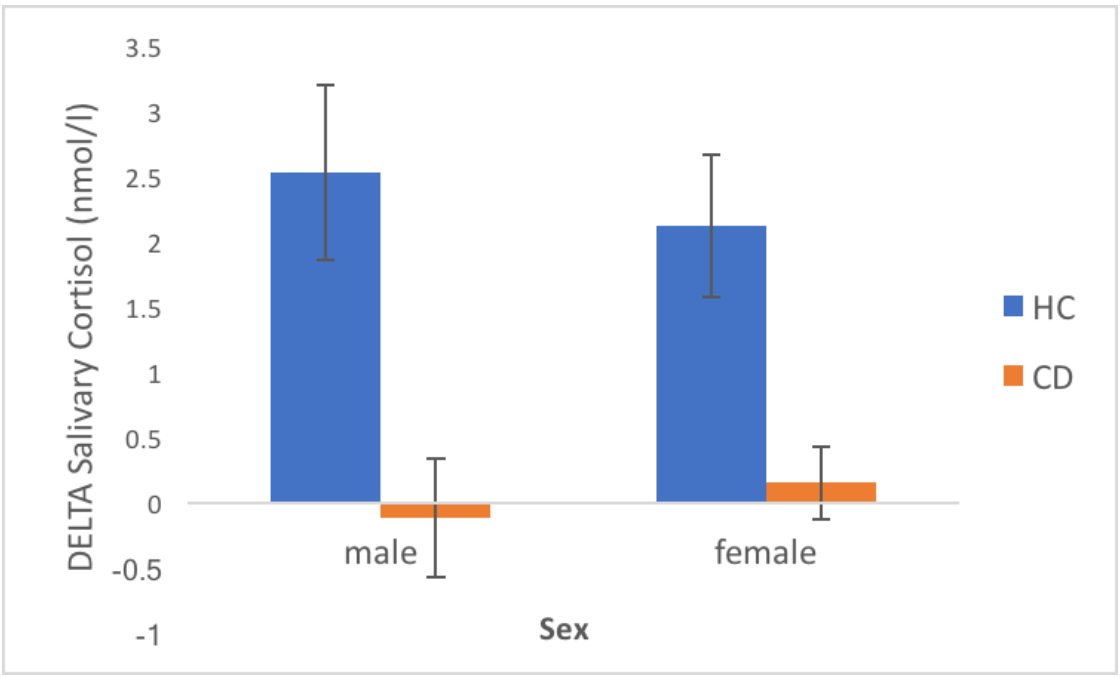


Figure 7.4 - Group differences in measures of stress reactivity

Cortisol at Time 2 minus pre-stress levels at Time 1. Mean and standard errors scores are indicated. HC; healthy control, CD; conduct disorder.

7.4.3 Confounding effects and correlations between cortisol reactivity and CD symptoms or psychopathic traits in the CD sample

The main effect of CD on cortisol reactivity remained significant after controlling for symptoms of general anxiety disorder, ADHD, age, puberty, and smoking – respectively. In addition, there were no significant correlations between CD symptoms, nor the three sub facets of psychopathic traits (impulsive/irresponsible, callous/unemotional, narcissistic/manipulative) within the CD sample.

7.4.4 Self-reported subjective feelings: Feelings of insecurity

A graphic representation of feelings of insecurity is provided in **Figure 7.5**. Repeated-measures MANOVA, with greenhouse-geisser correction, revealed a significant main effect of time ($F=39.37$, $p=0.0001$). No time by diagnosis interaction ($F=0.263$, $p=0.84$), nor time by sex by diagnosis interaction ($F=0.15$, $p=0.92$) were found. However, a significant time by sex interaction ($F=7.93$, $p<0.001$, $\eta p^2=0.10$) was revealed, as well as a significant main effect of sex ($F=9.40$, $p=0.003$, $\eta p^2=0.12$). This effect was driven by females, who in general, displayed stronger feelings of insecurity after the stress onset compared to males. There was no main effect of diagnosis ($F=0.307$, $p=0.58$), nor a significant sex by diagnosis interaction ($F=2.5$, $p=0.11$). Pair-wise comparisons, comparing basal scores on feelings of insecurity at time 1 with all other times, revealed that females and males with and without CD, had significantly increased feelings of insecurity following the stressor at time 2 ($p=0.001$), 3 ($p<0.001$), and 4 ($p<0.001$) and decreased feelings of insecurity at recovery period time 9 ($p=0.008$).

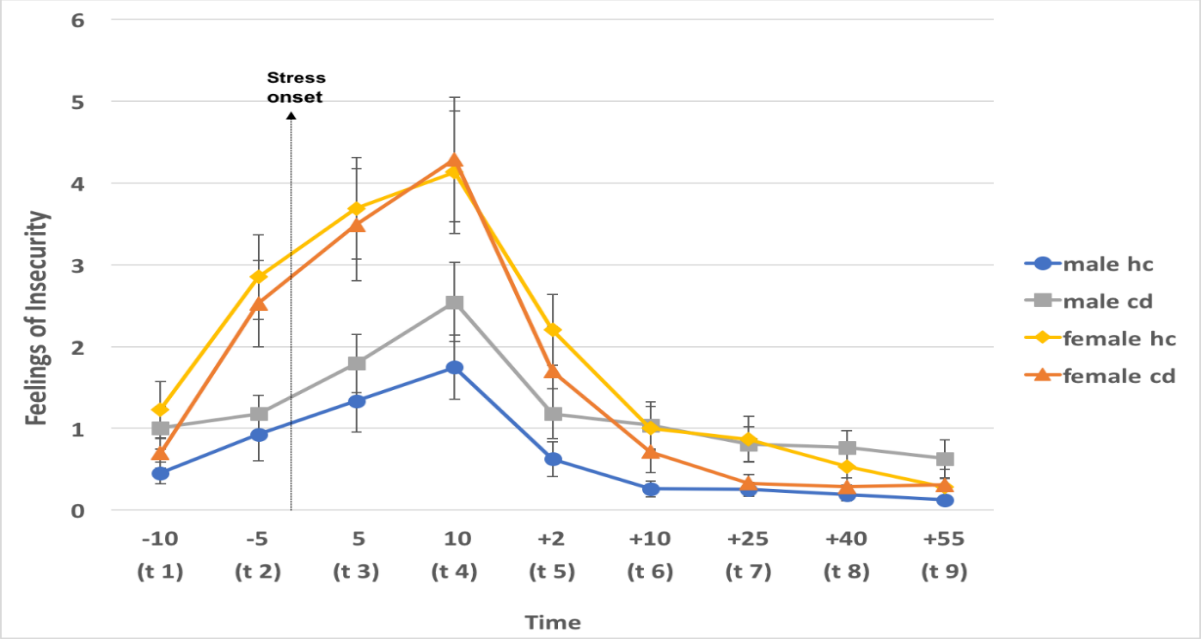


Figure 7.5 - Self-reported feeling of insecurity. Results are during baseline conditions, the preparation period, immediately after speaking, and again following stress. Means and standard error scores are indicated. Key: hc; healthy control, cd; conduct disorder, t; time

7.4.5 Self-reported subjective feelings: Feelings of Stress

A graphic representation of the data of subjective stress is provided in **Figure 7.6**. Repeated-measures ANOVA, with greenhouse-geisser correction, revealed a significant main effect of time ($F=80.36$, $p=0.0001$), but no time by diagnosis interaction ($F=1.32$, $p=0.26$) or time by sex by diagnosis interaction ($F=0.24$, $p=0.86$) was found. There was no main effect of diagnosis ($F=0.183$, $p=0.67$), nor a time by sex ($F=2.55$, $p=0.06$), or a time by sex by diagnosis interaction ($F=1.86$, $p=0.17$). However, there was a significant main effect of sex ($F=3.87$, $p=0.05$, $\eta p^2=0.05$), such that, in general, females showed increased feelings of stress compared to males. Pair-wise comparisons comparing basal scores on feelings of stress at time 1 with all other times, revealed that females and males with and without CD, had significantly increased feelings of stress following the stressor at time 2 ($p<0.001$), 3 ($p<0.001$), and 4 ($p<0.001$), 5 ($p<0.001$) and decreased feelings of stress at time 9 during the recovery period ($p=0.05$).

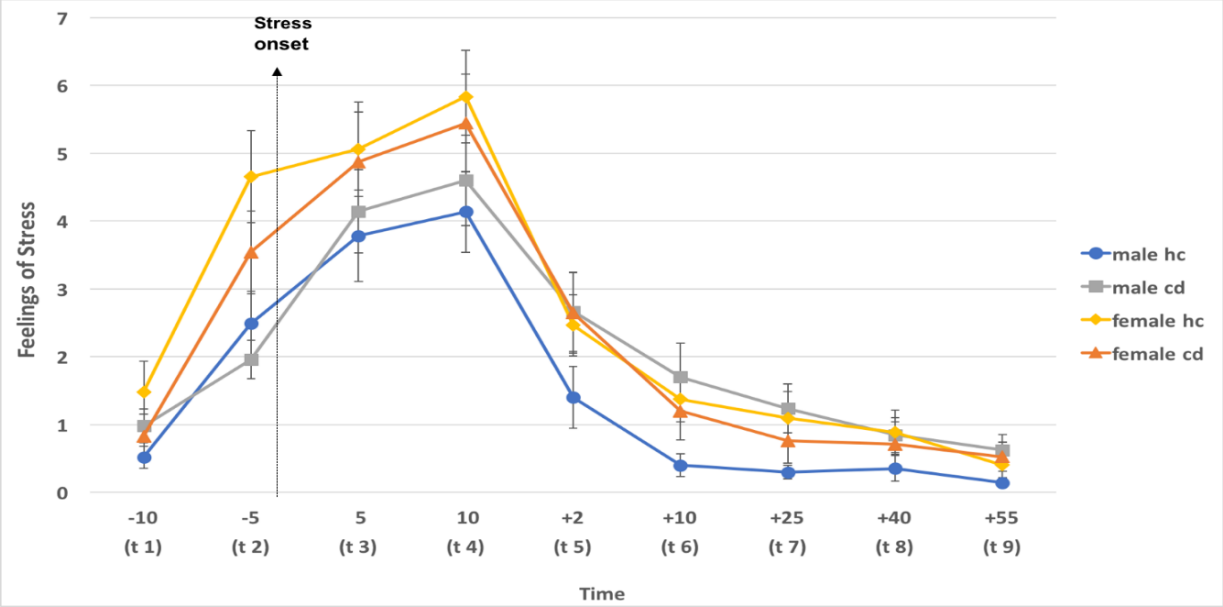


Figure 7.6 - Self-reported feeling of stress. Results are during baseline, preparation, speaking, and again after stress conditions. Means and standard error scores are indicated.

Key: hc; healthy control, cd; conduct disorder, t; time

7.4.6 Self-reported subjective feelings: Feelings of anxiety

A graphic representation of changes in feelings of anxiety during the TSST-C is provided in **Figure 7.7**. Repeated-measures MANOVA, with greenhouse-geisser correction, revealed a significant main effect of time (F=82.36, p=0.0001), but no time by diagnosis interaction (F=1.46, p=0.22), nor a time by sex by diagnosis interaction (F=0.73, p=0.54) was found. However, there was a significant time by sex interaction (F=3.37, p=0.02, ηp^2=0.05).

There was no main effect of diagnosis (F=0.91, p=0.34). However, there was a significant main effect of sex (F=5.02, p=0.03, ηp^2=0.07), such that, in general, females showed increased feelings of anxiety compared to males. There was also a significant sex by diagnosis interaction (F=4.13, p=0.05, ηp^2=0.06). Underlying these interactions, females with CD showed decreased feelings of anxiety at all time points, compared to females without CD, whereas males with CD showed increased feelings of anxiety (after the stress onset) compared to their healthy counterparts.

Pair-wise comparisons revealed that females and males with and without CD, had significant increased feelings of insecurity following the stressor at time 2 (p<0.001), 3 (p<0.001), and 4 (p<0.001), 5 (p<0.02) and decreased feelings of stress at recovery period time 8 (p=0.01), and 9 (p=0.001).

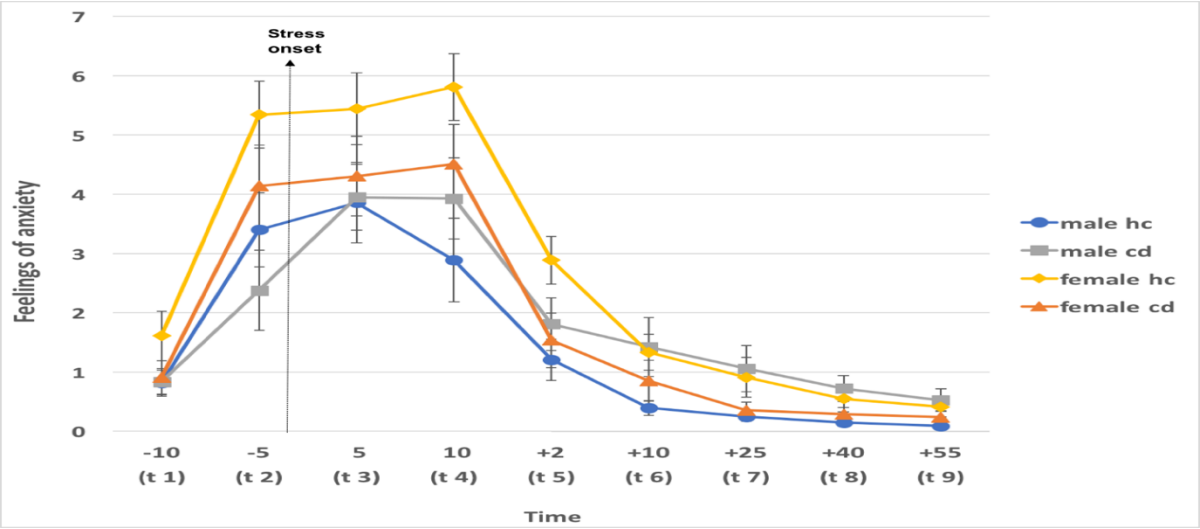


Figure 7.7 - Self-reported feeling of anxiety. Results are during baseline, preparation, speaking, and again after stress. Means and standard error scores are indicated. **Key**: hc; healthy control, cd; conduct disorder, t; time

7.4.7 Proof of concept study of the relationship between brain structure and cortisol reactivity

7.4.7.1 Cortical and subcortical volumes

For the sub-sample as a whole (n = 38), there were significant positive correlations between both left (r=0.46, p=0.004) and right (r=0.31, p=0.05) superior frontal gyrus (SFG) volume and delta cortisol during the TSST. There were no correlations for other regions. When looking at CD subjects and controls separately, we found positive associations between delta cortisol and bilateral SFG (left: r=0.70, p=0.002, right; r=0.59, p=0.01; **Figure 7.8**) in the CD group only and positive associations between delta cortisol and the volumes of the left hippocampus (r=0.56, p=0.01), and left SFG (r=0.51, p=0.02) in the healthy control group only.

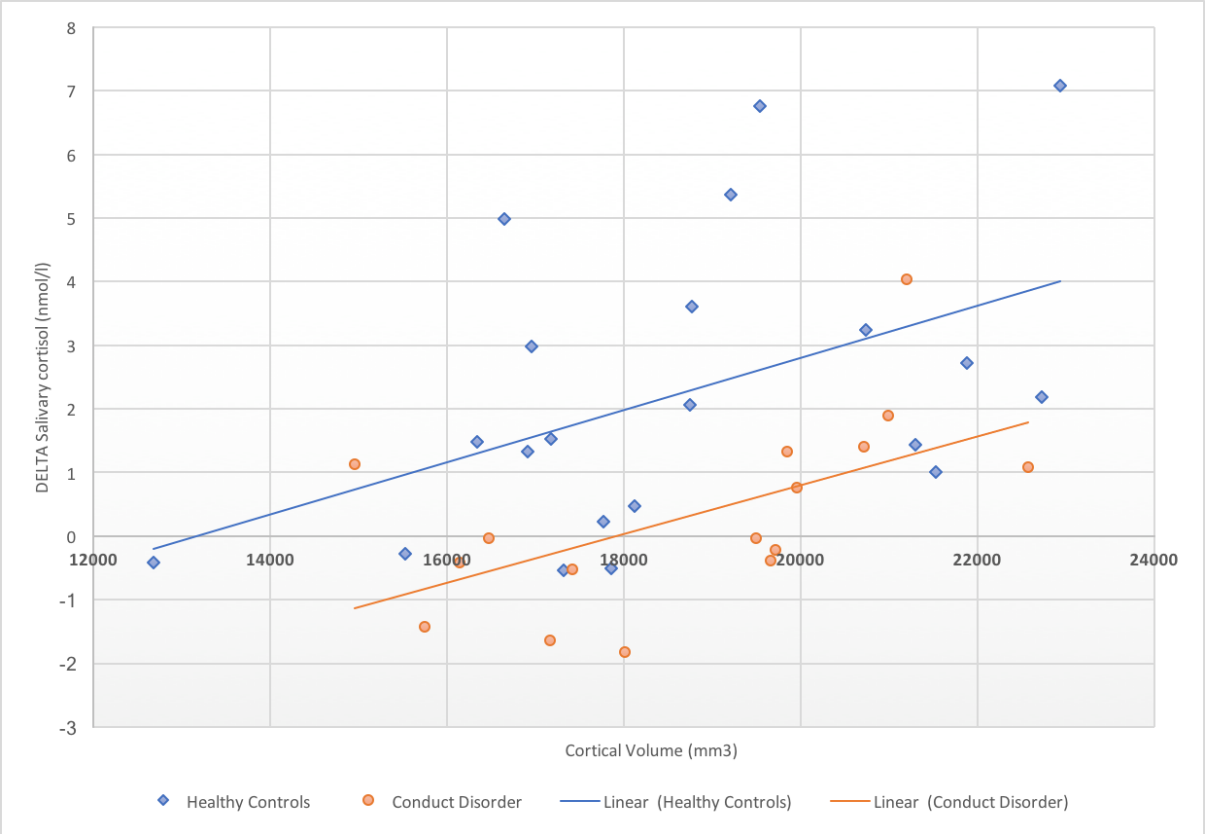


Figure 7.8 - Relationship between the cortical volume of the right superior frontal gyrus and cortisol reactivity. Circular markers (orange) display the association between cortisol reactivity and cortical volume of the right superior frontal gyrus in the conduct disorder group. This correlation showed to be significant. Diamond markers (blue) display the association between cortisol reactivity and cortical volume of the right superior frontal gyrus in the healthy control

7.4.7.2 White matter tracts

Across the whole sample for whom usable DTI data were available (n=29), cortisol reactivity during the TSST was significantly positively correlated with HMOA (r=0.49, p=0.007) and FA (r=0.43, p=0.02) and negatively correlated with radial diffusivity (RD) (r=-0.57, p=0.001) in the right subgenual cingulum. When the groups were analysed separately, we found a positive correlation between the FA (r=0.56, p=0.03) and a negative correlation between the radial diffusivity (r=-0.53, p=0.04) in the right subgenual cingulum and cortisol reactivity in the healthy control group only. No associations were found between parameters of white matter diffusion and cortisol reactivity in the CD group alone.

7.5 Discussion

This is the first study to investigate sex differences in cortisol reactivity to psychosocial stress in youths with CD. We aimed to replicate previous findings of hypocortisolism in response to psychosocial stress in adolescent males with CD and examine whether this pattern was also present in female adolescents with CD.

Our findings revealed that as predicted, CD was associated with a blunted cortisol response to stress. Our results extend the literature by revealing that females with CD also showed a blunted cortisol response compared to their healthy counterparts. Interestingly, both females and males with and without CD appeared to find the psychosocial test stressful and showed increases in feelings of insecurity, stress and anxiety after during the stressor. This is consistent with what previous studies have shown (Fairchild et al., 2008; Popma et al., 2006; van Goozen et al., 2000), demonstrating that the blunted cortisol response observed in CD individuals is not because the CD subjects did not find the TSST-C stressful. There were highly significant sex differences in levels of cortisol across the testing session – with girls having lower cortisol levels relative to males. Moreover, there were strong sex differences in subjective ratings with girls feeling more stressed, insecure and anxious than boys. Finally, there were some associations between measures of brain structure and HPA axis functioning which highlights the potential value of performing a full study on this topic in the future. More in depth interpretation of the findings will be provided below.

