# INVESTIGATING SEX DIFFERENCES IN STRUCTURAL AND FUNCTIONAL NEUROIMAGING CORRELATES OF EMPATHY IN CONDUCT DISORDER

by

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# ABSTRACT

Conduct Disorder (CD) is a childhood psychiatric disorder characterised by antisocial and aggressive behaviours. The overarching aim of this thesis was to investigate potential sex differences in the structural and functional neuroimaging correlates of empathy in CD. Studies in chapters 5 and 6 showed that youths with CD had decreased structural covariance between the anterior insula (AI) and thalamus compared to TD (typically developing) youths, but the groups did not differ in grey matter volume and no interactions with sex were observed. Studies in chapters 7 and 8 revealed that youths with CD exhibited reduced brain response in the AI and cerebellum when viewing others in pain. A sex-by-group interaction was also observed in the amygdala whereby females with CD had reduced brain response compared to TD females, with no differences between males with CD and TD males. Youths with CD exhibited decreased functional connectivity between the AI and the occipital lobe, but increased connectivity between the cerebellum and amygdala compared to TD youths. These findings suggest that structural and functional abnormalities in the AI are key features of both males and females with CD, while only females exhibit functional abnormalities in the amygdala when processing pain in others.

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# Dedication

This thesis is dedicated to my late grandfather, James M. Clanton, who taught me many things throughout my life, but mostly how to work hard and stay positive.

"*It is easier to build strong children than to repair broken men*"
– Frederick Douglass

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AI | Anterior Insula |
| ACC | Anterior Cingulate Cortex |
| ADHD | Attention Deficit Hyperactivity Disorder |
| CD | Conduct Disorder |
| CP | Conduct Problems |
| CU | Callous Unemotional (traits) |
| DARTEL | Diffeomorphic Anatomical Registration Through Exponential Lie Algebra |
| DSM | Diagnostic and Statistical Manual of Mental Disorders |
| FemNAT-CD | Female Neurobiology and Treatment of Conduct Disorder |
| FWE | Family Wise Error |
| fMRI | Functional Magnetic Resonance Imaging |
| GMV | Grey Matter Volume |
| HCU | High Levels of Callous-Unemotional Traits |
| ICU | Inventory of Callous-Unemotional Traits |
| IQ | Intelligence Quotient |
| IRR | Inter-Rater Reliability |
| K-SADS | Kiddie Schedule of Affective Disorders and Schizophrenia for School Age Children |
| LCU | Low Levels of Callous-Unemotional Traits |
| MNI | Montreal Neurological Institute |
| MRI | Magnetic Resonance Imaging |
| PPI | Psychophysiological Interaction |
| ROI | Region of Interest |
| RT | Reaction Time |
| SBM | Surface-Based Morphometry |
| sMRI | Structural Magnetic Imaging |

| | |
|---|---|
| SPM | Statistical Parametric Mapping |
| TD | Typically Developing |
| TE | Echo Time |
| TR | Repetition Time |
| VBM | Voxel-Based Morphometry |
| WASI | Wechsler Abbreviated Scales of Intelligence |
| YPI | Youth Psychopathic Traits Inventory |

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# CHAPTER 1. CONDUCT DISORDER AND EMPATHY: CHARACTERISTICS, DEVELOPMENT, AND SEX DIFFERENCES

## 1.1 General Introduction

Antisocial behaviour has a profound psychological and social impact on both the individual committing such acts, and society at large. Thus, understanding and preventing severe antisocial behaviour amongst youths and adults is an important challenge faced by modern societies. Conduct Disorder (CD) is a common childhood psychiatric disorder with a prevalence of approximately 10%, with an increased incidence in adolescence, meaning adolescent-onset cases are more frequently observed and diagnosed compared to childhood-onset. CD is characterised by "a repetitive and persistent pattern of behaviour by a child or teenager in which the basic rights of others or major age-appropriate societal norms or rules are violated" (p. 469, (American Psychiatric Association; APA, 2013). CD is known to have a significant impact on individuals' functioning, their family and interpersonal relationships, and society. It is a known risk factor for delinquency and maladjustment to adulthood (Hill, 2003), and severe behavioural problems in youths can lead to more serious offending patterns as they develop, which places a huge financial burden on society including educational and judicial facilities (Scott, Knapp, Henderson, & Maughan, 2001). Indeed, by the time they are 28 years old, individuals diagnosed with CD before the age of 10 cost society ten times as much as individuals who did not have conduct problems (CP) as children (Scott et al., 2001). In the US, annual healthcare costs for children with CD are $10,000 more than healthcare costs for healthy children (Merikangas, Nakamura, & Kessler, 2009), and estimates in the UK report the overall care of a child referred for persistent antisocial behaviour costs at nearly £6000 per annum (Romeo et al., 2006). Efforts to imprison and monitor parolees are

perceived as more expensive and time consuming than prevention strategies to identify early risk factors and tailor intervention for youths with CD (Hill, 2003).

The prevalence and the costs associated with CD in our society have prompted researchers and clinicians to investigate and identify developmental trajectories, predictors, and causes in the hope of developing effective interventions and preventions. This chapter will discuss the diagnosis of CD in terms of the most recent edition of the Diagnostic Statistical Manual (DSM-5), (APA, 2013). First, a brief historical overview of the diagnosis will be provided, followed by a description of the current diagnostic criteria as well as prevalence in community, clinical, and forensic settings. The second section will focus on the clinical presentation by focusing on the age of onset, level of callous-unemotional traits, sex differences and comorbidities.

CD is a heterogeneous disorder with different subtypes. A subgroup of individuals with CD also have high levels of callous-unemotional (CU) traits, otherwise referred to as the *limited prosocial emotions* specifier in the DSM-5. CU traits include lack of remorse or guilt, callousness-lack of empathy, lack of concern about performance in important activities, and shallow affect (American Psychiatric Association, 2013). The characteristics of youths with CD with high and low levels of CU traits will be discussed further in section 1.3.2. Researchers and clinicians have established lack of empathy is a key feature associated with CD, especially amongst youths with high levels of CU traits (Lahey et al., 1999), and it has been proposed that the root of evil and wrong-doing in the world stems from an erosion of empathy (Baron-Cohen, 2011). Empathy is one of the most important social constructs bringing humanity together, whilst CD, in which there is a significant lack of empathy, is one of the most common diagnoses found amongst youths in primary care settings (World Health Orgnisation, 2008). There is a large literature on the functional and structural neural correlates

of CD using magnetic resonance imaging. However, more work is needed due to discrepancies and inconsistencies, and the fact that relatively little is known about how sex might influence the neural correlates of empathy in CD. Therefore, the aim of this thesis is to replicate and extend previous findings via the investigation of sex differences in neural correlates of CD, with a specific focus on empathy. Sex differences in structural neural correlates will be reported, followed by structural covariance results derived from *a priori* seed-based region of interest analyses. The following chapters will cover sex differences in functional neuroimaging correlates of empathy and functional connectivity based on results from an *empathy for pain* MRI paradigm. Group and sex differences between males and females with CD and typically developing youths will be analysed.

## 1.2 Conduct Disorder

### *1.2.1 DSM Diagnostic Criteria*

The DSM-5 describes CD as a childhood psychiatric problem that has an increased incidence in adolescence (APA, 2013), and the International Classification of Diseases version 10 (ICD-10) defines CD as a repetitive and persistent aggressive or nonaggressive behaviour in which basic rights of others or social norms are violated (World Health Organization, 1992). The primary diagnostic features of CD are severe externalising behaviours including aggression to people or animals, destruction of property, deceitfulness or theft, and serious violations of rules. To receive a diagnosis of CD, a child must present with three out of 15 possible symptoms within the past 12 months, with one symptom present within the past six months (APA, 2013; see Table 1.1). Common symptoms include lying multiple times per week with the intention to manipulate or deceive others, truancy on more than two occasions, staying out late or overnight without permission on multiple occasions, and engaging in fighting or intimidating peers or adults. Individuals with CD often engage in

impulsive and risky activities and regularly have temper outbursts and/or defiant attitudes toward authority figures (Loeber, Burke, Lahey, Winters, & Zera, 2000). These youths also struggle with delayed gratification relative to typically developing (TD) youths, and they tend to choose immediate rewards over larger, delayed rewards (White et al., 2014). CD can be classified as mild, moderate or severe based on the type and severity of symptoms and the level of harm to others (APA, 2013). A diagnosis is made when the disturbance in behaviour causes clinically significant impairment in social, familial, academic, or occupational functioning.

*Table 1.1 DSM-5 Conduct Disorder Diagnostic Criteria*

| **Symptoms** | **Criteria** |
|---|---|
| Aggression to People and Animals | 1. Often bullies, threatens or intimidates others<br>2. Initiates physical fights<br>3. Has used a weapon to cause serious harm to others<br>4. Has been physically cruel to people<br>5. Has been physically cruel to animals<br>6. Has stolen whilst confronting a victim<br>7. Has forced someone into sexual activity |
| Destruction of Property | 8. Has engaged in fire setting with intention to damage<br>9. Has deliberately destroyed property (other than fire setting) |
| Deceitfulness or Theft | 10. Has broken into a house, car or building<br>11. Lies to obtain goods or avoid obligations<br>12. Has stolen items of non-trivial value without confronting a victim (shoplifting, forgery) |
| Serious Violation of Rules | 13. Stays out at night despite parental prohibitions, beginning before 13 years<br>14. Has run away from home overnight at least twice, or once having stayed away for a lengthy period of time<br>15. Often truants from school, beginning before 13 years |

### *1.2.2 Prevalence of CD in Community, Clinical, and Forensic Settings*

CD has a prevalence of approximately 9.5% in the US (Nock, Kazdin, Hiripi, & Kessler, 2006) and 5.8% in the UK (Green, McGinnity, Meltzer, Ford, & Goodman, 2005). Rates of CD amongst males are twice those seen in females (Maughan, Rowe, Messer, Goodman, & Meltzer, 2004). Discrepancies across countries may reflect a true difference, or may reflect a difference in methodologies used to assess prevalence (De Brito & Hodgins, 2009). CD and associated disruptive behaviour disorders (DBD) are the most common reason for child and adolescent referrals to mental health clinics; nearly 50% of child health referrals are for DBD and 30% of referrals to paediatricians are for DBD (National Institute for Health and Clinical Excellence, 2013). Furthermore, mental illness has been recognised in over half (53%) of individuals in juvenile detention centres with 40% of those meeting diagnostic criteria for DBD including oppositional defiant disorder (ODD) and CD (Shelton, 2001).

## 1.3 Clinical Presentation

### *1.3.1 Childhood-onset vs adolescent-onset*

One of the major criticisms of the diagnosis of CD is that it primarily indexes overt antisocial behaviour, which results in the identification of a highly heterogeneous group of youths with different temperamental and behavioural characteristics (Blair, Peschardt, Budhani, Mitchell, & Pine, 2006; Frick & Marsee, 2006). It has been proposed that these differences reflect distinct developmental pathways to CD (Frick & White, 2008). In the previous DSM version, DSM-4, this heterogeneity in CD was acknowledged for the first time, with the age-based distinction between *childhood-onset CD* (i.e., presence of at least one symptom before the age of 10) and *adolescent-onset CD* (i.e., absence of any symptom before the age of 10). The age-based distinction has been well supported for both females and males,

particularly in terms of its predictive validity and its ability to identify the characteristic problems of the two subtypes (Moffitt et al., 2008). Age of onset of CD predicts chronic delinquency (Moffitt, 2006), and it is one of the best predictors of antisocial behaviour in adulthood (Loeber, 1991). The younger the age of onset of CD is, the greater the number of aggressive behaviours and the higher the level of functional impairment (Lahey et al., 1999). Additionally, longitudinal evidence has shown children with the greatest number of antisocial behaviours before the age of 10 are more likely to have difficulties functioning in society by the time they near completion of adolescence and reach early adulthood (Fergusson, Horwood, & Ridder, 2005; Moffitt, Caspi, Dickson, Silva, & Stanton, 1996).

More than two decades of research have identified important differences between youths with childhood- and adolescent-onset CD. Those with childhood-onset CD have a higher genetic predisposition for antisocial behaviour, more frequently present with family dysfunction, antisocial parents, neurocognitive impairments such as lower IQs, and higher levels of comorbidities such as hyperactivity and inattention disorders compared to youths with adolescent-onset CD (Moffitt et al., 2008). These impairments are reflected in higher levels of school difficulties and peer relation problems. The adolescent-onset subtype, by contrast, tends to exhibit fewer difficulties in these areas (Moffitt et al., 2008). Research suggests that risk-taking and rebellious behaviour increase during teenage years within the general population and then decline in adulthood (Steinberg, 2004). However, youths with adolescent-onset CD exhibit a higher frequency and severity of CD symptoms compared to TD adolescents. Whilst childhood-onset CD is thought to have a neurocognitive basis, it has been posited that adolescent-onset CD is primarily associated with formation of relationships with other delinquent youths (Moffitt, 1993). This distinction has, however, recently been criticised by (Fairchild, Van Goozen, Calder, & Goodyer, 2013) who suggested that

differences between the two subtypes are quantitative rather than qualitative. Specifically, Fairchild and colleagues concluded that neurocognitive deficits are present in both subtypes and that environmental factors act as moderators between a child's predisposition and age of onset (Fairchild, Van Goozen, et al., 2013). The age-of-onset specification highlights one CD specifier, which is indicative of distinct profiles of youths with CD.

### *1.3.2 High vs. low callous-unemotional traits*

Psychopathic traits are a central feature of the adult syndrome of psychopathy (Cleckley, 1982), and *callous-unemotional (CU) traits* are the equivalent amongst youths. Youths with CD and high levels of CU traits (CD/HCU) are thought to be at an increased risk of developing psychopathy in adulthood, compared to youths with CD and low levels of CU traits (CD/LCU) (Blair, Leibenluft, & Pine, 2014; Frick, Ray, Thornton, & Kahn, 2014).

CU traits in late childhood and adolescence are positively correlated with psychopathy scores in adulthood, even after controlling for childhood CD and other childhood risk factors (Burke, Loeber, & Birmaher, 2002; Lynam, Caspi, Moffitt, Loeber, & Stouthamer-Loeber, 2007). Similar to CU traits, the adult personality disorder of psychopathy is associated with lack of empathy and high levels of instrumental aggression. High levels of CU traits are thought to be the antecedent of psychopathy, which is strongly associated with severe forms of Antisocial Personality Disorder (ASPD) in adulthood (Blair, Leibenluft, & Pine, 2014). For an adult to receive a diagnosis of ASPD, they must have received a diagnosis of CD before the age of 15, and childhood-onset CD is an established risk factor for life course delinquency (Moffitt, 2003).

There is overwhelming genetic and neurobiological evidence to support that HCU traits identify a particularly problematic subgroup of youths with CD who show a more severe,

stable, and aggressive pattern of antisocial behaviour than other youths with CD, (Frick et al., 2014), leading to the inclusion of the *limited prosocial emotions* CD specifier within DSM-5 (Pardini, Stepp, Hipwell, Stouthamer-Loeber, & Loeber, 2012; Vanwoerden, Reuter, & Sharp, 2016; APA, 2013). To qualify for this specifier, the individual has to meet full criteria for CD as well as persistently present with two of the following four characteristics in multiple relationships and settings over a minimum 12 month period: lack of remorse or guilt, callous lack of empathy, unconcern about performance at school or work, and shallow or deficient affect. Assessment of CU traits is currently done via questionnaires, primarily the Inventory of Callous-Unemotional Traits (ICU, Frick 1995) parent-report or child self-report measure, or the Youth Psychopathic Traits Inventory (YPI, Andershed et al 2002). There is not currently a standardised clinical interview in place to assess CU traits amongst youths which limits future scientific endeavours in regards to validity and replication of findings, meaning research studies may report on CU or psychopathic traits based on different measurements which can be confusing when attempting to interpret results. A standardised interview has been recently created and is being tested for clinical use called the Clinical Assessment of Prosocial Emotions (CAPE 1.1; Frick; 2013). This thesis will refer to CD as defined by a research diagnosis from semi-structured clinical interviews via the Kiddie Schedule for Schizophrenia and Affective Disorders Present and Lifetime versions (K-SADS; Kaufman, Birmaher, Rao, 1996), with CU traits measured via child-report Youth Psychopathic Traits Inventory (YPI) scores. A recent study examining the prevalence of this CU traits specifier amongst youths reported that in a community sample 10%-32% of those with CD met the CU specifier threshold, and these figures increased to 21%-50% of youths with CD from a clinical sample (Kahn, Frick, Youngstrom, Findling, & Youngstrom, 2012). Interestingly, across both community and clinic samples, between 2%-32% of youths without CD also met the

diagnostic threshold for the CU specifier, but those with CD/HCU showed consistently higher rates of aggression and cruelty than those with CD/LCU (Kahn et al., 2012).

Youths with CD/HCU and CD/LCU show distinct temperamental and affective profiles. Those with CD/HCU traits are insensitive to punishment but are more receptive to reward, consistent with a preference for novel and dangerous activities and impulsive behaviour (Blair, 2013; Frick & Marsee, 2006). In addition, they underestimate the likelihood of being punished for their actions relative to youths with other behaviour problems (Pardini, Lochman, & Frick, 2003). In contrast to youths with CD/LCU traits, those with CD/HCU traits show a weaker response to distress cues in others (Blair & Frith, 2000; Blair, 1999; Blair, Colledge, Murray, & Mitchell, 2001), and impairment in emotion recognition (Fairchild, Van Goozen, Calder, Stollery, & Goodyer, 2009; Sharp, Vanwoerden, Van Baardewijk, Tackett, & Stegge, 2015). There is also evidence that HCU traits are associated with lower levels of anxiety and fear, especially when controlling for impulsivity and conduct problems (Frick & Ellis, 1999; Pardini & Frick, 2013). By contrast, youths with CD/LCU traits do not show comparable punishment or reward sensitivities, but have problems regulating their emotions, displaying increased levels of anger and impulsivity (Frick & Marsee, 2006; Frick et al., 2014b). They are also more attuned to negative stimuli (Viding, Fontaine, & McCrory, 2012), and to the distress of others (Jones, Happé, Gilbert, Burnett, & Viding, 2010).

Given those distinct profiles, it is unsurprising that youths with CD/HCU traits and those with CD/LCU present with distinct patterns of antisocial and aggressive behaviour. The association between an early (i.e. school-age and early adolescence) stable presentation of antisocial behaviour and CU traits (Dandreaux & Frick, 2009; Frick & Viding, 2009; Rowe et al., 2010) has been linked to higher levels of aggression (Cornell et al., 1996; Frick et al.,

2003), over and above correlations with rule-breaking behaviours (e.g. Poythress, Dembo, Wareham, & Greenbaum, 2006). In a sample of high-risk males, HCU traits predicted an increased likelihood of violent and aggressive offending as an adult, even when controlling for age of onset (Loeber et al., 2005). Furthermore, compared to antisocial youths with LCU traits, those with HCU traits displayed a more varied and severe pattern of aggressive behaviours, including instrumental and premeditated aggression, akin to those observed in adult psychopathy at a younger age (Frick et al., 2003, Frick et al., 2014; Frick & Marsee, 2006). By contrast, youths with LCU traits have typically been found to be less aggressive, displaying predominantly threat-based reactive aggression (Viding & McCrory, 2012).

In summary, these findings support the view that the presence of CU traits designates a distinct group of children and adolescents who show a particularly severe, aggressive and stable pattern of antisocial behaviour (Frick, Ray, Thornton, & Kahn, 2014a; Frick et al., 2014b; Frick & White, 2008). Importantly, the positive association between CU traits and severity of antisocial and aggressive behaviour has been observed for both males (Kruh, Frick, & Clements, 2005) and females (Marsee & Frick, 2007), and in children as young as two (Waller et al., 2012) and three years old (Hyde et al., 2013; Kimonis et al., 2006).

### *1.3.3 Sex Differences*

There are well-documented sex differences in the prevalence of CD, which is more frequently diagnosed in males (Maughan et al., 2004), with a sex ratio of about 2:5 females to males (Moffitt & Caspi, 2001). The basis of these sex differences in the prevalence of CD remains unclear. Originally thought of as cultural bias (Robins, 1991), there has been much debate as to whether the diagnostic criteria for CD are tailored to fit predominantly overt antisocial male behaviours (e.g. aggression, theft, vandalism, rule-breaking), rather than more covert antisocial female behaviours (e.g. deceit, manipulation, bullying, lying, staying out

late) (Leadbeater, Kuperminc, Blatt, & Hertzog, 1999; Moffitt et al., 2008). For instance, amongst a community sample of young females with CD, the most common symptom reported by parents was lying to deceive others (Hipwell et al., 2002). As most female antisocial behaviour occurs in adolescence (Fontaine, Carbonneau, Vitaro, Barker, & Tremblay, 2009; Moffitt & Caspi, 2001), some researchers suggest that age of onset in females with CD is restricted to adolescence (Silverthorn & Frick, 1999). However, a more recent longitudinal study has shown that whilst females are more prone to develop adolescent-onset CD, they do in fact show both childhood and adolescent onset conduct problems (Odgers et al., 2008). Puberty has been considered to have an influential role on the development of CD, particularly early onset maturation (Keenan, Loeber, & Green, 1999). Early maturity in females has been associated with more externalising problems and higher rates of adolescent pregnancies (Lynne, Graber, Nichols, Brooks-Gunn, & Botvin, 2007). In males with CD, increased levels of testosterone after onset of puberty have been associated with adolescent-onset behaviours and group affiliation with delinquent peers (Rowe, Maughan, Worthman, Costello, & Angold, 2004), suggesting adolescent-onset CD in boys is primarily instigated by pubertal and hormonal changes.

CD in females has received increased attention recently following evidence documenting increased rates of conduct problems and antisocial behaviour in the US and UK, and a heightened number of arrests for crimes perpetrated by females in the past 10 years (Collishaw, Maughan, Goodman, & Pickles, 2004; Hawkins, Graham, Williams, & Zahn, 2009). Future research focusing on CD in females is of critical importance given its impact on social adjustment and implications for development of unhealthy interpersonal relationships with partners and offspring (Keenan et al., 1999). For example, females with CD tend to obtain lower levels of education, become a single parent at a younger age (Hill, 2003), or have

antisocial partners and raise children with behaviour problems (Keenan et al., 1999). They are also more likely to partake in risky activities, become targets for grooming and prostitution, and are more susceptible to drug abuse and contracting sexually transmitted diseases compared to TD females (Bardone et al., 1998; Pedersen & Mastekaasa, 2011).

Data from typically developing youths indicate that females show unique patterns of aggression, showing more relational or social aggression (e.g., gossiping, spreading rumours, and exclusion from social groups) than males (Crick & Grotpeter, 1995; Hipwell et al., 2002). Females also tend to display direct or overt aggressive behaviour when they are younger, and more indirect or covert aggressive behaviour during early adolescence; this switch in the type of aggression typically occurs earlier in females than in males (Wolke, Woods, Bloomfield, & Karstadt, 2001). Females with CP also present with lower levels of aggression and cause less damage and physical harm to other people than males with CP (Moffitt, Caspi, Rutter, & Silva, 2002). In forensic settings females held in juvenile detention centres tend to be more relationally aggressive and less physically aggressive than males (Marsee & Frick, 2007). Amongst these females, those with high levels of CU traits are more likely to exhibit proactive aggressive behaviour in social situations than those with LCU traits, who tend to exhibit reactive aggression socially (Marsee & Frick, 2007).

Aggressive, antisocial behaviours, are often observed less frequently amongst individuals with higher levels of empathy or prosocial behaviours. This makes the understanding of empathy and where it goes wrong amongst antisocial individuals an important endeavour.

## 1.4 Empathy

Empathy can be defined as the uniquely human ability to identify with another's experience or mental state, and consists of cognitive and affective components (Baron-Cohen,

2011). Cognitive empathy refers to the ability to comprehend another's thoughts and intentions, which is often regarded as interchangeable with theory of mind (Baron-Cohen, 2001). Affective empathy is the ability to feel what another is feeling emotionally, and is different from sympathy as one can experience sympathetic feelings without any impulse to react in a helpful way whereas affective empathy inspires emotional reaction to an event (Baron-Cohen, 2011). Feeling affective empathy is comparable to a mirroring phenomenon, whilst sympathy merely implies a person can understand and have pity for another's misfortune. This thesis will focus on the described construct of affective empathy as such.

Several factors influence the manifestation of empathetic behaviours in daily life, and are pertinent due to their influence on motivations behind basic cooperation and interaction (Rumble, Van Lange, & Parks, 2010). Concepts such as in-group and out-group experiences are important, in that group affiliation is known to influence empathy levels whereby members of a same group show higher levels of empathy and protection for each other than for members of another social group (Cikara, Bruneau, & Saxe, 2011). In-group and out-group identities have been associated with how individuals respond to experimental paradigms measuring empathic response. These phenomena will be reviewed further in the following chapter.

Empathy is crucial for constructing prosocial behaviours and facilitates interpersonal interactions useful for shared intentions and group functioning (Pfeifer, Iacoboni, Mazziotta, & Dapretto, 2008; Sturmer, Snyder, Kropp, & Siem, 2006). It can be related to altruism, or selflessness and desire to help others without expectation. It is still debated whether true altruism exists or if it's motivated by egoism, or desire to be seen by others in a favourable manner which would enhance social status, as altruistic behaviours can sometimes reduce the wellbeing of an individual to enhance another's wellbeing (Marsh et al., 2014). The

subsequent sections will first discuss the development of empathy in TD youths and review research investigating empathy in youths with CD. Next, sex differences in typically developing youths and youths with CD will be discussed.

### *1.4.1 Cognitive and affective components underlying empathy*

Investigation of the neural underpinnings of empathic response first requires an understanding of the development of certain cognitive and affective components (Shamay-Tsoory, 2011). Three cognitive mechanisms will be reviewed in this chapter including: separate understanding of self and other; contagious emotional and somatic states; and communication and interpretation of social cues (Decety & Meyer, 2008). Additionally, three emotional, or affective, mechanisms of empathy will be reviewed in this chapter including: affective sharing, empathic concern, and perspective taking (Decety & Cowell, 2015). Below, these mechanisms underlying empathy will be discussed followed by neuroimaging correlates of empathic response amongst healthy and conduct disordered individuals.

Understanding of self and others will be discussed first in consideration of development of empathic behaviour, as this is one of the earliest behaviours to manifest among individuals. This is due to empathy-related dependencies on limbic structures in the brain, which are the first to mature anatomically (Singer, 2006). Children are conscious of their own emotional states sometimes as early as two years of age when differences in levels of awareness of mental states start to emerge (Decety & Jackson, 2004). This is evidenced by a study wherein social behaviours were assessed amongst children ranging from low to high on empathy, and results showed those with the highest levels of empathy were more socially sensitive and aware of their own behaviours, as well as behaviours of others (Findlay, Girardi, & Coplan, 2006).

Emotional and somatic states are socially contagious and used for basic communication via functions such as facial expressions, eye contact, smiling, and even yawning (Iacoboni, 2009; Itier & Batty, 2009; Wild, Erb, Eyb, Bartels, & Grodd, 2003; Yoon & Tennie, 2010). While facial expressions themselves are not contagious, they have been deemed an important channel for emotional communication (Dimberg, Andréasson, & Thunberg, 2011). Facial expressions provide information to others regarding current emotional state including anger, happiness, fear, and sadness. Empathy is associated with strength of facial mimicry, in that higher levels of empathy are associated with enhanced ability to recognise and respond to facial expressions (Balconi & Canavesio, 2016; Dimberg et al., 2011; Rymarczyk, Zurawski, Jankowiak-Siuda, & Szatkowska, 2016). Eye contact has been described as one of the most powerful tools for human interaction, and typically developing infants show preference for mutual eye contact within the first few months of life, indicating a major foundation for development of social skills and communication (Farroni, Csibra, Simion, & Johnson, 2002). Furthermore, laughing, smiling, and yawning are unconscious primal actions, which are highly contagious and have been associated with rudimentary interspecies empathic abilities (Provine, 2005). Impairment in all of these socially beneficial communicative tools is evident amongst adults and youths with antisocial behaviours and psychopathic traits (Dadds et al., 2012; Marsh & Blair, 2008; Rundle, Vaughn, & Stanford, 2015).

The ability to communicate and interpret others' behaviours, the third cognitive mechanism mentioned, is noticeable as early as infancy (Kugiumutzakis, 1998), and mirroring and mimicking of facial expressions, gaze, and gestures can be considered a precursor to comprehension of more sophisticated abilities such as understanding others intentions, and responding empathetically to stressful events (Meltzoff & Decety, 2003). Animal research shows primates raised in isolated or neglectful environments are impaired at sending and

receiving emotional expressions to others compared to those raised in social settings (Harlow, 1965), suggesting early child-parent interaction is crucial and can have lasting impact on development of prosocial behaviours and empathy. Amongst humans, childhood neglect has also been found as negatively correlated with empathy, self-control, and social confidence (Dvir, Ford, Hill, & Frazier, 2014), and interestingly lower levels of empathy predict higher psychopathy scores on the Psychopathy Checklist: Youth Version (PCL: YV) amongst adolescents (Ometto et al., 2016). Also related to neglect, social exclusion has been found to be associated with detachment, numbness, and reduction in sensitivity to both physical and emotional pain (DeWall & Baumeister, 2006).

Another interesting discovery stemming from animal research is the mirror neuron system (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996), which includes the inferior parietal lobe, the ventral premotor cortex, and the inferior frontal gyrus as key areas recruited during social interaction and basic understanding of intention amongst macaques (Fabbri-Destro & Rizzolatti, 2008). Scientists have considered the macaque neural architecture to be homologous to that of the human brain (Passingham, 2009), and several neuroimaging studies have since been conducted amongst humans, which has resulted in the identification of regions of the brain associated with more sophisticated abilities, specifically empathy.

Emotional sharing, the first component involved in affective empathy, refers to detection of another's distress or discomfort (Decety & Cowell, 2014). The innate ability to be affected by emotional arousal of another can be observed as early as infancy when newborns cry in response to hearing another newborn cry (Geangu, Benga, Stahl, & Striano, 2010). Emotional sharing is important evolutionarily as it can trigger protective responses and inspires concern from in-group and out-group members which is the second component involved in affective empathy (Decety & Cowell, 2014).

Empathic concern is a highly important facet of affective empathy as humans depend on each other for survival, particularly infants and youths who rely on parental care and concern early in life (Decety, Norman, Berntson, & Cacioppo, 2012). Neurobiological systems including the limbic system, autonomic nervous system, hypothalamic-pituitary-adrenal axis, and associated hormonal systems involved in secretion of oxytocin and vasopressin are associated with social bonding and attachment behaviours (Keverne & Curley, 2004). These systems have been shown to be active when caring for or helping others, and they are also active during perception of pain to self amongst healthy individuals. This indicates an overlap in systems involved in both somatic and affective self-pain, and recruitment of these systems whilst showing concern for others (Eisenberger, 2011).

The third component of affective empathy is perspective taking, which can also be grouped with cognitive empathy due to the overlap of necessary skills and behaviours (Decety & Cowell, 2015). Empathy is generally prosocial, but sometimes it can impede on group coherence and functioning due to in-group bias. This means people who view themselves as similar are more likely to share emotional states and display empathic concern for each other (Castano, 2012). However, individuals who consider someone to be outside of their group or social circle may have more trouble with feeling empathy for out-group members. Perspective taking therefore breaks down barriers and allows for an individual to actively imagine himself or herself in another's situation, leading to empathic behaviours. Whilst perspective taking can be challenging, healthy individuals are capable of this, and it is effective in reducing conflict and facilitates prosocial interactions (Galinsky & Moskowitz, 2000). Neuroimaging evidence including these cognitive and affective components of empathy will be covered in the following chapter.

To overview, empathy is an important social construct with distinct underlying cognitive and emotional mechanisms. The healthy development of empathy begins as early as birth and can influence an individual's ability to perceive and attach to the environment, and has important implications for appropriate social interaction. Certain individuals in the population have empathy deficits, particularly children with a diagnosis of CD, indicating something has gone awry. With advances in neuroscience technology and research, MRI techniques have helped scientists explore neurobiological underpinnings of empathy and the next sections will focus on structural and functional neural correlates of empathy amongst healthy and conduct disordered individuals.

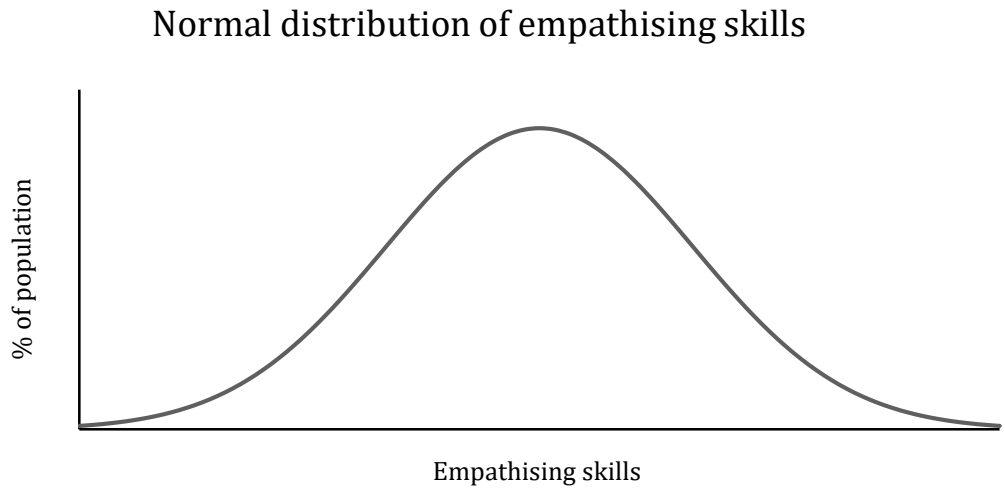



*Figure 1.1 Normal distribution of empathising skills (adapted from Baron-Cohen, 2004)*

### *1.4.2 Empathy in TD youths and youths with CD*

Individuals in the general population vary in their empathising skills, with a small percentage of people demonstrating low levels of empathy and a small percentage demonstrating high levels, the average person demonstrates normal levels, just between the two extremes (Baron-Cohen, 2004; Figure 1.1). Typically developing children show the

ability to empathise as young as two or three years of age as self-serving behaviours lessen and prosocial and caring behaviours become more apparent (Roth-Hanania, Davidov, & Zahn-Waxler, 2011; Zahn-Waxler, Robinson, & Emde, 1992). This indicates that empathic behaviours manifest early in life. Furthermore, twin studies show empathic traits are moderately heritable (Knafo, Zahn-Waxler, Van Hulle, Robinson, & Rhee, 2008) which provides evidence for a predisposition for empathic abilities. In comparison to TD youths, it has been established that youths with CD have lower levels of empathy (Blair, Leibenluft, & Pine, 2014; Kostic, Nesic, Stankovic, Zikic, & Markovic, 2016). CD involves infliction of harm to individuals or society via relational or physical means. This suggests aberrant affective abilities but intact cognitive abilities due to the recognition of how to gain something from another by understanding their psychological or physical weaknesses and limitations (Hare, Forth, & Hart, 1989). Animal literature supports this claim by showing deceitful behaviours amongst bonobos toward interspecies members, indicating use of basic intelligence and the ability to attribute motivations to others (Whiten & Byrne, 1988). Understandably, empathy is negatively correlated with externalising behaviours and aggression (de Kemp, Overbeek, de Wied, Engels, & Scholte, 2007), and instigation of physical fights and cruelty is correlated with desire to, and enjoyment of, inflicting pain upon others or displaying social dominance (Foulkes, McCrory, Neumann, & Viding, 2014). Interestingly, one study has shown that while there was no consistent relationship between empathy and aggression in younger children, a negative relationship was found between empathy and aggression in adolescents (Lovett & Sheffield, 2007). Cruelty to humans and animals, or moral transgressions, require a lack of remorse or guilt, which is also negatively correlated with empathy (Decety & Cowell, 2014; Tangney, 1995), and are central features of individuals with severe forms of CD and high levels of CU traits (Dadds, Turner, &

McAloon, 2002). Males with CD have reduced empathic responsiveness toward expressions of sadness and fear but not happiness compared to TD males, depending on situation or context (de Wied, Gispen-de Wied, & van Boxtel, 2010). However, empathy–inhibiting factors still need to be investigated in this population (de Wied et al, 2005). Additional evidence exists to support deficits in affective but not cognitive empathy amongst youths with CD and high levels of CU, based on data from an emotional affection video task (Schwenck et al., 2012).

### *1.4.3 Empathy & CU traits*

One of the defining features associated with high levels of CU traits is lack of empathy, with a strong negative relationship existing between the two (Frick et al., 2014b; Pasalich, Dadds, & Hawes, 2014). Higher levels of CU traits have also been associated with decreased morality and feelings of guilt and increased proactive aggression (Scheepers, Buitelaar, & Matthys, 2011). Adults with higher levels of empathy and prosocial behaviours have been found to have opposite patterns of intentions and biological motivations for their actions compared to those with high levels of psychopathic traits (Marsh et al., 2014). Furthermore individuals with higher levels of psychopathic traits tend to devalue relationships and prosocial behaviours (Foulkes, Seara-Cardoso, Neumann, Rogers, & Viding, 2014), and higher levels of CU traits amongst youths are negatively related to empathic traits, as CU traits manifest in narcissistic and selfish preferences (Foulkes, Seara-Cardoso, et al., 2014). A review of neuroimaging findings underlying these mechanisms will be discussed in more detail in the following chapter. Several theories have been posited as to why aberrant empathic response is observed in this population, with speculation regarding the existence of primary and secondary variants of CU traits. One theory posits CU traits comorbid with high levels of anxiety develop in response to chronic exposure to trauma, which is known as the

secondary variant (Kahn et al., 2013). This is supported by the fact that many children with CD/LCU traits have been victims of abuse, neglect, or other personally invasive crimes, especially from a young age (Kimonis et al., 2008), and in this case CU traits can be considered a protective mechanism against environmental stressors. Furthermore, considering the notion of "successful" psychopaths (Lilienfeld, Latzman, Watts, Smith, & Dutton, 2014), the presence of some psychopathic or callous-unemotional traits could be beneficial to an individual due to the learned preservation of emotional concern and reserved efforts toward others, resulting in more resources being allocated to personal survival, wellbeing, and prosperity. The other theory regarding CU traits is that CD / HCU traits comorbid with lower levels of anxiety are referred to as the primary variant, and because these traits have been identified in some children as early as three years of age (Hyde et al., 2013; Kimonis et al., 2006), and remain stable even into adulthood (Frick et al., 2003; Frick & White, 2008), this suggests neurobiological or genetic underpinnings (Blair, 2013).

### *1.4.4 Sex Differences in Empathy*

Females have been stereotyped as the more empathetic sex of the two (Lennon & Eisenberg, 1987), and within the general population females do indeed show greater levels of empathy than males (Toussaint & Webb, 2005). Females have higher scores on self-reported empathy (Baron-Cohen, 2004) and better performance on emotion recognition tasks than males (Barrett, Lane, Sechrest, & Schwartz, 2000). Females are also better at spontaneously empathising with others, whilst males have been described as better at systemising (Baron-Cohen, 2004). Interestingly, amongst toddlers, males show more callousness whilst females show higher levels of empathy suggesting females may develop empathic skills earlier than their male counterparts.

Relatively little research has been done on sex differences in empathy amongst youths with CD and CU traits. Cognitive empathy is understood to be intact amongst males with high levels of CU traits, but they struggle with affective empathy (Blair, 2001). More specifically, males with higher levels of CU traits show impairments in cognitive empathy in younger years and increase in their abilities after puberty (Dadds et al., 2009). Interestingly, Dadds et al (2009) also found females with high levels of CU traits show higher levels of affective empathy compared to males with high levels of CU traits, and it has been suggested there are developmental differences in the association between CU traits and cognitive empathy related to sex differences (Lui, Barry, & Sacco, 2016). More research is necessary to delineate sex differences in components of empathy amongst individuals with CD and varying levels of CU traits.

## 1.5 Overview/Upcoming Chapters

Chapter 1 has provided an overview of Conduct Disorder including diagnostic criteria, clinical presentation, prevalence, and subgroups. Sex differences in CD were then discussed followed by a review of empathy and callous-unemotional traits and their relationship to CD. Chapter 2 will provide a selective literature review on relevant structural and functional MRI correlates of CD, empathy, and sex differences. The succeeding chapters will overview experimental findings from four separate research studies, which include a subsample from a larger project (FemNAT-CD), with data analysed to address specific research questions for this thesis.

# CHAPTER 2. STRUCTURAL AND FUNCTIONAL NEURAL CORRELATES OF EMPATHY IN TYPICAL DEVELOPMENT AND IN CONDUCT DISORDER

## 2.1 Neuroimaging correlates of affective empathy

### *2.1.1 Typically developing*

The majority of functional MRI research on empathy has focused on responsivity to viewing others in pain, or anticipation of others in painful situations (Bernhardt & Singer, 2012). Meta-analytic evidence from 32 studies suggests there are neural correlates associated with empathy-evoking stimuli, comprising a network in the brain which primarily consists of the bilateral anterior insular cortex and medial and anterior cingulate cortex (Lamm, Decety, & Singer, 2011). Additional meta-analytic evidence supports the interplay of distinct networks, whereby the dorsal anterior cingulate cortex is associated with the cognitive-evaluative component of empathy, and the right anterior insula is associated with the affective-perceptual component of empathy (Fan, Duncan, de Greck, & Northoff, 2011). The unique interplay between the two regions is crucial for emotional and cognitive regulation (Medford & Critchley, 2010). Overall, evidence suggests the primary regions in the brain associated with empathy are the AI and the ACC, as they are commonly implicated in empathy for pain research, indicating an affective component to the neural response to pain (Lamm et al., 2011). The AI has been reported as necessary for empathic pain perception (Gu et al., 2012). The insula is largely associated with perception and self-awareness, and evidence has shown sharing emotionality involves extending first hand emotion (Craig, 2009). The ACC is also an area commonly implicated in empathy, as it is recruited during social interaction with others (Lavin et al., 2013), and has an influence on cognitive responses to emotional stimuli (Stevens, Hurley, & Taber, 2011). The ACC has been reported as an area

where executive and regulatory processes interact, evidenced by activation during emotion regulation in addition to attentional loading during cognitively demanding tasks (Medford & Critchley, 2010). A thorough review on the neural basis of empathy describes that whilst the perception of one's self and vicarious representations of self are necessary for empathy, different regions of the brain can be active simultaneously during empathic responsivity, and social information and environmental cues can modulate neuronal arousal (Bernhardt & Singer, 2012).

Areas hypothesised to be engaged in empathy, specifically limbic and paralimbic brain structures, are amongst the first to develop in a typically developing individual (Singer, 2006). Limbic structures are particularly vulnerable in the first few years of life, and abnormal development can lead to aberrant neural connections and potential difficulties with healthy social and emotional attachments (Joseph, 1999). Parent-child attachment, which involves healthy interaction and bonding behaviours, has a large influence on development of these brain structures. Early attachment difficulties have been linked to disruptive behaviours amongst young children including problems with socialisation and empathy for others (Theule, Germain, Cheung, Hurl, & Markel, 2016).

Furthermore, magnetoencephalography (MEG) results from a study including children, adolescents, and adults (n=209) showed that early signs of empathy for others' pain are observed amongst children in brain regions associated with basic sensory processing, whereas adolescents and adults process empathy for others' pain in frontal brain regions associated with a mature brain (Levy, Goldstein, Pratt, & Feldman, 2018). Thus, the results of that study suggest that the ability to empathasise begins early in childhood alongside normal neural network development and continues into adulthood when frontal regions, associated with

more sophisticated higher-order processing abilities, reach full maturity, which in turn leads to a better understanding of affect in others (Levy et al., 2018).

As mentioned in chapter 1, certain factors modulate empathic response, such as perspective-taking instructions. For example, amongst healthy adults, instructed perspective-taking has been associated with neural activation, in that imagining how someone else feels yields positive correlations between BOLD response and questionnaire-based empathy levels (Singer, 2004). Results from this study revealed that the higher a participant scored on an empathy questionnaire, the more BOLD response to perceptions of their partner's pain in the ACC and left AI. Additionally, Jackson, Meltzoff, & Decety (2005) found significant BOLD response in the ACC, AI, cerebellum, and thalamus during empathising with another's pain, but they did not find any correlations between empathy questionnaire scores and BOLD response. Selective empathy, or the tendency to attribute emotional concern toward others based on subconscious identification of similarities, has also been investigated via fMRI. Selective empathy results show stronger responses among healthy adults when viewing a loved one in pain compared to a stranger (Cheng, Chen, Lin, Chou, & Decety, 2010), and reduced responses toward outsiders, especially in other ethnic groups (Xu, Zuo, Wang, & Han, 2009). Also, reduced BOLD response has been observed while participants view someone in pain who they considered to have treated them unfairly previously (Singer, 2006).

In line with findings from healthy adults, typically developing youths exhibit increased BOLD response in both the insula, ACC, in addition to the somatosensory cortex, supplementary motor area, and the periaqueductal grey when viewing individuals experiencing pain inflicted both intentionally or unintentionally (Decety, Michalska, & Akitsuki, 2008). Furthermore, Decety and colleagues showed that viewing an individual intentionally causing harm to another is associated with increased response in additional areas

including the temporoparietal junction, paracingulate, orbitofrontal cortices, and amygdala. These data suggest substantial overlap between the network of regions recruited by adults and youths when processing pain experienced by others.

Overall, most research on functional neural correlates of empathic responsivity has focused on empathy for pain, and several factors can modulate neural responsivity influenced by painful stimuli amongst healthy individuals. Furthermore, separate neural regions may be recruited during empathy-related processing, including the AI and ACC, which demonstrates the existence of an empathy network (Fan et al., 2011).

### *2.1.2 Conduct Disorder & CU Traits*

In the last five years, an increasing number of fMRI studies have examined neural correlates of various forms of empathy in youths with CD and conduct problems. Some of these studies have investigated cognitive and affective components of empathy (e.g. O'Nions et al., 2014; Sebastian et al., 2012), whereas the majority of studies have investigated empathy for pain. Whilst cognitive empathy is intact, lack of affective empathy is a key feature associated with antisocial behaviour, and it may have neurobiological underpinnings (Blair, 2001). Regarding responsivity to empathic stimuli, youths with conduct problems have shown reduced activation whilst viewing pictures of strangers' in painful, versus non-painful, conditions in the AI, ACC, and inferior frontal gyrus (Lockwood et al., 2013), and interestingly, conduct problem symptoms in their sample were positively correlated with ACC response. Also, CU traits amongst participants in their sample were negatively associated with response in the AI and ACC (Lockwood et al., 2013). A more recent study found increased activation observed across all participants with CD symptoms and TD participants in the AI, amygdala, and temporal pole during a perceived intentional harm condition relative to unintentional harm to others (Michalska, Zeffiro, & Decety, 2016), which is consistent with

previous findings. However, Michalska et al. incorporated a different paradigm than Lockwood et al., in which participants viewed images of intentional and unintentional harm shown in succession to resemble animation, and the sample included both males and females unlike the male only sample in the Lockwood et al. paper. Participants in the Michalska et al. sample were younger as well with an age range of 9-11 ($M_{age}$=10), whereas participants in the Lockwood sample were 10-16 years of age ($M_{age}$=14). Within Lockwood et al.'s sample, the number of CD symptoms and severity of CU traits in this sample modulated BOLD response, in that a higher number of CD symptoms and greater severity of CU traits were associated with lower levels of activation in the right posterior insula during the harm condition compared to the no harm condition. Furthermore, there were negative correlations between CD severity, CU traits severity and activation in the AI, ACC, and superior temporal sulcus during the intentional harm condition. Very few studies have examined sex differences in functional neural empathic responsivity amongst youths with CD. However, Michalska et al (2016) observed sex-by-group interactions. The first implicated the right posterior superior temporal gyrus during the intentional vs. unintentional harm condition, whereby higher numbers of CP symptoms were associated with lower levels of BOLD response in females, but this effect was not observed in the males. The second sex-by-group interaction was observed in the posterior superior temporal sulcus and the middle frontal gyrus whereby conduct problems were negatively associated with BOLD response amongst females, but not males during the harm vs. no harm condition.

These studies are limited due to their samples consisting of youths with *conduct problems*, as in the Lockwood paper, and CD *symptoms* rather than CD *diagnoses* as in the Michalska paper. Results may be influenced by developmental maturation stage, or mean age of the participants. Additionally, a key difference between the two studies is the limiting male only

sample in the Lockwood paper, whereas a mixed sex sample was incorporated in the Michalska paper, which could potentially have interesting influences on the results. Future research should investigate neural underpinnings of empathic response amongst late adolescent CD participants whilst further examining sex differences.

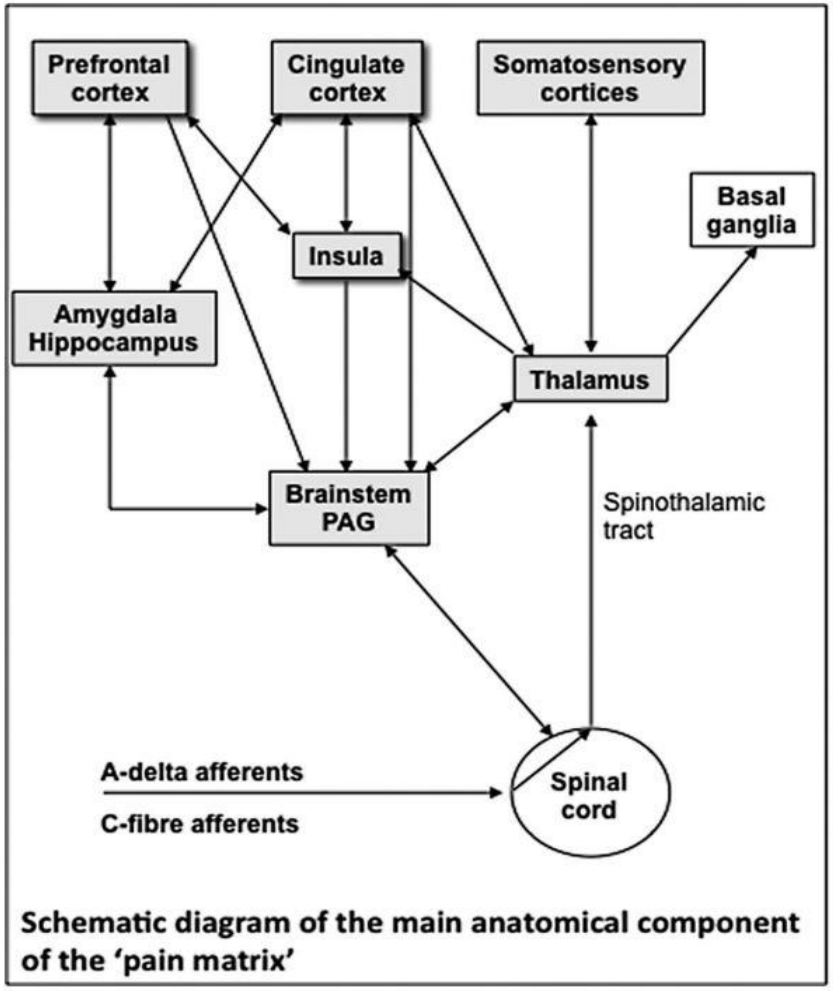
Interestingly, Decety, Michalska, Akitsuki, & Lahey (2009) studied a small sample of youths with childhood-onset aggressive CD (*n*=8) compared to TD youths (*n*= 8) ranging from 16-18 years of age, and BOLD response was stronger in youths with aggressive CD in the AI, aMCC, somatosensory cortex, and periaqueductal grey whilst viewing images of others in painful or potentially painful situations. Importantly, the authors have suggested the areas found to have increased activation are part of the neural pain matrix. The pain matrix is associated with empathy for others pain as indicated in Figure 2.1 (Decety, 2011).

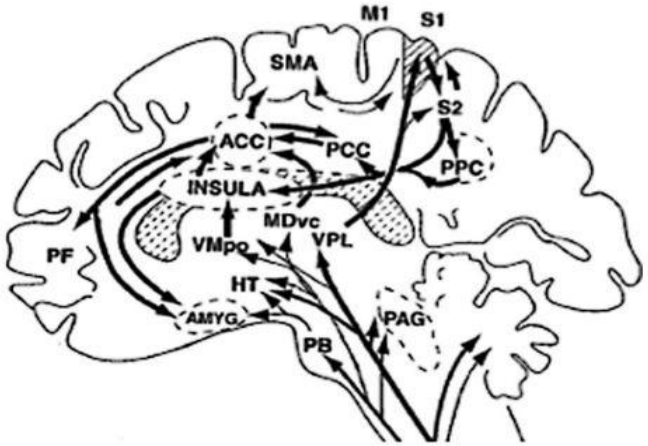
Reduced empathy amongst individuals with psychopathy and youths with CD/HCU traits has been thought to reflect amygdala dysfunction (Blair et al, 2014), and reduced responsivity in the amygdala to aversive affective stimuli such as fearful faces suggests dampened empathic abilities (Blair, 2008). Klapwijk et al. (2016) investigated empathic response in youths with Autism Spectrum Disorder (ASD) and CD/HCU, and found that both clinical groups had decreased BOLD response in the amygdala during emotional resonance, or judgment of their own emotion. However, whilst youths with CD/HCU had decreased responsivity in the AI and the inferior frontal gyrus during emotional resonance, youths with ASD showed decreased response in the hippocampus compared to TD youths. Individuals with ASD also show reduced activation in the vmPFC during facial recognition, indicating impairment in cognitive empathy amongst youths with ASD, which is not present amongst youths with CD/HCU (Klapwijk et al., 2016). It should however be noted that in this study the authors suggest recognition of facial expressions is equivalent to theory of mind, which is

not the typical measurement. Nevertheless, the authors argue these results provide evidence for dysfunctional emotion processing in addition to differentiation between neural mechanisms underlying CD/CU and ASD.

