Aggression in male adolescents
The role of genetic, hormonal, and cognitive factors

Mireille Janine Huvenaars-Bakker
Acknowledgements
This study was supported by Karakter, Child and Adolescent Psychiatry, University Center. The study is further supported by the European Union 7th Framework programs AGGRESSOTYPE (602805), MATRICS (603016), and TACTICS (278948), a Horizon 2020 Marie Skłodowska-Curie program MiND (643051), and the Innovative Medicines Initiative Joint Undertaking EU-AIMS (115300), resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007 - 2013) and the European Federation of Pharmaceutical Industries and Associations (EFPIA) companies’ in kind contribution and by a NWO Brain & Cognition grant (056-24-011). We are grateful to participating families.

Cover design & Layout by Andreas Huvenaars
Printed by ProefschriftMaken

ISBN: 978-94-6284-157-4

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Aggression in male adolescents:
The role of genetic, hormonal, and cognitive factors

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“First I drink the coffee, then I do the things”
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General introduction
Aggression

When most people think of aggression, they think of road rage, physical fights, and violent crime. However, not all aggression is bad. Aggression may be adaptive by for example, helping people and animals alike guard their homes from intruders and protect their children from threats. Problems arise when aggression is taken too far, escalating abnormally in a repeated way and becoming violent. Problems related to aggression in young people are traditionally included under the header of disruptive behaviour disorder (DBD), which include oppositional defiant disorder (ODD) and conduct disorder (CD). Both disorders are known to be highly complex and both clinically and aetiologically heterogeneous. More knowledge is needed about the genetic, biological, (neuro)cognitive, as well as behavioural bases of aggression in ODD and/or CD. In 2013, a new version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) was published and callous unemotional (CU) traits were added as a specifier for a more severe form of CD labelled ‘with limited prosocial emotions’. CU traits are described as: 1) lack of remorse or guilt, 2) callous lack of empathy, 3) lack of concern about performance, and 4) shallow or deficient affect (American Psychiatric Association [APA], 2013). This form of CD is associated with reductions in specific forms of empathy, in particular responding to the fear, sadness, pain and happiness of others (Blair, 2013). Decreased empathic functioning is also among the core symptoms of autism spectrum disorder (ASD) (Herpers, Klip, Rommelse, Greven, & Buitelaar, 2016; APA, 2013; Rogers, Viding, Blair, Frith, & Happe, 2006). Individuals with ASD also have elevated levels of aggressive behaviour compared to typically-developing individuals (Hill et al., 2014), although the aggression is not a core symptom of ASD and is typically less severe in ASD than ODD/CD (APA, 2013).

We only have limited understanding of the behavioural, (neuro)cognitive, and biological processes that underpin CU traits/diminished empathy and aggressive behaviour in ODD/CD and ASD. CU traits and aggression, and their underlying processes, may act as trans-diagnostic markers that form a bridge between disorders and biological substrates of behaviour (Rodriguez-Seijas, Eaton, & Krueger, 2015; Walkup, Mathews, & Green, 2017). This in turn, may clarify heterogeneity, comorbidity and inform cross-disorder interventions in clinical practice. This thesis examines proactive and reactive subtypes of aggression, aggression with or without CU traits, and their genetic, (neuro)endocrine, (neuro)cognitive, and behavioural underpinnings as transdiagnostic markers. For example, there is an ongoing discussion on the need for defining CU traits as a transdiagnostic construct (Herpers et al., 2016; Jambroes et al., 2016; Scheepers, Buitelaar, & Matthys, 2010).

The main aim of this thesis is to investigate the mechanisms of aggression and CU traits in a study that compares male adolescents with ODD/CD or ASD to typically developing individuals (TDI). Both, a categorical and a dimensional approach to ODD/CD and ASD, can provide new insights into the psychopathology of these three disorders. For example, the DSM-5, defines mental disorders as distinct binary constructs that are putatively unrelated to one another. In contrast, the Research Domain Criteria (RDoC) target specific feature domains as an approach to research across a range of mental disorders (Insel et al., 2010), which is an important consideration in light of the marked heterogeneity of ODD/CD, and ASD. RDoC integrates many levels of information (from genomics to self-report) to better understand basic dimensions of functioning underlying the full
range of human behaviour from normal to abnormal (Cuthbert & Insel, 2013; Insel et al., 2010), and seeks to unravel the fundamental mechanisms underlying psychopathology. In doing so, it has been creating a taxonomy of key functions (e.g. positive valence, social processes, cognition, arousal, regulatory systems) that, it is thought, correspond better to underlying mechanisms than does the existing system of discrete diagnoses. In the sections below, I will introduce the psychiatric disorders and theories of mechanisms underlying aggressive behaviour. To conclude, I will elaborate on the specific methods and aims of this thesis.

Oppositional Defiant Disorder and Conduct Disorder

The diagnostic criteria of ODD include a recurrent pattern of at least four symptoms from the following categories 1) angry/irritable mood, 2) argumentative/defiant behaviour, or 3) vindictiveness, lasting at least six months, and exhibited during interaction with at least one individual who is not a sibling (APA, DSM–5, 2013). Frequently occurring symptoms within each of these three categories are: 1) loses temper, touchy or easily annoyed, angry and resentful, 2) argues with authority figures, or for children and adolescents with adults, actively defies or refuses to comply with requests from authority figures or with rules, deliberately annoys others, blames others for his or her mistakes or misbehaviour, 3) has been spiteful or vindictive at least twice within the past six months. The behaviours do not occur exclusively during the course of a psychotic, substance use, depressive, or bipolar disorder. Also, in this instance the criteria are not met for disruptive mood dysregulation disorder. Severity of the disorder is specified as mild, moderate and severe, based on the presence of symptoms in a single setting, two or more than three settings (e.g. at home, at school, at work, with peers), respectively.

ODD is characterized by a repetitive and persistent pattern of anti-social and rule breaking behaviour. According to DSM-5, there are four generalized behavioural subtypes: 1) aggression to people and animals, 2) destruction of property, 3) deceitfulness or theft and 4) serious violations of rules (APA, 2013). The DSM-5 diagnostic criteria facilitate an age-related separation of CD cases, i.e. those with childhood onset (i.e. before 10 years of age) and adolescent onset (from 10 years of age) CD. Those with childhood onset CD have been found to display greater cognitive impairment, more psychiatric symptoms and to have committed more serious violent offences, compared to adolescent onset (Johnson, Kemp, Heard, Lennings, & Hickie, 2015; Moffitt, 2006). Some studies state that childhood-onset (but not adolescent-onset) CD is even a robust predictor of developing antisocial personality disorder in later life (Lahey, Loeber, Burke, & Applegate, 2005; Loeber, Burke, & Lahey, 2002; Zoccolillo, Pickles, Quinton, & Rutter, 1992). These studies seem to confirm the dual taxonomy model of Moffit (1993). Moffit states that life-course persistent antisocial behaviour begins in early childhood and continues throughout adulthood, while offenders with adolescent-onset antisocial behaviour refrain from offending in young adulthood. However, recent research has questioned Moffit’s model by showing that both childhood- and adolescent-onset CD, have severe executive impairment, which challenges the assumption that adolescent onset antisocial behaviour is a normative process (Fairchild, van Goozen, Calder, & Goodyer, 2013; Johnson et al., 2015).
Whether the underlying biological substrates of childhood- versus adolescent-onset CD are similar or different is not yet clear.

The DSM-5 further defines CD with or without CU traits. The latter marks a form of CD that is rather refractory to treatment and has a poor long-term prognosis (APA, 2013; Frick & White, 2008; Herpers et al., 2016; Scheepers et al., 2010) including elevated risk towards criminal activities and other negative outcomes in later life (Loeber & Stouthamer-Loeber, 1998). However, recent research has also suggested that children and adolescents with elevated CU traits are not “untreatable” and that these children can show reductions in both their CU traits and their antisocial behaviour (Wilkinson, Waller, & Viding, 2016), although they typically begin treatment with poorer premorbid functioning and can still end with higher levels of aggressive behaviour (Wilkinson et al., 2016).

In the current thesis, I will frequently refer to ODD and CD together for two reasons: First, in many cases, CD is preceded by ODD (Loeber, Burke, Lahey, Winters, & Zera, 2000), and both are part of the DBD header of DSM-5, and are frequently grouped together in the literature. Second, in the main study sample of this thesis, there were more individuals with ODD and rather fewer individuals with CD to investigate them as separate group. Nonetheless it is important to keep in mind that although ODD and CD are often described as different age-related manifestations of the same condition, with ODD preceding CD, a subgroup of children with ODD never transit to CD (50-76%) (Loeber & Farrington, 2000; Matthys & Lochman, 2010; Lahey, Waldman, & McBurnett, 1999; Rowe, Maughan, Pickles, Costello, & Angold, 2002; Speltz, Greenberg, & Deklyen, 1990).

**Autism Spectrum Disorder**

Individuals with ASD are characterised by impairments in reciprocal social interaction, deficits in verbal and non-verbal communication and presence of restricted or repetitive patterns behaviour, interests, and abnormal sensory behaviours that manifests in early childhood and persists through the entire life (APA, 2013). The previous version of DSM, DSM-IV-TR, had a category of Pervasive Developmental Disorders which included five separate diagnostic categories: Autistic Disorder, Asperger syndrome, Rett’s syndrome, Childhood Disintegrative Disorder, and pervasive developmental disorder not otherwise specified (PDDNOS) (APA, DSM-IV-TR, 2000). In the latest version of the DSM (DSM-5), Autistic Disorder, Asperger syndrome and PDDNOS were combined in one broad category of ASD with varying degrees of severity, and Rett’s syndrome and Childhood Disintegrative Disorder were removed. The rationale for this change of nomenclature is the high degree of similar clinical features between the disorders and concern for the validity of each of the disorders as separate entities, as it is now thought that the symptoms of the disorders are currently conceptualised to lie on a continuum of autism-specific traits. Additionally, a new diagnosis of Social Communication Disorder was added to include people who have persistent problems in social interaction, but do not meet the diagnostic criteria for ASD (APA, DSM-5, 2013). DSM-5 criteria for the diagnosis of ASD differ from the DSM-IV-TR in several aspects. First, DSM-5 combines the symptoms in two domains (i.e. 1) impairments in social interaction and communication, 2) repetitive and restricted behaviours and activities) instead of three (i.e. 1) social interaction, 3) language / communication, and 3) repetitive and restricted behaviours and activities). Impairments in social interaction and communication include behaviours such as reduced eye contact, failure to initiate or respond to social interaction, deficits
in maintaining social relationships. The second domain includes repetitive and restricted behaviours and activities.

Prevalence and comorbidity of ODD, CD, ASD

The community prevalence rates in children and adolescents between five and twenty years of age for ODD and CD vary from 4-14%, depending on the criteria used and population studied (Foster & Jones, 2005; Frick & Dickens, 2006; Frick & White, 2008; Scheepers et al., 2010). The ratio of boys to girls with ODD or CD falls between 4:1 and 2:1, but whether this represents a genuine prevalence difference across gender or a relative under diagnosis of girls due to a more covert aggression style in girls is debatable.

The population prevalence of ASD is about 1% (Matson & Shoemaker, 2009). Like ODD/CD, ASD is more prevalent in boys than in girls. Large-scale population-based studies have shown that two to three times more males than females are affected by ASD (Kim et al., 2011). In clinical ASD samples, male-female ratio estimates range up to four till five times more males than females with ASD (Fombonne, Quirke, & Hagen, 2009). In children/adolescents with ASD, a wide range of prevalence rates of comorbid ODD (4-75%) and CD (2-49%) have been reported (see for an overview van Steensel, Bogels, & de Bruin, 2013).

Although ODD, CD, and ASD are separate diagnostic categories, these disorders appear to frequently co-occur with each other and with other disorders. Research on rates of co-morbid ASD in children/adolescents with ODD/CD is hardly available. Far more commonly stated in research, is the role of comorbid ADHD which is present in about 50% of children and adolescents with disruptive behaviour disorder (Matthys & Lochman, 2010), and 30%-80% of children with ASD have comorbid ADHD (Matson, Rieske, & Williams, 2013). In addition, research has found that a number of children later diagnosed with ASD, previously had received a diagnosis of ODD/CD (12%) (Mandell, Ittenbach, Levy, & Pinto-Martin, 2007), likely due to overlapping difficulties in social interaction, which also complicates the correct differential diagnosis of ODD, CD, and ASD.

The majority of research on CU traits has been conducted in youths with conduct problems. However, little is known about the presence of CU traits in disorders other than CD and about their implications for severity of these disorders and functional adaptation (Herpers, Klip, Rommelse, Greven, & Buitelaar, 2016). Although prevalence rates will differ by population, one multi-site study estimated that 2% to 32% of community youth and 14% to 50% of clinic-referred youth meet the criteria for high CU traits, depending on whether or not they are diagnosed with CD and who the informant is (Kahn, Frick, Youngstrom, Findling, & Youngstrom, 2012). In community samples, high CU traits were associated with more global impairment (Ezpeleta, de la Osa, Granero, Penelo, & Domenech, 2013; Pardini, Stepp, Hipwell, Stouthamer-Loebel, & Loebel, 2012; Waschbusch et al., 2004), not only in the CD subsample, but in the no CD / high CU subsample as well (Pardini et al., 2012).
Treatments

Psychological treatments
The Guideline Development Group (2013), a subgroup of the National Institute for Health and Care Excellence (NICE), advocates the preferred treatment of disruptive behavioural problems to be psychological, with a particular emphasis on working with both parents (if possible) or guardians. In addition, other treatment approaches may include cognitive behavioural problem-solving and multi-systematic therapy. Evaluation and monitoring of these three different types of treatment programmes is important in establishing that such programmes are effective and acceptable to the patient population (National Institute for Health and Care Excellence, 2013). Despite evidence demonstrating overall low effect sizes of randomised controlled trials in treating aggression in children with these interventions, they remain paradoxically the treatment of choice (Bakker, Greven, Buitelaar, & Glennon, 2017; Conduct Problems Prevention Research, 1999, 2011; Scotto Rosato et al., 2012). However, many meta-analyses thus far have focussed on the treatment of aggression in general and are not CD-specific. Hence, in this thesis I will provide a systematic review and meta-analysis of nonpharmacological treatments, specifically on outcomes related to CD problems, and provide strategies for improving treatment (chapter 2).

Medical interventions
Clinical pharmacological interventions for conduct problems (e.g. aggression) have not been extensively developed (Smith & Coghill, 2010), there is no first line medication treatment licensed for aggressive symptoms. To date, medication approved for other indications (particularly ADHD) such as the psychostimulants (e.g. methylphenidate), alpha-2 agonists (e.g. guanfacine), and atypical antipsychotics (e.g. risperidone, aripiprazole, quetiapine) are the drug treatments of choice but these remain second-line treatment interventions to psychosocial approaches (Linton, Barr, Honer, & Procyslyn, 2013; Smith & Coghill, 2010). Within ASD, only risperidone and aripiprazole are Food and Drug Administration approved to treat aggressive behaviour and irritability (LeClerc & Easley, 2015). In addition, limited clinical data have supported treatment with SSRIs, CNS stimulants, NMDA-receptor antagonists, and other agents (LeClerc & Easley, 2015).

Models of aggression
Since Freud’s psychoanalytic theory (i.e. individual behaviours are motivated by sexual and instinctive drives and aggression was considered simply as a reaction to the blocking of libidinal impulses), a broad range of theories aim to explain the origins and triggers of human aggression. It is believed that a combination of 1) genetics, (neuro)endocrinological, and neural dysfunction (i.e. brain level), 2) (neuro) cognitive level (i.e. cognitive and affective factors), 3) behavioural level, and 4) environmental influences may explain the relationships among the various theories of aspects in aggressive individuals (Krol, Morton, & De Bruyn, 2004). The multiplicity of factors associated with the emergence, development and maintenance of aggressive behaviour suggests that aggression is a complex behaviour involving many
different processes and mechanisms. Additionally, these factors may all be associated with each other, which makes it even more difficult to reach a full understanding. Identification of the causal mechanisms that underlie ODD and CD is important to help inform prevention and treatment modalities. In the following sections these four factors will be briefly introduced.

1) Genetic, (neuro)endocrinological and neural dysfunction

Genetic factors play a role in the development or maintenance of aggressive behaviour, and it is unlikely to be explained by a few candidate genes (e.g. 5HTTLPR, COMT, and DRD4), but rather by a complex interaction between multiple genes (Vassos, Collier, & Fazel, 2014). In addition, the X-chromosomal MAOA gene, which encodes the enzyme monoamine oxidase A, responsible for the catabolism of dopamine, serotonin, as well as noradrenaline (Bortolato, Chen, & Shih, 2008), is believed to play a role in the development and maintenance of aggressive behaviour. The low activity variant of the MAOA gene is thought to be associated with reactive aggression particularly in those who have grown up in adverse environments (e.g. childhood maltreatment) Buckholtz & Meyer-Lindenberg, 2008). Accumulated evidence from quantitative and molecular genetic studies (Polderman et al., 2015; Rhee & Waldman, 2002) reveals the influence of genetic factors in the aetiology of antisocial behaviour problems. Genome-wide association studies indicate that antisocial behaviour may be highly polygenic and has potential heterogeneous genetic associations across gender (Tielbeek et al., 2017). Quantitative genetic results are based on research in twin studies and, to a lesser extent, adoption studies (Burt, 2009a; Rhee & Waldman, 2002). From twin studies we know that concordance rates regarding aggressive behaviour are higher for monozygotic than dizygotic twins, suggesting genetic influence on aggressive behaviour. Furthermore, approximately 50% of the variance in aggressive behaviour may be explained by genetic risk factors, whereas the remainder can be explained by a moderate influence of the non-shared environment and a small to modest influence of the shared environments (Burt, 2009a; Polderman et al., 2015; Rhee & Waldman, 2002). Also based on twin data, others have reported an association between antisocial behaviour and psychiatric traits (e.g. antisocial spectrum disorders and psychopathy), which was partially explained by common genetic factors (Gunter, Vaughn, & Philibert, 2010), suggestive of shared biological mechanisms between behaviour and traits. In addition, based on both twin and adoption studies, shared environmental influences accounted for 10-15%, non-shared environmental influences for 28-31%, and additive genetic influences for 57-59% of the variance in externalizing disorders (e.g. ODD problems and CD problems) (Burt, 2009b).

Heritability in ASD has been estimated at > 90% for classical autism (Abrahams & Geschwind, 2008; Freitag, 2007), although more recent studies tend to report lower heritability estimates (Hallmayer et al., 2011). The genetics of ASD is highly heterogeneous, and, like for ODD/CD, there are probably thousands of genetic variants that can contribute to a risk of developing ASD. Approximately 10% of individuals with ASD have an identifiable genetic aetiology corresponding to known chromosomal rearrangements of single gene disorders (e.g. Fragile X), another 7-10% carry monogenic forms due to the novo pathogenic mutations or copy number variances, yet the majority of ASD cases likely stem from multifactorial underpinnings involving several to many loci and gene-gene and gene-environment interactions (Abrahams & Geschwind, 2008; Berg & Geschwind, 2012;
In this thesis, I will provide a systematic review of genetic studies of aggressive behaviour in both humans and animal models, and examine to what degree prior studies have examined phenotypes that fit into the RDoC framework (chapter 3).

In addition to the genetic factors, neural substrates also play a role in the development or maintenance of aggressive behaviour. The brain is a complex organ and constitutes a broad range of complex mechanisms. Violent and irritable behaviour can be a result of damage to certain regions of the brain (e.g. the prefrontal cortex), and damage to brain circuits involved in moral judgments (Blair & Lee, 2013). Both, meta-analytic and narrative reviews showed evidence for smaller brain structures and lower brain activity in individuals with ODD/CD in mainly executive functional-related areas: bilateral amygdala, bilateral insula, right striatum, left medial/superior frontal gyrus, and left precuneus (Holz et al., 2017; Noordermeer, Luman, & Oosterlaan, 2016). Executive functioning, also seen as cold cognition, refers to goal-directed and problem-solving behaviours, as well as self-regulation, not involving motivational or affective aspects.

Furthermore, (neuro)endocrinological factors such as the hormones oxytocin, cortisol, and testosterone have also been implicated in aggressive behaviour. For instance, administration of intranasal oxytocin was found to decrease salivary cortisol concentrations during social stress (Linnen, Ellenbogen, Cardoso, & Joober, 2012), and dampen amygdala activity to negative stimuli or fear-inducing visual stimuli (Domes et al., 2007; Kirsch et al., 2005). Furthermore, lower salivary oxytocin concentrations predicted high teacher-rated CU traits (Levy et al., 2015). Effects of testosterone, cortisol and serotonin in the brain mostly implicate amygdala-prefrontal circuitry (Montoya, 2012).