7.5.1 Effects of diagnosis

The pattern of low cortisol reactivity has been explained by low arousal theories. According to these theories, patterns of low arousal in CD may indicate a lack of fear in situations where a mild stress response should be warranted. Consequentially, individuals with CD, are not concerned

about social cues (e.g., punishment). Thus, physiological under-arousal, and low levels of fearfulness may predispose youths with CD to engage in problematic behaviour. Conversely, individuals with low arousal may have increased thresholds for stress and participate in dangerous activities as a form of stimulation (Raine, 2002). Thus, since the findings of the current study, showed that both males and females with CD showed a reduced response to the psychosocial stressor, one could speculate that youths with CD developed altered stress responses based on social evaluative threat and that it fits with the low arousal theory. However, the differences in cortisol reactivity observed between the CD and healthy control groups were not explained by significantly increased or decreased feelings of anxiety, stress, or insecurity. Both groups showed a similar increase in self-reported measures of insecurity, stress and anxiety. In fact, there were slightly increased feelings of stress in males with CD compared to healthy male controls. Therefore, these findings are not in agreement with the idea that low arousal in CD is due to a lack of the ability to feel fear. On the contrary, there is a clear discrepancy, which may indicate less coordination between emotional and psychophysiological responsiveness in subjects with CD (Fairchild, van Goozen, et al., 2008). It also undermines the idea that cortisol is a good measure of state-related self-perceived emotional states.

Our findings of low cortisol reactivity in the CD group relative to the healthy control group is consistent with previous literature that reported an association between attenuated cortisol response to psychosocial stress and conduct problems (Van Goozen et al., 2007). In addition, our results are in agreement with previous studies in which attenuated cortisol levels in youths with CD were more evident during stress than during resting (i.e., basal) conditions (van Goozen et al., 1998; van Goozen et al., 2000). Furthermore, interestingly, this pattern has been found in at-risk sons of fathers with a psychoactive substance use disorder (Moss, Vanyukov, Yao, & Kirillova, 1999; Moss et al., 1995), in delinquent male adolescents (Popma, Jansen, Vermeiren, Steiner, Raine, Van Goozen, Van Engeland, et al., 2006) , and psychiatric groups with disruptive behaviour disorders (i.e., ODD, CD; Snoek & Goozen, 2004). In addition, our results were not explained by levels of callous unemotional traits. Thus, our findings support the suggestion that this trait could be considered as a neurobiological marker of antisocial behaviour in this population, which could help with future diagnosis (Popma et al., 2006).

It has been demonstrated that there is a stronger association between basal cortisol levels and youths with CP relative to associations with cortisol levels in response to stress (Alink et al., 2008). However, it has also been shown that when studies use stronger stressors (i.e., outcome uncontrollability and social-evaluative threat), the relationship between cortisol reactivity and conduct problems becomes stronger (Alink et al., 2008). Thus, our findings support the need of a strong enough stressor to elicit exaggerated feelings of stress and anxiety in the whole sample (CD and HC). The consistent use of a standardised and effective stress induction procedure, such as the

TSST-C, would facilitate the replication of findings, in which an association between conduct problems and low cortisol reactivity would be found more consistently.

In fact, it is important to consider that other studies using more naturalistic stressors have shown an opposite pattern of cortisol responsivity in youths with CP. For instance, adolescents with CP had reported an increased cortisol response to conflict discussion with caregivers compared to healthy controls (Kobak et al., 2009). However, the increased response appeared to be driven by females with CP relative to males. It is well known that harsh parental discipline or inadequate parental supervision is associated with the development of CP (B B. Lahey et al., 1999). Therefore, it may be that the stress response to conflict discussion with caregivers in youths without CP - whom may have experienced more supportive parenting - may not be as traumatic or threatening as for adolescents with CP. Moreover, girls in particular, might perceive this environmental stressor as being more challenging than boys. However, this study did not assess subjective feelings of stress, so the latter claim is hard to justify.

In the present study, there was no sex difference in the relationship between CD and blunted cortisol response to social evaluative stress. Interestingly, previous studies looking at associations between antisocial behaviour and heart rate revealed that both male and females and CD showed lower heart rates (Ortiz & Raine, 2004; Portnoy & Farrington, 2015). Thus, our results are in agreement with these results and are consistent with the idea that the same risk factors and biological mechanisms underlie CD in both males and females (Moffitt, Caspi, Rutter, & Silva, 2001). This finding raises questions regarding why males with CD display greater symptoms and higher prevalence than their female counterparts. Our finding of low stress reactivity in youths with CD of both sexes might suggest that both groups are under-aroused, or over-regulated, which leads to them becoming habituated to challenging and threatening situations (Fairchild, van Goozen, et al., 2008; Kruesi et al., 1989). For instance, individuals with CD may have experienced increased social adversity during development, or been exposed to more risky and challenging situations in the recent past, either as a result of maltreatment or negative experiences in their peer group or due to their reckless behaviour. This is important, as there is evidence that stress can have substantial effects on the developing neurobiological systems in the brain and the HPA axis (Lupien et al., 2009). However, responses to psychological stressors also involve the interaction between neurocognitive and affective processes (Dickerson & Kemeny, 2004). Lower cortisol levels have been associated with lower cognitive functioning (Suor, Sturge-Apple, Davies, Cicchetti, & Manning, 2016). Therefore, altered coordination between cognitive, emotional and physiological mechanisms may be related to blunted cortisol reactivity in youths with CD.

7.5.2 Sex Effects

Females showed lower cortisol levels than males at all time points. This finding is consistent with previous studies using a similar psychological stress of public speaking and mental arithmetic task in healthy individuals (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Kudielka & Kirschbaum, 2005). However, previous studies included young adults, whereas our study included adolescents aged between 12 to 18 years old. Thus, our results add to the literature by reporting a pattern of higher cortisol levels in a group of male adolescents compared with age-matched females (mean age:15 years old).

Females reported greater feelings of stress and anxiety than males. However, it is important to consider that regarding cortisol reactivity, there were no significant differences between the sexes. This means that both sexes responded equally to acute social stress, but the females simply started from a lower point. It has been shown that cortisol response to the circadian rhythm is slower and of lessened magnitude (less of a difference between morning and evening cortisol levels) in females than when compared to males (Cauter, Leproult, & Kupfer, 1996). This effect may be due to an increased sensitivity of the adrenal cortex in females compared to males (i.e., negative feedback mechanisms are more sensitive in females than in males resulting in modest cortisol release) (Roelfsema et al., 1993).

There is some indication that in the control group, typical males showed slightly greater reactivity than typical females. It has been argued that contraceptives may influence the neuroendocrine response to psychological stressors (i.e., as a result of estrogen) in females (Kirschbaum, Wust, & Hellhammer, 1992). It may be that the findings of this study were moderated by this factor, although females showed a similar degree of reactivity as the males- and it was just the starting point (baseline level) that differed. However, for the sake of clarification, our sensitivity analysis excluded female participants who were taking contraceptives, and the subsequent findings did not change. Therefore, our sex differences in overall cortisol levels across the testing session did not seem to be influenced by contraceptive use.

7.5.3 Sex by diagnosis interaction

Although a previous study investigated basal cortisol rather than cortisol reactivity to stress, it has been reported that inverse associations between CP and basal cortisol were observed in males, but not in females (Shirtcliff et al., 2005). However, the latter study was conducted in typically developing youths but with higher levels of CP. In addition, this study included younger participants (aged 6-16 years), and it is well known that there is an increased prevalence of antisocial behaviour amongst adolescent girls. Moreover, it has been shown that age moderates the relationship between conduct problems and cortisol (Alink et al., 2008). Thus, our findings

observed in females may be attributable to the older age of the subjects included in the present study. Nonetheless, it is worth mentioning that although our study demonstrated significant main effects of diagnosis in cortisol response, irrespective of sex, as well as significant subjective stress reactivity in both sexes with CD relative to typically developing peers, there was some indication that the reduction in cortisol reactivity was more pronounced in males with CD than their female counterparts.

7.5.4 Relationship between brain structure and HPA axis function

Although this is only a preliminary and small-scale study, the preliminary results of this pilot study are of interest. Firstly, we found associations between the volume of the superior frontal gyrus (SFG) and cortisol reactivity stress, which appeared to be of a stronger magnitude in the CD group. This finding is consistent with a previous study examining the relationship of cortisol awakening response and brain structure in a whole brain analysis. This study reported positive correlations between cortisol levels and SFG volumes (Valli et al., 2016). In addition, in Chapter 4, we showed higher cortical volume in the SFG of the HC group compared to the CD group. Hence, this preliminary result could indicate that those individuals within the CD group with higher cortical volumes in the SFG are more likely to show an intact response to stress. However, given the small sample size, it may be suggested that these associations are different in degree rather than present or absent. Similarly, given that an optimal cortisol response is beneficial and essential in helping organisms to cope with challenging situations, and since the TSST-C was intended to induce acute and short-term stress, the positive association between hippocampal volume and cortisol reactivity in the healthy control group may indicate a normal and functional HPA axis.

Finally, the positive associations observed for FA and negative associations for RD in the right subgenual cingulum (SGC) found in the healthy control group is of particular interest as the SGC is a WM tract connecting the anterior cingulate cortex with the amygdala, with some fibres reaching the hippocampus. Although a previous study used a different methodology to the one utilised in the present study, it is noteworthy that positive associations between the functional connectivity of the SGC and greater cortisol responsivity have been reported (Thomason, Hamilton, & Gotlib, 2011). Similar to the interpretation for CV, the positive association may indicate a healthy HPA axis function. However, the lack of relationships in the CD group may be due to insufficient variance in cortisol reactivity and SGC microstructure to detect any significant correlations.

7.5.5 Implications and future research

Reduced cortisol responses to stress may be a particular characteristic of CD (Van Goozen et al., 2007), and may help to identify those adolescents who are at risk of developing the disorder (Shoal, Giancola, & Kirillova, 2003). In addition, low HPA axis responsivity to stress in children with conduct problems has been associated with poor treatment outcomes (Van de Wiel, Van Goozaen, Mathhys, Snoek, & Van Engeland, 2004). Therefore, this cortisol measure could be used in the future to aid diagnosis and intervention (Van de Wiel et al., 2004). However, future research should aim to investigate associations between HPA (re)activity and different subtypes of CD. The low HPA axis activity relationship may differ between CD subgroups in a way that sheds light on clinical heterogeneity. For example, previous studies have shown that children with CD and anxiety show higher cortisol levels compared to those with CD but without anxiety (Schoorl et al., 2016). Although our findings reported that anxiety did not influence cortisol reactivity in males and females with and without CD, our sample was relatively small, and may not have provided enough power to reliably investigate this factor. Thus, future findings in this area might allow us to develop more effective and targeted interventions for different subgroups of CD individuals.

In addition, to have a better understanding of the nature of low HPA axis responsivity to stress in CD, it would be of interest to clarify whether this effect is unique to social and evaluative threats, or whether similar findings are observed for different types of stressors (e.g., conflict). For instance, studies using social evaluative stressors, but with a more naturalistic approach (e.g., arguments with parents) have shown increased, rather than decreased cortisol levels in response to conflict stress in youths with CP. Thus, we would recommend that future studies address whether attenuated cortisol responses to stress are a general characteristic of CD, or whether this feature is a specific state-like reaction to social evaluation. The use of non-psychological stressors (e.g., physical challenge) or even pharmacological challenges targeting the HPA axis would help to address this question. This would help us to have a better understanding of the different cognitive and affective processes that interplay with the psycho-physiological response to stress in females and males with CD.

7.5.6 Strenght and Limitations

This study has advantages over previous studies of cortisol reactivity in individuals with CD. For the first time, we examined the influence of sex in cortisol response to the TSST-C. By using the TSST-C, we ensured that the social stressor was robust to produce a stress response in the whole sample, compared to previous studies which have used a naturalistic approach. Additionally, we also measured subjective feelings of stress and anxiety, which allowed us to distinguish between physiological and cognitive-emotional processes. Moreover, in an attempt to control for the

varying cortisol levels of the natural circadian rhythm of the HPA axis we conducted the TSST-C between 1 and 4 pm. Finally, we also exanimated the influence of comorbid ADHD and anxiety disorder.

This study also has some limitations which should be noted. Firstly, due to the relatively small sample size, it was not possible to meaningfully examine potentially important variables which could affect our findings, such as, clinical heterogeneity in CD, internalising disorders, and age of onset. In addition, although we attempted to assess for potential correlations between CU traits and cortisol reactivity, we did not evaluate this factor as a categorical variable (e.g., CD with high vs low levels of callous unemotional traits). It is clear that future research with larger samples is warranted. In addition, due to difficulties in arranging testing dates, we were unable to test females in the same menstrual phase, and we did not have reliable information regarding their current menstrual phase, thus we were unable to control for this variable, even though previous research has shown differences between follicular and luteal phases in cortisol reactivity to stress (Kirschbaum et al., 1999). Finally, we did not control for symptoms of depression, and although there were comparatively low rates of comorbid internalising disorders in our sample, it is nevertheless important to assess the influence of this factor. However, recent research has suggested that contrary to what was previously thought, internalising problems may not lead to a stronger response to psychological stress (Van den Bos, Tops, & Westenberg, 2017). Further research investigating sex differences in the relationship between CD and stress reactivity should consider these issues of comorbidity and heterogeneity.

7.6 Conclusion

In conclusion, our results show for the first time that CD is associated with low HPA axis reactivity to social evaluative threat in both male and female adolescents. This is despite an intact subjective response to the stressor in the CD groups – all groups reported increases in feelings of insecurity, stress, and anxiety during the Trier Social Stress Test for Children. Future research should examine whether adolescents with CD show blunted cortisol responses to another type of stressors, such as non-social stress. This may provide a better understanding whether blunted cortisol reactivity is linked to specific effects of social-evaluative stress, or whether cortisol reactivity in CD is generally altered. In addition, larger samples are needed to assess possible differences in stress reactivity between the different subtypes of CD (e.g., CD/CU+ versus CD/CU-).

Chapter 8 General Discussion

8.1 Introduction

Although each of the empirical chapters in the present thesis (4-7) included a discussion of the specific findings, this final chapter will discuss the implications of the findings as a whole and how they fit into the literature on sex differences in CD. It will firstly start by briefly describing the overall objective of the thesis, followed by a summary of the key findings. Secondly, a synthesis of the emerging themes will be delineated. Thirdly, the results of this thesis will be considered together to see how they fit into the overall literature. It will further lay out the overall strengths and limitations of the series of studies included in the thesis. Finally, possible implications for research on CD and clinical practice will be described.

8.2 Aim of the thesis

This thesis was based on the dataset collected as part of the Neurobiology and Treatment of Adolescent Female Conduct Disorder study (FemNAT-CD), and the author made a significant contribution to the data collection. The overarching aim of this project is to improve our understanding of the role of genetic and environmental risk factors in the development of CD and, for the first time, examine sex differences in the neurobiology of CD.

This thesis, however, focused on investigating the fronto-limbic and hypothalamic-pituitary-adrenal (HPA) axis systems in youths with CD. Previous literature studying the neurobiology of CD and related phenotypes have predominantly reported brain abnormalities in regions of the prefrontal cortex (PFC) and limbic system, such as, the amygdala, ventromedial prefrontal cortex (vmPFC), anterior cingulate cortex (ACC), insula and striatum. Studies on the HPA axis have also revealed abnormal HPA axis activity in antisocial populations. However, research in youths with antisocial behaviour have been inconsistent. Studies had reported higher, lower and no differences in diffusivity (i.e., white matter microstructure) (Waller et al., 2017) , grey matter volume (Rogers & De Brito, 2016), as well as in cortisol levels (Alink et al., 2008). One possible reason for the inconsistencies between studies may be due to the use of small samples. In addition, there is a clear gap in the literature regarding sex differences in CD, as most of our knowledge concerning neurobiological mechanisms in CD comes from studies that have been restricted to male samples.

Therefore, the primary aim of this thesis was to specifically investigate the structure of the PFC, and limbic system by using different methodologies (e.g., surface based morphometry, shape analysis, and tractography) in a large sample of adolescents with and without CD. Given that the PFC and limbic system are strongly related to the functioning of the HPA axis, and altered HPA axis functioning has been found previously related to CD, we also aimed to investigate this neurobiological system in a sub-sample of the FemNAT-CD. A secondary, but no less important goal, was to investigate whether structural abnormalities in regions of the PFC, limbic system and HPA axis activity are also present in females with CD.

8.3 Summary of key findings

8.3.1 Chapter 4: Frontal and limbic, cortical and subcortical, structures in youths with conduct disorder

This chapter aimed to investigate sex differences in the structure of the key prefrontal and limbic brain regions that have previously been implicated in the pathophysiology of CD (e.g., amygdala and insula). To achieve this goal, we used surface-based morphometry (SBM) methods, which allowed us to examine distinct features of the cortex (e.g., cortical thickness (CT), surface area (SA), and cortical volume (CV)) in specific fronto-limbic areas (e.g., insula, anterior cingulate cortex). In addition, we also assessed the volume of sub-cortical structures that make up the limbic system, including the amygdala, hippocampus and striatum.

Contrary to our expectations, we observed few robust CD-related brain alterations in areas that have commonly been associated with CD, such as the amygdala, insula, and ACC. On the other hand, we found that irrespective of their sex, individuals with CD showed reduced CT, SA and CV in the superior frontal area, including the gyri and sulci. The superior frontal gyrus (SFG) is part of the dorsolateral prefrontal cortex (DL-PFC). The right DL-PFC has been implicated in cognitive control, decision making, and the ability to delay gratification (Baumgartner, Knoch, Hotz, Eisenegger, & Fehr, 2011; Knoch et al., 2006.; Shackman, Mcmenamin, Maxwell, Greischar, & Davidson, 2009). This is partly due to the contribution of the right SFG to response inhibition (Rubia et al., 2001). Response inhibition is defined as the suppression of inappropriate actions in a particular context, e.g., halting a motor movement. Interestingly, our findings also showed an inverse association between impulsive and psychopathic traits and SFG cortical thickness. This suggests that deficits in the SFG may particularly influence poor response inhibition in youths with CD and high levels of impulsive or psychopathic traits.

Furthermore, we did not find sex by diagnosis interactions in any of the regions of interest. These null results contradict those of a recent study from our lab, which found that CD males showed an increased, while CD females showed a decreased, SA in the SFG compared to sex-matched typically-developing controls (Smaragdi et al., 2017). Instead, the nominal results on sex by diagnosis interactions of SA in SFG, observed that males with CD displayed lower SA values in the SFG compared to the other groups. Thus, this may indicate that the effects in males are more pronounced than those seen in CD females. The discrepancy between the studies may be due to the use of slightly different samples, as well as the use of distinct methodologies (vertex-wise vs region of interest (ROI) approaches). The methods differ in that the ROI approach assesses a priori predicted region, and is sensitive to subtle differences in a large, well-defined area, while the vertex-wise analysis identifies greater differences in small areas anywhere in the brain. Also, the processing of the latter study was more comprehensive, in that segmentation errors were manually corrected. Although this improves the outputted data, this method is a time-consuming process and is not convenient for larger samples sizes, such as the one used in the present study.

8.3.2 Chapter 5: Morphology of the Amygdala, Hippocampus and Striatum in Youths with Conduct Disorder

Conduct Disorder has been consistently linked to volumetric abnormalities in limbic subcortical structures such as the amygdala. However, volumetric studies are not able to detect modest shape changes in specific localised areas. Given that subcortical structures are composed of sub regions, each region has different patterns of connectivity with the rest of the brain, and is responsible for a range of specific functions, subtle abnormalities of the shape of these structures may have functional significance (Andersen & Teicher, 2008; Raznahan et al., 2013). Thus, the aim of this chapter was for the first time to investigate whether CD is associated with alterations in the shape of subcortical brain structures such as the amygdala, hippocampus, caudate nucleus, nucleus accumbens, pallidum, putamen and thalamus. In addition, we aimed to assess sex differences in the relationship between CD and shape changes. We addressed this goal by using a vertex based analysis (FSL FIRST), which provides further delineation of localised shape changes.

We found that CD individuals exhibit alterations of shape (i.e., inward deformations) in the shell of the nucleus accumbens (NAcc). There were no other shape abnormalities in any other region, nor were there sex by diagnosis interactions in any region. Shape abnormalities in the shell of the NAcc are of particular interest due to its specific connections with the limbic system (Neto et al., 2008). Moreover, the shell of the NAcc has been strongly associated with reward processing and motivation (Pauli, O 'reilly, Yarkoni, & Wager, 2016). Thus, abnormalities in NAcc shape might map onto and underpin the neurocognitive deficits displayed by patients with CD, such as deficits in reward processing, decision-making and reinforcement learning.