Furthermore, perspective-taking instructions have yielded interesting results amongst youths with CU traits. Marsh et al. (2013) examined empathic response amongst youths with CD and high levels of CU traits while participants were instructed to imagine pain to self or pain to others, and results showed reduced BOLD response in the ACC, amygdala, and putamen amongst youths with CD/HCU compared to TD youths. Interestingly, the reduced amygdala activation was strongly associated with imagining pain to others compared to imagining pain to self. Severity of CU traits was also negatively correlated with empathic responsivity in the amygdala and ACC (Marsh et al., 2013).

Based on the studies reviewed, youths with CD and CP seem to have reduced empathic responsivity to viewing others in pain overall, however early-onset aggressive CD seems to be associated with increased empathic responsivity. CU traits and severity of CD symptoms also seem to modulate results, and further research should replicate and expand upon these findings with larger, mixed sex samples considering there is a dearth of research on sex differences in CD. The next section will review connectivity and structural covariance evidence on empathic response, drawing comparisons between healthy individuals and youths with CD. Relevant findings from studies including CU traits and sex differences will also be discussed.




➡ The primary (SI) and secondary (SII) sensory cortices are involved in the sensory-discriminative aspects of pain, e.g., the bodily location and intensity of the stimulus.

➡ ACC and anterior insula subserve the affective-motivational component, i.e., the evaluation of subjective discomfort and response preparation in the context of painful or aversive stimuli.

*Figure 2.1 Diagram of neural 'pain matrix'; Regions associated with empathy for pain; Indicating the role of the AI and ACC in affective-motivational component of empathy (taken from Decety et al., 2011)*

## 2.2 Neural Networks of Empathy

### *2.2.1 Typically developing*

A good understanding of the normal development of the neural network implicated in empathy is key to the study of the neural correlates of both antisocial and prosocial behaviours (Decety, 2010). The key meta-analyses on the neural correlates of empathy have focused on healthy adults and specifically excluded studies including patients and children. Whilst data on healthy adults provides insight into the neural networks implicated in a mature brain, information on how neural networks develop and function amongst youths is important, particularly when attempting to understand dysfunction amongst clinical populations. Evidence from one fMRI study suggests that healthy youths respond similarly to healthy adults during empathy for pain paradigms (Decety et al., 2008). Overall, healthy youths showed increased BOLD response in the insula, ACC, somatosensory motor area, and periacquductal grey when viewing others in pain caused by accident. The same study showed that processing pain inflicted intentionally to others was associated with increased BOLD response in the amygdala, tempoparietal junction, medial and orbitofrontal cortices. Finally, another study investigating neurodevelopment of empathy-related circuitry identified age-related changes in the amygdala, insula, and supplementary motor area in a sample of healthy individuals ranging from 7 to 40 years of age (Decety & Michalska, 2010).

fMRI analyses identify how neural regions differ in responsivity to various stimuli, providing insight into structural and functional *localisation*. However, advances in methodology have enabled investigation into functional connectivity of neural regions, resulting in functional *segregation* (Friston, 2011). Functional connectivity provides information regarding how specific regions and associated neural networks communicate, which helps to explain abnormalities in neural circuitry, especially in clinical populations.

Disruptions in one brain region may be influenced by another region, or may be mediated by external stimuli (Stephan & Friston, 2010). Therefore, activity-dependent connectivity analyses are beneficial to follow-up on fMRI analyses to identify how seed regions of interest are interacting with other regions in the brain during experimental procedures. Research into neural networks and connectivity of regions implicated in empathy has become increasingly popular as knowledge on human brain mapping expands, and complexities of interconnected regions associated with emotional responsivity have been explored.

Empathy for pain networks are dynamic, indicating the interaction of several networks, specifically the sensorimotor, affective/salience network, and high-order frontal areas (Betti & Aglioti, 2016). Resting state connectivity studies have shown the default mode network is impaired among healthy individuals with lower levels of empathy: specifically lower connectivity between the medial prefrontal cortex and the ACC (Kim et al., 2017). Based on structural and functional localisation studies (Lamm et al., 2011), the AI and ACC have been frequently identified in empathy-related studies, and therefore are typically included as seed regions of interest in connectivity studies. The AI and ACC communicate with other brain regions, evidenced by inter-individual functional connectivity and intra-individual psychophysiological interaction (PPI) analyses indicating the AI has connectivity with the midbrain and periaqueductal grey during pain to self, and the ACC has connectivity to the superior temporal sulcus, posterior cingulate, and precuneus during pain to others (Zaki, Ochsner, Hanelin, Wager, & Mackey, 2007). These findings were from a small sample of healthy adults showing connectivity between the AI, ACC, and the dorsal medial prefrontal cortex whilst viewing others in pain, implying an overlap in the operations of these separate brain regions. This means brain regions are multifaceted and do not act independently with only a singular purpose. However, increased connectivity was observed specifically in the AI

to the midbrain and PAG during self-pain. These results indicate that the AI and ACC have overlapping, yet distinct mechanisms in the role of empathic response to self and others. Limitations of these findings should be considered, as a small sample size was used and self-pain was administered via actual thermal physical stimuli whilst pain to others was merely viewed via video. It has also been found that activity in the inferior frontal operculum, which has been associated with empathy (Jabbi, Swart, & Keysers, 2007), is functionally connected to the inferior frontal gyrus whilst viewing videos of emotional expressions based on PPI and Granger causality modelling analyses (Jabbi & Keysers, 2008).

Structural covariance analyses have also been conducted on seed regions associated with empathy. The AI and ACC have been identified as hubs within prefrontal, temporolimbic, and midline structural covariance networks (Bernhardt, Klimecki, Leiberg, & Singer, 2014). Although not many studies have reported sex differences associated with functional connectivity or structural covariance correlates of empathy, structural covariance amongst a healthy female specific sample ($M_{age}$=24) was investigated, and higher empathy scores were associated with increased covariance of the dorsal AI to prefrontal and limbic regions, however they did not show the same association with the ACC (Bernhardt et al., 2014).

### *2.2.2 Conduct Disorder & CU Traits*

Resting state fMRI analyses on youths with CD have yielded results indicating decreased connectivity in resting state networks including the anterior default mode network, the somatosensory network, the lateral visual network, and the medial visual network, which provides evidence for abnormal connectivity in low-level and high-order networks in youths with CD (Lu et al., 2015). This sample consisted of adolescent males with pure CD, so research on sex differences is still necessary. Zhou et al (2015) reported aberrant connectivity within the default mode network, indicating youths with CD have reduced functional

connectivity in the bilateral posterior cingulate cortex, bilateral precuneus, and right superior temporal gyrus compared to TD youths (Zhou et al., 2015). Within their sample of youths with CD (*n*=18) compared to TD (*n*=18) participants, those with CD had lower amplitude of low-frequency fluctuations in the bilateral amygdala/parahippocampus, right insula, right cuneus, and right lingual gyrus. CD participants in this sample also had higher amplitude of low-frequency fluctuations in the right fusiform gyrus and right thalamus. Taken together, these results provide evidence for abnormal connectivity in low-level and high-order networks in youths with CD. Research on sex differences is still necessary, as these samples consisted of males with CD.

There are also recent data on preadolescents (*n* = 123, age 9-11, 60 females) indicating aberrant connectivity in youths with high levels of CU traits, particularly whilst viewing others in harm (Yoder, Lahey, & Decety, 2016). CU traits were associated with reduced functional connectivity between the ACC with the amygdala and the AI whilst viewing pictorial stimuli of others in pain, however CD symptoms in the same sample were positively correlated with insula connectivity to the temporoparietal junction (Yoder et al., 2016). Surprisingly, despite the fact that about half of the sample included females, sex was not included as a covariate of no interest and the influence of sex on the results was not investigated.

Future research should further examine sex differences in connectivity and structural covariance amongst youths with CD, as there are very few studies to date addressing these factors.

## 2.3 Structural neural correlates of empathy

### *2.3.1 Typically developing*

Several different methodologies have been used to investigate the structural neural correlates of empathy, and neuroscience evidence is strongly supported by lesion studies, which reinforce neural network findings highlighting primary regions of interest indicative of aberrant functional and structural integrity. Stroke and neurodegenerative disease patients often have reductions in cognitive and emotional empathy, and right hemisphere damage has been related to reductions in perspective-taking empathic processes post-stroke (Yeh & Tsai, 2014). Lesions in the medial prefrontal cortex, anterior insula, anterior cingulate cortex, anterior temporal cortex, and amygdala are commonly associated with reduced empathic abilities, consistent with functional MRI results (Hillis, 2014). Furthermore, the most commonly occurring form of focal epilepsy, refractory mesial temporal lobe epilepsy, is known to be associated with social cognition deficits, and lower levels of cognitive and affective empathy have been correlated with smaller gray matter volume in the right mesial temporal lobe amongst these patients (Toller et al., 2015). Morphometric methodologies have enabled researchers to investigate individual differences in brain surface, shape, thickness, folding, and volume associated with empathy. Voxel-based morphometry (VBM) has been an accessible and popular method for examining differences in brain atrophy between clinical populations and controls (Mechelli, Price, Friston, & Ashburner, 2005; Whitwell, 2009). Relevantly, VBM studies on healthy adults have shown affective empathy toward others is negatively correlated with grey matter volume in the precuneus, inferior frontal gyrus, and ACC, whereas self-oriented empathy was negatively correlated with GMV in the somatosensory cortex, and positively correlated with the insula (Banissy, Kanai, Walsh, & Rees, 2012). VBM studies have demonstrated grey matter differences in specific areas associated with affective and cognitive empathy, indicating higher levels of affective empathy are associated with greater grey matter density in the insula, whereas higher levels of

cognitive empathy are associated with greater grey matter density in the medial cingulate cortex/dorsolateral prefrontal cortex (Eres, Decety, Louis, & Molenberghs, 2015). Again, this study promotes the multifaceted nature of empathy with the existence of distinct regions related to certain aspects of empathy. Other neural areas involved in empathy include the superior temporal cortex, inferior frontal cortex, and the amygdala (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003). Structural imaging studies reinforce functional imaging findings to show impairments in certain regions are associated with impaired BOLD response in the same regions.

Worthy of consideration, vicarious responsivity, especially empathic response, is multifaceted, implying numerous factors influence the phenomenon. Therefore, context, interpersonal relationship between individuals, instructed perspective-taking, and selective empathy are all factors influencing empathic response that should be acknowledged (Bernhardt & Singer, 2012). This is important due to the complexity of empathy, and fMRI correlates of empathy will be influenced by these factors to a certain extent. The following section will provide an overview of brain regions found to be impaired amongst youths with CD and varying levels of CU traits.

### *2.3.2 Conduct Disorder & CU Traits*

Structural MRI evidence has suggested there are neural abnormalities amongst youths with CD, and the first VBM study on CD provided evidence for reduced GMV in the bilateral anterior insula and the left amygdala in a small sample of youths with CD relative to controls (Sterzer, Stadler, Poustka, & Kleinschmidt, 2007), and interestingly GMV in the AI was positively correlated with empathy scores amongst youths in the CD group. Since that study, meta-analytic evidence has provided support for structural deficits amongst youths with CD, congregating evidence from 13 VBM studies explaining general reduction in amygdala, AI,

ACC, fusiform gyrus, and prefrontal cortex (Rogers & Brito, 2016, but see also Raschele et al., 2015). Despite the fact that data on CU traits were only available for 6 of the thirteen studies, there was some tentative evidence that higher levels of CU traits were associated with lower GMV reduction in the left putamen. These results are somewhat consistent with an earlier study, which indicated increased grey matter concentration in the OFC and ACC and increased GMV in bilateral temporal lobes amongst males with CP/HCU compared to TD males (De Brito et al., 2009). By contrast, a more recent study reported reduced GMV in the left OFC in youths with CP/HCU compared to those with CP/LCU (Sebastian et al., 2016).

In contrast to VBM, which provides a composite measure of GMV, surface-based morphometry distinguishes amongst cortical thickness, surface area, and local gyrification, 3 cortical metrics that have been shown to have different etiologies and developmental trajectories (Panizzon et al., 2009). Reduced cortical thickness, thinning, and decreased grey matter densities have been identified in the cingulate, prefrontal, and insular cortices amongst youths with disruptive behaviour disorders compared to TD youths (Fahim et al., 2011), and (Hyatt et al., 2012) identified reduced cortical thickness amongst youths with CD in temporal and parietal lobes, and reduced folding in the AI, ACC, OFC, temporal, frontal, and parietal lobes compared to TD children. In addition to reduced volume in the amygdala and striatum, children with CD have shown reduced cortical thickness in the superior temporal cortex and ventromedial prefrontal cortex (Wallace et al., 2014). Interestingly, CU traits within this sample were significantly correlated with reduced cortical thickness in the right temporal gyrus. In a more recent study on a larger sample, CD diagnosis was associated with reduced cortical thickness in the temporal gyrus, and CD symptom severity was associated with reduced surface area in the OFC. Interestingly, increased cortical folding was revealed amongst participants with childhood onset and not adolescent onset CD (Fairchild et al.,

2015). No data are available on sex differences in brain structure amongst youths with CD, leaving a gap that should be addressed.

### *2.3.3 Sex Differences in CD*

There are only a few published structural MRI findings comparing females with CD to TD females. Fairchild et al (2013) found lower GMV in females with CD compared to healthy control females in the bilateral insula and right striatum. Furthermore, Dalwani et al (2015) reported reduced GMV in the PFC, vmPFC, ACC, OFC, and striatum. However, this sample consisted of females with comorbid CD and substance abuse, which makes the clinical group results more difficult to interpret. Many studies have included mixed-sex samples, however very few focus on specific analyses to examine sex differences due to small group sizes for the female CD groups.

### *2.3.4 White matter tracts in typically developing youths and youths with CD*

Whilst grey matter development can reveal important information about structural properties, white matter tract microstructure provides insight into structural connectivity, measured via Diffusion Tensor Imaging (DTI) (Bihan et al., 2001). Amongst typically developing youths, DTI results indicate white matter anisotrophy changes are associated with ageing in a sample of children and adolescents ranging from 6-19 years of age (Barnea-Goraly et al., 2005). Specifically within this sample of 34 youths, fractional anisotrophy values increased in prefrontal regions, the basal ganglia, thalamic pathways, visual pathways, and the corpus callosum. Overall, typically developing youths show increased fractional ansitrophy during development. A systematic review covering 22 different DTI studies throughout neurodevelopment showed that antisocial behaviour amongst adults is associated with greater diffusivity (poorer integrity) across many white matter tracts including uncinate fasciculus, thalamic radiations, corpus callosum, cingulum, inferior fronto-occipital fasciculus, and

corticospinal tract. The results were mixed in youths with antisocial behaviour, indicating lower and higher diffusivity across white matter tracts (Waller, Dotterer, Murray, Maxwell, & Hyde, 2017). However two recent studies obtained consistent results whereby both studies showed that youths with CD are characterised by lower diffusivity (potentially reflecting increased mylenation) in similar tracts as those observed among adults with antisocial behaviour. Those effects were mostly explained by CU traits (Puzzo et al., 2017; Sethi et al., 2018).

## 2.4 Conclusion, aim of thesis, and outline of remaining chapters

This chapter has reviewed structural and functional neuroimaging research on youths with CD, including relevant literature on the influence of empathy. There was a specific focus on findings relevant for youths with CD, CU traits, and any sex differences were reported.

The aim of this thesis is to investigate structural and functional neuroimaging correlates of empathy amongst males and females with CD. Chapter 3 will review neuroimaging methodologies, and chapter 4 will cover project design, assessment methods, and site qualification. The subsequent chapters will discuss experimental findings from four different analyses on the structural and functional neural correlates of empathy amongst male and females youths with CD compared to TD male and female youths. Chapter 5 will review structural MRI voxel-based morphometry (VBM) findings wherein I investigated structural differences and regions of interest associated with empathy. Chapter 6 is an extension on chapter 5, which will include an overview of structural covariance results based on seed regions associated with empathy. Chapter 7 will review functional MRI (fMRI) correlates of responsivity to an *empathy for pain* task, and chapter 8 is an extension of the fMRI analyses including functional connectivity psychophysiological interaction (PPI) results. Finally,

chapter 9 will include a summary and discussion of all the experimental chapters in relationship to the appropriate literature. Scientific and clinical implications will be discussed.

# CHAPTER 3. NEUROIMAGING METHODS

## 3.1 Introduction

The aim of this thesis is to investigate potential similarities and sex differences in the neuroimaging correlates of empathy in youths with Conduct Disorder. Therefore, this chapter will mostly cover the neuroimaging methodologies and steps used in this thesis, but will briefly refer to others where relevant. Neuroimaging is widely recognised as a popular and useful methodology for investigating biomarkers of psychiatric disorders (Malhi & Lagopoulos, 2008).This thesis focused exclusively on structural and functional magnetic resonance imaging (MRI) data. The following sections will review structural and functional MRI methodologies and aims of specific approaches.

## 3.2 Structural neuroimaging

MRI can be used to investigate structural brain properties and is ideal for working with humans due to its noninvasive nature. Alternatives exist within clinical settings such as computerised tomography (CT), which uses x-ray radiation. However, MRI is advantageous in research settings because it does not involve radiation exposure and instead utilises a magnetic field and radio waves, which pose less risk to individuals in a scanner, and allows for longer scanning sessions and the inclusion of children. Most researchers use 3 Tesla scanners which produce more detailed anatomical images and increased signal-to-noise ratio compared to scanners with lower field strength (Malhi & Lagopoulos, 2007). A variety of methods are available for measuring the following structural properties of the brain: cortical thickness, gyrification, grey matter volume, white matter volume, and total cranial size. These structural properties of the brain can be investigated via voxel-based morphometry,

deformation-based morphometry, and surface-based morphometry (May & Gaser, 2006). Voxel-based morphometry is a fully automated technique and can be used to measure differences in grey or white matter between groups. After extensive pre-processing procedures including segmentation and creation of sample-specific tissue probability maps, comparisons are made on standardised, spatially normalised brains at a voxel-wide basis (Ashburner & Friston, 2000). Alternatively deformation-based or tensor-based morphometry can be used to measure differences in brain shape rather than brain tissue after correction for overall brain size. This involves deformation fields to locate which neuroanatomical areas differ between subjects either globally, or at a voxel-wise basis. Surface-based morphometry analyses involve extraction of the cortical surface to measure differences in physical properties such as curvature and thickness after standardisation procedures (Greve, 2011). Advances in software development have led to the widespread availability of packages such as Statistical Parametric Mapping (SPM) (Friston, 2007), FMRIB Software Library (FSL) (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012), Analysis of Functional NeuroImages (AFNI) (Cox, 1996), and Free Surfer (Fischl, 2012). SPM and associated methodological toolboxes were used for data analysis in this thesis to compare differences in grey matter volume between youths with and without CD. This was in accordance with previous research studies in the field investigating neuroanatomical differences between CD and typically developing youths (e.g. Sterzer et al., 2007; De Brito et al., 2009; Sebastian et al., 2016). Structural MRI studies often investigate regions of interest, which can be defined either via manual tracing or through ROI definition using a toolbox such as aal PickAtlas (Maldjian, 1994) in SPM. Unlike manual ROI tracing, automated morphometry allows for examination of differences in several regions across the brain all within one analysis (Eckert et al., 2005), and VBM is ideal for local regional differences due to the greater sensitivity

compared to other techniques such as deformation based morphometry (Mechelli, Price, et al., 2005).The rationale behind VBM, including advantages and disadvantages of the method, will be reviewed in the following section.

### *3.2.1 Voxel based morphometry (VBM)*

VBM is used to analyse structural MRI data where a comparison happens at a voxel-wise level within specified brain tissue. During pre-processing of data, grey or white matter can be extracted, which allows for group comparisons of local composition of the tissue type of interest (Ashburner & Friston, 2000). VBM has been criticised as not sensitive enough due to potential limitations such as misregistration (Bookstein, 2001) and displacement of tissue during segmentation (Good et al, 2001), but to date it is one of the most statistically valid ways to preserve and compare total grey matter between clinical groups (Ashburner & Friston, 2001). There are ways to control for potential issues during pre-processing via use of the Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL; Ashburner, 2007) and customised tissue probability maps, which greatly reduces chances of the aforementioned errors.

### *3.2.2 Structural MRI Pre-processing*

Pre-processing procedures ensure normalisation for statistical analyses and comparisons between groups of subjects due to natural variation in shape and size of the human brain (Mechelli et al., 2005). To account for individual differences, spatial normalisation must therefore be done for imaging data to be in a standardised neuroanatomical space. Spatial normalisation corrects for global variation across subjects so that differences in local concentrations can be observed at a voxel-wise level (Mechelli et al., 2005). Figure 3.1 shows a standard VBM pre-processing pipeline.

As the brain consists of both grey and white matter in addition to cerebrospinal fluid, segmentation must be done in order to extract the tissue of interest for analyses. This is automated in VBM where a calculation is conducted to ascertain the probability of a voxel being grey matter or white matter. Use of a custom tissue probability map helps with this process, as the likelihood of a voxel being correctly categorised as grey or white matter is enhanced when matching subject data with previously collected structural images from individuals with the same age and gender (Mechelli et al., 2005).

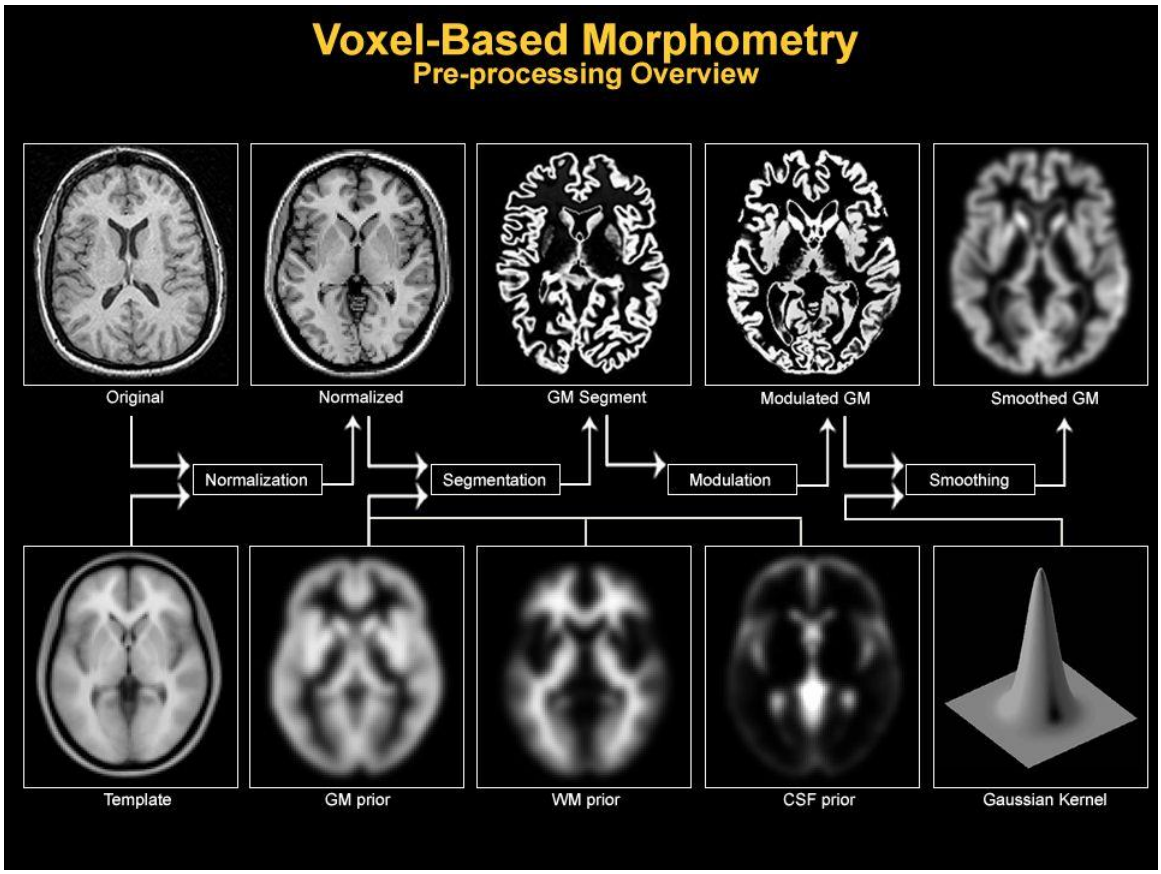



*Figure 3.1 VBM pre-processing pipeline*

All T1 images were assessed for quality and categorised as good, fair or poor by two independent raters (Roberta Clanton and Stephane De Brito). Poor scans refer to those with ringing, blurring, distortion, or neuroanatomical abnormalities, whereas good scans refer to those with clear details in brain regions and delineation of grey and white matter boundaries and gyri (see Figure 3.2). Only good T1 images were included in the analysis, therefore all participants included in this chapter. Images were then pre-processed using Statistical Parametric Mapping (SPM12, Wellcome Department of Imaging Neuroscience, University College London, UK) and Matlab (Mathworks, Natick, MA, USA). First, all scans were set to the anterior-posterior (AC-PC) commissure line at the origin [0 0 0 mm]. Age and sex specific tissue probability maps were then created with the template-o-matic toolbox (Wilke, Holland, Altaye, & Gaser, 2008) to account for the variance in child participants. Next, via the VBM8 and DARTEL toolboxes in SPM12, images were segmented into grey and white matter, and normalised with affine-only transformation. Grey matter segments were then smoothed with a 4mm Gaussian kernel. This size kernel was selected because DARTEL-based VBM analyses are better suited for smaller kernel sizes, especially with larger groups; notably, a 2-4 mm kernel has been suggested for use with groups over 50 participants (Shen & Sterr, 2013).

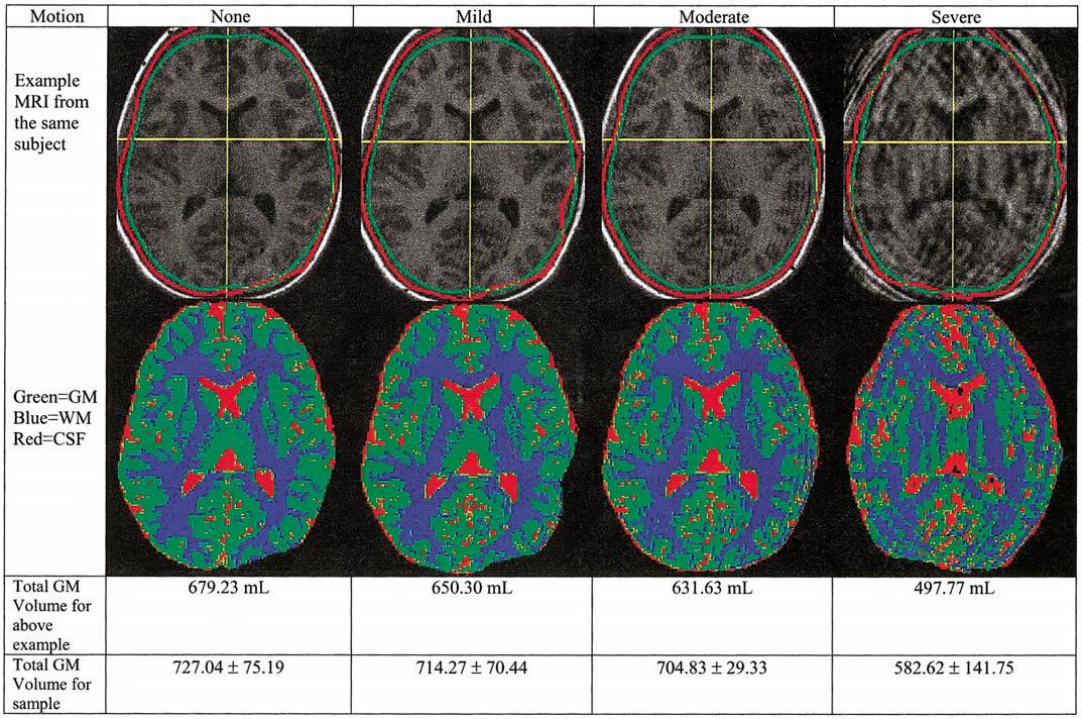



*Figure 3.2 Examples of varying degrees of movement artefacts in sMRI*

### *3.2.3 Structural MRI data analysis*

Using SPSS, effects of group, sex and site on overall GMV, white matter volume and cerebrospinal fluid were examined with 2 (Group: CD vs TD) x 2 (Sex: Females vs Males) x 2 (Site: Birmingham vs Southampton) ANCOVAs with age and IQ as covariates of no interest. Significance level was set at $p < .05$. Follow-up post-hoc Bonferroni pair-wise comparisons were used to compare means for any significant main effects or interactions.

VBM analyses of GMV were completed in SPM using a general linear model: a 2 (Group: CD vs TD) x 2 (Sex: male vs female) x 2 (Site: Birmingham vs Southampton) full factorial design was run using the smoothed gray matter images. Age and IQ were included as covariates of no interest. Using an absolute threshold of 0.1, regionally-specific between-group differences in GMV were assessed. Additional analyses were conducted comparing TD

youths to youths with CD/HCU traits and those with CD/LCU traits. Consistent with previous studies (e.g., Sebastian et al., 2016), the HCU and LCU group were formed based on median split (34) on the CU traits scores from the YPI questionnaire. Comparisons in GMV across the three groups were conducted with a one-way ANOVA. Consistent with previous VBM work on the FemNAT-CD sample (Rashle et al., 2018), a multiple regression with a sex-by-CU traits interaction term (mean-centered CU scores multiplied by the dichotomous variable sex) was conducted within the CD group only to examine whether sex and CU interacted to predict GMV. Age, IQ and sites were included as covariates of no interest. Using an absolute threshold of 0.1, regionally-specific between-group differences in GMV were assessed.

Whole-brain and region of interest analyses were conducted. Regions of interest were anatomically defined using the aal Atlas of the Wake Forest University PickAtlas (Maldjian, Laurienti, Kraft, & Burdette, 2003) and included bilaterally: the amygdala, anterior insula, anterior cingulate cortex, and ventromedial prefrontal cortex. For both whole-brain and ROI analyses, the height threshold within SPM was set at $p < 0.05$ family-wise error (FWE) corrected. For exploratory purposes, additional whole-brain analyses were also carried out using a reduced height threshold of $p < 0.001$, uncorrected with extend threshold of k=10 voxels.

#### *3.2.4 Structural covariance*

Structural covariance refers to the correlation of grey matter density in specific brain regions. Regional density can have an influence on other regions that are connected either functionally or structurally (Alexander-Bloch, Giedd & Bullmore, 2013). Structural properties of the brain are therefore complex, and this method enables to examine which regions are influenced by the same mechanisms whilst reinforcing available data on network structural connectivity

(Mechelli, Friston, Frackowiak, & Price, 2005). Structural covariance networks can provide support for functional network integrity, in that investigation into how structural neural networks co-vary can help explain how functional connections are formed and strengthened in the brain (Alexander-Bloch, Giedd, & Bullmore, 2013; Mechelli, Friston, et al., 2005). It is thought this is influenced by a combination of both genetic and environmental factors (Alexander-Bloch, Giedd & Bullmore, 2013).

Global and region-specific grey matter volume matures throughout childhood and adolescence, with some of the structural development necessary for large-scale functional integration not fully complete until late adolescence (Zielinski, Gennatas, Zhou, & Seeley, 2010). More specific networks, such as the salience network and CEN have shown structural covariance between regions is more advanced in late adolescence compared to early childhood (Zielinski et al., 2010). These neural networks are discussed in more detail in chapter 6, section 6.1.

### *3.2.5 Structural covariance pre-processing*

Data were pre-processed within the VBM8 toolbox in SPM12, and the GMV was extracted via the segmented and smoothed VBM data used in chapter 5. MNI coordinates for seed regions were then identified and entered into the MarsBar toolbox in SPM12 (Brett et al., 2002). The seed regions included left anterior insula (x = -40, y = 22, z = 0), right anterior insula (x = 39, y = 23, z = -4), and anterior cingulate cortex (x = -2, y = 23, z = 40), based on the MNI coordinates from meta-analytic evidence (Lamm et al., 2011). Consistent with previous structural covariance studies using GMV data from VBM (e.g. Heinze et al., 2015; Mechelli, Friston, et al., 2005), average regional grey matter volume intensities within the three ROIs were extracted from each participant's pre-processed VBM data using a 4mm

sphere defined within the SPM Marsbar toolbox, and centered around the peak coordinates of the three ROIs.

#### *3.2.6 Structural covariance data analysis*

Three (one for each of the three seed regions) two-way (Group: CD vs TD; Sex: Males vs Females) ANCOVAs models were created in SPM12, wherein the extracted grey matter intensities from the seed regions were contrasted against the whole brain. Separation of group and sex within the models, with a total of four groups, allowed investigation into possible main effects of group (TD vs CD) in addition to possible sex-by-group interactions. Covariates of no interest included global grey matter, age, IQ, and scanning site. The height threshold was set at $p < 0.05$, Family-Wise Error (FWE) corrected at whole-brain level, and results were displayed on a mean structural image of all the participants.

## 3.3 Functional neuroimaging

There are several ways to examine neural function including resting state fMRI and task-based fMRI (Huettel, Song, & McCarthy, 2004). Resting state fMRI provides insight into whole brain functional activity and neural network connectivity, which shows how various regions of the brain are interacting whilst an individual is at rest without immediate cognitive or attentional demands to influence responsivity. Transference of signal between brain regions during rest provides evidence for neural networks, or circuits, which are responsible for similar processes. This is important because aberrant network connectivity can be an indicator or biomarker for psychiatric disorders (Atluri, Padmanabhan, & Fang, et al., 2013).

By contrast, task-based functional MRI measures neural activation during a task designed to evoke cognitive or affective processes in response to stimuli. Broadly, fMRI results do not indicate changes in neuronal activity, but instead indicate changes in associated physiological

activity through blood oxygenation level dependent (BOLD) response (Huettel et al., 2004). BOLD refers to a contrast, and after several pre-processing steps, statistical parametric maps are produced to identify "increased" or "decreased" responsivity in brain regions. fMRI utilises a large magnetic field to measure a change in signal intensity reflecting deoxygenated hemoglobin in the brain, which has magnetic properties. This happens whilst humans view or undergo cognitively stimulating events, and is also known as the hemodynamic response function (HRF). Following exposure to a stimulus, the HRF lasts approximately 20 seconds and a peak response is usually observed after ~ 6 seconds. In MRI, the brain is divided into 3D pixels called voxels of several millimeters in size, each including millions of neurons. There are close to 100 billion neurons in the human brain, and when neurons are stimulated, glucose and oxygen are recruited via the bloodstream resulting in increased blood oxygen level (Huettel et al., 2004). Therefore, when a group of participants show "increased BOLD" response, this indicates that, on average, those participants experience a rush of oxygen in the blood to a cluster of neurons in a similar location, and the deoxygenated hemoglobin with magnetic properties is detected. A higher number of neurons recruited will result in an increased response.

fMRI cannot provide any useful information regarding a single individual, so a larger sample size of similar individuals is the only way to interpret any meaningful results. Due to the neurobiology of HRF, there are several caveats when considering fMRI design (Friston, Holmes, Poline et al., 1995; Henson, 2006). fMRI study design can have a large influence on results, and paradigms can be analysed via blocks or event-related designs (Amaro & Barker, 2006). In clinical samples, a block design is generally preferred as it involves averaging the BOLD response over a series of events and comparing it to other blocks of events, which increases statistical power and allows a smaller margin of error (Carter, Heckers, Nichols,

Pine, & Strother, 2008) . Event-related design requires precise timings across all participants as it measures the peak BOLD response to seeing an event. If timings are precise across all individuals, and there is no overlap in onset of a new event, this is fine. However, this is often a challenge and events in the paradigm can be poorly placed, which can be challenging for analysis because if new events are presented before 20 seconds, determining what the participants are actually responding to may prove difficult. Additionally, after 20 seconds it becomes a challenge to determine if a response is due to scanner drift, or external noise, or if it is indeed a true response (Aguirre, 2010). It is also important to pre-process the data to a high standard, and use appropriate statistical threshold to view results in order to balance risk for type I and type II errors (Lieberman & Cunningham, 2009). Whilst fMRI can provide information on which regions are activated separately during a task, functional connectivity shows how regions are transferring signals either during a task or during rest (Friston & Buchel, 2004).

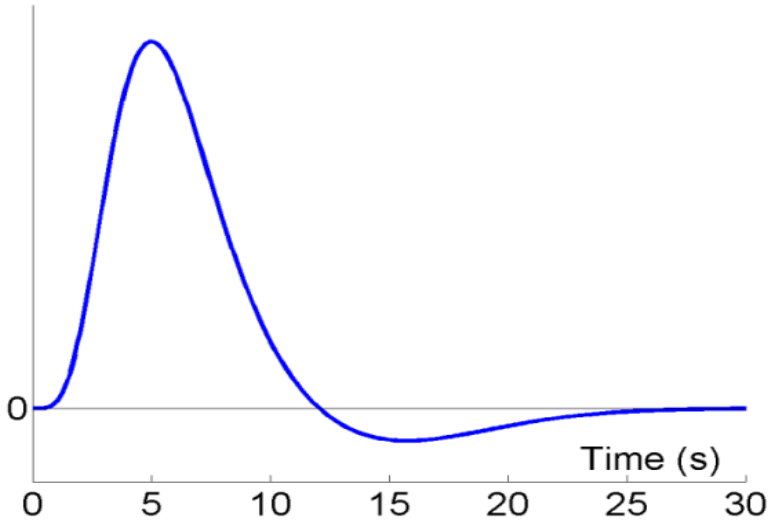



*Figure 3.3 Time course of the hemodynamic response function*

Based on previous studies, an empathy for pain fMRI task was used in this thesis. This task has been well validated and used in previous studies on healthy adults (Gu et al., 2010) and youths with conduct problems (e.g. Lockwood et al., 2013).

### *3.3.1 Functional MRI pre-processing*

Several pre-processing steps and quality control methods were used in accordance with typical fMRI analysis procedures to increase validity and standardisation of the data, taking into account the influence of external noise and physiological artefacts such as influence of data acquisition (e.g. scanner drift and thermal noise), and individual differences such as amount of movement.

After discarding the first 5 dummy scans for each participant, the remaining functional volumes were pre-processed within SPM12 (http://www.fil.ion.ucl.ac.uk/spm/), using Matlab. Voxel displacement maps were then created using the processed fieldmaps. To reduce the influence of individual movement during scanning, all functional scans were realigned to the reference scan in the time-series using rigid body transformation, producing a mean EPI for each participant. Mean realigned and unwarped EPIs were co-registered to the original T1 anatomical scans to allow for the final stage of normalisation to MNI space.

The normalisation procedure registered images from all participants to the same coordinates to ensure recognised voxels were the same regions for everyone. A customised tissue probability map was created within the Template-O-Matic toolbox (Wilke et al., 2008) to help account for the use of child data, which is an important step as previous fMRI studies on CD have not always included a customised TPM, and this could contribute to a mismatch between the recognised MNI coordinates and brain templates. The customised TPM accounted for age and sex of the participants, and matched the participant data with other paediatric brains

resulting in a specialised brain template to include in the next step, segmentation. All T1 images were segmented into grey and white matter using the VBM8 toolbox (Gaser, 2009), and individual native-space grey matter and white matter segments were normalised to the TPM using affine registration. A Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL; Ashburner, 2007) template was then created using the segmented grey and white matter images. All pre-processed image volumes were smoothed with a 6mm full-width/half-maximum kernel to increase signal-to-noise ratio (SNR), and final smoothed unwarped images were used for the first and second level analyses. All EPI volumes were also visually inspected, and any with excessive motion were discarded. Excessive motion was deemed to be more than 20 volumes, or having greater than 10% of volumes as outliers. To account for motion during scanning, artefact repair toolbox (ART; http://www.nitrc.org /projects/ artifact_detect/) was used for each participant, with a threshold of 1.5 mm. Any motion regressors detected by ART beyond 1.5 mm in terms of translation or rotation were included as covariates in all participants' first level GLM design.

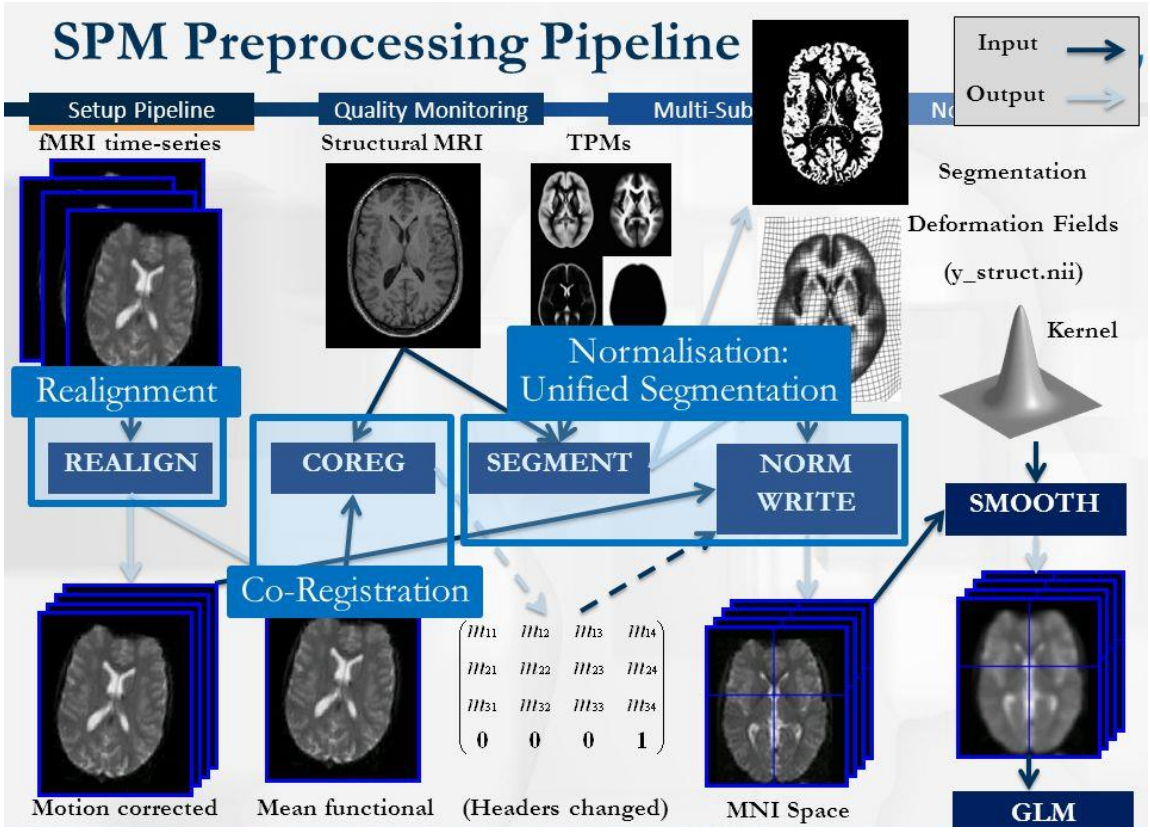



*Figure 3.4 fMRI pre-processing pipeline*

### *3.3.2 Functional MRI data analysis*

Following pre-processing of fMRI data, subject-specific first level general linear models (GLMs; Friston et al., 1994) were created consistent with block design including experimental conditions of pain, no pain, and fixation. Contrast images were created at the first level to assess the effect of 'pain > no pain' 'no pain > pain' 'pain > fixation' 'no pain > fixation'. Parameter estimates from each contrast were then used for second level analysis adopting a full factorial 2 (Group: CD vs TD) x 2 (Sex: Females vs Males) x 2 (Scanning site: Birmingham vs Southampton) design (ANCOVA) in SPM12, with age and IQ entered as covariates of no interest. After estimation of the models, results were then viewed at the whole brain level in addition to hypothesised regions of interest (ROIs), which were

anatomically defined using the aal Atlas of the Wake Forest University PickAtlas (Maldjian et al., 2003). The ROIs included, bilaterally, the anterior insula, amygdala, anterior cingulate cortex, orbitofrontal cortex, and ventromedial prefrontal cortex. ROIs were specified based on previous research identifying differences between youths with and without conduct problems during empathy-related tasks (e.g. Lockwood et al., 2013), and specific MNI coordinates reported in Lockwood's paper were used for the AI.

### *3.3.3 Functional connectivity*

fMRI analyses showed that compared to TD youths, those with CD had reduced activation in the left AI and cerebellum when processing stimuli depicting painful stimulation in others. To follow up on those results, functional connectivity between these regions of interest and the whole brain were conducted. Functional connectivity analyses provide information regarding interactions of neural regions and network organisation (Friston & Buchel, 2004). This is consistent with the shift from *functional localisation* to *functional integration* in human brain mapping, which has yielded a significant redirection of focus, and inclusion of connectivity in current neuroimaging research (Friston, 2011).

Functional integration can be divided into the two categories of functional connectivity and effective connectivity (Friston, 2011). Effective connectivity refers to the influence one brain region has on another and describes coupling between regions based on activity-dependent changes, whereas functional connectivity refers to correlations, but implies no information regarding mediation or causality (Friston, 2011). There are different techniques to measure functional connectivity, for example Granger Causality (Roebroeck, Formisano, & Goebel, 2005), Structural Equation Modeling (McIntosh & Gonzalez-Lima, 1994), or Dynamic Causal Modeling (Friston, Harrison, & Penny, 2003). This thesis used psychophysiological

interaction (PPI), which provides information on interactions between two regions activated simultaneously during fMRI (Friston et al., 1997; O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012), rather than identifying functional localisation as fMRI studies do. The connectivity between regions in the brain during the fMRI task (psychological term) and time series information from the specified seed regions (physiological term) creates the psychophysiological interaction of interest. The interaction term provides information regarding connections between regions as well as how certain regions can alter connectivity in a particular context such as a specific task's condition (e.g., pain vs no pain stimuli); (O'Reilly et al., 2012). PPI analyses are commonly used as a measure of functional connectivity following fMRI results (Table 8.3), and therefore serve as a useful tool to investigate task-based functional connectivity. However, in contrast to dynamic causal modeling, which is used to measure effective connectivity to make predictions regarding seed region input, PPI does not make such inferences (Friston et al., 2003). Finally, it is worth noting that other imaging modalities such as resting state fMRI are useful to examine connections during resting periods or non-activity related experiences, but these do not enable researchers to investigate how experimental manipulation influences connectivity (Friston, 2011).

### *3.3.4 Functional connectivity pre-processing*

fMRI results in this thesis revealed main effects of diagnosis (TD > CD/ pain > no pain) in the cerebellum (x = -9, y = -60, z = -33) and left anterior insula (x = -33, y = 24, z = 3). These two regions were used as seed regions for the PPI analysis. Several pre-processing steps and quality control methods were used in accordance with typical fMRI analysis procedures to increase validity and standarisation of the data, taking into account the influence of external noise and physiological artifacts such as influence of data acquisition (e.g. scanner drift and

thermal noise) and individual differences such as amount of movement. After discarding the first 5 dummy scans for each participant, the remaining functional volumes were pre-processed within SPM12 (http://www.fil.ion.ucl.ac.uk/spm/), using Matlab. Voxel displacement maps were then created using the processed fieldmaps. To reduce the influence of individual movement during scanning, all functional scans were realigned to the reference scan in the time-series using rigid body transformation, producing a mean EPI for each participant. Mean realigned and unwarped EPIs were co-registered to the original T1 anatomical scans to allow for the final stage of normalisation to MNI space. The normalisation procedure registered images from all participants to the same coordinates to ensure recognised voxels were the same regions for everyone.

A customised tissue probability map was created within the Template-O-Matic toolbox (Wilke et al., 2008) to help account for the use of child data, which is an important step as previous fMRI studies on CD have not always included a customised TPM, and this could contribute to a mismatch between the recognised MNI coordinates and brain templates. The customised TPM accounted for age and sex of the participants, and matched the participant data with other paediatric brains resulting in a specialised brain template to include in the next step, segmentation. All T1 images were segmented into grey and white matter using the VBM8 toolbox (Gaser, 2009), and individual native-space grey matter and white matter segments were normalized to the TPM using affine registration. A Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL; Ashburner, 2007) template was then created using the segmented grey and white matter images. All pre-processed image volumes were smoothed with a 6mm full-width/half-maximum kernel to increase signal-to-noise ratio (SNR), and final smoothed unwarped images were used for the first and second level analyses. All EPI volumes were also visually inspected and those with excessive motion

were discarded. Excessive motion was deemed to be more than 20 volumes, or having greater than 10% of volumes as outliers. To account for motion during scanning, artifact repair toolbox (ART; http://www.nitrc.org /projects/ artifact_detect/) was used for each participant, with a threshold of 1.5 mm. Motion regressors from ART were included in each participant's first level GLM design.

#### *3.3.5 Functional connectivity data analysis*

Within the first level fMRI design, new F contrasts were created for the effects of interest (pain, no pain, and fixation) for both seed regions (cerebellum and insula). Volumes of interest (VOI), or Eigenvariates, including time series information regarding effects of interest were then extracted from first level fMRI results for each participant with a sphere of 3 mm placed around the VOI. The VOI for each participant was entered into the PPI toolbox in SPM12 to generate new PPI regressors: *ppi* (psychophysiological interaction term), *y* (VOI, including time-series BOLD information), and *p* (psychological term, including task-based contrast information on pain > no pain). First level general linear models were then specified for each participant, with the new regressors including *ppi, y, and p.* After estimation of all first level designs, contrasts were created to look at the effect of the ppi interaction term. The PPI regression contrasts were then entered into a second level two-sample t-test (TD vs. CD) in SPM12 with age, IQ, scanning site, and sex as covariates of no interest. Based on previous results, ROIs were identified as the ACC, AI, amygdala, and thalamus. Exploratory analyses were also conducted at the whole brain level.

### 3.4 Considerations for use of MRI in research on clinical populations

There are several advantages and disadvantages of using MRI when researching clinical populations (Malhi & Lagopoulos, 2007). Benefits include identification of biomarkers, use

of “gold standard” technology for biological evidence of psychiatric disorders, and reduction in participant errors influencing results. Disadvantages include the relatively recent introduction of MRI for research purposes, software and statistical applications, and interpretation of results for clinical implications.

Firstly, psychiatric disorders are elusive because unlike many other medically recognised disorders, the signs and symptoms are often not visible or do not have a standardised medical test to categorise healthy or non-healthy. A substantial body of behavioural evidence exists to provide support for mental health disorders. However, biomarkers are crucial as they help determine cause of a disorder (Phillips, 2012). For example, when an individual has a broken bone, an x-ray and physical exam can quickly help identify location of the problem, diagnosis, and a specialised solution. Psychological biomarkers help clinicians to treat patients in a similar way, as understanding the cause of an issue can lead to improved strategies for prevention and intervention, even prior to adulthood (Venkatasubramanian & Keshavan, 2016).

Secondly, technological advancements have aided researchers and clinicians in identification of biomarkers, and MRI is considered the “gold standard” for psychology research (Savitz, Rauch, & Drevets, 2013). For many years psychologists had to depend on self-report and behavioural evidence. However, scientific breakthroughs have enabled researchers to adopt methods to measure biological evidence including heart rate and skin conductance signals, MR images, and EEG signals. MRI is considered by some as the best tool available for insight into the inner workings of the human brain (Linden & Fallgatter, 2009) .

Furthermore, MRI allows for less human interference. Although researchers may have to correct for motion, which is often the largest external artifact, or lack of attention whilst in

scanner, a MRI image is objective and the individual cannot bias an anatomical image of their brain (Phillips, 2012). Whilst the above sections cover clear benefits of MRI to research biomarkers amongst clinical populations, there are important limitations, which must be carefully considered during analysis and interpretation of MRI data.

First, MRI is a relatively recent methodology for research in psychology. It has clear clinical utility in hospital settings as it can show atrophy and identify blood flow complications in the brain. However, only in the past two decades have scientists begun using MRI for experimental research, and there is still plenty to investigate at a basic level, especially in terms of statistical analyses, and what can be derived from fMRI results. fMRI results from cross-sectional datasets only provide a limited amount of information, therefore it is important to collect longitudinal data to systematically investigate functional responsivity (Madhyastha et al., 2017) .