For example, gonadal (sex steroid) hormones are thought to have an important influence on the connection of the amygdala and the orbitofrontal cortex, influencing regulation of amygdala activity by the orbitofrontal cortex (van Wingen et al., 2011). Testosterone has been observed to increase amygdala activity (Derntl et al., 2009; Manuck et al., 2010; van Wingen et al., 2009) and to reduce orbitofrontal cortex coupling with the amygdala (Hermans et al., 2008; van Wingen et al., 2010), which can increase aggression (Mehta and Beer, 2010). Cortisol on the other hand has been shown to decrease amygdala activity (Henckens et al., 2010) (see also van Donkelaar, PhD Thesis “Genetic and neurobiological mechanisms underlying aggression subtypes”, 2018).

In this thesis, I will, for the first time, study three hormones (i.e. oxytocin, cortisol, and testosterone) together in one sample consisting of individuals with either ODD/CD, or ASD, compared to TDI (chapter 4).

2) Cognitive and affective factors
Cognitive models (e.g. cognitive behaviour therapy) assume that deficits in information processing are underlie increased aggression (Fossum, Handegard, Martinussen, & Morch, 2008; Goldstein, Glick, Reiner, Zimmerman, & Coultry, 1987). Dysfunctional cognitive processes and misperceptions that may lead to aggressive behaviour are described in two models 1) the social learning model (SLM) of Bandura (1978), and 2) the social information processing (SIP) model of Dodge & Coie (1987).

According to the model of Bandura (1978), aggression is initially learnt by modelling (e.g. through
observation and interaction with other people) and maintained by reinforcement, which encourages the further display of aggressive behaviour for personal gain, receiving few or no negative consequences. Alongside the SLM model, the SIP model is a circular depiction of the emotional and cognitive processes (i.e. deficient encoding, interpretation, preparation, planning and execution of response or behaviour) may lead to reduced emotional empathy, impaired decision making, and increased threat sensitivity (Crick & Dodge, 1994). At the core of the model is a database of memories, acquired social rules, social knowledge and schemata. For example: What do you do if someone pushes you? According to the SIP model, it is thought that individuals with aggressive behaviour have abnormal cognitive scripts for how to behave in social situations due to modified information processing (Crick & Dodge, 1996). These scripts are learnt from parents, media, and experience. The SIP consists of six steps from the encoding of social cues to evaluating and selecting responses. The processing of social cues can be affected by dysfunctional beliefs, social schemas (Gagnon, McDuff, Daelman, & Fournier, 2015), or cognitive biases. In response to the question at the beginning of this section, one could think of reasons for the aggressive act (e.g. why did the person push you?), but individuals with aggressive behaviour have the tendency to perceive these ambiguous actions by others as aggressive. More specific, aggressive individuals have inner biases that make them expect others to react aggressively; view ambiguous acts in a hostile manner; assume others act purposefully when they hurt or offend them (known as the hostile interpretation bias). This bias is considered to be an important cause as well as maintaining factor of aggressive behaviour (Crick & Dodge, 1996; Schonenberg & Jusyte, 2014).

Within this thesis, I will for the first time set out to investigate and directly compare emotional valence detection capacities (chapter 5), and reading faces (chapter 6) within and across clinical samples (i.e. ODD, CD, and ASD) and the general population sample.

3) Behavioural factors
Several clusters of behaviour, such as subtypes (i.e. reactive or proactive) of aggression have been found in individuals with severe aggressive behaviour (Blair, Veroude, & Buitelaar, 2016). Reactive aggression is known as an emotionally charged response to provocations or frustration and is also known as “impulsive”, “hot blooded” or “affective” aggression. Proactive aggression is defined as a conscious and planned act, used for personal gain or egocentric motives, also known as “premeditated”, “instrumental” or “cold-blooded” aggression (Smeets et al., 2017). These behaviours may be a result of frustration-based reactive aggression or threat-based reactive aggression. Berkowitz (1989) proposed the frustration-aggression hypothesis, stating that frustration leads to anger, and the more anger is generated (by means of unexpected frustration or if seen as unfair) the more aggression is generated. However, since we can apply our higher mental processes, such as thinking and reasoning, we do not necessarily always respond to frustration with aggression. We may do so, however, if our anger is great enough or if, for some reason, we cannot think rationally at that moment. CU traits may play a moderating role, and are associated with reductions in specific forms of emotional empathy, in particular, responding to the fear, sadness, pain and happiness of others (Blair, 2013).

In this thesis, I will examine the role of subtypes of aggression in relation to hormonal
concentrations (chapter 4), emotion processing (chapter 5), and reading faces (chapter 6) within and across clinical samples (i.e. ODD, CD, and ASD) and general population sample.

4) Environmental/ cultural factors

Several environmental risk factors have found to increase the risk for antisocial behaviour. These include prenatal factors, traumatic experiences, dysfunctional family life, or the way the child is raised and disciplined in the family (e.g. harsh and inconsistent parenting), abuse and neglect, and socially disadvantaged subcultures (i.e. deviant peers) (Blair, Leibenluft, & Pine, 2014; Carr, 2006; Humphreys & Zeanah, 2015; Hyde et al., 2016; Waller, Gardner, & Hyde, 2013). In addition, research supports a role of environmental adversity in particular for CU traits, stating two distinct CU subgroups (i.e. primary and secondary), with the secondary variant being specifically characterized by a maltreatment history (and high anxiety), compared to the primary variant (Kimonis, Frick, Cauffman, Goldweber, & Skeem, 2012; Kimonis, Skeem, Cauffman, & Dmitrieva, 2011). Alongside environmental factors, a new model has been proposed recently, the climate aggression and self-control in humans (CLASH) model, has been proposed in understanding aggression within and between countries (Van Lange, Rinderu, & Bushman, 2016). This model includes fast life strategy, short-term orientation, and poor self-control (i.e. no control of temptations and lack of long-term goals) as important determinants of aggressive behaviour. Fast life strategy is associated with short-term planning, greater risk taking, a focus on immediate gratification for short-term benefits, and more aggression (e.g. Frankenhuis, Panchanathan, & Nettle, 2016). Also, violent behaviour in individuals could be based on wounded pride, e.g. people think they are better than other people. Another cultural aspect is the role of honour: violent response to threats to one's honour is accepted in some cultures but not others. But also humiliation is a primary cause of violence and aggression in cultures of honour. Aggressive ‘cues’ may trigger aggression (Berkowitz, 1989), e.g. guns, knives, the colour black. Another aspect is, although strongly debated, is that violent media exposure tends to be associated with increased aggression (Black & Bevan, 1992; Bushman, 2016). Aggressive people view the world as more hostile than do non-aggressive people, but situational factors, such as media, may also play a role in this view (Bushman, 2016). The influence of genetic factors in interaction with (early) social risk factors can be catalyzed by a hostile environment, thus increasing the risk for the development of aggressive behaviour (Mendes, Mari Jde, Singer, Barros, & Mello, 2009). Other factors within the family life and / or subculture may be the maternal (mis)use of tobacco (Brennan, Grekin, Mortensen, & Mednick, 2002; Orlebeke, Knol, & Verhulst, 1997; Ruisch, Dietrich, Glennon, Buitelaar, & Hoekstra, 2017), or alcohol during pregnancy (Hawkins, Catalano, & Miller, 1992; Mendes et al., 2009) and malnutrition (Liu, Raine, Venables, & Mednick, 2004; Mendes et al., 2009; Ruisch et al., 2017).

The identification of relevant risk factors for ODD/CD and ASD has been a great challenge given their aetiological complexity. This involves both numerous genetic and environmental risk factors, as well as the interaction between these factors. For example, apparent environmental influences often also have a genetic component (known as the nature of nurture). Clever designs such as twin and adoption-at-conception designs (e.g. using a sample of families with children conceived through in vitro fertilization) can help disentangle genetic and environmental influences (e.g. Harold et al., 2013).

In this thesis, I will examine some demographic factors in relation to aggressive behaviour within and across clinical samples (i.e. ODD, CD, and ASD) and general population sample (chapter 4-6).
An integrated perspective of models in aggression

The schematic interpretation as shown in Figure 1, summarizes and integrates the above described models including genetic and (neuro)endocrine level, neural dysfunction level, cognitive and affective level, and environmental influences in the search of a better understanding of aggressive behaviour.

Figure 1. Model of aggression: consisting of a genetic and (neuro)endocrine level, neural dysfunction level, cognitive and affective level, behavioural level, and environmental influences. Adjusted and updated from Blair et. al., (2016).

Study sample

My thesis will focus on some aspects from the above models of aggression, such as broadly defined genetic, (neuro)endocrine, (neuro)cognitive, and behavioural underpinnings. This will be studied in the “psychopathology and the lack of empathy” sample.

The CU2 project enrolled 132 adolescents (see also Figure 2) with ASD, ODD, or CD diagnosis, who were recruited through clinical institutes across the Netherlands, specialized in disruptive behaviour problems (Hoenderloo Group, Otto Gerhard Heldring Foundation, and Woodbrookers) or psychiatric problems (Karakter Child and Adolescent Psychiatry), and through information leaflets that were sent to families via the Dutch federation of Autism (Nederlandse Vereniging voor Autisme). In
addition, typically developing individuals (TDI) were recruited via leaflets from a general community sample via city councils in the same geographical regions as the clinical groups. All adolescents were males and aged between 12 and 19 years old (M = 15.4, SD = 1.9), recruited between February 2011 and March 2015). The majority of the adolescents were non-Caucasian ethnicity (81%) and the mean estimated total IQ was M = 99.3 (SD = 12.2). Participants, parents and teachers were asked to complete several questionnaires concerning demographic and behavioural (i.e. CU traits, subtypes of aggressive behaviour) details before or during the testing day. In addition, only the adolescents were asked to complete a neuropsychological assessment during one or two testing days.

**Measures**

Various measurements were employed: self- and parent- rated questionnaires, neurocognitive computer tasks, eye-tracking, and saliva collection. Semi-structured interviews were used verify diagnosis. Self- and parent-rated questionnaires have been used to assess factors such as aggression subtype, and CU traits. The neurocognitive computer tasks focus on emotion valence detection, and processing of neutral and emotional faces. Finally, saliva collection was used as non-invasive method to collect hormonal concentrations of oxytocin, cortisol, and testosterone. More details on the sample and measures are given in Chapter 4-6.
Thesis aim and outline

The overall aim of this thesis was to examine proactive and reactive subtypes of aggression, aggression with or without CU traits, and their genetic, (neuro)endocrine, (neuro)cognitive, and behavioural underpinnings as transdiagnostic markers in a study that compares male adolescents with clinical disorders ODD/CD or ASD and typically developing individuals (TDI). These transdiagnostic markers between the clinical groups and biological substrate of behaviour are important to clarify heterogeneity, comorbidity and inform cross-disorder interventions in the clinical practice. This thesis also aimed to provide an overview of the efficacy of psychological treatments.

In chapter 2, a meta-analysis was conducted to evaluate the efficacy of nonpharmacological treatments for CD problems in children and adolescents, based on child, parent and teacher reports; articles published between January 1970 and March 2015. Results from this chapter raise questions on how to improve current or develop new treatments. In order to do so, better understanding of possible biological mechanisms involved in aggressive behaviour is required, which will be further explored in chapter 3. In chapter 3, a systematic review is described to examine the evidence for genetic underpinnings of aggression and to determine to what degree prior studies have examined aggression phenotypes that fit neatly, or at all, into the RDoC framework. This review focuses on three types of genetic studies: twin studies, human association studies of aggression, and animal model studies published between January 2009 and February 2015.

Hormones have been shown to play an important role in influencing reactions to other people, including aggression, affiliation and the stress system. In chapter 4, the (neuro)endocrinological mechanisms (i.e. hormones such as oxytocin, cortisol, and testosterone) will be examined in the possible relation with aggressive behaviour and/or empathy in TDI, and individuals with either ASD, or ODD / CD. The role of executive functioning (reflecting the higher order cognitive control of thought, action and emotion) are differentially associated with forms of antisocial behaviour and patterns of aggressive behaviour (Blair, 2013; Ogilvie, Stewart, Chan, & Shum, 2011). In chapter 5, emotion processing is investigated during a neuropsychological task (i.e., emotional Go/No-go) in TDI compared to individuals with either ODD/CD or ASD. Furthermore, whether CU traits moderate results in the ODD/CD group as well as the ASD group will be explored. Recognition of facial emotional expressions is an important aspect of social communication. Eye-tracking is a rather precise method to measure this aspect in empathy. In chapter 6, this method will be used to examine common and unique emotional face processing deficits (i.e. the time to first fixation and fixation duration) in TDI, compared to individuals with either ODD/ CD or ASD. Furthermore, the relationship of CU traits, psychopathic traits and aggressive behaviour with eye-tracking measures will be examined. The final chapter of this thesis, chapter 7, provides a summary of the main findings of each chapter. Limitations, directions for future research, and clinical implications are discussed.
References


Practitioner Review: Psychological treatments for children and adolescents with conduct disorder problems – a systematic review and meta-analysis

Published as:

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Practitioner Review: Psychological treatments for children and adolescents with conduct disorder problems – a systematic review and meta-analysis

Abstract

This meta-analysis evaluates the efficacy of nonpharmacological treatments for conduct disorder (CD) problems in children and adolescents, based on child, parent and teacher report. PubMed, PsycINFO and EMBASE were searched for peer-reviewed articles published between January 1970 and March 2015. Main inclusion criteria were nonpharmacological treatment, participants younger than 18 years, clinical CD problems/diagnosis, randomized controlled trials and inclusion of at least one CD problem-related outcome. Treatment efficacy is expressed in effect sizes (ESs) calculated for each rater (parent, teacher, self and blinded observer).

Of 1,549 articles retrieved, 17 (published between June 2004 and January 2014) describing 19 interventions met the inclusion criteria. All studies used psychological treatments; only three studies included a blinded observer to rate CD problems. Most studies were of very poor to fair quality. ESs were significant but small for parent-reported outcomes (0.36, 95% CI = 0.27–0.47), teacher-reported outcomes (0.26, 95% CI = 0.12–0.49) and blinded observer outcomes (0.26, 95% CI = 0.06–0.47), and they were nonsignificant for self-reported outcomes (0.01, 95% CI = 0.25 to 0.23). Comorbidity, gender, age, number of sessions, duration, intervention type, setting, medication use or dropout percentage did not influence the effect of treatment.

Psychological treatments have a small effect in reducing parent-, teacher- and observer-rated CD problems in children and adolescents with clinical CD problems/diagnosis. There is not enough evidence to support one specific psychological treatment over another. Future studies should investigate the influence of participant characteristics (e.g. age of CD onset), use more homogeneous outcome measures and allow better evaluation of study quality. Many reports failed to provide detailed information to allow optimization of psychological treatment strategies.
To date, studies of nonpharmacological treatments for disruptive behaviour have not specifically focused on clinically diagnosed conduct disorder (CD) nor related problem behaviour. Furthermore, these studies have often suffered from small sample sizes. The aims of this article are to provide an overview of nonpharmacological treatments for children and adolescents with CD and/or CD problems in the clinical range [e.g. including disruptive behaviour disorders (DBD) and oppositional defiant disorder (ODD)], to outline clinical implications for clinical practitioners, and make suggestions for future research to optimize clinical outcomes.

Conduct disorder is characterized by a repetitive and persistent pattern of antisocial and rule-breaking behaviour, categorized into four classes: (a) aggression to people and animals, (b) destruction of property, (c) deceitfulness or theft and (d) serious violations of rules (American Psychiatric Association, 2013). Aggressive and antisocial behaviour is heterogeneous, and it has been suggested that a distinction should be made between covert and overt aggression and between reactive or affective aggression and instrumental or proactive aggression (Buitelaar et al., 2013). The DSM-5 defines CD with and without callous-unemotional traits, whereby patients with high callous-unemotional traits are more refractory to treatment and have a poorer longterm prognosis (e.g. increased risk of criminal activity; American Psychiatric Association, 2013; Frick & White, 2008; Loeber & Stouthamer-Loeber, 1998; Scheepers, Buitelaar, & Matthys, 2010). Furthermore, individuals presenting with early-onset CD (i.e. before 10 years of age) may have a relatively poorer outcome than those with late-onset CD (i.e. onset after 10 years of age; Fairchild, van Goozen, Calder, & Goodyer, 2013; Moffitt, 2006; Moffitt, Caspi, Dickson, Silva, & Stanton, 1996) and are at greater risk of developing antisocial personality disorder in later life. In many cases, CD is comorbid with attention deficit hyperactivity disorder (ADHD) and is preceded by ODD (Loeber, Burke, Lahey, Winters, & Zera, 2000). This makes it important to develop effective treatment programmes for conduct problems.

Treatment for CD problems can be divided into pharmacological and nonpharmacological approaches. There is no first-line medication licensed for this age group, and all medication is primarily used off-label. Medications approved for other indications (particularly ADHD), such as stimulants (e.g. methylphenidate), alpha-2 agonists (e.g. guanfacine) and atypical antipsychotics (e.g. risperidone, aripiprazole and quetiapine), are currently the medical treatments of choice but are secondary to psychosocial interventions (Linton, Barr, Honer, & Procyshyn, 2013; Smith & Coghill, 2010). Six meta-analyses of psychological treatments for children and adolescents with (but not limited to) a CD diagnosis and/or CD problems have been published (Grove, Evans, Pastor, & Mack, 2008; Lundahl, Risser, & Lovejoy, 2006; McCart, Priester, Davies, & Azen, 2006; National Institute for Health and Clinical Excellence, 2013; Weisz et al., 2013; Wilson & Lipsey, 2007), and the aim of this article is to investigate the effectiveness of nonpharmacological interventions other than psychological ones for the treatment of CD and/or clinical CD problems.

The meta-analysis of Wilson and Lipsey (2007) focused on the efficacy of treatment in a population-based sample (e.g. through school-based programmes), and the other meta-analyses focused on prevention programmes for children and adolescents with disruptive and delinquent behaviour but without a manifest disorder (Grove et al., 2008), programmes for children and adolescents with internalizing (e.g. anxiety) and externalizing (e.g. misconduct, ADHD) problems
(Weisz et al., 2013), or specific treatment methods, such as parent training (Lundahl et al., 2006), or behavioural parental training and cognitive behavioural therapy (McCart et al., 2006). The National Institute for Health and Clinical Excellence (NICE) guidelines, published in 2013, provide a systematic review and meta-analysis of psychological treatments and conclude that psychosocial interventions have an overall small-to-moderate effect size (ES) compared with care as usual or no treatment in reducing antisocial behaviour in youths. The other five older metaanalyses showed that psychosocial treatment was modestly beneficial (overall ESs ranged from 0.17 to 0.42, including beyond the end) compared with care as usual or waiting-list control in children and adolescents with DBD/CD problems.

The present review included 17 studies (11 of which were not included in the NICE review). Although the NICE review included 154 studies, the current review extends the NICE guidelines in several ways. First, the NICE guidelines do not focus solely on clinical cases of CD/ODD but also on population-based samples and use broad definitions of psychopathy (e.g. the meta-analysis included samples in which only a minority had CD/ODD and/or that were not clinically at risk for CD/ODD). The inclusion of a mixed study population may affect the evaluated efficacy of psychological treatment, complicate the interpretation of findings and limit the generalizability of study outcomes. The current review focuses on nonpharmacological treatments in children and adolescents who have a clinical CD diagnosis and/or clinical CD problems (including DBD and ODD) and who have an IQ of minimally 80 points, compared with the minimally 60 points used as cut-off in the studies of the NICE guideline. Children and adolescents with an IQ lower than 80 have mild intellectual problems and may benefit from a different treatment approach from that for individuals with a higher IQ. The exclusion of children and adolescents with a lower IQ also increases the homogeneity of our study population. Third, the current review includes studies of nonpharmacological treatments involving children and adolescents and incorporates data of adolescents between 12 and 18 in contrast to the NICE guidelines in which the overall age range was between 7 and 14 years. Fourth, as recommended by Rosato et al. (2012), psychological treatments should be age specific, because developmental differences (i.e. in cognitive, behavioural, affective and communicative abilities) affect outcomes. Hence, the current review investigates the onset of CD as potential moderator of treatment effectiveness in reducing CD problems, an aspect that has not been addressed previously. Fifth, it is not always clear whether outcome measures are scored by blinded raters (McCart et al., 2006). Last, but not least, in contrast to the six metaanalyses, this review takes into account whether study participants are on stable medication during the study. In addition, not all previous metaanalyses took into account whether child and/or environmental factors in treatment efficacy into account.