8.3.3 Chapter 6. White matter microstructure of the extended limbic system in youths with conduct disorder

Abnormal structural connectivity has been reported in youths with CD. However, a number of diffusion tensor imaging studies investigating youths with CD have yielded inconsistent results. These inconsistencies may be partly due to different methodologies (e.g., tract-based spatial statistics (TBSS) vs tractography) and to the use of small study samples. It has previously been suggested that antisocial behaviour is a product of structural and functional abnormalities of the limbic system (Rubia, 2011). However, previous studies investigating the structural connectivity in CD have mainly focused on a particular fronto-temporal limbic white matter tract: the uncinate fasciculus (UF), leaving other key limbic white matter tracts uninvestigated. Thus, this study aimed to use advanced methodological approaches - spherical deconvolution tractography - to specifically investigate the UF and other limbic white matter tracts of interest, namely, the fornix, and cingulum bundles (i.e., subgenual cingulum, retrosplenial cingulum, and parahippocampal cingulum) in youths with and without CD and to examine sex differences.

We found main effects of diagnosis in the right retrosplenial cingulum (RSC). The CD group showed lower fractional anisotropy (FA) in participants with CD relative to controls. However, we observed sex by diagnosis interactions and recognised that the CD effect was driven by males with CD: males with CD showed reduced FA in the right RSC relative to typical males, whereas females with CD showed higher FA compared to typical females. In addition, we found that CD was associated with reduced axial and mean diffusivity in the left UF, but no sex by diagnosis interactions were observed. Effects in the UF did not survive correction for multiple comparisons.

The RSC connects the DL-PFC (Vann et al., 2009), ACC, and PCC (D K Jones, Christiansen, Chapman, & Aggleton, 2013). Thus, the RSC is the most interconnected structural tract with core areas involved in the default mode network (DMN). The RSC is related to self-referential processes. The medial prefrontal cortex hub appears to have a significant role in social cognition, while the PCC is associated with autobiographical memory and moral judgement (Talamini, Meeter, Elvevåg & Murre, 2005). Given the overlap of the RSC tract with brain regions that comprise the DMN, future studies may wish to investigate sex differences in the functional connectivity of this network in CD.

8.3.4 Chapter 7: Cortisol reactivity to stress in youths with conduct disorder

Previous studies examining the relationship between HPA axis (re)activity in response to stress and CD have consistently shown blunted cortisol responses to stress. However, HPA axis reactivity to stress effect has not been studied in females with CD. Therefore, this study aimed to investigate sex

differences in the cortisol response to stress in adolescents with CD. This was achieved by using a standardised and effective laboratory-based stress task – the Trier Social Stress Test for Children (TSST-C; (Buske-Kirschbaum et al., 1997)).

As predicted, CD was associated with a blunted cortisol response to stress. Our results extend the literature by revealing that females with CD also showed a blunted cortisol response compared to their healthy counterparts. Thus, these findings support the suggestion that this cortisol measure could be used to aid diagnosis and intervention (Van de Wiel et al., 2004), and provide further evidence that cortisol hypo-reactivity may be a characteristic feature or 'biomarker' of CD in both sexes. However, it would be of interest to address whether attenuated cortisol responses to stress are a general characteristic of CD, or whether this feature is a particular state-like reaction to social evaluation. The use of non-psychological stressors (e.g., physical challenge) or even pharmacological challenges targeting the HPA axis would help to address this question.

Interestingly, in contrast to the cortisol results, both females and males with and without CD appeared to find the psychosocial test equally stressful - reporting feelings of insecurity, stress and anxiety during and after the stressor. Thus, contrary to our expectations, the blunted cortisol response observed in CD individuals does not appear to be caused by a reduced psychological sensitivity to stress. This lack of a full mapping from biological to the psychological stress sensitivity challenge current idea about the role of cortisol in the experience of stress.

Finally, a proof of concept study revealed positive correlations between the cortical volume of the SFG and cortisol reactivity to stress, which appeared to be of a stronger magnitude in the CD group. Given that studies on healthy populations had shown that higher cortisol levels are associated with an increased cortical volume of the SFG (Valli et al., 2016), our results may indicate that those individuals within the CD group with higher cortical volume in the SFG are more likely to show an intact response to stress.

Table 8.1 – Summary of Key findings across the four chapters

Measure	Brain Structure	Parameter	Main effect of diagnosis	Main effect of Sex	Sex by diagnosis interaction
Surface based morphometry	Right Superior Frontal Gyrus	CT	**X**		
		CV	**X**	**X**	
	Right Superior frontal sulcus	SA	**X**		
Shape analysis	Right Nucleus accumbens	N/A	**X**	**X**	
SD tractography	Right retrosplenial cingulum	FA	**X**	**X**	**X**
DELTA cortisol	N/A	Nmol/l	**X**	**X**	

This table shows key findings for each chapter; surface based morphometry (chapter 4), shape analysis (chapter 5), SD tractography (chapter 6), DELTA cortisol (chapter 7); it is separated by main effects of diagnosis, sex, and sex by diagnosis interactions.
Key: SD, spherical deconvolution, CT, cortical thickness, CV, cortical volume, SA, surface area, FA, fractional anisotropy, N/A, not applicable, X=significant effect

8.4 Synthesis of key themes emerging across the studies

Although the four chapters included in the thesis employed different methodologies and had particular aims, it is of interest to note that there are some similarities between them in terms of themes that have emerged from their findings. In particular, taking all our findings together, we observed very few sex by diagnosis interactions suggesting that neurobiological alterations in CD are similar in male and female youth. Below we will firstly describe the relationship between the finding of the three neuroimaging chapters. It will be followed by discussing the association between stress reactivity and brain structure, and lastly, it will discuss how our findings contribute to the scientific and clinical debate on sex differences in CD.

8.4.1 Relationship between the neuroimaging findings

1. DLPFC and Executive control

Firstly, with regards to the three MRI chapters, our results confirm previous studies by showing CD-related effects in the dorsolateral prefrontal cortex (DL-PFC). Chapter 4 (SBM), revealed that CD individuals had reduced CT, SA, and CV in the SFG – which is a key region of the DL-PFC. Chapter 6 showed reduced fractional anisotropy (FA) in the retrosplenial cingulum (RSC) tract in participants with CD compared to healthy controls. The RSC connects regions of the DL-PFC (including the SFG) with the anterior and posterior cingulate cortex. More precisely, the SFG is divided into different subregions, namely the anteromedial, dorsolateral, and posterior frontal gyri. The anteromedial region of the SFG is particularly strongly connected to the middle anterior and mid-cingulate cortices (W. Li et al., 2013). Thus, it is worth noting that while the cortical structure of the SFG shows abnormalities in CD individuals relative to controls, a white matter tract connecting this region with the anterior and posterior part of the cingulate cortex was also found to be altered in CD participants relative to controls.

The DL-PFC has been implicated in aspects of executive functioning, such as sustained attention (W. Li et al., 2013). It has been suggested that the associations between DLPFC functioning and CD may be the result of the presence of comorbid ADHD (Rubia, 2011). However, in this thesis, as in previous studies with relatively large CD samples, impairments of the DL-PFC in CD individuals (i.e., the SFG), could not be explained by the presence of ADHD symptoms (Smaragdi et al., 2017). These findings are further supported by recent meta-analyses of structural and functional MRI studies of CD. The VBM meta-analysis indicated lower grey matter volume of the medial SFG in CD relative to controls (Noordermeer, Luman, & Oosterlaan, 2016; Raschle, Menks, Fehlbaum, Tshomba, & Stadler, 2015; Rogers & De Brito, 2016). The fMRI meta-analysis

showed reduced activation of the right DL-PFC region during emotion processing (Alegria et al., 2016). These studies highlight the possible role of the SFG in CD.

2. Heterogeneity

Of interest, the majority of our findings (besides the shape of the NAcc) were not explained by variation in the levels of CU traits in our CD sample. With regards to cortisol reactivity, our results are consistent with studies investigating youths with ADHD with and without CD, in which it was revealed that CU traits were not significantly associated with cortisol reactivity (Northover et al., 2016). Thus, our findings support the suggestion that conduct problems are a stronger predictor in elucidating differences in cortisol reactivity than variation in CU traits.

Similarly, the group differences in the SFG were related to impulsive traits, rather than CU traits. Studies of adults with antisocial personality disorder had suggested that DL-PFC abnormalities are associated with antisocial behaviour more in general because of its role in cognitive control (Andrea L Glenn & Raine, 2011). Thus, the structural abnormalities observed in the DL-PFC of individuals with CD could be more in general associated with conduct problems than with levels of CU traits. In contrast, given that psychopathy has previously been associated with an anatomically overlapping white matter tract (i.e., the dorsal cingulum) to the RSC tract, it would have been expected to find a significant relationship between the RSC and CU traits (Sethi et al., 2014). However, the structures interconnected by the RSC (i.e., mPFC and PCC), also play a significant role in moral decision-making (perhaps due to impairments in self-reflection) – which is a common feature of antisocial individuals (Raine & Yang, 2006). Future neuroimaging studies should consider the SFG and its connections as an emerging area of interest for the neural underpinnings of CD.

3. The Limbic system

Surprisingly, we did not find cortical or morphological abnormalities in areas that have commonly been associated with CD (e.g., amygdala, vmPFC). This, is surprising, given that several studies had consistently reported volumetric changes in these structures in individuals with CD relative to healthy controls (Rogers & De Brito, 2016). However, consistently with our results, two recent studies (one using an SBM and the other a VBM approach) with relatively large CD samples, were not able to identify grey matter changes in these structures (Michalska et al., 2015; Smaragdi et al., 2017). However, it is important to note, that several studies had treated distinct anatomical landmarks for the prefrontal cortex (Yang & Raine, 2009). For example, VBM studies have defined the anterior cingulate cortex by including distinct total numbers of voxels (1,399 vs 5,660)(Fairchild, Van Goozen, Stollery, & Goodyer, 2008; Sterzer et al., 2007). Therefore, it is difficult to conclude whether abnormalities in frontal areas such as in the vmPFC and anterior

cingulate cortex are present or absent. In addition, the discrepancies in studies investigating specific regions of interest may also be attributed to the use of different atlases. For instance, VBM atlases have mainly parcellated regions according to the terminology for sulci (Tzourio-Mazoyer et al., 2002). On the contrary, the present study used an atlas based on sulci and gyri (Destrieux et al., 2010). Therefore, this highlights the importance of investigating specific prefrontal sub regions using similar atlases. Bearing this in mind, given that the present study includes the largest sample of CD to date, these lacks of results should not be ignored, and it is suggested that future studies wanting to replicate these findings utilize the same atlas as the one used in Chapter 4 (Desikan et al., 2006; Destrieux et al., 2010).

In addition, the fact that we did not observe differences in these structures in a much larger sample of CD, may be related to the complex and heterogeneous nature of the disorder. For instance, although we attempted to assess for potential correlations between callous-unemotional traits with the cortical thickness of the SFG and white-matter microstructure in the RSC, we did not evaluate this factor as a categorical variable (e.g., CD with high vs low levels of callous unemotional traits). This may have shown whether CU traits are particularly influential in explaining previous findings observed in limbic structures (e.g., amygdala, ACC, insula). For instance, fMRI studies have found amygdala deactivations during negative emotions in individuals with CD and low CU traits, while increased activations were revealed in those with CD and high CU traits (Viding et al., 2012). Future research may want to detect whether abnormalities, for instance in the amygdala, are restricted to distinct sub-groups of CD individuals (e.g., those with high levels of CU traits).

4. Nucleus Accumbens and Reward Processing.

The study conducted in chapter 5 was the first study to reveal shape differences in the NAcc of youths with CD relative to healthy peers. Localised and subtle changes in the shape of the NAcc may inform us about alterations in particular anatomical connections and functions. Hence the observed deformations in the shell of the NAcc in CD individuals, allow us to better understand the potential role of this structure in the pathophysiology of CD. For instance, the shell of the NAcc has stronger connections with limbic areas, and in particular with the amygdala. Of interest, the NAcc and the amygdala are implicated in stimulus-reinforced associations, for both reward and punishment. However, the amygdala has a stronger role in the formation of stimulus-punishment, while the NAcc is involved in the formation of stimulus-reward (Ernst et al., 2005).

The NAcc has more commonly been identified in fMRI than sMRI studies. A possible explanation might be due to the distinct methodological approaches that have been employed thus far, as well as the fact that this region has not been defined as a region of interest in previous studies. However, when the striatum has been considered as a single structure, the ventral part – which includes the NAcc, has shown volumetric alterations (i.e., reduced) in individuals with CD compared to HC

(Fairchild et al., 2013). In addition, CD individuals show increased activations in the NAcc in response to outcomes of reward-directed behaviour, compared to healthy controls (Bjork, Chen, Smith, & Hommer, 2010). These findings support the notion that CD individuals exhibit increased reward sensitivity, which may lead to reward-seeking and poor decision making. Moreover, it has been suggested that abnormalities in the NAcc and the DL-PFC produce both hypersensitivity of the reward system (as observed with increased NAcc activation), and immature self-control (as observed with DL-PFC reductions) that might explain the reckless and impulsive style of decision-making observed in CD (Casey et al., 2008).

To summarise, CD is a heterogeneous disorder that manifests in different forms (e.g., CD with CU traits, comorbid anxiety, childhood-onset), however deficits in decision-making is one key characteristic shared by all the sub-groups. Interestingly, the structural brain changes that were observed in CD individuals are all regions associated with decision making. For instance, the SFG has been related to error detection and response inhibition. The retrosplenial cingulum (RSC) has been associated with self-referential processes and moral judgment, and the NAcc is related to motivation and reward-related behaviour. Perhaps the self-referential processes in CD individuals may influence hostile interpretational biases (e.g., the act of interpreting other's behaviours as having a hostile intent, even when the action is ambiguous or benign). In turn, the choices that individuals with CD take in a 'hostile world', are lacking pro-active or social motivation. Also, difficulties to reflect about the negative (or hostile) emotion related to the thought of breaking the rules or committing an immoral act, may lead to a tendency of making choices that have previously been punished, and fail to inhibit maladaptive responses, and neglect better outcomes or rewards.

8.4.2 Stress reactivity and brain structure

With regards to cortisol response to stress, our findings confirm those of previous studies and extend the findings to females with CD. However, given that individuals with CD reported high levels of subjective feelings of stress and anxiety during the psychosocial stress induction, the results challenge the fearlessness theory of CD. This theory argues that lower psychophysiological activity during basal and stress condition is due to fearlessness. To note, fearlessness has also been associated with abnormalities in the amygdala (R. J. R. Blair, 2008). However, our results found no associations between CD and this structure (see above). Thus, this null result (i.e., volumetric changes in the amygdala) supports our findings of increased anxiety and stress level in youths with CD during a social stress task - which is not what you would expect to see in a fearless individual.

As was discussed in Chapter 7, blunted cortisol levels and high levels of stress and anxiety in response to stress, may indicate a poorer coordination between cognitive-affective and psychophysiological mechanisms during stress in individuals with CD. As it was reviewed in the

introduction, the PFC and limbic system are high in glucocorticoid receptors, and previous studies have found that thinning in the SFG and alteration in the white matter of the right anterior cingulate were associated with HPA axis dysregulation in response to stress (Kern et al., 2008; Sheikh et al., 2014). Interestingly, although we assessed only a small sub-sample, we also observed an inverse association between stress reactivity with the SFG volume in individuals with CD. Perhaps, this preliminary result may provide some insights into the poorer cognitive-affective and psychophysiological coordination observed in CD individuals. It would be of interest to further investigate these associations to understand what drives the deficient coordination between cognitive-emotional and physiological systems.

8.4.3 Sex differences in conduct disorder

The fact that we found little evidence of sex differences in the relationships between CD and brain structure, white-matter microstructure or cortisol reactivity to stress is consistent with the suggestion that the biological risk factors that lead to CD are relatively similar for males and females (Moffitt et al., 2001). This claim has received further support from studies assessing physiological and biological factors. For instance, a recent eye-tracking study assessing emotion recognition in males and females with CD demonstrated that both sexes had difficulties in recognising emotional facial expression, although this effect was particularly stronger in males (Martyn-Key, Graf, Adams, & Fairchild, 2017). Furthermore, a meta-analysis assessing genetic and environmental influences on antisocial behaviour (AB), did not find significant differences between the sexes in the aetiology of AB (Burt, 2009). In addition, consistent with our findings, a previous meta-analysis investigating associations between heart rate level and antisocial behaviour in youths, reported that low heart rate was observed in both males and females with AB (Ortiz & Raine, 2004). Structural MRI studies had also shown that adolescent males and females with psychopathic traits share similar grey matter volume alterations in limbic areas (Lora M Cope et al., 2014). Finally, a recent study investigating sex differences in white matter microstructure in children with CD did not observe robust sex differences. Nevertheless, associations between higher axial and radial diffusivity in a range of white matter tracts and CD symptoms appeared to be stronger in females than in males (Decety, Yoder, & Lahey, 2015). However, contrary to the findings of this thesis, a recent SBM study reported sex differences in the relationship between CD and cortical structure in several brain regions such as the SFG (Smaragdi et al., 2017). However, the latter study used a different methodological approach to the one used in this thesis, which could explain the discrepancy between the findings (see Section 8.3.1 of this chapter for more details regarding the different methodologies).

Although we did not find sex by diagnosis interactions, it is important to note that there was some indication that although both sexes with CD showed reduced SA, CT and CV in the superior frontal area; the reductions were more pronounced in males with CD than in their female counterparts. Similarly, there was some indication that the observed blunted response to stress difference was larger in males with CD than in females with CD. Although it could be speculated that those results may explain sex differences in severity (males having more CD symptoms than females), none of our correlational analysis with CD symptoms proved to be significant.

In addition, it has previously been suggested that the large sex differences in the prevalence and aetiology of CD behaviour may be due to a gender paradox (Cloninger, 1978). This paradox argues that in disorders with a large difference in the proportion of males and female prevalence, such as CD, the sex in which the disorder is less common tends to be more severely affected. The findings of this thesis did not support this 'gender paradox'. On the contrary, our preliminary results showed that males with CD tended to show more pronounced alterations compared to females. Our findings may explain why males tend to exhibit poorer decision making (e.g., making more risky choices) compared to females with CD (Sidlauskaite et al., 2017).

8.4.4 Research Domain Criteria

In recent years there has been growing interest in the Research Domain Criteria (RDoC) initiative. This initiative was introduced to address concerns about the utility and reliability of the current diagnostic and classification systems (i.e. DSM and ICD), and the high levels of heterogeneity and/or comorbidity observed in many supposedly distinct disorders. The RDoC initiative aims to address these limitations by increasing our knowledge of the fundamental processes and circuits associated with observable behaviour and brain functions (National Institute of Mental Health, 2017). It also adopts a dimensional perspective on mental disorders, rather than taking a categorical approach. It does not aim to replace or compete with systems based on categorical models such as the DSM, however it argues that classifying disorders on this basis will be more informative – especially with regard to causal mechanisms - and could provide further insight into the classification and treatment of mental disorders. For example, it may allow researchers to develop targeted approaches that seek to alter activity in a given neural circuit or biological system that contributes to mental illness (e.g., the circadian clock). Some biological models of CD fit well with the RDoC approach, such as the fearlessness theory (see Chapter 2), however many of the current models have focused on CD with high CU traits rather than conduct problems in general (Fonagy & Luyten, 2017).

Although the methodology used in this thesis was not designed to use or test an RDoC approach, it potentially yields complementary information regarding CD symptoms. Firstly, we attempted to find correlations between brain structural cortical volume, cortisol, CD symptoms, and psychopathic traits. These correlations were only conducted in the CD group. However, there was considerable variability within our CD group in terms of CD symptoms (the number of symptoms endorsed ranged from 3 to 12 out of 15). Thus, it was feasible to use a dimensional approach for correlational analysis within the CD group only.

In addition, the results were interpreted by considering that brain regions work in conjunction, forming brain circuits. Each brain network modulates specific behavioural domains, such as reward learning, executive functions, and emotional processing. Our results indicate that individuals with CD have alterations in brain regions that are involved in executive, reward and default mode networks.

8.5 Strengths and limitations of the studies included in the thesis

Each chapter touched upon the strengths and limitations of each of the individual studies. However, there are some strengths and limitations that can be attributed to the overall work. The data used for this thesis, as it has been mentioned on numerous occasions, derived from the FemNAT-CD project. As with any multi-centre project, there are some strengths and some limitations with such multi-centre studies that should be acknowledged that may affect the interpretation of the findings. However, in our view, the strengths far outweigh the limitations. These will be described in more detail below.