Statistical software packages for MRI data analysis have many versions (e.g. SPM 12), which are based on complex algorithms and precise mathematics. This is often beyond the scope of typical training a psychologist receives, making researchers in the life sciences dependent on multi-disciplinary teams including physicists, engineers, and statisticians. This in itself is not a limitation, but it is important to remember the complexity of statistical analyses on MRI data. This variety of Statistical software packages for MRI data analysis can also account for lack of replications and variations in results between studies investigating particular construct or clinical group (Pauli et al., 2016). New software development and toolboxes will allow for more valid replication of previous findings, and a more fine-grained approach, as has been the case for the past decade.

Finally, the largest limitation of MRI is the fact that MRI cannot be used as a clinical diagnostic tool for psychiatric disorders (Weinberger & Radulescu, 2016). There is still a significant amount of research yet to be conducted on MRI and interpretation of BOLD response, particularly task-based fMRI, and whilst there have certainly been advancements in regards to aberrant network connectivity and biomarkers, definitive clinical utility of MRI for psychiatric groups is not yet possible.

## 3.5 Conclusions

This chapter has reviewed applications of structural and functional MRI for research on psychiatric populations. First structural MRI was discussed, with details of VBM and structural covariance including pre-processing and data analysis steps. Next, functional MRI was discussed with a particular focus on fMRI and PPI analyses, which were used in this thesis. Other methodological approaches were also briefly discussed, and methodological considerations for use of MRI for research on psychiatric populations were covered. The following four chapters will include studies focusing on the structural and functional MRI data collected from youths with and without CD.

# CHAPTER 4. FEMNAT-CD: PROJECT DESIGN, MEASUREMENTS, AND INDIVIDUAL CONTRIBUTION

## 4.1 Project Design

The Neurobiology and Treatment of Adolescent Female Conduct Disorder project, or FemNAT-CD (http://www.femnat-cd.eu/), is a European multi-site study the aim of which is to investigate potential sex differences amongst youths with CD by focusing on neurobiological, psychophysiological, genetic, and environmental components, in addition to treatment trails (Freitag, 2014). Across Europe there are six different work packages. In the United Kingdom the University of Birmingham and the University of Southampton are responsible for work package 6 (Fairchild & De Brito), which focuses on the functional and structural neuroimaging correlates of CD using MRI techniques.

## 4.2 Individual Input

The initial design and grant proposal for the project were completed by researchers leading the work packages at each site. As a PhD student, sponsored by the University of Birmingham College of Life and Environmental Science, I began working on the project after funding for the grant proposal was approved and as such I was not involved in the study design. Once in post my input included initial contact with recruitment sources, creation of scoring materials, and ordering of supplies. All data collection in Birmingham was completed by myself and colleagues from the University of Birmingham, and colleagues from the University of Southampton collected data in Southampton and Reading. Data sharing is central to the project, which is why half of the data included in the thesis comes from the University of Southampton.

## 4.3 Ethics

Ethical approval by the National Health Service (NHS) Research Ethics Committee was granted in January 2014, and data collection started on February of that year (NRES Committee West Midlands –Edgbaston; REC reference 13/WM/0483). All individuals included in the study under the age of 16 were required to provide assent, and also received parental consent for participation. Individuals aged 16 and above provided consent for themselves. Consent forms for the parents/caregivers were also obtained at the same time for their own participation. Participants were informed they were entitled to discontinue at any time.

## 4.4 Recruitment

All participants were recruited through a variety of sources in the community. Typically developing youths were recruited through mainstream schools and local youth groups. Youths with CD were recruited through Children and Adolescent Mental Health Services (CAMHS) within the National Health Service (NHS), youth offending services, charities working with at-risk youths, and schools for youths with social, emotional and behavioural difficulties. Visits by the study team were conducted at the recruitment sites to describe the project to staff and potential participants, during which information sheets and invitation letters were provided for parents/caregivers of the youths. Birmingham data were collected by me and my colleagues over the course of three sessions either at the participants' home or at the university. During the initial session, I obtained assent/consent from the participant and the parent/caregiver where appropriate. I then administered an IQ test, completed a semi-structured interview with the participant, and carried out MRI screening. During the second session, I collected questionnaire data from the participants, and the third session took place at the Birmingham University Imaging Centre to collect MRI data, including structural and

functional images. Participants, both youths and parents, were paid for taking part and the youths were also given a printed image of their brain after completion of the scanning session.

### 4.5 Assessment measures

Phone screening was completed with the participants, or with the parent/caregiver for youths under the age of 16, before scheduling an initial visit. During the phone screen age, sex, and current school information was collected in addition to questions about current or past mental illness, brain trauma, and eligibility to complete a MRI visit. Further screening and assessments were completed at the participants' homes or at the university to ensure all participants met the inclusion criteria, and none of the exclusion criteria. Table 3.1 provides information regarding inclusion / exclusion criteria for all participants. Inclusion criteria included a full-scale IQ ≥ 70, no current psychiatric illness for healthy controls, and no history of externalising disorders (e.g. ODD, CD, and ADHD). Exclusion criteria for all participants included a full IQ < 70, presence of serious psychiatric or developmental disorders including Autism, Bipolar, Schizophrenia, developmental disabilities, or genetic disorders. Neurological disorders, history of traumatic brain injuries, and inability to speak or understand English were also exclusion criteria. Female participants were also excluded if there was any possibility they could be pregnant. Only one child per family was accepted into the study to avoid any biases in the genetic analyses component of the FemNAT-CD study. Substance / drug abuse was screened for via the K-SADs, and only typically developing participants were excluded if they met thresholds for past or present substance abuse. Meticulous screening and assessment of participants was a crucial element of the study as it was a case-control study. Based on sample sizes from a previous study (Fairchild et al., 2013), power calculations were conducted to predict number of necessary participants. The effect size (f = .4) was calculated based on mean grey matter volume from the AI in the TD males

and females and CD males and females. On this basis, a total sample of 76 (19 per group) is required to detect group differences with 80% power and alpha set at .05. (analysis performed using G*Power 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007). Measurements used included semi-structured diagnostic interviews administered to both youths and parents, IQ testing, and self-report questionnaires assessing CU traits and empathy levels. The following sections will review the assessment and measurement tools used in each experimental chapter of this thesis.

*Table 4.1 Inclusion and exclusion criteria*

| **Criteria for all participants** | |
|---|---|
| *Inclusion* | *Exclusion* |
| Aged 9-18<br><br>IQ ≥ 70<br><br>No neurological disorders (e.g. cerebral palsy, motor problems due to motor or metabolic disorder, current treatment for epilepsy)<br><br>No traumatic brain injuries (defined as loss of consciousness for more than 1 hour)<br><br>Only one sibling per biological family<br><br>Speaks English | Monogenetic disorder, genetic syndrome (e.g. fragile-X-syndrome, Down's syndrome, Prader-Willi-syndrome metabolic disorder)<br><br>History of or current clinical diagnosis of Autism Spectrum Disorder (includes "Autism", "Asperger Syndrome", "Pervasive development disorder NOS", "Atypical Autism", and "Autism Spectrum Disorder"); Schizophrenia; Bipolar Disorder or Mania according to ICD-10, DSM-IV TD or DSM-5 criteria |
| **Additional criteria for typically developing participants** | |
| *Inclusion* | *Exclusion* |
| Not meeting current DSM-IV or DSM-5 criteria for any psychiatric disorder as assessed with the K-SADS-PL | |
| **Additional criteria for clinical participants** | |
| *Inclusion* | *Exclusion* |
| Diagnosis of CD; Co-occurring psychiatric disorders are acceptable (Depression, Anxiety, PTSD, ADHD, ODD) | Does not meet full threshold for CD or externalising behaviours and/or only meets thresholds for other psychiatric disorders; Current substance abuse |
| **Additional MRI exclusion criteria for all participants** | |
| Pregnancy or having recently given birth within last year<br><br>Metal embedded in body that cannot be removed (including piercings, braces, or ankle monitors)<br><br>History of claustrophobia (mock scanner available for participants to check comfortability prior to scanning session)<br><br>Significant visual impairment (all participants should be able to see stimuli in scanner with normal vision or corrected to normal vision) | |

### *4.5.1. Kiddie Schedule for Affective Disorders and Schizophrenia - Present and Lifetime version (K-SADS-PL; Kaufman et al, 1996)*

The K-SADS was the main instrument used to assign participants to either the CD or TD groups, and interviews typically lasted between one or two hours per participant. It is a well-validated semi-structured diagnostic interview created for assessment of past and current mental health disorder based on clinical thresholds according to DSM-IV criteria, wherein both the child and the parent report child behaviour and symptomology (Kaufman, Birmaher, Rao, 1996). DSM-5 classifications are used in research on clinical populations as many journals publish internationally recognised research based on these criteria supported by the APA. Importantly, the DSM has includes criteria on social consequences. Social impairments found in mental disorders are often a catalyst for further investigation into etiology, and the use of DSM criteria allows for this. Recently, mental health diagnoses have been shifting toward dimensional rather than categorical classifications, especially in consideration of personality disorders (Rutter, 2011), and advances in research have provided more neurobiological support for classification of mental illness, e,g, the Research Domain Criteria (RDoC) approach adopted by the NIH to provide evidence for biological indices of symptomology (e.g. Blair, White, Meffert, & Hwang, 2014). Future versions of the DSM and ICD will likely reflect these changes.

During the K-SADS semi-structured interview, participants are asked a series of screening questions regarding present or past symptoms and experiences related to every possible disorder. For each symptom within a disorder the individual is given a score of ‘3’ if the symptom is present and reaches the identified clinical threshold, a ‘2’ if the symptom is present but is sub-threshold, or a score of ‘1’ if the symptom is not present. The symptoms are scored for present, past 12 months, and lifetime. If the participant endorsed any of the

symptoms with a '3' for diagnoses not central to the study design, the interviewer completed the full K-SADS supplement for that specific disorder. However, full supplements for CD, ODD, and ADHD were completed for all participants. Importantly, symptoms or disorders can be endorsed by either the participant or the parent, meaning both interviewees do not have to be in agreement about the presence or absence of a behaviour, and the highest score (i.e. '3' versus '1') will be marked as the determinate when both interviews are compared. After comparing both interviews against one another, a summary is made using the highest scores, and a final research diagnosis is assigned. Inter-rater reliability analyses showed 92.0% agreement between two independent raters from a sub-sample of 16 participants from Birmingham (n=8) and Southampton (n=8). Participant interviews were completed by myself and other doctoral level trained colleagues, and the inter-rater reliability assessment was completed by myself and a colleague at the Birmingham site. After completion of the interviews, TD and youths with CD were separated into control or case groups respectively, and participants who did not meet criteria were excluded. The summaries took up to half an hour and were completed immediately after interviews concluded with both the child and the parent, with a total time of ~1- 2.5 hours from start of interview to final decision regarding diagnosis and inclusion.

#### *4.5.2 Wechsler Abbreviated Scale of Intelligence first edition (WASI; Wechsler, 1999)*

The WASI is a standardised measurement of IQ and consists of four tests: vocabulary, matrix reasoning, similarities, and block design. The two-subscales version (vocabulary and matrix reasoning skills) of the WASI was used in this thesis to provide an estimate for full-scale IQ. This was in accordance with previous research for consistency and comparability of various samples, as the WASI has been used in several publications focusing on the CD population (e.g. Fairchild et al., 2009; White et al., 2014). The WASI is a valid measurement

of estimated IQ, and is significantly correlated with the full WISC with inter-correlations ranging from .76-.87 on full, verbal, and performance scales (Scott, Austin, & Reid, 2007). The vocabulary subscale of the assessment requires participants to define increasingly difficult words, and are assigned '2','1', or '0' for the quality of their response. The matrix reasoning subscale requires participants to choose the correct item to complete a pattern, and receive '1' for correct or '0' for incorrect. The responses are then summed and the raw scores are converted to t-scores. Based on age and the t-score, a standardised IQ score is assigned. During both tests participants are given an unlimited response time although they generally respond within 10-30 seconds. Overall this assessment lasts approximately 15 minutes per participant. It is established IQ scores do differ significantly between youths with and without antisocial behaviour, and is a particularly robust finding amongst children with early-onset antisocial behaviour compared to adolescent-onset (Moffitt, 1993). This could be due to a combination of influences on genetics, neurobiology, and environment resulting in impaired neuropsychological functioning (Koenen, Caspi, Moffitt, Rijsdijk, & Taylor, 2006; Moffitt, 1993). Furthermore, language deficits are common amongst behaviourally disordered individuals (Moffitt, 1993).

#### *4.5.3. The Inventory of Callous-Unemotional Traits (ICU; Frick, 2003)*

The ICU is a parent-report questionnaire used to assess callous-unemotional traits in children. Development of this scale was based on extension of psychopathy research on adults, designed to specifically investigate the callous/unemotional facet of psychopathy amongst children. Four items were chosen from the CU subscale of the Antisocial Process Screening Device to create a foundation for the questionnaire (Frick & Hare, 2001). The ICU can be separated into three subscales: callousness, uncaring, and unemotional. The callousness subscale includes items such as "does not care who he/she hurts to get what

he/she wants." The uncaring subscale includes items such as "I try not to hurt others' feelings" (an example of a reversed scored item), and the unemotional subscale includes items such as "I hide my feelings from others". This questionnaire has been well-validated across different samples and cultures (Essau, Sasagawa, & Frick, 2006; Kimonis et al., 2008). The ICU parent version is a likert-scale 24-item assessment of behaviours and responses range from '0' (not true at all) to '3' (definitely true). Based on the four initial items used to develop the measure, three positively worded items and three negatively worded items were formulated resulting in the total of 24 items. Sub-scores are calculated for callousness, uncaring, unemotional, and total score, and the maximum possible total score is 72. Both parent and child versions were collected from the participants, however scores from the parent-report questionnaires are the preferred choice as self-report questionnaire responses tend to be more biased. The ICU has influenced inclusion of the "limited prosocial emotion" specifier in the DSM-V, however there is not a standardised assessment used yet, which is problematic for replication of results in research samples. The Clinical Assessment of Prosocial Emotions (CAPE 1.1; Frick, 2013) is currently being piloted and can be then used as a standardised clinical assessment of CU traits to meet DSM criteria for limited prosocial emotions specifier.

### *4.5.4 Youth Psychopathic Traits Inventory (YPI; Andershed et al., 2002)*

The YPI is a self-report measure of psychopathic traits in children and adolescents which measures the following three dimensions of psychopathy: grandiose manipulative, callous-unemotional, and impulsive irresponsible. These three dimensions are the key domains in psychopathy amongst adults. This is a 50-item questionnaire with ten subscales: dishonest charm, grandiosity, lying, manipulation, remorselessness, unemotionality, callousness, thrill-seeking, impulsivity, and irresponsibility. Participants respond to items on a 4-point Likert

scale, with options ranging from “does not apply at all” to “applies very well.” The questionnaire is reliable, with internal consistency for the subscales ranging from .66-.82, and is suitable for use with males and females within the community (Andershed et al, 2000) as well as delinquent adolescents (Skeem & Cauffman, 2003). Limitations of the YPI include bias from self-report, and since the questionnaire was originally designed for use with adolescents, or youths aged 12 and over (Andershed et al, 2000), it may be more difficult for younger participants under the age of 12 to resonate with and respond to the items.

### *4.5.5 Griffith Empathy Measurement (GEM; Dadds et al., 2008)*

The GEM is a parent-report questionnaire for assessment of affective and cognitive empathy levels in children (Dadds et al., 2008). The GEM is a likert-scale 23-item assessment of empathic responses ranging from ‘-4’ (strongly disagree) to ‘4’ (strongly agree). A total empathy score is calculated based on computing the sum of items from both affective and cognitive subscales and dividing by total number of items, resulting in a mean score. This measurement is based on Bryant’s Empathy Index for Children and Adolescents with changes from self-report to parent-report (Bryant, 1982), and examples of items include affective components such as “My child becomes sad when other children are sad” and cognitive components such as “my child can’t understand why other people get upset”, which can be divided into two factor or full item solutions. It has been validated and can be used across different ages and genders (Dadds et al, 2008).

### *4.5.6 Interpersonal Reactivity Index (IRI; Davis 1980)*

The IRI is a 28 item self-report questionnaire to assess empathy via a multi-dimensional approach with 4 subscales: perspective taking, fantasy, empathic concern, and personal distress. *Perspective-taking* refers to the tendency to adopt the view of others, for example “I sometimes find it difficult to see things from the other guy’s point of view”. *Fantasy* refers to

the tendency to transpose themselves imaginatively into the feelings and actions of fictitious characters in books, films, and plays, for example “I get really involved with the characters of a novel” (FS). *Empathic concern* refers to the “other-oriented” feelings of sympathy and concern for unfortunate others, for example “Sometimes I don’t feel very sorry for other people when they are having problems” (EC). *Personal distress* refers to “self-oriented” feelings of personal anxiety and unease in tense interpersonal settings, for example “When I see someone who badly needs help in an emergency, I go to pieces” (PD). Each subscale consists of 7 items, and responses are scored according to a 5-point Likert scale with options ranging from “does not describe me well” to “describes me very well.” The subscales have been proven reliable, with test-retest scores ranging from .62-.71 and internal reliability scores ranging from .71-.77. Significant sex differences have been identified as females consistently score higher than males on the four subscales (Davis, 1980; Tello et al., 2013).

### 4.6 Site qualification procedures: K-SADS and MRI

Both sites completed inter-rater reliability (IRR) assessments on the K-SADS interviews to ensure comparability of diagnostic measurement. Eight participants agreed to have two researchers in the room at once during the semi-structured interviews (three TD; five CD). This included having both researchers score the interview responses simultaneously and comparing results after for accuracy. There was a strong agreement between interviewers at both Birmingham and Southampton (92%).

MRI scans were completed at two different sites: Birmingham University Imaging Centre (BUIC) using a Phillips 3 Tesla scanner, and the Centre for Neuroscience and Neurodynamics (CINN) at Reading University using a Siemens 3 Tesla scanner, with a 32-channel head coil at both sites. Both sites had similar scanning parameters and underwent site qualification procedures by on-site physicists to ensure comparability. These included quality assurance

checks using an American College of Radiology (ACR) phantom, a Functional Biomedical Informatics Research Network phantom, and a human volunteer. The ACR phantom is designed to assess structural MRI sequences whereas the FBIRN is designed to assess scanning stability during functional MRI sequences, providing information regarding scanner drift, fluctuation in signal, signal-to-noise ratio and signal-to-fluctuation noise. The separate data-sets underwent quality assurance by Ali Chowdhury, a MRI physicist at Birmingham University Imaging Centre, whereby he adjusted parameters accordingly. MRI data acquisition began once physicists from both locations approved the site qualification procedures.

All participants underwent MRI screening assessments, and provided history of traumatic brain injury, surgeries or operations, metal inserts or foreign artefacts in or on the body, claustrophobia, and presence of removable piercings or tattoos. Participants recruited from the youth offending service also had to be screened for presence of ankle tracking devices. If participants successfully passed the MRI screening interview, they were invited for a scan where structural and functional images were collected. All participants filled in paperwork upon arrival to provide consent for scanning, and they were briefed on MRI safety regulations. After removal of metal and personal items, a metal detector was used for an additional check. Participants were also allowed to first spend time in a mock scanner to ensure they were comfortable with the procedure. Before entering the scanner, participants completed practice tasks on a laptop in the waiting area to ensure they understood fMRI task instructions. Participants were advised of the procedure if they wished to leave the scanner at any time, and they were instructed to move as little as possible and to not cross arms or legs. All scans were completed by two individuals including one fully trained scanner operator and one student operator. For continuity and rapport purposes, the participants were usually

scanned by the same individuals who completed the K-SADS interview with them. Throughout the scanning session, one of the operators spoke to the participant over the intercom to ensure they were comfortable and paying attention. Participants were also given reminders about task instructions. The scanning session typically lasted approximately 1.5-2 hours per participant.

## 4.7 Conclusion

This chapter reviewed the FemNAT-CD project, my individual contribution, assessment measures, and site qualification procedures. As stated, I joined the project after initial design, but all data included in this thesis was collected by myself and colleagues working on the project. After initial screening, participants completed three sessions in total. However, only a subset of the data collected is included in this thesis. The following chapters will cover experimental analyses from a subsample of youths from the project to address sex differences in neuroimaging correlates of CD and empathy.

# CHAPTER 5. GREY MATTER CORRELATES OF CONDUCT DISORDER: INVESTIGATING POTENTIAL SEX DIFFERENCES

## 5.1 Introduction

As discussed in the introductory chapters, behavioural and experimental studies on youths have identified a number of impairments associated with CD. Recent neuroimaging research has sought to discover neural correlates of these impairments. As discussed in chapter 2, fMRI studies have shown that youths with CD exhibit abnormal BOLD response in a number of brain regions during affective and cognitive processing (Alegria, Radua, & Rubia, 2016). In particular, this research indicates that key features of CD such as difficulties with emotion recognition based on facial expressions, reduced empathy, aggressive tendencies, as well as decision-making and reward processing may have neurobiological underpinnings. Hypoactivation in the amygdala in response to emotional faces, particularly fearful expressions, has been found to be more pronounced amongst youths with CP compared to TD youths (Jones, Laurens, Herba, Barker, & Viding, 2009; Passamonti et al., 2010). Evidence exists to suggest youths with conduct problems have reduced empathy, indicated by decreased BOLD response in regions associated with empathy, namely the anterior insula, anterior cingulate cortex, and inferior frontal gyrus whilst viewing others in pain (Lockwood et al., 2013). In contrast, other studies on youths with aggressive and more severe forms of CD have reported these youths show increased activation in the pain matrix (insula, anterior cingulate cortex) relative to TD youths (Decety et al., 2009). Support from meta-analyses, including data from nine functional and eight structural imaging studies, suggests there are differences in limbic, temporal, and prefrontal cortices between children with aggressive, disruptive behaviours and TD children (Raschle et al., 2015). Additionally, a recent meta-analysis of 24

different fMRI studies on youths with disruptive behaviour disorders showed decreased activation in the medial prefrontal cortex and the rostral and dorsal anterior cingulate, as well as the ventral caudate, relative to TD youths (Alegria et al., 2016). These fMRI studies provide useful insight into neural correlates of key features associated with CD.

Structural neural correlates of CD have since been explored within the key brain areas identified in fMRI studies employing a variety of techniques. Early studies examining structural correlates in youths with CD used manual tracing of regions of interest (ROI). For example, Bussing, et al. (2002) reported that youths with ADHD and comorbid CD had reduced grey matter volume in the posterior, superior, and inferior lobes of the vermis. Another study showed that youths with CD had grey matter reductions in the temporal lobe bilaterally (Kruesi, Casanova, Mannheim, & Johnson-Bilder, 2004). Other studies have reported that reduced grey matter volume in the anterior cingulate cortex is associated with aggressive and defiant behaviour amongst TD males, but not females (Boes, Tranel, Anderson, & Nopoulos, 2008). Consistent with those findings, reduced GMV in the ventromedial prefrontal cortex has been associated with low impulse control amongst typically developing males when focusing on amygdala, ACC, and vmPFC as regions of interest (Boes et al., 2009). However, manual tracing and automated ROI definition techniques suffer from a number of limitations. First, because manual measurement of ROIs is time-consuming, studies have generally relied on small samples. Second, these techniques are associated with increased difficulty in replicating results across studies, as structural region boundaries become increasingly challenging to define at different testing sites (Blair, 2009). Finally, these techniques require precise skill and knowledge regarding neuroanatomical location, and regions of interest must be large enough to have clear borders,

which is often not the case for very small structures such as the amygdala (Eckert et al., 2008).

A more recent method used for the current study is VBM, which is a popular way to conduct voxel-wise comparison of grey matter between groups (Ashburner & Friston, 2000). It is a helpful tool to differentiate grey matter from white matter, and while it has been criticised as not sensitive enough due to potential limitations such as misregistration (Bookstein, 2001) and displacement of tissue during segmentation (Good et al, 2001), to date it is one of the most statistically valid ways to preserve and compare total grey matter between clinical groups (Ashburner & Friston, 2001). There are ways to control for potential issues during pre-processing via use of the Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL; Ashburner, 2007) and customised tissue probability maps, which greatly reduces chances of the aforementioned errors. Unlike manual ROI tracing, automated morphometry allows for examination of differences in several regions across the brain all within one analysis (Eckert et al., 2005), and VBM is ideal for local regional differences due to the greater sensitivity compared to other techniques such as deformation based morphometry (Mechelli, Price, et al., 2005). The first published VBM study showed that compared to TD youths, those with CD had reduced GMV in the left amygdala and the anterior insula bilaterally, and that GMV in the anterior insula was positively correlated with empathy scores (Sterzer et al., 2007). Additional studies have identified reduced GMV in the left orbitofrontal cortex, amygdala, hippocampus, and bilateral temporal lobes in youths with CD (Huebner et al., 2008). Based on three recent meta-analysis results including grey matter volume studies, the regions that have been most consistently identified as reduced in volume in youths with conduct problems are the amygdala, insula, medial cingulate cortex, and temporal lobes (Raschle et al., 2015; Rogers & Brito, 2016).

More recently a number of studies have relied on surface-based morphometry, another whole-brain automated technique, which focuses on three different properties of the cortex: cortical thickness, cortical folding, and surface area (Greve, 2011). A number of SBM studies have shown that youths with CD exhibit: reduced cortical thickness in the temporal and parietal lobes in addition to the superior sulcus; reduced surface area in the orbitofrontal cortex; reduced gyrification in the insular cortex, orbitofrontal cortex, ventromedial prefrontal cortex, anterior cingulate cortex, orbitofrontal cortex, and temporal cortex (Hyatt et al., 2012; Wallace et al., 2014). Conversely, increased gyrification has found been in the insula amongst youths with CD relative to TD youths (Fairchild et al., 2015). These studies suggest that youths with CD have reduced cortical thickness and gyrification in regions generally identified as abnormal according to sMRI and fMRI studies. Interestingly the AI, a key region implicated in empathy (Gu et al., 2012), has repeatedly been identified in the literature (Sterzer et al., 2007), but the results to date have been inconsistent regarding direction of the effect. For example, Fairchild et al. found increased folding in the insula, whereas Hyatt et al. (2012) found reduced folding in this area. This could be related to differences in age (range: 16-21 years, $M_{age}$ = 18.5 compared to 12-18, $M_{age}$=16.2, respectively). Furthermore, these previous studies included small sample sizes so replication is needed with larger, age-matched samples.

Aside from small sample sizes, few studies have examined the influence of sex, with the majority of previous sMRI studies on CD primarily focusing on males. However a number of recent studies have provided preliminary evidence to suggest there may be sex differences regarding structural neural correlates of CD and aggressive behaviour. For example, both males and females with CD have shown reduced GMV in the amygdala compared to their TD peers, but a significant sex-by-group interaction in the anterior insula has been observed,

indicating that females with CD had reduced GMV bilaterally in the anterior insula compared to healthy control females, while males with CD had increased GMV in the left operculum/insula relative to healthy control males (Fairchild et al, 2013). Psychopathic traits amongst incarcerated females have been associated with decreased GMV in the orbitofrontal cortex, temporal poles, and hippocampus (Cope, Ermer, Nyalakanti, Calhoun, & Kiehl, 2014). These findings are consistent with those on males with high levels of psychopathic traits (Ermer, Cope, Nyalakanti, Calhoun, & Kiehl, 2013). Interestingly, Michalska et al (2014) did not initially find any significant differences in GMV between CD and TD youths in a large mixed sex sample (*n*= 53 males and 58 females). Furthermore, females with conduct problems and comorbid substance abuse have been found to exhibit reduced GMV in key decision-making areas such as the prefrontal cortex compared to healthy control females (Dalwani et al., 2015).

Related studies have focused on brain structure differences between healthy youths, or youths not diagnosed with a disorder as such, but divided between personality traits or behaviours such as low versus high levels of aggression. For example, higher levels of aggression have been shown to be associated with increased GMV in the hippocampus amongst TD females relative to TD males, whereas GMV asymmetry (greater right but smaller left) in the anterior cingulate cortex was associated with higher levels of aggression in TD males, but not TD females (Visser et al., 2014). Studies on grey matter volume in healthy populations support the idea that certain anatomical regions serve as endophenotypes for mental illness. A positive relationship has been found between amygdala volume, which is associated with fear processing (Adolphs, Tranel, & Damasio, 1998), and fearfulness among healthy girls, but not boys (van der Plas, Boes, Wemmie, Tranel, & Nopoulos, 2010). This implies two things: 1) sex differences exist in the amygdala, occurring separately from

clinical classification; and 2) brain volume and structure alone cannot provide information regarding diagnosis of a psychiatric disorder, as genetic and environmental influences on neuroanatomical properties of individuals are difficult to parse.. However, abnormally small or large grey matter volumes in certain brain regions could place an individual at risk, predisposing them to disruptive and maladaptive behaviours or psychiatric illnesses. Therefore, when a person is exposed to certain triggers or environmental stressors, this may cause abnormal functioning in their brain but may not necessarily impact brain structure. Chronic exposure to certain experiences such as maltreatment can alter GMV leading to atrophy (Lim, Radua, & Rubia, 2014). However, this also occurs in the other direction, in that chronic exposure to positive experiences such as learning (Maguire, Woollett, & Spiers, 2006), proper nutrition (Taki et al., 2010), and exercise (Killgore, Olson, & Weber, 2013) can increase grey matter volume. The amalgamation of positive experiences alongside individual personality traits can be protective, resulting in resilience. Neurogenesis throughout the lifespan allows for axonal myelination, meaning over time the developing brain forms new neurons and synaptic connections (Eriksson et al., 1998). This further supports the notion of neuroplasticity (Kolb & Gibb, 2011), which accounts for gene-environment interaction.

Overall, functional and structural MRI studies to date have shown that youths with CD have abnormal brain responses and structures compared to TD youths. Whilst meta-analysis evidence has shown GMV reduction across cortical and subcortical regions, there are still some inconsistencies suggesting replication is necessary with larger sample sizes. Crucially, very few studies have examined the influence of sex on the structural brain correlates of empathy in youths with CD. Therefore, the aim of the current study was to apply VBM to a large sample of sMRI data of males and females with CD to identify similarities and differences in GMV abnormalities between males and females. The two CD groups were

compared to age and sex-matched TD males and females. Region of interest and whole brain analyses were conducted. ROIs were selected based on recent structural meta-analytic evidence and reviews identifying those regions as abnormal in youths with CD compared to TD peers (Raschle et al., 2015; Rogers & De Brito, 2016; Noordermeer et al, 2016; Baker & Clanton et al, 2015), and as being implicated in empathy (Singer & Lamm, 2009). Those ROIs included bilaterally: the amygdala, anterior insula, anterior cingulate cortex, and ventromedial prefrontal cortex.

## 5.2 Methods

### *5.2.1 Recruitment*

A sample of 159 youths (67 females) aged 9-18 years of age ($M_{age}$=15.09, SD = 2.07) was recruited from a variety of sources within the community in Southampton (n=79) and Birmingham (n=80). Participants were divided into 4 groups: TD females (n=42), TD males (n=37), females with CD (n=25), males with CD (n=55). TD youths were recruited from mainstream schools and youth groups, whereas participants with CD were recruited from youth offending teams, schools for youths with emotional, social and behavioural difficulties, and Child and Adolescent Mental Health Services. Table 5.1 displays basic demographic information for the sample, and Table 5.2 provides sample characteristics including age, IQ, CD symptomology, ADHD symptomology, empathy scores, and YPI scores.

### *5.2.2 Assessment: Interview and Questionnaires*

All assessment instruments were reviewed in chapter 3. For this specific chapter, all participants were assessed via the Kiddie Schedule for Affective Disorders and Schizophrenia Present and Lifetime Version (K-SADS-PL; Kaufman et al. 1996) - a semi-structured interview with both the participant and the parent. Interviews were conducted by trained research students and staff, and inter-rater reliability on diagnoses was conducted across sites

to ascertain consistency across raters, as reported in the previous chapter. The vocabulary and matrix reasoning subtests of the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999) were administered to obtain an estimate of full-scale IQ. Medical history, including data on perinatal and participants first years of life, was collected from parents. Self-report empathy scores were also collected via the Interpersonal Reactivity Index (IRI).

*Table 5.1 Birmingham and Southampton participants' demographic and clinical characteristics*

| | Southampton (*n*=79) | Birmingham (*n*=80) | p values |
|---|---|---|---|
| | M (SD) | | |
| Age (years) | 15.16(2.07) | 15.03(2.08) | t(157) = -0.42, p= 0.67[a] |
| Total IQ | 95.24(13.82) | 95.85(11.74) | t(157)=0.30, p=0 .77[a] |
| Sex (% females) | 41% | 44% | p=0.68[b] |
| Groups (% CD) | 52% | 49% | p=0.69[b] |

[a] t-tests

[b] Chi-square

*Table 5.2 Demographic and clinical characteristics of the sample*

| | TD | | CD | | | | |
|---|---|---|---|---|---|---|---|
| Variable | TD Females 1 (*n*=42) | TD Males 2 (*n*=37) | CD Females 3 (*n*=25) | CD Males 4 (*n*=55) | *F*group (*p*) | *F*sex (*p*) | *F*groupXsex (*p*) |
| | M (SD) | | | | | | |
| Age (years) | 15.02(2.04) | 15.52(2.02) | 15(2.13) | 15.02(2.10) | F < 1 (0.62) | F < 1(0.55) | F= 0.41(.74) |
| Estimated IQ | 100.9(10.08) | 103.43(10.49) | 87.6(13.56) | 89.76(10.82) | F=55.29(<0.001) (TD > CD) | F < 1 (0.74) | F= 18.96(<0.001) (1&2 > 3&4) |
| Verbal IQ | 100.95(12.90) | 103.78(12.56) | 89.36(16.63) | 86.47(13.15) | F= 41.14 (<0.001) (TD > CD) | F= 1.67(0.20) | F=16.56(<0.001) (1&2 > 3&4) |
| Performance IQ | 100.38(11.52) | 103.38(10.89) | 85.60(15.17) | 93.60(12.94) | F=27.87 (<0.001) (TD > CD) | F=1.42 (0.23) | F=12.37(<0.001) (1&2 > 3&4) |
| Current CD Symptoms | 0.12(0.32) | 0.24(0.49) | 4.92(2.90) | 5.78(2.43) | F=324.45 (<0.001) (TD < CD) | F=10.45 (0.01) (F < M) | F=110.64(<0.001) (1&2 < 3&4) |
| Past CD Symptoms | 0.12(0.40) | 0.27(0.56) | 5.76(3.06) | 6.38(2.90) | F=318.71(<0.001) (TD < CD) | F=8.70(0.004) (F < M) | F=106.48(<0.001) (1&2 < 3&4) |
| Current ADHD Symptoms | 0.48(0.22) | 0.27(0.16) | 1.60(2.29) | 5.24(5.57) | F=50.62 (<0.001) (TD < CD) | F=15.74 (<.001) (F < M) | F=25.25(<0.001) (1, 2, 3 < 4) |
| Past ADHD Symptoms | 0.24(0.15) | 0.05(0.23) | 2.28(3.25) | 6.05(5.93) | F=60.91(<0.001) (TD < CD) | F=15.48(<.001) (F < M) | F=28.20(<0.001) (1,2.3 < 4) |
| Empathic Concern IRI | 18.83(3.88) | 17.62(3.49) | 19.08(4.85) | 12.56(6.47) | F=18.18 (<.001) (TD > CD) | F=25.63 (<0.001) (F > M) | F = 17.22(<0.001) (1,2,3 > 4) |
| Total YPI Score | 90.17(18.15) | 96.98(16.35) | 117.16(16.48) | 114.09(24.71) | F=45.78(<0.001) (TD < CD) | F=3.31(.07) (F < M) | F = 16.21(<0.001) (1&2 < 3&4) |

Note: ADHD = attention-deficit/hyperactivity disorder; CD = conduct disorder; TD=typically developing; IQ = intelligence quotient (measured using the WASI). Group, sex, and sex-by-group interactions were computed using between-group ANOVAs and Chi square tests. Bonferroni correction was used to make pairwise comparisons.

### *5.2.3 MRI Acquisition*

High resolution T1-weighted scans were acquired using similar acquisition sequences on 3T scanners (Philips in Birmingham, and Siemens in Southampton) and 32-channel head coils. The T1-weighted scans were collected using a magnetization prepared rapid acquisition gradient-echo sequence (Echo time (Philips)= 3.7, Echo time (Siemens)=3.4, flip angle= 9 degrees, Foot to Head and Anterior to Posterior field of view= 256, Right to Left field of view= 192, matrix= 256, voxel size=1×1×1 mm, sagittal slices= 192, bandwidth (Philips) = 174 hz/pix, bandwidth (Siemens)= 180 Hz/pix, total scan time = 4 min 26 sec (Siemens) or 6 min 5 sec (Philips). After registration anatomical scans were collected, and if the quality was deemed to be fair or poor by the scanner operator, a second anatomical scan was collected.

### *5.2.4 Data Pre-processing*

All T1 images were assessed for quality categorised as *good*, *fair* or *poor* by two independent raters (Roberta Clanton and Stephane De Brito). *Poor* scans refer to those with ringing, blurring, distortion, or neuroanatomical abnormalities, whereas *good* scans refer to those with clear details in brain regions and delineation of grey and white matter boundaries and gyri. Only *good* T1 images were included in the analysis, therefore all participants included in this chapter were agreed as having *good* T1 images. Images were then pre-processed using Statistical Parametric Mapping (SPM8, Wellcome Department of Imaging Neuroscience, University College London, UK) and Matlab (Mathworks, Natick, MA, USA). First, all scans were set to the anterior-posterior (AC-PC) commissure line at the origin [0 0 0 mm]. Age and sex specific tissue probability maps were then created with the template-o-matic toolbox (Wilke et al., 2008) to account for the variance in child participants. Next, via the VBM8 and DARTEL toolboxes in SPM12, images were segmented into grey and white matter using the custom made tissue probability maps, and normalised with affine-only

transformation. Grey matter segments were then smoothed with a 4mm Gaussian kernel. This size kernel was selected because DARTEL-based VBM analyses are better suited for smaller kernel sizes, especially with larger groups; notably, a 2-4 mm kernel has been suggested for use with groups over 50 participants (Shen & Sterr, 2013).

### *5.2.5 Data Analysis*

Effects of group, sex and site on overall GMV, white matter volume and cerebrospinal fluid were examined with 2 (Group: CD vs TD) x 2 (Sex: Females vs Males) x 2 (Site: Birmingham vs Southampton) ANCOVAs with age and IQ as covariates of no interest. Significance level was set at $p < .05$. Follow-up post-hoc Bonferroni pair-wise comparisons were used to compare means for any significant main effects or interactions.

After pre-processing, statistical analyses were completed in SPM8 using a general linear model: a 2 (group: CD vs TD) x 2 (sex: male vs female) x 2 (site: Birmingham vs Southampton) full factorial design was run using the smoothed gray matter images. Age and IQ were included as covariates of no interest. Whole-brain and region of interest analyses were conducted. Regions of interest were anatomically defined using the aal Atlas of the Wake Forest University PickAtlas (Maldjian et al., 2003) and included bilaterally: the amygdala, anterior insula, anterior cingulate cortex, and ventromedial prefrontal cortex. For both whole-brain and ROI analyses, the height threshold within SPM was set at $p < 0.05$ family-wise error (FWE) corrected. For exploratory purposes, additional whole-brain analyses were also carried out using a reduced height threshold of $p < 0.001$, uncorrected with extent threshold of k=10 voxels.

Additional analyses were conducted comparing TD youths to youths with CD/HCU traits and those with CD/LCU traits. Consistent with previous studies (e.g., Sebastian et al., 2016), the HCU and LCU group were formed based on a median split (34) on the CU traits scores

from the YPI questionnaire. Comparisons in GMV across the three groups were conducted with a one-way ANOVA. Consistent with previous VBM work on the FemNAT-CD sample (Rashle et al., 2018), a multiple regression with a sex-by-CU traits interaction term (mean-centered CU scores multiplied by the dichotomous variable sex) was conducted within the CD group only to examine whether sex and CU interacted to predict GMV. Age, IQ and sites were included as covariates of no interest. Using an absolute threshold of 0.1, regionally-specific between-group differences in GMV were assessed.

## 5.3 Results

### *5.3.1 Overall Grey Matter Volume*

Analyses yielded no main effects ($F$ = 1.07, $p$ = .38). There were no significant differences in overall grey matter volume between the groups: (TD females: 759.45 ml ±73.46, TD males: 755.08 ml ± 84.05, CD females: 752.06 ml ± 55.28, CD males: 733.68 ml ± 85.64), $F$ = 1.08, $p$ = 0.34. There were no significant differences in grey matter volume between males and females (Females: 756.68 ml ± 66.9; Males: 743.95 ml ± 85.19), $F$ = 1.09, $p$ = .29. There were no significant differences in grey matter volume between scanning sites (Birmingham: 751.51 ml ± 71.95; Southampton: 745.17 ml ± 84.23); $F$ = < 1; $p$ = 0.65. There were no significant interactions.

CD / LCU vs CD / HCU vs. TD: A one-way ANOVA revealed no significant differences ($F$ = 2.94, $p$ = .28) in overall grey matter volume between the groups (TD: 757.39 ml ±78.11, CD/LCU: 752.57 ml ±81.02, CD/HCU: 728.67 ±78.11).

### *5.3.2 Overall White Matter Volume*

Analyses yielded a significant main effect of group ($F$ = 2.12, $p$ = .05). Males with CD had less overall white matter volume (444.57 ml ± 56.17) in comparison to both the TD females (483 ml ± 62.05) and TD males (478.25 ml ± 59.28), $F$ = 4.5, $p$ = 0.005; CD males vs. TD males ($p$ =0.04); CD males vs. TD females ($p$= 0.009), CD females (453.71 ml ± 53.05), ($p$ = 1). There were no significant differences in white matter volume between males and females (Females: 472.07 ± 10.16, Males: 458.12 ± 59.48) $F$ = <1, $p$ = .42. There were no significant differences in white matter volume between scanning sites (Birmingham: 466.13 ml ± 60.04, Southampton: 461.84 ± 60.22) $F$ = <1, $p$ = .71. There were no significant interactions.

CD / LCU vs. CD / HCU vs TD: A one-way ANOVA revealed no significant differences ($F$ = 1.46, $p$ = .59) in overall white matter volume between the groups (TD: 480.77 ml ±60.43, CD/LCU: 452.75 ml ±52.25, CD/HCU: 443.97 ±59.98).

### *5.3.3. Overall Cerebral Spinal Fluid*

Analyses yielded no main effects $F$= <1, $p$ = 0.48. There were no significant differences in overall cerebrospinal fluid between the groups (TD females: 195.63 ml ± 32.48, TD males: 190.91 ml ± 28.8, CD females: 207.48 ml ± 27.92, CD males: 197.89 ml ± 35.25), $F$ = 1.38, $p$ = 0.25. There were no significant differences in cerebral spinal fluid between males and females (Males: 195.08 ml ± 31.18, Females: 200.05 ± 31.18), $F$ = 2, $p$ = 0.16. There were no significant differences in cerebral spinal fluid between scanning sites (Birmingham: 198.62 ± 31.23, Southampton: 195.72 ± 33.17), $F$ = <1, $p$ = 0.47. There were no significant interactions.

CD / LCU vs. CD / HCU vs TD: A one-way ANOVA revealed no significant differences ($F$ = 1.46, $p$ = .59) in cerebral spinal fluid volume between the groups (TD: 193.42 ml ±30.71, CD/LCU: 199.55 ml ±27.45, CD/HCU: 201.99 ±37.63).

### *5.3.4 Whole Brain*

Comparison of the CD and TD groups at the whole brain level yielded no significant group differences, and no group by sex interactions at $p < 0.05$ FWE. Regions showing group differences or interactions at $p < 0.001$, uncorrected, $k = 10$, are reported in the appendix.

Comparison of the CD/HCU, CD/LCU, and TD groups at the whole brain level yielded no significant group differences at $p < 0.05$ FWE. Multiple regression analyses yielded no significant CU trait –by- sex interactions in the participants with CD at $p < 0.05$ FWE.

### *5.3.5 Region of Interest*

Comparison of the CD and TD groups using region of interest restricted analyses yielded no significant group differences, and no group by sex interactions at $p < 0.05$, FWE. Regions showing group differences or interactions at $p < 0.001$, uncorrected, $k=10$, are reported in the appendix.

Comparison of the CD/HCU, CD/LCU, and TD groups within the ROIs yielded no significant group differences at $p < 0.05$ FWE. Multiple regression analyses yielded no significant CU trait –by- sex interactions in the participants with CD at $p < 0.05$ FWE.

## 5.4 Discussion

The aim of this study was to replicate and extend upon previous research by using VBM to compare GMV between youths with CD and TD youths, and to examine the potential influence of sex. Whole-brain and regions of interest analyses were conducted, focusing on four ROIs identified in previous meta-analyses of sMRI data investigating the neural correlates of conduct problems and of empathy: bilateral amygdala, anterior insula, anterior cingulate cortices, and ventromedial prefrontal cortices. Analyses did not identify any statistically significant main effects of group, or interactions between group and sex. Below, I discuss some possible explanations for these results, put them in the context of previous sMRI studies that have examined youths with CD, identify the strengths and weaknesses of this study, and finally formulate implications for future studies.

There are a number of possible explanations for the reported results, which are related to the sample. This is a unique sample in some very important ways, including sex and age of all participants, and non-specification of the CD participants. First, the majority of previous studies did not specifically investigate sex differences. The mixed sex sample includes the full

spectrum of the disorder, with a variety of both male and female youths. Since previous studies were mostly limited to males with CD, and therefore can only inform on neural correlates of CD amongst males, this study examined GMV in both pre and post-adolescent males *and* females with CD. Also, the results are based on a very heterogeneous sample, as this is a large sample recruited from multiple community and clinical sites. Previous studies have recruited more 'homogeneous' samples from the community (Boes et al., 2008; Hyatt et al., 2012), clinical (Sterzer et al., 2007) or forensic settings (Ermer et al., 2013; Cope et al., 2014), but youths in this sample came from all of these sources. These samples were also more homogeneous in terms of age range and sex. Perhaps a more homogeneous sample with consideration of severity of CD symptoms and subtypes of CD such as CU traits and age of onset would prove to be more useful when examining structural differences of youths with CD.

Second, the large age range (9-18 years) of this particular sample could have limited the ability to detect group differences, as GMV is still developing in childhood and adolescence until the twenties (Giedd, 2004), and decreases as individuals age (Ge et al., 2002). For example, frontal GMV has been found to peak in females at 11 years of age compared to 12 years among males (Giedd, 2004). Therefore, perhaps GMV has not developed at the same pace for all participants, making it more difficult to compare groups including participants from both sexes. For example, Fairchild and colleagues examined sex differences in brain structure amongst CD youths, but included post-adolescent females ($M_{age}$ CD=17.23; $M_{age}$ TD=17.55) (Fairchild, Hagan, et al., 2013). Regions involved in more basic functions develop fully before regions involved in more complex behaviours such as decision-making and socioemotional processing (Gogtay et al., 2004), so younger participants may not have had a chance to completely develop areas of brain that are being compared to the older participants.

Michalska et al. (2015) also included a large mixed-sex sample of youths (age range: 9-11) with conduct problems and also failed to find significant group differences in GMV.

Third, previous studies have conducted analyses on accompanying CD specifiers and personality traits such as callous-unemotional traits, severity of CD symptoms, or empathy levels. In these instances, findings may represent a separate sub-sample of the population of interest, as they included CD specifications, suggesting the importance of heterogeneity. More specifically other studies have included additional group specifications such as callous-unemotional traits (De Brito et al., 2009; Sebastian et al., 2016), age-of-onset division (Fairchild et al, 2011), regressions based on levels of empathy (Sterzer et al., 2007) or aggression (Boes et al., 2008). Furthermore, antisocial adult offenders with higher levels of psychopathic traits have been found to have decreased levels of GMV compared to antisocial offenders with lower levels of psychopathic traits and non-offenders (Gregory et al., 2012). These factors reflect the importance of sub-grouping. Also, considering symptomology on a continuum rather than binary clinical group versus control group will an important avenue for future research. There were no group differences when dividing the CD group according to CU traits, and no interaction between sex and CU traits were observed in the CD group. Possible explanations might be the large age range of the participants in combination with a mixed sex samples. It is also possible that previous studies including small samples reported false positive results (Button et al., 2013; Fusar-Poli et al., 2014; Ioannidis, 2011).

In summary, there are several external factors contributing to neuroanatomical differences between individuals. Further research is necessary to replicate and extend findings with large samples of male and female youths with CD. It is important to remember MRI is still maturing as a research tool rather than a purely clinically informative device, therefore future research should focus on eliminating potential confounds to derive any meaningful results

from MRI data collected from psychiatric patients. Diagnosis may or may not have a causal influence on neuroanatomy, but individual differences such as hydration levels, head motion during scanning sessions, and average time spent on daily physical activity can also affect both brain structure and function (Weinberger & Radulescu, 2016) regardless of categorical patient or non-patient status. Research to date suggests there are certain brain structures that are impaired in youths with CD, but this study did not yield any significant differences in brain structure between youths with CD and TD youths. There could be various reasons for this, as discussed in the sections above. Importantly, the brain is a complex bundle of neurons and is rapidly changing not only due to biological development timelines, but also due to new information and learning (Alexander-Bloch et al., 2013). These factors can increase grey matter or cause atrophy depending on environmental context, and since regions are in constant communication it is highly unlikely a few regions are impaired independently. The amygdala, for example, has consistently been identified in both structural and functional studies on CD (Fairchild et al., 2011; Raine, 2011), but certainly there are other brain regions in communication with the amygdala that may influence its structure and function. To address this, future studies should investigate structural and functional connectivity amongst youths with CD. Additional research could use SBM in a large sample of youths (Fairchild et al., 2015), which would allow for examination of different metrics of the cortex thereby providing a more fine grained approach to assess what properties of the cortex might be altered in CD.

### *5.4.1 Strengths and Limitations*

The current study had several strengths. Firstly, this is the first VBM study on CD that has included a large sample of male and female youths with CD to examine similarities and differences in whole-brain and regional GMV. Second, the sample was very well characterised as participants were recruited from a variety of sources including the

community, schools, and clinical and forensic sites, and they were assessed via semi-structured interviews with both the youth and parent/caregiver. Third, participants were well matched on average age, and are representative of youths throughout the country as they came from both the Midlands and Southampton.

This study was not without limitations, including a large age range, and no consideration of comorbidities or personality traits. Further research could conduct separate analyses with an age split, in addition to age-of-onset analyses. Also, data could be analysed according to levels of CU traits or empathy scores rather than CD and TD. Regression analyses could also be run on specific ROIs to assess whether higher levels of personality traits or symptoms are associated with volume of neuroanatomical regions (e.g. Sterzer et al., 2007).

### *5.4.2 Implications for Future Research*

It is important for future researchers to use similar methods and comparable samples as this will avoid confusion and misleading results. Grouping "disruptive behaviour disorders" and "conduct problems" together to inform on the clinically recognised diagnosis Conduct Disorder is not completely accurate, and researchers should come to a consensus on: 1) How to measure the clinical group, and 2) which participants actually meet these requirements. Parsing scattered findings from so many diverse samples is a challenge the field is facing. Therefore, future research should include large mixed sex samples of youths who meet the clinical diagnosis of CD. Comorbidities and personality traits should then be considered. Finally, future studies using neuroimaging to investigate CD or clinical populations including youths should take into account the impact age has on the development and possible connectivity of the brain.

## 5.4.3 Conclusion

Overall, results from this chapter suggest there are no significant differences in GMV between youths with CD and their TD peers within my sample. Whilst results are indeed inconsistent with previous research, this is likely due to my sample, which is characterised as heterogeneous, large, mixed-sex, with a wide age range compared to more homogenous samples included in previous studies. Given the importance of CU traits on the structural and functional brain correlates of CD, it will be important for future work to examine how they are associated with GMV in male and female youths with CD. Further investigation could provide more information regarding communication between key regions in the brain, including structural covariance and functional responsivity, which will be addressed in the following chapters of this thesis. The next chapter will cover differences in whole brain structural covariance in regions associated with empathy between CD and TD youths.

# CHAPTER 6. WHOLE BRAIN STRUCTURAL COVARIANCE: INVESTIGATING SEED REGIONS ASSOCIATED WITH EMPATHY FOR PAIN

## 6.1 Introduction

There is a plethora of evidence from meta-analyses of fMRI (Alegria et al., 2016; Noordermeer, Luman, & Oosterlaan, 2016; Raschle et al., 2015) and sMRI studies (Aoki, Inokuchi, Nakao, & Yamasue, 2014; Noordermeer et al., 2016; Rogers & Brito, 2016) indicating that a number of distributed cortical and subcortical regions are implicated in CD. These data suggest that interconnected networks of regions, rather than focal regions, might be associated with the disorder. This is in line with the now widely recognised view that psychiatric disorders are considered to be disorders of brain circuits (or networks) rather than brain regions (Insel et al., 2010). Understanding how grey matter volume in various brain regions co-vary with each other can reveal information about neural organisation and how different regions are connected. Structural regions within the brain have been identified as part of large-scale networks, which transfer information and signals back and forth quickly (Clayden, 2013). Specific brain regions therefore act interdependently and are connected via network hubs. Structural covariance networks can provide support for functional network integrity, in that investigation into how structural neural networks co-vary can help explain how functional connections are formed and strengthened in the brain (Alexander-Bloch et al., 2013; Mechelli, Friston, et al., 2005).