Although evidence for the efficacy of psychological treatments for CD problems is limited, these remain the treatment of choice (Conduct Problems Prevention Research Group, 1999, 2011; Rosato et al., 2012). The NICE guidelines recommend a psychosocial approach to the treatment of CD problems, with emphasis on working with parents (if possible) or guardians. Both the NICE guideline (2013) and Eyberg, Nelson, and Boggs (2008) advocate three treatment approaches, depending on the age of the affected individual. For children aged 3–11 years, parenting training programmes to improve the parenting skills of parents are recommended. These programmes typically consist of 10–12 parents in a group and involve 10–16 meetings (each lasting 90–120 min) in which modelling
(e.g. imitating the child), rehearsal (e.g. parents rehearse new skills in session) and feedback (e.g. therapists discuss and, if necessary, modify parents’ behaviours; Pilling, Gould, Whittington, Taylor, & Scott, 2013) are used to improve parenting skills. For children aged 9–14 years, cognitive behavioural approaches are the treatment of choice and consist of 10–18 weekly meetings (lasting 2 hr) during which modelling (e.g. imitating peers), rehearsal (e.g. the child rehearses new skills) and feedback (e.g. therapists discuss the child’s behaviour, with a view to modifying it; Pilling et al., 2013) are used to improve the child’s skills. And for adolescents aged 11–17 years, multimodal programmes (e.g. multisystemic therapy) are preferred, consisting of 3–4 meetings a week for 3–5 months. These programmes are based on a social learning model with interventions provided at different levels (i.e. individual, family, school, criminal justice and community) and have an explicit and supportive focus on the family. They are provided by appropriately trained case managers (Pilling et al., 2013).

An up-to-date comparison of nonpharmacological treatments targeting specifically CD or related problems is needed. This systematic review and metaanalysis of nonpharmacological treatments for CD, specifically on outcomes related to CD problems, provides strategies for improvement.

Methods

Inclusion and exclusion criteria
A search of the PubMed, EMBASE and PsycINFO databases for peer-reviewed papers published up to March 2015, using the following keywords: (((conduct disorder OR CD OR disruptive behavio* AND disorder) OR (disruptive behavio* AND disorders)) NOT (disease)), identified 1,549 articles. The titles and abstracts of the retrieved articles were read by at least two of the authors (MJB and JKB), and reference lists and relevant published reviews (i.e. Von Sydow, Retzlaff, Beher, Haun, & Schweitzer, 2013; Woolfenden, Williams, & Peat, 2001) were hand-searched to identify additional related publications. Books and unpublished articles were not included. Authors were contacted to gather further information about details not reported in the selected papers. Then, the following inclusion criteria were applied: (a) participants younger than 18 years with CD and/or ODD diagnosis, or scores on a dimensional construct of CD problems in the clinical range; (b) at least one quantitative measure of CD problem outcome reported (e.g. rating scale or observation scale pre- and/or post-measurements, follow-up); (c) study published in a peer-reviewed journal; and (d) randomized controlled trial of nonpharmacological intervention versus control (placebo, waiting list, no treatment or treatment as usual). Comorbidity and use of medications were not exclusion criteria; articles not written in English, case reports and review articles were excluded. In each article, all outcome measures relevant to CD problems were selected.

Data extraction and statistics
In total, 1,549 articles were retrieved and an additional 42 were identified from reference lists. Ultimately, 17 articles describing 19 interventions met the inclusion and exclusion criteria (Figure 1). These articles were published between June 2004 and January 2014. The following information
was extracted: age range or mean if available, clinical diagnosis and/or diagnostic criteria used, sample size, gender distribution, treatment name, treatment duration, setting (e.g. outpatient, clinic), outcomes (in terms of CD-related problems), medication use, rater (e.g. parental report, teacher report and self-report) and rater blinding to treatment allocation. All study characteristics and participant details of the included studies are summarized in Table 1. Study quality was assessed by two authors (MJB and JG), using the standard definition for randomization; missing data were accounted for as described by Jadad et al. (1996), with the following scores for randomized controlled trial quality: 5 = excellent, 4 = good, 3 = fair, 2 = poor, 1 = very poor (Crowther, Lim, & Crowther, 2010; Jadad et al., 1996). Two points were awarded for (appropriate) randomization, two points for (appropriate) blinding and one point for reporting the fate of all patients (including dropouts).

Treatment efficacy is expressed in terms of the ES. Per study, for each instrument and rater, the ES was calculated by using the unbiased estimate of ES (dppc') developed by Morris (2008).
Within brackets, the Mpost/pre, T and Mpost/pre, C refer to the mean CD problem score, for the post- and pre-test for treatment and control group, respectively. SDpre is referred to the standard deviation of the pretest and Cp is the sample size bias correction, based on the n in the treatment group (Nt) and the n in the control group (Nc; Morris, 2008).

\[ \text{Cp} = \frac{1}{4(Nt + Nc - 2)} \]

Cp is defined as the standardized difference between preand post-treatment and was subtracted from the standardized mean difference (SMD) of the control condition. Because we were interested in a sustained effect of the interventions, we included (where possible) the follow-up outcomes when calculating ESs. By using the SMD, we could take into account the use of different instruments to monitor CD-related problem behaviours. The SMD and 95% confidence interval were calculated using Review Manager 5.3 (‘Review Manager (RevMan)’, 2014). A SMD higher than zero indicates that the active treatment is better than the control condition in reducing problem behaviour in children and adolescents diagnosed with CD. An overall ES (indicated as ES-all) was calculated per rater if multiple outcome measures were used in a study. In addition, as an exploratory step, the data obtained with the lowest and the highest scoring instruments were calculated per rater (indicated as ES-low and ES-high). One study (McDonald, Dodson, Rosenfield, & Jouriles, 2011) did not provide data to enable us to calculate the ES as described above, and so we used an online calculator to calculate an ES (Thalheimer & Cook, 2002; see Table 1). In accordance with the literature, an ES of 0.2 was considered to be small, 0.5 to be moderate and ≥ 0.8 to be large (Thalheimer & Cook, 2002). Heterogeneity was calculated using chi-square (\( \chi^2 \)) and I-squared (\( I^2 \)) tests. If heterogeneity was present, metaregression analyses were performed with possible moderators, such as participant or treatment characteristics, using Bonferroni correction for multiple testing; the adjusted pvalue was .003.

Results

The 17 studies recruited 1,999 participants (of which 73.4% boys), with a mean age of 7.5 years (range 2.8–16.8 years). Nine studies included participants (n = 782) diagnosed according to DSM-IV or DSM-III with CD and/or ODD; the remaining eight studies included participants at risk of conduct problems or with externalizing problems in the clinical range. ADHD was the most common comorbidity (n = 6); eight studies did not report comorbidity data. The 17 studies examined the effect
of 19 different psychological interventions with a median duration of 14 hr for children/adolescents and 21.8 hr for parents. Only half of the studies provided information about the duration of teacher involvement. More participants were allocated to the intervention arm than to the control arm (1,225 and 774, respectively); overall, the median dropout was 17.6%. Interventions were given in a clinical setting (52.6%), home, school or a combination of locations. Control conditions were treatment as usual (n = 1) or a waiting-list control group (n = 9). Ten studies focused on group interventions and seven on individual interventions; four studies used a combination. For nine interventions, pre- and post-treatment outcomes were recorded (x = 16 weeks), and for 10 interventions, pretreatment and follow-up outcomes were recorded (x = 1.2 years). Details on intervention duration are provided in Table 1. Two interventions did not have a set duration – Eyberg, Boggs, and Jaccard (2014) continued treatment until the parent’s skills reached a preset level, and Sundell et al. (2008) did not regulate contact with therapists, who were available 24/7. Two interventions made use of audiovisual material (Jones et al., 2014; Scott & O’Connor, 2012). Most interventions (n = 17) made use of parent-reported information; seven interventions made use of teacher-reported information, and 10 interventions made use of self-reported information [Child Behaviour Check List (CBCL) – Externalizing subscale, n = 5; Achenbach, 1991a; Eyberg Child Behaviour Inventory (ECBI) – Intensity and Problem Behaviour subscales, n = 8, Robinson, Eyberg, & Ross, 1980]. Two studies collected parent-reported information on specific aggression-related outcomes (e.g. callous-unemotional traits; McDonald et al., 2011; Somech & Elizur, 2012) and one study collected similar teacher-reported information (Kolko, Lindhiem, Hart, & Bukstein, 2013). None of the parent-scored instruments assessed subtypes of aggression, such as reactive and proactive aggression; only one teacher-scored instrument did (van Manen, Prins, & Emmelkamp, 2004). The most widely used instrument to score teacher-rated aggression was the Teacher Report Form – Externalizing subscale (n = 2; Achenbach, 1991b). A wide range of instruments was used for child-rated CD problem outcomes (n = 5). Only two studies included blinded observer-rated outcomes (Jouriles et al., 2009; Perrin, Sheldrick, McMenamy, Henson, & Carter, 2014). Four of the 19 interventions allowed participants to use medication: in three, ADHD medication (not further specified) was used, and in one, no details were provided (see also Table 1). Information about whether medication use was stable was not provided in these studies. On the basis of the Jadad scale score, most studies were of ‘very poor’ or ‘poor’ quality (n = 12); five studies were of ‘fair’ quality (Table 1).

Effectiveness of psychosocial treatment – parent report

Seventeen interventions made use of parent-reported information (Figure 2). Thirteen interventions were focused on parent management skills (e.g. on proactive and nurturing parenting, effective limit setting and handling misbehaviour) and psycho-education (Bagner, Sheinkopf, Vohr, & Lester, 2010; Boylan, Macpherson, & Fristad, 2013; Drugli & Larsson, 2006; Eyberg et al., 2014; Hanisch et al., 2010; Jones et al., 2014; Jouriles et al., 2009; Kolko, Campo, Kelleher, & Cheng, 2010; Kolko et al., 2013; McDonald et al., 2011; Perrin et al., 2014; Somech & Elizur, 2012; Sundell et al., 2008). Some interventions focused on additional aspects such as mandated participation of the father (Somech & Elizur, 2012), stress of the parent (Boylan et al., 2013; Eyberg et al., 2014), and instrumental and emotional support to mothers (Jouriles et al., 2009; McDonald et al., 2011). On the basis of
posttreatment/follow-up data, the weighted mean ES-all was 0.37 (95% CI = 0.27–0.47; random effect models), indicating that psychosocial treatment had a small but significant effect in reducing CD problems in children/adolescents with clinical CD problems and/or a CD diagnosis. Because the 95% confidence interval did not contain zero, the null hypothesis that \( \mu_{dppc} = 0 \) was rejected at the 0.05 level. In addition, the weighted mean ES based on the lowest parent-reported score in each study was 0.30 (95% CI = 0.20–0.40; random effect models) and that based on the highest parent-reported score was 0.42 (95% CI = 0.33–0.52; random effect models). Heterogeneity was calculated in RevMan 5.3 (Review Manager (RevMan), 2014), using the I-squared and chi-square test for the lowest and highest ES parent-reported scores in each study. If the mean scores of different samples differ, then the samples may originate from different populations (heterogeneity). Heterogeneity ranged from \( I^2 = 62\% \) (\( v^2 = 40, p = .0005 \)) to \( I^2 = 63\% \) (\( v^2 = 40.13, p = .0004 \)), which shows inconsistency of study results (Higgins, Thompson, Deeks, & Altman, 2003). This necessitated the use of a random effect model, which corrects for heterogeneity, to test for differences in outcomes in the meta-analysis (Borenstein, Hedges, Higgins, & Rothstein, 2010; Ried, 2006). As seen in Table 1, the ES reported in the study by Bagner et al. (2010) deviated substantially from that reported in other studies. Therefore, this study was excluded from the sensitivity analysis, which showed that specific participant and intervention characteristics did not affect treatment efficacy.

### Effectiveness of psychosocial treatment – teacher report

Seven interventions made use of teacher-reported information (Figure 3). Teachers were not always directly involved in an intervention other than filling out some questionnaires. Two parent-focused programmes also included teacher report alongside parent report (Hanisch et al., 2010; Kolko et al., 2010). The follow-up study by Kolko et al. (2013) and the study by van Manen et al. (2004) included teacher-reported information on the effect of a cognitive behavioural intervention. One study involved a multimodal programme (including the child, parents and teachers; Owens, Murphy, Richerson, Girio, & Himawan, 2008), and another involved two types of interventions, namely, a multimodal programme and a separate parent-focused programme, and included teacher-reported ratings (Drugli & Larsson, 2006). On the basis of available teacher-reported posttreatment/follow-up outcomes, the weighted mean ES-all was 0.26 (95% CI = 0.12–0.49; random effect models), indicating a small but significant effect of the intervention in reducing CD problems in children/adolescents with clinical CD problems and/or a CD diagnosis. In addition, the weighted mean ES-low was 0.18 (95% CI = 0.00–0.36; random effect models) and ES-high was 0.27 (95% CI = 0.08–0.46; random effect models). Heterogeneity ranged from \( I^2 = 32\% \) (\( v^2 = 10.3, p = .05 \)) to \( I^2 = 39\% \) (\( v^2 = 11.5, p < .006 \)), which shows inconsistency of study results. Therefore, the random effect model was used.

### Effectiveness of psychosocial treatment – self-report

Two interventions made use of self-reported information (Figure 4). These were multimodal programmes involving children and parents (Sundell et al., 2008) and/or family, school and courts (Hendriks, van der Schee, & Blanken, 2012). In these cases, the focus was on the training of specific skills by means of cognitive behavioural therapy (Boylan et al., 2013; Drugli & Larsson, 2006; Kolko...
Table 1: Study and Participant Characteristics of Included Randomized Controlled Trials.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Intervention</th>
<th>Reported Design Quality</th>
<th>Effect Size Based on Last Time Range</th>
<th>Averaged Effect size Regardless of Rater</th>
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<tr>
<td>Bagner et al., 2010</td>
<td>2-5 EP (n.r.)</td>
<td>WL 14 (14)</td>
<td>Overall</td>
<td>Parent-Child Interaction Therapy</td>
<td>Clinic 3</td>
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<td>Perrin et al., 2014</td>
<td>2-4 DBI (n.r.)</td>
<td>WL IYP 89</td>
<td>17</td>
<td>Incredible Years Program-Parent Training (IYP) &amp; Not Randomized IYP (NR-IYP)</td>
<td>Clinic 3</td>
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<td>Somech &amp; Elizur, 2012</td>
<td>3-5 DBS (n.r.)</td>
<td>AC 140 (69)</td>
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<td>Hitkashrut – Parent Training</td>
<td>Control 2 (n.r.)</td>
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<td>Hanisch et al., 2010</td>
<td>3-6 ODD (ADHD)</td>
<td>TAU 91 (64)</td>
<td>74</td>
<td>Prevention Program for Externalizing Problem Behaviour</td>
<td>Kindergarten 2</td>
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<td>Eyberg et al., 2014</td>
<td>3-6 ODD (ADHD)</td>
<td>TAU 31 (30)</td>
<td>14 (13)</td>
<td>Overall Parent-Child Interaction Therapy + Maintenance Treatment (PCIT-MT)</td>
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<td>Jones et al., 2014</td>
<td>3-8 DB* (n.r.)</td>
<td>AC 7 (8)</td>
<td>Overall</td>
<td>Helping the Non-compliant Child (HNC) + Technology Enhanced (TE)</td>
<td>Clinic 2</td>
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<td>3-min video’s (number n.r.), mid-week video-calls (n.r), 8-12 videotaped home practice (n.r.)</td>
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<td></td>
</tr>
<tr>
<td>Study</td>
<td>Sample</td>
<td>Treatment</td>
<td>Length</td>
<td>Follow-Up</td>
<td>Outcomes</td>
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<tr>
<td>(Scott &amp; O'Connor, 2012)</td>
<td>4-6</td>
<td>CP and/or ODD (n.r.)</td>
<td>ED 36 (32)</td>
<td>n.r.</td>
<td>ED 75 (73)</td>
</tr>
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<td>(Drugli &amp; Larsson, 2006)</td>
<td>4-8</td>
<td>ODD / CD (ADHD)</td>
<td>PT 47 (28)</td>
<td>23</td>
<td>80 (n.r.)</td>
</tr>
<tr>
<td>Jouriles et al., 2009</td>
<td>4-9</td>
<td>ODD / CD (n.r.)</td>
<td>TAU 32 (34)</td>
<td>5 (5)</td>
<td>58 (41)</td>
</tr>
<tr>
<td>(McDonald et al., 2011)</td>
<td>4-9</td>
<td>CD / ODD (n.r.)</td>
<td>TAU 32 (34)</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td>Owens, Murphy, Richerson, Girio, &amp; Himawan, 2008</td>
<td>6-10</td>
<td>CP / ODD (ADHD)</td>
<td>WL 91 (26)</td>
<td>n.r.</td>
<td>78 (73)</td>
</tr>
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<td>(Kolko et al., 2010)</td>
<td>6-11</td>
<td>CP and/or ODD (ADHD)</td>
<td>AC 83 (80)</td>
<td>9 (14)</td>
<td>65 (65)</td>
</tr>
<tr>
<td>Boylan et al., 2013</td>
<td>8-11</td>
<td>ODD/CD (Mood disorders)</td>
<td>WL+ TAU 78 (87)</td>
<td>17 (26)</td>
<td>76 (71)</td>
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</table>

<sup>a</sup> Additional interventions or modifications.
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Setting</th>
<th>N Sessions (Duration)</th>
<th>Time to Follow-up</th>
<th>Design</th>
<th>Quality</th>
<th>Effect Size Based Averaged on Last Time</th>
<th>Male</th>
<th>Effect Size</th>
<th>Interval</th>
<th>Regard</th>
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<tr>
<td>van Maren et al., 2004</td>
<td>CD / ODD (Clinic Setting)</td>
<td>9-13</td>
<td>CD</td>
<td>SC11 (70 min)</td>
<td>Weekly by phone (n.r);</td>
<td>Clinic 2</td>
<td>SC</td>
<td>SS</td>
<td>SS</td>
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<td>CBCL-O/A/E</td>
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<td>(Kolko et al., 2013)</td>
<td>CD / ODD (ADHD)</td>
<td>10-14</td>
<td>CD</td>
<td>SC11 (70 min)</td>
<td>86</td>
<td>Booster Treatment at 11 weeks</td>
<td>Maximum of 1 year</td>
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<td>(Saud et al., 2008)</td>
<td>CD / ODD (Control)</td>
<td>12-17</td>
<td>CD</td>
<td>SC11 (70 min)</td>
<td>24</td>
<td>Booster Treatment at 11 weeks</td>
<td>Maximum of 1 year</td>
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<td>(Hardies et al., 2009)</td>
<td>CD / ODD (Dependence)</td>
<td>13-18</td>
<td>CD</td>
<td>SC11 (70 min)</td>
<td>8</td>
<td>Booster Treatment at 11 weeks</td>
<td>Maximum of 1 year</td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations participants: CD = Conduct Disorder; ODD = Oppositional Defiant Disorder; ADHD = Attention Deficit Hyperactivity Disorder; NR = Not reported; M = Male; F = Female. Abbreviations comparison group: AC = Active Control; TAU = Treatment As Usual; WL = Wait List. Abbreviations N treatment (control): AC = Active Control; TAU = Treatment As Usual; WL = Wait List. Abbreviations outcome measures: CBCL-O/A/E = Child Behaviour Checklist-Overall/Aggression/Externalizing; TRF-E = Teacher Report Form-Externalizing; VPC = Violent/Property Crimes past 90 days. Abbreviations study population: CD prob = Conduct Disorder probability; E = Externalizing; CP = Conduct Problems. Abbreviations study design: SC = Single Case; SS = Social Skills; WL = Wait List. Abbreviations quality: C = Clinical; R = Randomized; D = Double Blind. Abbreviations design quality: Jadad ratings: 5 = excellent, 4 = good, 3 = fair, 2 = poor, 1 = very poor.
et al., 2010, 2013) or on improving motivation (Hendriks et al., 2012; van Manen et al., 2004). On the basis of self-reported posttreatment/follow-up outcomes, the weighted mean ES-all was 0.01 (95% CI = 0.25 to 0.23; random effect models), indicating that neither intervention reduced CD problems. Only Sundell et al. (2008) included multiple self-report information, which yielded ESSs ranging from $d = 0.12$ (95% CI = 0.44 to 0.19; random effect models) to $d = 0.01$ (95% CI = 0.22 to 0.41; random effect models).

**Figure 2.** Forest plot conduct disorder problems, comparison of effect sizes per study based on parent report.
The inclusion of multiple reports resulted in a weighted mean ES-low of 0.00 (95% CI = 0.02 to 0.21; random effect models) and ES-high of 0.10 (95% CI = 0.11 to 0.31; random effect models). Heterogeneity was $I^2 = 0\%$ ($v^2 = 1.72, p = .63$), which shows consistency of study results. Nevertheless, the random effect model was used to correct for the different outcome measures used in the metaanalysis.