8.5.1 Strengths

Use of A Unique And Well-Characterised Sample

Given that this thesis combined data from several sites across Europe (especially Germany and the UK), our sample size was significantly larger than previous studies of CD populations. This should have helped to reduce the presence of false positives and negatives – that are often found in small samples. In addition, the large sample size permitted me to allocate the participants to groups that were matched for age, IQ and sex – which is typically challenging considering that previous studies have shown a robust association between CD and low IQ (Moffitt et al., 2001). Moreover, and most importantly, it also allowed us to test large numbers of males and females with and without

CD. This enabled us to test for sex by diagnosis interactions and permitted us to assess sex differences more accurately.

In addition, we assessed for diagnoses of CD and comorbid disorders such as ADHD and depression using a standardised semi-structured interview, the K-SADS-PL, Rather than self- or parent-report questionnaires (e.g., the Child Behaviour Checklist). This approach has several advantages over questionnaire-based methods because an interview allows the researcher to expand or clarify any question if needed. It is also likely that the respondents think more carefully about the questions if asked them in an interview format. Another strength of this study is that only those participants with a current diagnosis of CD were included - those with lifetime or past CD were excluded.

Originality

This thesis contributed not only to the debate of whether the neurobiological basis of CD differs between the sexes. We also addressed innovative questions, such as whether there are morphological changes in subcortical structures (e.g., nucleus accumbens), or whether there are specific white matter alterations in an extended limbic network (e.g., retrosplenial cingulum). In a proof of concept analysis, we also investigated the link between cortisol reactivity and brain structure. We addressed these questions by using innovative neuroimaging methods (e.g., shape analysis, spherical deconvolution tractography).

8.5.2 Limitations

Differences Between Sites And Countries In Recruitment And Referral Routes

To overcome sample size limitations, a multi-centre approach to data collection was employed. However, this procedure also has limitations. For instance, as multiple sites were involved in data collection, a larger number of interviewers were required to perform the K-SADS-PL, which is likely to introduce or increase subjective biases. However, to reduce this bias, all sites went through systematic inter-rater reliability checks, and this showed high reliability and agreement between different raters (see Chapter 3 for further details).

Another limitation regarding recruitment across multiple sites is that each country had different sources of recruitment. For instance, while the UK sites largely recruited from the community or specialist schools, the German sites mainly recruited participants with CD from specialised mental health clinics. This generated a more heterogeneous sample in that some participants had more severe forms of CD (i.e., higher symptom count), or in that some were significantly more impaired

than others (e.g., needing medical assistance). However, it could be argued that this means our results are more generalizable to the whole CD population, rather than specific sub-groups or settings. In addition, there were some differences between the sites in the age of the participants: the German sites recruited younger participants, and the UK sites recruited older participants. Also, there were differences in gender (e.g., more females were recruited in Basel).

Effects of Scanning site

Since MRI data were acquired across multiple sites, this could have increased the risk that scanner hardware and software differences introduced unwanted noise into the data. However, to reduce artefacts, we standardised the protocol for image acquisition and went through a site qualification process in which phantoms were scanned at each site and the pilot data were reviewed by an experienced MR physicist before proper data collection commenced. However, even when data acquisition methods are standardised across sites, there is still a risk of significant site-specific effects. Nevertheless, the benefit of larger samples probably outweighs the potentially confounding effects of including data from several locations (Gountouna et al., 2010; Nieuwenhuis et al., 2017). Moreover, in an attempt to reduce the impact of site effects, we controlled for the site as a factor in all our statistical models.

8.6 Implications

8.6.1 Research

This thesis focused on brain regions that are potentially of critical relevance in CD. These areas were selected based on previous neuropsychological, endocrinological and neuroimaging findings. Surprisingly, regarding brain structure (i.e., cortical thickness, cortical volume, surface area and shape) we did not find structural alterations in some of the most consistently reported brain areas, such as the amygdala, insula, and ventromedial prefrontal cortex. Therefore, given the scale of this cohort, the failure to support previous findings suggests that there is still much to learn about neurobiological changes in youths with CD relative to controls. Perhaps findings from previous studies were false positives, and those results may have been influenced by the use of small samples. Future neuroimaging studies may want to investigate other brain regions, beyond the frontal and limbic brain regions, such as parietal and occipital areas. In addition, it would be very useful for future studies to increase their sample size, report effect sizes, and try to reduce the impact of potential confounding variables, such as comorbid ADHD, and group differences in age, IQ, and sex composition to obtain more robust results.

In addition, understanding the functional contribution to CD symptomatology of the superior frontal gyrus, retrosplenial cingulum and nucleus accumbens would be of interest. As discussed in the general introduction to this thesis (Chapter 2), stress can affect reward-based decision making, and this ability has been linked with the DL-PFC and NAcc. Therefore, it would be of interest to study the association between stress, cortisol response and these structures with decision making in individuals with CD.

8.6.2 Clinical

Although there are no direct clinical implications in terms of diagnosis or treatment that can be drawn from the present results, it is important to gain insight into the neurobiological mechanisms of CD, which may inform further studies aiming to identify treatments for CD that are based on modifying the underlying pathophysiology (e.g., enhancing DL-PFC function via transcranial direct current stimulation). Additionally, knowing that there are neurobiological mechanisms involved in the development of CD, should inform the development of causal models that seek to explain the condition.

If individuals with CD present with neuroanatomical changes compared to their typically developing peers, we need first to identify and learn more about not only the brain areas but also the connections that are involved and investigate how these areas could change with treatment. Therefore, by enhancing our understanding of the neurobiology of CD, we could support better-targeted treatments.

Finally, in the last few years, there has been a growing interest in the neuroimaging field in machine learning approaches. This technique learns brain networks, and it further classifies the pathological change of the brain anatomy to classify subjects into a patient and healthy group. Several studies have assessed the diagnostic value of these techniques (e.g. mild cognitive impairment (Teipel et al., 2007) and have shown promising findings. By investigating the key brain areas involved in the pathophysiology of CD, we might be able to develop a machine-learning algorithm to have more evidence for more accurate diagnosis and assessments of prognosis.

Regarding treatment and interventions, our findings suggest that the same type of treatment might be effective in both males and females with CD. Perhaps, given that the structures found to have significant alterations are associated with decision-making, interventions aiming to improve reward-based decision making could be considered. For instance, interventions in CD could be targeted to improving executive functioning (EF), or in stimulant drugs to address EF deficits. However, it is clear that better understanding of the functional and structural consequences of alterations in these structures is needed. In addition, measures of cortisol reactivity may be used clinically to measure response to therapeutic intervention. Finally, given that there were indications

that males with CD exhibited more pronounced structural alterations than their female counterparts, our results are in agreement with recent suggestions that males with CD may need longer or more comprehensive interventions than females with CD (Martyn-Key et al., 2017).

8.7 General conclusions

The present thesis investigated sex differences in the relationship between CD and alterations in frontal and limbic brain networks and the HPA axis. We found main effects of CD relation to prefrontal cortex cortical thickness, volume and surface area in the superior frontal area. The SFG has been associated with cognitive control and response inhibition. We also found that when comprehensively investigating subcortical structures, group effects were only observed in the nucleus accumbens. Interestingly, when examining limbic white matter tracts, we found sex by diagnosis interactions in the right retrosplenial cingulum; males with CD showed lower, while females with CD showed higher fractional anisotropy relative to sex-matched healthy control groups. Finally, we also found that both males and females with CD exhibit blunted cortisol responses to stress relative to sex-matched healthy controls. Taking all of our findings together, we conclude that the neurobiological basis of CD is broadly similar for males and females, although some outcome measures showed nominally larger CD-related effects (e.g., cortisol reactivity) and others showed smaller group differences (e.g., cortical structure). We tentatively conclude that previous findings based on male only samples are likely to be applicable to females with CD, although future studies should still attempt to test for interactions between CD status and gender and avoid collapsing across the sexes in studies with small sample sizes.

Appendix A Documents used for recruitment

A.1 Flyer used for the Fem-NAT-CD study

Project Title: Understanding sex differences in disruptive behaviour in children and teenagers: The FemNAT-CD Study (Ethics number: 8215, Version 1, 16/10/2013)

FemNAT CD

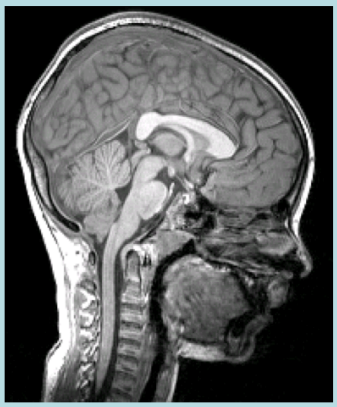
E-mail A.Smaragdi@soton.ac.uk

Are you between 9 and 18 years old?

Would you like a picture of your brain?

Take part in our study !

- You will be paid up to £55 for taking part
- You will get a picture of your brain as a thank you!



UNIVERSITY OF Southampton

The study involves:

- An interview at your home asking about your typical feelings, thoughts, and behaviours (1 ½ hours; £10)
- A visit to Southampton University to do face recognition and learning tasks. You will be asked to give a saliva sample, and have your heart rate measured using sensors (3 hours; £20).
- A brain scan at Reading University (1 ½ hours; £25)

Interested? Ask your case worker for more details or call us on 07979072331 or 07979073512

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 602407

A.2 Information Sheet for the Fem-NAT-CD study

Project Title: Understanding sex differences in disruptive behaviour in children and teenagers: The FemNAT-CD study. **Ethics number:** 8215

Please read this sheet carefully before deciding to take part in this study. If your child is happy to take part, you and they will be asked to sign a consent form.

What is the research about?

We are interested in studying why boys and girls behave differently, and particularly why boys are more likely than girls to show disruptive behaviour. We are recruiting boys and girls aged between 9 and 18 years to take part in a study investigating these issues. The aim of our research is to help reduce disruptive behaviour in schools and local communities.

The first part of the study involves meeting up with you and your son or daughter; we will ask you and your son or daughter some questions about their typical feelings, behaviours, and lifestyle (including questions about things like alcohol use). This would normally happen at your home, but we could meet at the University or your child's school if you prefer. If your son or daughter is eligible to take part in the main study, we will invite them to the University to fill in questionnaires and complete computerised tasks that will test their ability to recognise emotions in faces, control their emotions, and learn to choose some shapes while avoiding others. We will ask them to give us some saliva (spit) samples, which will allow us to measure the structure of their DNA (genes) and their stress hormone levels. We will also place plastic sensors on their chest and back, and fingertips to measure their heart rate and tiny changes in sweat production. On another day, we will invite them to Reading University for a magnetic resonance imaging (MRI) brain scan. We will take images of the size and shape of their brain and ask them to complete face processing and decision-making tasks while they are in the scanner to measure their brain activity.

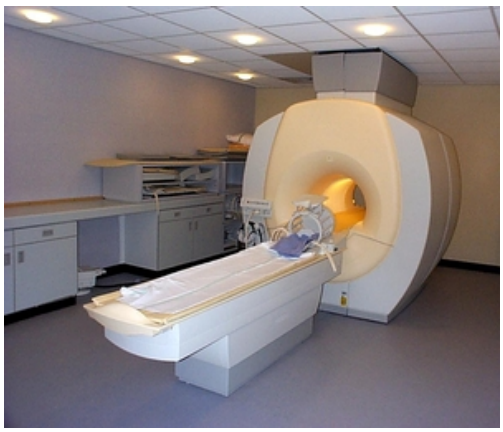
Why has my child been chosen?

We are recruiting children and teenagers aged between 9 and 18 years through schools, colleges, education centres, Child and Adolescent Mental Health Services in the NHS, Families Matter, and the Hampshire, Southampton and Reading Youth Offending Services. Our sample is what is called an 'opportunity' sample – anyone who chooses to take part can do so, providing they are not affected by the study's exclusion criteria (such as pregnancy, having autism, or being older than our age limit of 18). Your child does not have to have disruptive behaviour to take part – we are interested in recruiting young people <u>without</u> disruptive behaviour, as well as those who may have been in trouble a lot at school, or with the police. If your son or daughter decides to take part, you will be given this information sheet and both of you will be asked to sign a consent form. They will still remain free to stop taking part at any time, without giving a reason. They can also choose to opt out of any part of the study (e.g. the MRI brain scan or the spit

samples), if they wish.

What is magnetic resonance imaging or MRI?

Magnetic resonance imaging (MRI) is a technique that uses strong magnetic fields to create detailed images of the size and shape of the brain, or measure brain activity. By taking a series of pictures of your child's brain while they perform a task (e.g., recognising faces), we can study which brain areas are involved in doing that task. The scan does not involve any medications, injections or X-rays, and MRI is generally thought to be a very safe, non-invasive method, but because of the strong magnets involved we have to be careful not to let any metal objects (e.g. dental braces) into the scanner environment. This means that anything made of metal has to be removed before they go into the scanner. MRI is suitable and safe for children and teenagers.



What will happen if my son or daughter decides to take part?

We will meet up with you and your son or daughter to explain in detail what the study involves and find out whether they are eligible to take part (for example, they cannot be pregnant or have metal plates in their head). We will ask both of you questions about your child's typical moods, behaviours, and lifestyle, ideally in separate rooms, so that the information you give us is private. This part of the study should take around one and a half hours and you and your son or daughter would be paid for your time (**£10 each**).

The main study is split into two parts that will take place on two separate days. We would like your son or daughter to take part in both parts, but they can choose to opt out of the brain scan or the University visit if they wish. The visit to Reading University for the brain scan will last around 1.5 hours, with around 50 minutes being spent inside the scanner itself (plus travel time). Before their scan, a radiographer will ask your son or daughter some questions to check that they have no metal on or within their body before they enter the scanner's magnetic field. The tasks that they will perform whilst in the scanner will be explained to them and we will answer any questions that you or they may have. They will then be asked to lie still while we take pictures of their brain and the connections between different brain areas. They will also do a task that involves deciding whether the faces they see are male or female, a task that involves making choices to win or avoid losing money, and a task that shows people in mild pain (finger trapped in a door). They will receive **£25 and a picture of their own brain** for this part of the study.

The second part of the study involves completing questionnaires and computerised tasks at Southampton or Reading University. All the tasks will be quite easy and will be explained to your son or daughter in detail before they start. They will have the

opportunity to practise them and ask questions. There will be tasks measuring facial recognition, emotional control, and learning to choose or avoid shapes leading to different outcomes. They will also be asked to complete some questionnaires that measure personality traits, well-being and life events. We will ask you to fill in similar questionnaires in a separate room, some of which ask about negative life experiences. We will also ask your son or daughter to spit in a plastic pot to measure their stress hormone levels and their DNA (genes). Lastly, we will attach plastic sensors to their fingers, chest, and back to measure heart rate and tiny changes in sweat production (this will be done by a female researcher if the volunteer is female). You and your child will be given detailed information about the purpose of the tasks and the goals of the research at the end of the study. They will receive **£20** for this part of the study (3 hours), and we will also cover all of your travel expenses.

Are there any benefits in my child taking part?

We will reimburse them for their time and travel (£10 for the initial meeting, £25 for the brain scan, and £20 for the visit to the University), and they will have made an important contribution to our understanding of sex differences in disruptive behaviour. However, their participation in this study is not intended to have any direct clinical or medical benefits.

Are there any risks involved?

The scanner can be loud when it is working, so your child will be given headphones and earplugs to block out the sound. In addition, the scanner environment is quite small and confined, so people who are uncomfortable in small spaces may not be able to take part. Otherwise MRI is considered safe, and there are no known risks or side effects. However, if your child feels uncomfortable, they may stop the scan. There is a small chance that you or they may find some of the questionnaires or computerised tasks upsetting – if this is the case, they will be offered the chance to take a break, leave out questions, or stop the task or questionnaire you are doing. You will also be able to leave out questions or stop filling in the questionnaires, if you wish. Your child can also stop taking part in the study at any time.

Will my child's participation be confidential?

Yes, your child's identity will be protected by changing their name to a subject code, which will be used on all questionnaires, data files, and samples. Records of their personal details will only be kept if you both agree to this. We can assure you that any information you both give us will be strictly confidential, and you will both be able to leave out questions you are uncomfortable with. The only exception to this is when you or they give us information that makes us concerned about their well-being or safety or that of others – in this case, we will discuss the different options with you or them, such as contacting their GP or a counsellor and making a referral. All questionnaires will be number coded and if the study is written up for publication, the article(s) will not include

people's names.

As this study is part of a larger project involving Universities in seven other European countries, some of the biological samples that are collected in Southampton will be transferred to other European countries for analysis. However, your child's identity and personal details will always be protected. In addition, the researchers working on the project will share information and transfer data between countries to carry out their statistical analysis, although none of the data files that will be shared will contain personal details or people's names. Finally, all study data will be protected against unauthorised access.

What happens if my child changes their mind or I change my mind about taking part?

You and your child will be able to stop taking part in the study at any time, without explaining why. If they don't object, we may use any data collected up to the point of withdrawal (when they stop taking part). They can also opt out of any part of the study.

Will this be the only time my child takes part in the study?

Most young people will only take part in the study once, so after they have had a brain scan and come for their testing session at the University, their involvement in the study will be over. However, we will be running a follow-up study, therefore we will be contacting the young people who took part in the first part of the study in around 18 months' time. We will write to or call them to ask whether they would be willing to be interviewed and come to the University again, and have another brain scan. As before, your son or daughter would be paid for their time and travel expenses, and they can opt out of any part of the study.

What happens if something goes wrong?

If you or your child have a concern or complaint regarding any aspect of this study, you can contact the Research Governance Office at the University (02380 595058, rgoinfo@soton.ac.uk) who will be happy to help or discuss your concerns.

Where can I get more information?

Thank you for taking the time to read this information sheet. If you or your son or daughter would like to ask us further questions before making a decision about taking part, please feel free to contact us by e-mail or phone (Karen: k.gonzalez@soton.ac.uk - you can also call Areti or Karen on 07979 072331 or 02380 596652).

Appendix B Diagnostic interviews to assess for psychiatric disorders

B.1 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

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?

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?

C	P	No information
0	0	Not at all or less than once a week.
1	1	Subthreshold: Often experiences dysphoric mood at least 3 times a week for more than 3 hours each time.
2	2	
3	3	Threshold: Feels "depressed" most of the day more days than not.

(II) Irritability and anger

	C	P	
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?* *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?*	0	0	No information
	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.
	3	3	Threshold: Feels irritable/angry daily, or almost daily, at least 50% of awake time. Or often shouts, loses temper.

DMDD

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

C	P
0	0
1	1
2	2
3	3

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

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?

C	P	
		No information
0	0	Not present
1	1	Subthreshold: Several activities definitely less pleasurable or interesting. Or bored or apathetic at least 3 times a week during activities.
2	2	
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.

(IV) Recurrent thoughts of death

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

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".

(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	
0	0	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?

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
1	
2	
3	

(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
		Subthreshold: Racing thoughts cause minor distress or impairment.
		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 delusional
3	3	Threshold: Definite 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

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?

Did you try to avoid___? Were there times you could___? If someone was with you, could you___?

C	P	
0	0	No information
1	1	Not present
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

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.

C	P	
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

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.

C	P	
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

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?

C	P	
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	
		No information
1	1	Not present
		Subthreshold: Frequently nervous/anxious (more than 1 time per week), somewhat of a problem.
		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 Not present
1	1	Subthreshold: Only mildly inflexible, or inflexibility not evident in early childhood. 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

Eye to Eye Gaze: Do you frequently have to remind your child to look at you or the person s/he is talking to?
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?
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

C	P	
0	0	No information
1	1	Not present
2	2	Subthreshold: Subtle problems in one or more area, which is evident to family members and professionals but not to teachers or classmates.
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
a. Ever smoked 0 1 2
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
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?

C	P	
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

B.2 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 0 1 2

Did you do some of the things we talked about with your friends and others on your own?

(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.

B.3 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

B.4 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	
		No information.
0	0	Not present.
		Subthreshold: Occasionally has difficulty waiting his/her turn. Problem has only minimal effect on functioning.
		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	
		No information.
0	0	Not present.
		Subthreshold: Occasionally interrupts others.
1	1	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.
		Not present.
		Subthreshold: Occasionally engages in activities that are physically dangerous.
		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 C Questionnaires

C.1 Youth Psychopathic traits 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.	❑	❑	❑	❑

C.2 Reactive Proactive Questionnaire

ID:_____ Date Of Birth:_____Today's Date:_____

Instructions: There are times when most of us feel angry, or have done things we should not have done. Please rate each of the items below by putting a circle around 0 (never), 1 (sometimes), or 2 (often). Do not spend a lot of time thinking about the items—just give your first response. Make sure you answer all the items (see below).