Previous studies on healthy adults and children have identified the existence of large-scale brain networks (Bressler & Menon, 2010; Mechelli, Friston, et al., 2005; Zielinski et al., 2010), three of which are particularly relevant in relation to antisocial behaviour and CD: the default mode network (DMN) , the central executive network (CEN), and the salience

network. The DMN, which is the brain network most active whilst individuals are at rest, consists of the ventromedial prefrontal cortex, the dorsal medial prefrontal cortex, and the posterior cingulate cortex (Raichle, 2015). The CEN, associated with executive control and attention, consists of the dorsolateral prefrontal cortex and posterior parietal cortex (Bressler & Menon, 2010). The salience network is involved in social behaviour, self-awareness, and emotional processing (Menon & Uddin, 2010), and primarily consists of the anterior insula, and dorsal anterior cingulate cortex, and secondarily includes the amygdala, ventral striatum, and the ventral tegmental regions (Menon, 2015), brain regions that have been implicated in the pathophysiology of CD. Reduced connectivity within the DMN has been observed amongst youths with conduct problems and psychopathic traits (Broulidakis et al., 2016; Cohn et al., 2015; Dalwani et al., 2014; Zhou et al., 2015). Disruption in the CEN has also been observed amongst youths with CD since areas within the frontal lobe, specifically the superior frontal gyrus, are involved in both the DMN and the CEN, and has been associated with decreased network connectivity compared to TD youths (Jiang et al., 2016). Resting state analyses on fMRI data provides additional support to suggest networks and regions are disrupted amongst youths with pure CD, specifically in the temporal-parietal-limbic cortices and the cerebellum (Wu, Zhang, Dong, Wang, & Yao, 2017).

Global and region-specific grey matter volume matures throughout childhood and adolescence, with some of the structural development necessary for large-scale functional integration not fully complete until late adolescence (Zielinski et al., 2010). More specific networks, such as the salience network and CEN have shown that structural covariance between regions is more advanced in late adolescence compared to early childhood (Zielinski et al., 2010). Whilst structural covariance has been examined amongst healthy children and adolescents (DuPre & Spreng, 2017) and in psychiatric populations including disorders such

as autism, ADHD (Bethlehem, Romero-Garcia, Mak, Bullmore, & Baron-Cohen, 2017) and psychosis (Heinze et al., 2015), only one study to date has examined structural covariance amongst youths with CD. Fairchild et al. (2016) compared youths with childhood-onset CD or adolescent-onset CD to TD youths and investigated interregional correlations in cortical thickness using a technique called Mapping Anatomical Correlations Across Cerebral Cortex (MACACC). MACACC provides information on correlations between anatomical regions, and it is usually done with *a priori* seed based hypotheses to investigate differences across cortical networks (Lerch et al., 2006). Results showed that participants with childhood-onset CD had significantly more correlations within and across occipital, temporal, parietal, and frontal cortices than participants with adolescent-onset CD or TD youths, and youths with adolescent-onset CD had fewer correlations across the whole brain than TD youths (Fairchild et al., 2016). Whilst cortical thickness data were used in Fairchild et al.'s study via Freesurfer software, the methodology can also be applied to VBM data, which allows for replication and extension of these findings. These results suggest neuroanatomical differences between youths with CD and TD youths are distributed throughout the brain, affecting several neural networks rather than individual regions, supporting whole brain network-based approaches rather than structural or functional localisation approaches. Furthermore, these data distinguish neural correlates of childhood-onset CD from adolescent-onset CD, which is important when considering heterogeneity of the disorder.

In the previous chapter using VBM, no significant differences in GMV were found between youths with CD and TD youths. However, exploring the structural covariance between GMV of regions could provide further information on how the brain is connected and organised in youths with CD relative to TD youths. Although there were no significant region-specific differences, there may be differences in global brain development, based on

the consideration of the brain as a segregated network rather than independent localised areas. Based on evidence of empathy impairments in youths with CD, the aim of this study was to investigate whole-brain structural covariance patterns of the neural empathy network in youths with CD and TD youths. Specifically, patterns of whole brain grey matter volume covariance were examined using seed regions that have been shown to be central to the empathy for pain network in previous fMRI studies (Lamm et al., 2011). The following three seed regions were selected based on Lamm et al.'s meta-analysis of 32 studies on empathy for pain: left anterior insula, right anterior insula, and anterior cingulate cortex. The left and right anterior insula are associated with the affective-perceptual facet of empathy (Bernhardt & Singer, 2012; Decety & Michalska, 2010; Medford & Critchley, 2010), whilst the anterior cingulate cortex is associated with the cognitive-evaluative facet of empathy (Vogt, Sikes, & Vogt, 1993). Structural connectivity-based and resting state fMRI research amongst healthy individuals has shown the insula to be connected to the orbitofrontal cortex, temporal pole, amygdala, and the thalamus (Cerliani et al., 2012; Cloutman, Binney, Drakesmith, Parker, & Lambon Ralph, 2012; Jakab, Molnár, Bogner, Béres, & Berényi, 2012; Wiech, Jbabdi, Lin, Andersson, & Tracey, 2014). Whereas structural connectivity results have shown that the anterior cingulate cortex is connected to the prefrontal cortex, midline thalamus, and brainstem (Paus, 2001). In consideration of evidence that youths with CD have abnormal patterns of brain response during affective empathy, but not during cognitive empathy (Sebastian et al., 2012; Lockwood et al., 2013), it was hypothesised that youths with CD would have reduced whole brain structural covariance with the left anterior insula, right anterior insula, and anterior cingulate cortex seed regions compared to the TD youths. Sex-by-group interactions were also hypothesised in the AI bilaterally, considering interactions have been found in previous literature in this region. Specifically, males with CD have shown

increased GMV compared to TD males, and females with CD showed decreased GMV compared to TD females bilaterally in the AI (Fairchild, Hagan, et al., 2013).

## 6.2 Methods

### *6.2.1 Participants*

A representative sample of youths was recruited from a variety of sources in the community in Southampton and Birmingham. TD youths were recruited from mainstream schools and youth groups, whereas participants with CD were recruited from youth offending teams, schools for youths with emotional, social and behavioural difficulties, and Child and Adolescent Mental Health Services. Data were shared across two sites from the University of Birmingham and the University of Southampton (see Table 6.1), and included=159 youths (67 females) aged 9-18 years of age ($M_{age}$=15, SD = 2.07). Four groups were included in the total sample: TD females (n=42), TD males (n=37), CD females (n=25), CD males (n=55) (see Table 6.2).

*Table 6.1 Birmingham and Southampton participants' demographic and clinical characteristics*

| | Southampton (*n*=79) | Birmingham (*n* = 80) | p values |
|---|---|---|---|
| | M (SD) | | |
| Age (years) | 15.16(2.07) | 15.03(2.08) | t(157) = -0.42, p= 0.67[a] |
| Total IQ | 95.24(13.82) | 95.85(11.74) | t(157)=0.30, p=0 .77[a] |
| Sex (% females) | 41% | 44% | p=0.68[b] |
| Groups (% CD) | 52% | 49% | p=0.69[b] |

[a] t-tests

[b] Chi-square

*Table 6.2 Demographic and clinical characteristics of the sample*

| | **TD** | | **CD** | | | | |
|---|---|---|---|---|---|---|---|
| Variable | TD Females 1 (*n*=42) | TD Males 2 (*n*=37) | CD Females 3 (*n*=25) | CD Males 4 (*n*=55) | *F*group (*p*) | *F*sex (*p*) | *F*groupXsex (*p*) |
| | M (SD) | | | | | | |
| Age (years) | 15.02(2.04) | 15.52(2.02) | 15(2.13) | 15.02(2.10) | F < 1 (0.62) | F < 1(0.55) | F= 0.41(.74) |
| Estimated IQ | 100.9(10.08) | 103.43(10.49) | 87.6(13.56) | 89.76(10.82) | F=55.29(<0.001) (TD > CD) | F < 1 (0.74) | F= 18.96(<0.001) (1&2 > 3&4) |
| Verbal IQ | 100.95(12.90) | 103.78(12.56) | 89.36(16.63) | 86.47(13.15) | F= 41.14 (<0.001) (TD > CD) | F= 1.67(0.20) | F=16.56(<0.001) (1&2 > 3&4) |
| Performance IQ | 100.38(11.52) | 103.38(10.89) | 85.60(15.17) | 93.60(12.94) | F=27.87(<0.001) (TD > CD) | F=1.42(0.23) | F=12.37(<0.001) (1&2 > 3&4) |
| Current CD Symptoms | 0.12(0.32) | 0.24(0.49) | 4.92(2.90) | 5.78(2.43) | F=324.45(<0.001) (TD < CD) | F=10.45 (0.01) (F < M) | F=110.64(<0.001) (1&2 < 3&4) |
| Past CD Symptoms | 0.12(0.40) | 0.27(0.56) | 5.76(3.06) | 6.38(2.90) | F=318.71(<0.001) (TD < CD) | F=8.70(0.004) (F < M) | F=106.48(<0.001) (1&2 < 3&4) |
| Current ADHD Symptoms | 0.48(0.22) | 0.27(0.16) | 1.60(2.29) | 5.24(5.57) | F=50.62(<0.001) (TD < CD) | F=15.74(<.001) (F < M) | F=25.25(<0.001) (1, 2, 3 < 4) |
| Past ADHD Symptoms | 0.24(0.15) | 0.05(0.23) | 2.28(3.25) | 6.05(5.93) | F=60.91(<0.001) (TD < CD) | F=15.48(<.001) (F < M) | F=28.20(<0.001) (1,2.3 < 4) |
| Empathic Concern IRI | 18.83(3.88) | 17.62(3.49) | 19.08(4.85) | 12.56(6.47) | F=18.18(<.001) (TD > CD) | F=25.63(<0.001) (F > M) | F =17.22(<0.001) (1,2,3 > 4) |
| Total YPI Score | 90.17(18.15) | 96.98(16.35) | 117.16(16.48) | 114.09(24.71) | F=45.78(<0.001) (TD < CD) | F=3.31(.07) (F < M) | F =16.21(<0.001) (1&2 < 3&4) |

Note: ADHD = attention-deficit/hyperactivity disorder; CD = conduct disorder; TD=typically developing; IQ = intelligence quotient (measured using the WASI). Group, sex, and sex-by-diagnosis interactions were computed using between-group ANOVAs and Chi square tests. Bonferroni correction was used to make pairwise comparisons.

### *6.2.2 Data pre-processing*

Data were pre-processed within the VBM8 toolbox in SPM12, and the GMV was extracted via the segmented and smoothed VBM data used in the previous chapter. MNI coordinates for seed regions were then identified and entered into the MarsBar toolbox in SPM12 (Brett et al., 2002). The seed regions included left anterior insula (x = -40, y = 22, z = 0), right anterior insula (x = 39, y = 23, z = -4), and anterior cingulate cortex (x = -2, y = 23, z = 40), based on the MNI coordinates from meta-analytic evidence (Lamm et al., 2011). Consistent with previous structural covariance studies using GMV data from VBM (e.g. Heinze et al., 2015; Mechelli, Friston, et al., 2005), average regional grey matter volume intensities within the three ROIs were extracted from each participant's pre-processed VBM data using a 4mm sphere defined within the SPM Marsbar toolbox, and centered around the peak coordinates of the three ROIs.

### *6.2.3 Data Analysis*

Three (one for each of the three seed regions) two-way (Group: CD vs TD; Sex: Males vs Females) ANCOVAs models were created in SPM12, wherein the extracted grey matter intensities from the seed regions were contrasted against the whole brain. Separation of group and sex within the models, with a total of four groups, allowed investigation into possible main effects of group (TD vs CD) in addition to possible sex-by-group interactions. Covariates of no interest included global grey matter, age, IQ, and scanning site. The height threshold was set at $p < 0.05$, Family-Wise Error (FWE) corrected at whole-brain level, and results were displayed on a mean structural image.

## 6.3 Results

Analyses showed that youths with CD had significantly less structural covariance between the left anterior insula and the thalamus (x = 16, y= -12, z = 18; k = 6; z = 5.49, $p < 0.05$, FWE) compared to the TD youths (Figure 5.1). No group by sex interactions were observed at $p < 0.05$, FWE. Trends were observed at a reduced threshold ($p < 0.001$) for each of the seed regions (see appendix).

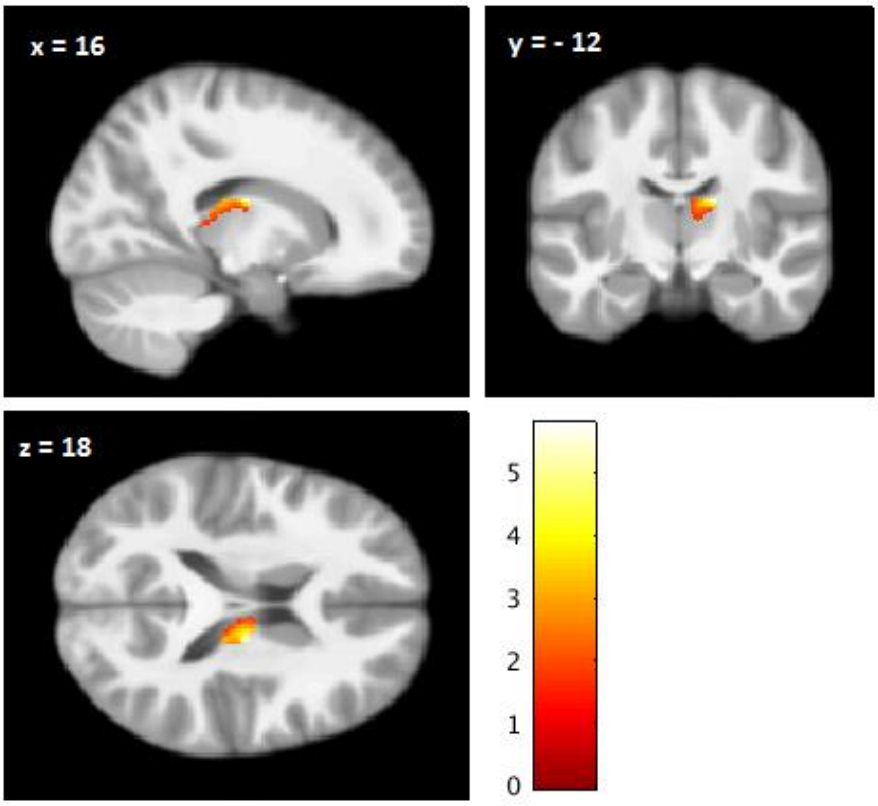



*Figure 5.1 Main effect of diagnosis CD < TD from right thalamus (x=16, y= -12, z= 18, t=5.79, k=6) covariance with Seed Region left anterior insula (x=-40, y=22, z=0). Results shown at p < 0.05 (k=669) using anatomical mask for right thalamus for display purposes. Colour bar represents t-statistic. Results overlaid on mean structural scan of all participants. Note: The effect is bilateral at a reduced threshold, but only statistical tests on the right thalamus survived FWE correction.*

## 6.4 Discussion

This study aimed to characterise whole-brain structural covariance patterns of the empathy for pain network in a sample of youths with CD compared with TD youths. Based on evidence that youths with CD have difficulties empathising with others' pain (Kostic, Nesic, Stankovic, Zikic, & Markovic, 2016) and that they are characterised by functional and structural brain differences in brain regions implicated in empathy for pain (Sterzer et al., 2007; Lockwood et al., 2013), it was hypothesised that youths with CD would have reduced structural covariance within the empathy for pain network compared with TD youths. In line with the hypothesis, results revealed significantly decreased covariance between the left anterior insula and the right thalamus in youths with CD relative to TD youths. Against predictions, no sex-by-group interactions were observed.

The thalamus has been described as an important neural hub (Hwang, Bertolero, Liu, & D'Esposito, 2017), possessing several nuclei, which deliver sensory information from subcortical regions to cortical regions for further processing. In particular, the thalamus sends information to the cerebral cortex regarding sensory input, and after processing the brain is able to better identify where the sensation came from and how to react (Jones, 2012). Pertinent to the current results and thesis, the thalamus has also been considered as a part of the neural pain matrix, as it is involved in receiving information regarding pain (Jackson et al., 2005; Yen & Lu, 2013), and is functionally connected to the insula (O'Muircheartaigh, Keller, Barker, & Richardson, 2015). It has been suggested that a midbrain-medial thalamic pathway is implicated in modulation of perception of endogenous pain, expectancy of pain,

and interaction between pain and anxiety (Lorenz, Minoshima, & Casey, 2003), which could be associated with internal processing of empathy for pain in oneself and others. Additionally, youths with CD experience an increased threshold or tolerance for sensitivity to pain compared to TD youths (Northover, Thapar, Langley, & Van Goozen, 2015), which could be related to initial communication in the brain between the thalamus and areas associated with pain and empathy.

This finding is in line with previous research showing that, compared to TD youths, those with conduct problems have reduced neural response to painful compared to non-painful stimuli in both the anterior insula and the thalamus (Lockwood et al., 2013). Importantly, lesions in the right thalamus have been associated with impaired empathy levels (Hillis, 2014), and previous research on healthy populations has shown the AI is necessary for empathy for pain (Gu et al., 2012). Taken together, these data indicate that both the right thalamus and left anterior insula are associated with empathy and pain processing. The difference in structural covariance of GMV in these regions adds to the burgeoning body of evidence on neural circuitry, as the AI and thalamus are key areas involved in empathy, and may reflect a lack of input of the thalamus to the AI amongst youths with CD. Youths with CD struggle to immediately process information in the anterior insula and the thalamus compared to TD youths (Decety & Michalska, 2010), therefore reduced structural covariance between these two areas could be associated with how the two areas are connected, either structurally or functionally. However, structural covariance is not necessarily a measure of connectivity. Whilst covariance between GMV has been shown to overlap with connectivity within white matter tracts, they do measure separate properties of the brain (Gong, He, Chen, & Evans, 2012). Therefore, structural covariance indicates a correlation between the amount of GMV in two separate regions which can be informative in regards to formation of neural

networks and can have potential implications on how neural networks are connected (Alexander-Bloch et al., 2013). Moreover, reduced structural covariance between the anterior insula and the thalamus among youths with CD could be linked to their diminished responsiveness to painful or emotional salient stimuli for both themselves and for others. Based on my finding, it is critical to consider whether youths with CD struggle to appreciate or understand pain in others due to a fundamental problem with experiencing pain themselves, perhaps reflecting their heightened threshold for pain (Northover et al., 2015), or if they simply have difficulties with affective pain rather than physical pain. Future studies should therefore focus on differentiating responsiveness to physical and affective painful stimuli amongst youths with CD.

Overall it is unclear what may be causing the reduced structural covariance observed amongst the youths with CD. Whilst many morphometric properties within the brain are genetically based and thereby heritable (Batouli, Trollor, Wen, & Sachdev, 2014), properties such as grey matter volume are susceptible to individual differences such as environment, experience, and learning (Alexander-Bloch et al, 2013). As discussed in the previous chapter, these external factors (e.g. environment, experiences, learning) may have an influence on structural properties and covariance patterns, but likely have a larger immediate influence on connections formed and strengthened in brain than overall morphology. This may partially explain the VBM results in the previous chapter, and it is possible that functional measures are more sensitive than structural measures at detecting neural differences. Functional studies examining neural correlates of empathy could shed more light on this matter therefore the following chapters of this thesis will explore functional responsivity during an empathy for pain task in addition to functional connectivity from follow up psychophysiological interaction analyses.

There were no other significant results when using the reported threshold, suggesting structural networks are relatively intact amongst the youths with CD in my sample, which is supported by the VBM results. Interestingly, there were no sex-by-group interactions. However, based on previous VBM literature indicating sex-by-group interactions in the AI (Fairchild et al., 2013), and a recent fMRI study which reported a sex-by-group interaction in temporal lobes amongst youths with CD in an empathy for pain paradigm (Michalska et al., 2016), differences in brain response in males and females with CD were hypothesised. Perhaps previous sex-by-group interactions could be associated with hormonal influences on neurobiology related to age differences, as Fairchild et al. (2013) included participants with an age range of 14-20 years and Michalska et al. (2016) 9-11 years. Thus, the participants in the Fairchild and colleagues study were of post-pubertal age, and the participants in the Michalska and colleagues study were likely pre-pubertal given their age range. This suggests their samples were more homogenous regarding age and pubertal status, increasing the likelihood that sex-by-group interactions could be observed. Post-pubertal females' brain structures are more developed compared to pre-pubertal females and males, which could influence whether sex-by-group interactions are detected in a sample with a large age range and varying stages of puberty. Furthermore, the age of pubertal onset has decreased amongst girls in recent generations (Kaplowitz, Slora, Wasserman, Pedlow, & Herman-Giddens, 2001; Lee & Styne, 2013), and start of puberty is associated with increased levels of antisocial behaviours amongst females (Caspi, Lynam, Moffitt, & Silva, 1993; Fontaine et al., 2009). These factors complicate the investigation of sex differences in the brain amongst youths, especially if females with CD are more likely to have started puberty at a younger age than the other groups. The participants in my sample varied on pubertal status, although they were mostly post-pubertal considering the average age (see Table 5.2). Moreover, recent evidence

shows hormonal and pubertal influences could have a strong influence on structural covariance and neural development, implying psychiatric disorders are not the only influence on abnormal brain structure or connectivity (Nguyen et al., 2016).

### *6.4.1 Future Directions*

Future research should focus on a few different avenues. First, consideration of callous-unemotional traits rather than diagnosis as a grouping determinant might prove useful. For example, a comparison of youths with HCU and LCU traits could provide further information regarding the consideration of severity of symptoms rather than grouping based on receipt of a disorder diagnosis. Second, symptom count and severity could also be useful ways to compare participants. For example, youths with severe forms of CD, or higher numbers of symptoms/more aggressive symptoms, compared to youths with less severe symptoms or fewer symptoms would add to the current literature. Third, more seed regions would allow further investigation across the brain, as this study focused on a few empathy-specific regions. However, CD is a heterogeneous disorder and there are many other aspects and neural networks to investigate besides empathy. Reward processing, for example, which is disrupted amongst youths with CD (White et al., 2014) could be another avenue, as the thalamus, insula, and nucleus accumbens have been implicated in neural processing of reward (Cho et al., 2013). My finding therefore provides evidence for overlapping neural correlates of both empathy and reward processing, considering the involvement of both the thalamus and the insula in anticipation for pain and incentives. Fourth, considering evidence that hormones influence brain organisation (Barth, Villringer, & Sacher, 2015), future studies should link hormone levels and brain structure. Levels of certain hormones such as testosterone and estrogen affect brain organisation and behaviour (Patchev, Hayashi, Orikasa, & Almeida, 1995), which is particularly pertinent to youths with CD who are prone to aggressive

behaviours. Higher levels of testosterone are associated with higher levels of aggressive behaviours (Archer, 1991), and testosterone has been shown to regulate the organisation and function of regions such as the amygdala (Nguyen et al., 2016), which is impaired in youths with CD. This is just one example of hormonal influences brain development. Puberty and other biological mechanisms such as secretion of oxytocin could be key when considering sex differences, including examination of the limbic system. Another interesting avenue would be to examine the volume of the thalamus and hypothalamus in relation to levels of oxytocin, since these brain regions are directly involved in secretion of oxytocin, a neuropeptide that is central in empathy and human bonding (Shamay-Tsoory & Abu-Akel, 2016). For example, a variant in the oxytocin receptor gene (OXYR) has been associated with amygdala volume (Furman, Chen, & Gotlib, 2011), and similar investigations of the relationship between GMV and OXYR amongst youths with CD would provide more information on how genetics, hormones, and neuroanatomical regions are interacting. Fifth, in utero studies will be highly beneficial as early differences can be detected whilst a baby is developing, and prenatal maternal stress and generated estrogen-dependent processes have a large impact on infant and later childhood brain development (Kinsella & Monk, 2009). Therefore, measuring hormone levels of pregnant women and following up on toddler outcomes regarding aggression levels and hormonal levels could be an interesting avenue to pursue (Beyer, 1999; Monk, Spicer, & Champagne, 2012; Suurland et al., 2016). Additionally, MRI studies including women in during early stages of pregnancy on deprived women exposed to stressors such as domestic violence and financial hardship could also provide important information, as it can show how maternal environment and cortisol levels can influence fetal brain development (Anderson & Thomason, 2013).

### *6.4.2 Strengths and Limitations*

It is important to consider the strengths and limitations of this study. First, the strengths include the methodology; only one other study has utilised structural covariance thus far in the current literature on CD (Fairchild et al., 2016), but with pre-processed cortical thickness data rather than GMV data. Therefore, incorporating the data from the segmented grey matter volume used in the previous chapter constitutes a good follow-up on the previous VBM analysis. Second, another strength is the well-characterised sample, as it is a large, mixed-sex sample with enough of power to address the hypotheses. The participants also completed semi-structured diagnostic interviews, and most of the time diagnoses were made based on information from both the youths and their parents/caregivers. The participant groups are well matched on average age, which is very important when considering structural integrity, as discussed earlier in the chapter. Third, an *a priori* seed region hypothesis on the empathy for pain network is a strong approach considering this thesis aimed to address neural correlates of empathy among youths with CD. Due to the limited number of studies employing structural covariance to investigate the disorder of CD, perhaps further investigations should focus on an exploration of large-scale network integrity in the population, specifically, structural covariance of the salience network especially since crucial areas documented as abnormal amongst youths with CD are included in the salience network, such as the anterior insula and amygdala (e.g. Fairchild et al, 2011). This could be achieved by selecting seed regions from the salience network and analysing covariance locally between the regions, as well as globally across the whole brain.

The study is limited in that it does not address the influences of comorbidities, or other psychiatric symptoms such as ADHD or anxiety. The cross-sectional design is also a limitation, whereas a prospective longitudinal design would allow for comparisons over time.

It is important to compare differences in brain structure at two separate time points amongst developing youths as this could provide more insight into how psychiatric symptomology amongst youths predicts mental health outcomes later in life (Muetzel et al., 2017).

### *6.4.3 Conclusions*

Overall, this study suggests decreased structural covariance between the left AI and the right thalamus among youths with CD relative to TD youths, but otherwise intact whole brain structural covariance with the empathy for pain network. This could imply abnormal integrity in a key network associated with empathy. The next chapter will cover task-based fMRI results from an *empathy for pain* paradigm, which will provide further insight into neural correlates of empathy amongst youths with CD.

# CHAPTER 7. FUNCTIONAL NEURAL CORRELATES OF EMPATHY FOR PAIN IN MALE AND FEMALE YOUTHS WITH CONDUCT DISORDER

## 7.1 Introduction

Youths with CD are prone to commit violent transgressions toward others and engage in self-serving antisocial behaviours, thereby demonstrating reduced levels of empathy for others (Blair, 2005; Lovett & Sheffield, 2007). Investigating the functional neural correlates of empathic processing in youths with CD is an important endeavour to clarify the underpinnings of their lack of prosocial behaviours (Foulkes, McCrory, et al., 2014). Previous fMRI studies in healthy individuals have examined neural correlates of empathy using various tasks (Decety & Michalska, 2010; Gu & Han, 2007; Singer, 2004) the most commonly used paradigm probes the neural correlates of empathy for others' pain, where brain response while viewing others in painful situations is compared to brain response when viewing others in non-painful situations (Bernhardt & Singer, 2012; Jackson et al., 2005). Studies on healthy individuals have identified increased BOLD response to pain in the anterior cingulate cortex, anterior insula, cerebellum, amygdala, and thalamus, key regions for empathy and part of the pain matrix network (Craig, 2009; Fan et al., 2011; Gu et al., 2010; Jackson et al., 2005; Singer & Lamm, 2009). Based on two different meta-analyses including 28 and 32 fMRI studies respectively, the anterior insula and ACC were the most commonly reported regions activated during paradigms probing empathy for others' pain (Gu et al., 2012; Lamm et al., 2011). Furthermore, lesion studies have provided additional evidence for the involvement of the AI in empathy. Lesions in the AI have been associated with reduced levels of empathy (Hillis, 2014), and decreased accuracy and slower reaction times when completing an empathy for pain task (Gu et al., 2012). Furthermore, a study including stroke patients

showed the AI and the temporal pole, but not the ACC and other hypothesised regions, were significantly associated with impairments in affective empathy (Leigh et al., 2013).

Although behavioural and self-report evidence suggests that females have higher levels of empathy and greater accuracy when judging intentionality of harm (Friesdorf, Conway, & Gawronski, 2015; Fumagalli et al., 2010), there is very little evidence to suggest that sex differences exist in responsivity to empathy-evoking stimuli (Baez et al., 2017). Both men and women have shown increased activation in the insula and ACC whilst viewing empathy-related stimuli, however reduced activation has been observed in men whilst instructed to view an unfair individual in a painful situation (Singer, 2006), indicating an ability to regulate empathy may exist in men but not women based on perceived social biases. In adult forensic settings, psychopathic male offenders have shown decreased activation to viewing others' pain in brain regions including the ACC, AI, but this effect was reduced when participants were instructed to imagine themselves as the other person (Meffert, Gazzola, Den Boer, Bartels, & Keysers, 2013), further suggesting that men may be able to regulate empathic responsivity. This has yet to be addressed amongst adult female offenders.

Six previous fMRI studies that have examined the neural correlates of empathy for pain in youths with conduct problems have shown that youths with conduct problems and CU traits exhibit decreased neural responsivity compared to TD youths in the AI, ACC, amygdala, and temporoparietal junction (Dong et al., 2017; Lockwood et al., 2013; Marsh et al., 2013; Michalska et al., 2016; Yoder et al., 2016), but one study reported increased responsivity in youths with aggressive forms of CD compared to TD youths (Decety et al., 2009). However, some regions typically associated with empathy have not been consistently shown across studies. For example, Marsh et al (2013) reported that youths with conduct problems have reduced neural responsivity to empathic stimuli in the amygdala and the anterior cingulate

cortex, but not the AI. Differences in the results could be due to differences across paradigms, which have relied on video clips of accidental or intentional harm to others (Michalska et al., 2016), dynamic images of accidental or intentional harm to others (Yoder et al., 2016), images of others' in pain (Lockwood et al., 2013), or manipulations of instructions (e.g. imagine self vs. imagine other) (Marsh et al., 2013). Another potential explanation for inconsistent results might be because of variations in the samples' demographic and clinical features. While Dong et al (2017) included adolescents with CD compared to typically developing adolescents, other samples included aggressive forms of CD (Decety et al., 2009), youths with some symptoms of CD rather than a diagnosis of CD (Michalska et al., 2016), conduct problems and CU traits (Marsh et al., 2013; Lockwood et al., 2013; Yoder et al., 2016). These samples also varied on mean age and age range of participants. Furthermore, most studies included males only, but a few had mixed sex samples (e.g. Michalska et al., 2016; Yoder et al., 2016), although only Michalska reported sex differences.

Overall, results suggest reduced BOLD response in the AI, ACC, amygdala, temporoparietal junction, and inferior frontal gyrus (a region also associated with mirror neurons; Rizzolatti, 2005) in youths with CP relative to TD youths (Michalska et al., 2016; Lockwood et al., 2013; Dong et al., 2017), whilst increased BOLD response amongst males with aggressive forms of CD has been identified in the amygdala, ventral striatum, and temporal pole compared to TD males (Decety et al., 2009).

To date only one study within the CD literature has examined sex in relation to neural responsivity to empathy (Michalska et al., 2016). This was a large sample (*n*=169) with pre-adolescent males and females (9-11 years of age), which was unique as it was the first study to include a pre-adolescent sample. In addition, in contrast to all previous studies, video clips were used to measure empathic responsivity to harm vs. no harm and intentional harm vs.

non-intentional harm. Two sex-by-CP symptoms interactions were identified. The first implicated the right posterior superior temporal gyrus during the intentional vs. unintentional harm condition, whereby higher numbers of CP symptoms were associated with lower levels of BOLD response in females, but this effect was not observed in the male. The second sex-by-CP symptoms interaction was observed in the posterior superior temporal sulcus and the middle frontal gyrus, whereby conduct problems were negatively associated with BOLD response amongst females but not males during the harm > no harm condition. The authors suggested that, should this effects be replicated, they could help understand sex differences in the etiology and prevalence of CP. Overall, inconsistencies between the studies to date may be due to differences in samples, such as variance due to heterogeneity of the disorder or age, as well as differences in measurement of empathy such as task design. Finally, mixed-sex samples could influence differences, therefore sex differences should be examined in further detail. The first aim of the current study was to clarify the neural correlates of empathy for pain in a sample of youths with a diagnosis of CD. The second aim was to examine potential similarities and differences across sexes amongst a large, mixed sex, sample of youths with CD compared to TD youths. It was hypothesised that, compared to TD youths, those with CD would show reduced BOLD response to pain in others in in the bilateral anterior insula, anterior cingulate cortex, and amygdala, orbitofrontal cortex, and ventromedial prefrontal cortex, key regions identified in previous studies related to empathy. Additionally, I examined if sex as a potential exploratory variable and diagnosis would interact to modulate brain response in youths with CD.

## 7. 2 Methods

### *7.2.1 Recruitment, sample characteristics, and questionnaires*

A representative sample of youths was recruited from a variety of sources: mainstream schools, youth groups, youth offending services, and child and adolescent mental health services in the UK. Participants were 106 youths (46 females) aged 9-18 years of age ($M_{age}$=14.9, SD = 2.19) recruited across two sites: The University of Birmingham (n=62) and The University of Southampton (n=44). Basic demographic information can be seen in Table 7.1, and sample characteristics are displayed in Table 7.2 including age, IQ, CD symptomology, ADHD symptomology, empathy, and YPI scores. Fifty-three of the 159 participants from the structural analyses were not included in this sample due to not completing the empathy for pain task or missing data (n=20), excessive motion (n=4), missing behavioural responses (n=4), and poor first level mask (n=25) resulting in the final sample size of 106 (Appendix 7.1). All participants were assessed with the K-SADS to establish diagnostic grouping, and IQ subtests were administered for vocabulary and matrix reasoning. General inclusion and exclusion criteria were identical to those of the previous experimental chapters, and are described in chapter 4.

*Table 7.1 Birmingham and Southampton participants' demographic and clinical characteristics*

| | Southampton (*n*=44) | Birmingham (*n* =62) | p value |
|---|---|---|---|
| | M (SD) | | |
| Age (years) | 15.05(2.38) | 14.95(2.08) | .83[a] |
| Total IQ | 97.43(13.60) | 98.05(11.15) | .79[a] |
| Sex (% females) | 45% | 42% | .72[b] |
| Groups (% CD) | 36% | 45% | .37[b] |

[a] t-tests

[b] Chi-square

*Table 7.2 Demographic and clinical characteristics of the sample*

| | TD | | CD | | | | |
|---|---|---|---|---|---|---|---|
| Variable | TD Females 1 (*n*=33) | TD Males 2 (*n*=29) | CD Females 3 (*n*=13) | CD Males 4 (*n*=31) | *F*group (*p*) | *F*sex (*p*) | *F*groupXsex (*p*) |
| | M (SD) | | | | | | |
| Age (years) | 14.64(2.34) | 15.06(2.31) | 15.92(1.44) | 14.9(2.17) | F = < 1 (0.4) | F = > 1 (0.97) | F= 1.09(0.35) |
| Estimated IQ | 103.12(9.9) | 103.79(9.27) | 88.54(11.61) | 90.39(10.94) | F=45.83(<0.001) (TD > CD) | F = < 1 (0.37) | F= 15.16(<0.001) (1&2 > 3&4) |
| Verbal IQ | 103.85(2.04) | 103.93(2.23) | 91.08(4.09) | 86.68(2.43) | F=40.47 (<0.001) (TD > CD) | F = 3.28 (0.07) | F=13.74(<0.001) (1&2 > 3&4) |
| Performance IQ | 101.73(2.04) | 103(2.14) | 85.62(4.28) | 93.71(1.97) | F = 21.3(<0.001) (TD > CD) | F = 0.15 (0.69) | F=8.72(<0.001) (1&2 > 3&4) |
| Current CD Symptoms | 0.09(0.29) | 0.28(0.53) | 4.85(2.82) | 5.81(2.74) | F = 224.9(<0.001) (TD < CD) | F = 7.8 (0.006) (F < M) | F=76.44(<0.001) (1&2 < 3&4) |
| Past CD Symptoms | 0.09(0.09) | 0.28(0.59) | 5.78(3.06) | 6.23(2.86) | F = 251.14(<0.001) (TD < CD) | F = 6.17 (0.02) (F < M) | F=82.87(<0.001) (1&2 < 3&4) |
| Current ADHD Symptoms | 0.06(0.24) | 0 | 1(1.68) | 3.97(5.41) | F= 25.15 (<0.001) (TD < CD) | F = 6.94 (0.01) (F < M) | F=11.95(<0.001) (1, 2, 3 < 4) |
| Past ADHD Symptoms | 0.03(0.17) | 0.03(0.19) | 1.62(2.18) | 5.13(5.95) | F=35.74 (<0.001) (TD < CD) | F = 8.46 (0.004) (F < M) | F=16.3(<0.001) (1,2.3 < 4) |
| Empathic Concern IRI | 18.21(4.65) | 17.51(3.68) | 20.15(4.81) | 13.16(5.29) | F=7.15(0.009) (TD > CD) | F=13.17(<0.001) (F > M) | F = 9.89(<0.001) (1,2,3 > 4) |
| Total YPI Score | 91.30(16.03) | 98.58(18.15) | 115.07(16.13) | 113.55(23.14) | F=26.58(<0.001) (TD < CD) | F=4.12(0.05) (F < M) | F = 9.67(<0.001) (1&2 < 3&4) |

Note: ADHD = attention-deficit/hyperactivity disorder; CD = conduct disorder; HC = healthy control; IQ = intelligence quotient (measured using the WASI). Group, sex, and sex-by-group interactions were computed using between-group ANOVAS and Chi square tests. Pair-wise comparisons were Bonferroni corrected.

### *7.2.2 fMRI Task*

The task was identical to previous empathy for pain studies on healthy participants (Gu et al., 2010) and boys with conduct problems (Lockwood et al., 2013), and included 192 images of either hands or feet with painful or non-painful context. There were 96 pictures per condition, with four different possible conditions (pain/hand; no pain/hand; pain/foot; no pain/foot). Examples of painful stimuli included an image of a hand being closed in a car door, or a knife stabbing a foot. Non-painful stimuli included a hand resting next to a door, or a nail a few inches away from a foot (Figure 6.1). All images were tested in a previous *empathy for pain* study, and validated as empathy–evoking (Gu et al., 2010). Images were presented for 20 second blocks, wherein eight separate images were displayed for 2000 ms each with a 500 ms interval between stimuli. Rest periods, marked by a fixation cross, were displayed after every 6 blocks for 15 seconds. There were a total of four runs, with six blocks per run, and four rest blocks. The block design was pseudo-randomised, in that conditions were never presented in the same order twice. Total task time was nine minutes and 17.5 seconds. Participants were instructed to press a button to indicate whether they saw a hand (press with index finger) or foot (press with middle finger) to ensure attention each time stimuli appeared on the screen. Reaction time and accuracy data were collected throughout the task. All participants completed a condensed practice version outside of the scanner just before entering to ensure they understood the task and instructions.

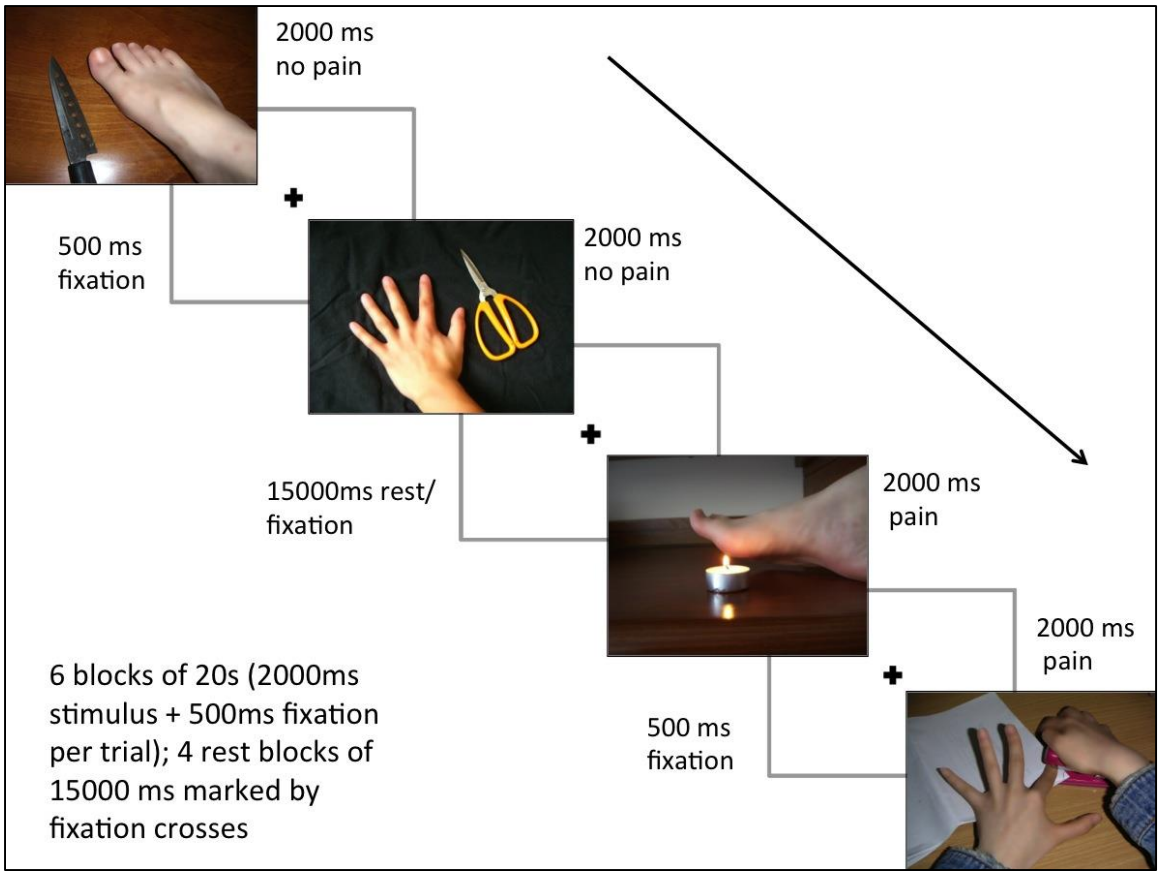



*Figure 7.1 Empathy for pain task including timing information and examples of painful and non-painful stimuli*

### *7.2.3 Scan acquisition*

Functional and anatomical scans were collected via a Phillips 3T scanner at the Birmingham University Imaging Centre, and a 3T Siemens Tim Trio scanner at Reading Imaging Centre. Scanning parameters were identical at both sites, including a 32-channel head coil. Each site underwent a site qualification procedure by physicists to ensure data would be comparable. Functional data were acquired in a single run of 9 minutes and 17.5 seconds, with 218 volumes plus 5 dummy volumes (223 total). $T_2^*$-weighted EPI data sensitive to BOLD were acquired (TR = 2500 ms; TE=30 ms; flip angle = 83 degrees, slices = 41, voxel size= 3x3x3 mm; slice

thickness= 2 mm; Right to Left and Anterior to Posterior field of view = 192; Foot to Head Field of view = 122). Fieldmaps were collected to remove distortion caused by magnetic field inhomogeneity, and collection of the fieldmap lasted approximately one minute including 46 slices; TE 1= 4.6ms; TE 2 = 6.9 ms; TR=500 ms; matrix size =64x64. T1-weighted images were included from the structural analyses. Acquisition of T1 scans included a gradient-echo sequence ET=3.7 at UOB; ET=3.4 at UOS; Repetition time = 1.9; flip angle = 9 degrees; foot to head and anterior to posterior field of view = 256; right to left field of view = 192; matrix = 256; voxel size = 1x1x1mm; sagittal slices = 192; bandwidth at UOB = 174 hz/pix and 180/Hz/pix at UOS. Total anatomical scan time was 4 minutes and 26 seconds at UOS and 6 minutes and 5 seconds at UOB. Participants were provided with cushioning around their heads to minimise head movement, and anatomical scans were collected before functional images. In the case the where T1 scan was deemed unusable at the time of scanning, a second anatomical scan was collected.

### *7.2.4 Data pre-processing*

Several pre-processing steps and quality control methods were used in accordance with typical fMRI analysis procedures to increase validity and standardisation of the data, taking into account the influence of external noise and physiological artefacts such as influence of data acquisition (e.g. scanner drift and thermal noise), and individual differences such as amount of movement. After discarding the first 5 dummy scans for each participant, the remaining functional volumes were pre-processed within SPM12 (http://www.fil.ion.ucl.ac.uk/spm/), using Matlab. Voxel displacement maps were then created using the processed fieldmaps. To reduce the influence of individual movement during scanning, all functional scans were realigned to the reference scan in the time-series using rigid body transformation, producing a mean EPI for each

participant. Mean realigned and unwarped EPIs were co-registered to the original T1 anatomical scans to allow for the final stage of normalisation to MNI space. The normalisation procedure registered images from all participants to the same coordinates to ensure recognised voxels were the same regions for everyone. A customised tissue probability map was created within the Template-O-Matic toolbox (Wilke et al., 2008) to help account for the use of child data, which is an important step as previous fMRI studies on CD have not always included a customised TPM, and this could contribute to a mismatch between the recognised MNI coordinates and brain templates. The customised TPM accounted for age and sex of the participants, and matched the participant data with other paediatric brains resulting in a specialised brain template to include in the next step, segmentation. All T1 images were segmented into grey and white matter using the VBM8 toolbox (Gaser, 2009), and individual native-space grey matter and white matter segments were normalised to the TPM using affine registration. A Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL; Ashburner, 2007) template was then created using the segmented grey and white matter images. All pre-processed image volumes were smoothed with a 6mm full-width/half-maximum kernel to increase signal-to-noise ratio (SNR), and final smoothed unwarped images were used for the first level analyses. All EPI volumes were also visually inspected, and any with excessive motion were discarded. Excessive motion was deemed to be more than 20 volumes, or having greater than 10% of volumes as outliers. Any participants with excessive motion were excluded. To account for motion during scanning, artefact repair toolbox (ART; http://www.nitrc.org /projects/ artifact_detect/) was used for each participant included in the study, with a threshold of 1.5 mm. Motion regressors from ART were then entered into first level GLMs for each participant along with timing information.

### *7.2.5 Data Analysis*

For behavioural data, effects of group, sex, and site on reaction time and accuracy data were examined with 2 (Group: CD vs TD) x 2 (Sex: Females vs Males) x 2 (Scanning site: Birmingham vs Southampton) mixed model ANCOVAs with age and IQ as covariates of no interests. Significance level was set at $p < .05$. Follow-up post-hoc Bonferroni pair-wise comparisons were used to compare means for any significant main effects or interactions.

Following pre-processing of fMRI data, subject-specific first level general linear models (GLMs; Friston et al., 1994) were created consistent with block design including experimental conditions of pain, no pain, and fixation. Contrast images were created at the first level to assess the effect of 'pain > no pain' 'no pain > pain' 'pain > fixation' 'no pain > fixation'. Parameter estimates from each contrast were then used for second level analysis adopting a full factorial 2 (Group: CD vs TD) x 2 (Sex: Females vs Males) x 2 (Scanning site: Birmingham vs Southampton) design (ANCOVA) in SPM12, with age and IQ entered as covariates of no interest. After estimation of the models, results were then viewed at the whole brain level in addition to hypothesised regions of interest (ROIs), which were created in the Wfupickatlas automated anatomical labeling toolbox (aal.02; Tzourio-Mazoyer et al., 2002). The ROIs included, bilaterally, the anterior insula, amygdala, anterior cingulate cortex, orbitofrontal cortex, and ventromedial prefrontal cortex. ROIs were specified based on previous research identifying differences between youths with and without conduct problems during empathy-related tasks (e.g. Lockwood et al., 2013), and specific MNI coordinates reported in Lockwood's paper were used for the AI. All results are reported at a threshold of $p < .05$, FWE, whole brain or small

volume corrected. Results for the main effects of group at a threshold of p < .001 uncorrected with a cluster size (k) of 10 voxels or more are reported in the appendix.

## 7.3 Results

### *7.3.1 Behavioural results*

**Reaction times:** There was a significant effect of condition indicating that all participants were slower to respond to painful stimuli than non-painful stimuli ($F$ = 6.47, $p$ = 0.01). There were no main effects of group for reaction time between all four groups ($F$ = < 1, $p$ = 0.45). There were no main effects of sex ($F$ < 1, $p$ = 0.51). There were no main effects of site ($F$ = 1.3, $p$ = 0.25). There were no interactions ($F$ < 1, $p$ = 0.78).

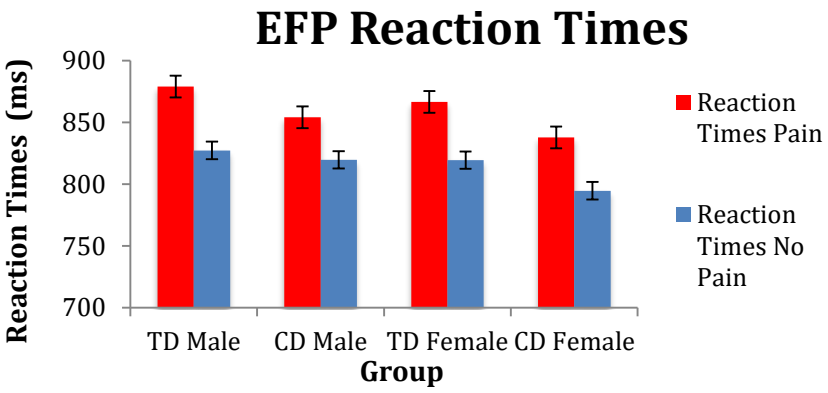



*Figure 7.2 Reaction times during the pain and no pain conditions*

**Accuracy:** There was no significant main effect of condition ($F$ = <1, $p$ = 0.72). There were no significant effects of group for accuracy during painful and non-painful stimuli between all four groups ($F$ < 1, $p$ = 0.66). There were no significant effects of sex ($F$ = 5, $p$ = 0.06). There were

no main effects of site ($F < 1$, $p = 0.93$). There were no interactions ($F < 1$, $p = 0.59$). No participants missed more than 20% of responses.

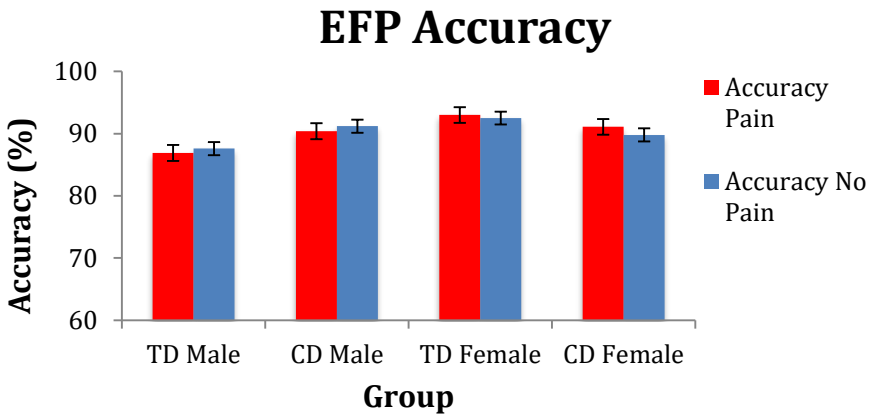



*Figure 7.3 Accuracy (% correct) during the pain and no pain conditions*

### *7.3.2 fMRI results*

Across the whole sample, all participants exhibited increased BOLD response whilst viewing painful compared to non-painful stimuli (Figure 7.4), and results replicate what has been found in similar studies using the same task (Figure 7.5). Although the activation intensity is lower in this sample as seen in Figure 7.5, indicated by the smaller *F* values, this is likely due to a less homogeneous sample in terms of age, sex, and combination of data from two different sites.

**Whole-brain analysis:** For the contrast pain > no pain, youths with CD had reduced brain response in the left cerebellum (x = -9, y = -33, z = -60; $t$=5.14; k=4; $p$=0.01, FWE-corrected across the whole brain at the peak voxel level) in comparison to the TD youths (Figure 7.6). No differences were found at p < 0.05, FWE whole-brain corrected for CD > TD.

**Region of interest analyses:** For the contrast pain > no pain, a significant main effect of group was found within the left anterior insula (x = -33, y = 24, z = 3, *t* = 3.35, k = 35, *p* = 0.02, S.V.C) whereby the youths with CD showed lower response compared to the TD youths (Figure 7.7). This was based on an a priori hypothesis using MNI coordinates for the left anterior insula (y= -30, x=16, z= 2) from the Lockwood et al., 2013 paper reported in the pain > no pain contrast table. Other regions of interest were tested using the same method, including the ACC, but did not reach statistical significance. A sex-by-group interaction was found in the right amygdala (x = 30, y = 3, z = -21, *t* = 3.16, k = 1; *p* = 0.04), where females with CD showed reduced activation relative to TD participants, but TD males and males with CD did not differ (Figure 7.8). No other differences were found at p < 0.05, FWE corrected for CD > TD within the ROIs.