**Effectiveness of psychosocial treatment – observer report**

Three interventions also made use of blinded observers (not further specified) to score the children/adolescents’ CD problems (Jouriles et al., 2009; Perrin et al., 2014) (Figure 5). The weighted mean ES-all was 0.26 (95% CI = 0.06–0.47; random effect models), indicating a moderate but significant effect in reducing CD problems. As both studies reported only one observer-rated outcome, it was not appropriate to calculate the weighted mean ES-low and ES-high. In these two studies, the mean weighted ES-all based on parent-reported information was higher than that based on observer-reported information ($d = 0.41$, 95% CI = 0.21–0.62).
Moderators of treatment effect

In order to explain the heterogeneity in outcomes, we investigated whether ESs (per ES level and per rater) were moderated by specific participant and intervention characteristics. However, analyses revealed no effect of comorbidity, gender, age, type of control (i.e. waiting-list control, treatment as usual or active control group), number of sessions, duration (hours), intervention type (i.e. parent-focused, child-focused or multimodal programmes), group size (i.e. group, individual or combination), setting (i.e. school, clinic, home or combination) or dropout percentage (all p-values were at least >.07). Individual studies, in particular of children aged 10 years or older, did not report whether their participants had early- or late-onset CD, with the exception of Kolko et al. (2010, 2013). However, we found a trend towards smaller ESs in studies involving children aged 10 years or older compared with those of studies involving children younger than 10 years (i.e. average treatment efficacy based on all parentreported information was 0.21 and 0.52, respectively). A similar trend was seen with teacherreported information (average ES = 0.32 in children aged <10 years and average ES = 0.11 in children aged >10 years). It was not possible to compare treatment efficacy in the two age groups based on self-reported and observer-reported information because of the limited data available. Treatment efficacy appeared not to be influenced by whether CD had been formally diagnosed or by early- versus late-onset CD. Moreover, timing of assessment (posttreatment or follow-up) did not influence results: ES ranged from 0.06 to 1.46 for interventions with posttreatment assessment (n = 9) and from 0.01 to 0.76 for interventions with a follow-up assessment (n = 10). This was the case for

*Figure 4.* Forest plot CD problems, comparison of effect sizes per study based on self-report.

**Abbreviations studies:** NR Not Randomized to Treatment Group
- [Name study]. High Weighted effect size based on single and highest aggression outcome.
- [Name study]. Low Weighted effect size based on single and lowest aggression outcome
- [Name study]. All Weighted effect size based on multiple aggression outcomes.
parent-, teacher-, self- and observer-rated outcomes. As most studies (n = 15) did not provide details about the medication used, it was not possible to perform sensitivity analyses for medication use. Lastly, trials with high Jadad ratings did not necessarily yield large ESs, although the statistical power to identify such effects was relatively low, as reported in another study (Field & Gillett, 2010).

Discussion

We carried out this systematic review and meta-analysis to investigate the effect of nonpharmacological interventions for CD problems and to provide recommendations for future research, with a view to optimizing clinical outcomes in children/adolescents with CD. Although we specifically searched databases for nonpharmacological treatments other than psychological treatments, all identified studies used psychological treatments. Overall, we found that CD is more common among males than females and that based on information provided by teachers, parents and blinded observers, psychosocial treatments have a small but significant effect in reducing CD problems in children/adolescents. However, based on the children/adolescents’ ratings, these interventions were not effective. Within the same rater and between raters, different instruments evaluating the same construct of CD problems yielded widely different ESs. Treatment effects remained significant when we focused on the instrument with the lowest ES, to account for this variability. While our findings support the use of psychological treatments for CD, there is a lack of evidence about what the best treatment is. While gender, age, number of sessions, duration, intervention type, setting, medication use or dropout percentage did not influence treatment efficacy, our findings suggested

Figure 5. Forest plot CD problems, comparison of effect sizes per study based on blinded rater report.
that treatment was less effective in older children (>10 years) than in younger children (<10 years), based on parent-rated outcomes. It is possible that the diminished efficacy of treatment in older children is because treatment is often delayed in children with early-onset CD and/or CD problems. An alternative explanation is that different interventions are used at different ages. For example, systematic treatment of children older than 10 years might be more effective if accompanied by parent training.

Our findings regarding the efficacy of psychological treatment for CD-related problems are partly in line with the current literature, in which small-to-moderate effects have been reported (Excellence, 2013; Grove et al., 2008; Lundahl et al., 2006; McCart et al., 2006; Weisz et al., 2013; Wilson & Lipsey, 2007). The studies included in this analysis made use of different raters (teacher, parent, children and blinded observers) and different assessment instruments. We noted that parents tended to consider treatment more effective than did teachers or blinded observers. This might be because, unlike blinded observers, parents expect treatment to be effective (Sonuga-Barke et al., 2013). That teachers considered treatment to be less effective than parents did, might be because children are slower to implement acquired skills in the classroom than at home or because peer-pressure at school prevents them from doing so. Therefore, psychological treatment should not only impart skills but also take into account the school environment, so that children are encouraged to use these skills (Berryhill & Prinz, 2003). Interventions may be more effective if they also target teachers (e.g. helping teachers adapt their behaviour so that negative interactions with children are reduced) or peers.

In contrast, on the basis of self-report information provided by the children/adolescents, we did not find psychological interventions to have a significant effect. This might be because children/adolescents are usually less inclined to report on their externalizing behaviour (Smith, Pelham, Gnagy, Molina, & Evans, 2000). The self-report measures of CD problems used in the two studies that included self-reported data (Hendriks et al., 2012; Sundell et al., 2008) have been shown to have a high internal consistency and re-test validity. The review by Grove et al. (2008) suggested that children who have completed psychological treatment are better at avoiding being caught by parents, teachers and schools than before participation. Therefore, children might continue to exhibit antisocial behaviour at the same rate and intensity as before treatment but they are not caught doing so; however, they continue to report exhibiting this behaviour.

Conduct disorder behaviour may be exhibited in different ways (e.g. covert vs. overt aggression) and in different contexts (e.g. home vs. school). In the studies included in this review, CD problem outcomes were mainly scored with the CBCL and the ECBI questionnaires. In the CBCL, information about misconduct behaviour (both aggression and delinquency) is represented in the Externalizing scale, and in the ECBI, the Intensity scale represents misconduct behaviour. Unfortunately, the two scales are not comparable at the level of individual items. Furthermore, the ECBI lacks information about how children or adolescents act at home and at school, whereas the CBCL lacks information on the frequency of misconduct behaviour. Thus, while the two questionnaires are complementary in observing and rating CD problem behaviour, they may miss important information about function (e.g. covert vs. overt; Olson et al., 2013) and subtypes of aggression (e.g. proactive vs. reactive; Raine et al., 2006). The predominant subtype of aggression may influence the focus of treatment.
focus, which in turn may lead to better outcomes. For example, in the case of predominant reactive aggression, treatment could be focused on improving cognitive control and improving strategies for controlling negative emotions of distress, frustration and anger. In the case of predominant proactive aggression, the focus of treatment could be on improving sensitivity to moral issues and moral reasoning and on increasing emotional empathy (Blair, 2013).

Although we did not find the dropout rate to influence treatment efficacy, the dropout varied considerably in the different studies. Unfortunately, the studies did not provide information about the characteristics of the dropouts, so we could not analyse whether dropout is related to the subtype of aggression. Interventions for young children (aged < 10 years) have the benefit that the involvement of parents and/or guardians means that they can make sure the child attends treatment sessions. This is in contrast to interventions for older children (> 10 years), where treatment compliance is less likely to be under parent and/or guardian control. For this reason, the dropout rate might be higher among older children.

We did not find treatment duration to influence treatment efficacy. Thus, while one could infer that shorter treatments are as effective as longer treatments, this would need to be confirmed in a cost–benefit analysis that includes the actual cost of treatment over time and the individual’s involvement in different systems.

Limitations
This review overcame some of the shortcomings of previous studies by including randomized controlled trials with a specific target group and incorporating sensitivity analyses to take possible moderators (e.g. study quality) into account. However, we could not control for other potential modifying factors because of a lack of information or a small number of studies, such as those involving an active control intervention (e.g. treatment as usual). For instance, we were unable to control for the following aspects: (a) potential effects of psychiatric medication used by participants, (b) potential gender effects primarily due to the small sample sizes of the CD cohorts in general and of females in particular, (c) blinded versus unblinded raters, because only two primary studies used blinded observer report, (d) the influence of callous-unemotional traits and/or subtypes of CD problems (e.g. covert), and (e) onset of CD (i.e. early or late onset), which was reported in only one individual study. Surprisingly, nearly half of the included studies did not report data on comorbidity, not even on ADHD, which is the most common comorbidity in cases with CD.

Another limitation is that our standardized quality assessment (Jadad scale) reflects the information provided in individual study reports and may not fairly represent the trials themselves (Jadad et al., 1996). For example, there is some debate about the use of the Jadad scale, which includes double blinding, because the design of many psychological trials makes it difficult to blind patients or use blinded raters.

Recommendations for future research
The small ES of psychological interventions may lead to two conclusions that are not necessarily opposing, namely that CD problems are persistent and rather refractory to treatment and that psychological interventions for CD problems could be improved, to make them more effective. Future
studies should address a number of shortcomings identified in our review. First, we found evidence for rater effects so that future studies should integrate information from multiple informants (e.g. parents, teachers and blinded observers) and assess CD problems in more than one environment (e.g. home and school situations). Second, we need to understand whether treatments are more effective in certain subgroups, classified by the time of onset, the presence and severity of callous-unemotional symptoms and the subtype of aggression. Third, future randomized controlled trials should be more precise in reporting their methods of randomization and blinding, the fate of all patients (including dropouts) within the trial and the medication used by participants, all of which might influence the effect of treatment on CD outcomes. Fourth, more randomized controlled trials with large samples are needed because there have been relatively few such studies, which limits the generalizability of findings and makes it difficult to evaluate possible moderators and mechanisms of change. Larger, possibly multisite, studies are needed to optimize psychological treatment efficacy and maintenance. Fifth, as the nonpharmacological interventions in the current meta-analysis were behavioural/psychosocial treatments, there is a need to investigate the effectiveness of other psychological interventions, such as dietary interventions and cognitive training. One study suggested that food intolerance (based on primarily ADHD and/or CD) and deficient intake of either micronutrients (e.g. vitamins) or fatty acids (e.g. omega-3) may predispose imprisoned delinquents to aggressive behaviour (Benton, 2007). Finally, while we found that intervention duration (in hours) did not moderate study outcomes, few studies explicitly investigated this and future studies should establish whether there is an optimal treatment duration. To the best of our knowledge, guidelines are not consistent about the duration of treatment, and so we would suggest a standardized treatment duration of 3 months, in order to allow interstudy comparison of treatment efficacy.

Implications for healthcare policy and clinical practice

This review suggests that psychological treatments have modest efficacy in reducing problems in children/adolescents with diagnosed CD or with clinical CD problems and that treatments appear to be more effective in younger children (<10 years) than in older children (>10 years). The limited evidence based on parent-reported information suggests that, regardless of group- or individual-focused sessions, the following treatment programmes may be especially effective in reducing CD problems: (a) the parent–child-based intervention: ‘Parent–Child Interaction Therapy’, and the multicomponent intervention ‘Incredible Years Program plus Child Literacy Program’ [in an emotionally dysregulated (e.g. angry and resentful) subgroup as opposed to a headstrong (e.g. arguing with adults) subgroup]; and (b) two specific parent-focused interventions: ‘Parent Training Hitkashrut’ and ‘Project Support’. In addition, teacher-reported information suggested that a multicomponent group-focused treatment programme such as ‘Parent Training plus Child Training’ is also effective in reducing CD problems. However, as to date only single trials of specific types of treatment for children/adolescents with particular clinical CD diagnoses and/or CD problems in the clinical range have been performed, there is a need for further research. The finding that psychological interventions had a modest effect not only on CD symptoms but also on CD-related problems, such as academic performance, in both the home and school environments is promising and shows that such interventions are broadly beneficial.
Conclusion
The current review demonstrates that psychological treatments for children/adolescents with CD and CD-related problems are modestly effective, as rated by parents, teachers and blinded observers, but not by children; however, the ES varied according to the instrument used. There is not enough evidence to support one type of psychological treatment over another. We could not compare treatment efficacy between children/adolescents based on self-report and blinded observer report because of the limited data available. In addition, too few studies examined the possible moderating role of participant and study characteristics, and so more research is needed in this area. The overall quality of the studies was poor, and many studies failed to provide important details, such as on subtypes of aggression and presence of callous-unemotional traits, information that is needed to further optimize psychological treatment strategies. Future studies of CD should pay close attention to these details, be of high quality and be adequately powered.
### Key Points

**Practitioner message**

Psychological treatments appear to be effective in reducing conduct disorder (CD) problems in children and adolescents with clinically elevated CD problems and/or CD diagnosis.

Effect sizes (ESs) are small, but significant, based on parent report (effect size, ES = 0.36), teacher report (ES = 0.21) and blinded observer report (ES = 0.26). This suggests that these treatments are effective in reducing CD problems across different raters and situations (e.g. home and school environments). However, this is not the case when children/adolescents rated their CD problems; then psychological treatments appear not to be effective.

Effects are not limited to CD symptoms, but include a range of CD problems including frequency of misconduct behaviour, academic problems, and how children/adolescents behave at home and at school. Nevertheless, ESs varied within the same rater and between raters, when different instruments measuring the same construct of CD problems were used.

Comorbidity, gender, age, number of sessions, duration, intervention type, setting, medication use or dropout percentage do not appear to influence the effect of treatment.

There is tentative evidence that treatment may be more effective in children younger than 10 years.

In the light of current evidence, psychological treatment is recommended for children/adolescents with CD problems. Future studies should address a number of key shortcomings to further bolster this recommendation.

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### Areas for future research

There is not enough evidence to support one specific psychological treatment over another. More research is needed comparing specific interventions.

Studies included in this meta-analysis used a range of different outcome measures for CD problems. While this allows generalization of treatment effects across a range of CD problems, future studies should use a more homogeneous set of outcome measures to improve comparability across studies.

To allow better evaluation of the quality of studies, future randomized controlled trials should provide detailed information on their methods of randomization and blinding and on the fate of all trial participants (including dropouts).

In order to improve treatment efficacy, future studies should pay greater attention to the role of participant characteristics, such as CD onset, presence of and severity of callous-unemotional traits, subtype of aggression (e.g. proactive vs. reactive aggression) and severity of aggression. The nonpharmacological interventions included in the meta-analysis were psychosocial treatments. More research is needed into the effectiveness of other intervention types, such as diet and cognitive training.
References


Genetics of aggressive behaviour: an overview

Published as:
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Genetics of aggressive behaviour: an overview

American Journal of Medical Genetics Part B 171B:3–43
Abstract

The Research Domain Criteria (RDoC) address three types of aggression: frustrative non-reward, defensive aggression, and offensive/proactive aggression. This review sought to present the evidence for genetic underpinnings of aggression and to determine to what degree prior studies have examined phenotypes that fit into the RDoC framework. Although the constructs of defensive and offensive aggression have been widely used in the animal genetics literature, the human literature is mostly agnostic with regard to all the RDoC constructs. We know from twin studies that about half the variance in behaviour may be explained by genetic risk factors. This is true for both dimensional, trait-like, measures of aggression and categorical definitions of psychopathology. The non-shared environment seems to have a moderate influence with the effects of shared environment being unclear. Human molecular genetic studies of aggression are in an early stage. The most promising candidates are in the dopamergic and serotonergic systems along with hormonal regulators. Genome-wide association studies have not yet achieved genome-wide significance, but current samples are too small to detect variants having the small effects one would expect for a complex disorder. The strongest molecular evidence for a genetic basis for aggression comes from animal models comparing aggressive and non-aggressive strains or documenting the effects of gene knockouts. Although we have learned much from these prior studies, future studies should improve the measurement of aggression by using a systematic method of measurement such as that proposed by the RDoC initiative.
During the early stages of human evolution, aggression was probably an adaptive trait, as it is for many animals in the wild today. It seems logical that during this period of time people who had the variants of genes that promoted aggression were more likely to survive than other people. These variants have persisted in the human genome and partly explain why some people exhibit aggressive behaviors.

Although the word “irascibilem” comes from the Latin “irascibilem”, meaning “to attack,” in current language aggression means much more. In the genetics literature aggression has been operationalized in many ways. As a categorical disorder it has been studied as conduct disorder (CD), oppositional defiant disorder (ODD) and antisocial personality disorder (APD). These categories are convenient for diagnosticians because other work suggests aggression to be a quantitative trait that is better operationalized on dimensions of externalizing behavior, rule breaking, psychopathy and violence.

A dimensional view of aggression is consistent with the approach taken by the NIMH Research Domain Criteria (RDoC) Initiative [Sanislow et al., 2010]. RDoC seeks to focus researchers on the fundamental mechanisms underlying psychopathology. In doing so, it has been creating a dimensional taxonomy of behaviors that, hopefully, corresponds better to underlying mechanisms than does a system of discrete diagnoses.

In the RDoC nomenclature, aggression is categorized into three areas: frustrative non-reward, defensive aggression and offensive (or proactive) aggression. Frustrative non-reward refers to behaviors that correspond to the withdrawal or prevention of reward. This derives from human and animal studies showing that aggression occurs after repeated, failed attempts to obtain rewards even after sustained efforts. Defensive aggression refers to behaviors caused by the perception of an immediate threat, which have the goal of eliminating the threat. Offensive (or proactive) aggressive behaviors are instrumental behaviors aimed at achieving a positive goal, often in the face of competition or in the context of social hierarchies.

The long-term goal of RDoC is to map RDoC phenotypes to underlying mechanisms. In this review, we sought to present the evidence for genetic underpinnings of aggression and to determine to what degree prior studies have examined phenotypes that fit neatly, or at all, into the RDoC framework. We focus the review on three types of genetic studies: twin studies, human association studies of aggression and animal model studies.

**Twin studies of aggression**

This section outlines recent findings from twin studies on aggression and related psychopathology, i.e. ODD, CD and APD. Studies using the classical twin design estimate heritability by comparing the covariation between monozygotic (MZ; identical) and dizygotic (DZ; fraternal) twins [Plomin et al., 1994; Boomsma et al., 2002]. MZ twins are assumed to share 100% of their genetic material while DZ twins share 50% of their genetic material, and both types of twins share a common environment [Posthuma et al., 2003]. Under an ACE model [Neale and Cardon, 1992], the correlation (r) between phenotypes of MZ twin pairs encompasses additive genetic factors (a^2 or h^2; heritability) plus
common environmental factors \( (c^2) \), that is \( r_{MZ} = h^2 + c^2 \). For DZ twin pairs who share 50% of their segregating genetic material, \( r_{DZ} = 0.5 \times h^2 + c^2 \). This gives the following formula to calculate the fraction of phenotypic variance accounted for by genetic factors: \( h^2 = 2(r_{MZ} - r_{DZ}) \). The influence of the common environment \( c^2 \) can be derived as follows: \( r_{MZ} - h^2 \) (or \( 2 \times r_{DZ} - r_{MZ} \)). Genetic influences can also be non-additive \( (d^2) \), but these effects cannot be estimated simultaneously with \( c^2 \) if only using data from twin pairs who are raised together. Accordingly, variance within twin pairs that is not explained by genetic factors or the common environment, is attributed to influence of the non-shared environment, \( e^2 = 1 - r_{MZ} \), which also includes measurement error \([\text{Holzinger, 1929; Falconer, 1960}]\). It is important to note here that the non-shared (unique) environment includes all experiences that contribute to differences between children in the same family, i.e. a common event (for example parents’ divorce) can affect siblings differently.

Twin studies have investigated aggression from different perspectives, e.g. as a personality trait \([\text{Miles and Carey, 1997}]\), as antisocial behaviour \([\text{Rhee and Waldman, 2002}]\) or as a symptom of childhood and adolescent psychopathology. Previous reviews of twin studies and adoption studies on aggression have estimated heritability up to 0.50, with an additional large role for non-shared environmental influences and a small influence of the shared environment \([\text{Viding et al., 2008; Tuvblad and Baker, 2011}]\). Genetic effects seem to predominantly account for phenotypic correlations between different forms of aggression, such as reactive (defensive) and proactive (offensive) aggression, although few studies have examined this \([\text{Rhee and Waldman, 2011}]\). To update these prior reviews, we conducted a systematic search for studies in the period January 2009 until February 2015. PubMed and PsycINFO were searched for peer-reviewed papers to identify studies of twins with characteristics of externalizing behaviour and psychopathy, regardless of age. We used the following search strategy: aggress OR antisocial behav OR aggressive trait OR behaviour problem OR behaviour problem OR problem behavi OR CD OR conduct disorder OR conduct problem OR crime OR criminal OR delinquen OR disruptive behav OR ODD OR oppositional defiant disorder OR antisocial personality OR psychopathy OR sociopathy AND heritabilit.

A total of 254 records were retrieved. Neither books nor unpublished articles were retrieved from the references. Titles and abstracts were read by at least two of the authors (MJB and KV); article selection is summarized in Figure 1. Articles were retained if they: 1) included constructs related to aggression, i.e. aggressive traits, externalizing/impulsive-antisocial behaviour and violent criminality/offences/delinquency or diagnostic categories ODD/CD/APD 2) reported univariate heritability estimates 3) had been published in peer-reviewed journals from January 2009 onwards. Reference lists from the identified articles were manually searched for relevant publications. Articles were excluded if they were not written in English, were a case-report, were review articles, reported only multivariate analyses, or were not specifically focused on aggression, e.g. publications about substance abuse, victimization, or sexual risk behaviour.