How often have you. . .	Never	Sometimes	Often
1. Shouted at others when they have annoyed you	0	1	2
2. Had fights with others to show them who was the boss	0	1	2
3. Reacted angrily when provoked by others	0	1	2
4. Taken things from other people	0	1	2
5. Gotten angry when frustrated	0	1	2
6. Vandalised something for fun	0	1	2
7. Had temper tantrums	0	1	2
8. Damaged things because you felt angry	0	1	2
9. Had a group fight to be cool	0	1	2
10. Hurt others to win a game	0	1	2
11. Become angry or mad when you don't get your way	0	1	2
12. Used physical force to get others to do what you want	0	1	2
13. Gotten angry or mad when you lost a game	0	1	2
14. Gotten angry when others threatened you	0	1	2
15. Used force to obtain money or things from others	0	1	2
16. Felt better after hitting or yelling at someone	0	1	2
17. Threatened and bullied someone	0	1	2
18. Made obscene phone calls for fun	0	1	2
19. Hit others to defend yourself	0	1	2
20. Gotten others to gang up on someone	0	1	2
21. Carried a weapon to use in a fight	0	1	2
22. Gotten angry or mad or hit others when teased	0	1	2
23. Yelled at others so they would do things for you	0	1	2

C.3 Pubertal Developmental Scale (males)

Date completed: _____

ID_____

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

C.4 Pubertal Developmental Scale (females)

Date completed: _____

ID :_____

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

C.5 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.

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.)

C.6 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) ……

Appendix D Supplementary tables of Chapter 4

D.1 Supplementary Table S4.3: Effects of diagnosis on Cortical Volume

Brain Region	Hemisphere	Healthy Controls	Conduct disorder	Statistics			
		Mean±SD N=159	Mean±SD N=156	T	P	Corrected p value	*Cohen's d*
Transverse frontopolar gyri and sulci	Left	2007.6855±442.5568	1968.1538±389.1654	-1.37	0.17	0.64	0.09
Anterior part of the cingulate gyrus and sulcus (ACC)		5387.4214±883.9551	5309.0449±782.0003	-1.93	0.05	0.63	0.09
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		3190.7547±586.2894	3117.1795±635.9166	-0.52	0.6	0.94	0.12
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		2796.8994±429.4498	2778±411.2955	0.06	0.95	0.95	0.04
Posterior-dorsal part of the cingulate gyrus (dPCC)		1664.8491±429.6133	1615.0705±365.9169	-1.69	0.09	0.64	0.12
Posterior-ventral part of the cingulate gyrus (vPCC)		649.1069±200.4324	642.2885±201.0527	-0.47	0.64	0.94	0.03
Opercular part of the inferior frontal gyrus		4216.0566±731.8734	4125.6346±690.9536	-1.33	0.18	0.64	0.13
Orbital part of the inferior frontal gyrus		982.717±249.3694	963.1667±236.1953	-0.2	0.84	0.95	0.08
Triangular part of the inferior frontal gyrus		3262.5912±715.5983	3221.8269±678.3844	-0.83	0.41	0.72	0.06
Middle frontal gyrus		12752.9245±2455.7391	12727.7436±2331.9946	-0.83	0.41	0.72	0.01
Superior frontal gyrus		20178.7044±2521.8956	19891.8526±2630.6045	-0.87	0.39	0.72	0.11
Long insular gyrus and central sulcus of the insula		1251.5472±257.7095	1198.7372±243.6916	-1.89	0.06	0.63	0.21
Short insular gyri		2352.1824±450.9393	2329.8397±379.0441	-0.43	0.67	0.94	0.05
Parahippocampal gyrus		3633.9119±757.9481	3545.0897±705.2795	-1.11	0.27	0.72	0.12
Anterior segment of the circular sulcus of the insula		1014.5849±203.7142	1013.6667±219.7019	0.14	0.89	0.95	<0.01
Inferior segment of the circular sulcus of the insula		2452.956±408.4993	2472.1667±432.861	0.91	0.36	0.72	0.05
Superior segment of the circular sulcus of the insula		2979.3082±455.7692	2944.2756±415.0483	-1	0.32	0.72	0.08
Inferior frontal sulcus		4128.2704±908.6586	4029.3526±806.3643	-1.36	0.17	0.64	0.12
Middle frontal sulcus		2824.9497±681.9297	2831.8013±727.271	0.07	0.95	0.95	0.01
Superior frontal sulcus		5487.9434±1013.7356	5388.8141±1003.1087	0.23	0.82	0.95	0.1
Ventromedial PFC		14474.1572±2037.5054	14370.7244±1979.8777	-0.31	0.76	0.95	0.05

Supplementary Table S4.3: Effects of diagnosis on Cortical Volume continued

Brain Region	Hemisphere	Healthy Controls	Conduct disorder	Statistics			
		Mean±SD N=159	Mean±SD N=156	T	P	Corrected p value	*Cohen's d*
Transverse frontopolar gyri and sulci	Right	3020.1258±579.4024	2987.9808±569.7304	-1.12	0.26	0.51	0.06
Anterior part of the cingulate gyrus and sulcus (ACC)		6133.9748±950.7386	6029±875.1541	-1.11	0.27	0.51	0.11
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		3546.9497±619.8341	3420.0192±568.8965	-2.01	0.04	0.24	0.21
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		3169.805±532.8292	3034.25±512.032	-2.65	0.01	0.09	0.26
Posterior-dorsal part of the cingulate gyrus (dPCC)		1563.8176±352.9836	1531.7436±354.4252	-0.88	0.38	0.63	0.09
Posterior-ventral part of the cingulate gyrus (vPCC)		731.1258±208.6674	729.8333±193.0682	-0.13	0.9	0.93	0.01
Opercular part of the inferior frontal gyrus		3744.8176±698.5251	3705.75±712.525	-1.13	0.26	0.51	0.06
Orbital part of the inferior frontal gyrus		1090.2075±238.6488	1072.609±251.6199	-0.79	0.43	0.64	0.07
Triangular part of the inferior frontal gyrus		3203.805±740.6858	3222.0449±756.0975	0.21	0.84	0.92	-0.02
Middle frontal gyrus		11761.3333±2194.0134	11556.7949±2143.7958	-1.33	0.18	0.51	0.09
Superior frontal gyrus		19628.7044±2552.5319	18965.2628±2483.8038	-3.47	<0.01	0.01	0.26
Long insular gyrus and central sulcus of the insula		1351.7484±310.3558	1311.3205±258.894	-0.86	0.39	0.63	0.14
Short insular gyri		2118.7736±417.1853	2120.2949±352.3593	0.57	0.57	0.73	0
Parahippocampal gyrus		4039.8742±854.7572	3838.9231±739.0426	-1.19	0.23	0.51	0.25
Anterior segment of the circular sulcus of the insula		1172.1698±221.0362	1164.4679±227.744	-1.39	0.16	0.51	0.03
Inferior segment of the circular sulcus of the insula		2089.7862±398.6662	2087.2628±397.4033	0.08	0.93	0.93	0.01
Superior segment of the circular sulcus of the insula		2370.717±430.6177	2377.609±389.2199	-0.54	0.59	0.73	-0.02
Inferior frontal sulcus		3660.7358±747.1034	3679.2885±763.5605	-0.73	0.47	0.65	-0.02
Middle frontal sulcus		3729.1321±784.9737	3766.609±748.1068	-0.4	0.69	0.8	-0.05
Superior frontal sulcus		5025.9623±971.4593	4836.4936±864.1912	-2.3	0.02	0.16	0.21
Ventromedial PFC		14343±2000.2497	14168.8205±1909.718	-1.7	0.09	0.38	0.09

D.2 Supplementary table S4.4: Effects of diagnosis on surface area

Brain Region	Hemisphere	Healthy Controls	Conduct disorder	Statistics			
		Mean±SD N=159	Mean±SD N=156	T	P	Corrected p value	*Cohen's d*
Transverse frontopolar gyri and sulci	Left	510.5849±113.1106	502.0385±91.9756	-1.56	0.12	0.68	0.08
Anterior part of the cingulate gyrus and sulcus (ACC)		1679.3459±284.193	1645.3333±274.7583	-1.61	0.11	0.68	0.12
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		1019±178.2508	991.5±191.1297	-0.89	0.37	0.83	0.15
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		953.9874±133.6671	950.5641±131.1072	-0.05	0.96	0.98	0.03
Posterior-dorsal part of the cingulate gyrus (dPCC)		416.2075±103.5718	407.0321±88.112	-1.34	0.18	0.68	0.1
Posterior-ventral part of the cingulate gyrus (vPCC)		215.4654±62.3103	216.5641±72.4555	0.08	0.94	0.98	-0.02
Opercular part of the inferior frontal gyrus		1036.8742±167.9489	1013.2949±165.4435	-1.19	0.24	0.71	0.14
Orbital part of the inferior frontal gyrus		223.3145±51.2253	214.0577±52.8138	-0.71	0.48	0.83	0.18
Triangular part of the inferior frontal gyrus		812.8931±170.3007	802.3333±165.9806	-0.72	0.47	0.83	0.06
Middle frontal gyrus		3276.8994±606.9091	3246.5±576.6688	-0.61	0.54	0.83	0.05
Superior frontal gyrus		5107.3585±636.7277	5037.6731±670.5237	-0.35	0.72	0.98	0.11
Long insular gyrus and central sulcus of the insula		314.805±56.1441	301.6346±56.0384	-1.47	0.14	0.68	0.23
Short insular gyri		471.6038±92.0531	454.6218±75.8056	-1.3	0.19	0.68	0.2
Parahippocampal gyrus		835.2075±144.1681	827.9038±170.6057	-1.61	0.11	0.68	0.05
Anterior segment of the circular sulcus of the insula		371.3962±72.0198	368.2372±73.0953	0.03	0.98	0.98	0.04
Inferior segment of the circular sulcus of the insula		947.9434±122.9388	946.9038±120.8277	0.24	0.81	0.98	0.01
Superior segment of the circular sulcus of the insula		1259.9623±165.4687	1246.4167±154.0015	-0.98	0.33	0.83	0.08
Inferior frontal sulcus		1710.6352±324.1743	1685.4167±292.0763	-0.75	0.45	0.83	0.08
Middle frontal sulcus		1149.9937±253.758	1153.5128±262.0674	0.24	0.81	0.98	-0.01
Superior frontal sulcus		2133.1761±378.0679	2094.6795±349.5488	0.21	0.84	0.98	0.11
Ventromedial PFC		4621.5157±632.6946	4560.359±598.2308	-0.59	0.55	0.83	0.1
Transverse frontopolar gyri and sulci	Right	825.4654±150.9328	819.141±158.7069	-1.18	0.24	0.84	0.04
Anterior part of the cingulate gyrus and sulcus (ACC)		1961.7484±282.0219	1940.3846±297.8604	-0.53	0.6	0.88	0.07
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		1108.5031±180.7072	1071.9615±172.1454	-1.92	0.06	0.39	0.21
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		1060.5849±165.6439	1030.1346±157.9913	-2.05	0.04	0.39	0.19
Posterior-dorsal part of the cingulate gyrus (dPCC)		387.3836±86.7555	382.4231±82.9565	-0.78	0.44	0.88	0.06
Posterior-ventral part of the cingulate gyrus (vPCC)		191.7547±49.6606	190.9487±43.7649	-0.09	0.93	0.97	0.02
Opercular part of the inferior frontal gyrus		916.327±165.4383	916.5962±170.0522	-0.63	0.53	0.88	0
Orbital part of the inferior frontal gyrus		254.8868±54.08	254.1538±58.5044	-0.23	0.82	0.95	0.01

Supplementary table S4.4: Effects of diagnosis on surface area continued

Brain Region	Hemisphere	Healthy Controls	Conduct disorder	Statistics			
		Mean±SD N=159	Mean±SD N=156	T	P	Corrected p value	*Cohen's d*
Triangular part of the inferior frontal gyrus	Right	805.761±182.3282	809.75±192.4465	0.39	0.7	0.92	-0.02
Middle frontal gyrus		2960.2013±514.1647	2924.0897±520.4325	-0.48	0.63	0.88	0.07
Superior frontal gyrus		4889.9811±616.934	4726.859±614.7893	-3.04	0.003	0.05	0.26
Long insular gyrus and central sulcus of the insula		348.2453±72.5236	330.5±62.2081	-1.79	0.07	0.39	0.26
Short insular gyri		424.8239±97.3712	416.5±73.4326	-0.28	0.78	0.95	0.1
Parahippocampal gyrus		951.3962±186.593	917.891±176.0277	-0.5	0.62	0.88	0.18
Anterior segment of the circular sulcus of the insula		427.4277±76.1112	426.2372±87.3584	-0.94	0.35	0.88	0.01
Inferior segment of the circular sulcus of the insula		828.044±128.1541	826.0513±118.3265	-0.14	0.89	0.97	0.02
Superior segment of the circular sulcus of the insula		988.0566±148.2795	986.6346±142.0862	-0.7	0.49	0.88	0.01
Inferior frontal sulcus		1547.0943±272.9808	1550.2051±278.1854	-0.65	0.52	0.88	-0.01
Middle frontal sulcus		1554.4654±289.3311	1592.0833±300.209	-0.01	0.99	0.99	-0.13
Superior frontal sulcus		1934±333.229	1892.9487±308.8496	-1.04	0.3	0.88	0.13
Ventromedial PFC		4503.6478±610.3801	4461.7308±619.9243	-1.19	0.24	0.84	0.07

D.3 Supplementary table S4.5: Effects of diagnosis on Cortical Thickness

Brain Region	Hemisphere	Healthy Controls	Conduct disorder	Statistics			
		Mean±SD N=159	Mean±SD N=156	T	P	Corrected p value	*Cohen's d*
Transverse frontopolar gyri and sulci	Left	2.9088±0.28792	2.8867±0.25655	-0.1	0.92	0.97	0.08
Anterior part of the cingulate gyrus and sulcus (ACC)		2.9389±0.18962	2.9502±0.19999	-0.16	0.87	0.97	-0.06
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		2.9528±0.16363	2.972±0.17374	1.41	0.16	0.56	-0.11
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		2.7926±0.14881	2.7858±0.16055	-0.01	0.99	0.99	0.04
Posterior-dorsal part of the cingulate gyrus (dPCC)		3.1365±0.21086	3.1287±0.22336	-0.86	0.39	0.89	0.04
Posterior-ventral part of the cingulate gyrus (vPCC)		2.5894±0.28887	2.5648±0.31545	-1.04	0.3	0.79	0.08
Opercular part of the inferior frontal gyrus		3.0259±0.20617	3.0336±0.18183	-0.1	0.92	0.97	-0.04
Orbital part of the inferior frontal gyrus		3.0829±0.2941	3.1401±0.26857	1.72	0.09	0.56	-0.2
Triangular part of the inferior frontal gyrus		2.9501±0.19825	2.9526±0.20395	-0.47	0.64	0.97	-0.01
Middle frontal gyrus		2.896±0.18174	2.9106±0.18678	-0.21	0.83	0.97	-0.08
Superior frontal gyrus		3.1391±0.1818	3.1414±0.19403	-0.24	0.81	0.97	-0.01
Long insular gyrus and central sulcus of the insula		3.3876±0.31874	3.4214±0.28395	0.21	0.83	0.97	-0.11
Short insular gyri		3.7321±0.29523	3.7877±0.26286	1.43	0.15	0.56	-0.2
Parahippocampal gyrus		3.1783±0.31508	3.1572±0.28612	1.08	0.28	0.79	0.07
Anterior segment of the circular sulcus of the insula		3.0462±0.22639	3.0288±0.23645	-1.57	0.12	0.56	0.08
Inferior segment of the circular sulcus of the insula		2.9637±0.24195	3.0007±0.23369	1.96	0.05	0.56	-0.16
Superior segment of the circular sulcus of the insula		2.7549±0.16813	2.7412±0.16926	-0.45	0.65	0.97	0.08
Inferior frontal sulcus		2.4531±0.1578	2.4331±0.16738	-1.61	0.11	0.56	0.12
Middle frontal sulcus		2.4213±0.19155	2.4179±0.20148	-0.73	0.47	0.89	0.02
Superior frontal sulcus		2.5952±0.15289	2.589±0.16664	-0.75	0.46	0.89	0.04
Ventromedial PFC		5.4872±0.30009	5.5068±0.34537	0.45	0.65	0.97	-0.06
Transverse frontopolar gyri and sulci	Right	2.7698±0.22946	2.7603±0.23805	-0.24	0.81	0.95	0.04
Anterior part of the cingulate gyrus and sulcus (ACC)		2.8694±0.20542	2.8483±0.20555	-1.4	0.16	0.48	0.1
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		2.9664±0.1585	2.9558±0.17501	-0.71	0.48	0.84	0.06
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		2.8043±0.14086	2.7767±0.16326	-2.14	0.03	0.28	0.18
Posterior-dorsal part of the cingulate gyrus (dPCC)		3.1234±0.202	3.102±0.21494	-0.12	0.9	0.95	0.1
Posterior-ventral part of the cingulate gyrus (vPCC)		2.9247±0.32466	2.9195±0.29721	-0.26	0.8	0.95	0.02
Opercular part of the inferior frontal gyrus		3.0576±0.18868	3.0265±0.17358	-1.93	0.05	0.28	0.17
Orbital part of the inferior frontal gyrus		3.0595±0.27493	3.0421±0.30393	-0.41	0.68	0.89	0.06

Supplementary table S4.5: Effects of diagnosis on Cortical Thickness continued

Brain Region	Hemisphere	Healthy Controls	Conduct disorder	Statistics			
		Mean±SD N=159	Mean±SD N=156	T	P	Corrected p value	*Cohen's d*
Triangular part of the inferior frontal gyrus	Right	2.9346±0.2248	2.9221±0.21326	-0.99	0.32	0.68	0.06
Middle frontal gyrus		2.8943±0.16613	2.886±0.18263	-1.33	0.18	0.48	0.05
Superior frontal gyrus		3.1196±0.17806	3.1126±0.18447	-0.51	0.61	0.85	0.04
Long insular gyrus and central sulcus of the insula		3.4281±0.37373	3.5097±0.33982	1.76	0.08	0.34	-0.23
Short insular gyri		3.6588±0.30341	3.7015±0.26112	1.26	0.21	0.49	-0.15
Parahippocampal gyrus		3.2333±0.27677	3.2119±0.26172	-0.02	0.99	0.99	0.08
Anterior segment of the circular sulcus of the insula		3.0476±0.25198	3.0402±0.23738	-0.55	0.59	0.85	0.03
Inferior segment of the circular sulcus of the insula		2.9177±0.22463	2.948±0.22501	1.53	0.13	0.44	-0.13
Superior segment of the circular sulcus of the insula		2.7966±0.20179	2.818±0.16857	0.58	0.56	0.85	-0.12
Inferior frontal sulcus		2.4205±0.16368	2.4157±0.15231	-0.77	0.44	0.84	0.03
Middle frontal sulcus		2.3672±0.18123	2.3255±0.16737	-1.95	0.05	0.28	0.24
Superior frontal sulcus		2.6245±0.14817	2.5869±0.16652	-3.1	0.002	0.04	0.24
Ventromedial PFC		5.5136±0.34308	5.5035±0.35332	-0.12	0.9	0.95	0.03