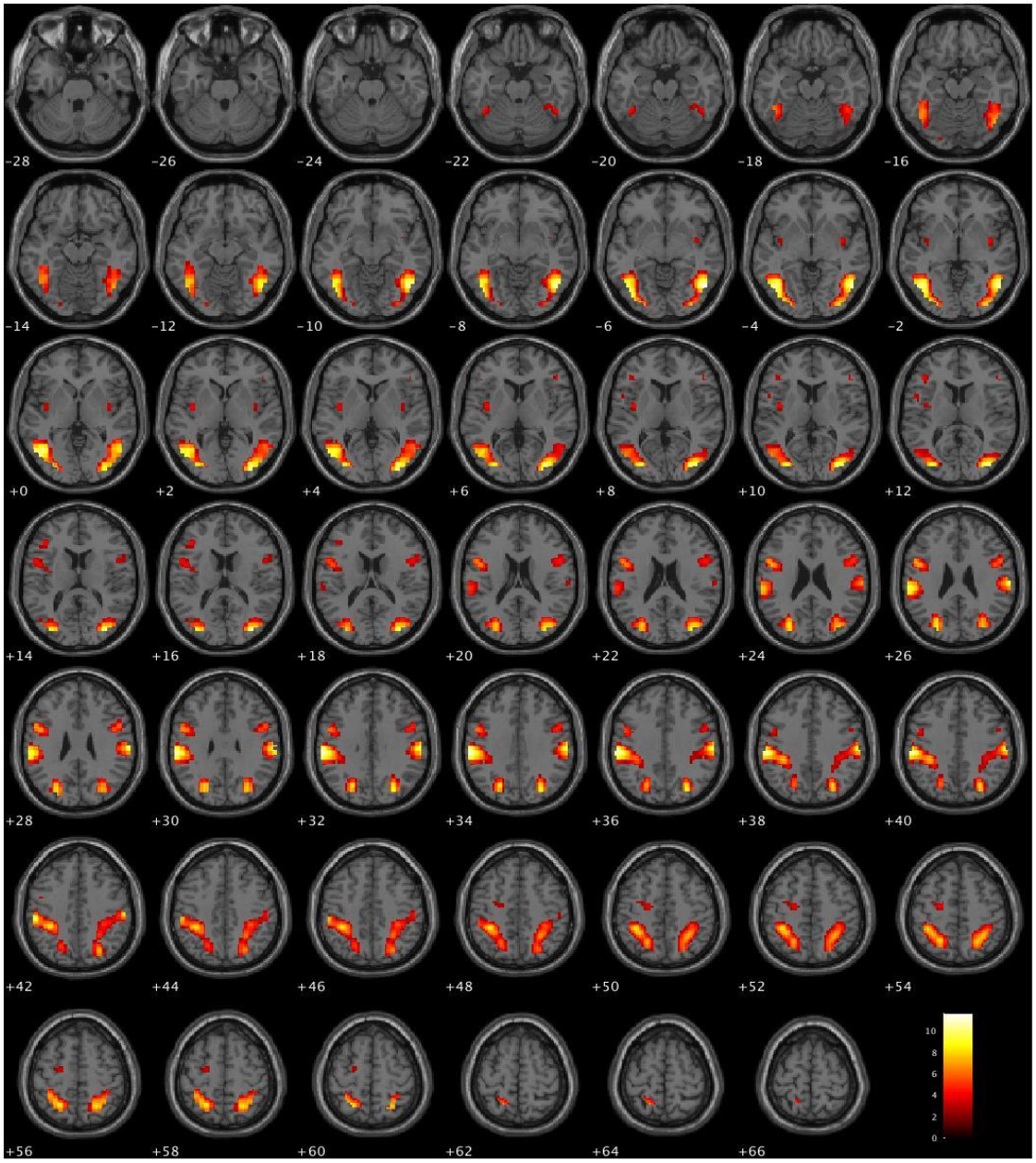



*Figure 7.4 Axial view of contrast pain > no pain for all participants, displayed at uncorrected statistical threshold ($p < 0.001$), colour bar represents t-statistic*

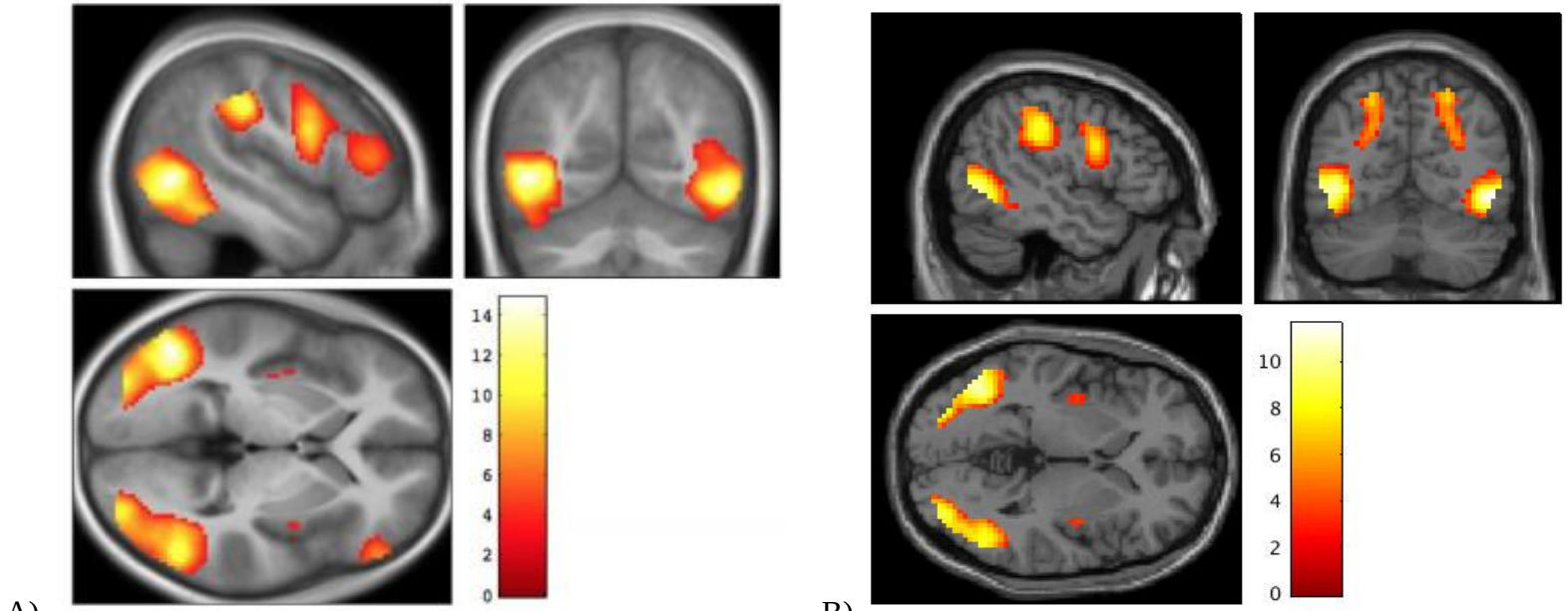



*Figure 7.5 Comparison of the pain > no pain contrast for all participants between A) Lockwood et al. (2013) and B) This sample; Results displayed at uncorrected threshold (p < 0.001) for pain > no pain contrast only. This figure highlights the similarities in results between the studies, as this thesis aimed to replicate and extend previous findings. Colour bars represents t-statistics.*

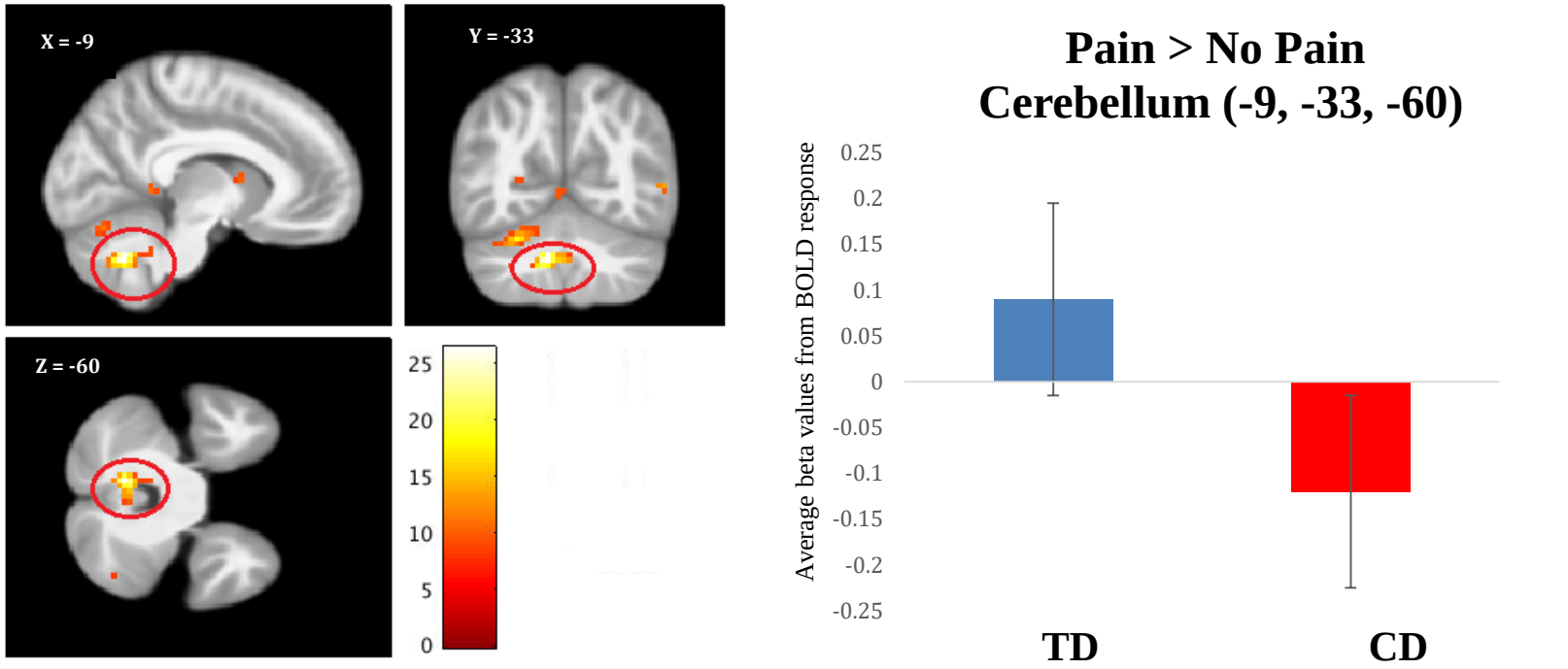



*Figure 7.6 Pain > No Pain; TD > CD;(x = -9, y = -60, z = -33; t=5.14; k=4; p=0.01, FWE-corrected across the whole brain at the peak voxel level); F-test shown at p=.005 for display purposes and overlaid on mean structural T1 from all participants. Youths with CD exhibited significantly reduced BOLD response while viewing images of others in painful situations in the left cerebellum compared to TD youths (indicated on left). Beta values extracted from peak cluster within the pain > no pain contrast second level GLM are shown on right. Colour bar represents t-statistic.*

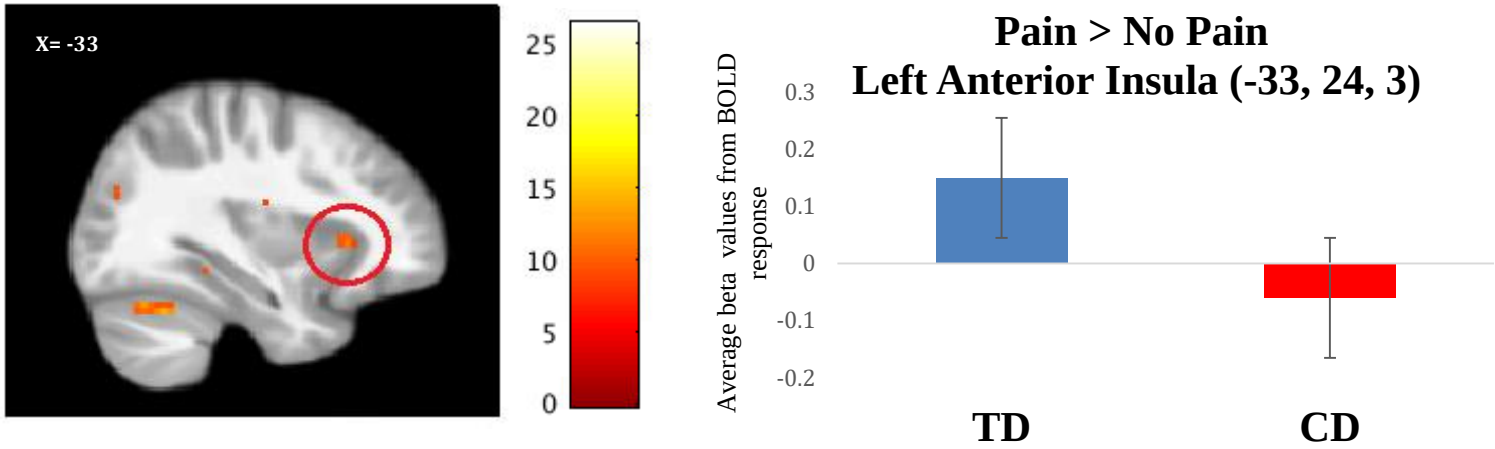
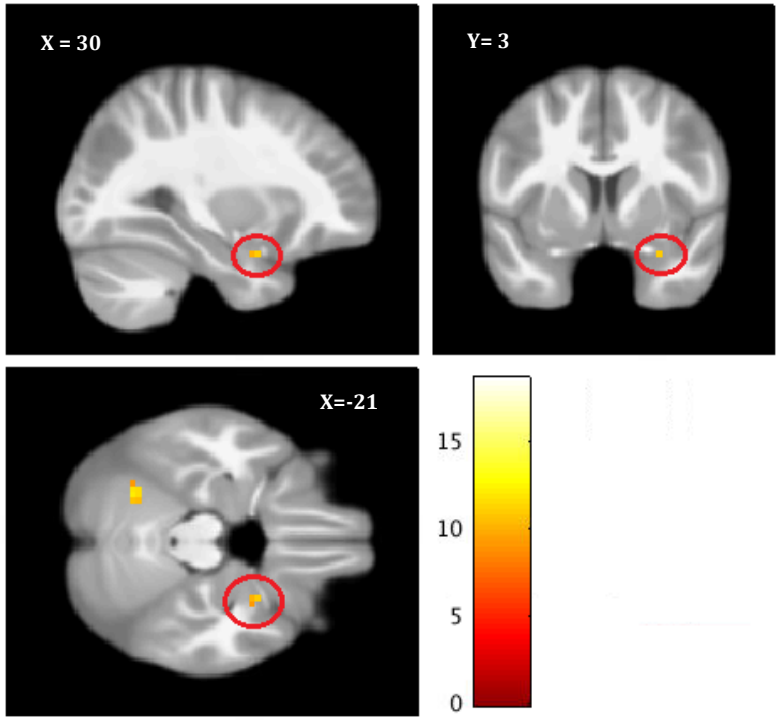



*Figure 7.7 Pain > No Pain, TD > CD (x = -33, y = 24, z = 3,* t *= 3.35, k = 35,* p *= 0.02, S.V.C); F-test shown at p = .005 for display purposes and overlaid on mean structural T1 from all participants. Youths with CD exhibited significantly reduced BOLD response while viewing images of others in painful situations in the left anterior insula compared to TD youths (indicated on left). Beta values extracted from peak cluster within the pain > no pain contrast second level GLM are shown on right. Colour bar represents t-statistic.*



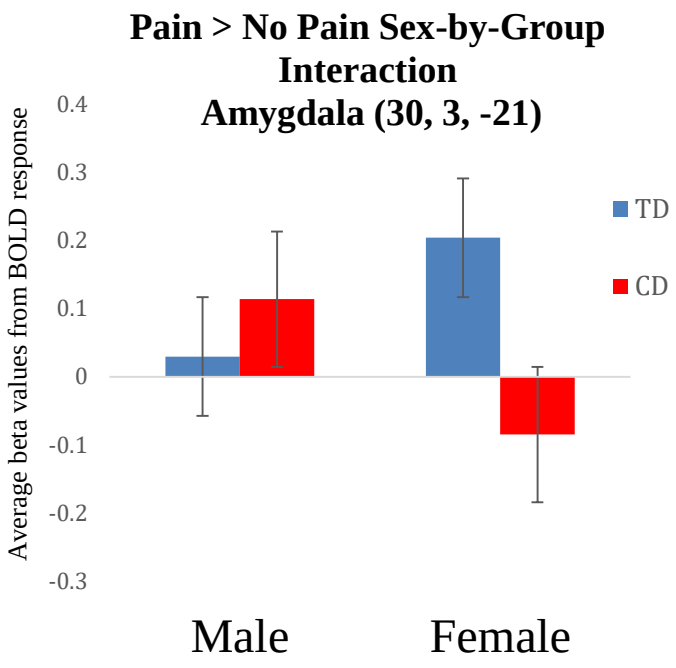




*Figure 7.8 Pain > No Pain; Sex –by- Group interaction (x = 30, y = 3, z = -21,* t = *3.16, k = 1;* p = *0.04); F-test shown at p=.005 for display purposes and overlaid on mean structural T1 from all participants are shown on the left. Beta values extracted from peak cluster within interaction during the pain > no pain contrast second level GLM are shown on right. Colour bar represents t-statistic.*

## 7.4 Discussion

The main aim of this study was to examine the neural correlates of empathy for pain in youths with CD, and investigate whether males and females with CD exhibit similar or different brain response compared to TD males and females. Based on previous literature, it was hypothesised that, compared to TD youths, those with CD would have reduced response in the bilateral AI, ACC, and amygdala when viewing others in pain. I also aimed to examine any potential sex-by-group interactions. In partial support for the hypotheses, ROI analyses revealed that, in response to the painful stimuli, youths with CD had reduced response within the left anterior insula compared to the TD youths. In addition, for the same contrast, the whole brain analysis revealed that youths with CD had reduced response in the left cerebellum. Finally, a sex-by-group interaction was also identified in the right amygdala for the same contrast, whereby females with CD showed significantly reduced BOLD response in the amygdala compared to TD females while TD males and males with CD did not differ. Taken together, these results, which identify reduced anterior insula response in youth with CD, are consistent with previous findings within the literature on CD and conduct problems (Lockwood et al., 2013; Michalska et al., 2016), but they also extend the current evidence base in two ways. First, this study identifies the cerebellum as an additional region implicated in the blunted empathy for pain response in youths with CD. Second, in line with recent sMRI (Fairchild, Hagan, et al., 2013) and fMRI (Michalska et al., 2016) studies, the sex-by-group interaction identified in the amygdala suggests differences in neural responsivity between males and females with CD. The following sections will review the results from the AI, cerebellum, and amygdala. Strengths and limitations of the study will then be discussed, followed by future directions.

The AI is an integral neural region for empathy (Gu et al., 2000), and the decreased BOLD response in youths with CD identified in this study supports previous findings (Lockwood et al., 2013; Michalska et al., 2016). Given that previous studies differed in numerous ways due to their sample characteristics and methodology, these results, together with previous studies, provide strong support for reduced AI as central to the empathy for others' pain impairment seen in youths with CD (Kostic et al., 2016). In contrast, Decety et al. (2009) found an opposite pattern of results, but the sample size was small and highly likely to be influenced by comorbidities or CU traits, with the latter not being measured leaving the possibility that the sample included a majority of youths with low levels of CU traits who have been found to exhibit heightened response to negative stimuli compared to TD youths (e.g., Viding et al., 2012; Sebastian et al., 2013).

Interestingly, my whole-brain analysis for the pain > no pain contrast also revealed that youths with CD showed a very robust (surviving FWE correction for multiple comparisons across the entire brain) reduced response within the left cerebellum compared to the TD youths. The cerebellum was not included specifically as an ROI, but it is indeed implicated in the pain matrix (Moulton et al., 2011), and has been associated with increased levels of BOLD response during exposure to pain-related stimuli amongst healthy individuals (Jackson, Meltzoff, & Decety, 2005; Lang, Yu, Markl, Müller, & Kotchoubey, 2011), and the opposite for youths with CD (Lockwood et al., 2013). The cerebellum has more commonly been associated with motor function and learning behaviours (Della-Maggiore, Scholz, Johansen-Berg, & Paus, 2009), but recent evidence supports its role in a social and emotional context (Bernard et al., 2012). For example, a case study from a cerebellar stroke patient has shown that disruption to the blood flow through the

cerebellum was associated with alterations in ability to empathise (Gerschcovich et al., 2011), and because the cerebellum is associated with the limbic system, it can be linked to cognitive and affective processes such as empathy (Schmahmann & Sherman, 1998; Wolf, Rapoport, & Schweizer, 2009). Similarly, Cerebellar Cognitive Affective Syndrome, a disease manifesting in the cerebellum, is associated with deficits in language, blunted affect, problems with self-control, and the cerebellum has been put forth as a modulator of neurocognitive and affective processing (Manto & Mariën, 2015). While trends have been observed in previous empathy-related studies on CD, the cerebellum has not shown such a strong effect before. Importantly, given that white matter connections exist between the cerebellum and large brain networks, such as the salience network (Habas et al., 2009), it will be important for future studies to replicate my finding of reduced cerebellar response in youths with CD and further examine its role in CD through the examination of its connectivity with other neural networks implicated in the pathophysiology of CD.

Finally, a sex-by-group interaction was observed in the right amygdala whereby females with CD showed reduced activation relative to the TD females while TD males and males with CD did not differ. Interestingly, Michalska et al. (2016) also identified a sex-by-group interaction, but in the superior temporal sulcus where CD symptoms and BOLD response were negatively correlated among females, but not in males. These results are consistent with my finding of reduced BOLD response among CD females, but not males with CD. Furthermore, a previous sMRI study has also identified a similar sex-by-group interaction, but in the AI where females with CD had significantly reduced GMV compared to TD females, with an opposite pattern among males (Fairchild, Hagan, et al., 2013). Differences were expected within the amygdala due

to evidence of reductions in GMV in the amygdala (Sterzer et al., 2007; Huebner et al., 2008; Fairchild et al., 2011) and abnormal BOLD response (either increased or decreased) in youths with CD during empathic processing (Decety et al., 2009; Marsh et al., 2013). The observed sex-by-group interaction in this region is consistent with data showing that the amygdala mediates sex differences in emotion-related behaviours (Hamann, 2005), and this could be related to hormonal influences on neural structure and function (Davidson et al., 2002). It will be important for future studies on CD to consider the influence of sex, as this can provide important information regarding similarities and differences on the neural correlates of the disorder.

Surprisingly, contrary to my hypothesis, no group differences were observed in the ACC. Lesion research has shown the AI is more strongly implicated in empathy for pain than the ACC (Gu et al., 2012). The AI has been associated with emotional awareness (Gu, Hof, Friston, & Fan, 2013) and affective empathy (Lamm & Singer, 2010), but the ACC is associated more frequently with motivational and action components of empathy (Bernhardt & Singer, 2012; Craig, 2009). Whilst the ACC has been identified in previous studies on youths with CP (Lockwood et al., 2013; Marsh et al., 2013; Michalska et al., 2016), Decety et al. (2009) and Dong et al. (2017) did not find reduced activation amongst youths with CD in the ACC. Evidence exists to suggest functional dissociation between the AI and ACC amongst healthy individuals showing increased activation in the AI during empathy to others' pain but not the ACC (Gu et al., 2010). This highlights discrepancies in the existing literature, especially regarding the recruitment of both the AI and ACC during empathy-related tasks, and could account for why in my study significant group differences were only observed within the AI, but not the ACC. Also in line with my

findings, the ACC has not been consistently affected in sMRI studies on youths with CP (Rogers & De Brito, 2016).

### *7.4.1 Strengths and Limitations*

The strengths of this study are the use of a large, well-characterised sample, including enough males and females to address effects of sex, and a well-validated task. The research addressed important gaps in the literature, and adds a novel contribution to the field. First, the sample was well-characterised, considering all participants in the clinical group met the threshold for a diagnosis of CD after an in-depth clinical interview with both the participant and their parent/caregiver, whereas some previous studies (e.g., Lockwood et al., 2013) used a variety of parent and teacher questionnaires to assess participants, which might provide a less reliable assessment of CD. For example, to measure conduct problems Lockwood et al. (2013) used parent and teacher ratings on the Child and Adolescent Symptom Inventory Conduct Disorder Scale (CASI; Gadow & Sprafkin, 2009), which assesses behaviour problems, but does not provide such a detailed assessment of CD symptoms as a semi-structured interview with two informants. Michalska et al (2016) used the Diagnostic Interview Schedule for Children (DISC; Shaffer et al., 2000), but included in their sample participants with *some* CD symptoms within their clinical group compared to *no* CD symptoms in their control group. Decety et al (2009) only included participants in their sample with the highest levels of aggression. Second, the sample size in the current study was larger in comparison to all, but one (Michalska et al., 2016). Third, the influence of sex was examined in this study which has only been investigated once before (Michalska et al., 2016), thereby addressing a gap in the current literature on the influence of sex on brain response and empathic responsivity amongst youths with CD. The combination of my

findings alongside previous results provides evidence to suggest there are slight sex differences present among youths with CD, which requires replication and further examination. Finally, the task has been well-validated (Gu et al., 2010; Lockwood et al., 2013) which helps to replicate and extend upon previous findings.

There are also a number of limitations that should be discussed. First, the participants in my study spanned a large age range (9-18 years) between late childhood and late adolescence. The mean age of previous samples has varied, with some studies including a broad range (e.g. 10-16 years; Lockwood et al., 2013), whilst others have included a more limited age range including a group of post-adolescent males with an age range of 16-18 years (Decety et al., 2009), or pre-adolescent youths with an age range of 9-11 years (Michalska et al., 2016). Future research should carefully consider the age range of participants, as to eliminate any potential confounds. Second, the influence of personality traits such as levels of CU and aggressive tendencies was not considered, despite evidence that they have an influence on BOLD response in previous studies on empathy. For example, the aggressive participants in Decety's sample exhibited increased BOLD response during an empathy for pain task, whereas participants with high levels of CU/psychopathic traits exhibited decreased BOLD response during an empathy for pain task (Lockwood et al., 2013; Marsh et al., 2013; Yoder et al., 2016). Whilst the main aim of this thesis was to examine the influence of sex on neural correlates in CD, heterogeneity of the disorder is an important consideration, whereby different subgroups included in the disorder as a whole will likely display distinct neural correlates. Finally, the paradigm used in my study essentially provides information on neural activation whilst viewing body parts (e.g. hands and feet) receiving pain, rather than seeing facial expressions during painful experiences (Decety et al.,

2009), or videos of other people in harm (Michalska et al., 2016). Future research should focus on parsing neural correlates of physical pain and affective or emotional pain, as well as pain to self and others, as these are important facets of empathic responsivity (Lockwood et al., 2016).

### *7.4.2 Future Directions*

First, future work should systematically investigate the respective contribution of CU traits and CD symptoms, given evidence from previous studies showing that these clinical features exert opposing relationships with the neural correlates of empathy for pain in youths with CD/CP (Lockwood et al., 2013; Yoder et al., 2016). This work would be beneficial to further delineate the heterogeneity of CD and facilitate the understanding of subtypes. Second, in consideration of the various regions and direction of effects identified to date, future work should focus on recruitment of specific regions during empathic responsivity, with particular focus on delineation of AI and ACC (Lockwood, 2016) as it is currently unclear to what extent they are both structurally and functionally impaired amongst youths with CD and CU traits. Third, sex differences should be examined in more depth, considering my finding of a sex-by-group interaction in the amygdala, and previous evidence for sex-by-group interactions in frontal and temporal regions (Michalska et al., 2016). Fourth, functional connectivity between brain regions during empathic processing will provide further insight into the neural underpinnings of CD, as this could extend the current knowledge on activation and connection of functionally segregated regions. The next chapter will build on the results of this chapter by examining functional connectivity of the left anterior insula and cerebellum.

### *7.4.3 Conclusions*

To summarise, youths with and without CD completed an empathy for pain fMRI task, and functional neural correlates were examined. This study addressed gaps in the current literature by examining effects on a large, well-characterised mixed-sex sample of youths with diagnoses of CD. Results show that, in response to the pain of others, youths with CD have reduced activation in the left AI and cerebellum compared to youths without CD. Furthermore, a sex-by-group interaction was observed in the right amygdala. These results are in line with previous work, but they also add to the literature by identifying the cerebellum as an important novel region to consider, and by suggesting that there might be sex differences in neural correlates of empathic responsivity among youths with CD.

# CHAPTER 8. PSYCHOPHYSIOLOGICAL INTERACTIONS AMONGST YOUTHS WITH CONDUCT DISORDER AND TYPICALLY DEVELOPING YOUTHS

## 8. 1 Introduction

In the previous chapter I found that compared to TD youths, those with CD had reduced activation in the left AI and cerebellum when processing stimuli depicting painful stimulation in others. A useful and common way to follow up on those results is to investigate functional connectivity of these regions of interest with the whole brain. This is consistent with the shift from *functional localisation* to *functional integration* in human brain mapping, which has yielded a significant redirection of focus, and inclusion of connectivity in current neuroimaging research (Friston, 2011).

Functional integration can be divided into the two categories of functional connectivity and effective connectivity (Friston, 2011). Effective connectivity refers to the influence one brain region has on another and describes coupling between regions based on activity-dependent changes, whereas functional connectivity refers to correlations, but implies no information regarding mediation or causality (Friston, 2011). There are different techniques to measure functional connectivity, for example Granger Causality (Roebroeck et al., 2005), Structural Equation Modeling (McIntosh & Gonzalez-Lima, 1994), or Dynamic Causal Modeling (Friston, Harrison, & Penny, 2003). This chapter will focus on psychophysiological interaction (PPI), which provides information on interactions between two regions activated simultaneously during fMRI (Friston et al., 1997; O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012), rather than identifying functional localization as fMRI studies do. The connectivity between regions in

the brain during the fMRI task (psychological term) and time series information from the specified seed regions (physiological term) creates the psychophysiological interaction of interest, and provides information regarding connections between regions as well as how certain regions can alter connectivity in a particular context such as a specific task's condition (e.g., pain vs no pain stimuli); (O'Reilly et al., 2012). PPI analyses are commonly used as a measure of functional connectivity following fMRI results (Table 7.3), and therefore serve as a useful tool to investigate task-based functional connectivity. However, in contrast to dynamic causal modeling, which is used to measure effective connectivity to make predictions regarding seed region input, PPI does not make such inferences (Friston et al., 2003). Finally, it is worth noting that other imaging modalities such as resting state fMRI are useful to examine connections during resting periods or non-activity related experiences, but these do not enable researchers to investigate how experimental manipulation influences connectivity (Friston, 2011).

Evidence from studies that have examined connectivity in youths with CD suggests that the disorder is associated with aberrant functional connectivity during both resting state fMRI (Aghajani et al., 2017; Lu et al., 2015; Lu, Zhou, Wang, Xiang, & Yuan, 2017; Uytun et al., 2016; Zhou et al., 2015) and task-based fMRI (Marsh et al., 2008; Decety et al., 2009; Marsh et al., 2011; Finger et al., 2012; Yoder et al., 2016). Lu et al. (2015) investigated resting state functional connectivity amongst youths with CD, and found that they had decreased connectivity in the anterior default mode network (left middle frontal gyrus), somatosensory network (bilateral supplementary motor area and right postcentral gyrus), lateral visual network (left superior occipital gyrus), and the medial visual network (right fusiform, left lingual gyrus and right calcarine) compared to TD youths. Another study, which employed functional connectivity density mapping, which is a quick method for mapping distribution of local functional

connectivity between clusters with high concentrations of activation (Tomasi & Volkow, 2010), reported increased connectivity among youths with CD compared to TD youths in the bilateral precuneus and posterior cingulate cortex. These results were positively correlated with levels of impulsivity (Lu et al., 2017). In another recent study, youths with CD/HCU show decreased functional connectivity in the orbitofrontal and ventromedial prefrontal cortices, but increased connectivity in the ACC, prefrontal cortices, posterior cingulate, striatal regions, and sensory areas relative to youths with CD/LCU and TD youths (Aghajani et al., 2017).

Over the past decade, there have been a number of relevant studies investigating task-based functional connectivity using PPI (Table 7.3). These studies have focused on empathy among healthy and antisocial individuals (Decety, Michalska, Akitsuki, & Lahey, 2009; Decety et al., 2013; Yoder, Lahey, & Decety, 2016; Zaki, Ochsner, Hanelin, Wager, & Mackey, 2007), moral judgments in youths with CD (Marsh et al., 2011), fear processing in youths with CD (Marsh et al., 2008), and passive avoidance learning in youths with CD (Finger et al., 2012).

Amongst healthy adults, increased connectivity has been observed between the AI and the midbrain and periaqueductal grey during an empathy for pain task while individuals received pain themselves, whereas increased connectivity was observed between the ACC and dorsal medial prefrontal cortex whilst viewing others in pain (Zaki et al., 2007). This provides evidence for increased connectivity during empathy- related fMRI tasks among healthy adults in the AI and ACC, key areas associated with empathy. It also suggests dissociation between the AI and ACC, implying the ACC and AI have separate patterns of functional connectivity during empathic processing for *self* and *others*. This provides evidence for different responsivity in the AI and ACC based on who the pain is inflicted upon (Lockwood et al., 2013). Decreased functional connectivity between the amygdala and the AI has been observed amongst adult male

offenders (Decety et al., 2013), and a similar pattern of connectivity has been observed among youths with aggressive CD between the amygdala and prefrontal cortex (OFC) (Decety et al., 2009).

In a recent study, Yoder et al. (2016) focused on the functional connectivity of key seed regions in the salience network in youths with conduct problems whilst they performed an empathy for pain task. Results revealed that higher numbers of conduct problem symptoms were associated with increased functional connectivity of the insula with the temporoparietal junction. By contrast, youths with higher levels of CU traits exhibited decreased functional connectivity of the ACC with the amygdala and anterior insula (Yoder et al., 2016). This again highlights the heterogeneity of the disorder, and suggests that youths with high levels of CU traits have distinct neural correlates. The authors interpreted these results as supporting the hypothesis that individuals with high levels of CU traits have reduced responsivity to viewing others in distress in key areas associated with empathy. A few key aspects of this study include use of the DISC for CP symptoms rather than including youths with clinical diagnoses of CD, a young age range of participants (9-11; $M_{age}$=10.5), and the use of a socially relevant empathy task rather than images of strangers' hands and feet. Images of others' body parts in pain may influence distinct responsivity and neural correlates separate from viewing images or videos of self other people in painful situations. This highlights the difference between this thesis and the existing literature, and suggests a few factors that may influence findings. Overall, results suggest youths with CD have abnormal functional connectivity patterns compared to typically developing youths. However, few studies have included participants with a clinical diagnosis of CD, and most studies have focused on male samples only.

The aim of the current study was to follow up on task-based empathy for pain fMRI results using PPI to explore functional connectivity. The AI and cerebellum were selected as seed regions, given the significant differences reported in these regions between the youths with CD and TD participants in the previous chapter. Although there was a sex-by-group interaction identified in the amygdala in chapter 7, the amygdala was not selected as a seed region for PPI for two reasons. First, these follow-up analyses were focused on group differences rather than sex-by-group differences. Second, the amygdala was not included to avoid potential type-I error due to low number of females with CD. It was hypothesised that relative to TD youths, youths with CD would have aberrant connectivity between the left AI and left cerebellum with the following regions of interest bilaterally: amygdala, AI, ACC, and thalamus. These regions of interest were selected for three reasons: 1) their involvement in the empathy for pain network (Decety, 2011) 2) they have been identified in previous experimental chapters of this thesis (Chapters 6 and 7), and 3) they are structurally connected with the insula and the cerebellum (Blatt, Oblak, & Schmahmann, 2013). Exploratory whole brain analyses were also conducted, with a hypothesis of reduced connectivity of the insula and the cerebellum among youths with CD compared to TD youths.

## 8. 2 Methods

### *8.2.1 Recruitment and sample characteristics*

A representative sample of youths was recruited from a variety of sources such as mainstream schools, youth groups, youth offending services, and child and adolescent mental health services in the United Kingdom. Data were shared across two sites from University of Birmingham (n=62) and University of Southampton (*n*=44) to produce a final sample of 106 (46 females)

participants aged 9-18 years of age ($M_{age}$=14.9, SD = 2.19) (table 6.3). 53 of the 159 participants from the structural analyses were not included in this sample because: 1) they did not perform the empathy for pain task (*n*=20); 2) had excessive motion (*n*=4); 3) had missing behavioural responses (*n*=4), and 4) had poor first-level mask (*n*=25). All participants were interviewed with the K-SADS to establish diagnostic grouping, and IQ subtests were administered for vocabulary and matrix reasoning. General inclusion and exclusion criteria are identical to the previous experimental chapters and described in Chapter 3.

*Table 8.1 Birmingham and Southampton participant information*

| | Southampton (*n*=44) | Birmingham (*n*=62) | p value |
|---|---|---|---|
| | M (SD) | | |
| Age (years) | 15.05(2.38) | 14.95(2.08) | .83[a] |
| Total IQ | 97.43(13.60) | 98.05(11.15) | .79[a] |
| Sex (% females) | 45% | 42% | .72[b] |
| Groups (% CD) | 36% | 45% | .37[b] |

[a] t-tests

[b] Chi-square

*Table 8.2 Demographic and clinical characteristics of the sample*

| | TD | | CD | | | | |
|---|---|---|---|---|---|---|---|
| Variable | TD Females 1 (*n*=33) | TD Males 2 (*n*=29) | CD Females 3 (*n*=13) | CD Males 4 (*n*=31) | *F*group (*p*) | *F*sex (*p*) | *F*groupXsex (*p*) |
| | M (SD) | | | | | | |
| Age (years) | 14.64(2.34) | 15.06(2.31) | 15.92(1.44) | 14.9(2.17) | F = < 1 (0.4) | F = > 1 (0.97) | F= 1.09(0.35) |
| Estimated IQ | 103.12(9.9) | 103.79(9.27) | 88.54(11.61) | 90.39(10.94) | F=45.83(<0.001) (TD > CD) | F = < 1 (0.37) | F= 15.16(<0.001) (1&2 > 3&4) |
| Verbal IQ | 103.85(2.04) | 103.93(2.23) | 91.08(4.09) | 86.68(2.43) | F=40.47 (<0.001) (TD > CD) | F = 3.28 (0.07) | F=13.74(<0.001) (1&2 > 3&4) |
| Performance IQ | 101.73(2.04) | 103(2.14) | 85.62(4.28) | 93.71(1.97) | F = 21.3(<0.001) (TD > CD) | F = 0.15 (0.69) | F=8.72(<0.001) (1&2 > 3&4) |
| Current CD Symptoms | 0.09(0.29) | 0.28(0.53) | 4.85(2.82) | 5.81(2.74) | F = 224.9(<0.001) (TD < CD) | F = 7.8 (0.006) (F < M) | F=76.44(<0.001) (1&2 < 3&4) |
| Past CD Symptoms | 0.09(0.09) | 0.28(0.59) | 5.78(3.06) | 6.23(2.86) | F = 251.14(<0.001) (TD < CD) | F = 6.17 (0.02) (F < M) | F=82.87(<0.001) (1&2 < 3&4) |
| Current ADHD Symptoms | 0.06(0.24) | 0 | 1(1.68) | 3.97(5.41) | F= 25.15 (<0.001) (TD < CD) | F = 6.94 (0.01) (F < M) | F=11.95(<0.001) (1, 2, 3 < 4) |
| Past ADHD Symptoms | 0.03(0.17) | 0.03(0.19) | 1.62(2.18) | 5.13(5.95) | F=35.74 (<0.001) (TD < CD) | F = 8.46 (0.004) (F < M) | F=16.3(<0.001) (1,2.3 < 4) |
| Empathic Concern IRI | 18.21(4.65) | 17.51(3.68) | 20.15(4.81) | 13.16(5.29) | F=7.15(0.009) (TD > CD) | F=13.17(<0.001) (F > M) | F = 9.89(<0.001) (1,2,3 > 4) |
| Total YPI Score | 91.30(16.03) | 98.58(18.15) | 115.07(16.13) | 113.55(23.14) | F=26.58(<0.001) (TD < CD) | F=4.12(0.05) (F < M) | F = 9.67(<0.001) (1&2 < 3&4) |

Note: ADHD = attention-deficit/hyperactivity disorder; CD = conduct disorder; HC = healthy control; IQ = intelligence quotient (measured using the WASI). Group, sex, s and sex-by-group interactions were computed using between-group ANOVAS and Chi square tests. Pair-wise comparisons were Bonferroni corrected.

### *8.2.2 Scan acquisition*

Anatomical and functional scans were collected via a Phillips 3T scanner at the Birmingham University Imaging Centre and a 3T Siemens Tim Trio scanner at Reading Imaging Centre. Scanning parameters were similar at both sites, including a 32-channel head coil. Each site underwent a site qualification procedure by physicists to ensure data would be comparable. Functional data were acquired in a single run of 9 minutes and 17.5 seconds, with 218 volumes plus 5 dummy volumes (223 total). $T_2^*$-weighted EPI data sensitive to BOLD were acquired (TR = 2500 ms; TE=30 ms; flip angle = 83, slices = 41). T1-weighted images were collected to aid the normalisation of the EPI data. Acquisition of T1 scans included a gradient-echo sequence ET=3.7 at UOB; ET=3.4 at UOS; Repetition time = 1.9; flip angle = 9 degrees; foot to head and anterior to posterior field of view = 256; right to left field of view = 192; matrix = 256; voxel size = 1x1x1mm; sagittal slices = 192; bandwidth at UOB = 174 hz/pix and 180/ Hz/pix at UOS. Total anatomical scan time was 4 minutes and 26 seconds at UOS and 6 minutes and 5 seconds at UOB. Participants were provided with cushioning around their heads to minimize head movement, and anatomical scans were collected before functional images. In the case the T1 scan was deemed unusable at the time of scanning, a second anatomical scan was collected.

### *8.2.3 Data analysis*

fMRI results from the previous chapter revealed main effects of diagnosis (TD > CD/ pain > no pain) in the cerebellum (x = -9, y = -60, z = -33) and left anterior insula (x = -33, y = 24, z = 3). These two regions were used as seed regions for the PPI analysis. Several pre-processing steps and quality control methods were used in accordance with typical fMRI analysis procedures to increase validity and standarisation of the data, taking into account the influence of external noise

and physiological artifacts such as influence of data acquisition (e.g. scanner drift and thermal noise) and individual differences such as amount of movement. After discarding the first 5 dummy scans for each participant, the remaining functional volumes were pre-processed within SPM12 (http://www.fil.ion.ucl.ac.uk/spm/), using Matlab. Voxel displacement maps were then created using the processed fieldmaps. To reduce the influence of individual movement during scanning, all functional scans were realigned to the reference scan in the time-series using rigid body transformation, producing a mean EPI for each participant. Mean realigned and unwarped EPIs were co-registered to the original T1 anatomical scans to allow for the final stage of normalisation to MNI space. The normalisation procedure registered images from all participants to the same coordinates to ensure recognised voxels were the same regions for everyone. A customised tissue probability map was created within the Template-O-Matic toolbox (Wilke et al., 2008) to help account for the use of child data, which is an important step as previous fMRI studies on CD have not always included a customised TPM, and this could contribute to a mismatch between the recognised MNI coordinates and brain templates. The customised TPM accounted for age and sex of the participants, and matched the participant data with other paediatric brains resulting in a specialised brain template to include in the next step, segmentation. All T1 images were segmented into grey and white matter using the VBM8 toolbox (Gaser, 2009), and individual native-space grey matter and white matter segments were normalized to the TPM using affine registration. A Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL; Ashburner, 2007) template was then created using the segmented grey and white matter images. All pre-processed image volumes were smoothed with a 6mm full-width/half-maximum kernel to increase signal-to-noise ratio (SNR), and final smoothed unwarped images were used for the first and second level analyses. All EPI volumes

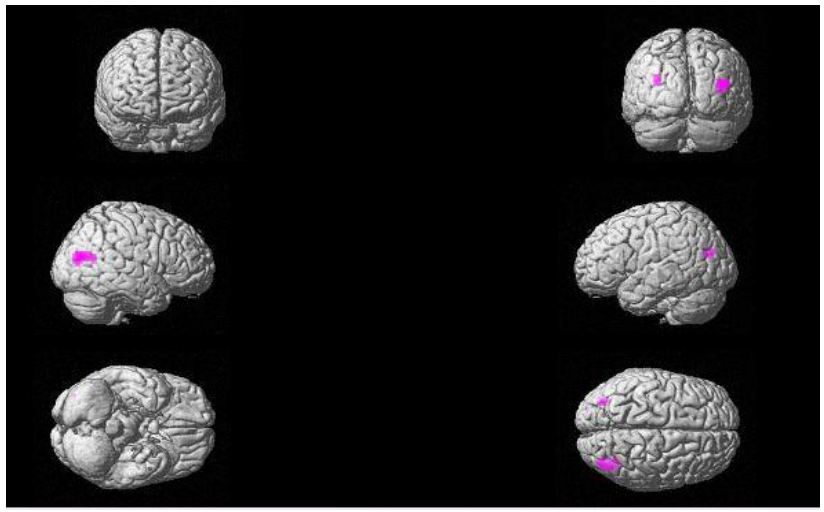
were also visually inspected, and any with excessive motion were discarded. Excessive motion was deemed to be more than 20 volumes, or having greater than 10% of volumes as outliers. To account for motion during scanning, artifact repair toolbox (ART; http://www.nitrc.org /projects/ artifact_detect/) was used for each participant, with a threshold of 1.5 mm. Motion regressors detected by ART were included in all participants' first level GLM design. Within the first level fMRI design, new F contrasts were created for the effects of interest (pain, no pain, and fixation) for both seed regions (cerebellum and insula). Volumes of interest, or Eigenvariates, including time series information regarding effects of interest were then extracted from first level fMRI results for each participant with a sphere of 3 mm placed around the VOI. The VOI for each participant was entered into the PPI toolbox in SPM12 to generate new PPI regressors: *ppi* (psychophysiological interaction term), *y* (VOI, including time-series BOLD information), and *p* (psychological term, including task-based contrast information on pain > no pain). First level general linear models were then specified for each participant, with the new regressors including *ppi, y, and p.* After estimation of all first level designs, contrasts were created to look at the effect of the ppi interaction term. The PPI regression contrasts were then entered into a second level two-sample t-test (TD vs. CD) in SPM12 with age, IQ, scanning site, and sex as covariates of no interest. Based on previous results, ROIs were identified as the ACC, AI, amygdala, and thalamus. These ROIs were anatomically defined using the PickAtlas aal toolbox in SPM. Exploratory analyses were also conducted at the whole brain level. All results for both ROI and whole brain analyses are reported at an uncorrected threshold of $p < 0.005$ for participants. This height threshold is in line with previous studies investigating PPI functional connectivity in youths with CD or conduct problems (Table 7.3).

## 8.3 Results

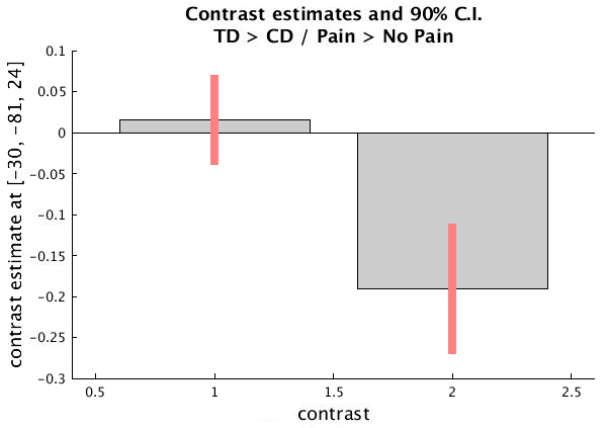
For the left anterior insula seed region (x= -33, y = 24, z = 3), the two groups exhibited a different pattern of connectivity with the occipital lobe bilaterally (left: x = -30, y = -81, z = -24, *t* =3.07, k =13, p= 0.001; right: x = 39, y = -75, z = 18, *t* =3.66, k = 69,p <0.001). Plotting the functional connectivity pattern for the two groups separately revealed less connectivity between the left AI and the occipital lobe amongst the youths with CD, whereas the TD youths did not exhibit difference in coupling between the two regions for the contrast pain > no pain (see Figure 7.1).

For the left cerebellum seed region (x= -9, y = -60, z = -33) the two groups exhibited a different pattern of connectivity with the left amygdala, (x = -21, y = -3, z= -18, *t* = 2.94, k = 4, p <0.001. Plotting the connectivity pattern for the two groups separately revealed that the TD youths had decreased connectivity between the left cerebellum and the left amygdala, whereas the CD youths exhibited increased connectivity between the two regions for the contrast pain > no pain.