From the literature search, which generated 254 hits, 80 articles were identified of which 40 articles were eligible for review according to the above guidelines. All included studies were published as articles in scientific journals. Online publication dates ranged from January 2009 to November 2014. The following information was extracted from the articles: sample size, age range (or mean if unavailable), clinical diagnostic criteria used, instruments used to measure the construct of aggression and key findings. A portion of the studies used interviews or reports to assess diagnoses of ODD,
CD or APD based on the Diagnostic and Statistical Manual of Mental Disorders (DSM; APA, 2001) while other studies employed questionnaires and rating scales to assess aggressive symptoms on a continuum. All characteristics and details of the included studies are summarized in Table 1. We discuss the findings below, starting with research on aggression as a dimensional measure followed by research on diagnostic categories. Within these subsections, results are ordered (where possible) on the basis of age.

Twin Studies of Aggression as a Dimension of Behaviour

Aggression in children and adolescents.
Researchers have explored the etiology of aggressive behavior in children as young as two years of age [Gagne et al., 2011]. The authors reported that more than half of the variance of externalizing behavior problems could be explained by genetic factors, and around one quarter by shared environmental influences. A genetic correlation between externalizing behavior and inhibitory control
was also observed, pointing to deficient inhibitory control as a risk factor for aggressive traits. At age 4, somewhat lower heritability estimates for externalizing behavior have been found (0.39, 95% CI = 0.25–0.54; Tucker-Drob and Harden, 2013). The influence of the non-shared environment was of equal size as the genetic influences. Interestingly, the amount of variance accounted for by shared environmental factors changed with age depending on preschool enrollment. For 5-year-old children that attended preschool, there was no contribution of shared environment while heritability estimates increased. For children who did not attend preschool, the influence of the shared environment was more than 50% and the influence of additive genetic factors decreased. Another study in 4-year-olds from the same cohort found a gene-environment interaction [Boutwell et al., 2012]. In the context of maternal disengagement, genetic risk factors had a strong effect on externalizing behavior problems. Genetic risk did not play a role in behavior problems when maternal disengagement was low, i.e. when children were securely attached. Remarkably, other researchers showed that genetic effects explained the correlation between negative parenting and conduct problems around age 6, but only for low levels of negative parenting [Fujisawa et al., 2012]. For high levels of negative parenting, there was a larger non-shared environmental correlation between negative parenting and conduct problems. To summarize, the reviewed twin studies in children between age 2 and 6 have focused on externalizing and conduct problems in a broad sense. Heritability estimates ranged from 0.39 to 0.60 with variation contingent upon the school and home environment.

From about the time when children start primary school, aggression can be operationalized more specifically. Self-report, parent-report or teacher ratings have been used to assess externalizing and aggressive behavior, with different measures leading to slightly different findings. Both the Twins Early Development Study (TEDS) from the UK and the Netherlands Twin Register (NTR) included twin pair ratings by the same teacher as well as by different teachers. Same teacher ratings provided larger heritability estimates (0.69, 95% CI = 0.57–0.76 – 0.82, 95% CI = 0.79–0.85) than different teacher ratings (0.40, 95% CI = 0.20–0.52 – 0.47, 95% CI = 0.38–0.55; Barker et al., 2009; Lamb et al., 2012). Also, heritability estimates of conduct problems based on parent-report were higher compared to estimates from self-report [Trzaskowski et al., 2013]. Several studies focused on callous-unemotional (CU) traits, which are considered a genetic risk for antisocial behavior [Viding and McCrory, 2012; Blair, 2013]. Distinct developmental trajectories have been found in 7 to 12 year olds, with the largest heritability for boys who have stable high CU traits (0.78, 95% CI = 0.42–0.88; Fontaine et al., 2010). Composite scores across ages confirmed high heritability of CU traits, while heritability estimates were close to zero in a Genome-Wide Complex Trait Analysis [GCTA; Viding et al., 2013]. Contrary to Fontaine et al. [2010], Ficks et al. [2014] observed no sex differences in genetic and environmental influences on CU traits, although nonshared environmental influences on impulsivity were larger in boys. For parent ratings of conduct problems, the Child Behavior Checklist [CBCL; Achenbach and Rescorla, 2001] is often employed. Scores are taken from the DSM-Oriented Scale (DOS) for conduct problems [Spatola et al., 2010; Bertoletti et al., 2014] or the externalizing scale of the CBCL encompassing the aggression and rule-breaking subscales [Burt and Klump, 2012; Robbers et al., 2012; Nikolas et al., 2013]. Meta-analyses have shown a distinction between aggression and rule-breaking, with the former primarily influenced by genetics and the latter by the shared environment [Burt, 2009, 2013]. In summary for children between 6 to 14 years old, the heritability of parental
reports of aggression-related phenotypes ranged from 0.46 to 0.60. The estimates for non-shared environmental influences were between 0.18 and 0.48.

Some twin studies collected longitudinal data to examine stability and change in the etiology of behavior over time. In the Risk Factors for Antisocial Behavior twin study, children age 9–10 were followed into adolescence. Separate genetic and non-shared environmental influences were found on aggression versus rule breaking during childhood, in addition to joint influences on a latent common factor of antisocial behavior [Niv et al., 2013]. At age 14–15, novel genetic influences on the latent factor of general antisocial behavior were observed. In the same project, a link between adolescent aggression and brain functioning at age 9–10 was demonstrated [Niv et al., 2015]. The power of alpha waves, brain oscillations of 8–13 Hz measurable by electroencephalography (EEG), is a biomarker of low arousal. This intermediate phenotype was explored based on theories stating that low arousal evokes externalizing behavior to reach a higher, optimal level of arousal. Indeed, alpha power recorded over the frontal cortex at age 9–10 predicted aggression at age 14–15. The correlation could be explained by genetic factors and was shown in males but not females, and for aggressive behavior but not for rule-breaking.

In Swedish twins, followed from age 8 to 20, a latent factor representing persistent antisocial behavior was found as well as novel shared environmental influences on aggression and delinquency at age 13–14 [Tuvblad et al., 2011]. Within the same twin registry, self-reports of antisocial behavior and related traits at age 16–17 reflected shared environmental risk for criminality [Kendler et al., 2013]. Analyzing parent-reports in addition to self-reports revealed genetic continuity but also novel genetic influences at age 13–14 and 16–17, plus novel unique environmental influences for early adolescents [Wichers et al., 2013]. Data from the Add Health project suggested that for young adults (age 18 to 26), genetic influences on criminal behavior were smaller than those on self-reported delinquency in adolescence [Vaske et al., 2012]. An analysis combining CBCL data from 1022 Swedish twin pairs aged 7–9 years and 501 British twin pairs aged 8–16 years concluded that the etiologies of aggressive and nonaggressive antisocial behavior differ for males and females [Eley et al., 1999].

Interestingly, a meta-analysis reported an age-related increase in heritability estimates of externalizing behaviors [Bergen et al., 2007]. It has been suggested that this increase may be specific to rule-breaking and delinquency, while the magnitude of genetic and environmental influences on aggression only is stable across adolescence [Burt and Klump, 2009; Burt and Neiderhiser, 2009]. However, Tuvblad and colleagues probed reactive (impulsive; defensive) and proactive (instrumental; offensive) aggression and found larger heritability estimates in early adolescence than in childhood for both subtypes of aggression [Tuvblad et al., 2009a]. Altogether, aggression is heritable across development (range 0.38–0.88) but the magnitude of genetic and environmental influences varies according to age and assessment method.

**Aggression in adults**

A few extant twin studies focused specifically on aggressive traits in adults, some of which have used retrospective measures. With conviction of violent crime as a dichotomous variable, heritability estimates were comparable to previous heritability findings of self-reported anti-social behavior [Frisell et al., 2012]. Estimates for this outcome in the classic twin design were similar in a sibling model.
<table>
<thead>
<tr>
<th>Study</th>
<th>Twin registry</th>
<th>N</th>
<th>Age</th>
<th>Measure</th>
<th>a²</th>
<th>c²</th>
<th>e²</th>
<th>Other findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gagne et al., 2011</td>
<td>Boston University Twin Project</td>
<td>291</td>
<td>2</td>
<td>CBCL externalizing scale</td>
<td>0.54</td>
<td>0.27</td>
<td>0.19</td>
<td>Genetic correlation with inhibitory control</td>
</tr>
<tr>
<td>Tucker-Drob &amp; Harden, 2013</td>
<td>Early Childhood Longitudinal</td>
<td>600</td>
<td>4+5</td>
<td>parent-ratings externalizing</td>
<td>0.39</td>
<td>0.23</td>
<td>0.37</td>
<td>GxE interaction at age 5 with preschool attendance</td>
</tr>
<tr>
<td></td>
<td>Study Birth Cohort</td>
<td></td>
<td></td>
<td>problems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boutwell et al, 2012</td>
<td>Early Childhood Longitudinal</td>
<td>1600</td>
<td>4</td>
<td>parent-ratings externalizing</td>
<td>-0.60</td>
<td>-0.08</td>
<td>-0.32</td>
<td>GxE interaction with maternal disengagement</td>
</tr>
<tr>
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<td>Study Birth Cohort</td>
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<td>problems</td>
<td></td>
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<tr>
<td>Fujisawa et al., 2012</td>
<td>Tokyo Twin Cohort Project &amp; Cross Sectional Survey</td>
<td>1677</td>
<td>6</td>
<td>SDQ conduct problems</td>
<td>0.46</td>
<td>-</td>
<td>0.54</td>
<td>Covariance with negative parenting Moderation by hyperactivity/inattention problems</td>
</tr>
<tr>
<td>Lamb et al., 2012</td>
<td>Netherlands Twin Registry</td>
<td>3000</td>
<td>7+10+12</td>
<td>TRF externalizing scale</td>
<td>0.82</td>
<td>-</td>
<td>0.18</td>
<td>Lower a² for girls and for ratings by different teachers Similar effects at age 10 and 12</td>
</tr>
<tr>
<td>Barker et al., 2009</td>
<td>Twins Early Development Study</td>
<td>872</td>
<td>9</td>
<td>SDQ + APSD aggression teacher rating</td>
<td>0.69</td>
<td>0.04</td>
<td>0.27</td>
<td>Lower a² and higher e² for ratings by different teachers</td>
</tr>
<tr>
<td>Trzaskowski et al., 2013</td>
<td>Twins Early Development Study</td>
<td>2500</td>
<td>12</td>
<td>SDQ conduct problems parent-and selfreport</td>
<td>0.55</td>
<td>0.22</td>
<td>0.23</td>
<td>Lower a² and higher e² for ratings by different teachers No genetic influence in GCTA</td>
</tr>
<tr>
<td>Fontaine et al., 2010</td>
<td>Twins Early Development Study</td>
<td>9462</td>
<td>7+9+12</td>
<td>SDQ + APSD CU traits teacher rating</td>
<td>0.78</td>
<td>0.01</td>
<td>0.21</td>
<td>Boys with stable high CU over time Lower h² and higher c² for girls</td>
</tr>
<tr>
<td>Viding et al., 2013</td>
<td>Twins Early Development Study</td>
<td>2886</td>
<td>7+9+12</td>
<td>SDQ + APSD CU traits teacher rating</td>
<td>0.64</td>
<td>-</td>
<td>0.36</td>
<td>No genetic influence in GCTA</td>
</tr>
<tr>
<td>Ficks et al., 2014</td>
<td>twins born in Georgia</td>
<td>885</td>
<td>4+17</td>
<td>APSD CU traits parent rating</td>
<td>0.49</td>
<td>0.19</td>
<td>0.32</td>
<td>No sex differences for CU traits Higher e² for impulsivity in boys</td>
</tr>
<tr>
<td>Study</td>
<td>Registry/Location</td>
<td>N</td>
<td>Age Range</td>
<td>Measure</td>
<td>a²</td>
<td>b²</td>
<td>c²</td>
<td>Notes</td>
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<tr>
<td>Robbers et al., 2012</td>
<td>Netherlands Twin Registry</td>
<td>4592</td>
<td>3-12</td>
<td>CBCL externalizing</td>
<td>0.60</td>
<td>0.18</td>
<td>0.22</td>
<td>Lower a² for girls and higher a² for children from divorced families</td>
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<td>Nikolas et al., 2013</td>
<td>Michigan State University Twin Registry</td>
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<td>6-10</td>
<td>CBCL externalizing</td>
<td>0.46</td>
<td>0.20</td>
<td>0.34</td>
<td>Covariation with children's perception of inter-parental conflict</td>
</tr>
<tr>
<td>Burt &amp; Klump, 2012</td>
<td>Michigan State University Twin Registry</td>
<td>312</td>
<td>6-10</td>
<td>CBCL aggressive scale</td>
<td>0.56</td>
<td>d²   -0.13</td>
<td>0.32</td>
<td>Nuclear twin family model</td>
</tr>
<tr>
<td>Bertoletti et al., 2014</td>
<td>Italian Twin Registry</td>
<td>100</td>
<td>6-14</td>
<td>CBCL DOS Conduct Problems</td>
<td>0.52</td>
<td>-</td>
<td>0.48</td>
<td>Genetic correlation (r = -0.33) with P300 ERP amplitude</td>
</tr>
<tr>
<td>Spatola et al., 2010</td>
<td>Italian Twin Registry</td>
<td>796</td>
<td>8-11+</td>
<td>CBCL DOS Conduct Problems</td>
<td>0.57</td>
<td>-</td>
<td>0.43</td>
<td>Larger h² for 12-17 year olds</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12-17</td>
<td></td>
<td></td>
<td></td>
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<td>Different computational methods</td>
</tr>
</tbody>
</table>

**Dimensional Measures of Aggression in Adolescents**

<table>
<thead>
<tr>
<th>Study</th>
<th>Registry/Location</th>
<th>N</th>
<th>Age Range</th>
<th>Measure</th>
<th>a²</th>
<th>b²</th>
<th>c²</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td>Niv et al., 2013</td>
<td>University of Southern California Twin Study</td>
<td>602</td>
<td>9-10+</td>
<td>CBCL aggressive scale ABCL rule-breaking scale</td>
<td>0.41</td>
<td>0.40</td>
<td>0.19</td>
<td>Latent factor antisocial behavior Novel genetic influences at 14-15</td>
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<td></td>
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<td>14-15</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Niv et al., 2015</td>
<td>University of Southern California Twin Study</td>
<td>383</td>
<td>14-15</td>
<td>CBCL aggressive scale ABCL rule-breaking scale</td>
<td>0.65</td>
<td>-</td>
<td>0.35</td>
<td>Genetic correlation (r = 0.22) with Frontal Alpha Power at age 9-10</td>
</tr>
<tr>
<td>Tuvblad et al., 2011</td>
<td>Swedish Twin Registry</td>
<td>2600</td>
<td>8-20</td>
<td>CBCL at age 8-9 self-report delinquency</td>
<td>0.67</td>
<td>0.26</td>
<td>0.07</td>
<td>Latent factor antisocial behavior Age-specific c² at 13-14 years old</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(4 ages)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Wichers et al., 2013</td>
<td>Swedish Twin Registry</td>
<td>1480</td>
<td>8-20</td>
<td>CBCL and YSR ABCL and ASR</td>
<td>0.88</td>
<td>0.04</td>
<td>0.08</td>
<td>Novel e² at 13-14 years old Novel a² at age 13-14 and 16-17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4 ages)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Kendler et al., 2013</td>
<td>Swedisch Twin Registry</td>
<td>442</td>
<td>16-17</td>
<td>CBCL externalizing criminal behavior</td>
<td>0.38</td>
<td>0.10</td>
<td>0.52</td>
<td>Self-report measures correlate with criminal behavior due to a² and c²</td>
</tr>
<tr>
<td>Burt &amp; Klump, 2009</td>
<td>Michigan State University Twin Registry</td>
<td>252</td>
<td>10-15</td>
<td>CBCL aggressive scale ABCL rule-breaking scale</td>
<td>0.49</td>
<td>0.22</td>
<td>0.29</td>
<td>Stability across age for aggression Increased genetic influences on rule-breaking</td>
</tr>
<tr>
<td>Burt &amp; Neiderhiser, 2009</td>
<td>Nonshared Environment in Adolescent Development</td>
<td>192</td>
<td>10-18</td>
<td>parent- and self-report aggression, delinquency</td>
<td>0.60</td>
<td>-</td>
<td>0.40</td>
<td>Twin/sibling design Stability over age for aggression Increase in h² on delinquency Novel e¹ and a¹ at age 11-14 Also for proactive aggression</td>
</tr>
<tr>
<td>Tuvblad et al., 2009</td>
<td>University of Southern California Twin Study</td>
<td>607</td>
<td>9-10+</td>
<td>RPQ parent-report</td>
<td>0.43</td>
<td>0.15</td>
<td>0.42</td>
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<tr>
<td></td>
<td></td>
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<td>11-14</td>
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</table>
Table 1. Twin studies on aggression, ODD/CD and AAB/APD (continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Twin registry</th>
<th>N</th>
<th>Age</th>
<th>Measure</th>
<th>a²</th>
<th>c²</th>
<th>e²</th>
<th>Other findings</th>
</tr>
</thead>
</table>
| Vaske et al., 2012           | Add Health               | 784  | 12-20+       | self-reported delinquency and criminal behavior | 0.40| -   | 0.60| Smaller a² in young adults  
Correlation with violent victimization due to a² and e² |
| Frisell et al., 2012         | Swedish total population register | 36877| 18+          | conviction violent crime dichotomous variable | 0.49| 0.15| x   | GLMM without e² estimation  
Similar in sibling model  
Smaller a² and c² for adoptees |
| Yeh et al., 2010             | PennTwins Cohort         | 1470 | 2.6-42       | Life History of Aggression questionnaire     | 0.54| -   | 0.64| General aggression factor  
Smaller a² for Physical aggression |
| Brook et al., 2010           | Vietnam Era Twin Registry| 272  | 41-58        | MPQ dimension Impulsive-Antisocial           | 0.32| -   | 0.68| Genetic correlation with Fearless-Dominant dimension |
| Veselka et al., 2011         | Canada and US Residents  | 456  | 17-92        | Self-Report Psychopathy Scale                | 0.34| 0.22| 0.44| Correlation with Supernumerary Personality Inventory traits |

### Dimensional Measures of Aggression in adults

<table>
<thead>
<tr>
<th>Study</th>
<th>Twin registry</th>
<th>N</th>
<th>Age</th>
<th>Measure</th>
<th>a²</th>
<th>c²</th>
<th>e²</th>
<th>Other findings</th>
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Smaller a² for Physical aggression |
| Brook et al., 2010           | Vietnam Era Twin Registry| 272  | 41-58        | MPQ dimension Impulsive-Antisocial           | 0.32| -   | 0.68| Genetic correlation with Fearless-Dominant dimension |
| Veselka et al., 2011         | Canada and US Residents  | 456  | 17-92        | Self-Report Psychopathy Scale                | 0.34| 0.22| 0.44| Correlation with Supernumerary Personality Inventory traits |
| ODD/CD                       |                          |      |              |                                              |     |     |     |                                                                                 |
| Tuublad et al., 2009         | University of Southern California Twin Study | 605  | 9-10         | DISC CD and ODD interview child and parent   | 0.39| 0.32| 0.28| Slightly larger h² in girls  
Latent factor for CD, ODD, ADHD |
| Anckarsäter et al., 2011     | Child and Adolescent Twin Study in Sweden | 8610 | 9/12         | Conduct module parental phone interview      | 0.60| 0.03| 0.37| No c² for mental problems, except for conduct problems in girls |
| Bornovalova et al., 2010     | Minnesota Twin Family Study | 1069 | 11           | DISCA CD and ODD interview child and parent also parental symptoms | 0.51| 0.30| 0.19| Larger h² and no c² for ODD  
Latent factor for CD, ODD, ADHD  
Heritable liability for externalizing |
| Singh & Waldman, 2010        | Georgia Twin Registry    | 838  | 4-17         | parent-report CD, ODD DSM-based questionnaires | 0.52| d²=0.27| 0.21| AE model for ODD  
Link with negative emotionality |
| Waldman et al., 2010         | Tennesse Twin Study      | 1981 | 6-18         | parent-report CD DSM-based questionnaires and interview child | 0.70| 0.04| 0.26| Smaller h² for self-ratings  
Link with negative emotionality, daring and prosociality |
<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Description</th>
<th>N</th>
<th>Age</th>
<th>Measure</th>
<th>Score</th>
<th>Heritability</th>
<th>Shared Environment</th>
<th>Nonshared Environment</th>
<th>Genotype-Environment Interaction</th>
<th>Results/Notes</th>
</tr>
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<tbody>
<tr>
<td>Lahey et al., 2011</td>
<td>Tennessee Twin Study</td>
<td>1571</td>
<td>6-18</td>
<td>DISC-based CD and ODD Interview child and parent</td>
<td>0.76</td>
<td>-</td>
<td>0.24</td>
<td></td>
<td>Multivariate shows $a^2$ differentiation by global $e^2$ in ODD</td>
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<tr>
<td>Young et al., 2009</td>
<td>Colorado Longitudinal Twin Study</td>
<td>293</td>
<td>12+17</td>
<td>DISC CD, CBCL and TRF externalizing</td>
<td>0.70</td>
<td>0.11</td>
<td>0.19</td>
<td></td>
<td>Smaller $h^2$ at age 17 Link with behavioral disinhibition</td>
<td></td>
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<tr>
<td>Schulz-Heik et al., 2010</td>
<td>Add Health</td>
<td>753</td>
<td>12-20</td>
<td>DSM-based self-report Conduct problems</td>
<td>0.41</td>
<td>$t^2=0.17$</td>
<td>0.42</td>
<td></td>
<td>Twin environment = $t^2$ Twin/sibling design Small correlation maltreatment</td>
<td></td>
</tr>
</tbody>
</table>

**AAB/APD**

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Description</th>
<th>N</th>
<th>Age</th>
<th>Measure</th>
<th>Score</th>
<th>Heritability</th>
<th>Shared Environment</th>
<th>Nonshared Environment</th>
<th>Genotype-Environment Interaction</th>
<th>Results/Notes</th>
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</thead>
<tbody>
<tr>
<td>Hicks et al., 2009</td>
<td>Minnesota Twin Family Study</td>
<td>1315</td>
<td>17</td>
<td>DSM AAB</td>
<td>0.76</td>
<td>-</td>
<td>0.24</td>
<td></td>
<td>GxE interaction effects with academic achievement and engaging pro-social peers, mother-child relational father-child relationship problems.</td>
<td></td>
</tr>
<tr>
<td>Hicks et al., 2013</td>
<td>Minnesota Twin Family Study and Sibling Interaction and Behavior Study</td>
<td>1999</td>
<td>26</td>
<td>CD AAB</td>
<td>0.35</td>
<td>0.26</td>
<td>0.39</td>
<td></td>
<td>Similar for AAB High $a^2$ and moderate $c^2$, $e^2$ in general externalizing liability</td>
<td></td>
</tr>
<tr>
<td>Meier et al., 2011</td>
<td>Australian Twin Registry</td>
<td>6383</td>
<td>adults</td>
<td>DSM CD ASB interview</td>
<td>0.32</td>
<td>-</td>
<td>0.68</td>
<td></td>
<td>Similar for females Stability greater for males</td>
<td></td>
</tr>
<tr>
<td>Torgersen et al., 2012</td>
<td>Norwegian Institute of Public Health Twin Panel</td>
<td>&gt;2800</td>
<td>adults</td>
<td>APSD - cluster B self-report interview</td>
<td>0.69</td>
<td>-</td>
<td>0.31</td>
<td></td>
<td>Latent factor Method specificity resulting in no $a^2$ for interview only</td>
<td></td>
</tr>
</tbody>
</table>

**Note.**

**Variance explained**: $a^2$ = heritability estimate; $c^2$ = influence of shared environment; $e^2$ = influence of nonshared environment; $d^2$ = non-additive genetic influences.