D.4 Supplementary table S4.6: Effects of diagnosis on Subcortical Volume

Brain Region	Hemisphere	Healthy Controls	CD	Statistics			
		Mean±SD N=159	Mean±SD N=156	T	P	Corrected p value	Cohen's *d*
Accumbens	Left	561.5377±132.4835	572.691±144.9638	1.99	0.05	0.39	-0.1
Amygdala		1581.0698±195.8952	1551.4141±231.8287	-1.16	0.25	0.58	0.1
Caudate		4060.7503±640.0787	3964.9641±664.9739	0.68	0.5	0.78	0.1
Hippocampus		4298.7189±492.6977	4248.241±597.0613	0.42	0.67	0.85	0.1
Pallidum		1875.5881±403.2319	1897.9449±453.1808	1.91	0.06	0.39	-0.1
Putamen		5913.4421±797.0701	5890.609±840.3463	0.11	0.91	0.93	0
Thalamus		8801.4717±1566.697	8762.9045±1610.1345	-0.76	0.45	0.78	0
Accumbens	Right	618.0145±113.9552	599.7064±112.0458	-1.28	0.2	0.58	0.2
Amygdala		1585.5918±254.1187	1563.1744±252.9977	-1.43	0.15	0.58	0.1
Caudate		4088.4019±591.2555	3972.5885±656.2242	-0.14	0.89	0.93	0.2
Hippocampus		4312.6899±487.643	4240.1288±449.2148	-1.87	0.06	0.39	0.2
Pallidum		1681.6283±281.467	1692.3859±325.0563	1.18	0.24	0.58	-0.04
Putamen		5771.0421±733.6677	5763.6449±770.4226	0.21	0.84	0.93	0.01
Thalamus		8065.7057±1421.5061	8038.9859±1425.9403	-0.54	0.59	0.8	0.02

D.5 Supplementary table S4.7: Effects of sex on Cortical Volume

Brain Region	Hemisphere	Males Mean±SD N=159	Females Mean±SD N=156	Statistics			
				T	P	Corrected p value	*Cohen's d*
Transverse frontopolar gyri and sulci	Left	2064.7692±422.4472	1912.8931±398.2972	-1.37	0.17	0.36	0.37
Anterior part of the cingulate gyrus and sulcus (ACC)		5605.9423±896.1754	5096.1258±683.3278	-2.17	0.03	0.23	0.64
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		3272.5449±632.9541	3038.3208±568.1369	0.43	0.66	0.8	0.39
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		2879.7756±442.9426	2697.044±376.0894	0.45	0.65	0.8	0.44
Posterior-dorsal part of the cingulate gyrus (dPCC)		1746.1603±438.5428	1536.2327±326.3804	-1.22	0.22	0.43	0.54
Posterior-ventral part of the cingulate gyrus (vPCC)		714.9679±200.0337	577.7987±176.6924	-2.52	0.01	0.23	0.73
Opercular part of the inferior frontal gyrus		4270.6538±787.1109	4073.7736±617.2257	0.13	0.9	0.9	0.28
Orbital part of the inferior frontal gyrus		1022.8846±254.3976	924.1258±220.8041	-0.9	0.37	0.51	0.41
Triangular part of the inferior frontal gyrus		3374.641±775.5131	3112.6604±583.2409	-0.97	0.33	0.51	0.38
Middle frontal gyrus		13387.75±2495.56	12105.3711±2106.3644	-1.37	0.17	0.36	0.56
Superior frontal gyrus		20917.4872±2775.0748	19172.4214±2029.0793	-0.98	0.33	0.51	0.72
Long insular gyrus and central sulcus of the insula		1280.391±263.1407	1171.434±228.5111	-1.68	0.09	0.25	0.44
Short insular gyri		2456.3205±461.7396	2228.0881±330.7958	-2.02	0.04	0.23	0.57
Parahippocampal gyrus		3657.8141±798.6636	3523.3145±656.9809	-0.3	0.76	0.8	0.18
Anterior segment of the circular sulcus of the insula		1078.2436±220.5013	951.2264±181.9731	-1.96	0.05	0.23	0.63
Inferior segment of the circular sulcus of the insula		2542.4872±467.009	2383.9623±352.6841	0.39	0.7	0.8	0.38
Superior segment of the circular sulcus of the insula		3102.0897±458.4385	2824.4717±364.3427	-1.85	0.07	0.23	0.67
Inferior frontal sulcus		4285.9487±982.4198	3876.5157±661.8507	-1.71	0.09	0.25	0.49
Middle frontal sulcus		2956.1603±725.0688	2702.9371±660.479	-0.93	0.35	0.51	0.37
Superior frontal sulcus		5713.9231±1099.1522	5168.9686±828.5397	0.31	0.76	0.8	0.56
Ventromedial PFC		15015.6923±2170.6498	13841.3585±1640.946	-1.9	0.06	0.23	0.61
Transverse frontopolar gyri and sulci	Right	3123.391±567.3807	2887.2704±557.7253	-2.069	0.04	0.11	0.42
Anterior part of the cingulate gyrus and sulcus (ACC)		6444.0449±951.665	5726.761±718.2391	-3.086	0.002	0.02	0.85
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		3642.2692±665.5711	3328.8931±475.6108	-1.022	0.31	0.38	0.54
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		3259.0705±571.1413	2949.2264±427.094	-1.742	0.08	0.16	0.61
Posterior-dorsal part of the cingulate gyrus (dPCC)		1644.0513±368.4612	1453.6289±311.5847	-0.576	0.56	0.56	0.56
Posterior-ventral part of the cingulate gyrus (vPCC)		772.4423±224.1787	689.3208±165.4245	-1.403	0.16	0.24	0.42
Opercular part of the inferior frontal gyrus		3845.9679±780.0821	3607.2453±601.1446	-0.933	0.35	0.41	0.34
Orbital part of the inferior frontal gyrus		1136.4167±236.5163	1027.6038±241.7423	-1.45	0.15	0.24	0.46

Supplementary table S4.7: Effects of sex on Cortical Volume continued

Brain Region	Hemisphere	Males Mean±SD N=159	Females Mean±SD N=156	Statistics			
				T	P	Corrected p value	*Cohen's d*
Triangular part of the inferior frontal gyrus	Right	3415.9231±812.6553	3013.5849±617.3057	-1.98	0.05	0.11	0.56
Middle frontal gyrus		12234.4423±2365.1535	11096.4717±1791.654	-1.232	0.22	0.31	0.54
Superior frontal gyrus		20157.391±2642.5855	18459.0692±2121.0385	-2.597	0.01	0.05	0.71
Long insular gyrus and central sulcus of the insula		1390.4423±302.3579	1274.1195±257.7998	-1.193	0.23	0.31	0.41
Short insular gyri		2237.7244±420.7805	2003.5597±307.9331	-1.975	0.05	0.11	0.64
Parahippocampal gyrus		4058.1346±837.2412	3824.7987±756.142	-0.806	0.42	0.47	0.29
Anterior segment of the circular sulcus of the insula		1225.1154±232.8015	1112.6667±200.7482	-2.118	0.03	0.11	0.52
Inferior segment of the circular sulcus of the insula		2174.7179±431.3704	2003.9811±341.7503	-0.688	0.49	0.52	0.44
Superior segment of the circular sulcus of the insula		2526.4359±400.9312	2224.6981±361.9428	-2.686	0.008	0.05	0.79
Inferior frontal sulcus		3860.3654±845.8711	3483.0755±597.8585	-2.425	0.02	0.07	0.52
Middle frontal sulcus		3915.9872±759.2672	3582.5723±738.2265	-1.732	0.08	0.16	0.45
Superior frontal sulcus		5154.1346±968.9647	4714.3145±822.6777	-1.477	0.14	0.24	0.49
Ventromedial PFC		14846.4487±2095.569	13678.1572±1614.1979	-3.308	0.001	0.02	0.62

D.6 Supplementary table S4.8: Effects of sex on Surface Area

Brain Region	Hemisphere	Males Mean±SD N=159	Females Mean±SD N=156	Statistics			
				T	P	Corrected p value	*Cohen's d*
Transverse frontopolar gyri and sulci	Left	523.5769±104.0802	489.4528±99.609	-1.48	0.14	0.21	0.33
Anterior part of the cingulate gyrus and sulcus (ACC)		1735.0705±298.8422	1591.3019±239.7684	-0.91	0.36	0.5	0.53
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		1039.3462±187.7161	972.0566±176.5286	0.48	0.63	0.52	0.37
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		979.4679±138.6219	925.6289±120.1652	0.49	0.62	0.52	0.42
Posterior-dorsal part of the cingulate gyrus (dPCC)		436.2821±102.8723	387.5094±82.5718	-1.24	0.22	0.21	0.52
Posterior-ventral part of the cingulate gyrus (vPCC)		233.7628±60.1568	198.5912±69.7615	-0.92	0.36	0.5	0.54
Opercular part of the inferior frontal gyrus		1052.4487±178.8735	998.4591±149.9828	0.26	0.79	0.61	0.33
Orbital part of the inferior frontal gyrus		228.2436±56.9846	209.3962±45.1713	-0.41	0.68	0.6	0.37
Triangular part of the inferior frontal gyrus		840.5064±188.5528	775.4403±138.1931	-1.09	0.28	0.21	0.39
Middle frontal gyrus		3435.0449±603.0926	3091.9119±528.7144	-0.96	0.34	0.21	0.61
Superior frontal gyrus		5309.3782±670.7115	4840.7799±546.0406	-1.13	0.26	0.21	0.77
Long insular gyrus and central sulcus of the insula		324.2949±58.6153	292.5723±49.4695	-1.35	0.18	0.21	0.58
Short insular gyri		488.7436±92.0566	438.1258±68.2969	-1.57	0.12	0.13	0.62
Parahippocampal gyrus		851.0769±158.9698	812.4717±154.3709	-1.05	0.29	0.21	0.25
Anterior segment of the circular sulcus of the insula		390.1026±73.4855	349.9434±65.8176	-1.36	0.17	0.21	0.58
Inferior segment of the circular sulcus of the insula		967.5±131.5276	927.7358±108.0628	0.86	0.39	0.5	0.33
Superior segment of the circular sulcus of the insula		1302.8269±160.9922	1204.6164±143.1928	-1.86	0.06	0.13	0.64
Inferior frontal sulcus		1756.2756±352.9051	1641.1132±245.5765	-0.59	0.55	0.5	0.38
Middle frontal sulcus		1207.8333±266.7807	1096.6981±236.2172	-0.93	0.35	0.32	0.44
Superior frontal sulcus		2214.8526±404.0668	2015.2704±288.9925	0.25	0.8	0.61	0.57
Ventromedial PFC		4730.9359±651.8816	4454.1572±546.0696	-0.44	0.66	0.55	0.46
Transverse frontopolar gyri and sulci	Right	850.1154±151.1705	795.0755±153.5639	-1.7	0.09	0.21	0.36
Anterior part of the cingulate gyrus and sulcus (ACC)		2047.1218±300.9879	1857.0252±244.7794	-1.39	0.17	0.32	0.69
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		1137.1218±194.4341	1044.5723±145.0392	-0.92	0.36	0.5	0.54
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		1087.2308±169.9309	1004.566±143.7133	-1.06	0.29	0.5	0.53
Posterior-dorsal part of the cingulate gyrus (dPCC)		408.0769±88.1114	362.2138±75.0026	-0.93	0.35	0.5	0.56
Posterior-ventral part of the cingulate gyrus (vPCC)		200.2308±53.9312	182.6478±36.5796	-0.81	0.42	0.52	0.38
Opercular part of the inferior frontal gyrus		941.9359±184.0119	891.4654±145.7792	-0.5	0.61	0.61	0.3
Orbital part of the inferior frontal gyrus		268.8526±53.7436	240.4654±55.2047	-1.84	0.07	0.21	0.52

Supplementary table S4.8: Effects of sex on Surface Area continued

Brain Region	Hemisphere	Males Mean±SD N=159	Females Mean±SD N=156	Statistics			
				T	P	Corrected p value	*Cohen's d*
Triangular part of the inferior frontal gyrus	Right	858.9359±202.3148	757.5031±155.8921	-1.82	0.07	0.21	0.56
Middle frontal gyrus		3090.6859±551.0367	2796.7484±435.6459	-0.61	0.54	0.6	0.59
Superior frontal gyrus		5030.3141±618.0901	4592.2516±542.4384	-2.56	0.01	0.13	0.75
Long insular gyrus and central sulcus of the insula		355.5385±70.5423	323.6792±61.8498	-1	0.32	0.5	0.48
Short insular gyri		448.5705±98.5238	393.3585±61.3496	-1.76	0.08	0.21	0.67
Parahippocampal gyrus		975.1026±192.6099	895.2642±161.8732	-0.84	0.4	0.52	0.45
Anterior segment of the circular sulcus of the insula		447.9038±88.4651	406.1698±68.8091	-1.8	0.07	0.21	0.53
Inferior segment of the circular sulcus of the insula		850.3141±132.4128	804.239±109.0988	-0.72	0.47	0.55	0.36
Superior segment of the circular sulcus of the insula		1038±140.7202	937.6604±131.7162	-2.51	0.01	0.13	0.69
Inferior frontal sulcus		1610.9551±302.4534	1487.4906±230.4409	-1.64	0.1	0.21	0.54
Middle frontal sulcus		1641.9103±292.1725	1505.5786±282.5334	-1.78	0.08	0.21	0.53
Superior frontal sulcus		1987.5±340.4525	1841.2327±284.7613	-0.54	0.59	0.61	0.56
Ventromedial PFC		4651.9167±653.1066	4317.0503±525.4657	-1.97	0.05	0.21	0.38

D.7 Supplementary table S4.9: Effects of sex on Cortical Thickness

Brain Region	Hemisphere	Males Mean±SD N=159	Females Mean±SD N=156	Statistics			
				T	P	Corrected p value	*Cohen's d*
Transverse frontopolar gyri and sulci	Left	2.9206±0.27951	2.8755±0.26467	-0.14	0.89	0.93	0.17
Anterior part of the cingulate gyrus and sulcus (ACC)		2.9613±0.2005	2.9281±0.18779	-2.28	0.02	0.13	0.17
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		2.9784±0.18501	2.9465±0.14995	-1.1	0.27	0.63	0.19
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		2.8104±0.14944	2.7684±0.15704	-0.92	0.36	0.68	0.27
Posterior-dorsal part of the cingulate gyrus (dPCC)		3.1479±0.23415	3.1177±0.19797	-0.42	0.68	0.89	0.14
Posterior-ventral part of the cingulate gyrus (vPCC)		2.6167±0.3261	2.5386±0.27203	-2.69	0.007	0.08	0.26
Opercular part of the inferior frontal gyrus		3.0348±0.19778	3.0247±0.19117	-0.44	0.66	0.89	0.05
Orbital part of the inferior frontal gyrus		3.1317±0.2741	3.0911±0.29044	-0.03	0.97	0.97	0.14
Triangular part of the inferior frontal gyrus		2.9692±0.20701	2.9338±0.1935	-0.93	0.35	0.68	0.18
Middle frontal gyrus		2.9019±0.19847	2.9046±0.16945	-0.35	0.72	0.89	-0.01
Superior frontal gyrus		3.1384±0.18822	3.142±0.1877	0.24	0.81	0.89	-0.02
Long insular gyrus and central sulcus of the insula		3.3844±0.33125	3.424±0.26988	-0.3	0.76	0.89	-0.13
Short insular gyri		3.7439±0.29636	3.775±0.26427	0.45	0.66	0.89	-0.11
Parahippocampal gyrus		3.1509±0.29861	3.1845±0.30293	1.64	0.1	0.31	-0.11
Anterior segment of the circular sulcus of the insula		3.0645±0.25228	3.0113±0.20592	-2.25	0.03	0.13	0.23
Inferior segment of the circular sulcus of the insula		3.0051±0.25765	2.9594±0.21593	0.48	0.63	0.89	0.19
Superior segment of the circular sulcus of the insula		2.7749±0.184	2.7218±0.14781	-0.31	0.75	0.89	0.32
Inferior frontal sulcus		2.4844±0.17578	2.4027±0.13764	-3.22	0.001	0.03	0.52
Middle frontal sulcus		2.446±0.20336	2.3938±0.18599	-1.7	0.09	0.31	0.27
Superior frontal sulcus		2.6134±0.17925	2.5713±0.13502	-1.33	0.18	0.48	0.27
Ventromedial PFC		5.5467±0.33724	5.4481±0.3014	-1.96	0.05	0.21	0.31
Transverse frontopolar gyri and sulci	Right	2.7903±0.23751	2.7403±0.22739	-1.15	0.25	0.4	0.22
Anterior part of the cingulate gyrus and sulcus (ACC)		2.8947±0.21549	2.8238±0.18921	-3.478	0.0006	0.01	0.35
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		2.9778±0.17514	2.9448±0.15682	-1.22	0.22	0.4	0.2
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		2.8174±0.15808	2.7643±0.14301	-2.19	0.03	0.1	0.35
Posterior-dorsal part of the cingulate gyrus (dPCC)		3.1346±0.23454	3.0914±0.17736	0.12	0.9	0.96	0.21
Posterior-ventral part of the cingulate gyrus (vPCC)		2.9533±0.30628	2.8914±0.31327	-1.32	0.19	0.4	0.2
Opercular part of the inferior frontal gyrus		3.0581±0.18122	3.0266±0.18146	-1.58	0.12	0.27	0.17
Orbital part of the inferior frontal gyrus		3.0265±0.26445	3.0748±0.31079	1.03	0.3	0.43	-0.17
Triangular part of the inferior frontal gyrus		2.9416±0.2328	2.9155±0.20425	-1.2	0.23	0.4	0.12

Supplementary table S4.9: Effects of sex on Cortical Thickness continued

Brain Region	Hemisphere	Males Mean±SD N=159	Females Mean±SD N=156	Statistics			
				T	P	Corrected p value	*Cohen's d*
Middle frontal gyrus	Right	2.8902±0.18257	2.8902±0.16629	-1.06	0.29	0.43	0
Superior frontal gyrus		3.1201±0.18642	3.1122±0.17605	-0.19	0.85	0.96	0.04
Long insular gyrus and central sulcus of the insula		3.4612±0.37977	3.4756±0.33867	0.01	0.99	0.99	-0.04
Short insular gyri		3.6744±0.28623	3.6855±0.28182	-0.1	0.92	0.96	-0.04
Parahippocampal gyrus		3.1999±0.27435	3.2452±0.26299	0.25	0.8	0.96	-0.17
Anterior segment of the circular sulcus of the insula		3.0644±0.26567	3.0239±0.22079	-1.69	0.09	0.24	0.17
Inferior segment of the circular sulcus of the insula		2.9717±0.22762	2.8945±0.21631	-0.45	0.65	0.85	0.35
Superior segment of the circular sulcus of the insula		2.8503±0.18719	2.7649±0.17554	-1.79	0.07	0.22	0.47
Inferior frontal sulcus		2.4428±0.16274	2.394±0.14965	-2.35	0.02	0.1	0.31
Middle frontal sulcus		2.3785±0.17976	2.3153±0.16585	-2.22	0.03	0.1	0.37
Superior frontal sulcus		2.6276±0.16395	2.5846±0.15023	-2.23	0.03	0.1	0.27
Ventromedial PFC		5.5499±0.36224	5.4681±0.32888	-2.19	0.03	0.1	0.24

D.8 Supplementary table S4.10: Effects of sex on subcortical volume

Brain Region	Hemisphere	Males	Females	Statistics			
		Mean±SD N=159	Mean±SD N=156	T	P	Corrected p value	Cohen's *d*
Accumbens	Left	592.4429±141.1442	542.1585±132.0067	1.16	0.25	0.78	0.4
Amygdala		1637.3455±221.1966	1496.7597±183.5438	-1.29	0.2	0.58	0.7
Caudate		4098.0724±722.5116	3930.1535±567.3658	2.48	0.01	0.39	0.3
Hippocampus		4398.6359±583.9589	4151.1616±478.2041	0.65	0.51	0.85	0.5
Pallidum		1959.9878±441.7081	1814.7157±402.9878	-1.54	0.13	0.58	0.3
Putamen		6142.5571±887.9717	5666.2478±664.6544	-0.7	0.48	0.8	0.6
Thalamus		8948.3532±1758.3247	8619.522±1382.6413	-1.46	0.15	0.58	0.2
Accumbens	Right	643.5833±112.1161	574.9654±103.8788	-0.91	0.37	0.78	0.6
Amygdala		1658.8282±276.6955	1491.7428±196.4669	-2.16	0.03	0.39	0.7
Caudate		4107.9135±670.8754	3955.6302±570.645	1.99	0.05	0.58	0.2
Hippocampus		4394.8865±495.1478	4160.8522±413	-0.87	0.38	0.78	0.5
Pallidum		1749.7321±322.3889	1625.3642±270.6788	-0.55	0.58	0.93	0.4
Putamen		6030.1917±783.4959	5509.5245±619.0803	-2.03	0.04	0.58	0.7
Thalamus		8243.4365±1558.9886	7865.1126±1249.0414	-2.28	0.02	0.39	0.3