A)



B)




C)

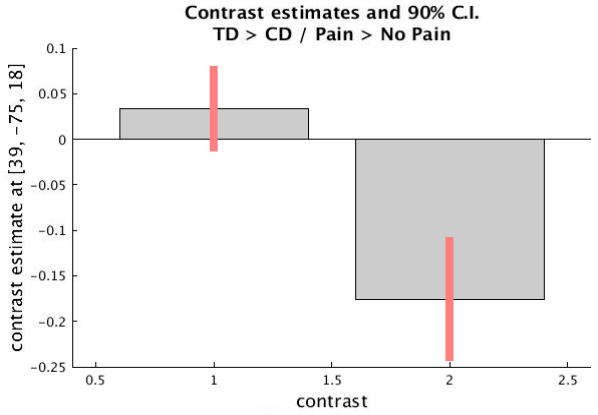



*Figure 8.1 Psychophysiological interaction between seed region left anterior insula and bilateral occipital lobe A) Bilateral occipital lobe overlaid on rendered brain B) left occipital lobe plot (x=-30, y=-81, z= 24, p < 0.001) C) right occipital lobe plot (x=39, y=-75,z=18, p < 0.001)*

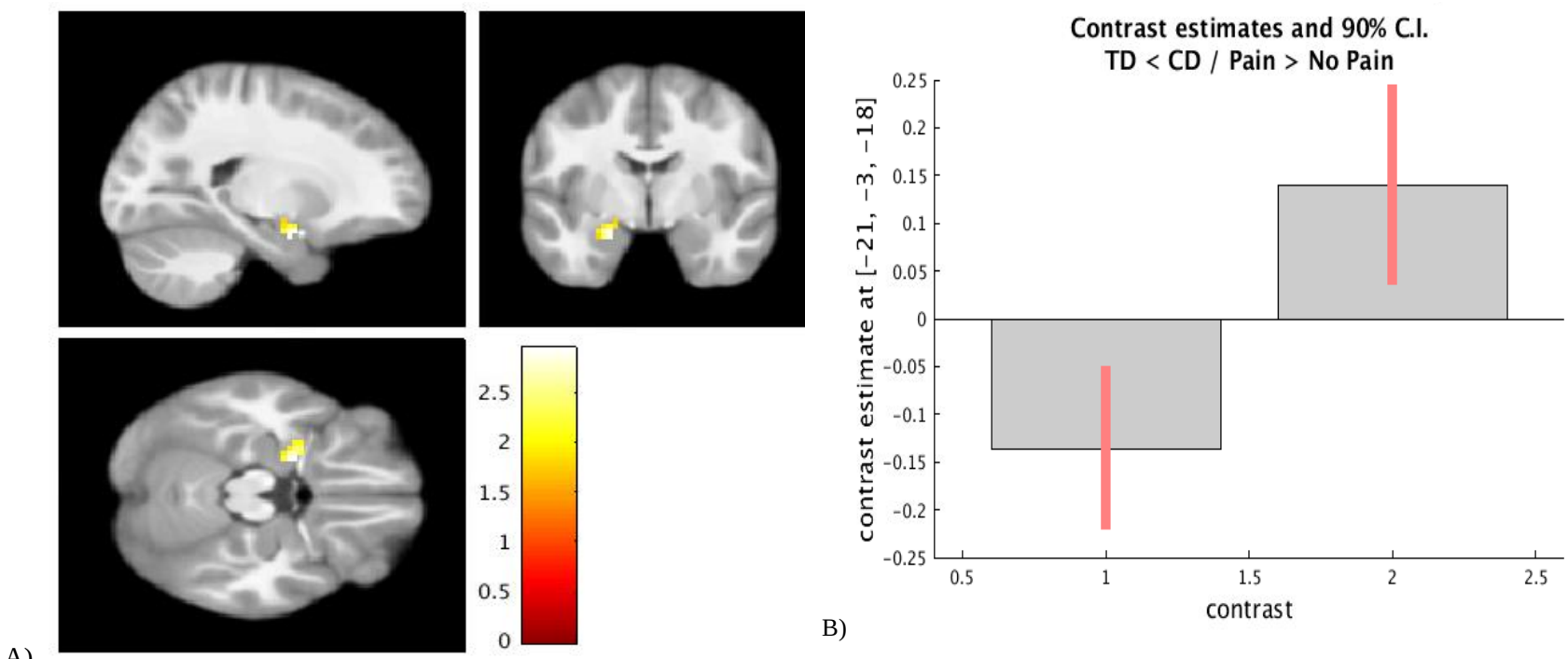



*Figure 8.2 Psychophysiological interaction between seed region left cerebellum and left amygdala*

*A) Left amygdala overlaid on brain B) Left amygdala plot (x=-21, y=-3, z=-18, p < 0.005); Colour bar represents t-statistic.*

*Table 8.3 Relevant studies including psychophysiological interactions*

| Study (year) | fMRI | PPI |
|---|---|---|
| **Zaki et al. (2007)** | **Paradigm:** Empathy - receiving thermal pain to self vs. watching video clips of others in pain<br>**Sample characteristics:** Healthy adults<br>**Results:** Increased BOLD response was observed in the AI, ACC, middle frontal gyrus, premotor cortex, and thalamus during both receipt of self pain and viewing others in pain | **Seed regions / ROIs:** AI and ACC; threshold: p = .005 (k=10)<br>**Results:** Greater connectivity was observed between the AI and the midbrain and periaqueductal gray during self-pain compared to viewing others in pain; Greater connectivity was observed between the ACC and the dorsal medial prefrontal cortex while viewing others in pain compared to self-pain |
| **Marsh et al. (2008)** | **Paradigm:** Fear processing (images of fearful expressions on faces of strangers)<br>**Sample characteristics:** 12 youths with disruptive behaviour disorder and high levels of CU traits; 12 youths with ADHD; 12 typically developing youths (Age range: 10-17)<br>**Results:** Youths with DBD and high levels of CU traits showed reduced BOLD response in the amygdala compared to youths with ADHD and typically developing youths | **Seed regions / ROIs:** Amygdala and ventromedial prefrontal cortex; threshold: p = .001<br>**Results:** Increased connectivity between amygdala and ventromedial prefrontal cortex among youths with ADHD and typically developing youths relative to youths with disruptive behaviour disorders and high levels of CU traits; Connectivity was negatively correlated with symptom severity |
| **Decety et al. (2009)** | **Paradigm:** Empathy for pain vs. no pain (animated images of others in pain)<br>**Sample characteristics:** 8 adolescents with aggressive CD; 8 typically developing adolescents (Age range: 16-18)<br>**Results:** Increased BOLD response among youths with CD in amygdala, striatum, and temporal pole whilst viewing others in pain | **Seed regions / ROIs:** Amygdala, prefrontal and orbitofrontal cortices; threshold: p = .001 (k=10) for whole-brain analyses; p = .005 (k=10) for ROI analyses<br>**Results:** Youths with CD exhibited decreased amygdala/prefrontal connectivity whilst viewing others in pain relative to TD youths |
| **Marsh et al. (2011)** | **Paradigm:** Moral judgment (legal vs. illegal implicit associations)<br>**Sample characteristics:** 14 youths with disruptive behaviour disorders and high levels of CU traits; 14 typically developing youths (Age range: 10-16)<br>**Results:** Youths with disruptive behaviour disorders and high levels of CU traits exhibited reduced BOLD response in the amygdala during legal actions compared to typically developing youths | **Seed regions / ROIs:** Amygdala, orbitofrontal, dorsomedial, and lateral frontal cortices; threshold: p= .005 (k=10)<br>**Results:** Youths with DBD and high levels of CU traits showed reduced connectivity between the amygdala and the orbitofrontal cortex compared to typically developing youths |

| | | |
|---|---|---|
| **Finger et al. (2012)** | **Paradigm:** Follow-up functional connectivity analyses from fMRI learning task (published previously)<br>**Sample characteristics:** 12 youths with disruptive behaviour disorders and high levels of CU traits; 14 typically developing youths<br>**Results:** Youths with DBD and high levels of CU traits demonstrated reduced BOLD response in the amygdala and orbitofrontal cortex compared to typically developing youths | **Seed regions / ROIs:** Amygdala, threshold: p = .005<br>**Results:** Reduced connectivity among youths with disruptive behaviour disorders and high levels of psychopathic traits between amygdala and rostral anterior cingulate cortex, insula, superior temporal gyrus, and caudate |
| **Decety et al. (2013)** | **Paradigm:** Empathy for pain vs. no pain with manipulation in task instructions (imagine self vs. other)<br>**Sample characteristics:** Incarcerated males (Age range: 18-50)<br>**Results:** Participants with higher levels of psychopathic traits exhibited increased BOLD response in the anterior medial cingulate cortex, anterior insula, inferior frontal gyrus, and right posterior temporal sulcus and temporoparietal junction compared to participants with lower levels of psychopathic traits | **Seed regions / ROIs:** Amygdala & AI, threshold: p = .001 (k=10)<br>**Results:** "imagine self" revealed reduced connectivity between the AI and the hippocampus and orbitofrontal cortex among participants with lower levels of psychopathy and reduced connectivity between the AI and right posterior temporal sulcus among participants with high levels of psychopathy whereas during the "imagine other" there was negative connectivity between the AI and the orbitofrontal and posterior cingulate cortices; "imagine other" yielded decreased connectivity between the amygdala and the orbitofrontal and dorsolateral prefrontal cortices among participants with higher levels of psychopathy |
| **Yoder et al (2016)** | **Paradigm:** Empathy for pain vs. no pain (images of harm vs. no harm to other people)<br>**Sample characteristics:** 53 "low risk" – typically developing and 53 "high risk" – youths with conduct problems and high levels of CU traits (Age range: 9-11); n = 123 (60 females); Pre-adolescent<br>**Results:** Increased BOLD response in amygdala was associated with high levels of CU traits | **Seed regions/ ROIs:** AI and ACC, threshold: p=.05, FDR<br>**Results:** Youths with high levels of CU traits demonstrated decreased connectivity between the ACC and amygdala and AI; Typically developing youths demonstrated increased connectivity between both the AI and ACC and the temporoparietal junction |

## 8.4 Discussion

This study aimed to examine functional connectivity among youths with CD compared to TD youths. Seed regions of interest were the left AI and the left cerebellum because in the previous task-based fMRI empathy for pain chapter I reported that youths with CD showed decreased activity in those regions compared to the TD youths. Regions of interest included the amygdala, AI, ACC, and thalamus. These regions of interest were selected for three reasons: 1) their involvement in the empathy for pain network (Decety, 2011), 2) they have been identified in previous experimental chapters of this thesis (Chapters 6 and 7), 3) they have structural connectivity with the insula and the cerebellum (Blatt et al., 2013). The whole-brain analyses revealed that youths with CD exhibited decreased functional connectivity between the left AI and the bilateral occipital lobe compared to TD youths. ROIs analyses showed that youths with CD exhibited increased functional connectivity, between the left cerebellum and the left amygdala whereas TD youths showed decreased functional connectivity between the two regions.

In line with previous studies youths with CD demonstrated reduced functional connectivity compared to TD youths between the AI (Yoder et al., 2016), and for the first time, the occipital cortex. In line with findings from antisocial adults, youths with CD demonstrated increased functional connectivity compared to TD youths between the cerebellum and the amygdala (Leutgeb et al., 2016). These findings will be discussed in the following sections.

### *8.4.1 Insula connectivity*

The insula is connected to many cortical and subcortical brain structures, with recent evidence suggesting connections with the cingulate, parahippocampal, supramarginal and angular gyri, precuneus, and occipital regions (Ghaziri et al., 2017). It has evolved functional purpose in

social awareness and empathy (Allen, Damasio, & Grabowski, 2002; Kaas, 2013; Semendeferi & Damasio, 2000). The AI has been found to have significantly increased functional connectivity with the midbrain and periaqueductal grey among healthy individuals during empathy-related fMRI tasks (Zaki et al., 2007), and significantly decreased functional connectivity among individuals with high levels of psychopathic and CU traits (Decety et al., 2013; Yoder et al., 2016). Results from my study provide additional support for abnormal functional connectivity in the left AI amongst youths with CD relative to TD youths. Decreased connectivity in the AI could be associated with difficulties empathizing with others due to its established involvement in empathy and associated networks.

### *8.4.2 Cerebellum connectivity*

The cerebellum is traditionally thought to be involved in motor function and learning behaviours (Della-Maggiore et al., 2009), but recent evidence suggests it plays a role in social/emotional contexts (Bernard et al., 2012). Interestingly, a case study from a cerebellar stroke patient has shown that damage to the blood flow through the cerebellum was associated with alterations in the ability to empathasise (Gerschcovich et al., 2011), and the cerebellum is structurally connected with the limbic system (Blatt et al., 2013). The cerebellum is also implicated in cognitive and affective processes (Schmahmann & Sherman, 1998; Wolf et al., 2009). Due to reported statistical tests surviving FWE from the cerebellum at the whole-brain level in the previous fMRI chapter, it was included as a seed region to explore connectivity with other regions of the brain. ROI analyses from this study indicated youths with CD had increased connectivity between the cerebellum and the amygdala, whereas TD youths had decreased connectivity between the two regions. Interestingly, violent high-risk offenders have exhibited

the same pattern of increased connectivity between the cerebellum and the amygdala (Leutgeb et al., 2016). This was during resting state fMRI, and the authors speculate this finding might be related to moral processing. Moreover, the cerebellum is increasingly put forward as an area associated with emotional experiences and empathy processing (Turner et al., 2007; Lang et al., 2011).

### *8.4.3 Amygdala connectivity*

A recent meta-analysis of 49 PPI studies showed that cortical and subcortical regions, including the medial frontal gyrus, bilateral insula, anterior cingulate, fusiform gyrus, parahippocampal gyrus, thalamus, and basal ganglia are characterized by task-modulated connectivity with the amygdala. Functional connectivity can be modulated by type of fMRI task, recruiting neural activation in different areas for fear processing, emotion regulation, or face processing (Di, Huang, & Biswal, 2017). The amygdala is recognised as being involved in emotional processing, especially fear and threat perception (Phan, Wager, Taylor, & Liberzon, 2002), and fear –related tasks show increased connectivity in the insula, ACC, and fusiform gyrus (Vytal & Hamann, 2010). The amygdala has also been involved in aberrant functional connectivity between areas associated with empathy amongst youths with CD and high levels of CU traits (e.g. Decety et al., 2009; Yoder et al., 2016). Interestingly, youths with CD in this thesis exhibited increased connectivity between the cerebellum and the amygdala. This could be due to heterogeneity of the disorder, as CD symptoms were also associated with increased functional connectivity in Yoder et al.'s sample, and aggressive CD symptoms have been associated with increased BOLD response in the amygdala (Decety et al., 2009).

In summary, these findings suggest youths with CD have reduced connectivity between the insula and the occipital lobe, but greater connectivity between the cerebellum and the amygdala compared to TD youths. Based on those results, it could be hypothesised that viewing others in pain amongst TD youths is associated with normal signaling or communication from the occipital lobe to the insula, which is involved in empathy processing, whereas in youths with CD there is neural dysregulation of this response as suggested by the decreased connectivity between these regions. These hypotheses are formulated due to the recruitment of the occipital lobe whilst processing visual stimuli, and the anterior insula during empathy processing. Whereas perhaps when youths with CD view someone in pain they are less likely to have normal or increased connectivity between regions facilitating empathy, but more likely to perceive threat and thus recruit areas of the brain involved in threat processing such as the amygdala. This could also be associated with aberrant moral or empathy processing, as Leutgeb et al. (2016) suggested.

### *8.4.4 Strengths and Limitations*

The strengths of this study include the use of a large, well-characterised sample, and pre-identified seed regions to examine task-based functional connectivity. Limitations of the study include no consideration of CU traits, comorbidities, or sex. The sample included in this study is large with enough power to address the hypothesis. The youths included were also assessed thoroughly via the K-SADS to ensure diagnostic criteria were met according to information from both the youth and their parent/carergiver. The AI and cerebellum served as the seed regions of interest, which demonstrates a strong point of the thesis as they were found to have significantly reduced BOLD response among youths with CD compared to TD youths in the previous fMRI chapter on empathy for pain. Finally, sex differences have yet to be addressed. Sex was not

included in this sample as the seed regions identified were selected based on group differences between youths with CD and TD youths at the second level in the fMRI analysis from chapter 6. This study does extend on previous results in that my sample included both males and females, whereas the majority of previous studies have included males only.

### *8.4.5 Future directions*

Future studies could include subtypes of CD to address heterogeneity of the disorder, sex differences, and more seed regions. Additionally, resting state fMRI would be beneficial to examine functional connectivity amongst youths with CD since task-based connectivity limits the results to the model of the paradigm and particular time-series information (O'Reilly et al., 2012). Future functional connectivity studies should also investigate other seed regions, namely the amygdala, anterior cingulate cortex, thalamus given their involvement in empathy as well as other emotional processes thought to be impaired in youths with CD. As seen in Table 7.3, previous studies have focused on psychopathic or CU traits or severity/number of CD symptoms rather than CD without specifications. Therefore examining differences in connectivity between youths with high versus low CU traits as well as higher levels of aggressive symptoms or lower levels of empathic traits would be useful.

### *8.4.6 Conclusions*

Overall, results from this study show that youths with CD exhibited reduced functional connectivity between the left AI and the bilateral occipital lobe compared to TD youths. Results also revealed that youths with CD exhibited increased connectivity between the left cerebellum and left amygdala, whereas the opposite pattern was observed amongst TD youths. These findings provide evidence for differences in functional connectivity between youths with CD and

TD youths. Future studies should seek to examine the influence of CD specifiers (e.g. age-of-onset and CU traits) as well as sex differences.

# CHAPTER 9. GENERAL DISCUSSION

## 9.1 Review

The aim of this thesis was to investigate structural and functional neuroimaging correlates of empathy amongst male and female youths with CD compared to TD youths. This chapter will first review the key findings from the preceding experimental chapters including interpretation of findings collectively, and how these results extend current knowledge. Secondly, themes within and across the studies will be explored with consideration of how this thesis relates to current trends in the literature on neuroimaging in CD, empathy, and sex differences. Finally, strengths and limitations will be reviewed, followed by directions for future research. Scientific and clinical implications will be discussed.

## 9.2 Summary of key findings

### *9.2.1 Voxel-based morphometry (Chapter 5)*

In chapter 4, VBM analyses yielded no significant differences between youths with CD and TD youths. In this study the aim was to replicate previous findings on structural differences between youths with CD and TD youths, and to explore the influence of sex. Furthermore, ROIs were selected based recent meta-analytic evidence of VBM studies in youths with CD (Rogers & De Brito, 2015), and regions associated with empathic processing (Sterzer et al., 2007). Contrary to previous results from structural MRI studies on youths with CD (Table 4.3, appendix), no main effects of group or sex were observed. As reviewed in chapter 4, this could be due to many factors such as differences in sample characteristics or methodological approaches between studies.

### *9.2.2 Structural covariance (Chapter 6)*

In chapter 6, structural covariance analyses expanded upon the results from chapter 5. The smoothed grey matter images from the VBM analyses were used to examine whole brain covariance by focusing on the bilateral AI and the anterior cingulate cortex, three seed regions central to the empathy for pain network (Lamm et al., 2011). Youths with CD exhibited reduced structural covariance between the left AI and the right thalamus compared to TD youths. No other main effects of group or sex by group interactions were observed at the specified statistical threshold, but trends were observed at a reduced threshold (Table 5.3, appendix). Only one other study to date has investigated differences in structural covariance among youths with CD (Fairchild et al., 2016). Fairchild and colleagues showed that youths with childhood-onset CD had significantly more correlations within and across occipital, temporal, parietal, and frontal cortices than participants with adolescent-onset CD or typically developing participants. By contrast, youths with adolescent-onset CD had fewer correlations across the whole brain than TD youths, a pattern that is somewhat consistent with my results. My finding could be associated with impairments in processing affective empathy for pain in addition to physical pain, since youths with CD have an increased threshold for pain (Northover et al., 2015).

### *9.2.3 Functional MRI empathy for pain (Chapter 7)*

In chapter 7, task-based fMRI analyses showed that youths with CD exhibited reduced BOLD response in the left cerebellum and left AI compared to TD youths whilst viewing others in painful situations compared to non-painful situations. Additionally, there was a sex-by-group interaction in the right amygdala indicating females with CD had significantly reduced BOLD response in the right amygdala compared to TD females, whereas BOLD response was not

significantly different between males with CD and TD males. These results are consistent with previous studies on empathy for pain in CD youths, specifically reduced BOLD response in the AI among CD youths compared to TD youths (Lockwood et al., 2013; Michalska et al., 2016). Previous research has found the cerebellum to be implicated in the pain matrix (Bushnell, Čeko, & Low, 2013; Loggia, Mogil, & Catherine Bushnell, 2008) and decreased BOLD response among youths with conduct problems compared to TD youths (Lockwood et al., 2013). However, this was the first study to find a significant decrease in BOLD response in the cerebellum among youths with CD compared to TD youths. This is also the first study to demonstrate a sex-by-group interaction in the amygdala during empathy processing amongst youths with CD. This suggests males and females with CD may have different neural correlates associated with empathy processing, which would have implications for intervention programs aimed at improving empathy skills amongst CD individuals.

### *9.2.4 Psychophysiological interactions (Chapter 8)*

In chapter 8, time-series data from regions with significant group differences in chapter 7 were included in PPI analyses to measure connectivity with identified ROIs in addition to the whole brain. The left cerebellum and left AI were selected as seed regions based on fMRI results form chapter 6. ROIs included the AI, ACC, amygdala, and thalamus. . PPI analyses indicated youths with CD exhibited significantly decreased connectivity between the left AI and the bilateral occipital lobe compared to TD youths. However, youths with CD also exhibited significantly increased connectivity between the left cerebellum and left amygdala compared to TD youths. These results extend upon previous findings showing youths with conduct problems have decreased connectivity between the AI and other regions (Marsh et al., 2008; Decety et al.,

2009; Finger et al., 2012; Yoder et al., 2016). They also are in line with a resting state fMRI finding indicating high-risk violent adult offenders exhibit increased connectivity between the amygdala and the cerebellum (Leutgeb et al., 2016).

## 9.3 Identifying common brain regions across studies

The following sections will review brain regions identified throughout this thesis that demonstrated significant group differences or sex-by-group interactions. The key regions identified in the experimental chapters of this thesis include the anterior insula, amygdala, thalamus, and cerebellum, areas that are recruited during emotion processing and empathic responsivity. These areas are also involved in the neural pain matrix (Bushnel et al., 2013; Figure 9.1). Measuring neural correlates of empathy for pain is currently one of the most common ways to investigate empathic responsivity (Lamm et al., 2011). Therefore, results from the four experimental chapters included in this thesis add to the evidence on the neural system involved in empathy for pain, which is impaired amongst youths with CD (Lockwood et al., 2013; Michalska et al., 2016; Yoder et al., 2016).

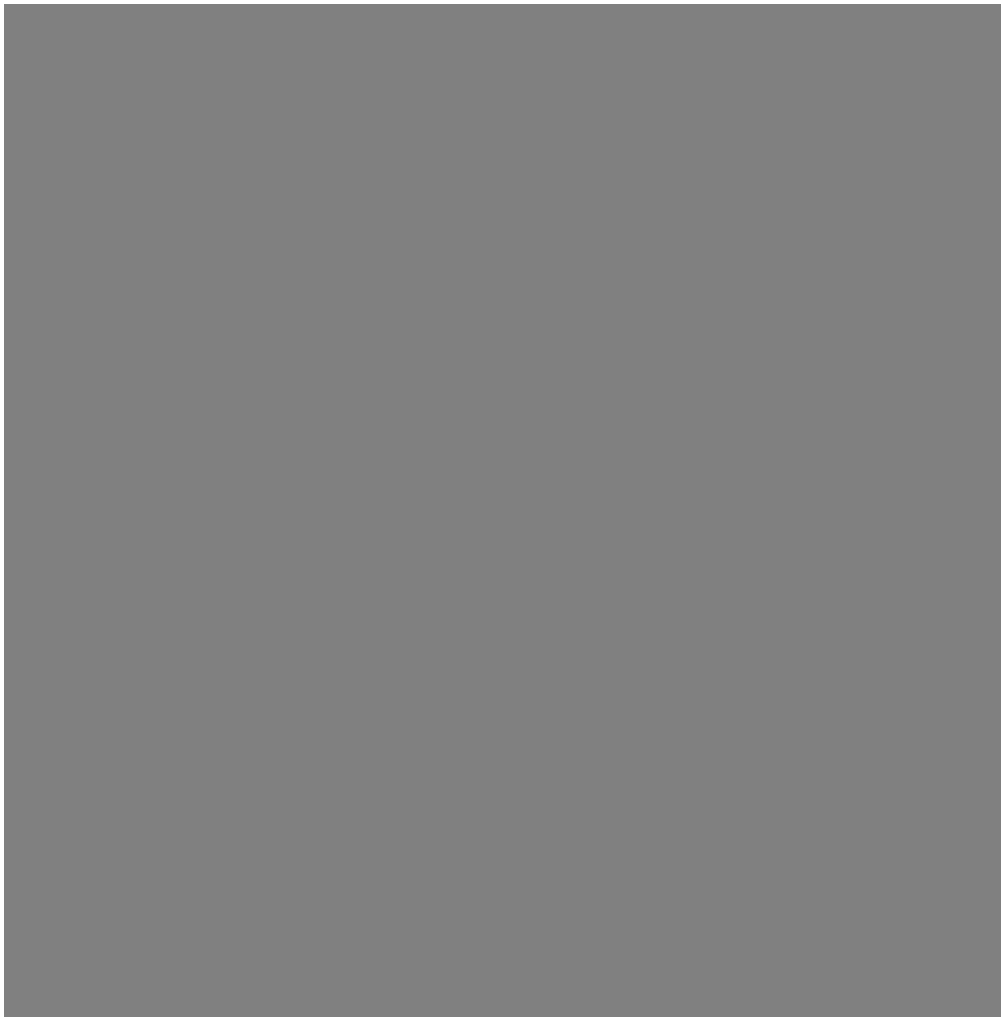


*Figure 9.1 Neural afferent pain pathways from Bushnell et al. (2013) in Nature Reviews including key regions implicated in the pain matrix. PFC=prefrontal cortex; ACC = anterior cingulate cortex; AMY=amygdala; PAG = periaqueductal grey; PB= parabrachial nucleus; BG = basal ganglia; S1= primary somatosensory cortex; S2=secondary somatosensory cortex*

### *9.3.1 Anterior Insula*

The anterior insula is a key region implicated in empathic processing (Gu et al., 2010; Lamm et al., 2011), hence its selection as a region of interest in all experimental chapters of this thesis. Overall, GMV in the insula was not found to be significantly different between groups, but I

found youths with CD had significantly less structural covariance between the left anterior insula and the right thalamus compared to TD youths. Based on previous studies indicating reduced BOLD response in the left anterior insula amongst youths with conduct problems (Lockwood et al., 2013), the region was included as a ROI in an empathy for pain fMRI task, and results indicated youths with CD responded with significantly reduced BOLD response in the left AI relative to TD youths. Finally, PPI analyses showed youths with CD had significantly less connectivity between the anterior insula and the occipital cortex compared to TD youths. Taken together, these results suggest aberrant structural covariance, functional responsivity, and functional connectivity in the anterior insula amongst youths with CD, which reinforces findings from previous studies (Lockwood et al., 2013; Yoder et al., 2016). Furthermore, females with CD have been found to show decreased grey matter volume in the AI compared to TD females (Fairchild et al., 2013a). My findings add to the current evidence to suggest that the AI is central to the pathophysiology of CD. Due to the association between the insula and empathy processing, these results also suggest deficiencies in empathic responsivity amongst youths with CD.

### *9.3.2 Thalamus*

The thalamus is an important neural hub as it receives sensory input and relays the information on to the appropriate regions for processing (Hwang et al., 2017). The thalamus is also implicated in the pain matrix as evidenced by increased responsivity amongst healthy individuals during empathy for pain fMRI tasks (Jackson et al., 2005). In chapter 5, decreased structural covariance was reported between the thalamus and the AI amongst youths with CD compared to TD youths. The thalamus, insula, and nucleus accumbens are functionally connected (Cho et al., 2013). These areas are also implicated in reward processing, which could indicate an

overlap in the network disruption involved in both empathy and reward processing amongst youths with CD. Decreased structural covariance between the AI and the thalamus could indicate impairment in the salience network (Uddin, 2017). Additionally, abnormal thalamic covariance could be associated with limbic system disruption amongst youths with CD, which also involves the amygdala (e.g. Finger et al., 2012).

### *9.3.3 Amygdala*

The amygdala is implicated in emotion processing including responsivity to fear and threat (Phelps & LeDoux, 2005), and as such it is crucial for survival (Mobbs, Hagan, Dalgleish, Silston, & Prévost, 2015). It is connected to the hippocampus, which is primarily associated with memory and learning behaviours, and the two areas communicate regarding learning to avoid or approach certain situations or people (Phelps, 2004). Over the past decade researchers have provided evidence to suggest youths with CD have abnormalities in the amygdala both structurally and functionally (Alegria et al., 2016; Rogers & Brito, 2016). Within this thesis there were no group differences in amygdala structure. However, there was a sex-by-group interaction found in the right amygdala indicating females with CD had reduced BOLD response relative to TD females during the empathy for pain fMRI task. PPI analyses indicated the CD group also exhibited increased connectivity between the amygdala and the cerebellum during the empathy for pain task. These findings are in line with previous research showing males and females have different patterns of amygdala responsivity depending on the stimuli presented (Hamann, 2005).

### *9.3.4 Cerebellum*

Trends in decreased BOLD response in the cerebellum have been observed amongst youths with conduct problems (Lockwood et al., 2013). However, significantly reduced BOLD response

was found in the cerebellum amongst youths with CD during a task-based fMRI empathy for pain task in this thesis. Furthermore, increased functional connectivity between the cerebellum and the amygdala was observed in the youths with CD compared to TD youths. There is an increasing amount of evidence to suggest cerebellar recruitment during empathy-evoking tasks amongst healthy individuals (Lang et al., 2016). Therefore the role of the cerebellum should be further investigated regarding its involvement in empathy in addition to neural differences in youths with CD. My findings in the cerebellum may be indicative of problems empathising with others, however future research should aim to replicate these findings.

## 9.4 Neural correlates of empathy in CD

Overall, the regions discussed above are recruited during processing of pain and are implicated in empathic responsivity (Bushnel et al., 2013; Lamm et al., 2011). This thesis provides evidence to support there is indeed empathy impairment amongst youths with CD due to the impairment in regions associated with empathy processing. Importantly, both the amygdala and the thalamus are part of the limbic system (Rajmohan & Mohandas, 2007). The limbic system is also comprised of the cingulate gyrus, hippocampus, and hypothalamus, and together these regions interact as a network to control emotional processes (McLachlan, 2009). The most widely recognised emotional processes associated with the limbic system include social bonding behaviours such as reproduction and early parent-child attachment and healthy social interactions with other humans (Insel & Fernald, 2004). These activities are related to secretion of oxytocin during birth and breast-feeding (C.S. Carter, Williams, Witt, & Insel, 1992; Ross & Young, 2009) and normal levels of oxytocin alongside proper limbic system functioning are associated with typical empathic responsivity (Shirtcliff et al., 2009). Moreover, disrupted processing in

neural regions known to have overlap in both the empathy for pain network and the limbic system in youths with CD provides support for underlying neural mechanisms associated with antisocial behaviours and reduced levels of empathy. Additionally, presence of a large cavum septum pellucidum is considered a neurodevelopmental marker for limbic maldevelopment, and both antisocial adults and antisocial youths have large CSPs (Raine, Lee, Yang, & Colletti, 2010; White et al., 2013) which contributes additional evidence to support aberrant limbic system development amongst youths with CD.

Impairment in overlapping brain regions and neural networks suggests empathy processing is only a part of the problem, as these brain regions are also involved in responding to other salient and emotional stimuli such as reward processing. However, investigating neural correlates of empathy is of the utmost importance as individuals with significantly decreased levels of empathic responsivity can have a serious and harmful impact on society.

### 9.5 Sex differences in CD

Previous studies have investigated sex differences in CD in regards to risk factors and clinical outcomes (Berkout, Young, & Gross, 2011). However, very few studies have investigated neural correlates of sex differences in CD. Existing neuroimaging studies on CD have primarily included samples with males only. There is a dearth of research on this topic, and to date one other study has investigated neuroimaging correlates of sex differences in empathy amongst youths with conduct problems (Michalska et al., 2016). Results from that study indicated two sex-by-group interactions were observed. The first implicated the right posterior superior temporal gyrus during the intentional vs. unintentional harm condition, whereby higher numbers of CP symptoms were associated with lower levels of BOLD response in females, but this effect

was not observed in males. The second sex-by-group interaction was observed in the posterior superior temporal sulcus and the middle frontal gyrus, whereby conduct problems were negatively associated with BOLD response amongst females, but not males during the harm vs. no harm condition. However, the participants in that sample did not have a full diagnosis of CD, so the groups were comprised of individuals with *some* CD symptoms or *no* CD symptoms. They were also pre-adolescent (9-11 years of age). This thesis explored neural correlates of empathy in a large sample of pre and post-adolescent males and females meeting full criteria for CD thus addressing an important gap in the literature. I found a sex-by-group interaction in the amygdala during an empathy for pain task-based fMRI study indicating females with CD had reduced BOLD response relative to TD females whilst viewing others in painful situations. Males with CD and TD males did not differ. This is an interesting finding that could suggest reduced threat or fear-based responsivity amongst females with CD due to decreased response to viewing others in pain in a region heavily affiliated with emotion processing. This could suggest different biomarkers for males and females in this clinical group. Future studies should aim to investigate sex differences in the amygdala amongst youths with CD.

Overall, results from this thesis alongside findings in the current literature suggest males and females with CD have relatively similar neural structure and function. However, the sex-by-group interaction in the amygdala is an interesting finding suggesting there may be differences in how emotional stimuli are processed between the two groups. The direction of this effect is in line with structural MRI findings indicating females with CD have reduced GMV in the amygdala, anterior insula, and striatum compared to TD females (Fairchild et al., 2013). Given the behavioural differences discussed in chapter 1, (covert behaviours compared to overt

behaviours in males), perhaps neuroimaging research should focus on how to delineate these differences in covert and overt symptomology. There may be general overlap, with perhaps a few nuances underlying these differences. For example, one study showed the most common symptom reported amongst females with CD was lying to deceive others (Hipwell et al., 2002). General investigations on empathy could encompass neural correlates involved in deceitful behaviours. But a more specific paradigm focusing on how the brain responds to moral situations and overt compared to covert symptoms could be informative.

The results presented in this thesis suggest that males and females with CD have similar brain structure and function. There were no sex-by-group interactions apart from one interaction observed in the amygdala during the fMRI empathy for pain task in chapter 6. This result could add to the other evidence to suggest partial sex differences in CD (e.g Michalska et al., 2016; Fairchild et al., 2013; Smaragdi et al., in press). However, this could also be due to a variety of reasons unrelated to CD diagnosis. Ultimately it is premature to suggest sex differences exist in the neurobiology of CD, and more research is necessary to properly address this issue.

## 9.6 General strengths and limitations

The following two sub-sections will review strengths and limitations of this thesis, at both the micro and macro levels. Strengths will be reviewed first followed by limitations. In consideration of the topics covered, the following section will then provide suggestions for future directions.

### *9.6.1 Strengths*

First, a large sample with enough power to address hypotheses was used in this thesis. This is important because previous studies aiming to explore similar research questions have included

much smaller samples (e.g. Sterzer et al., 2007; Decety et al., 2009), which in effect may limit abilities to make generalisations extended to the population.

Second, the sample is well characterised, as the participants met full diagnostic criteria for CD and the K-SADS was used for assessment, which is a thorough and reliable assessment tool for clinical classification in research. In-depth interviews were completed with both the participants and their parents/ guardians where appropriate, which assures thorough screening. Not all previous studies have relied upon a standardised interview, and instead opted for questionnaire data. Ensuring participants meet criteria for the disorder is of the utmost important as only then can inferences be made regarding the population as a whole.

Third, this thesis provided evidence to replicate and extend upon findings from research on both the neural pain matrix and the empathy for pain matrix amongst youths with CD. Abnormal neural correlates of empathy in a clinical population require further investigation and provide insight into neural underpinnings of the phenotype.

Finally, this thesis addressed gaps in the existing literature on sex differences among youths with CD. To date there are only a few studies that have examined sex differences amongst youths with CD (e.g. Fairchild et al., 2013; Michalska et al., 2016), therefore this thesis contributed evidence from a sample of both male and female youths with CD.

### *9.6.2 Limitations*

First, the large age range (9-18) of participants should be considered as youths within this range are at different pubertal and developmental stages. Hormonal changes during puberty have an effect on brain structure (Goddings et al., 2014) therefore a group including both pre and post-

pubertal youths is less homogeneous, and this complicates the ability to ascertain meaningful differences between the groups.

Second, CU traits, personality traits, and comorbidities were not addressed in this thesis. These factors clearly have an influence on the effects observed at the neural level among youths with CD, and distinct neuroimaging correlates are observed for youths with different clinical subtypes. Addressing the influence of CD specifiers or subtypes was not in the scope of this thesis as I aimed to address sex differences and variations in empathic responsivity.

Third, a number of the participants with CD had missing or limited information from their parents or guardians, resulting in challenges interpreting assessments and questionnaires. For example, the ICU parent-report questionnaire has been used in previous studies (e.g. Lockwood et al., 2013) to analyse levels of CU traits amongst participants, but due to a large number of clinical participants in this sample with missing parent-report ICU data, the YPI self-report questionnaire was used to measure psychopathic traits instead. Self-report data, especially from a clinical group known for narcissistic and deceitful tendencies (Barry, Frick, & Killian, 2003; Nock et al., 2006) can be biased therefore third party or parent report data is more desirable.

Fourth, this sample includes a relatively small number of females with CD compared to the other three experimental groups (males with CD, typically developing males, and typically developing females). Considering the prevalence of females with CD is claimed to be underestimated (Delligatti, Akin-Little, & Little, 2003), it is unclear if the small group size in my thesis is an accurate reflection of the population, or if females with CD are merely more difficult to recruit. That being said, the majority of the females with CD included in this thesis were not

referred from clinical or forensic settings, but rather through word of mouth. Although schools, clinics, and forensic locations did indeed contain females with behaviour problems, some ultimately declined invitations to participate in the study.

Fifth, as discussed in chapter 3 the data included in this thesis constitute a subsample from the larger FemNAT-CD project, of which data collection is ongoing. Researchers working on the project until completion will be better equipped to address gaps, and can then assess the full scope of study aims once all data are collected and all key factors are taken into consideration.

Additional limitations include possible influence of comorbid disorders and substance misuse amongst participants. ADHD is a common co-occurring disorder with CD (Loeber et al., 2000), and to control for this, the analyses in this thesis were run with and without ADHD symptoms as covariates of no interest. Results were not significantly different, suggesting the reported effects were not due to ADHD symptoms consistent with recent sMRI and fMRI meta-analyses on conduct problems (Alegria et al., 2016; Rogers & Brito, 2016). Typically developing participants were not included in the study if they reported past or present substance misuse, but this was not an exclusion criteria for the participants with CD, as the clinical reality is that youths with CD often do have comorbid disorders, including substance misuse. Therefore, not controlling for this provides an accurate reflection of the clinical population. However, it leaves open the possibility that some of the reported results might have been influenced by group differences in substance misuse ( see Dalwani et al., 2014, 2015).

Finally, the cross-sectional design of this study is a limitation. Longitudinal designs allow for researchers to compare brain structure and function over time and analyse how psychiatric

symptomology predicts mental health outcomes (Muetzel et al., 2017). This is also particularly important in a group of youths at differing levels of maturation and developmental stages, as individual differences in neural properties can be compared at a later developmental stage.

## 9.7 Future directions

There are many potential avenues to explore in the future including analyses related to CD specifiers and age. Several studies to date have examined how these variables modulate effects as reviewed in chapter 2. However, more research is necessary on neural correlates of sex differences among youths with CD and the associated subtypes. Firstly, CU traits or the *limited prosocial emotion* specifier recently added to the DSM-5 should be investigated as trends in neuroimaging research suggest there to be different structural and functional correlates of youths with high and low levels of CU traits, further supporting the heterogeneity of the disorder. Another CD specifier, age of onset should also be investigated. Finally, personality characteristics such as aggression and empathy levels should be taken into account, as examining how variations in these individual traits correlate with neuroimaging data will provide insight into how behaviours and clinical presentation map onto the organisation of the human cortex.

Regarding sex differences in CD, future studies should aim to closely examine neural correlates of specific behaviours observed as different between males and females. For example in chapter 1, males were characterised by overt aggressive tendencies and females were characterised by covert relationally aggressive tendencies. Also, the common symptom reported amongst females with CD was lying to deceive others (Hipwell et al., 2002). Empathy is a vague and complex construct, and detection of underlying neural differences between males and

females with CD could include more symptom-specific paradigms examining relational compared to physical aggression could help address this gap.

## 9.8 Scientific and clinical implications

### *9.8.1 Scientific implications*

Results previously identified in the existing literature were replicated in this thesis, and novel research was carried out, offering new contributions to the field. The information reviewed thus far suggests neuroimaging investigations on CD should carefully examine subtypes and specifiers of CD in addition to the influence of symptomology from a dimensional approach. Further research is also necessary into sex differences in CD. Whilst this thesis explored empathy correlates, other studies should examine sex differences in separate constructs and emotional processes that have been associated with impairment in CD including aggression, decision-making, and reward processing. The increased incidence of CD amongst females should prompt investigations into factors such as puberty and associated hormonal changes that seem to happen at an earlier age over generations, which is associated with antisocial behaviours (Keenan et al., 1999; Fontaine et al., 2009).

### *9.8.2 Clinical implications*

Youths with CD in this sample demonstrated relatively intact structural neural correlates, but more pronounced functional impairment, which may have advantageous clinical implications. As discussed in chapters 4 and 5, structural properties of the brain are heritable and can be altered over time due to influences on neuroplasticity. However, changes in GMV and brain structure happen over extended periods of time, influenced by circumstances such as child development, learning, or chronic exposure to positive or negative events. In contrast, the functioning brain is more sensitive to external factors, especially task-based functional MRI and connectivity. Perhaps the majority of youths with CD without a severe classification of the disorder are receptive to early intervention and therapeutic strategies before significant differences in brain structure emerge (Finger et al., 2012). Additionally, due to impaired structural covariance between regions associated with processing pain and reduced BOLD response during empathic processing, youths with CD could benefit from empathy training. Preemptive empathy training could be offered to enhance abilities to recognise distress in others, and Empathic-Emotion Recognition training has indeed been an effective technique to help reduce the number of conduct problems (Dadds et al., 2012). Importantly, this implies empathy can be taught. Whilst individuals will inevitability vary on levels of empathy and CU traits, parents, teachers, and clinicians can be valuable resources for infants and youths to learn attachment, social bonding behaviours, and prosocial attitudes which may in turn reduce chances of developing antisocial behaviours and the neural impairments associated with antisocial behaviours and deficient empathy processing.

Importantly, therapeutic strategies can significantly reduce number of symptoms and lead to decreased neural functional impairment, as evidenced by Cognitive Behaviour Therapy outcomes amongst individuals with psychosis (Kumari et al., 2011). This demonstrates another benefit of longitudinal research designs as long-term effects of treatment on neural functioning of individuals can be assessed. Randomised clinical trials with various types of therapy targeted at improving empathy processing amongst youths with CD would provide more information regarding response to treatment and how this maps onto the brain.

In chapter 1 observable behavioural differences amongst males and females with CD were discussed including overt and covert aggression. Whilst males are known to be more physically aggressive, females are more relationally aggressive (Marsee & Frick, 2007). Therefore perhaps the criteria for CD could be reassessed to include aggressive subtypes or subtypes and symptoms better suited to encompass all phenotypes of the disorder. However, without further research clarification on this matter remains elusive.

## 9.9 General Conclusion

Overall, I investigated structural and functional neural correlates of empathy among males and females with CD. Results revealed youths with CD have relatively intact brain structure relative to TD youths within this sample, whereas functional impairment between the groups was more pronounced. These findings add to the existing literature as sex differences were addressed in a large well-powered sample, novel methodological approaches were used, and differences were found in brain regions previously identified which indicates replication, in addition to regions not previously identified, which yields a unique contribution to the field.

# APPENDICES

*Appendix 5.1 Voxel-based morphometry whole brain analyses uncorrected results*

| Brain Region | BA | L/R | Peak voxel MNI coordinates | | | k | *t* |
|---|---|---|---|---|---|---|---|
| **TD > CD** | | | | | | | |
| Temporal_Mid | 22 | R | 68 | -36 | 4 | 55 | 4.46 |
| Frontal_Sup | 9 | L | -26 | 50 | 42 | 22 | 4.30 |
| Temporal_Mid | 21 | L | -68 | -2 | -16 | 21 | 3.98 |
| Temporal_Sup | 22 | R | 58 | -15 | -4 | 35 | 3.89 |
| Occipital_Mid | 19 | R | 45 | -84 | 8 | 41 | 3.83 |
| Temporal_Mid | 21 | R | 58 | -10 | -12 | 14 | 3.83 |
| Frontal_Sup | 8 | L | -12 | 20 | 57 | 64 | 3.77 |
| Cingulum_Mid | 23 | - | 0 | -15 | 44 | 16 | 3.72 |
| Parietal_Inf | 40 | L | -52 | -34 | 45 | 17 | 3.69 |
| Cerebellum | 19 | R | 36 | -81 | -18 | 15 | 3.66 |
| Precuneus | 7 | R | 10 | -72 | 51 | 14 | 3.65 |
| Frontal_Mid | 45 | R | 46 | 44 | 30 | 13 | 3.63 |
| Cingulum_Mid | 23 | R | 2 | -8 | 45 | 13 | 3.60 |
| Fusiform | 19 | L | -30 | -66 | -16 | 34 | 3.59 |
| Frontal_Inf_Orb | 38 | L | -52 | 26 | -10 | 17 | 3.59 |
| Paracentral_Lobule | - | - | 0 | -24 | 56 | 32 | 3.57 |
| Precuneus | 7 | R | 16 | -75 | 50 | 12 | 3.51 |
| Frontal_Sup | 8 | R | 16 | 30 | 52 | 11 | 3.49 |
| Fusiform | 37 | R | 34 | -46 | -16 | 12 | 3.46 |
| Precuneus | 5 | L | -2 | -38 | 66 | 11 | 3.39 |
| Paracentral_Lobule | 6 | L | -6 | -15 | 72 | 11 | 3.35 |
| **TD < CD** | | | | | | | |
| Temporal_Mid | 22 | R | 68 | -36 | 4 | 55 | 4.46 |
| Frontal_Sup | 9 | L | -26 | 50 | 42 | 22 | 4.30 |
| Temporal_Mid | 21 | L | -68 | -2 | -16 | 21 | 3.98 |
| Temporal_Sup | 22 | R | 58 | -15 | -4 | 35 | 3.89 |
| Occipital_Mid | 19 | R | 45 | -84 | 8 | 41 | 3.83 |
| Temporal_Mid | 21 | R | 58 | -10 | -12 | 14 | 3.83 |
| Frontal_Sup | 8 | L | -12 | 20 | 57 | 64 | 3.77 |
| Cingulum_Mid | 23 | - | 0 | -15 | 44 | 16 | 3.72 |
| Parietal_Inf | 40 | L | -52 | -34 | 45 | 17 | 3.69 |
| Cerebellum | 19 | R | 36 | -81 | -18 | 15 | 3.66 |
| Lingual (Frontal Lobe) | 18 | L | -14 | -75 | 3 | 13 | 3.57 |

Height threshold = p <.001, uncorrected.
Extent threshold = 10 voxels.
Group differences in ANCOVA co-varying out the effect of age, IQ, and for the contrasts CD > TD, CD < TD only.
MNI = Montreal Neurological Institute.
TD = Typically Developing; CD = Conduct Disorder

*Appendix 5.2 Structural MRI studies of disruptive behaviour disorders in youths*

| Study | Nature of sample | Participants | Methods | Main Results[a] |
|---|---|---|---|---|
| Sterzer *et al*. (2007) | Clinical | 24 males (12 CD/childhood-onset; 12 TD controls) aged 12 **years** (M: 12.6) | VBM | CD < Controls: Significantly reduced grey matter in left amygdala and bilateral anterior insula. In the CD group, bilateral anterior insula grey matter volume correlated positively with empathy score. |
| Huebner *et al*. (2008) | Clinical | 46 males (23 CD/childhood-onset (17 co-morbid for ADHD); 23 TD controls) aged 12-17 **years** (M: 14.4) | VBM | CD < Controls: 6% reduction in average grey matter volume. Significantly reduced grey matter volume in bilateral temporal cortex, left amygdala, left hippocampus, orbitofrontal & ventromedial regions. CD symptom severity inversely correlated with grey matter volume within limbic structures.<br>CD > Controls: Significant increase in grey matter volume within cerebellum bilaterally. |
| De Brito *et al.* (2009) | Community | 48 males (23 CP/HCU traits; 25 TD controls) aged 10-13.3 **years** (M: 11.7) | VBM | CP > Controls: Boys with elevated CU traits showed increased grey matter concentration within medial OFC and ACC. Increased grey matter volume and concentration observed in bilateral temporal regions. |
| De Brito *et al*. (2011) | Community | 48 males (23 CP/HCU traits; 25 TD controls) aged 10-13.3 **years** (M: 11.7) | VBM | CP < Controls: Significantly decreased white matter concentration in right superior frontal lobe, right dorsal ACC, right superior temporal gyrus and left precuneus.<br>CP > Controls: Increased white matter concentration within bilateral middle frontal gyrus. |
| Dalwani *et al.* (2011) | Clinical | 44 males (25 antisocial substance dependence; 19 TD controls) aged 14-18 **years** (M: 16.6) | VBM | Patients < Controls: Whole-brain analysis revealed significantly reduced grey matter volume in left DLPFC, right lingual gyrus and bilateral cerebellum. Grey matter volume in left DLPFC negatively associated with severity of substance dependence.<br>Patients > Controls: Significantly increased grey matter volume in right precuneus. |
| Fahim *et al*. (2011) | Community | 47 males (22 DBD; 25 TD controls) aged 8 **years** (M:8.4) | VBM & SBM | DBD < Controls: Significantly decreased grey matter density in left medial PFC, cingulate, and bilateral insula cortices.<br>Increased DBD score associated with decreased grey matter density in left medial middle and superior frontal cortex, precuneus, and right superior temporal and occipital/cuneus regions. |

| | | | | |
|---|---|---|---|---|
| Fairchild *et al.* (2011) | Community | 90 males (36 CD/childhood-onset; 27 CD/adolescent-onset; 27 TD controls) aged 16-21 **years** (M: 18.0) | VBM | CD < Controls: Reduced grey matter volume in amygdala bilaterally (including the insula).<br>CD (adolescent-onset) < Controls: Reduced amygdala and right insula grey matter volume.<br>CD (childhood-onset) < Controls: Reduced amygdala grey matter volume. Right insula volume was negatively correlated with CD-symptom severity in both sub-groups. |
| Hyatt *et al.* (2012) | Community | 43 male/female adolescents (19 CD (10 male); 24 TD control (14 male**)** aged 12-18 **years** (M: 16.2) | SBM | CD < Controls: Reduced cortical thickness mainly in several posterior brain regions across the temporal and parietal lobes. Reduced gyrification primarily located in anterior brain regions (insula, inferior and dorsal frontal regions including lateral OFC, VMPFC and ACC), but also in the temporal and parietal lobes. |
| Stevens *et al*. (2012) | Community | 72 male/female adolescents (24 CD; 24 ADHD; 24 TD controls; 51Male) aged 15-16 **years** (M: 15.9) | VBM | CD < Controls: 13% reduction in grey matter volume reflecting deficits in frontal, temporal, parietal and subcortical regions. Increased grey matter in right OFC, bilateral amygdala and bilateral temporal cortices associated with increased symptom severity in CD adolescents. |
| White *et al.* (2013) | Community | 59 adolescents (32 DBD (25 male): 27 TD controls (19 male) (M age: 14.9) | Manual Tracing | Large cavum septum pellucidum observed in 7/32 DBD adolescents but not for any of the controls. No correlation between size of cavum septum pellucidum and CU traits. |
| Ermer *et al.* (2013) | Prison | 191 male prisoners (M age: 17.3). | VBM | Psychopathic trait scores negatively associated with grey matter volume in regions of interest: PCC and OFC (extending into parahippocampal cortex and temporal poles). Increased grey matter volume in pre-frontal cortex positively associated with psychopathic traits. |
| Fairchild *et al*. (2013) | Community | 42 females (22 CD, 17 adolescent-onset; 20 TD controls) aged 14-20 **years** (M: 17.3) | VBM | CD < Controls: Significantly reduced bilateral anterior insula and right striatal grey matter volumes. Right DLPFC volume was negatively correlated with CD symptom severity whilst CU traits were positively correlated with bilateral OFC volume. |

| | | | | |
|---|---|---|---|---|
| Olvera *et al.* (2014) | Prison | 72 male/female adolescents (24 CD with bipolar disorder (16 male); 24 CD (21 male); 24 TD controls (16 male) (M age: 15.8) | VBM | CD + bipolar disorder < Controls: Decreased grey matter volume of the right medial PFC, superior and inferior frontal gyrus, ACC and temporal gyrus.<br>CD only = Controls: No differences in brain volume. |
| Cope *et al.* (2014) | Prison | 39 female prisoners (M age: 17.6) | VBM | Negative correlations between CU traits and grey matter volume in limbic and paralimbic regions, including OFC, parahippocampal cortex, temporal poles and hippocampus. |
| Wallace *et al.* (2014) | Community | 49 male/female adolescents (22 CD; 27 TD controls) aged 10-18 **years** (M: 14.9) | SBM | CD < Controls: Reduced cortical thickness in superior temporal cortex and reduced gyrification in the VMPFC. Amygdala and striatum (pallidum and putamen) cortical volumes also reduced. Right temporal cortical thickness was inversely correlated with CU trait severity. |

Anatomical abbreviations: ACC – anterior cingulate cortex, DLPFC – dorsolateral prefrontal cortex, OFC – orbitofrontal cortex, PCC – posterior cingulate cortex, PFC – prefrontal cortex, VMPFC – ventromedial prefrontal cortex.
Note: ADHD – Attention-deficit hyperactivity-disorder, CD – conduct disorder, CP – conduct problems, DBD – disruptive behaviour disorder, SBM – surface based morphometry, TD – typically developing, VBM – voxel based morphometry.
[a]CU traits in the Main Results refer to lack of empathy and guilt, and shallow affect. Individual authors may have used a different label, i.e. psychopathic traits, in their paper, but for ease of reading we have used CU traits consistently. The Measures of CU traits column lists the specific measure used.