**Diagnosis**: CD = Conduct Disorder; ODD = Oppositional Defiant Disorder; ADHD = Attention Deficit Hyperactivity Disorder; APD = Antisocial Personality Disorder; ASB = Adult Antisocial Behavior.

**Measure**: ABCL = Adult Behavior Checklist; APSD = Antisocial Process Screening Device; ASR = Adult Self Report; CBCL = Child Behavior Checklist; DICA = Diagnostic Interview for Children and Adolescents; DSM = Diagnostic Statistical Manual of Mental Disorders; DISC = Diagnostic Interview Schedule for Children; DOS = DSM-Oriented Scale; MPQ = Multidimensional Personality Questionnaire; RPQ = Reactive Proactive Aggression Questionnaire; SDQ = Strengths and Difficulties Questionnaire; TRF = Teacher Report Form; YSR = Youth Self Report.

**Other**: CU = callous-unemotional; ERP = Event-Related Potential; GCTA = Genome-wide Complex Trait Analysis; GLMM = Generalized Linear Mixed Model; GxE = gene-environment interaction; N = number of twin pairs; $a^2$ = approximately; $x$ = not included.
but for adoptees, genetic and shared environmental influences appeared smaller. Using the Lifetime History of Aggression Questionnaire [LHA; Coccara et al., 1997], two factors were distinguished [Yeh et al., 2010]; general aggression (temper tantrums, verbal and indirect aggression) plus physical aggression (fighting and physical assault). Genetic influences were larger for general aggression while non-shared environmental influences were larger for physical aggression, pointing to the importance of subtyping aggressive behavior. Two studies in adult twins have used questionnaires to measure the construct of psychopathy. Brook and colleagues administered the Multidimensional Personality Questionnaire [MPQ; Tellegen, 1982] to middle-aged males. On the impulsive-antisocial dimension, heritability was 0.32 (95% CI = 0.18–0.45), and a strong influence of the non-shared environment was reported with no effect of the shared environment [Brook et al., 2010]. Non-shared environmental factors also explained the correlation between the impulsive-antisocial dimension and the fearless-dominant dimension of psychopathy. On the Self-Report Psychopathy scale [SRP; Hare, 1985], heritability was 0.34 (95% CI = 0.10–0.69) and genetic plus non-shared environmental factors explained the phenotypic correlation of psychopathy with risk-taking, among other variables [Veselka et al., 2011].

Overall, in adult twin studies based on a dimensional approach to aggression, as in studies with children, various definitions and measures have been used. It is therefore difficult to compare results and to make a link with the RDoC classification [Sanislow et al., 2010]. In the next section, we will describe research that focused on diagnostic categories related to DSM criteria [APA, 2000].

Aggressive Psychopathology

Oppositional defiant disorder (ODD) and conduct disorder (CD) in children and adolescents. Several studies of twin children and adolescents (N = 12, age range: 4–23 years) have focused on aggression expressed in childhood and adolescent psychopathology (e.g. CD or ODD). All these studies were characterized by a wide age range, encompassing both childhood and adolescence. For example, Singh and Waldman [2010] focused on an age range from 4 to 17 years in a sample characterized by symptoms of ODD and CD rated by the parent [Singh and Waldman, 2010]. Based on a univariate standard ACDE model (95% CI’s not provided), both disorders showed a different model of best fit, in which heritability was roughly the same. An AE model was the best fit for ODD, in which two thirds of variance was accounted for by genetic effects. While an ADE model was a best fit for CD: nearly half of the variance was explained by additive genetic factors, followed by non-additive genetic and non-shared environment effects. In the Tennessee Twin Study, high heritabilities were reported for CD 0.70 (95% CI = 0.44–1.00; Waldman et al., 2011) and confirmed by Lahey et al. [2011]. In addition, for ODD symptoms heritability was 0.69 (95% CI’s not provided; Lahey et al., 2011). However, selfreports showed a reduction in variance explained by genetic influences 0.39 (95% CI = 0.16–0.72) and a small to moderate role for the common 0.14 (95% CI = 0.004–0.47) and non-shared environment 0.47 (95% CI = 0.38–0.57) effects [Waldman et al., 2011]. In contrast, Lahey et al. [2011] reported strong genetic influences and moderate non-shared environmental influences for both CD and ODD based on combined adult caretaker- and youthreports. In addition, a multivariate model
based on a global factor for internalizing and externalizing disorders showed moderate genetic and non-shared environmental effects of the externalizing factor in both CD and ODD. The non-shared environment effect was moderate in ODD and small in CD.

As these few studies mentioned above show, there are mixed results for CD and ODD; some studies favor an ACE/ADE model and others an AE model. Another example of an ACE model is a study (N = 605 twin pairs) of Tuvblad et al. [2009a,b]. Both CD and ODD symptoms were assessed with the DISC-IV structured interview. The authors found unique genetic and environmental influences for each set of symptoms, which suggests unique influences of the two disorders. Moreover, the relative effects of genetic, shared, and non-shared environmental factors were similar between CD and ODD. Furthermore, it has been suggested that both the genetic (95% CI_f = 0.17–0.74, 95% CI_m = 0.12–0.70) and nonshared environmental (95% CI_f = 0.23–0.39, 95% CI_m = 0.22–0.37) influences on CD are slightly higher in girls (f) than boys (m) and slightly lower for shared environment (95% CI_f = 0.00–0.50, 95% CI_m = 0.03–0.56). Furthermore, common influences have been reported based on a latent externalizing behaviour factor, indicating high genetic and moderate non-shared environmental influences. Anckarster et al. [2011] reported that both CD and ODD are more influenced by genetic (95% CI_f = 0.13–0.36, 95% CI_m = 0.61–0.67) factors in boys (m) than in girls (f). In contrast, the influence of shared environment was negligible (95% CI_f = 0.17–0.35, 95% CI_m = 0.00–0.02), the one exception being conduct problems in girls.

Bornovalova et al. [2010] studied a large sample of twin pairs (aged 11 years) in which an ACE model was the best fit. A higher heritability of 0.73 (95% CI = 0.59–0.79) and nonshared environmental influences of 0.24 (95% CI = 0.21–0.26) was found for ODD compared with CD, in which heritability was 0.51 (95% CI = 0.39–0.63) and common environment was 0.30 (95% CI = 0.18–0.41). In addition, common environment was significant for CD only. In the longitudinal study of Young et al. [2009], twin pairs were assessed at 12 and 17 years of age on both childhood and adolescent psychopathology and aggressive traits (CBCL and TRF- externalizing behaviour). They reported smaller genetic 0.49 (95% CI = 0.25–0.76) and nonshared environmental 0.25 (95% CI = 0.20–0.32) influences at age 17 compared with age 12 (a^2 = 0.70, 95% CI = 0.46–0.85; e^2 = 0.19, 95% CI = 0.15–0.24). This AE model was linked to structural stability of behavioural and response disinhibition across adolescence, and this relationship was primarily genetic in origin.

To conclude this section on developmental psychopathology in childhood and adolescence, one large study in adolescents reported an AE model with moderate genetic effects in conduct problems [Schulz-Heik et al., 2010].

**Aggressive psychopathology in older adolescents and adults.**

Among the studies of CD or ODD, two also reported on Adult Antisocial Behaviour (AAB) [Hicks et al., 2009, 2013]. For AAB, Hicks et al. [2009] reported strong genetic influences (95% CI = 0.65–0.79) and moderate non-shared environment influences (95% CI = 0.21–0.26). Across six environmental risk factors (low academic achievement and engagement, antisocial peers, lack of prosocial peers, mother-child relationship problems, father-child relationship problems, stressful life events), genetic variance in externalizing disorders increased in the context of greater environmental adversity. This indicates that as environmental stress increases genetic differences among young adults become...
more important in the etiology of externalizing disorders. Three studies focused on adults with CD and AAB [Meier et al., 2011; Hicks et al., 2013] and cluster B personality antisocial personality disorder [Torgersen et al., 2012]. Hicks et al. [2013] focused on both biological twins and non-biological siblings. They reported for both CD and AAB moderate genetic (95% CI = 0.35–0.52), shared (95% CI = 0.11–0.25) and non-shared environmental influences (95% CI = 0.34–0.42). Meier et al. [2011] reported approximately two thirds of the variance explained by nonshared environmental influences (95% CI = 0.63–0.74), followed by genetic effects (95% CI = 0.26–0.37) in CD regardless of gender. No gender differences were reported for AAB for which the non-shared environment explained two thirds of the variance followed by genetic influences. However, males showed greater stability in antisocial behaviour from childhood to adulthood. As for the study on cluster B personality [Torgersen et al., 2012], one-third of the variance was explained by genetic influences and two thirds by non-shared environment based on interview measures of personality disorders. These findings were method specific, since the magnitude of the genetic component varied by type of interview compared to self-reported questionnaires. Thus, differences in twin studies on AAB and APD may be due to gender or to differences in measurement methods.

Overall, the non-shared environmental effects are less strong compared to genetic effects. Furthermore, a risk of bias arises in the cited studies, given that the power to detect shared environmental influences is often low in biometric analyses of twin data and these studies assume that the environmental effects are free of influence by genetic effects [Burt, 2013]. Therefore, results should be interpreted with caution.

Summary: Twin Studies of Aggressive Behaviour and Psychopathology
Recent publications about twin data on aggression-related problems suggest that around 50% of the variance in aggressive behaviour may be explained by genetic influences. The non-shared environment seems to have a moderate influence. With regard to the shared environment, findings are mixed: About half of the reviewed studies report no influence while other studies indicate estimates between 0.15 and 0.35. The former is in line with a previous review that showed the presence of only non-shared environmental and genetic influences of 0.50 each [Tuvblad and Baker, 2011]. Although a meta-analysis demonstrated increased heritability estimates for externalizing with age [Bergen et al., 2007], this pattern was not evident in the current review. However, most of the included articles examined children and adolescents, and only a few articles focused specifically on adults. An effect of gender has occasionally been observed [Tuvblad et al., 2009b; 2011; Meier et al., 2011; Lamb et al., 2012; Robbers et al., 2012] but, for most studies, similar models for boys and girls were suitable. Hence, heritability estimates may be comparable between males and females despite the finding that aggression occurs more often in males, particularly direct, overt aggression as opposed to relational aggression [Ligthart et al., 2005]. Of note, genetic influences on aggressive behaviour might depend on the environment, as gene-environment interaction appears to play an important role.

The operationalization of the construct aggression differed widely across the reviewed articles. Some researchers investigated aggression as a trait in the general population while others focused on DSM-based psychopathology, i.e. ODD, CD and AAB/APD. Both the dimensional and the categorical approaches yielded heritability estimates ranging from approximately 0.30 to 0.80. Several studies
found a latent factor of externalizing/antisocial behaviour with unique genetic or environmental influences on specific forms of aggression [Bornovalova et al., 2010; Yeh et al., 2010; Lahey et al., 2011; Tuvblad et al., 2011; Niv et al., 2013]. Thus, a limitation of the current state of the field is that researchers do not use common definitions with regard to aggression, which makes it difficult to compare studies. Future studies may improve the measurement of aggression by using dimensional constructs from the RDoC framework, i.e. defensive aggression, offensive aggression and frustrative non-reward (http://www.nimh.nih.gov/researchpriorities/rdoc/negative-valence-systems-workshop-proceedings.shtml). These constructs are defined and will be continuously refined based on multiple units of analysis, such as genes, brain circuits and behaviour, to better integrate clinical findings with neuroscience [Sanislow et al., 2010; Cuthbert and Insel, 2013]. Discovering genes that are related to various aggression dimensions is one step towards advanced understanding of psychopathology.

**Human association studies of aggression**

Based on previous searches performed by Vassos et al. [2014] and Gunter et al. [2010] we searched articles on PubMed using the terms “(aggression OR aggressivity OR aggressive OR anger OR hostility OR irritability OR violence OR convict OR crimin OR offend OR externalizing OR conduct OR antisocial OR impulsive aggression OR psychopathy OR ODD OR oppositional defiant OR callous unemotional) AND (genetics OR gene OR polymorphism OR genotype OR allele OR genome OR haplotype)” to update their searches from December 2009 until February 2015, with an output of 7,202 articles. Subsequently, we filtered works written in English language, performed in humans, including sample characteristics and performing genetic association studies that had been published as articles in scientific journals. We selected 268 potential articles within this range of dates and some additional 263 articles from a previous review [Gunter et al., 2010] and a meta-analysis [Vassos et al., 2014]. From these 531 articles we selected those studies that included traits related to aggression (aggressiveness, anger, externalizing behaviour, impulsive aggression, criminality, violence or delinquency), or diagnostic categories of ODD, CD, antisocial behaviour or ASPD, callous unemotional or psychopathy. Also, we excluded studies assessing aggressive or antisocial traits in drug use or dependence cohorts, or samples of other psychiatric disorders (e.g. schizophrenia, bipolar disorder, major depression). A total of 277 articles were finally considered for this review. Our selection process is described in Figure 2.

Most association studies exploring the genetic susceptibility to aggression have focused on candidate genes (candidate gene association studies, CGAS), especially those related to serotonergic and dopaminergic neurotransmission. Additionally, a few genomewide association studies (GWAS) have been performed and will also be reviewed. These studies have used either trait measures of aggression (Table II) or measures of aggression psychopathology (Tables 3 and 4). Candidate gene association studies have often rendered conflicting results, since in several cases associations were identified with different alleles of the same variation or could not be replicated in the same phenotype. In addition, many of the CGAS were performed in small samples that often lead to false positive or false negative findings due to lack of statistical power. Finally, GWAS of aggression phenotypes have not identified genome-wide significant associations so far. In consequence, results obtained in previous association studies, either CGAS or GWAS, must be taken with caution.
Candidate Genes Studied Across the Lifespan

The MAOA and 5HTT genes have been studied quite extensively in aggressive traits in children, adolescents and adults (Table 2), and also in diagnostic categories of aggression in children (Table III) and adults (Table 4). MAOA encodes the enzyme monoamine oxidase A, responsible for the catabolism of dopamine, serotonin and other neurotransmitters. An upstream polymorphism consisting of a variable number of tandem repeats (uVNTR) located in the promoter region of the gene, with an effect on transcription, has been extensively studied. In children, several studies identified the uVNTR variants determining low gene expression levels associated with aggression, anger, externalizing behaviour and delinquency, especially in high risk environments (maltreatment or low maternal sensitivity) [Weder et al., 2009; Edwards et al., 2010; Pickles et al., 2013]. In adolescents and young adults, low activity variants were found associated with increased aggressive reactions, violent delinquency and even the use of weapons, stabbing and shooting [Guo et al., 2008; Kuepper et al., 2013; Beaver et al., 2010a,b, 2014]. In adults, many studies have associated the low activity variants with aggression, impulsivity, hostility and violent criminal and delinquent behaviours [Manuck et al., 2000, 2002; Eisenberger et al., 2007; Frazzetto et al., 2007; Reif et al., 2007; Gallardo-Pujol et al., 2013; Armstrong et al., 2014; Gorodetsky et al., 2014; Tiihonen et al., 2014]. Only a few studies have failed to replicate these results or have identified high activity variants as risk alleles for these phenotypes [Huizinga et al., 2006; Yang et al., 2007; van der Vegt et al., 2009; Perroud et al., 2010; Verhoeven et al., 2012]. Thus, the bulk of the evidence indicates that low activity alleles of the MAOA-
uVNTR are probably associated with aggressive traits. Interestingly, the MAOA gene has not been associated with CD or ODD in children. Indeed, it has only been associated with CD in the presence of an adverse childhood environment [Caspi et al., 2002; Foley et al., 2004; Haberstick et al., 2005; Kim-Cohen et al., 2006; Young et al., 2006; Prom-Wormley et al., 2009; Qian et al., 2009; Wakschlag et al., 2010; Kieling et al., 2013]. Many studies assessing MAOA in adults identified associations with antisocial behaviour, conduct problems and psychopathy in the presence of adverse childhood environment, most of them identifying the shorter variant of the uVNTR as the risk allele [Lu et al., 2003; Widom and Brzustowicz, 2006; Prichard et al., 2007; Flowler et al., 2009; Williams et al., 2009; Beach et al., 2010; Derringer et al., 2010; Fergusson et al., 2011, 2012; Philibert et al., 2011; Reti et al., 2011; McGrath et al., 2012; Sadeh et al., 2013; Byrd and Manuck, 2014; Ficks and Waldman, 2014; Haberstick et al., 2014].

Studies of the MAOA-uVNTR and aggression have usually been restricted to males; since this is an X-linked gene. Because information on the inactivation of the locus is not available, association results are difficult to interpret in females. Other MAOA variants, such as the single nucleotide polymorphisms (SNPs) rs5906957, rs909525, rs5323, and rs2064070, have been associated with physical aggression in boys or anger in male adults [Antypa et al., 2013; Pingault et al., 2013]. Also, another VNTR (10 bp) in this gene was found associated with ASPD [Philibert et al., 2011].

The SLC6A4 or 5HTT gene, which encodes the serotonin transporter, has been associated with several aggressive phenotypes. A functional polymorphism in the promoter, called 5HTTLPR for 5HTT-Linked Polymorphic Region, has been associated in children and adolescents with aggression, violence, delinquency and externalizing behaviour, although with contradictory results regarding the identity of the risk variant and the associated genotypes [Zalsman et al., 2001; Cadoret et al., 2003; Gerra et al., 2005; Beitchman et al., 2006; Haberstick et al., 2006; Hohmann et al., 2009; Zimmermann et al., 2009; Aslund et al., 2013]. In contrast, many studies of adults have found the short variant (S) of 5HTTLPR to drive lower transcription levels of the gene and to be associated with aggression, anger, hostility, neuroticism, violence and criminality [Greenberg et al., 2000; Liao et al., 2004; Retz et al., 2004; Verona et al., 2006; Reif et al., 2007; Gonda et al., 2009; Sysoeva et al., 2009; Conway et al., 2012; Gyurak et al., 2013; LopezCastroman et al., 2014].

The shorter variant of 5HTTLPR has been associated with conduct problems and CD [Sakai et al., 2006, 2007, 2010; Brody et al., 2011]. The 5HTTLPR has been associated with psychopathy and antisocial behaviour, although with conflicting results [Flowler et al., 2009; Garcia et al., 2010; Sadeh et al., 2013; Ficks and Waldman, 2014].