D.9 Supplementary table S4.11: Sex by diagnosis interactions on Cortical Volume

Brain Region	Hemisphere	Healthy Controls		Conduct disorder		Statistics			
		Males (n=79) Mean ± SD	Females (n =80) Mean ± SD	Males (n =77) Mean ± SD	Females (n=79) Mean ± SD	T	P	Corrected p value	$\eta\pi^2$
Transverse frontopolar gyri and sulci	Left	2117.7975±434.4035	1898.95±425.7905	2010.3636±405.43	1927.0127±370.575	1.32	0.19	0.83	0.006
Anterior part of the cingulate gyrus and sulcus (ACC)		5737.2785±976.4689	5041.9375±614.8669	5471.1948±789.3376	5151±746.2418	2.19	0.03	0.61	0.016
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		3314.962±603.782	3068.1±544.8581	3229.026±662.6771	3008.1646±592.7298	-0.09	0.93	0.95	<0.001
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		2888.7468±425.1787	2706.2±416.6523	2870.5714±463.0737	2687.7722±332.4385	-0.25	0.8	0.89	<0.001
Posterior-dorsal part of the cingulate gyrus (dPCC)		1803.1772±462.4635	1528.25±346.3232	1687.6623±407.2888	1544.3165±306.8725	1.44	0.15	0.83	0.007
Posterior-ventral part of the cingulate gyrus (vPCC)		727.3671±209.9994	571.825±156.9458	702.2468±189.7956	583.8481±195.4981	0.68	0.5	0.83	0.002
Opercular part of the inferior frontal gyrus		4355.5696±800.7206	4078.2875±632.1038	4183.5325±768.3303	4069.2025±605.7919	0.9	0.37	0.83	0.003
Orbital part of the inferior frontal gyrus		1031.0253±279.4473	935.0125±206.4696	1014.5325±227.3885	913.1013±235.2406	-0.3	0.76	0.89	<0.001
Triangular part of the inferior frontal gyrus		3436.4304±798.7499	3090.925±578.0778	3311.2468±750.833	3134.6709±591.2941	0.95	0.34	0.83	0.003
Middle frontal gyrus		13602.557±2668.4347	11913.9125±1896.3576	13167.3636±2301.3856	12299.2532±2295.5086	1.55	0.12	0.83	0.008
Superior frontal gyrus		21148.8228±2745.3461	19220.7125±1846.0495	20680.1429±2803.2306	19123.519±2209.862	0.42	0.67	0.89	0.001
Long insular gyrus and central sulcus of the insula		1318.8481±279.5667	1185.0875±216.0044	1240.9351±240.6044	1157.6076±241.1071	0.75	0.46	0.83	0.002
Short insular gyri		2487.1519±508.6693	2218.9±339.1422	2424.6883±408.9835	2237.3924±324.0235	0.66	0.51	0.83	0.001
Parahippocampal gyrus		3742.6076±836.157	3526.575±659.6819	3570.8182±753.7948	3520.0127±658.4327	0.85	0.4	0.83	0.002
Anterior segment of the circular sulcus of the insula		1082.0127±200.377	948±185.2566	1074.3766±240.6729	954.4937±179.7109	0.07	0.95	0.95	<0.001
Inferior segment of the circular sulcus of the insula		2533.4177±470.6652	2373.5±319.616	2551.7922±466.1281	2394.557±385.0603	-0.34	0.73	0.89	<0.001
Superior segment of the circular sulcus of the insula		3149.1266±464.2585	2811.6125±381.2182	3053.8312±450.2893	2837.4937±348.3721	1.16	0.25	0.83	0.004
Inferior frontal sulcus		4396.5823±1041.3115	3863.3125±661.5394	4172.4416±910.9085	3889.8861±666.123	1.19	0.23	0.83	0.005
Middle frontal sulcus		2971.7342±696.8015	2680±638.6296	2940.1818±757.2196	2726.1646±685.1918	0.31	0.76	0.89	<0.001
Superior frontal sulcus		5728.6456±1138.478	5250.25±812.6767	5698.8182±1064.5483	5086.6582±841.4329	-0.99	0.32	0.83	0.003
Ventromedial PFC		15140.6709±2170.6808	13815.975±1663.6456	14887.4675±2177.3113	13867.0633±1627.8577	0.44	0.66	0.89	0.001
Transverse frontopolar gyri and sulci	Right	3180.519±558.4372	2861.7375±558.909	3064.7792±574.1232	2913.1266±558.8925	1.2	0.23	0.44	0.005
Anterior part of the cingulate gyrus and sulcus (ACC)		6535.4557±1030.9709	5737.5125±661.8174	6350.2597±859.4261	5715.8734±775.3102	0.69	0.49	0.74	0.002
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		3741.7342±665.1	3354.6±506.184	3540.2208±654.6925	3302.8608±444.2297	1	0.32	0.56	0.003
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		3366.8101±556.5506	2975.2625±429.941	3148.5325±568.2745	2922.8608±425.2967	1.38	0.17	0.44	0.006
Posterior-dorsal part of the cingulate gyrus (dPCC)		1678.2025±392.2222	1450.8625±267.0198	1609.013±341.3848	1456.4304±352.7463	0.78	0.44	0.71	0.002
Posterior-ventral part of the cingulate gyrus (vPCC)		779.0633±233.6011	683.7875±169.2282	765.6494±215.4023	694.9241±162.3689	0.39	0.7	0.86	<0.001
Opercular part of the inferior frontal gyrus		3914.6329±763.3633	3577.125±585.8442	3775.5195±795.6692	3637.7468±618.4895	1.2	0.23	0.44	0.005
Orbital part of the inferior frontal gyrus		1151.0759±228.5862	1030.1±234.4198	1121.3766±244.967	1025.0759±250.4139	0.39	0.69	0.86	0.001
Triangular part of the inferior frontal gyrus		3422.7342±807.7335	2987.6125±598.1601	3408.9351±822.9129	3039.8861±638.8493	0.23	0.82	0.95	<0.001
Middle frontal gyrus		12502.2658±2365.6184	11029.6625±1735.2168	11959.6623±2348.242	11164.1266±1855.684	1.34	0.18	0.44	0.006
Superior frontal gyrus		20769.5443±2649.0774	18502.125±1874.912	19529.3377±2500.1441	18415.4684±2355.5834	2.31	0.02	0.44	0.017
Long insular gyrus and central sulcus of the insula		1414.4937±319.1642	1289.7875±290.2419	1365.7662±284.0504	1258.2532±220.9038	0.02	0.98	0.98	<0.001

Supplementary table S4.11: Sex by diagnosis interactions on Cortical Volume continued

Brain Region	Hemisphere	Healthy Controls		Conduct disorder		Statistics			
		Males (n=79) Mean ± SD	Females (n =80) Mean ± SD	Males (n =77) Mean ± SD	Females (n=79) Mean ± SD	T	P	Corrected p value	$\eta\pi^2$
Short insular gyri	Right	2243.3038±462.2823	1995.8±325.9842	2232±376.4401	2011.4177±290.3869	-0.05	0.96	0.98	<0.001
Parahippocampal gyrus		4150.3165±919.0315	3930.8125±776.5627	3963.5584±738.1563	3717.443±724.0033	-0.52	0.61	0.85	0.001
Anterior segment of the circular sulcus of the insula		1252.4684±217.9965	1092.875±194.8793	1197.0519±245.3236	1132.7089±205.8144	1.87	0.06	0.44	0.011
Inferior segment of the circular sulcus of the insula		2183.7595±398.4212	1996.9875±378.9297	2165.4416±465.2066	2011.0633±301.7477	0.12	0.9	0.98	<0.001
Superior segment of the circular sulcus of the insula		2556.1772±414.0581	2187.575±365.0044	2495.9221±387.322	2262.2911±357.1879	1.5	0.13	0.44	0.007
Inferior frontal sulcus		3911.1772±830.8511	3413.425±556.8606	3808.2338±863.3205	3553.6076±632.4168	1.42	0.16	0.44	0.007
Middle frontal sulcus		3956.4051±718.5665	3504.7±787.3406	3874.5195±801.4758	3661.4304±680.8525	1.28	0.2	0.44	0.005
Superior frontal sulcus		5330.9494±1028.2706	4724.7875±811.5653	4972.7273±873.9808	4703.7089±838.8337	1.58	0.11	0.44	0.008
Ventromedial PFC		15117.8734±2113.2444	13577.8125±1548.991	14567.974±2053.7023	13779.7722±1681.4165	1.95	0.05	0.44	0.012

D.10 Supplementary table S4.12: Sex by diagnosis interactions on Surface Area

Brain Region	Hemisphere	Healthy Controls		Conduct disorder		Statistics			
		Males (n=79) Mean ± SD	Females (n =80) Mean ± SD	Males (n =77) Mean ± SD	Females (n=79) Mean ± SD	T	P	Corrected p value	$\eta\pi^2$
Transverse frontopolar gyri and sulci	Left	539.5443±109.4934	481.9875±109.9279	507.1948±96.186	497.0127±88.0012	1.91	0.06	0.6	0.012
Anterior part of the cingulate gyrus and sulcus (ACC)		1776.3544±311.8404	1583.55±216.3657	1692.7143±280.6167	1599.1519±262.5139	1.56	0.12	0.84	0.008
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		1056.8354±183.003	981.6375±166.2389	1021.4026±191.9636	962.3544±186.9347	0.14	0.89	0.97	<0.001
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		983.3291±138.4705	925.0125±122.8467	975.5065±139.5728	926.2532±118.1694	0.09	0.93	0.97	<0.001
Posterior-dorsal part of the cingulate gyrus (dPCC)		447.9873±109.6676	384.825±87.0184	424.2727±94.6104	390.2278±78.2743	1.27	0.2	0.97	0.005
Posterior-ventral part of the cingulate gyrus (vPCC)		235.8861±68.3584	195.3±48.1938	231.5844±50.7464	201.9241±86.5062	0.51	0.61	0.97	0.001
Opercular part of the inferior frontal gyrus		1071.3418±172.7679	1002.8375±156.7851	1033.0649±184.034	994.0253±143.6321	0.63	0.53	0.97	0.001
Orbital part of the inferior frontal gyrus		232.2405±58.3274	214.5±41.6015	224.1429±55.6544	204.2278±48.2345	-0.42	0.68	0.97	0.001
Triangular part of the inferior frontal gyrus		854.9747±187.5911	771.3375±140.4994	825.6623±189.6058	779.5949±136.5886	0.83	0.4	0.97	0.002
Middle frontal gyrus		3482.3418±656.0881	3074.025±477.2724	3386.5195±543.4229	3110.0253±578.6844	0.84	0.4	0.97	0.002
Superior frontal gyrus		5351.3038±672.0728	4866.4625±496.5936	5266.3636±670.9659	4814.7722±594.0012	-0.15	0.88	0.97	<0.001
Long insular gyrus and central sulcus of the insula		331.519±60.1332	298.3±46.6784	316.8831±56.4454	286.7722±51.7947	-0.04	0.97	0.97	<0.001
Short insular gyri		499.5949±102.0096	443.9625±71.5268	477.6104±79.7217	432.2152±64.7806	0.24	0.81	0.97	<0.001
Parahippocampal gyrus		876.6076±162.8301	794.325±109.4732	824.8831±151.5133	830.8481±188.2924	2.5	0.01	0.27	0.02
Anterior segment of the circular sulcus of the insula		391.9873±68.7745	351.0625±69.7205	388.1688±78.4316	348.8101±62.0414	-0.15	0.88	0.97	<0.001
Inferior segment of the circular sulcus of the insula		970.6835±134.7927	925.4875±106.1182	964.2338±128.8928	930.0127±110.6286	0.09	0.93	0.97	<0.001
Superior segment of the circular sulcus of the insula		1320.3165±163.1943	1200.3625±145.6874	1284.8831±157.7425	1208.9241±141.4207	1.15	0.25	0.97	0.004
Inferior frontal sulcus		1784.2658±374.9557	1637.925±246.1517	1727.5584±328.7229	1644.3418±246.5229	0.71	0.48	0.97	0.002
Middle frontal sulcus		1212.8734±256.1407	1087.9±236.9608	1202.6623±278.8642	1105.6076±236.6385	0.24	0.81	0.97	<0.001
Superior frontal sulcus		2220.9241±432.7817	2046.525±292.6403	2208.6234±375.071	1983.6203±283.5878	-1.04	0.3	0.97	0.004
Ventromedial PFC		4784.519±646.6447	4460.55±578.7406	4675.961±656.8666	4447.6835±514.5004	0.41	0.68	0.97	0.008
Transverse frontopolar gyri and sulci	Right	868.2025±148.4095	783.2625±142.0731	831.5584±152.6866	807.038±164.4288	0.11	1.62	0.54	0.009
Anterior part of the cingulate gyrus and sulcus (ACC)		2066.4937±300.5877	1858.3125±219.2202	2027.2468±302.0638	1855.7215±269.6168	0.71	0.37	0.9	<0.001
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		1165.8608±189.7891	1051.8625±152.3403	1107.6364±195.9449	1037.1899±137.8307	0.33	0.97	0.67	0.003
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		1113.9114±170.8539	1007.925±142.837	1059.8571±165.6336	1001.1646±145.4281	0.21	1.24	0.56	0.005
Posterior-dorsal part of the cingulate gyrus (dPCC)		415.5823±95.3632	359.5375±67.0345	400.3766±79.885	364.9241±82.6379	0.35	0.94	0.67	0.003
Posterior-ventral part of the cingulate gyrus (vPCC)		202.038±56.979	181.6±38.9445	198.3766±50.922	183.7089±34.2353	0.73	0.35	0.9	<0.001
Opercular part of the inferior frontal gyrus		952.3924±182.3627	880.7125±138.9939	931.2078±186.2682	902.3544±152.4545	0.3	1.05	0.67	0.004
Orbital part of the inferior frontal gyrus		270.1013±52.2829	239.8625±51.8683	267.5714±55.5161	241.0759±58.717	0.82	0.23	0.96	<0.001
Triangular part of the inferior frontal gyrus		858.6329±202.777	753.55±142.5946	859.2468±203.1685	761.5063±169.1203	0.99	-0.01	0.99	<0.001
Middle frontal gyrus		3129.3797±544.2413	2793.1375±423.5522	3050.987±558.6792	2800.4051±450.2419	0.62	0.49	0.9	0.001
Superior frontal gyrus		5167.1772±612.2518	4616.25±488.255	4889.8961±595.8076	4567.9494±594.4622	0.08	1.78	0.54	0.01
Long insular gyrus and central sulcus of the insula		365.6582±73.3215	331.05±67.8875	345.1558±66.4504	316.2152±54.488	0.9	0.12	0.99	<0.001
Short insular gyri		453.7848±114.1807	396.225±66.5065	443.2208±79.7464	390.4557±55.9221	0.94	-0.07	0.99	<0.001
Parahippocampal gyrus		988.2152±197.9278	915.0375±168.1071	961.6494±187.3315	875.2405±153.7802	0.49	-0.69	0.83	0.002

Supplementary table S4.12: Sex by diagnosis interactions on Surface Area continued

Brain Region	Hemisphere	Healthy Controls		Conduct disorder		Statistics			
		Males (n=79) Mean ± SD	Females (n =80) Mean ± SD	Males (n =77) Mean ± SD	Females (n=79) Mean ± SD	T	P	Corrected p value	$\eta\pi^2$
Anterior segment of the circular sulcus of the insula	Right	455.3544±76	399.85±65.8247	440.2597±99.58	412.5696±71.5536	0.15	1.46	0.54	0.007
Inferior segment of the circular sulcus of the insula		855.3671±126.6647	801.0625±124.5887	845.1299±138.7028	807.4557±91.4773	0.7	0.39	0.9	<0.001
Superior segment of the circular sulcus of the insula		1050.1266±141.1954	926.7625±128.9676	1025.5584±140.0556	948.6962±134.3581	0.15	1.43	0.54	0.007
Inferior frontal sulcus		1629.7848±304.2833	1465.4375±209.8351	1591.6364±301.3231	1509.8228±248.9346	0.2	1.28	0.56	0.005
Middle frontal sulcus		1651.0886±269.1749	1459.05±278.0856	1632.4935±315.5312	1552.6962±280.9004	0.11	1.6	0.54	0.008
Superior frontal sulcus		2022.1899±365.1093	1846.9125±273.8759	1951.9091±311.5189	1835.481±297.0201	0.51	0.65	0.83	0.001
Ventromedial PFC		4723.3038±637.9582	4286.7375±497.3875	4578.6753±664.4551	4347.7468±553.9363	0.13	1.52	0.54	0.001

D.11 Supplementary table S4.13: Sex by diagnosis interactions on Cortical Thickness

Brain Region	Hemisphere	Healthy Controls		Conduct disorder		Statistics			
		Males (n=79) Mean ± SD	Females (n =80) Mean ± SD	Males (n =77) Mean ± SD	Females (n=79) Mean ± SD	T	P	Corrected p value	$\eta\pi^2$
Transverse frontopolar gyri and sulci	Left	2.9165±0.2984	2.9012±0.27886	2.9249±0.2606	2.8495±0.24854	-0.9	0.37	0.75	0.001
Anterior part of the cingulate gyrus and sulcus (ACC)		2.9605±0.18955	2.9176±0.18845	2.9621±0.2124	2.9386±0.18773	0.58	0.56	0.75	0.002
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		2.961±0.18156	2.9448±0.14446	2.9962±0.18798	2.9484±0.15622	-0.88	0.38	0.75	0.001
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		2.8114±0.13231	2.774±0.16217	2.8095±0.16607	2.7628±0.15251	-0.3	0.76	0.89	0.008
Posterior-dorsal part of the cingulate gyrus (dPCC)		3.1623±0.20401	3.111±0.21566	3.1331±0.26204	3.1244±0.17943	0.86	0.39	0.75	0.001
Posterior-ventral part of the cingulate gyrus (vPCC)		2.6395±0.30058	2.54±0.26963	2.5932±0.35079	2.5371±0.27616	0.69	0.49	0.75	<0.001
Opercular part of the inferior frontal gyrus		3.0385±0.21193	3.0135±0.20088	3.031±0.18344	3.0361±0.18139	0.58	0.56	0.75	0.005
Orbital part of the inferior frontal gyrus		3.0958±0.2914	3.0701±0.29803	3.1686±0.25173	3.1124±0.28286	-0.57	0.57	0.75	<0.001
Triangular part of the inferior frontal gyrus		2.9761±0.21492	2.9245±0.17793	2.9622±0.19974	2.9432±0.20881	0.7	0.48	0.75	0.002
Middle frontal gyrus		2.9056±0.18503	2.8866±0.17909	2.8981±0.21253	2.9228±0.15815	1.03	0.31	0.75	0.007
Superior frontal gyrus		3.1424±0.17823	3.1358±0.18634	3.1343±0.19903	3.1484±0.19004	0.47	0.64	0.79	0.001
Long insular gyrus and central sulcus of the insula		3.3865±0.35718	3.3887±0.2779	3.3822±0.3047	3.4596±0.25838	1.04	0.3	0.75	0.001
Short insular gyri		3.7197±0.32797	3.7443±0.26041	3.7687±0.25981	3.8062±0.26614	0.07	0.95	0.97	<0.001
Parahippocampal gyrus		3.1261±0.28681	3.2299±0.33453	3.1764±0.31005	3.1386±0.26135	-2.13	0.03	0.71	0.001
Anterior segment of the circular sulcus of the insula		3.093±0.24455	3±0.19775	3.0352±0.25828	3.0227±0.21453	1.55	0.12	0.75	0.001
Inferior segment of the circular sulcus of the insula		2.9763±0.27	2.9512±0.21163	3.0347±0.24254	2.9676±0.22125	-1.03	0.3	0.75	0.002
Superior segment of the circular sulcus of the insula		2.7828±0.17575	2.7274±0.15651	2.7669±0.19292	2.7162±0.13922	0.04	0.97	0.97	0.001
Inferior frontal sulcus		2.5022±0.15836	2.4046±0.14231	2.4662±0.19135	2.4008±0.13363	1.01	0.31	0.75	0.002
Middle frontal sulcus		2.4547±0.18616	2.3884±0.19223	2.437±0.22049	2.3992±0.18052	0.74	0.46	0.75	<0.001
Superior frontal sulcus		2.6254±0.16897	2.5653±0.12938	2.601±0.18953	2.5773±0.14106	0.92	0.36	0.75	0.018
Ventromedial PFC		5.5349±0.31711	5.44±0.2762	5.5587±0.35841	5.4562±0.32652	-0.15	0.88	0.97	<0.001
Transverse frontopolar gyri and sulci	Right	2.7882±0.21764	2.7516±0.24055	2.7925±0.25772	2.7289±0.21416	-0.41	0.68	0.88	0.003
Anterior part of the cingulate gyrus and sulcus (ACC)		2.9128±0.21936	2.8265±0.18204	2.8762±0.21128	2.821±0.19734	0.85	0.39	0.88	0.001
Middle-anterior part of the cingulate gyrus and sulcus (aMCC)		2.9878±0.17113	2.9451±0.14286	2.9674±0.17969	2.9446±0.17071	0.51	0.61	0.88	0.003
Middle-posterior part of the cingulate gyrus and sulcus (pMCC)		2.8442±0.13386	2.765±0.13724	2.79±0.17625	2.7637±0.1495	1.53	0.13	0.88	<0.001
Posterior-dorsal part of the cingulate gyrus (dPCC)		3.1397±0.23773	3.1073±0.15898	3.1294±0.23266	3.0753±0.19391	-0.55	0.58	0.88	0.002
Posterior-ventral part of the cingulate gyrus (vPCC)		2.9572±0.29071	2.8925±0.35392	2.9494±0.32334	2.8904±0.2682	0.1	0.92	0.98	0.002
Opercular part of the inferior frontal gyrus		3.0862±0.17264	3.0294±0.20038	3.0294±0.18635	3.0238±0.16131	1.21	0.23	0.88	0.001
Orbital part of the inferior frontal gyrus		3.0357±0.2347	3.083±0.30929	3.0171±0.29312	3.0665±0.31406	-0.01	0.99	0.99	0.001