*Appendix 6.1 Structural covariance left anterior insula whole brain analyses uncorrected results*

| Brain Region | BA | L/R | Peak voxel MNI coordinates | | | k | *t* |
|---|---|---|---|---|---|---|---|
| **TD > CD** | | | | | | | |
| Thalamus | - | R | 16 | -12 | 18 | 251 | 5.79 |
| Insula | 48 | L | -40 | -16 | 24 | 25 | 4.64 |
| Thalamus | - | L | -14 | -18 | 20 | 176 | 4.49 |
| Precentral (Frontal Lobe) | 6 | R | 33 | -24 | 69 | 50 | 4.35 |
| Frontal_Inf_Tri | 45 | L | -52 | 24 | 22 | 49 | 4.21 |
| Temporal_Sup | 21 | R | 64 | -28 | -4 | 16 | 4.00 |
| Thalamus | - | R | 22 | -26 | 8 | 109 | 3.98 |
| Frontal_Mid_Orb | 11 | L | -30 | 44 | -10 | 10 | 3.78 |
| Precentral (Frontal Lobe) | 4 | L | -33 | -30 | 60 | 15 | 3.70 |
| Precuneus | - | R | 9 | -42 | 57 | 23 | 3.65 |
| ParaHippocampal | 27 | L | -21 | -38 | -2 | 34 | 3.58 |
| Corpus Callosum | - | R | 2 | -18 | 26 | 12 | 3.43 |
| **TD < CD** | | | | | | | |
| Frontal_Sup | 9 | R | 24 | 46 | 40 | 54 | 4.53 |
| Brain Stem | - | L | -3 | -12 | -15 | 95 | 4.39 |
| Precentral (Frontal Lobe) | 6 | R | 45 | 2 | 52 | 133 | 4.37 |
| Frontal_Mid | 9 | R | 33 | 32 | 46 | 11 | 3.75 |
| Frontal_Mid | 9 | L | -30 | 34 | 48 | 12 | 3.69 |
| Temporal_Pole_Sup | - | R | 44 | 15 | -16 | 10 | 3.59 |
| Cerebellum | - | L | -3 | -46 | -14 | 24 | 3.41 |
| Temporal Lobe | 48 | R | 38 | -2 | 20 | 22 | 3.40 |

Height threshold = p <.001, uncorrected.
Extent threshold = 10 voxels.
Group differences from ANCOVA co-varying out the effect of age, IQ, global grey matter and for the contrasts CD > TD, CD < TD only.
MNI = Montreal Neurological Institute.
TD = Typically Developing; CD = Conduct Disorder

*Appendix 6.2 Structural covariance right anterior insula whole brain analyses uncorrected results*

| Brain Region | BA | L/R | Peak voxel MNI coordinates | | | k | *t* |
|---|---|---|---|---|---|---|---|
| **TD > CD** | | | | | | | |
| Caudate | - | L | -4 | 10 | 12 | 472 | 4.56 |
| Caudate | - | R | 16 | -6 | 16 | 96 | 4.34 |
| Temporal_Mid | 21 | R | 66 | -28 | -3 | 47 | 4.25 |
| Hippocampus | - | L | -22 | -33 | 9 | 72 | 4.07 |
| Corpus Callosum | - | R | 2 | -16 | 26 | 11 | 4.06 |
| Thalamus | - | R | 6 | -28 | 14 | 21 | 3.95 |
| Postcentral (Parietal Lobe) | 3 | L | -28 | -36 | 70 | 15 | 3.53 |
| **TD < CD** | | | | | | | |
| Frontal_Mid | 6 | R | 40 | 2 | 51 | 18 | 3.76 |
| Supp_Motor_Area | 6 | R | 9 | -4 | 50 | 15 | 3.71 |
| Frontal_Sup_Medial (Frontal Lobe) | 9 | R | 9 | 51 | 45 | 16 | 3.56 |
| Temporal_Inf | 20 | L | -58 | -28 | -27 | 19 | 3.46 |

Height threshold = p <.001, uncorrected.
Extent threshold = 10 voxels.
Group differences from ANCOVA co-varying out the effect of age, IQ, global grey matter and for the contrasts CD > TD, CD < TD only.
MNI = Montreal Neurological Institute.
TD = Typically Developing; CD = Conduct Disorder

*Appendix 6.3 Structural covariance anterior cingulate cortex whole brain analyses uncorrected results*

| Brain Region | BA | L/R | Peak voxel MNI coordinates | | | k | *t* |
|---|---|---|---|---|---|---|---|
| **TD > CD** | | | | | | | |
| Precuneus | 7 | R | 8 | -62 | 40 | 86 | 4.28 |
| Cingulum_Mid | - | L | -2 | -42 | 52 | 117 | 4.24 |
| Temporal_Mid | 21 | L | -48 | -2 | -16 | 22 | 4.05 |
| Temporal_Mid | 20 | L | -70 | -39 | -6 | 39 | 4.03 |
| Temporal_Mid | 21 | R | 56 | -18 | -10 | 57 | 3.90 |
| Angular gyrus (Parietal Lobe) | 19 | L | -44 | -70 | 36 | 14 | 3.90 |
| Frontal_Mid_Orb | 11 | L | -27 | 40 | -9 | 18 | 3.85 |
| Parietal_Inf | 40 | L | -36 | -39 | 50 | 21 | 3.81 |
| Temporal_Mid | 20 | R | 50 | -8 | -14 | 30 | 3.74 |
| Cingulum_Mid | - | L | -2 | -38 | 44 | 16 | 3.67 |
| Angular gyrus (Parietal Lobe) | 39 | R | 63 | -57 | 36 | 12 | 3.58 |
| Caudate | - | R | 16 | -14 | 20 | 14 | 3.56 |
| Postcentral gyrus (Parietal Lobe) | 3 | R | 20 | -38 | 62 | 10 | 3.44 |
| **TD < CD** | | | | | | | |
| Frontal_Mid | 46 | L | -38 | 51 | 28 | 52 | 4.06 |
| Frontal_Mid | 9 | L | -33 | 33 | 51 | 10 | 3.98 |
| Supp_Motor_Area | 6 | L | -4 | -10 | 60 | 21 | 3.66 |

Height threshold = p <.001, uncorrected.
Extent threshold = 10 voxels.
Group differences from ANCOVA co-varying out the effect of age, IQ, global grey matter and for the contrasts CD > TD, CD < TD only.
MNI = Montreal Neurological Institute.
TD = Typically Developing; CD = Conduct Disorder

*Appendix 7.1 Participants excluded from functional MRI analyses*

| *n* = 53 | TD Females UOS | TD Females UOB | TD Males UOS | TD Males UOB | CD Males UOS | CD Males UOB | CD Females UOS | CD Females UOB | Total Number |
|---|---|---|---|---|---|---|---|---|---|
| Did not complete task / Missing data | 1 | 1 | 2 | 0 | 7 | 3 | 5 | 1 | 20 |
| Excessive motion | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 4 |
| Missing behavioural responses | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 4 |
| Bad first level mask | 4 | 3 | 2 | 2 | 6 | 4 | 4 | 0 | 25 |

*Appendix 7.2 fMRI pain > no pain for all participants whole brain analyses uncorrected results*

| Brain Region | BA | L/R | Peak voxel MNI coordinates | | | k | *t* |
|---|---|---|---|---|---|---|---|
| Temporal_Inf | 37 | R | 51 | -63 | -6 | 1427 | 11.65 |
| SupraMarginal | 48 | L | -60 | -24 | 33 | 1487 | 11.19 |
| Frontal_Inf_Oper | 44 | L | -48 | 6 | 24 | 159 | 7.55 |
| Precentral (Frontal Lobe) | 44 | R | 51 | 6 | 27 | 108 | 6.18 |
| Insula | 48 | L | -39 | -6 | 6 | 22 | 4.43 |
| Frontal_Inf_Tri (Frontal Lobe) | 45 | L | -42 | 33 | 12 | 17 | 4.29 |
| Sub-Gyral (Frontal Lobe) | 6 | L | -21 | -12 | 54 | 18 | 3.99 |
| Insula | 48 | R | 39 | -3 | -3 | 10 | 3.93 |

Height threshold = p <.001, uncorrected.
Extent threshold = 10 voxels.
MNI = Montreal Neurological Institute.
Results from ANCOVA co-varying out the effects of age and IQ, and for the contrast pain > no pain only.

*Appendix 7.3 fMRI pain > no pain, effect of diagnosis, whole brain analyses uncorrected results*

| Brain Region | BA | L/R | Peak voxel MNI coordinates | | | k | *t* |
|---|---|---|---|---|---|---|---|
| **TD > CD** | | | | | | | |
| Cerebellum | - | L | -9 | -60 | -33 | 65 | 5.14 |
| Cerebellum | 19 | R | 30 | -81 | -24 | 26 | 4.39 |
| Cerebellum | 19 | L | -27 | -60 | -24 | 38 | 4.25 |
| Sub-Gyral Frontal Lobe | 48 | R | 30 | -15 | 30 | 23 | 4.07 |
| Lingual (Occipital Lobe) | 18 | R | 15 | -75 | -15 | 17 | 3.90 |
| Cerebellum | 37 | R | 36 | -51 | -27 | 13 | 3.89 |
| Sub-Gyral Parietal Lobe | 48 | R | 39 | -30 | 24 | 12 | 3.52 |
| Frontal_Inf_Tri (Frontal Lobe) | 48 | R | 36 | 15 | 24 | 10 | 3.56 |

Height threshold = p <.001, uncorrected.
Extent threshold = 10 voxels.
MNI = Montreal Neurological Institute.
Group differences from ANCOVA co-varying out the effects of age and IQ, and for the contrast pain > no pain only. There were no effects observed for TD < CD.

# REFERENCES


Adolphs, R., Tranel, D., & Damasio, A. R. (1998). The human amygdala in social judgment. *Nature*, *393*(6684), 470–474. https://doi.org/10.1038/30982

Aghajani, M., Klapwijk, E. T., van der Wee, N. J., Veer, I. M., Rombouts, S. A. R. B., Boon, A. E., … Colins, O. F. (2017). Disorganized Amygdala Networks in Conduct-Disordered Juvenile Offenders With Callous-Unemotional Traits. *Biological Psychiatry*, *82*(4), 283–293. https://doi.org/10.1016/j.biopsych.2016.05.017

Aguirre, G. K. (2010). Experimental design and data analysis for fMRI. In *Functional MRI* (pp. 55–69). https://doi.org/10.1007/978-1-4419-0345-7

Alegria, A. A., Radua, J., & Rubia, K. (2016). Meta-analysis of fmri studies of disruptive behavior disorders. *American Journal of Psychiatry*, *173*(11), 1119–1130. https://doi.org/10.1176/appi.ajp.2016.15081089

Alexander-Bloch, A., Giedd, J. N., & Bullmore, E. (2013). Imaging structural co-variance between human brain regions. *Nat Rev Neurosci*, *14*(5), 322–336. https://doi.org/10.1038/nrn3465

Allen, J. S., Damasio, H., & Grabowski, T. J. (2002). Normal neuroanatomical variation in the human brain: An MRI-volumetric study. *American Journal of Physical Anthropology*, *118*(4), 341–358. https://doi.org/10.1002/ajpa.10092

American Psychiatric Association. (2013). American Psychiatric Association. Diagnostic and statistical manual of mental disorders. *Washington DC*. https://doi.org/10.1176/appi.books.9780890425596.744053

Anderson, A. L., & Thomason, M. E. (2013). Functional plasticity before the cradle: A review of neural functional imaging in the human fetus. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2013.03.013

Aoki, Y., Inokuchi, R., Nakao, T., & Yamasue, H. (2014). Neural bases of antisocial behavior: a voxel-based meta-analysis. *Social Cognitive and Affective Neuroscience*, *9*(8), 1223–1231. https://doi.org/10.1093/scan/nst104

Archer, J. (1991). The influence of testosterone on human aggression. *British Journal of Psychology (London, England : 1953)*, *82 ( Pt 1)*, 1–28. https://doi.org/10.1111/j.2044-8295.1991.tb02379.x

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *NeuroImage*, *38*(1), 95–113. https://doi.org/10.1016/j.neuroimage.2007.07.007

Ashburner, J., & Friston, K. J. (2000). Voxel-Based Morphometry—The Methods. *NeuroImage*, *11*(6), 805–821. https://doi.org/10.1006/nimg.2000.0582

Ashburner, J., & Friston, K. J. (2001). Why Voxel-Based Morphometry Should Be Used. *NeuroImage*, *14*(6), 1238–1243. https://doi.org/10.1006/nimg.2001.0961

Baez, S., Flichtentrei, D., Prats, M., Mastandueno, R., Garc?a, A. M., Cetkovich, M., & Ibanez, A. (2017). Men, women?who cares? A population-based study on sex differences and gender roles in empathy and moral cognition. *PLOS ONE*, *12*(6), e0179336. https://doi.org/10.1371/journal.pone.0179336

Balconi, M., & Canavesio, Y. (2016). Is empathy necessary to comprehend the emotional faces? The empathic effect on attentional mechanisms (eye movements), cortical correlates (N200 event-related potentials) and facial behaviour (electromyography) in face processing. *Cognition and Emotion*, *30*(2), 210–224. https://doi.org/10.1080/02699931.2014.993306

Banissy, M. J., Kanai, R., Walsh, V., & Rees, G. (2012). Inter-individual differences in empathy are reflected in human brain structure. *NeuroImage*, *62*(3), 2034–2039.

https://doi.org/10.1016/j.neuroimage.2012.05.081
Bardone, A. M., Moffitt, T. E., Caspi, A., Dickson, N., Stanton, W. R., & Silva, P. A. (1998). Adult physical health outcomes of adolescent girls with conduct disorder, depression, and anxiety. *Journal of the American Academy of Child and Adolescent Psychiatry*, *37*(6), 594–601. https://doi.org/10.1097/00004583-199806000-00009
Barnea-Goraly, N., Menon, V., Eckert, M., Tamm, L., Bammer, R., Karchemskiy, A., … Reiss, A. L. (2005). White matter development during childhood and adolescence: A cross-sectional diffusion tensor imaging study. *Cerebral Cortex*. https://doi.org/10.1093/cercor/bhi062
Baron-Cohen, S. (2001). Theory of Mind in Normal Development and Autism. *Prisme*, *34*, 174–183. Retrieved from http://www.autism-community.com/wp-content/uploads/2010/11/TOM-in-TD-and-ASD.pdf
Baron-Cohen, S. (2004). Essential Difference: Male and Female Brains and the Truth about Autism. *The Essential Difference: The Truth about the Male and Female Brain.*, (January 2003), 290. https://doi.org/eBook ISBN: 9780786740314
Baron-Cohen, S. (2011). *Zero degrees of empathy: a new theory of human cruelty*. London: Allen Lane.
Barrett, L. F., Lane, R. D., Sechrest, L., & Schwartz, G. E. (2000). Sex Differences in Emotional Awareness. *Personality and Social Psychology Bulletin*, *26*(9), 1027–1035. https://doi.org/10.1177/01461672002611001
Barry, C. T., Frick, P. J., & Killian, A. L. (2003). The relation of narcissism and self-esteem to conduct problems in children: a preliminary investigation. *Journal of Clinical Child and Adolescent Psychology : The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*, *32*(1), 139–52. https://doi.org/10.1207/S15374424JCCP3201_13
Barth, C., Villringer, A., & Sacher, J. (2015). Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2015.00037
Batouli, S. A. H., Trollor, J. N., Wen, W., & Sachdev, P. S. (2014). The heritability of volumes of brain structures and its relationship to age: A review of twin and family studies. *Ageing Research Reviews*. https://doi.org/10.1016/j.arr.2013.10.003
Berkout, O. V., Young, J. N., & Gross, A. M. (2011). Mean girls and bad boys: Recent research on gender differences in conduct disorder. *Aggression and Violent Behavior*. https://doi.org/10.1016/j.avb.2011.06.001
Bernard, J. a, Seidler, R. D., Hassevoort, K. M., Benson, B. L., Welsh, R. C., Wiggins, J. L., … Peltier, S. J. (2012). Resting state cortico-cerebellar functional connectivity networks: a comparison of anatomical and self-organizing map approaches. *Frontiers in Neuroanatomy*, *6*(August), 31. https://doi.org/10.3389/fnana.2012.00031
Bernhardt, B. C., Klimecki, O. M., Leiberg, S., & Singer, T. (2014). Structural covariance networks of the dorsal anterior insula predict females' individual differences in empathic responding. *Cerebral Cortex*, *24*(8), 2189–2198. https://doi.org/10.1093/cercor/bht072
Bernhardt, B. C., & Singer, T. (2012). The Neural Basis of Empathy. *Annual Review of Neuroscience*, *35*(1), 1–23. https://doi.org/10.1146/annurev-neuro-062111-150536
Bethlehem, R. A. I., Romero-Garcia, R., Mak, E., Bullmore, E. T., & Baron-Cohen, S. (2017). Structural covariance networks in children with autism or ADHD. *Cerebral Cortex*, *27*(8), 4267–4276. https://doi.org/10.1093/cercor/bhx135
Betti, V., & Aglioti, S. M. (2016). Dynamic construction of the neural networks underpinning empathy for pain. *Neuroscience and Biobehavioral Reviews*.

https://doi.org/10.1016/j.neubiorev.2016.02.009
Beyer, C. (1999). Estrogen and the developing mammalian brain. *Anatomy and Embryology*. https://doi.org/10.1007/s004290050236
Blair, J., & Frith, U. (2000). Neurocognitive explanations of the antisocial personality disorders. *Criminal Behaviour and Mental Health*, *10*(SPEC.ISS.), S66–S81. https://doi.org/10.1002/cbm.344
Blair, R. (2001). Neurocognitive models of aggression, the antisocial personality disorders, and psychopathy. *Journal of Neurology, Neurosurgery & Psychiatry*, *71*(6), 727–731. https://doi.org/10.1136/jnnp.71.6.727
Blair, R. J. R. (1999). Responsiveness to distress cues in the child with psychopathic tendencies. *Personality and Individual Differences*, *27*(1), 135–145. https://doi.org/10.1016/S0191-8869(98)00231-1
Blair, R. J. R. (2005). Responding to the emotions of others: Dissociating forms of empathy through the study of typical and psychiatric populations. *Consciousness and Cognition*. https://doi.org/10.1016/j.concog.2005.06.004
Blair, R. J. R. (2008). Fine cuts of empathy and the amygdala: Dissociable deficits in psychopathy and autism. In *Quarterly Journal of Experimental Psychology* (Vol. 61, pp. 157–170). https://doi.org/10.1080/17470210701508855
Blair, R. J. R. (2009). Too much of a good thing: Increased grey matter in boys with conduct problems and callous-unemotional traits. *Brain*. https://doi.org/10.1093/brain/awp051
Blair, R. J. R. (2013). The neurobiology of psychopathic traits in youths. *Nature Reviews Neuroscience*, *14*(11), 786–799. https://doi.org/10.1038/nrn3577
Blair, R. J. R., Colledge, E., Murray, L., & Mitchell, D. G. V. (2001). A selective impairment in the processing of sad and fearful expressions in children with psychopathic tendencies. *Journal of Abnormal Child Psychology*, *29*(6), 491–498. https://doi.org/10.1023/A:1012225108281
Blair, R. J. R., Leibenluft, E., & Pine, D. S. (2014). Conduct disorder and callous-unemotional traits in youth. *The New England Journal of Medicine*, *371*(23), 2207–16. https://doi.org/10.1056/NEJMra1315612
Blair, R. J. R., Peschardt, K. S., Budhani, S., Mitchell, D. G. V, & Pine, D. S. (2006). The development of psychopathy. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. https://doi.org/10.1111/j.1469-7610.2006.01596.x
Blair, R. J., White, S. F., Meffert, H., & Hwang, S. (2014). Disruptive behavior disorders: Taking an RDoC(ish) approach. *Current Topics in Behavioral Neurosciences*, *16*, 319–336. https://doi.org/10.1007/7854_2013_247
Blatt, G. J., Oblak, A. L., & Schmahmann, J. D. (2013). Cerebellar Connections with Limbic Circuits: Anatomy and Functional Implications. In *Handbook of the Cerebellum and Cerebellar Disorders* (pp. 479–496). https://doi.org/10.1007/978-94-007-1333-8
Boes, A. D., Bechara, A., Tranel, D., Anderson, S. W., Richman, L., & Nopoulos, P. (2009). Right ventromedial prefrontal cortex: A neuroanatomical correlate of impulse control in boys. *Social Cognitive and Affective Neuroscience*, *4*(1), 1–9. https://doi.org/10.1093/scan/nsn035
Boes, A. D., Tranel, D., Anderson, S. W., & Nopoulos, P. (2008). Right anterior cingulate: A neuroanatomical correlate of aggression and defiance in boys. *Behavioral Neuroscience*, *122*(3), 677–684. https://doi.org/10.1037/0735-7044.122.3.677
Bookstein, F. L. (2001). "Voxel-Based Morphometry" Should Not Be Used with Imperfectly Registered Images. *NeuroImage*, *14*(6), 1454–1462. https://doi.org/10.1006/nimg.2001.0770

Bressler, S. L., & Menon, V. (2010). Large-scale brain networks in cognition: emerging methods and principles. *Trends in Cognitive Sciences*. https://doi.org/10.1016/j.tics.2010.04.004

Broulidakis, M. J., Fairchild, G., Sully, K., Blumensath, T., Darekar, A., & Sonuga-Barke, E. J. S. (2016). Reduced Default Mode Connectivity in Adolescents With Conduct Disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, *55*(9), 800–808.e1. https://doi.org/10.1016/j.jaac.2016.05.021

Burke, J.D., Loeber, R., & Birmaher, B. (2002). Oppositional Defiant Disorder and Conduct Disorder: A Review of the Past 10 Years, Part II. *Journal of the American Academy of Child & Adolescent Psychiatry*, *41*(11), 1275–1293. https://doi.org/10.1097/00004583-200211000-00009

Bushnell, M. C., Čeko, M., & Low, L. A. (2013). Cognitive and emotional control of pain and its disruption in chronic pain. *Nature Reviews Neuroscience*, *14*(7), 502–511. https://doi.org/10.1038/nrn3516

Bussing, R., Grudnik, J., Mason, D., Wasiak, M., & Leonard, C. (2002). ADHD and Conduct Disorder: An MRI Study in a Community Sample. *The World Journal of Biological Psychiatry*, *3*(4), 216–220. https://doi.org/10.3109/15622970209150624

Button, K. S., Ioannidis, J. P. A., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S. J., & Munafò, M. R. (2013). Power failure: Why small sample size undermines the reliability of neuroscience. *Nature Reviews Neuroscience*, *14*(5), 365–376. https://doi.org/10.1038/nrn3475

Carr, L., Iacoboni, M., Dubeau, M.-C., Mazziotta, J. C., & Lenzi, G. L. (2003). Neural mechanisms of empathy in humans: A relay from neural systems for imitation to limbic areas. *Proceedings of the National Academy of Sciences*, *100*(9), 5497–5502. https://doi.org/10.1073/pnas.0935845100

Carter, C. S., Heckers, S., Nichols, T., Pine, D. S., & Strother, S. (2008). Optimizing the Design and Analysis of Clinical Functional Magnetic Resonance Imaging Research Studies. *Biological Psychiatry*. https://doi.org/10.1016/j.biopsych.2008.06.014

Carter, C. S., Williams, J. R., Witt, D. M., & Insel, T. R. (1992). Oxytocin and Social Bonding. *Annals of the New York Academy of Sciences*, *652*(1), 204–211. https://doi.org/10.1111/j.1749-6632.1992.tb34356.x

Caspi, A., Lynam, D., Moffitt, T. E., & Silva, P. A. (1993). Unraveling girls' delinquency: Biological, dispositional, and contextual contributions to adolescent misbehavior. *Developmental Psychology*, *29*(1), 19–30. https://doi.org/10.1037/0012-1649.29.1.19

Castano, E. (2012). Antisocial Behavior in Individuals and Groups: An Empathy-Focused Approach. In *The Oxford Handbook of Personality and Social Psychology*. https://doi.org/10.1093/oxfordhb/9780195398991.013.0017

Cerliani, L., Thomas, R. M., Jbabdi, S., Siero, J. C. W., Nanetti, L., Crippa, A., … Keysers, C. (2012). Probabilistic tractography recovers a rostrocaudal trajectory of connectivity variability in the human insular cortex. *Human Brain Mapping*, *33*(9), 2005–2034. https://doi.org/10.1002/hbm.21338

Cheng, Y., Chen, C., Lin, C. P., Chou, K. H., & Decety, J. (2010). Love hurts: An fMRI study. *NeuroImage*, *51*(2), 923–929. https://doi.org/10.1016/j.neuroimage.2010.02.047

Cho, Y. T., Fromm, S., Guyer, A. E., Detloff, A., Pine, D. S., Fudge, J. L., & Ernst, M. (2013). Nucleus accumbens, thalamus and insula connectivity during incentive anticipation in typical adults and adolescents. *NeuroImage*, *66*, 508–521. https://doi.org/10.1016/j.neuroimage.2012.10.013

Cikara, M., Bruneau, E. G., & Saxe, R. R. (2011). Us and Them: Intergroup Failures of

Empathy. *Current Directions in Psychological Science*, *20*(3), 149–153. https://doi.org/10.1177/0963721411408713
Clayden, J. D. (2013). Imaging connectivity: MRI and the structural networks of the brain. *Functional Neurology*. https://doi.org/10.11138/FNeur/2013.28.3.197
Cleckley, H. (1982). *The Mask of Sanity*. *Southern Medical Journal* (Vol. 44). Saint Louis, United States: The C.V. Mosby Company. https://doi.org/10.1097/00007611-195105000-00028
Cloutman, L. L., Binney, R. J., Drakesmith, M., Parker, G. J. M., & Lambon Ralph, M. A. (2012). The variation of function across the human insula mirrors its patterns of structural connectivity: Evidence from in vivo probabilistic tractography. *NeuroImage*, *59*(4), 3514–3521. https://doi.org/10.1016/j.neuroimage.2011.11.016
Cohn, M. D., Pape, L. E., Schmaal, L., van den Brink, W., van Wingen, G., Vermeiren, R. R. J. M., … Popma, A. (2015). Differential relations between juvenile psychopathic traits and resting state network connectivity. *Human Brain Mapping*, *36*(6), 2396–2405. https://doi.org/10.1002/hbm.22779
Collishaw, S., Maughan, B., Goodman, R., & Pickles, A. (2004). Time trends in adolescent mental health. *Journal of Child Psychology and Psychiatry*, *45*(8), 1350–1362. https://doi.org/10.1111/j.1469-7610.2004.00842.x
Cope, L. M., Ermer, E., Nyalakanti, P. K., Calhoun, V. D., & Kiehl, K. A. (2014). Paralimbic gray matter reductions in incarcerated adolescent females with psychopathic traits. *Journal of Abnormal Child Psychology*, *42*(4), 659–668. https://doi.org/10.1007/s10802-013-9810-4
Cornell, D. G., Warren, J., Hawk, G., Stafford, E., Oram, G., & Pine, D. (1996). Psychopathy in instrumental and reactive violent offenders. *Journal of Consulting and Clinical Psychology*, *64*(4), 783–790. https://doi.org/10.1037/0022-006X.64.4.783
Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research, an International Journal*. https://doi.org/10.1006/cbmr.1996.0014
Craig, A. D. (2009). How do you feel — now? The anterior insula and human awareness. *Nature Reviews Neuroscience*, *10*(1), 59–70. https://doi.org/10.1038/nrn2555
Crick, N. R., & Grotpeter, J. K. (1995). Relational Aggression, Gender, and Social-Psychological Adjustment. *Child Development*, *66*(3), 710–722. https://doi.org/10.1111/j.1467-8624.1995.tb00900.x
Dadds, M. R., Allen, J. L., Oliver, B. R., Faulkner, N., Legge, K., Moul, C., … Scott, S. (2012). Love, eye contact and the developmental origins of empathy v. psychopathy. *British Journal of Psychiatry*, *200*(3), 191–196. https://doi.org/10.1192/bjp.bp.110.085720
Dadds, M. R., Hawes, D. J., Frost, A. D. J., Vassallo, S., Bunn, P., Hunter, K., & Merz, S. (2009). Learning to "talk the talk": The relationship of psychopathic traits to deficits in empathy across childhood. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *50*(5), 599–606. https://doi.org/10.1111/j.1469-7610.2008.02058.x
Dadds, M. R., Hunter, K., Hawes, D. J., Frost, A. D. J., Vassallo, S., Bunn, P., … Masry, Y. El. (2008). A measure of cognitive and affective empathy in children using parent ratings. *Child Psychiatry and Human Development*, *39*(2), 111–122. https://doi.org/10.1007/s10578-007-0075-4
Dadds, M. R., Turner, C. M., & McAloon, J. (2002). Developmental Links between Cruelty to Animals and Human Violence. *Australian and New Zealand Journal of Criminology*, *35*(3), 363–382. https://doi.org/10.1375/acri.35.3.363

Dalwani, M. S., McMahon, M. A., Mikulich-Gilbertson, S. K., Young, S. E., Regner, M. F., Raymond, K. M., … Sakai, J. T. (2015). Female adolescents with severe substance and conduct problems have substantially less brain gray matter volume. *PLoS ONE*, *10*(5). https://doi.org/10.1371/journal.pone.0126368

Dalwani, M. S., Tregellas, J. R., Andrews-Hanna, J. R., Mikulich-Gilbertson, S. K., Raymond, K. M., Banich, M. T., … Sakai, J. T. (2014). Default mode network activity in male adolescents with conduct and substance use disorder. *Drug and Alcohol Dependence*, *134*(1), 242–250. https://doi.org/10.1016/j.drugalcdep.2013.10.009

Dandreaux, D. M., & Frick, P. J. (2009). Developmental Pathways to conduct problems: A further test of the childhood and adolescent-onset distinction. *Journal of Abnormal Child Psychology*, *37*(3), 375–385. https://doi.org/10.1007/s10802-008-9261-5

De Brito, S. A., & Hodgins, S. (2009). Antisocial Personality Disorder. In M. McMurran & R. Howard (Eds.), *Personality, Personality Disorder and Violence* (42nd ed., pp. 133–153). United Kingdom.

De Brito, S. A., Mechelli, A., Wilke, M., Laurens, K. R., Jones, A. P., Barker, G. J., … Viding, E. (2009). Size matters: Increased grey matter in boys with conduct problems and callousunemotional traits. *Brain*, *132*(4), 843–852. https://doi.org/10.1093/brain/awp011

de Kemp, R. A. T., Overbeek, G., de Wied, M., Engels, R. C. M. E., & Scholte, R. H. J. (2007). Early adolescent empathy, parental support, and antisocial behavior. *Journal of Genetic Psychology*, *168*, 5–18. https://doi.org/10.3200/GNTP.168.1.5-18

de Wied, M., Gispen-de Wied, C., & van Boxtel, A. (2010). Empathy dysfunction in children and adolescents with disruptive behavior disorders. *European Journal of Pharmacology*. https://doi.org/10.1016/j.ejphar.2009.10.016

Decety, J. (2010). The neurodevelopment of empathy in humans. *Developmental Neuroscience*, *32*(4), 257–267. https://doi.org/10.1159/000317771

Decety, J. (2011). The neuroevolution of empathy. *Annals of the New York Academy of Sciences*, *1231*(1), 35–45. https://doi.org/10.1111/j.1749-6632.2011.06027.x

Decety, J., & Cowell, J. M. (2014). Friends or Foes: Is Empathy Necessary for Moral Behavior? *Perspectives on Psychological Science*, *9*(5), 525–537. https://doi.org/10.1177/1745691614545130

Decety, J., & Cowell, J. M. (2015). Empathy, justice, and moral behavior. *AJOB Neuroscience*, *6*(3), 3–14. https://doi.org/10.1080/21507740.2015.1047055

Decety, J., & Jackson, P. L. (2004). The Functional Architecture of Human Empathy. *Behavioral and Cognitive Neuroscience Reviews*, *3*(2), 71–100. https://doi.org/10.1177/1534582304267187

Decety, J., & Meyer, M. (2008). From emotion resonance to empathic understanding: A social developmental neuroscience account. *Development and Psychopathology*, *20*(4), 1053–1080. https://doi.org/10.1017/S0954579408000503

Decety, J., & Michalska, K. J. (2010). Neurodevelopmental changes in the circuits underlying empathy and sympathy from childhood to adulthood. *Developmental Science*, *13*(6), 886–899. https://doi.org/10.1111/j.1467-7687.2009.00940.x

Decety, J., Michalska, K. J., & Akitsuki, Y. (2008). Who caused the pain? An fMRI investigation of empathy and intentionality in children. *Neuropsychologia*, *46*(11), 2607–2614. https://doi.org/10.1016/j.neuropsychologia.2008.05.026

Decety, J., Michalska, K. J., Akitsuki, Y., & Lahey, B. B. (2009). Atypical empathic responses in adolescents with aggressive conduct disorder: A functional MRI investigation. *Biological Psychology*, *80*(2), 203–211.

https://doi.org/10.1016/j.biopsycho.2008.09.004
Decety, J., Norman, G. J., Berntson, G. G., & Cacioppo, J. T. (2012). A neurobehavioral evolutionary perspective on the mechanisms underlying empathy. *Progress in Neurobiology*. https://doi.org/10.1016/j.pneurobio.2012.05.001
Della-Maggiore, V., Scholz, J., Johansen-Berg, H., & Paus, T. (2009). The rate of visuomotor adaptation correlates with cerebellar white-matter microstructure. *Human Brain Mapping*, *30*(12), 4048–4053. https://doi.org/10.1002/hbm.20828
Delligatti, N., Akin-Little, A., & Little, S. G. (2003). Conduct disorder in girls: Diagnostic and intervention issues. *Psychology in the Schools*. https://doi.org/10.1002/pits.10080
DeWall, C. N., & Baumeister, R. F. (2006). Alone but feeling no pain: Effects of social exclusion on physical pain tolerance and pain threshold, affective forecasting, and interpersonal empathy. *Journal of Personality and Social Psychology*, *91*(1), 1–15. https://doi.org/10.1037/0022-3514.91.1.1
Di, X., Huang, J., & Biswal, B. B. (2017). Task modulated brain connectivity of the amygdala: a meta-analysis of psychophysiological interactions. *Brain Structure and Function*, *222*(1), 619–634. https://doi.org/10.1007/s00429-016-1239-4
Dimberg, U., Andréasson, P., & Thunberg, M. (2011). Emotional empathy and facial reactions to facial expressions. *Journal of Psychophysiology*, *25*(1), 26–31. https://doi.org/10.1027/0269-8803/a000029
Dong, D., Ming, Q., Wang, X., Yu, W., Jiang, Y., Wu, Q., … Yao, S. (2017). Temporoparietal junction hypoactivity during pain-related empathy processing in adolescents with conduct disorder. *Frontiers in Psychology*, *7*(JAN). https://doi.org/10.3389/fpsyg.2016.02085
DuPre, E., & Spreng, R. N. (2017). Structural covariance networks across the lifespan, from 6-94 years of age. *Network Neuroscience*, 1–38. https://doi.org/10.1162/NETN_a_00016
Dvir, Y., Ford, J. D., Hill, M., & Frazier, J. A. (2014). Childhood Maltreatment, Emotional Dysregulation, and Psychiatric Comorbidities. *Harvard Review of Psychiatry*, *22*(3), 149–161. https://doi.org/10.1097/HRP.0000000000000014
Eckert, M. A., Leonard, C. M., Wilke, M., Eckert, M., Richards, T., Richards, A., & Berninger, V. (2005). Anatomical Signatures of Dyslexia in Children: Unique Information from Manual and Voxel Based Morphometry Brain Measures. *Cortex*, *41*(3), 304–315. https://doi.org/10.1016/S0010-9452(08)70268-5
Eckert, M. A., Lombardino, L. J., Walczak, A. R., Bonihla, L., Leonard, C. M., & Binder, J. R. (2008). Manual and automated measures of superior temporal gyrus asymmetry: Concordant structural predictors of verbal ability in children. *NeuroImage*, *41*(3), 813–822. https://doi.org/10.1016/j.neuroimage.2008.03.002
Eres, R., Decety, J., Louis, W. R., & Molenberghs, P. (2015). Individual differences in local gray matter density are associated with differences in affective and cognitive empathy. *NeuroImage*, *117*, 305–310. https://doi.org/10.1016/j.neuroimage.2015.05.038
Eriksson, P. S., Perfilieva, E., Björk-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine*, *4*(11), 1313–7. https://doi.org/10.1038/3305
Ermer, E., Cope, L. M., Nyalakanti, P. K., Calhoun, V. D., & Kiehl, K. A. (2013). Aberrant paralimbic gray matter in incarcerated male adolescents with psychopathic traits. *Journal of the American Academy of Child and Adolescent Psychiatry*, *52*(1), 94–103. https://doi.org/10.1016/j.jaac.2012.10.013
Essau, C. A., Sasagawa, S., & Frick, P. J. (2006). Callous-Unemotional Traits in a Community Sample of Adolescents. *Assessment*, *13*(4), 454–469.

https://doi.org/10.1177/1073191106287354
Fabbri-Destro, M., & Rizzolatti, G. (2008). Mirror Neurons and Mirror Systems in Monkeys and Humans. *Physiology*, *23*(3), 171–179. https://doi.org/10.1152/physiol.00004.2008
Fahim, C., He, Y., Yoon, U., Chen, J., Evans, A., & Pérusse, D. (2011). Neuroanatomy of childhood disruptive behavior disorders. *Aggressive Behavior*, *37*(4), 326–337. https://doi.org/10.1002/ab.20396
Fairchild, G., Hagan, C. C., Walsh, N. D., Passamonti, L., Calder, A. J., & Goodyer, I. M. (2013a). Brain structure abnormalities in adolescent girls with conduct disorder. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *54*(1), 86–95. https://doi.org/10.1111/j.1469-7610.2012.02617.x
Fairchild, G., Van Goozen, S. H. M., Calder, A. J., & Goodyer, I. M. (2013b). Research Review: Evaluating and reformulating the developmental taxonomic theory of antisocial behaviour. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *54*(9), 924–940. https://doi.org/10.1111/jcpp.12102
Fairchild, G., Passamonti, L., Hurford, G., Hagan, C. C., Von Dem Hagen, E. A. H., Van Goozen, S. H. M., … Calder, A. J. (2011). Brain structure abnormalities in early-onset and adolescent-onset conduct disorder. *American Journal of Psychiatry*, *168*(6), 624–633. https://doi.org/10.1176/appi.ajp.2010.10081184
Fairchild, G., Toschi, N., Hagan, C. C., Goodyer, I. M., Calder, A. J., & Passamonti, L. (2015). Cortical thickness, surface area, and folding alterations in male youths with conduct disorder and varying levels of callous-unemotional traits. *NeuroImage: Clinical*, *8*, 253–260. https://doi.org/10.1016/j.nicl.2015.04.018
Fairchild, G., Toschi, N., Sully, K., Sonuga-Barke, E. J. S., Hagan, C. C., Diciotti, S., … Passamonti, L. (2016). Mapping the structural organization of the brain in conduct disorder: replication of findings in two independent samples. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *57*(9), 1018–1026. https://doi.org/10.1111/jcpp.12581
Fairchild, G., Van Goozen, S. H. M., Calder, A. J., Stollery, S. J., & Goodyer, I. M. (2009). Deficits in facial expression recognition in male adolescents with early-onset or adolescence-onset conduct disorder. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *50*(5), 627–636. https://doi.org/10.1111/j.1469-7610.2008.02020.x
Fallgatter, A. J. (2009). Neuroimaging in psychiatry: from bench to bedside. *Frontiers in Human Neuroscience*, *3*. https://doi.org/10.3389/neuro.09.049.2009
Fan, Y., Duncan, N. W., de Greck, M., & Northoff, G. (2011). Is there a core neural network in empathy? An fMRI based quantitative meta-analysis. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2010.10.009
Farroni, T., Csibra, G., Simion, F., & Johnson, M. H. (2002). Eye contact detection in humans from birth. *Proceedings of the National Academy of Sciences*, *99*(14), 9602–9605. https://doi.org/10.1073/pnas.152159999
Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*. https://doi.org/10.3758/BF03193146
Fergusson, D. M., Horwood, L. J., & Ridder, E. M. (2005). Show me the child at seven: The consequences of conduct problems in childhood for psychosocial functioning in adulthood. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *46*(8), 837–849. https://doi.org/10.1111/j.1469-7610.2004.00387.x
Findlay, L. C., Girardi, A., & Coplan, R. J. (2006). Links between empathy, social behavior, and social understanding in early childhood. *Early Childhood Research Quarterly*, *21*(3),

347–359. https://doi.org/10.1016/j.ecresq.2006.07.009
Finger, E. C., Marsh, A., Blair, K. S., Majestic, C., Evangelou, I., Gupta, K., … Blair, R. J. (2012). Impaired functional but preserved structural connectivity in limbic white matter tracts in youth with conduct disorder or oppositional defiant disorder plus psychopathic traits. *Psychiatry Research - Neuroimaging*, *202*(3), 239–244. https://doi.org/10.1016/j.pscychresns.2011.11.002
Fischl, B. (2012). FreeSurfer. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2012.01.021
Fontaine, N., Carbonneau, R., Vitaro, F., Barker, E. D., & Tremblay, R. E. (2009). Research review: A critical review of studies on the developmental trajectories of antisocial behavior in females. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. https://doi.org/10.1111/j.1469-7610.2008.01949.x
Foulkes, L., McCrory, E. J., Neumann, C. S., & Viding, E. (2014). Inverted social reward: Associations between psychopathic traits and self-report and experimental measures of social reward. *PLoS ONE*, *9*(8). https://doi.org/10.1371/journal.pone.0106000
Foulkes, L., Seara-Cardoso, A., Neumann, C. S., Rogers, J. S. C., & Viding, E. (2014). Looking after number one: Associations between psychopathic traits and measures of social motivation and functioning in a community sample of males. *Journal of Psychopathology and Behavioral Assessment*, *36*(1), 22–29. https://doi.org/10.1007/s10862-013-9381-2
Frick, P. J., Cornell, A. H., Bodin, S. D., Dane, H. E., Barry, C. T., & Loney, B. R. (2003). Callous-unemotional traits and developmental pathways to severe conduct problems. *Developmental Psychology*, *39*(2), 246–260. https://doi.org/10.1037/0012-1649.39.2.246
Frick, P. J., & Ellis, M. (1999). Callous-unemotional traits and subtypes of conduct disorder. *Clinical Child and Family Psychology Review*, *2*(3), 149–168. https://doi.org/10.1023/A:1021803005547
Frick, P. J., & Hare, R. D. (2001). The antisocial process screening device. *Toronto: Multi-Health Systems*. https://doi.org/10.1037/t00032-000
Frick, P. J., & Marsee, M. A. (2006). Psychopathy and Developmental Pathways to Antisocial Behavior in Youth. In *Handbook of psychopathy*. (pp. 353–374). Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=2006-01001-018&site=ehost-live&scope=site
Frick, P. J., Ray, J. V., Thornton, L. C., & Kahn, R. E. (2014a). Annual research review: A developmental psychopathology approach to understanding callous-unemotional traits in children and adolescents with serious conduct problems. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. https://doi.org/10.1111/jcpp.12152
Frick, P. J., Ray, J. V., Thornton, L. C., & Kahn, R. E. (2014b). Can callous-unemotional traits enhance the understanding, diagnosis, and treatment of serious conduct problems in children and adolescents? A comprehensive review. *Psychological Bulletin*, *140*(1), 1–57. https://doi.org/10.1037/a0033076
Frick, P. J., & Viding, E. (2009). Antisocial behavior from a developmental psychopathology perspective. *Development and Psychopathology*, *21*(4), 1111. https://doi.org/10.1017/S0954579409990071
Frick, P. J., & White, S. F. (2008). Research Review: The importance of callous-unemotional traits for developmental models of aggressive and antisocial behavior. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. https://doi.org/10.1111/j.1469-7610.2007.01862.x
Frick, P., & Marsee, M. (2006). Psychopathy and developmental pathways to antisocial behavior in youth. *Handbook of Psychopathy*.

Friesdorf, R., Conway, P., & Gawronski, B. (2015). Gender Differences in Responses to Moral Dilemmas: A Process Dissociation Analysis. *Personality and Social Psychology Bulletin*, *41*(5), 696–713. https://doi.org/10.1177/0146167215575731

Friston, K., & Buchel, C. (2004). Functional connectivity. In *Human Brain Function* (Vol. 19, pp. 391–397). Retrieved from http://discovery.ucl.ac.uk/65621/

Friston, K. J. (2011). Functional and effective connectivity: A synthesis. *Brain Connectivity*, *1*(1), 13–36. https://doi.org/10.1089/brain.2011.0008

Friston, K. J., Buechel, C., Fink, G. R., Morris, J., Rolls, E., & Dolan, R. J. (1997). Psychophysiological and modulatory interactions in neuroimaging. *NeuroImage*, *6*(3), 218–229. https://doi.org/10.1006/nimg.1997.0291

Friston, K. J., Harrison, L., & Penny, W. (2003). Dynamic causal modelling. *NeuroImage*, *19*(4), 1273–1302. https://doi.org/10.1016/S1053-8119(03)00202-7

Fumagalli, M., Ferrucci, R., Mameli, F., Marceglia, S., Mrakic-Sposta, S., Zago, S., … Priori, A. (2010). Gender-related differences in moral judgments. *Cognitive Processing*, *11*(3), 219–226. https://doi.org/10.1007/s10339-009-0335-2

Furman, D. J., Chen, M. C., & Gotlib, I. H. (2011). Variant in oxytocin receptor gene is associated with amygdala volume. *Psychoneuroendocrinology*, *36*(6), 891–897. https://doi.org/10.1016/j.psyneuen.2010.12.004

Fusar-Poli, P., Radua, J., Frascarelli, M., Mechelli, A., Borgwardt, S., Di Fabio, F., … David, S. P. (2014). Evidence of reporting biases in voxel-based morphometry (VBM) studies of psychiatric and neurological disorders. *Human Brain Mapping*, *35*(7), 3052–3065. https://doi.org/10.1002/hbm.22384

Galinsky, A. D., & Moskowitz, G. B. (2000). Perspective-taking: Decreasing stereotype expression, stereotype accessibility, and in-group favoritism. *Journal of Personality and Social Psychology*, *78*(4), 708–724. https://doi.org/10.1037//0022-3514.78.4.708

Gallese, V., Fadiga, L., Fogassi, L., & Rizzolatti, G. (1996). Action recognition in the premotor cortex. *Brain : A Journal of Neurology*, (2), 593–609. https://doi.org/10.1093/brain/119.2.593

Ge, Y., Grossman, R. I., Babb, J. S., Rabin, M. L., Mannon, L. J., & Kolson, D. L. (2002). Age-related total gray matter and white matter changes in normal adult brain. Part I: volumetric MR imaging analysis. *AJNR. American Journal of Neuroradiology*, *23*(8), 1327–1333.

Geangu, E., Benga, O., Stahl, D., & Striano, T. (2010). Contagious crying beyond the first days of life. *Infant Behavior and Development*, *33*(3), 279–288. https://doi.org/10.1016/j.infbeh.2010.03.004

Ghaziri, J., Tucholka, A., Girard, G., Houde, J.-C., Boucher, O., Gilbert, G., … Nguyen, D. K. (2017). The Corticocortical Structural Connectivity of the Human Insula. *Cerebral Cortex*, *27*(2), 1216–1228. https://doi.org/10.1093/cercor/bhv308

Giedd, J. N. (2004). Structural magnetic resonance imaging of the adolescent brain. In *Annals of the New York Academy of Sciences* (Vol. 1021, pp. 77–85). https://doi.org/10.1196/annals.1308.009

Goddings, A. L., Mills, K. L., Clasen, L. S., Giedd, J. N., Viner, R. M., & Blakemore, S. J. (2014). The influence of puberty on subcortical brain development. *NeuroImage*, *88*, 242–251. https://doi.org/10.1016/j.neuroimage.2013.09.073

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, a C., … Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(21), 8174–9. https://doi.org/10.1073/pnas.0402680101

Gong, G., He, Y., Chen, Z. J., & Evans, A. C. (2012). Convergence and divergence of thickness correlations with diffusion connections across the human cerebral cortex. *NeuroImage*, *59*(2), 1239–1248. https://doi.org/10.1016/j.neuroimage.2011.08.017

Green, H., McGinnity, A., Meltzer, H., Ford, T., & Goodman, R. (2005). *Mental Health of Children and Young People in Great Britain, 2004*. *Child and Fa* (Vol. 14). https://doi.org/10.1037/e557702010-001

Gregory, S., Ffytche, D., Simmons, A., Kumari, V., Howard, M., Hodgins, S., & Blackwood, N. (2012). The antisocial brain: Psychopathy matters: A structural mri investigation of antisocial male violent offenders. *Archives of General Psychiatry*, *69*(9), 962–972. https://doi.org/10.1001/archgenpsychiatry.2012.222

Greve, D. N. (2011). An Absolute Beginner's Guide to Surface- and Voxel-based Morphometric Analysis. *Proceedings of the International Society for Magnetic Resonance in Medicine*, *i*, 1–7.

Gu, X., Gao, Z., Wang, X., Liu, X., Knight, R. T., Hof, P. R., & Fan, J. (2012). Anterior insular cortex is necessary for empathetic pain perception. *Brain*, *135*(9), 2726–2735. https://doi.org/10.1093/brain/aws199

Gu, X., & Han, S. (2007). Attention and reality constraints on the neural processes of empathy for pain. *NeuroImage*, *36*(1), 256–267. https://doi.org/10.1016/j.neuroimage.2007.02.025

Gu, X., Hof, P. R., Friston, K. J., & Fan, J. (2013). Anterior insular cortex and emotional awareness. *Journal of Comparative Neurology*, *521*(15), 3371–3388. https://doi.org/10.1002/cne.23368

Gu, X., Liu, X., Guise, K. G., Naidich, T. P., Hof, P. R., & Fan, J. (2010). Functional Dissociation of the Frontoinsular and Anterior Cingulate Cortices in Empathy for Pain. *Journal of Neuroscience*, *30*(10), 3739–3744. https://doi.org/10.1523/JNEUROSCI.4844-09.2010

Habas, C., Kamdar, N., Nguyen, D., Prater, K., Beckmann, C. F., Menon, V., & Greicius, M. D. (2009). Distinct cerebellar contributions to intrinsic connectivity networks. *Journal of Neuroscience*, *29*(26), 8586–8594. https://doi.org/10.1523/JNEUROSCI.1868-09.2009

Hamann, S. (2005). Sex differences in the responses of the human amygdala. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *11*(4), 288–93. https://doi.org/10.1177/1073858404271981

Hare, R. D., Forth, A. E., & Hart, S. D. (1989). The psychopath as prototype for pathological lying and deception. In *Credibility assessment* (pp. 25–49). https://doi.org/10.1007/978-94-015-7856-1_2

Harlow, H. F. (1965). Total Social Isolation: Effects on Macaque Monkey Behavior. *Science*, *148*(3670), 666. https://doi.org/10.1126/science.148.3670.666-a

Hawkins, S. R., Graham, P. W., Williams, J., & Zahn, M. A. (2009). Resilient girls-factors that protect against delinquency. *Office of Juvenile Justice and Delinquency Prevention*, 1–14.

Heinze, K., Reniers, R. L. E. P., Nelson, B., Yung, A. R., Lin, A., Harrison, B. J., … Wood, S. J. (2015). Discrete alterations of brain network structural covariance in individuals at ultra-high risk for psychosis. *Biological Psychiatry*, *77*(11), 989–996. https://doi.org/10.1016/j.biopsych.2014.10.023

Hill, J. (2003). Early identification of individuals at risk for antisocial personality disorder. *The British Journal of Psychiatry*, *182*(44), 11s–14. https://doi.org/10.1192/bjp.182.44.s11

Hillis, A. E. (2014). Inability to empathize: Brain lesions that disrupt sharing and

understanding another's emotions. *Brain*. https://doi.org/10.1093/brain/awt317
Hipwell, A. E., Loeber, R., Stouthamer-Loeber, M., Keenan, K., White, H. R., & Kroneman, L. (2002). Characteristics of girls with early onset disruptive and antisocial behaviour. *Criminal Behaviour and Mental Health : CBMH*, *12*(1), 99–118. https://doi.org/10.1002/cbm.489
Huebnerm T., Vloet, T.D., Marx, I., Konrad, K., Fink, G.R., Herpertz, S.C., & Herpertz-Dahlmnn, B. (2008). Morphometric Brain Abnormalities in Boys With Conduct Disorder. *Journal of the American Academy of Child & Adolescent Psychiatry*, *47*(5), 540–547. https://doi.org/10.1097/CHI.0b013e3181676545
Huettel, S. A., Song, A. W., & McCarthy, G. (2004). *Functional magnetic resonance imaging*. *Magnetic Resonance Imaging*.
Hwang, K., Bertolero, M. A., Liu, W. B., & D'Esposito, M. (2017). The Human Thalamus Is an Integrative Hub for Functional Brain Networks. *The Journal of Neuroscience*, *37*(23), 5594–5607. https://doi.org/10.1523/JNEUROSCI.0067-17.2017
Hyatt, C. J., Haney-Caron, E., & Stevens, M. C. (2012). Cortical thickness and folding deficits in conduct-disordered adolescents. *Biological Psychiatry*, *72*(3), 207–214. https://doi.org/10.1016/j.biopsych.2011.11.017
Hyde, L. W., Shaw, D. S., Gardner, F., Cheong, J., Dishion, T. J., & Wilson, M. (2013). Dimensions of callousness in early childhood: links to problem behavior and family intervention effectiveness. *Development and Psychopathology*, *25*(2), 347–63. https://doi.org/10.1017/S0954579412001101
Iacoboni, M. (2009). Imitation, Empathy, and Mirror Neurons. *Annual Review of Psychology*, *60*(1), 653–670. https://doi.org/10.1146/annurev.psych.60.110707.163604
Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D., Quinn, K., … Wang, P. (2010). Research Domain Criteria ( RDoC ): Toward a new classification framework for research on mental disorders. *Am J Psychiatry*, *167*(7), 748–751. https://doi.org/10.1176/appi.ajp.2010.09091379
Insel, T., & Fernald, R. (2004). How the brain processes social information: searching for the social brain. *Annual Review of Neuroscience*, *27*, 697–722. https://doi.org/10.1146/annurev.neuro.27.070203.144148
Ioannidis, J. P. A. (2011). Excess significance bias in the literature on brain volume abnormalities. *Archives of General Psychiatry*, *68*(8), 773–780. https://doi.org/10.1001/archgenpsychiatry.2011.28
Itier, R. J., & Batty, M. (2009). Neural bases of eye and gaze processing: The core of social cognition. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2009.02.004
Jabbi, M., & Keysers, C. (2008). Inferior frontal gyrus activity triggers anterior insula response to emotional facial expressions. *Emotion (Washington, D.C.)*, *8*(6), 775–780. https://doi.org/10.1037/a0014194
Jabbi, M., Swart, M., & Keysers, C. (2007). Empathy for positive and negative emotions in the gustatory cortex. *NeuroImage*, *34*(4), 1744–1753. https://doi.org/10.1016/j.neuroimage.2006.10.032
Jackson, P. L., Meltzoff, A. N., & Decety, J. (2005). How do we perceive the pain of others? A window into the neural processes involved in empathy. *NeuroImage*, *24*(3), 771–779. https://doi.org/10.1016/j.neuroimage.2004.09.006
Jakab, A., Molnár, P. P., Bogner, P., Béres, M., & Berényi, E. L. (2012). Connectivity-based parcellation reveals interhemispheric differences in the insula. *Brain Topography*, *25*(3), 264–271. https://doi.org/10.1007/s10548-011-0205-y

Jenkinson, M., Beckmann, C. F., Behrens, T. E. J., Woolrich, M. W., & Smith, S. M. (2012). FSL. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2011.09.015

Jiang, Y., Liu, W., Ming, Q., Gao, Y., Ma, R., Zhang, X., … Huang, B. (2016). Disrupted Topological Patterns of Large-Scale Network in Conduct Disorder. *Scientific Reports*, *6*(October), 37053. https://doi.org/10.1038/srep37053

Jones, A. P., Happé, F. G. E., Gilbert, F., Burnett, S., & Viding, E. (2010). Feeling, caring, knowing: different types of empathy deficit in boys with psychopathic tendencies and autism spectrum disorder. *Journal of Child Psychology and Psychiatry*, *51*(11), 1188–1197. https://doi.org/10.1111/j.1469-7610.2010.02280.x

Jones, A. P., Laurens, K. R., Herba, C. M., Barker, G. J., & Viding, E. (2009). Amygdala hypoactivity to fearful faces in boys with conduct problems and callous-unemotional traits. *American Journal of Psychiatry*, *166*(1), 95–102. https://doi.org/10.1176/appi.ajp.2008.07071050

Joseph, R. (1999). Environmental influences on neural plasticity, the limbic system, emotional development and attachment: A review. *Child Psychiatry and Human Development*. https://doi.org/10.1023/A:1022660923605

Kaas, J. H. (2013). The evolution of brains from early mammals to humans. *Wiley Interdisciplinary Reviews: Cognitive Science*. https://doi.org/10.1002/wcs.1206

Kahn, R. E., Frick, P. J., Youngstrom, E. A., Kogos Youngstrom, J., Feeny, N. C., & Findling, R. L. (2013). Distinguishing primary and secondary variants of callous-unemotional traits among adolescents in a clinic-referred sample. *Psychological Assessment*, *25*(3), 966–978. https://doi.org/10.1037/a0032880

Kahn, R. E., Frick, P. J., Youngstrom, E., Findling, R. L., & Youngstrom, J. K. (2012). The effects of including a callous-unemotional specifier for the diagnosis of conduct disorder. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *53*(3), 271–282. https://doi.org/10.1111/j.1469-7610.2011.02463.x

Kaplowitz, P. B., Slora, E. J., Wasserman, R. C., Pedlow, S. E., & Herman-Giddens, M. E. (2001). Earlier onset of puberty in girls: relation to increased body mass index and race. *Pediatrics*, *108*(2), 347–353. https://doi.org/10.1542/peds.108.2.347

Kaufman, Birmaher, Rao, R. (1996). Diagnostic Interview Kiddie-Sads-Present and Lifetime Version (K-SADS-PL). *October*, *135*(October), 153–62. https://doi.org/10.1097/00004583-199707000-00021

Keenan, K., Loeber, R., & Green, S. (1999). Conduct Disorder in Girls: A Review of the Literature. *Clinical Child & Family Psychology Review*, *2*(1), 3–19. https://doi.org/10.1023/A:1021811307364

Keverne, E. B., & Curley, J. P. (2004). Vasopressin, oxytocin and social behaviour. *Current Opinion in Neurobiology*. https://doi.org/10.1016/j.conb.2004.10.006

Killgore, W. D. S., Olson, E. A., & Weber, M. (2013). Physical exercise habits correlate with gray matter volume of the hippocampus in healthy adult humans. *Scientific Reports*, *3*, 3457. https://doi.org/10.1038/srep03457

Kim, S., Kim, S., Kim, H., Han, K., Jeong, B., Kim, J., … Kim, J. (2017). Altered functional connectivity of the default mode network on low-empathy subjects. *Yonsei Medical Journal*, *58*(5).