SNP rs25531 modifies the transcription of 5HTTLPR: The long 5HTTLPR allele with a G (Lg) at rs25531 drives low transcription levels, similar to the short allele (S), whereas the La allele at rs25531 determines higher transcription levels. This could explain contradictory association results. Beitchman et al., [2006] considered this SNP when analyzing 5HTTLPR genotypes, identifying association between lower transcription genotypes (S/S, S/Lg and Lg/Lg) and childhood aggression [Beitchman et al., 2006].

Several meta-analyses have evaluated the contribution of the MAOA-uVNTR and 5HTTLPR to aggressive behaviour. Vassos et al. [2014] assessed these two variants, among others, in a total of 31 genes, and did not observe any significant contribution to the phenotype for any of the variants.
<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Phenotype</th>
<th>Study</th>
<th>References</th>
</tr>
</thead>
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<td><strong>Children and adolescents</strong></td>
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<td>AVP</td>
<td>arginine vasopressin</td>
<td>Aggression</td>
<td>CGAS</td>
<td>(Malik et al., 2014)</td>
</tr>
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<td>arginine vasopressin receptor 1A</td>
<td>Aggression</td>
<td>CGAS</td>
<td>(Malik et al., 2014)</td>
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<tr>
<td>AVPR1B</td>
<td>arginine vasopressin receptor 1A</td>
<td>Aggression</td>
<td>CGAS</td>
<td>(Luppino et al., 2014; Zai et al., 2012b)</td>
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<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
<td>Aggressive behavior</td>
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<td>CGAS</td>
<td>(Dick et al., 2011; Latendresse et al., 2011)</td>
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<td>(Miodovnik et al., 2012)</td>
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<td>DRD2</td>
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<td>(Guo et al., 2007; Zai et al., 2012a)</td>
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<td>dopamine receptor D4</td>
<td>Aggression, externalizing behavior and delinquency</td>
<td>CGAS</td>
<td>(Buchmann et al., 2014; Dmitrieva et al., 2011; Farbiash et al., 2014; Hohmann et al., 2009; Nobile et al., 2007; Schlomer et al., 2015)</td>
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<td>MAOA</td>
<td>monoamine oxidase A</td>
<td>Aggression, anger, externalizing behavior and delinquency</td>
<td>CGAS</td>
<td>(Beaver et al., 2014; Edwards et al., 2010; Guo et al., 2008; Pickles et al., 2013; Pingault et al., 2013; van der Vegt et al., 2009; Weder et al., 2009)</td>
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<td>ESR1</td>
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<td>LRRC7</td>
<td>leucine rich repeat containing 7</td>
<td>Aggressive behavior</td>
<td>GWAS</td>
<td>(Mick et al., 2011)</td>
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<td>OXTR</td>
<td>oxytocin receptor</td>
<td>Aggression</td>
<td>CGAS</td>
<td>(Malik et al., 2012; Malik et al., 2014)</td>
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<td>SLC6A4 (5HTT)</td>
<td>solute carrier family 6 (neurotransmitter transporter), member 4 (serotonin transporter)</td>
<td>Aggression, violence, delinquency and externalizing behavior</td>
<td>CGAS</td>
<td>(Aslund et al., 2013; Beitchman et al., 2006; Gador et al., 2003; Gerra et al., 2005; Haberstick et al., 2006; Hohmann et al., 2009; Zalsman et al., 2001; Zimmermann et al., 2009)</td>
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<td>SLC6A3 (DAT1)</td>
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<td>Externalizing behavior, pathological violence, serious delinquency and criminal conduct</td>
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<td>GWAS</td>
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<td>TMEM132D</td>
<td>transmembrane protein 132D</td>
<td>Aggressive behavior</td>
<td>GWAS</td>
<td>(Mick et al., 2011)</td>
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<td><strong>Adults</strong></td>
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<td>AR</td>
<td>androgen receptor</td>
<td>Violent criminal behavior, aggression, impulsivity and neuroticism</td>
<td>CGAS</td>
<td>(Aluja et al., 2011; Cheng et al., 2006; Jonsson et al., 2001; Rajender et al., 2008; Westberg et al., 2009)</td>
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<td>ABCG1</td>
<td>ATP-binding cassette, sub-family G (WHITE), member 1</td>
<td>Aggression and anger</td>
<td>CGAS</td>
<td>(Gietl et al., 2007)</td>
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<td>AKAP5</td>
<td>A kinase (PRKA) anchor protein 5</td>
<td>Anger</td>
<td>CGAS</td>
<td>(Richter et al., 2011)</td>
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<td>ANK3</td>
<td>ankyrin 3, node of Ranvier (ankyrin G)</td>
<td>Externalizing behavior</td>
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<td>(Logue et al., 2013)</td>
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<td>CDH13</td>
<td>cadherin 13</td>
<td>Violent behavior</td>
<td>CGAS</td>
<td>(Tiihonen et al., 2014)</td>
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<td>cholinergic receptor, muscarinic 2</td>
<td>Externalizing behavior</td>
<td>CGAS</td>
<td>(Dick et al., 2008)</td>
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<td>COMT</td>
<td>catechol-O-methyltransferase</td>
<td>Aggression, externalizing and anger</td>
<td>CGAS</td>
<td>(Kulikova et al., 2008; Perraud et al., 2010; Shehzad et al., 2012; Wagner et al., 2010)</td>
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<td>CRHR1</td>
<td>corticotropin releasing hormone receptor 1</td>
<td>Aggressive behavior</td>
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<td>(Chen et al., 2014)</td>
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<td>CYP2D6</td>
<td>Aggression</td>
<td>CGAS</td>
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### Table 2. Genes Associated with Aggression Trait Measures. (continued)

<table>
<thead>
<tr>
<th>Gene symbol</th>
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<th>Phenotype</th>
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<th>References</th>
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<td>DARPP32</td>
<td>protein phosphatase 1, regulatory (inhibitor) subunit 1B</td>
<td>Anger</td>
<td>CGAS</td>
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<td>DBH</td>
<td>dopamine beta-hydroxylase (dopamine beta-monoxygenase)</td>
<td>Aggressive hostility, impulsivity and neuroticism</td>
<td>CGAS</td>
<td>(Hess et al., 2009)</td>
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<tr>
<td>FYN</td>
<td>FYN proto-oncogene, Src family tyrosine kinase</td>
<td>Anger</td>
<td>GWAS</td>
<td>(Mick et al., 2014)</td>
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<td>HTR1B</td>
<td>5-hydroxytryptamine (serotonin) receptor 1B, G protein-coupled</td>
<td>Aggressive behavior, anger and hostility</td>
<td>CGAS</td>
<td>(Conner et al., 2010; Hakulinen et al., 2013; Zouk et al., 2007)</td>
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<td>HTR2A</td>
<td>5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled</td>
<td>Aggression, anger, hostility and criminality</td>
<td>CGAS</td>
<td>(Banlaki et al., 2015; Berggard et al., 2003; Dijkstra et al., 2013; Giegling et al., 2006; Keltikangas-Jarvinen et al., 2008)</td>
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<td>MAOA</td>
<td>monoamine oxidase A</td>
<td>aggression, impulsivity, hostility, use of weapons and violent criminal and delinquent behaviors</td>
<td>CGAS</td>
<td>(Antypa et al., 2013; Armstrong et al., 2014; Beaver et al., 2010a; Beaver et al., 2010b; Eisenberger et al., 2007; Frazzetto et al., 2007; Gallardo-Pujol et al., 2013; Gorodetsky et al., 2014; Huang et al., 2004; Kinnally et al., 2009; Kuepper et al., 2013; Manuck et al., 2000; Manuck et al., 2002; Reif et al., 2007; Tiitinen et al., 2014; Verhoeven et al., 2012)</td>
</tr>
<tr>
<td>NOS1</td>
<td>nitric oxide synthase 1 (neuronal)</td>
<td>Impulsive aggressivity and aggression</td>
<td>CGAS</td>
<td>(Reif et al., 2009; Retz et al., 2010; Rujescu et al., 2008)</td>
</tr>
<tr>
<td>NOS3</td>
<td>nitric oxide synthase 3 (endothelial cell)</td>
<td>Aggressive behavior</td>
<td>CGAS</td>
<td>(Rujescu et al., 2008)</td>
</tr>
<tr>
<td>SLC6A4 (5HTT)</td>
<td>solute carrier family 6 (neurotransmitter transporter), member 4 (serotonin transporter)</td>
<td>Aggression, anger, hostility, neuroticism, violence and criminality</td>
<td>CGAS</td>
<td>(Conway et al., 2012; Gonda et al., 2009; Greenberg et al., 2000; Gyurak et al., 2013; Liao et al., 2004; Lopez-Castroman et al., 2014; Reif et al., 2007; Retz et al., 2004; Silva et al., 2007; Sysoeva et al., 2009; Verona et al., 2006)</td>
</tr>
<tr>
<td>TBX19</td>
<td>T-box 19</td>
<td>Angry hostility</td>
<td>CGAS</td>
<td>(Wasserman et al., 2007)</td>
</tr>
<tr>
<td>TH</td>
<td>tyrosine hydroxylase</td>
<td>Angry hostility and neuroticism</td>
<td>CGAS</td>
<td>(Persson et al., 2000)</td>
</tr>
<tr>
<td>TPH1</td>
<td>tryptophan hydroxylase 1</td>
<td>Aggression, aggressive behavior, anger and violence</td>
<td>CGAS</td>
<td>(Evans et al., 2000; Hennig et al., 2005; Manuck et al., 1999; Reuter and Hennig, 2005; Rotondo et al., 1999; Rujescu et al., 2002; Yang et al., 2010)</td>
</tr>
<tr>
<td>TPH2</td>
<td>tryptophan hydroxylase 2</td>
<td>Anger</td>
<td>CGAS</td>
<td>(Ke et al., 2006; Mann et al., 2008; Yang et al., 2010; Yoon et al., 2012)</td>
</tr>
</tbody>
</table>

CGAS: candidate gene association study; GWAS: genome-wide association study
### Table 3. Genes associated with Aggression Psychopathology Measures in Children and Adolescents.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Phenotype</th>
<th>Study</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBFOX1 (A2BP1)</td>
<td>RNA binding protein, fox-1 homolog (C. elegans) 1</td>
<td>Conduct problems and CD</td>
<td>GWAS</td>
<td>(Anney et al., 2008; Sonuga-Barke et al., 2008)</td>
</tr>
<tr>
<td>ADH1C</td>
<td>alcohol dehydrogenase 1C (class I), gamma polypeptide</td>
<td>CD</td>
<td>GWAS</td>
<td>(Sonuga-Barke et al., 2008)</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
<td>ODD and CU</td>
<td>CGAS</td>
<td>(Willoughby et al., 2013)</td>
</tr>
<tr>
<td>MYRFL (C12orf28)</td>
<td>myelin regulatory factor-like</td>
<td>Conduct problems</td>
<td>GWAS</td>
<td>(Anney et al., 2008)</td>
</tr>
<tr>
<td>COMT</td>
<td>catechol-O-methyltransferase</td>
<td>CD</td>
<td>CGAS</td>
<td>(Caspi et al., 2008; DeYoung et al., 2010; Qian et al., 2009)</td>
</tr>
<tr>
<td>DRD4</td>
<td>dopamine receptor D4</td>
<td>CD, ODD and CU</td>
<td>CGAS</td>
<td>(Kirley et al., 2004; Nikitopoulos et al., 2014; Zohsel et al., 2014)</td>
</tr>
<tr>
<td>KIAA2012 (FLJ39061)</td>
<td>KIAA2012</td>
<td>Conduct problems</td>
<td>GWAS</td>
<td>(Anney et al., 2008)</td>
</tr>
<tr>
<td>HTR1B</td>
<td>5-hydroxytryptamine (serotonin) receptor 1B, G protein-coupled</td>
<td>CD and CU</td>
<td>CGAS</td>
<td>(Jensen et al., 2009; Moul et al., 2013; Viding et al., 2010)</td>
</tr>
<tr>
<td>HTR2A</td>
<td>5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled</td>
<td>CU</td>
<td>CGAS</td>
<td>(Jensen et al., 2009; Moul et al., 2013)</td>
</tr>
<tr>
<td>KIRREL</td>
<td>kin of IRRE like (Drosophila)</td>
<td>Conduct problems</td>
<td>GWAS</td>
<td>(Anney et al., 2008)</td>
</tr>
<tr>
<td>RPS24P4 (LOC729257)</td>
<td>ribosomal protein S24 pseudogene 4</td>
<td>Conduct problems</td>
<td>GWAS</td>
<td>(Anney et al., 2008)</td>
</tr>
<tr>
<td>MAOA</td>
<td>monoamine oxidase A</td>
<td>CD and ODD with adverse childhood environment</td>
<td>CGAS</td>
<td>(Caspi et al., 2002; Foley et al., 2004; Haberstick et al., 2005; Kieling et al., 2013; Kim-Cohen et al., 2006; Prom-Wormley et al., 2009; Qian et al., 2009; Wakschlag et al., 2010; Young et al., 2006)</td>
</tr>
<tr>
<td>MFHAS1</td>
<td>malignant fibrous histiocytoma amplified sequence 1</td>
<td>CD</td>
<td>GWAS</td>
<td>(Sonuga-Barke et al., 2008)</td>
</tr>
<tr>
<td>OXTR</td>
<td>oxytocin receptor</td>
<td>CD and CU</td>
<td>CGAS</td>
<td>(Beitchman et al., 2012)</td>
</tr>
<tr>
<td>PAWR</td>
<td>PRKC, apoptosis, WT1, regulator</td>
<td>Conduct problems</td>
<td>GWAS</td>
<td>(Anney et al., 2008)</td>
</tr>
<tr>
<td>PKD1L2</td>
<td>polycystic kidney disease 1-like 2 (gene/pseudogene)</td>
<td>Conduct problems</td>
<td>GWAS</td>
<td>(Anney et al., 2008)</td>
</tr>
<tr>
<td>PKD1L3</td>
<td>polycystic kidney disease 1-like 3</td>
<td>Conduct problems</td>
<td>GWAS</td>
<td>(Anney et al., 2008)</td>
</tr>
<tr>
<td>RGL1</td>
<td>ral guanine nucleotide dissociation stimulator-like 1</td>
<td>Conduct problems</td>
<td>GWAS</td>
<td>(Anney et al., 2008)</td>
</tr>
<tr>
<td>RIT1</td>
<td>Ras-like without CAAX 1</td>
<td>CD</td>
<td>GWAS</td>
<td>(Sonuga-Barke et al., 2008)</td>
</tr>
<tr>
<td>ROBO2</td>
<td>roundabout, axon guidance receptor, homolog 2 (Drosophila)</td>
<td>CU</td>
<td>GWAS</td>
<td>(Viding et al., 2010)</td>
</tr>
<tr>
<td>SLC6A1 (DAT1)</td>
<td>solute carrier family (neurotransmitter transporter, member 1 (GABA transporter)</td>
<td>CD</td>
<td>GWAS</td>
<td>(Sonuga-Barke et al., 2008)</td>
</tr>
<tr>
<td>SLC6A4 (5HTT)</td>
<td>solute carrier family 6 (neurotransmitter transporter, member 4 (serotonin transporter)</td>
<td>CD and conduct problems</td>
<td>CGAS</td>
<td>(Brody et al., 2011; Sakai et al., 2010; Sakai et al., 2007; Sakai et al., 2006)</td>
</tr>
<tr>
<td>SLC6A3 (DAT1)</td>
<td>solute carrier family 6 (neurotransmitter transporter, member 3 (dopamine transporter)</td>
<td>ODD and conduct problems</td>
<td>CGAS</td>
<td>(Burt and Mikolajewski, 2008; Lee, 2007 #276; Todd et al., 2005; Young et al., 2002)</td>
</tr>
</tbody>
</table>

CGAS: candidate gene association study; GWAS: genome-wide association study; CD: conduct disorder; ODD: oppositional defiant disorder; CU: callous-unemotional
Table 4. Genes Associated with Aggression Psychopathology Measures in Adults.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Phenotype</th>
<th>Study</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>androgen receptor</td>
<td>Antisocial behavior</td>
<td>CGAS</td>
<td>(Prichard et al., 2007)</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
<td>Psychopathy</td>
<td>CGAS</td>
<td>(Kourmouli et al., 2013)</td>
</tr>
<tr>
<td>DYRK1A</td>
<td>dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A</td>
<td>Antisocial behavior</td>
<td>GWAS</td>
<td>(Tielbeek et al., 2012)</td>
</tr>
<tr>
<td>ESR1</td>
<td>estrogen receptor 1</td>
<td>Antisocial behavior, neuroticism and psychoticism</td>
<td>CGAS</td>
<td>(Prichard et al., 2007; Westberg et al., 2003)</td>
</tr>
<tr>
<td>HTR2A</td>
<td>5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled</td>
<td>Antisocial behavior</td>
<td>CGAS</td>
<td>(Burt and Mikolajewski, 2008)</td>
</tr>
<tr>
<td>MAOA</td>
<td>monoamine oxidase A</td>
<td>Antisocial behavior, conduct problems and psychopathy</td>
<td>CGAS</td>
<td>(Beach et al., 2010; Byrd and Manuck, 2014; Derringer et al., 2010; Fergusson et al., 2011; Fowler et al., 2009; McGrath et al., 2012; Philibert et al., 2011; Reti et al., 2011; Sadeh et al., 2013; Williams et al., 2009)</td>
</tr>
<tr>
<td>NR4A2</td>
<td>nuclear receptor subfamily 4, group A, member 2</td>
<td>Antisocial behavior</td>
<td>CGAS</td>
<td>(Prichard et al., 2007)</td>
</tr>
<tr>
<td>SLC6A4 (5HTT)</td>
<td>solute carrier family 6 (neurotransmitter transporter), member 4 (serotonin transporter)</td>
<td>Psychopathy and antisocial behavior</td>
<td>CGAS</td>
<td>(Ficks and Waldman, 2014; Fowler et al., 2009; Garcia et al., 2010; Sadeh et al., 2013)</td>
</tr>
<tr>
<td>SNAP25</td>
<td>synaptosomal-associated protein, 25kDa</td>
<td>Antisocial personality disorder</td>
<td>CGAS</td>
<td>(Basoglu et al., 2011)</td>
</tr>
<tr>
<td>TFAP2B</td>
<td>transcription factor AP-2 beta (activating enhancer binding protein 2 beta)</td>
<td>Antisocial behavior</td>
<td>CGAS</td>
<td>(Prichard et al., 2007)</td>
</tr>
</tbody>
</table>

CGAS: candidate gene association study; GWAS: genome-wide association study

assessed. Heterogeneity (I²) for the uVNTR and LPR was higher than 50% (P < 0.01). In contrast, the meta-analysis of Ficks and Waldman [2014] identified an association between aggressive behaviours and the low activity alleles of the MAOA-uVNTR (OR = 1.14; P = 1.37e-06) and the short allele of the 5HTTLPR (OR = 1.52; P = 7.59e-11). Also, Byrd and Manuck [2014] found the low activity alleles of the MAOA-uVNTR to be associated with aggressive behaviours in the presence of childhood maltreatment (P = 8e-07).

Candidate Genes Studied in Children and Adolescents

Association studies assessing aggressive traits in children and adolescents have also considered other candidate genes (Table 2). Thus, a 48-bp VNTR polymorphism in intron 3 of DRD4, encoding the dopamine receptor D4, has been studied. Carriers of the 7-repeat (7R) allele showed higher levels of aggression, externalizing behaviour and delinquency [Nobile et al., 2007; Hohmann et al., 2009; Dmitrieva et al., 2011; Buchmann et al., 2014; Farbiash et al., 2014; Schlomer et al., 2015]. Interestingly, an epistatic effect of this allele and the S allele of 5HTTLPR has been reported for aggressive and delinquent behaviour [Hohmann et al., 2009]. Also, polymorphic variants within the dopamine transporter gene (SLC6A3 or DAT) and the dopamine receptor 2 gene (DRD2) have also been associated with aggressive behaviour, externalizing behaviour, violence, criminal conduct and violent delinquency in children and adolescents [Young et al., 2002; Chen et al., 2005; Guo et
al., 2007; Beaver et al., 2008; Zai et al., 2012a]. The genes for vasopressin and for the oxytocin and vasopressin receptors (AVP, OXTR, AVPR1A and AVPR1B) have been associated with aggression in children [Malik et al., 2012, 2014; Zai et al., 2012b; Luppino et al., 2014]. Oxytocin and vasopressin encode neurohypophysial hormones with primary roles in sexual reproduction and in water retention, respectively, but they have also been related with different behavioural traits. Associations with other less studied genes were identified in children and adolescent samples, such as BDNF with aggressive behaviour [Kretschmer et al., 2014; Musci et al., 2014], CHRM2 and CYP19 with externalizing behaviour [Dick et al., 2011; Latendresse et al., 2011; Miodovnik et al., 2012] or SLIT2 and ESR1 with anger [Sokolowski et al., 2010; Vermeersch et al., 2013].