Supplementary table S4.13: Sex by diagnosis interactions on Cortical Thickness continued

Brain Region	Hemisphere	Healthy Controls		Conduct disorder		Statistics			
		Males (n=79) Mean ± SD	Females (n =80) Mean ± SD	Males (n =77) Mean ± SD	Females (n=79) Mean ± SD	T	P	Corrected p value	$\eta\pi^2$
Triangular part of the inferior frontal gyrus	Right	2.9581±0.23471	2.9114±0.21349	2.9247±0.23113	2.9196±0.19573	0.83	0.41	0.88	0.002
Middle frontal gyrus		2.9094±0.15624	2.8795±0.17506	2.8705±0.20532	2.901±0.15729	1.49	0.14	0.88	0.003
Superior frontal gyrus		3.1278±0.16418	3.1114±0.19147	3.1121±0.20756	3.113±0.16015	0.41	0.69	0.88	0.001
Long insular gyrus and central sulcus of the insula		3.4128±0.38786	3.4431±0.36106	3.5109±0.36716	3.5085±0.31324	-0.41	0.68	0.88	0.004
Short insular gyri		3.6491±0.29451	3.6684±0.31351	3.7003±0.277	3.7028±0.24642	-0.3	0.76	0.89	<0.001
Parahippocampal gyrus		3.2027±0.27614	3.2636±0.27575	3.197±0.27427	3.2265±0.24977	-0.61	0.54	0.88	0.015
Anterior segment of the circular sulcus of the insula		3.0757±0.27256	3.0199±0.2282	3.0527±0.25967	3.028±0.21441	0.55	0.59	0.88	0.008
Inferior segment of the circular sulcus of the insula		2.9503±0.22403	2.8856±0.22194	2.9936±0.23064	2.9035±0.21148	-0.73	0.46	0.88	0.003
Superior segment of the circular sulcus of the insula		2.8446±0.19023	2.7492±0.20283	2.8562±0.18509	2.7808±0.14228	0.43	0.67	0.88	<0.001
Inferior frontal sulcus		2.4511±0.16209	2.3902±0.16055	2.4342±0.16401	2.3977±0.13866	0.73	0.47	0.88	0.003
Middle frontal sulcus		2.4018±0.18238	2.3331±0.17458	2.3545±0.17498	2.2972±0.15554	0.37	0.71	0.88	0.002
Superior frontal sulcus		2.6675±0.14282	2.5821±0.14179	2.5868±0.17476	2.5871±0.15919	2.37	0.02	0.39	0.003
Ventromedial PFC		5.5534±0.35063	5.4742±0.33298	5.5462±0.37605	5.4618±0.32669	-0.09	0.93	0.98	<0.001

D.12 Supplementary table S4.14: Sex by diagnosis interactions on subcortical volume

Brain Region	Hemisphere	Healthy Controls		Conduct disorder		Statistics			
		Males (n=79) Mean ± SD	Females (n =80) Mean ± SD	Males (n =77) Mean ± SD	Females (n=79) Mean ± SD	T	P	Corrected p value	$\eta\pi^2$
Accumbens	Left	578.3468±123.0506	544.9388±139.9729	606.9052±157.0714	539.343±124.253	-1.61	0.11	0.3	0.008
Amygdala		1662.2405±196.2416	1500.9137±160.0985	1611.8039±242.8125	1492.5532±205.5352	0.72	0.47	0.58	0.002
Caudate		4102.4671±733.5556	4019.555±533.5264	4093.5636±715.7843	3839.6203±589.2903	-2.09	0.04	0.3	<0.001
Hippocampus		4406.3506±553.9064	4192.4325±399.4359	4390.7208±616.8186	4109.3684±545.9957	-1.11	0.27	0.39	0.004
Pallidum		1941.3152±412.2666	1810.6825±385.7376	1979.1455±471.9565	1818.8±422.1703	-1.17	0.24	0.39	0.004
Putamen		6169.4582±840.5979	5660.6262±665.366	6114.9571±938.8229	5671.9405±668.1358	0.05	0.96	0.96	<0.001
Thalamus		9079.3215±1781.2948	8527.095±1273.8903	8813.9831±1735.7472	8713.119±1487.0024	1.7	0.09	0.3	0.009
Accumbens	Right	654.9848±105.2224	581.5062±111.011	631.8857±118.3189	568.3418±96.3772	0.31	0.76	0.85	<0.001
Amygdala		1686.9481±260.0076	1485.5025±204.9064	1629.9779±291.7098	1498.062±188.6343	1.39	0.17	0.3	0.006
Caudate		4141.0342±654.7653	4036.4275±519.9707	4073.9325±689.6404	3873.8101±610.1715	-1.43	0.15	0.3	0.007
Hippocampus		4469.7481±490.9992	4157.595±434.208	4318.0805±490.7073	4164.1506±393.1107	1.49	0.14	0.3	0.007
Pallidum		1741.9304±297.8111	1622.08±252.2921	1757.7364±347.5893	1628.6899±289.6948	-0.69	0.49	0.58	0.002
Putamen		6047.1646±716.5528	5498.3713±646.5708	6012.7779±851.1151	5520.819±593.8669	0.05	0.96	0.96	<0.001
Thalamus		8345.143±1555.2584	7789.7613±1223.9093	8139.0883±1566.0662	7941.4177±1277.2363	1.39	0.16	0.3	0.006

Appendix E Additional material for the TSST-C study

E.1 Information Sheet for the TSST-C study

Information Sheet for young people who have previously taken part in the FemNAT-CD study, Version 3, 28/04/2015

Project Title: Understanding how young people respond to stress: The FemNAT-CD study.
Ethics number: 12177

Please read this sheet carefully before deciding to take part in this study. If you are happy to take part, you will be asked to sign a consent form.

What is the research about?

We would like to extend our study entitled "Understanding sex differences in disruptive behaviour in children and teenagers". In this part of the study, we are interested in studying how people cope with stress and how their body reacts to stress by producing hormones that can be measured in saliva (chewing a small piece of cotton). Hormones are molecules that are released by our bodies when we feel stressed. There are also changes in our hormone levels across the day. In this study we are interested in studying cortisol, which is sometimes called the "stress hormone". We are interested to see whether there is any link between how people cope with stress and disruptive behaviour or anger, and also whether there are differences between boys and girls in how they cope with stress.

In this study we want to test how your hormones react while being exposed to a stressful or challenging task. We can't tell you exactly what the challenging task involves, as we want to measure your natural response to the task. However, we can briefly tell you that you will be expected to speak for a few minutes in front of a panel. Although it is possible that you might find this task stressful or distressing, it is short and you will have time to calm down and recover once the task has finished. It is also true that some young people don't find it very stressful. We also want to measure how your stress hormones change over the day, as you go about your normal routine. Therefore, we will ask you to collect several saliva samples during a visit to the University and on two weekdays at home.

Why have I been chosen?

We are inviting participants that took part in our previous study entitled "Understanding sex differences in disruptive behaviour" to take part in this study. We are recruiting participants

aged between 12 and 18 years through schools, colleges, education centres, Child and Adolescent Mental Health Services in the NHS, Families Matter, and the Hampshire, Southampton and Reading Youth Offending Services. Our sample is what is called an 'opportunity' sample – anyone who chooses to take part can do so, providing they are not affected by the study's exclusion criteria (such as pregnancy, having autism, or being older than our age limit of 18). You do not have to have disruptive behaviour to take part – we are interested in recruiting young people without disruptive behaviour, as well as those who may have been in trouble a lot at school, or with the police. If you decide to take part, you will be given this information sheet and you and your parent or carer will be asked to sign a consent form. You are still free to stop taking part at any time, without giving a reason. You can also choose to opt out of any part of the study.

What will happen to me if I decide to take part?

We will meet up with you and your parent/carer to explain in detail what the study involves and find out whether you are eligible to take part (for example, you cannot be pregnant).

As mentioned above, we want to study hormone levels in two different conditions: as you go about your normal routine at home/school and again while you take part in a stressful or challenging task at the University of Southampton. Therefore, this study is divided in two parts. One part will require you to come to the University. We will ask you to chew on a piece of cotton several times so we can collect your saliva. We will also attach sensors to your chest and back to measure your heart rate. The challenging task will only last around 10-15 minutes. But we will ask you to arrive at the University one hour before and to stay for one hour after the task. This is because we would like to measure your hormone levels and heart rate before and after the task. In addition, hormones react very slowly. That's why it is important that you stay at the University for one hour after the stressful task. Before and after the task you can just relax and enjoy yourself. You will receive **£20** for this part of the study.

The second part will involve collecting saliva samples at your home. We will ask you to collect saliva samples across two typical school/college or work days to assess how your hormone levels change across the day. Levels of stress hormones are usually much higher in the morning than the evening, but we want to see whether some young people show different patterns of hormone production. Furthermore, as it is very important for us to collect the saliva samples at the right times during the day, if this is okay with you, we will agree a time when you want to be woken up for the following two days of the study. The time will be registered and you or your parent/carer will receive a text message at that time followed by

two text messages: 30 minutes after waking and 60 minutes after waking. The same procedure (reminder text messages) will be followed for the following samples. We will also give you an instruction sheet on how to collect your saliva. You will receive **£10** for this part. If you complete both stages successfully you will get **a £5** pound bonus. We will also cover all travel expenses.

Are there any benefits in my taking part?

There are potential benefits for the volunteers. First, the participants will be paid for their time (£10 for the home task, £20 for the visit to the University, and £5 as a bonus) Secondly, you will gain an insight into the research process by taking part in the study, which might inspire you to learn more about psychology or how the brain works. Third, we hope that understanding more about the causes of disruptive behaviour in children and teenagers will lead to new interventions for disruptive behaviour, and you will be contributing to this knowledge. Finally, we can send you information about the results of the study once the project has finished.

Are there any risks involved?

No - we will be collecting saliva using a non-invasive technique. However, there is a possibility that some young people might become upset during or after the stressful task, but we will give everyone time to calm down and recover before they leave the University.

If the researchers feel that a young person has become very upset during the task, they will stop the task immediately. However, if you feel extremely distressed while doing the task and the researchers do not notice it, please tell them. You will be asked to stay for the recovery period (one hour), but only if you feel that you can cope with it.

Will my participation be confidential?

Yes, your identity will be protected. We will change your name to a subject code, which will be used on all the samples. Records of your personal details will only be kept if you agree to this. We can assure you that any information you give us will be strictly confidential, not shown to your parent or carer, and you can leave out questions if you wish.

If the study is written up for publication, the article(s) will not include people's names. As this study is part of a larger project involving Universities in seven other European countries, the saliva samples that are collected in Southampton will be transferred to a University in Germany for analysis. However, your identity and personal details will always be protected. In addition, the researchers working on the project will share information and transfer data between countries to carry out their statistical analysis, although none of the data files that

will be shared will contain personal details or people's names. Finally, all study data will be protected against unauthorized access.

What happens if I change my mind?

You will be able to stop taking part in the study at any time, without explaining why. If you don't object, we may use any data collected up to the point of withdrawal (when you stop). You can also opt out of any part of the study.

What happens if something goes wrong?

If you have a concern or complaint regarding any aspect of this study, you can contact the Research Governance Office at the University. Phone number: 02380 595058, Email address: rgoinfo@soton.ac.uk They will be happy to help or discuss your concerns.

Where can I get more information?

Thank you for taking the time to read this information sheet. If you or your parent or carer would like to ask me any further questions before making a decision about taking part, please feel free to contact me by e-mail or phone (Karen: K.gonzalez@soton.ac.uk, or call: 07551 077571).

E.2 Sports and substance form

Subject ID: ..... Temperature: .....

Date: ..... Humidity: .....

Recording time (start/end): ..... (hh:mm) / ..... (hh:mm)

Past hour

- How many cigarettes have you smoked in the past hour? …….
- Did you practice any sports in the past hour? * YES / NO

Past 24 hours

- How many caffeine-containing drinks (e.g. coffee, tea, coke, energy drinks) have you consumed in the past 24 hours?

  ……..……..…… …… (cups/glasses)

- How many alcoholic drinks have you consumed in the past 24 hours?

  …… …… (drinks/glasses)

- Have you used any drugs in the past 24 hours? *

  YES / NO What did you take?

  Soft drugs (cannabis/hash/weed/YES / NO marihuana)

Stimulantia (speed, amphetamine, uppers)	YES / NO
Sedatives (benzodiazepines, valium)	YES / NO
Cocaine	YES / NO
Opiates (heroine, morphine, methadone)	YES / NO
Hallucinogens (LSD, psychedelics, mescaline)	YES / NO

In general

- How many hours a week do you practice sports? ……. (hours/week)
- How many cigarettes do you smoke on an average day? …… (cigarettes/day)
- Have you had any heart problems in the past?

  YES / NO (arrythmia / heart surgery)
- Have you had any head injury? YES / NO

  (brain trauma; abrasions on the head are not considered 'injury')
- Have you been pregnant? Or have you had a miscarriage or abortion? YES / NO When was this?

  ……

  …….. (report day participant gave birth or had abortion/miscarriage (dd/mm/yyyy))

  Check with exclusion criteria on page 2 of the SOP psychophysiological measurement.

NOTES:.....
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NB:

* For the following conditions, consider whether the measurement will be valid or needs to be postponed:
  - Use of any substance longer than **24 hours ago**; proceed. Report detailed information

on the use/condition of the subject.

- Use of **caffeine/cigarettes** on the **day of assessment**; proceed. Report detailed information on the use/condition of the subject.
- Use of **caffeine/cigarettes during the past hour**; try to postpone the measurement for half an hour.
  Report detailed information of the consumption.
- Intense **physical exercise during the past hour**: try to postpone the measurement for half an hour.
  Normal walking/lifting objects is not considered 'intense physical exercise'.
- Use of **soft- or harddrugs**, the **day before assessment**; proceed. Report detailed info on use!
- Use of **soft- or harddrugs** on the **day of assessment**; try to postpone the assessment to another day. For each substance/condition: always report detailed information on what was used, when and how much.

E.3 Instructions for the Trier social stress test

Script for Panel Members of the TSST study

To conduct this experiment **three researchers** are needed:

Test leader: Timing saliva samples, explaining procedure and task instructions, accompanying subject during relaxation periods, connecting VU-Ams

Panel member 1: Taking notes during public speaking task which can be used by the test leader to provide the subject feedback, g iving instructions for mental arrhythmic task and sending 2 markers during mental arrhythmic task.

- *Panel member 2*: taking notes during public speaking task, which can be used by the test leader to provide the subject feedback, sending 2 markers in the movie speech, competitions of VAS 3 and 4 by subject.

Note: Panel members should act in a neutral way during the speech. They should neither provide active encouragement, nor discouragement, but keep observing the participant and making notes.
Start of TSST: panel members enter the room
Panel member 1: sits down at the table and panel member instructs the subject: *"Are you ready? Please stand here, in front of the camera."*
*Panel member 2: pushes the **marker button**, or asks the subject to push it, and registers the marker (**start speech**) on the feedback & marker log form.*
Panel members 2 sits down and instructs the subject to start :
"What movie have you chosen to speak about?
Please can you start now?"
*Panel member 1: **starts the stopwatch**.*
Both panel members take notes during the speech to provide feedback for the subject (feedback & marker log form). Only positive feedback is reported. Please make the comments as clear as possible, because the test leader, who won't have watched the speech, will have to report the feedback to the subject.
*Panel member 1: **When speech is too short, PM1 comments:***

- *"Please continue, your speech has to be 5 minutes long."*
- *"Can you explain us why this is your favourite movie?"*
- *"Can you tell a little bit more about …" (an aspect of the movie the subject touched during his/her speech)*
- *"What things about this movie made you like it so much?"*
- *"What scenes or parts of the movie did you especially like?"*
- *Tell a little bit more about how you felt when you watched this movie for the first time."*
- *For older boy/girls: "What do you think the director/maker of this movie was trying to say by making the movie?"*

*Panel member 1: When **5 minutes** are over, **stop the stopwatch**:*
"Your time is up. Thank you very much for your speech."

*Panel member 2: pushes the **marker button**, or asks the subject to push it, and registers the marker (**end speech**) on the feedback & marker log form.*
Hand over VAS 3: *"Please fill in this short questionnaire." –*
When the subject has completed VAS 3, the instructions for the mental arithmetic task can be presented.
Panel member 1: "Now you will be told about the second task.
We will give you a certain number and ask you to subtract a fixed number from it.
So your number is … . Please subtract .. from it and continue by subtracting .. from your result. Is this clear to you?
Please do this as fast and accurately as possible. If you make a mistake, we will correct you."

***9 – 11** years old*	*start with **758** and subtract steps of **7***
***12 – 14** years old*	*start with **1023** and subtract steps of **13***
***15 – 17** years old*	*start with **1023** and subtract steps of **17***
***18** years old or older*	*start with **2023** and subtract steps of **17***

*Panel member 1 pushes the **marker button**, or asks the subject to push it, and registers the marker (**start mental arithmetic task**) on the feedback & marker log form.*
*"Please start now." **Start** the **stopwatch**.*
Panel member 2: The subject has to perform this mental arithmetic task for 5 minutes. He/she should continue by subtracting the number after a correct answer. On every failure, the subject has to restart at 758/1023/2023 depending on the age category (see above).
When the answer is correct: "Correct. Now continue by subtracting .. from your result." OR: "Please continue."When the answer is incorrect: "Your answer is incorrect. Please start again. .. subtracted from .. is?" OR: "Stop. Please start again."

-The panel member could switch to a lower level if the subject gets really frustrated. However, a heightened level of frustration/stress is what we're aiming for, so switch to a lower level only when you think the subject cannot tolerate this level of frustration any longer.
"We'll try another number. Please subtract .. from .. . What is the result?"

- *After **5 minutes** the task will be finished, regardless of the amount of correct/incorrect answers.*

*Panel member 1: pushes the **marker button**, or asks the subject to push it, and registers the marker (**end mental arithmetic task**) on the feedback & marker log form.*
Panel member 1: tells the subject she has completed the task and did very well.
Both panel members 1 and 2, smile to the subject when the tasks are finished, as a signal of positive, non-verbal feedback.
Panel member 2: "Thank you very much. You did very well. I will now call the task leader, so she can take you to the relaxation room."
*The panel members let the test leader enter the room or call the test leader and **hand over the feedback & marker log forms**.*
The test leader accompanies the subject to the relaxation room.
Importantly, panel members will be duty bound to stop the TSST immediately, not only when the participants disclose feelings of distress, but also when the participants show signs of significant distress or extreme discomfort such as, crying, hyperventilation or trying to leave the room.

E.4 Visual Analogue Scale for TSST-C

VAS

Subject ID: ……..

☐ VAS 1 ☐ VAS 2 ☐ VAS 3 ☐ VAS 4 ☐ VAS 5 ☐ VAS 6 ☐ VAS 7 ☐ VAS8

Time: …….

How do you feel?

- Do you feel anxious?

No, not at all — Yes, very much

- Do you feel (emotionally) insecure?

No, not at all — Yes, very much

- Do you feel stressed?

No, not at all — Yes, very much

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