Kimonis, E. R., Frick, P. J., Boris, N. W., Smyke, A. T., Cornell, A. H., Farrell, J. M., & Zeanah, C. H. (2006). Callous-unemotional features, behavioral inhibition, and parenting: Independent predictors of aggression in a high-risk preschool sample. *Journal of Child and Family Studies*, *15*(6), 741–752. https://doi.org/10.1007/s10826-006-9047-8

Kimonis, E. R., Frick, P. J., Skeem, J. L., Marsee, M. A., Cruise, K., Munoz, L. C., … Morris,

A. S. (2008). Assessing callous-unemotional traits in adolescent offenders: Validation of the Inventory of Callous-Unemotional Traits. *International Journal of Law and Psychiatry*, *31*(3), 241–252. https://doi.org/10.1016/j.ijlp.2008.04.002

Kinsella, M. T., & Monk, C. (2009). Impact of Maternal Stress, Depression & Anxiety on Fetal Neurobehavioral Development. *Clinical Obstetrics and Gynecology*, *52*(3), 425–440. https://doi.org/10.1097/GRF.0b013e3181b52df1.Impact

Klapwijk, E. T., Aghajani, M., Colins, O. F., Marijnissen, G. M., Popma, A., Lang, N. D. J., … Vermeiren, R. R. J. M. (2016). Different brain responses during empathy in autism spectrum disorders versus conduct disorder and callous- unemotional traits. *Journal of Child Psychology and Psychiatry*. https://doi.org/10.1111/jcpp.12498

Knafo, A., Zahn-Waxler, C., Van Hulle, C., Robinson, J. L., & Rhee, S. H. (2008). The developmental origins of a disposition toward empathy: Genetic and environmental contributions. *Emotion*, *8*(6), 737–52. https://doi.org/10.1037/a0014179

Koenen, K. C., Caspi, A., Moffitt, T. E., Rijsdijk, F., & Taylor, A. (2006). Genetic influences on the overlap between low IQ and antisocial behavior in young children. *Journal of Abnormal Psychology*, *115*(4), 787–797. https://doi.org/10.1037/0021-843X.115.4.787

Kolb, B., & Gibb, R. (2011). Brain Plasticity and Behaviour in the Developing Brain. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*. https://doi.org/10.1016/B978-0-444-63327-9.00005-9

Kostic, J. ; Nesic, M. ; Stankovic, M. ; Zikic, O. & Markovic, J. (2016). Evaluating empathy in adolescents with conduct disorders. *Vojnosanitetski Pregled*, *73*(5), 429–434. https://doi.org/10.2298/VSP150121031K

Kruesi, M. J. P., Casanova, M. F., Mannheim, G., & Johnson-Bilder, A. (2004). Reduced temporal lobe volume in early onset conduct disorder. *Psychiatry Research - Neuroimaging*, *132*(1), 1–11. https://doi.org/10.1016/j.pscychresns.2004.07.002

Kruh, I. P., Frick, P. J., & Clements, C. B. (2005). Historical and Personality Correlates to the Violence Patterns of Juveniles Tried as Adults. *Criminal Justice and Behavior*, *32*(1), 69–96. https://doi.org/10.1177/0093854804270629

Kugiumutzakis, G. (1998). Neonatal imitation in the intersubjective companion space. *Intersubjective Communication and Emotion in Early Ontogeny*, (October), 63–88.

Lahey, B. B., Goodman, S. H., Waldman, I. D., Bird, H., Canino, G., Jensen, P., … Applegate, B. (1999). Relation of age of onset to the type and severity of child and adolescent conduct problems. *Journal of Abnormal Child Psychology*, *27*(4), 247–260. https://doi.org/10.1023/a:1022661224769

Lamm, C., Decety, J., & Singer, T. (2011). Meta-analytic evidence for common and distinct neural networks associated with directly experienced pain and empathy for pain. *NeuroImage*, *54*(3), 2492–2502. https://doi.org/10.1016/j.neuroimage.2010.10.014

Lamm, C., & Singer, T. (2010). The role of anterior insular cortex in social emotions. *Brain Structure and Function*, 1–13. https://doi.org/10.1007/s00429-010-0251-3

Lang, S., Yu, T., Markl, A., Müller, F., & Kotchoubey, B. (2011). Hearing others' pain: neural activity related to empathy. *Cognitive, Affective, & Behavioral Neuroscience*, *11*(3), 386–395. https://doi.org/10.3758/s13415-011-0035-0

Lavin, C., Melis, C., Mikulan, E., Gelormini, C., Huepe, D., & Ibañez, A. (2013). The anterior cingulate cortex: an integrative hub for human socially-driven interactions. *Frontiers in Neuroscience*, *7*. https://doi.org/10.3389/fnins.2013.00064

Le Bihan, D., Mangin, J. F., Poupon, C., Clark, C. a, Pappata, S., Molko, N., & Chabriat, H. (2001). Diffusion tensor imaging: concepts and applications. *Journal of Magnetic Resonance Imaging : JMRI*. https://doi.org/10.1002/jmri.1076 [pii]

Leadbeater, B. J., Kuperminc, G. P., Blatt, S. J., & Hertzog, C. (1999). A multivariate model of gender differences in adolescents' internalizing and externalizing problems. *Developmental Psychology*, *35*(5), 1268–1282. https://doi.org/10.1037/0012-1649.35.5.1268

Lee, Y., & Styne, D. (2013). Influences on the onset and tempo of puberty in human beings and implications for adolescent psychological development. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2013.03.014

Leigh, R., Oishi, K., Hsu, J., Lindquist, M., Gottesman, R. F., Jarso, S., … Hillis, A. E. (2013). Acute lesions that impair affective empathy. *Brain*, *136*(8), 2539–2549. https://doi.org/10.1093/brain/awt177

Lennon, R., & Eisenberg, N. (1987). Gender and age differences in empathy and sympathy. In *Empathy and its development* (pp. 195–217).

Lerch, J. P., Worsley, K., Shaw, W. P., Greenstein, D. K., Lenroot, R. K., Giedd, J., & Evans, A. C. (2006). Mapping anatomical correlations across cerebral cortex (MACACC) using cortical thickness from MRI. *NeuroImage*, *31*(3), 993–1003. https://doi.org/10.1016/j.neuroimage.2006.01.042

Leutgeb, V., Wabnegger, A., Leitner, M., Zussner, T., Scharmüller, W., Klug, D., & Schienle, A. (2016). Altered cerebellar-amygdala connectivity in violent offenders: A resting-state fMRI study. *Neuroscience Letters*, *610*, 160–164. https://doi.org/10.1016/j.neulet.2015.10.063

Levy, J., Goldstein, A., Pratt, M., & Feldman, R. (2018). Maturation of pain empathy from child to adult shifts from single to multiple neural rhythms to support interoceptive representations. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-19810-3

Lilienfeld, S. O., Latzman, R. D., Watts, A. L., Smith, S. F., & Dutton, K. (2014). Correlates of psychopathic personality traits in everyday life: Results from a large community survey. *Frontiers in Psychology*, *5*(JUL). https://doi.org/10.3389/fpsyg.2014.00740

Lim, L., Radua, J., & Rubia, K. (2014). Gray matter abnormalities in childhood maltreatment: A voxelwise metaanalysis. *American Journal of Psychiatry*, *171*(8), 854–863. https://doi.org/10.1176/appi.ajp.2014.13101427

Lockwood, P. L., Sebastian, C. L., McCrory, E. J., Hyde, Z. H., Gu, X., De Brito, S. A., & Viding, E. (2013). Association of callous traits with reduced neural response to others' pain in children with conduct problems. *Current Biology*, *23*(10), 901–905. https://doi.org/10.1016/j.cub.2013.04.018

Loeber, R. (1991). Antisocial behavior: More enduring than changeable? *Journal of the American Academy of Child and Adolescent Psychiatry*. https://doi.org/10.1097/00004583-199105000-00007

Loeber, R., Burke, J.D., Lahey, B.B., Winters, A., & Zera, M. (2000). Oppositional Defiant and Conduct Disorder: A Review of the Past 10 Years, Part I. *Journal of the American Academy of Child & Adolescent Psychiatry*, *39*(12), 1468–1484. https://doi.org/10.1097/00004583-200012000-00007

Loeber, R., Pardini, D., Homish, D. L., Wei, E. H., Crawford, A. M., Farrington, D. P., … Rosenfeld, R. (2005). The prediction of violence and homicide in young men. *Journal of Consulting and Clinical Psychology*, *73*(6), 1074–88. https://doi.org/10.1037/0022-006X.73.6.1074

Loggia, M. L., Mogil, J. S., & Catherine Bushnell, M. (2008). Empathy hurts: Compassion for another increases both sensory and affective components of pain perception. *Pain*, *136*(1–2), 168–176. https://doi.org/10.1016/j.pain.2007.07.017

Lorenz, J., Minoshima, S., & Casey, K. L. (2003). Keeping pain out of mind: The role of the

dorsolateral prefrontal cortex in pain modulation. *Brain*, *126*(5), 1079–1091. https://doi.org/10.1093/brain/awg102
Lovett, B. J., & Sheffield, R. A. (2007). Affective empathy deficits in aggressive children and adolescents: A critical review. *Clinical Psychology Review*. https://doi.org/10.1016/j.cpr.2006.03.003
Lu, F. M., Zhou, J. S., Wang, X. P., Xiang, Y. T., & Yuan, Z. (2017). Short- and long-range functional connectivity density alterations in adolescents with pure conduct disorder at resting-state. *Neuroscience*, *351*, 96–107. https://doi.org/10.1016/j.neuroscience.2017.03.040
Lu, F. M., Zhou, J. S., Zhang, J., Xiang, Y. T., Liu, Q., Wang, X. P., & Yuan, Z. (2015). Functional connectivity estimated from resting-state fMRI reveals selective alterations in male adolescents with pure conduct disorder. *PLoS ONE*, *10*(12). https://doi.org/10.1371/journal.pone.0145668
Lui, J. H. L., Barry, C. T., & Sacco, D. F. (2016). Callous-unemotional traits and empathy deficits: Mediating effects of affective perspective-taking and facial emotion recognition. *Cognition and Emotion*, *30*(6), 1049–1062. https://doi.org/10.1080/02699931.2015.1047327
Lynam, D. R., Caspi, A., Moffitt, T. E., Loeber, R., & Stouthamer-Loeber, M. (2007). Longitudinal evidence that psychopathy scores in early adolescence predict adult psychopathy. *Journal of Abnormal Psychology*, *116*(1), 155–165. https://doi.org/10.1037/0021-843X.116.1.155
Lynne, S. D., Graber, J. A., Nichols, T. R., Brooks-Gunn, J., & Botvin, G. J. (2007). Links between Pubertal Timing, Peer Influences, and Externalizing Behaviors among Urban Students Followed Through Middle School. *Journal of Adolescent Health*, *40*(2). https://doi.org/10.1016/j.jadohealth.2006.09.008
Madhyastha, T., Peverill, M., Koh, N., McCabe, C., Flournoy, J., Mills, K., … McLaughlin, K. A. (2017). Current methods and limitations for longitudinal fMRI analysis across development. *Developmental Cognitive Neuroscience*. https://doi.org/10.1016/j.dcn.2017.11.006
Maguire, E. A., Woollett, K., & Spiers, H. J. (2006). London taxi drivers and bus drivers: A structural MRI and neuropsychological analysis. *Hippocampus*, *16*(12), 1091–1101. https://doi.org/10.1002/hipo.20233
Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*, *19*(3), 1233–1239. https://doi.org/10.1016/S1053-8119(03)00169-1
Malhi, G. S., & Lagopoulos, J. (2008). Making sense of neuroimaging in psychiatry. *Acta Psychiatrica Scandinavica*. https://doi.org/10.1111/j.1600-0447.2007.01111.x
Manto, M., & Mariën, P. (2015). Schmahmann's syndrome - identification of the third cornerstone of clinical ataxiology. *Cerebellum & Ataxias*, *2*, 2. https://doi.org/10.1186/s40673-015-0023-1
Marsee, M. A., & Frick, P. J. (2007). Exploring the cognitive and emotional correlates to proactive and reactive aggression in a sample of detained girls. *Journal of Abnormal Child Psychology*, *35*(6), 969–981. https://doi.org/10.1007/s10802-007-9147-y
Marsh, A. A., & Blair, R. J. R. (2008). Deficits in facial affect recognition among antisocial populations: A meta-analysis. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2007.08.003
Marsh, A. A., Finger, E. C., Fowler, K. A., Adalio, C. J., Jurkowitz, I. T. N., Schechter, J. C., … Blair, R. J. R. (2013). Empathic responsiveness in amygdala and anterior cingulate

cortex in youths with psychopathic traits. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *54*(8), 900–910. https://doi.org/10.1111/jcpp.12063
Marsh, A. A., Finger, E. C., Fowler, K. A., Jurkowitz, I. T. N., Schechter, J. C., Yu, H. H., … Blair, R. J. R. (2011). Reduced amygdala-orbitofrontal connectivity during moral judgments in youths with disruptive behavior disorders and psychopathic traits. *Psychiatry Research - Neuroimaging*, *194*(3), 279–286. https://doi.org/10.1016/j.pscychresns.2011.07.008
Marsh, A. A., Stoycos, S. A., Brethel-Haurwitz, K. M., Robinson, P., Vanmeter, J. W., Cardinale, E. M., & Gazzaniga, M. S. (n.d.). Neural and cognitive characteristics of extraordinary altruists. https://doi.org/10.1073/pnas.1408440111
Maughan, B., Rowe, R., Messer, J., Goodman, R., & Meltzer, H. (2004). Conduct Disorder and Oppositional Defiant Disorder in a national sample: Developmental epidemiology. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *45*(3), 609–621. https://doi.org/10.1111/j.1469-7610.2004.00250.x
May, A., & Gaser, C. (2006). Magnetic resonance-based morphometry: A window into structural plasticity of the brain. *Current Opinion in Neurology*. https://doi.org/10.1097/01.wco.0000236622.91495.21
McIntosh, A. R., & Gonzalez-Lima, F. (1994). Structural equation modeling and its application to network analysis in functional brain imaging. *Human Brain Mapping*, *2*(1–2), 2–22. https://doi.org/10.1002/hbm.460020104
McLachlan, R. S. (2009). A brief review of the anatomy and physiology of the limbic system. *The Canadian Journal of Neurological Sciences. Le Journal Canadien Des Sciences Neurologiques*, *36 Suppl 2*, S84-7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19760912
Mechelli, A., Friston, K. J., Frackowiak, R. S., & Price, C. J. (2005). Structural Covariance in the Human Cortex. *Journal of Neuroscience*, *25*(36), 8303–8310. https://doi.org/10.1523/JNEUROSCI.0357-05.2005
Mechelli, A., Price, C. J., Friston, K. J., & Ashburner, J. (2005). Voxel-based morphometry of the human brain: methods and applications. *Current Medical …*, *1*(2), 105–113. https://doi.org/10.2174/1573405054038726
Medford, N., & Critchley, H. D. (2010). Conjoint activity of anterior insular and anterior cingulate cortex: awareness and response. *Brain Structure and Function*, pp. 1–15. https://doi.org/10.1007/s00429-010-0265-x
Meffert, H., Gazzola, V., Den Boer, J. A., Bartels, A. A. J., & Keysers, C. (2013). Reduced spontaneous but relatively normal deliberate vicarious representations in psychopathy. *Brain*, *136*(8), 2550–2562. https://doi.org/10.1093/brain/awt190
Meltzoff, A. N., & Decety, J. (2003). What imitation tells us about social cognition: a rapprochement between developmental psychology and cognitive neuroscience. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *358*(1431), 491–500. https://doi.org/10.1098/rstb.2002.1261
Menon, V. (2015). Salience Network. In *Brain Mapping: An Encyclopedic Reference* (Vol. 2, pp. 597–611). https://doi.org/10.1016/B978-0-12-397025-1.00052-X
Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Structure and Function*, 1–13. https://doi.org/10.1007/s00429-010-0262-0
Merikangas, K. R., Nakamura, E. F., & Kessler, R. C. (2009). Epidemiology of mental disorders in children and adolescents. *Dialogues in Clinical Neuroscience*. https://doi.org/10.2964/jsik.kuni0223

Michalska, K. J., Zeffiro, T. A., & Decety, J. (2016). Brain response to viewing others being harmed in children with conduct disorder symptoms. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *57*(4), 510–519. https://doi.org/10.1111/jcpp.12474

Mobbs, D., Hagan, C. C., Dalgleish, T., Silston, B., & Prévost, C. (2015). The ecology of human fear: Survival optimization and the nervous system. *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2015.00055

Moffitt, T., Caspi, a, Rutter, M., & Silva, P. (2002). Sex differences in antisocial behaviour: Conduct disorder, delinquency and violence in the Dunedin longitudinal study. *Psychological Medicine*, *32*(8), 1475–1476. https://doi.org/10.1017/CBO9780511490057

Moffitt, T. E. (1993). Adolescence-limited and life-course-persistent antisocial behavior: a developmental taxonomy. *Psychological Review*, *100*(4), 674–701. https://doi.org/10.1037/0033-295X.100.4.674

Moffitt, T. E. (2003). Life-course-persistent and adolescence-limited antisocial behavior: A 10-year research review and a research agenda. In *Causes of conduct disorder and juvenile delinquency* (pp. 49–75).

Moffitt, T. E. (2006). Life-Course-Persistent versus Adolescence-Limited Antisocial Behavior. In *Developmental Psychopathology: Second Edition* (Vol. 3, pp. 570–598). https://doi.org/10.1002/9780470939406.ch15

Moffitt, T. E., Arseneault, L., Jaffee, S. R., Kim-Cohen, J., Koenen, K. C., Odgers, C. L., … Viding, E. (2008). Research Review: DSM-V conduct disorder: Research needs for an evidence base. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. https://doi.org/10.1111/j.1469-7610.2007.01823.x

Moffitt, T. E., & Caspi, A. (2001). Childhood predictors differentiate life-course persistent and adolescence-limited antisocial pathways among males and females. *Development and Psychopathology*, *13*, 355–375. https://doi.org/10.1017/S0954579401002097

Moffitt, T. E., Caspi, A., Dickson, N., Silva, P., & Stanton, W. (1996). Childhood-onset versus adolescent-onset antisocial conduct problems in males: Natural history from ages 3 to 18 years. *Development and Psychopathology*, *8*(2), 399–424. https://doi.org/10.1017/S0954579400007161

Monk, C., Spicer, J., & Champagne, F. A. (2012). Linking prenatal maternal adversity to developmental outcomes in infants: the role of epigenetic pathways. *Development and Psychopathology*, *24*(4), 1361–76. https://doi.org/10.1017/S0954579412000764

Moulton, E. a, Elman, I., Pendse, G., Schmahmann, J., Becerra, L., & Borsook, D. (2011). Aversion-related circuitry in the cerebellum: responses to noxious heat and unpleasant images. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(10), 3795–804. https://doi.org/10.1523/JNEUROSCI.6709-10.2011

Muetzel, R. L., Blanken, L. M. E., van der Ende, J., El Marroun, H., Shaw, P., Sudre, G., … White, T. (2017). Tracking Brain Development and Dimensional Psychiatric Symptoms in Children: A Longitudinal Population-Based Neuroimaging Study. *American Journal of Psychiatry*, appi.ajp.2017.1. https://doi.org/10.1176/appi.ajp.2017.16070813

National Institute for Health and Clinical Excellence. (2013). *Antisocial behaviour and conduct disorders in children and young people: Recognition, intervention and management*. *Nice Clinical Guideline*. https://doi.org/10.1111/cch.12074_5

Nock, M. K., Kazdin, A. E., Hiripi, E., & Kessler, R. C. (2006). Prevalence, subtypes, and correlates of DSM-IV conduct disorder in the National Comorbidity Survey Replication. *Psychological Medicine*, *36*(5), 699. https://doi.org/10.1017/S0033291706007082

Noordermeer, S. D. S., Luman, M., & Oosterlaan, J. (2016). A Systematic Review and Meta-

analysis of Neuroimaging in Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD) Taking Attention-Deficit Hyperactivity Disorder (ADHD) Into Account. *Neuropsychology Review*. https://doi.org/10.1007/s11065-015-9315-8

Northover, C., Thapar, A., Langley, K., & Van Goozen, S. H. M. (2015). Pain sensitivity in adolescent males with attention-deficit/hyperactivity disorder: Testing for associations with conduct disorder and callous and unemotional traits. *PLoS ONE*, *10*(7). https://doi.org/10.1371/journal.pone.0134417

O'muircheartaigh, J., Keller, S. S., Barker, G. J., & Richardson, M. P. (2015). White matter connectivity of the thalamus delineates the functional architecture of competing thalamocortical systems. *Cerebral Cortex*, *25*(11), 4477–4489. https://doi.org/10.1093/cercor/bhv063

O'Nions, E., Sebastian, C. L., McCrory, E., Chantiluke, K., Happé, F., & Viding, E. (2014). Neural bases of Theory of Mind in children with autism spectrum disorders and children with conduct problems and callous-unemotional traits. *Developmental Science*, *17*(5), 786–796. https://doi.org/10.1111/desc.12167

O'Reilly, J. X., Woolrich, M. W., Behrens, T. E. J., Smith, S. M., & Johansen-Berg, H. (2012). Tools of the trade: Psychophysiological interactions and functional connectivity. *Social Cognitive and Affective Neuroscience*, *7*(5), 604–609. https://doi.org/10.1093/scan/nss055

Odgers, C. L., Moffitt, T. E., Broadbent, J. M., Dickson, N., Hancox, R. J., Harrington, H., … Caspi, A. (2008). Female and male antisocial trajectories: From childhood origins to adult outcomes. *Development and Psychopathology*, *20*(2), 673–716. https://doi.org/10.1017/S0954579408000333

Ometto, M., de Oliveira, P. A., Milioni, A. L., dos Santos, B., Scivoletto, S., Busatto, G. F., … Cunha, P. J. (2016). Social skills and psychopathic traits in maltreated adolescents. *European Child and Adolescent Psychiatry*, *25*(4), 397–405. https://doi.org/10.1007/s00787-015-0744-y

Panizzon, M. S., Fennema-Notestine, C., Eyler, L. T., Jernigan, T. L., Prom-Wormley, E., Neale, M., … Kremen, W. S. (2009). Distinct genetic influences on cortical surface area and cortical thickness. *Cerebral Cortex (New York, N.Y. : 1991)*, *19*(11), 2728–35. https://doi.org/10.1093/cercor/bhp026

Pardini, D. a, Lochman, J. E., & Frick, P. J. (2003). Callous/unemotional traits and social-cognitive processes in adjudicated youths. *Journal of the American Academy of Child and Adolescent Psychiatry*, *42*(3), 364–371. https://doi.org/10.1097/00004583-200303000-00018

Pardini, D., & Frick, P. J. (2013). Multiple developmental pathways to conduct disorder: Current conceptualizations and clinical implications. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *22*(1), 20–25.

Pardini, D., Stepp, S., Hipwell, A., Stouthamer-Loeber, M., & Loeber, R. (2012). The clinical utility of the proposed DSM-5 callous-unemotional subtype of conduct disorder in young girls. *Journal of the American Academy of Child and Adolescent Psychiatry*, *51*(1). https://doi.org/10.1016/j.jaac.2011.10.005

Pasalich, D. S., Dadds, M. R., & Hawes, D. J. (2014). Cognitive and affective empathy in children with conduct problems: Additive and interactive effects of callous-unemotional traits and autism spectrum disorders symptoms. *Psychiatry Research*, *219*(3), 625–630. https://doi.org/10.1016/j.psychres.2014.06.025

Passamonti, L., Fairchild, G., Goodyer, I. M., Hurford, G., Hagan, C. C., Rowe, J. B., & Calder, A. J. (2010). Neural Abnormalities in Early-Onset and Adolescence-Onset

Conduct Disorder. *Archives of General Psychiatry*, *67*(7), 729–738. https://doi.org/10.1001/archgenpsychiatry.2010.75
Passingham, R. (2009). How good is the macaque monkey model of the human brain? *Current Opinion in Neurobiology*. https://doi.org/10.1016/j.conb.2009.01.002
Patchev, V. K., Hayashi, S., Orikasa, C., & Almeida, O. F. (1995). Implications of estrogen-dependent brain organization for gender differences in hypothalamo-pituitary-adrenal regulation. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *9*(5), 419–23. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7896013
Pauli, R., Bowring, A., Reynolds, R., Chen, G., Nichols, T. E., & Maumet, C. (2016). Exploring fMRI Results Space: 31 Variants of an fMRI Analysis in AFNI, FSL, and SPM. *Frontiers in Neuroinformatics*, *10*. https://doi.org/10.3389/fninf.2016.00024
Paus, T. (2001). Primate anterior cingulate cortex: where motor control, drive and cognition interface. *Nature Reviews Neuroscience*, *2*(6), 417–424. https://doi.org/10.1038/35077500
Pedersen, W., & Mastekaasa, A. (2011). Conduct disorder symptoms and subsequent pregnancy, child-birth and abortion: a population-based longitudinal study of adolescents. *Journal of Adolescence*, *34*(5), 1025–1033. https://doi.org/10.1016/j.adolescence.2010.11.005
Pfeifer, J. H., Iacoboni, M., Mazziotta, J. C., & Dapretto, M. (2008). Mirroring others' emotions relates to empathy and interpersonal competence in children. *NeuroImage*, *39*(4), 2076–2085. https://doi.org/10.1016/j.neuroimage.2007.10.032
Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *NeuroImage*, *16*(2), 331–48. https://doi.org/10.1006/nimg.2002.1087
Phelps, E. A. (2004). Human emotion and memory: Interactions of the amygdala and hippocampal complex. *Current Opinion in Neurobiology*. https://doi.org/10.1016/j.conb.2004.03.015
Phelps, E. A., & LeDoux, J. E. (2005). Contributions of the amygdala to emotion processing: From animal models to human behavior. *Neuron*. https://doi.org/10.1016/j.neuron.2005.09.025
Phillips, M. L. (2012). Neuroimaging in psychiatry: Bringing neuroscience into clinical practice. *British Journal of Psychiatry*. https://doi.org/10.1192/bjp.bp.112.109587
Poythress, N. G., Dembo, R., Wareham, J., & Greenbaum, P. E. (2006). Construct validity of the Youth Psychopathic Traits Inventory (YPI) and the Antisocial Process Screening Device (ASPD) with justice-involved adolescents. *Criminal Justice and Behavior*, *33*(1), 26–55.
Provine, R. R. (2005). Contagious yawning and laughing: Everyday imitation- and mirror-like behavior. *Behavioral and Brain Sciences*, *28*(2), 142. https://doi.org/10.1017/S0140525X05390030
Puzzo, I., Seunarine, K., Sully, K., Darekar, A., Clark, C., Sonuga-Barke, E. J. S., & Fairchild, G. (2017). Altered White-Matter Microstructure in Conduct Disorder Is Specifically Associated with Elevated Callous-Unemotional Traits. *Journal of Abnormal Child Psychology*, pp. 1–16. https://doi.org/10.1007/s10802-017-0375-5
Raichle, M. E. (2015). The Brain's Default Mode Network. *Annual Review of Neuroscience*, *38*(1), 433–447. https://doi.org/10.1146/annurev-neuro-071013-014030
Raine, A. (2011). An amygdala structural abnormality common to two subtypes of conduct disorder: A neurodevelopmental conundrum. *American Journal of Psychiatry*, *168*(6),

569–571. https://doi.org/10.1176/appi.ajp.2011.11030416
Raine, A., Lee, L., Yang, Y., & Colletti, P. (2010). Neurodevelopmental marker for limbic maldevelopment in antisocial personality disorder and psychopathy. *British Journal of Psychiatry*, *197*(3), 186–192. https://doi.org/10.1192/bjp.bp.110.078485
Rajmohan, V., & Mohandas, E. (2007). The limbic system. *Indian Journal of Psychiatry*, *49*(2), 132–9. https://doi.org/10.4103/0019-5545.33264
Raschle, N. M., Menks, W. M., Fehlbaum, L. V., Tshomba, E., & Stadler, C. (2015). Structural and functional alterations in right dorsomedial prefrontal and left insular cortex co-localize in adolescents with aggressive behaviour: An ALE meta-analysis. *PLoS ONE*, *10*(9). https://doi.org/10.1371/journal.pone.0136553
Rizzolatti, G. (2005). The mirror neuron system and its function in humans. In *Anatomy and Embryology* (Vol. 210, pp. 419–421). https://doi.org/10.1007/s00429-005-0039-z
Robins, L. N. (1991). Conduct Disorder. *Journal of Child Psychology and Psychiatry*, *32*(1), 193–212. https://doi.org/10.1111/j.1469-7610.1991.tb00008.x
Roebroeck, A., Formisano, E., & Goebel, R. (2005). Mapping directed influence over the brain using Granger causality and fMRI. *NeuroImage*, *25*(1), 230–242. https://doi.org/10.1016/j.neuroimage.2004.11.017
Rogers, J. C., & Brito, S. A. De. (2016). Cortical and Subcortical Gray Matter Volume in Youths With Conduct Problems A Meta-analysis. *JAMA Psychiatry*, *73*(1), 64–72. https://doi.org/10.1001/jamapsychiatry.2015.2423
Roldan Gerschcovich, E., Cerquetti, D., Tenca, E., Leiguarda, R., Gerschcovich, R. E., Cerquetti, D., … Leiguarda, R. (2011). The impact of bilateral cerebellar damage on theory of mind, empathy and decision making. *Neurocase*, *17*(3), 270–275. https://doi.org/10.1080/13554791003730618
Romeo, R., Knapp, M., & Scott, S. (2006). Economic cost of severe antisocial behaviour in children--and who pays it. *The British Journal of Psychiatry : The Journal of Mental Science*, *188*, 547–553. https://doi.org/10.1192/bjp.bp.104.007625
Ross, H. E., & Young, L. J. (2009). Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Frontiers in Neuroendocrinology*. https://doi.org/10.1016/j.yfrne.2009.05.004
Roth-Hanania, R., Davidov, M., & Zahn-Waxler, C. (2011). Empathy development from 8 to 16 months: Early signs of concern for others. *Infant Behavior and Development*, *34*(3), 447–458. https://doi.org/10.1016/j.infbeh.2011.04.007
Rowe, R., Maughan, B., Moran, P., Ford, T., Briskman, J., & Goodman, R. (2010). The role of callous and unemotional traits in the diagnosis of conduct disorder. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *51*(6), 688–695. https://doi.org/10.1111/j.1469-7610.2009.02199.x
Rowe, R., Maughan, B., Worthman, C. M., Costello, E. J., & Angold, A. (2004). Testosterone, antisocial behavior, and social dominance in boys: Pubertal development and biosocial interaction. *Biological Psychiatry*, *55*(5), 546–552. https://doi.org/10.1016/j.biopsych.2003.10.010
Rumble, A. C., Van Lange, P. A. M., & Parks, C. D. (2010). The benefits of empathy: When empathy may sustain cooperation in social dilemmas. *European Journal of Social Psychology*, *40*(5), 856–866. https://doi.org/10.1002/ejsp.659
Rundle, B. K., Vaughn, V. R., & Stanford, M. S. (2015). Contagious yawning and psychopathy. *Personality and Individual Differences*, *86*, 33–37. https://doi.org/10.1016/j.paid.2015.05.025
Rutter, M. (2011). Research review: Child psychiatric diagnosis and classification: Concepts,

findings, challenges and potential. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. https://doi.org/10.1111/j.1469-7610.2011.02367.x
Rymarczyk, K., Zurawski, Ł., Jankowiak-Siuda, K., & Szatkowska, I. (2016). Emotional empathy and facial mimicry for static and dynamic facial expressions of fear and disgust. *Frontiers in Psychology*, *7*(NOV). https://doi.org/10.3389/fpsyg.2016.01853
Savitz, J. B., Rauch, S. L., & Drevets, W. C. (2013). Clinical application of brain imaging for the diagnosis of mood disorders: The current state of play. *Molecular Psychiatry*. https://doi.org/10.1038/mp.2013.25
Scheepers, F. E., Buitelaar, J. K., & Matthys, W. (2011). Conduct Disorder and the specifier callous and unemotional traits in the DSM-5. *European Child and Adolescent Psychiatry*, *20*(2), 89–93. https://doi.org/10.1007/s00787-010-0149-x
Schmahmann, J. D., & Sherman, J. C. (1998). The cerebellar cognitive affective syndrome. *Brain*, *121*(4), 561–579. https://doi.org/10.1093/brain/121.4.561
Schwenck, C., Mergenthaler, J., Keller, K., Zech, J., Salehi, S., Taurines, R., … Freitag, C. M. (2012). Empathy in children with autism and conduct disorder: Group-specific profiles and developmental aspects. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *53*(6), 651–659. https://doi.org/10.1111/j.1469-7610.2011.02499.x
Scott, S., Knapp, M., Henderson, J., & Maughan, B. (2001). Financial cost of social exclusion: follow up study of antisocial children into. *BMJ*, *323*(7306), 191–191. https://doi.org/10.1136/bmj.323.7306.191
Scott, W., Austin, D., & Reid, D. (2007). Investigation of the WISC-III and WASI in clinical child populations. *Canadian Journal of School Psychology*, *22*(2), 249–254.
Sebastian, C. L., De Brito, S. A., McCrory, E. J., Hyde, Z. H., Lockwood, P. L., Cecil, C. A. M., & Viding, E. (2016). Grey Matter Volumes in Children with Conduct Problems and Varying Levels of Callous-Unemotional Traits. *Journal of Abnormal Child Psychology*, *44*(4), 639–649. https://doi.org/10.1007/s10802-015-0073-0
Sebastian, C. L., Fontaine, N. M. G., Bird, G., Blakemore, S.-J., De Brito, S. A., McCrory, E. J. P., & Viding, E. (2012). Neural processing associated with cognitive and affective Theory of Mind in adolescents and adults. *Social Cognitive and Affective Neuroscience*, *7*(1), 53–63. https://doi.org/10.1093/scan/nsr023
Semendeferi, K., & Damasio, H. (2000). The brain and its main anatomical subdivisions in living hominoids using magnetic resonance imaging. *Journal of Human Evolution*, *38*(2), 317–332. https://doi.org/10.1006/jhev.1999.0381
Sethi, A., Sarkar, S., Dell'Acqua, F., Viding, E., Catani, M., Murphy, D. G. M., & Craig, M. C. (2018). Anatomy of the dorsal default-mode network in conduct disorder: Association with callous-unemotional traits. *Developmental Cognitive Neuroscience*, *30*, 87–92. https://doi.org/10.1016/j.dcn.2018.01.004
Shamay-Tsoory, S. G. (2011). The Neural Bases for Empathy. *The Neuroscientist*, *17*(1), 18–24. https://doi.org/10.1177/1073858410379268
Shamay-Tsoory, S. G., & Abu-Akel, A. (2016). The Social Salience Hypothesis of Oxytocin. *Biological Psychiatry*. https://doi.org/10.1016/j.biopsych.2015.07.020
Sharp, C., Vanwoerden, S., Van Baardewijk, Y., Tackett, J. L., & Stegge, H. (2015). Callous-unemotional traits are associated with deficits in recognizing complex emotions in preadolescent children. *Journal of Personality Disorders*, *29*(3), 347–359. https://doi.org/10.1521/pedi_2014_28_161
Shelton, D. (2001). Emotional disorders in young offenders. *Journal of Nursing Scholarship*, *33*, 259–263. https://doi.org/10.1111/j.1547-5069.2001.00259.x
Shen, S., & Sterr, A. (2013). Is DARTEL-based voxel-based morphometry affected by width

of smoothing kernel and group size? A study using simulated atrophy. *Journal of Magnetic Resonance Imaging*, *37*(6), 1468–1475. https://doi.org/10.1002/jmri.23927
Shirtcliff, E. A., Vitacco, M. J., Graf, A. R., Gostisha, A. J., Merz, J. L., & Zahn-Waxler, C. (2009). Neurobiology of empathy and callousness: Implications for the development of antisocial behavior. *Behavioral Sciences and the Law*. https://doi.org/10.1002/bsl.862
Silverthorn, P., & Frick, P. J. (1999). Developmental pathways to antisocial behavior: The delayed-onset pathway in girls. *Development and Psychopathology*, *11*(1), 101–126. https://doi.org/10.1017/S0954579499001972
Singer, T. (2004). Empathy for Pain Involves the Affective but not Sensory Components of Pain. *Science*, *303*(5661), 1157–1162. https://doi.org/10.1126/science.1093535
Singer, T. (2006). The neuronal basis and ontogeny of empathy and mind reading: Review of literature and implications for future research. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2006.06.011
Singer, T., & Lamm, C. (2009). The social neuroscience of empathy. *Annals of the New York Academy of Sciences*, *1156*, 81–96. https://doi.org/10.1111/j.1749-6632.2009.04418.x
Steinberg, L. (2004). Risk taking in adolescence: What changes, and why? In *Annals of the New York Academy of Sciences* (Vol. 1021, pp. 51–58). https://doi.org/10.1196/annals.1308.005
Stephan, K. E., & Friston, K. J. (2010). Analyzing effective connectivity with fMRI. *Wiley Interdisciplinary Reviews. Cognitive Science*, *1*(3), 446–459. https://doi.org/10.1002/wcs.58
Sterzer, P., Stadler, C., Poustka, F., & Kleinschmidt, A. (2007). A structural neural deficit in adolescents with conduct disorder and its association with lack of empathy. *NeuroImage*, *37*(1), 335–342. https://doi.org/10.1016/j.neuroimage.2007.04.043
Stevens, F. L., Hurley, R. A., & Taber, K. H. (2011). Anterior Cingulate Cortex: Unique Role in Cognition and Emotion. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *23*(2), 121–125. https://doi.org/10.1176/jnp.23.2.jnp121
Sturmer, S., Snyder, M., Kropp, A., & Siem, B. (2006). Empathy-Motivated Helping: The Moderating Role of Group Membership. *Personality and Social Psychology Bulletin*, *32*(7), 943–956. https://doi.org/10.1177/0146167206287363
Suurland, J., van der Heijden, K. B., Smaling, H. J. A., Huijbregts, S. C. J., van Goozen, S. H. M., & Swaab, H. (2016). Infant autonomic nervous system response and recovery: Associations with maternal risk status and infant emotion regulation. *Development and Psychopathology*, 1–15. https://doi.org/10.1017/S0954579416000456
Taki, Y., Hashizume, H., Sassa, Y., Takeuchi, H., Asano, M., Asano, K., & Kawashima, R. (2010). Breakfast staple types affect brain gray matter volume and cognitive function in healthy children. *PLoS ONE*, *5*(12). https://doi.org/10.1371/journal.pone.0015213
Tangney, J. P. (1995). Recent Advances in the Empirical Study of Shame and Guilt. *American Behavioral Scientist*, *38*(8), 1132–1145. https://doi.org/10.1177/0002764295038008008
Theule, J., Germain, S. M., Cheung, K., Hurl, K. E., & Markel, C. (2016). Conduct Disorder/Oppositional Defiant Disorder and Attachment: A Meta-Analysis. *Journal of Developmental and Life-Course Criminology*, *2*(2), 232–255. https://doi.org/10.1007/s40865-016-0031-8
Toller, G., Adhimoolam, B., Grunwald, T., Huppertz, H. J., Kurthen, M., Rankin, K. P., & Jokeit, H. (2015). Right mesial temporal lobe epilepsy impairs empathy-related brain responses to dynamic fearful faces. *Journal of Neurology*, *262*(3), 729–741. https://doi.org/10.1007/s00415-014-7622-2

Tomasi, D., & Volkow, N. D. (2010). Functional connectivity density mapping. *Proc Natl Acad Sci U S A*, *107*(21), 9885–9890. https://doi.org/10.1073/pnas.1001414107
Toussaint, L., & Webb, J. R. (2005). Gender Differences in the Relationship Between Empathy and Forgiveness. *The Journal of Social Psychology*, *145*(6), 673–685. https://doi.org/10.3200/SOCP.145.6.673-686
Uddin, L. Q. (2017). Salience Network Dysfunction. In *Salience Network of the Human Brain* (pp. 23–28). https://doi.org/10.1016/B978-0-12-804593-0.00005-9
Uytun, M. C., Karakaya, E., Oztop, D. B., Gengec, S., Gumus, K., Ozmen, S., … Ozsoy, S. D. (2016). Default mode network activity and neuropsychological profile in male children and adolescents with attention deficit hyperactivity disorder and conduct disorder. *Brain Imaging and Behavior*, pp. 1–10. https://doi.org/10.1007/s11682-016-9614-6
van der Plas, E. A. A., Boes, A. D., Wemmie, J. A., Tranel, D., & Nopoulos, P. (2010). Amygdala volume correlates positively with fearfulness in normal healthy girls. *Social Cognitive and Affective Neuroscience*, *5*(4), 424–431. https://doi.org/10.1093/scan/nsq009
Vanwoerden, S., Reuter, T., & Sharp, C. (2016). Exploring the clinical utility of the DSM-5 conduct disorder specifier of "with limited prosocial emotions" in an adolescent inpatient sample. *Comprehensive Psychiatry*, *69*, 116–131. https://doi.org/10.1016/j.comppsych.2016.05.012
Venkatasubramanian, G., & Keshavan, M. S. (2016). Biomarkers in psychiatry – A critique. *Annals of Neurosciences*. https://doi.org/10.1159/000443549
Viding, E., Fontaine, N. M. G., & McCrory, E. J. (2012). Antisocial behaviour in children with and without callous-unemotional traits. *Journal of the Royal Society of Medicine*, *105*(5), 195–200. https://doi.org/10.1258/jrsm.2011.110223
Viding, E., & McCrory, E. J. (2012). Why should we care about measuring callous-unemotional traits in children? *British Journal of Psychiatry*. https://doi.org/10.1192/bjp.bp.111.099770
Visser, T. A. W., Ohan, J. L., Whittle, S., Yücel, M., Simmons, J. G., & Allen, N. B. (2014). Sex differences in structural brain asymmetry predict overt aggression in early adolescents. *Social Cognitive and Affective Neuroscience*, *9*(4), 553–560. https://doi.org/10.1093/scan/nst013
Vogt, B. A., Sikes, R. W., & Vogt, L. J. (1993). Anterior Cingulate Cortex and the Medial Pain System. In *Neurobiology of Cingulate Cortex and Limbic Thalamus: A Comprehensive Handbook* (pp. 313–344). https://doi.org/10.1007/978-1-4899-6704-6_11
Vytal, K., & Hamann, S. (2010). Neuroimaging Support for Discrete Neural Correlates of Basic Emotions: A Voxel-based Meta-analysis. *Journal of Cognitive Neuroscience*, *22*(12), 2864–2885. https://doi.org/10.1162/jocn.2009.21366
Wallace, G. L., White, S. F., Robustelli, B., Sinclair, S., Hwang, S., Martin, A., & Blair, R. J. R. (2014). Cortical and subcortical abnormalities in youths with conduct disorder and elevated callous-unemotional traits. *Journal of the American Academy of Child and Adolescent Psychiatry*, *53*(4). https://doi.org/10.1016/j.jaac.2013.12.008
Waller, R., Dotterer, H. L., Murray, L., Maxwell, A. M., & Hyde, L. W. (2017). White-matter tract abnormalities and antisocial behavior: A systematic review of diffusion tensor imaging studies across development. *NeuroImage: Clinical*. https://doi.org/10.1016/j.nicl.2017.01.014
Waller, R., Gardner, F., Hyde, L. W., Shaw, D. S., Dishion, T. J., & Wilson, M. N. (2012). Do

harsh and positive parenting predict parent reports of deceitful-callous behavior in early childhood? *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *53*(9), 946–953. https://doi.org/10.1111/j.1469-7610.2012.02550.x

Weinberger, D. R., & Radulescu, E. (2016). Finding the elusive psychiatric "lesion" with 21st-century neuroanatomy: A note of caution. *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.2015.15060753

White, S. F., Brislin, S., Sinclair, S., Fowler, K. A., Pope, K., & Blair, R. J. R. (2013). The relationship between large cavum septum pellucidum and antisocial behavior, callous-unemotional traits and psychopathy in adolescents. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *54*(5), 575–581. https://doi.org/10.1111/j.1469-7610.2012.02603.x

White, S. F., Clanton, R., Brislin, S. J., Meffert, H., Hwang, S., Sinclair, S., & Blair, R. J. R. (2014). Reward: Empirical Contribution: Temporal Discounting and Conduct Disorder in Adolescents. *Journal of Personality Disorders*, *28*(1), 5–18. https://doi.org/10.1521/pedi.2014.28.1.5

Whiten, A., & Byrne, R. W. (1988). The Machiavellian intelligence hypotheses: editorial. In *Machiavellian intelligence: Social expertise and the evolution of intellect in monkeys, apes, and humans* (pp. 1–9).

Whitwell, J. L. (2009). Voxel-Based Morphometry: An Automated Technique for Assessing Structural Changes in the Brain. *Journal of Neuroscience*, *29*(31), 9661–9664. https://doi.org/10.1523/JNEUROSCI.2160-09.2009

(WHO), W. H. O. (2008). Integrating mental health into primary care: A global perspective. *WHO Library*. https://doi.org/10.1097/00001504-199403000-00013

Wiech, K., Jbabdi, S., Lin, C. S., Andersson, J., & Tracey, I. (2014). Differential structural and resting state connectivity between insular subdivisions and other pain-related brain regions. *Pain*, *155*(10), 2047–2055. https://doi.org/10.1016/j.pain.2014.07.009

Wild, B., Erb, M., Eyb, M., Bartels, M., & Grodd, W. (2003). Why are smiles contagious? An fMRI study of the interaction between perception of facial affect and facial movements. *Psychiatry Research - Neuroimaging*, *123*(1), 17–36. https://doi.org/10.1016/S0925-4927(03)00006-4

Wilke, M., Holland, S. K., Altaye, M., & Gaser, C. (2008). Template-O-Matic: A toolbox for creating customized pediatric templates. *NeuroImage*, *41*(3), 903–913. https://doi.org/10.1016/j.neuroimage.2008.02.056

Wolf, U., Rapoport, M. J., & Schweizer, T. A. (2009). Evaluating the affective component of the cerebellar cognitive affective syndrome. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *21*(3), 245–53. https://doi.org/10.1176/jnp.2009.21.3.245

Wolke, D., Woods, S., Bloomfield, L., & Karstadt, L. (2001). Bullying involvement in primary school and common health problems. *Archives of Disease in Childhood*, *85*(3), 197–201. https://doi.org/10.1136/adc.85.3.197

World Health Organization. (1992). The ICD-10 Classification of Mental and Behavioural Disorders. *International Classification*, *10*, 1–267. https://doi.org/10.1002/1520-6505(2000)9:5<201::AID-EVAN2>3.3.CO;2-P

Wu, Q., Zhang, X., Dong, D., Wang, X., & Yao, S. (2017). Altered spontaneous brain activity in adolescent boys with pure conduct disorder revealed by regional homogeneity analysis. *European Child and Adolescent Psychiatry*, pp. 1–11. https://doi.org/10.1007/s00787-017-0953-7

Xu, X., Zuo, X., Wang, X., & Han, S. (2009). Do You Feel My Pain? Racial Group Membership Modulates Empathic Neural Responses. *Journal of Neuroscience*, *29*(26),

8525–8529. https://doi.org/10.1523/JNEUROSCI.2418-09.2009
Yeh, Z. T., & Tsai, C. F. (2014). Impairment on theory of mind and empathy in patients with stroke. *Psychiatry and Clinical Neurosciences*, *68*(8), 612–620. https://doi.org/10.1111/pcn.12173
Yen, C. T., & Lu, P. L. (2013). Thalamus and pain. *Acta Anaesthesiologica Taiwanica*. https://doi.org/10.1016/j.aat.2013.06.011
Yoder, K. J., Lahey, B. B., & Decety, J. (2016). Callous traits in children with and without conduct problems predict reduced connectivity when viewing harm to others. *Scientific Reports*, *6*(1), 20216. https://doi.org/10.1038/srep20216
Yoon, J. M. D., & Tennie, C. (2010). Contagious yawning: a reflection of empathy, mimicry, or contagion? *Animal Behaviour*, *79*(5). https://doi.org/10.1016/j.anbehav.2010.02.011
Zahn-Waxler, C., Robinson, J. L., & Emde, R. N. (1992). The Development of Empathy in Twins. *Developmental Psychology*, *28*(6), 1038–1047. https://doi.org/10.1037/0012-1649.28.6.1038
Zaki, J., Ochsner, K. N., Hanelin, J., Wager, T. D., & Mackey, S. C. (2007). Different circuits for different pain: patterns of functional connectivity reveal distinct networks for processing pain in self and others. *Social Neuroscience*, *2*(January 2015), 276–291. https://doi.org/10.1080/17470910701401973
Zhou, J., Yao, N., Fairchild, G., Cao, X., Zhang, Y., Xiang, Y. T., … Wang, X. (2015). Disrupted default mode network connectivity in male adolescents with conduct disorder. *Brain Imaging and Behavior*, 995–1003. https://doi.org/10.1007/s11682-015-9465-6
Zielinski, B. A., Gennatas, E. D., Zhou, J., & Seeley, W. W. (2010). Network-level structural covariance in the developing brain. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(42), 18191–6. https://doi.org/10.1073/pnas.1003109107