Candidate gene association studies evaluating CD and ODD in children and adolescents have also considered other genes related to serotonergic and dopaminergic neurotransmission (Table III). The COMT Val/Val genotype of the p.Val158Met polymorphism was found associated with CD [Caspi et al., 2008; Qian et al., 2009; DeYoung et al., 2010]. COMT encodes the enzyme catechol-o-methyltransferase, involved in the degradation of dopamine, epinephrine and norepinephrine. Also, the DRD4-7R allele was found associated with ODD, CD and callous unemotional (CU) traits [Kirley et al., 2004; Nikitopoulos et al., 2014; Zohsel et al., 2014]. DAT has been associated with ODD and conduct problems [Lee et al., 2007; Burt and Mikolajewski, 2008]. The genes for the serotonergic receptors HTR1B and HTR2A have been associated with CD and CU [Jensen et al., 2009; Moul et al., 2013]. Several variants within the OXTR gene have been associated with CD and CU [Beitchman et al., 2012; Malik et al., 2012; Sakai et al., 2012; Dadds et al., 2014; Smearman et al., 2015]. Also, associations have been described for BDNF with ODD and CU [Willoughby et al., 2013].

Candidate Genes Studied in Adults

Association studies with aggression traits in adults are summarized in Table 2. The Val/Val genotype of the p.Val158Met (rs4680G>A) polymorphism in the COMT gene has been associated with aggression, externalizing behaviour and anger. It has also been found to moderate the influence of childhood sexual abuse in these traits [Kulikova et al., 2008; Perroud et al., 2010; Shehzad et al., 2012]. However, other studies did not replicate these results [Flory et al., 2007; Kang et al., 2008; Albaugh et al., 2010]. Several associations have been reported for the serotonin receptor genes HTR1B and HTR2A in adult samples. [Berggard et al., 2003; Giegling et al., 2006; Zouk et al., 2007; Keltikangas-Jarvinen et al., 2008; Conner et al., 2010; Dijkstra et al., 2013; Hakulinen et al., 2013; Banlaki et al., 2015], but no significant associations were identified for HTR1A or HTR2C [Serretti et al., 2007; Keltikangas-Jarvinen et al., 2008; Perroud et al., 2010]. No consistent results were obtained for TPH1 and TPH2 genes in the susceptibility to aggressive behaviours [Manuck et al., 1999; Rotondo et al., 1999; Evans et al., 2000; Rujescu et al., 2002; Hennig et al., 2005; Reuter and Hennig, 2005; Mann et al., 2008; Yang et al., 2010; Yoon et al., 2012]. Associations with the nitric oxide synthase genes NOS1 and NOS3 have been reported for aggressive behaviours [Rujescu et al., 2008; Reif et al., 2009; Retz et al., 2010]. An androgen receptor (AR) haplotype has been associated with aggression, impulsivity, violent criminal behaviour and neuroticism, mostly in adult males [Jonsson et al., 2001; Cheng et al., 2006; Rajender et al., 2008; Westberg et al., 2009; Aluja et al., 2011]. Other less studied genes in adult samples are: ABCG1, AKAP5, ANK3, CDH13, CHRM2, CRHR1, CYP2D6, DARPP32,
DBH, TBX19, and TH. These have been associated with aggressive behaviours in one or a few studies [Persson et al., 2000; Gietl et al., 2007; Wasserman et al., 2007; Dick et al., 2008; Gonzalez et al., 2008; Hess et al., 2009; Reuter et al., 2009; Richter et al., 2011; Logue et al., 2013; Chen et al., 2014; Tiihonen et al., 2014].

Only a few association studies have been performed for antisocial behaviour and psychopathy (Table IV). Studies in which antisocial behaviour was assessed in alcoholic individuals or as an outcome of drug use are not considered here. Other less studied genes showed association with antisocial behaviour, conduct problems or psychopathy in adults are the ones encoding the androgen receptor (AR) and the estrogen receptor 1 (ESR1), and also BDNF, HTR2A, NR4A2, SNAP25 and TFAP2B [Westberg et al., 2003; Prichard et al., 2007; Burt and Mikolajewski, 2008; Basoglu et al., 2011; Kourmouli et al., 2013].

Genome-Wide Association Studies (GWAS)

GWAS studies of aggression have highlighted genes involved in synaptic plasticity, which had previously not been assessed by any candidate gene association study (Tables 2–4). None of the association signals reached genome-wide significance, but suggestive associations at $P = 1e-05$ will be discussed. Two GWAS have been performed on aggressive traits (Table 2). Mick et al. identified several genes that were nominally associated with aggressive behaviour scores in children, such as LRRC7 and STIP1. These genes are involved in neuronal excitability and astrocyte differentiation, respectively [Mick et al., 2011]. Another GWAS was performed in adults and identified 11 nominal association signals with anger ($P \leq 1e-05$). The most significant association was found with the FYN gene, involved in calcium influx and release in the postsynaptic density and also in long-term potentiation [Mick et al., 2014]. The long-term potentiation pathway could play a role in aggressive behaviours both in children and in adults, since FYN, LRRC7 and STIP1, as well as other nominally associated genes in the children GWAS, such as BDNF, NTRK2, and CAMK2A, are mediators in this pathway [Mick et al., 2011, 2014]. Another study assessed hostility in adolescents and in adult males and identified several SNPs that showed nominal associations with anger, some of them in the PURG and SHISA6 genes. However, little is known about the function of these genes [Merjonen et al., 2011].

GWAS studies in children have been performed for CD and CU traits (Table 3). Anney et al. performed a family-based genomewide study and identified nine genes that were associated with conduct problems: A2BP1, c12orf28, FLJ39061, KIRREL3, LOC729257, PAWR, PKD1L2, PKD1L3, and RGL1 [Anney et al., 2008]. A2BP1 and KIRREL3 encode proteins involved in neuron development and synaptic plasticity, respectively, and PAWR participates in the regulation of dopamine receptor D2 signaling. However, little is known about the function of the other genes in the brain. Another GWAS studied the interaction between genes and environmental risk factors (GxE). It found nominal associations between CD and mother’s warmth interacting with several variants in five genes: RIT1, ADH1C, SLC6A1, A2BP1, and MFHAS1 [Sonuga-Barke et al., 2008]. SLC6A1 codes for a GABA transporter, and the proteins encoded by RIT1 and A2BP1 are involved in neuronal development and regeneration. Interestingly, the latter also shows suggestive associations with CD the GWAS discussed above [Anney et al., 2008]. Hamshere et al. performed a meta-analysis of ADHD GWAS
data and observed that polygenic risk for ADHD was higher in ADHD with CD, and that was mainly associated with aggression [Hamshere et al., 2013]. Regarding CU, Viding et al. performed a two-stage GWAS, identifying several suggestive associations. Some SNPs that were associated with psychopathic traits in the discovery sample (all of them showing $10^{-5} < P < 0.05$) and that were nominally replicated were located in neurodevelopmental genes, such as ROBO2 [Viding et al., 2010]. One of the genes within the top-30 list is close to the serotonin receptor HTR1B, which had previously been found associated with CU traits, CD, childhood aggressive behaviour, impulsive aggression, anger and hostility [Zouk et al., 2007; Jensen et al., 2009; Conner et al., 2010; Hakulinen et al., 2013; Moul et al., 2013]. Finally, a GWAS that assessed antisocial behaviour in adults (Table 4) identified association with DYRK1A, which encodes a kinase with a role in synaptic plasticity and brain development [Tielbeek et al., 2012].

**Summary: Genetic Association Studies of Aggression**

Both CGAS and GWAS approaches have identified potential susceptibility genes for aggressive behaviours. Candidate gene studies have focused mainly in dopaminergic and serotonergic genes and have identified several associations in these (MAOA, 5HTT, HTR1B, HTR2A, DAT, DRD2, DRD4, etc.) and other systems (e.g., hormone-related genes like ESR1, AR, AVP or OXTR). However, most of these associations showed contradictory results or were identified in underpowered samples. Thus these results should be interpreted with caution. On the other hand, genome-wide studies, although not reaching genome-wide significance, have highlighted genes involved in neurodevelopmental processes and synaptic plasticity, not previously considered in candidate gene studies. This may indicate that aggressive behaviour does not only involve neurotransmitters or hormonal functions, but also molecules involved in establishing neuronal circuits, neuron-to-neuron connectivity and brain plasticity.

The lack of genome-wide significant findings in the GWAS and the variable results obtained from many of the GCAS is likely due to the small sample sizes of these studies and also to clinical and etiological heterogeneity of the patient groups studied. When assessing aggression-related phenotypes it may be relevant to separate the different phenotypes into more homogeneous groups (e.g., reactive versus proactive aggression) rather than considering them as a whole, since variability in the causes of each type of aggressive behaviour may dilute genetic susceptibility effects. In this review we have considered only those data obtained from studies in which aggressive behaviours could not be attributed to other psychiatric conditions, such as drug dependence, bipolar disorder or schizophrenia. For instance, a recent meta-analysis of violent or aggressive behaviours considered 277 associations in 31 genes and did not find any significant result, although GxE interactions were not considered. However, this meta-analysis included data from studies with very different phenotypic traits, psychiatric and neurological disorders, and probably that may have prevented from identifying significant associations [Vassos et al., 2014]. On the other hand, other meta-analyses identified associated the MAOA-uVNTR and 5HTTLPR polymorphisms [Byrd and Manuck, 2014; Ficks and Waldman, 2014].
Animal model studies of aggression

Aggression is an evolutionarily conserved behaviour that has been studied in many non-human species. This section of the review focuses on four species of animal models that have helped tremendously to shape the basis of our current understanding of neurobiological and molecular mechanisms underlying aggression: avian models, zebrafish, rodents and drosophila models. We particularly emphasize the utilities and potential of these animal model organisms for future genetic studies of aggression.

Avian Models of Aggression

As one of the earliest species used to study the biological basis of aggression, songbirds, demonstrate rich social behaviours such as territoriality, flock hierarchies and male dominance, as well as breeding and parenting behaviours. Most studies focused on offensive behaviour associated with territoriality. Defensive behaviours have been studied using intruders or subordinate birds. Study of songbirds behaviour and their hormonal and neuronal correlates have shaped our basic understanding of aggressive behaviour including, for example, the roles of plasma testosterone and hypothalamo-pituitary-gonadal (HPG) axis (see reviews [Adkins-Regan, 2005; Maney and Goodson, 2011]), and the serotonin and dopamine systems. In contrast to the large amount of behavioural, neurochemical and endocrine studies of songbirds over the last several decades, dissecting the genetic underpinnings of aggression has been scarce.

A naturally occurred segregation of high vs. low aggression with a plumage polymorphism in white-throated sparrow offers a unique opportunity for identifying causal genetic factors responsible for aggressive songbird phenotypes [Thorneycroft, 1966; Ficken et al., 1978]. Half of white-throated sparrows are heterozygous carriers of a rearranged chromosome 2 (ZAL2m); they have a white stripe in the crown and show high aggressive and poor parenting behaviours. Another half are homozygous for wild-type chromosomes (ZAL2); they are less aggressive, show normal parenting and have a tan stripe in the crown. Heterozygotes almost always mate with wild-type birds, which maintains the population structure. Horton et al reported a behavioural characterization of a homozygote female, demonstrating extremely aggressive and dominating behaviour and supporting the causal role of rearranged chromosome 2 in increased aggression [Horton et al., 2013]. However, it has taken nearly 30 years after the discovery of this phenotype to describe causal genes and variants in the affected regions [Davis et al., 2011; Huynh et al., 2011]. Among them a prime candidate gene is estrogen receptor 1 (ESR1), in which promoter polymorphisms linked with the rearranged chromosome were shown to regulate brain region-specific expression of ESR1 which was correlated with aggressive behaviour [Horton et al., 2014].

Rodent Models of Aggression

Rodents, including mouse, rat, hamster and prairie vole, are wellstudied models for aggressive behaviour due to controlled breeding, and their rich repertoire of species-specific social behaviours. Similar to many birds, rodents are also territorial. Adult male mice or rats will establish a territory when given sufficient living space and attack unfamiliar males intruding in their home cage. The
intruders will show defensive behaviours in response to the offensive attacks by the resident. In this classic resident-intruder test setting, both offensive aggression (resident) and defensive behaviour (intruder) can be studied [Mineur and Crusio, 2002]. Usually, the latency to initiate the first attack from the resident from the first sniff of the opponent is indicative of the aggressiveness of the resident.

Variations of the resident-intruder test are often used to evaluate the factors influencing aggressive behaviour. For example, social isolation (individual housing from days to weeks) can increase offensive aggression of male mice towards group-housed strangers [DaVanzo et al., 1986]. However isolation can also induce timidity in a small but considerable percentage of mice, which show alert and defensive postures, and behaviours such as running away, nonagonistic social interactions rather than delivering attack bites [Krsiak, 1975; DaVanzo et al., 1986]. The difference in social isolation induced abnormal aggressive behaviour in mice provides a model to study underlying genetic, hormonal and environmental factors. For example, cannabinoid CB1 receptor (CB1r) knockout mice showed lack of isolation-induced aggression, which was associated with higher expression of 5HT1Br, COMT and MAO-A in amygdala [Rodriguez-Arias et al., 2013]. Social isolation also disrupts immune function and enhances agonistic behaviour in prairie voles [Scotti et al., 2015]. Social-isolated rats show hyper-aroused behaviour during aggressive contacts, respond inappropriately to species-typical social cues and attack more aggressively by aiming at vulnerable body parts such as head, throat and belly. The enhanced abnormal aggressive behaviour was associated with significantly increased activation of brain regions that are known to regulate intermale aggression in rats [Toth et al., 2012].

For female mice or rats, a well-studied aggressive behaviour is maternal aggression. Female mice show enhanced aggression during the first two weeks of the post-partum period. The lactating female will attack male and female intruders to protect her litter. The attack bites of dominant females are usually directed towards the head and snout of opponents [Miczek et al., 2001]. These offensive attacks are usually fast and rarely preceded by anogenital investigation or threats; although sniffing the intruder’s genital area after an attack is also considered offensive aggressive behaviour. Sometimes, highly aggressive females will attack this vulnerable part. Maternal aggressive behaviour can also be defensive, for example piloerection and an upright posture in front of the intruder, boxing and holding down the intruder with her front legs, etc [Bosch and Meddle, 2005]. Neural manipulation studies showed that disrupting offensive attacks may not affect defensive expressions and vice versa, suggesting that the two categories of maternal aggressive behaviour are neurobiologically dissociable domains. However, some argued that all maternal aggression can be collectively categorized as defensive because the ultimate goal of such behaviour was to defend and protect the litter [Lonstein and Gammie, 2002]. Lonstein et al. thoroughly reviewed the neural circuitry underlying the maternal aggression and the sensory, hormonal and neurochemical control of the behaviour [Lonstein and Gammie, 2002]. A large number of studies have evaluated the roles of neuropeptides such as oxytocin, vasopressin and opioid, neurotransmitter systems such as dopamine, serotonin, GABA, as well as corticotrophin releasing hormone and nitric oxide in contributing to the presentation of maternal aggression in rodents (reviewed [Lonstein and Gammie, 2002].

Noxious and painful stimuli (for example electric shock) have been used to induce aggressive
bites in rodents, even in nonaggressive strains. However, the validity of such approaches is questioned in regard to human aggression. The tube dominance test is another standardized laboratory test that is commonly used to measure aggression and social dominance in rodents [Lindzey et al., 1961]. The test employs a transparent tube that allows two animals (mice or rats) to enter from opposite ends face to face and to interact in the center. Dominant animals will force the opponent to completely retreat from the tube. The numbers of winning vs. losing interactions are indicative of the dominance status. Defensive burying refers to a stereotypical response in rodents to a noxious stimuli (such as an electric shock-probe), demonstrated by shoving bedding material to bury the threats. Behaviours observed in a standardized shock-probe/defensive bury test such as burying, freezing, rearing, grooming and exploration are often used to measure anxiety levels and different coping strategies that are correlated with aggression phenotypes.

Strain differences in rodents (particularly mice) have clearly shown that aggressive phenotypes are inherited. Several genetic tools have been developed for rodent models to study the molecular and biological mechanisms underlying aggressive behaviour. The earliest one was artificial breeding. Using standardized behavioural testing paradigms, artificial selective breeding was carried out to produce contrasting inbred strains with high vs. low aggression scores. These inbred strains include the Finland Turku aggressive (TA) and non-aggressive (TNS) strains [Sandnabba, 1996], the North Carolina NC900 and NC100 strains [Caramaschi et al., 2007], and the Netherlands short attack latency (SAL) and long attack latency (LAL) mice [van Oortmerssen and Bakker, 1981]. Cross-fostering and the post-natal environment do not alter the development of aggression in these mouse lines, further supporting the genomic etiology of their aggression. The TA and TNS lines demonstrated Mendelian segregation and autosomal inheritance [Sandnabba, 1996]. The Y chromosome was found to play a role in the difference of attack latencies between the SAL and LAL lines [Sluyter et al., 1995; Sluyter et al., 1997]. Several naturally developed inbred lines with different levels of aggression were also recognized as useful models for studying the genetics of aggression. For example, the FVB/NtacfBR male shows more aggression toward females when compared with C57BL/6J males [Canastar and Maxson, 2003]; the NZB/B1NJ strain shows extremely high inter-male aggression, whereas A/J mice rarely show any aggressive behaviour [Roubertoux and Guillot, 2005]. A useful summary of commonly used inbred mouse lines was provided by Crawley et al. who compared a wide variety of behavioural traits including aggression, anxiety and parental behaviours based on an extensive literature review [Crawley et al., 1997].

Like the studies of songbirds, studies of these inbred strains in the past three decades have helped our understanding of neural circuitry, hormonal and neurochemical correlates for different domains of aggressive behaviour. See reviews [Miczek et al., 2001]. However, identification of causal genetic determinants has not been fruitful. A few attempts have been made to identify quantitative trait loci (QTLs) underlying differences in aggressive phenotypes between inbred lines. QTL analysis showed that aggressive attacks measured in different testing conditions, for example the inter-male aggression and isolation induced aggression, have overlapping, yet different genetic contributions [Roubertoux and Guillot, 2005]. This observation supports the distinction of different domain/categories of aggressive behaviour and highlights the complexity of underlying genetic causality. However, we are still far away from pinpointing the causal genes within these QTL regions which
often contain hundreds of genes. New analytic methodologies have recently been used to uncover such complex genetic causes of aggression. Malki et al. [2014] used a weighted gene co-expression network analysis (WGCNA) method to examine transcriptome-wide differences between the three inbred mouse lines with high vs. low aggression levels. They uncovered two important pathways involving NF-kB and MAPKs. The study also yielded 14 differentially expressed genes from the two significant pathways as plausible candidates and some of them, such as Adbk2, had previously been implicated in aggressive behaviour. Since gene expression is an unbiased approach, identifying previously implicated candidate genes confirms the biological relevance of those co-expression networks in mouse aggressive phenotypes. Although we still have not pinpointed the genetic determinants underlying the differences in aggression between those inbred models, we are one step closer towards understanding the complex genetic networks that are underlying the phenotypes.

Another useful genetic approach is single gene manipulation, i.e., transgenic and gene knockout or mutations, particularly in mice. A detailed review of earlier genetic knockout studies has been provided elsewhere [Takahashi and Miczek, 2014]. We performed an updated PubMed search using keywords of “Knockout AND (Mice OR Mouse) AND (aggressive behaviour) OR aggression)” and retrieved 265 articles on non-human animals. After filtering through title, abstract and full texts, we summarized 85 genes that altered one or more subtypes of aggressive behaviour in knockout mice (or were silenced by siRNA, see Table 5). Many of these genes regulate sensory, hormonal and neurochemical/neurotransmitter systems and neurodevelopmental processes. KO mice phenotype information can also easily searched through databases such as Mouse Phenome Database at The Jackson Laboratory and currently 50 strains of mutant mice with abnormal aggressive behaviour are available from the Jackson Laboratory inventory.

In this section, we give some classical examples and highlight the advantages and limitations of the single gene approach. For example, gene knockouts of 5-HT neuron-specific transcription factor Pet-1 or tryptophan hydroxylases 2 (TPH2) lead to enhanced offensive aggression in resident-intruder tests accompanied by reduced 5-HT content or 5-HT neural activities [Hendricks et al., 2003; Alenina and Kikic, 2009; Angoa-Perez et al., 2012; Mosienko et al., 2012]. Knockout of alpha-calcium-calmodulin– dependent kinase II (a-CaMKII) induced a decreased fear response and an increase in defensive aggression accompanied by reduced serotonin release in dorsal raphe neurons [Chen et al., 1994]. In contrast, knockout of the monoamine oxidase A (MAOA) gene increased brain 5-HT content. In humans, deficiency of MAOA causes Brunner syndrome characterized by impulsive aggressiveness [Brunner and Nelen, 1993]. MAOA knockout mice also display enhanced aggression toward intruder mice [Scott et al., 2008], but reduced defensive behaviour in the presence of predatorrelated cues [Godar et al., 2011]. These examples show the complexity of the genetic mechanisms underlying different aggression domains and also highlight the limitations of the single gene approach.

Manipulation of a single gene produces a cascade of expression and biochemical changes during development, which interact with environmental factors and other genetic factors. For example MAOA knockout mice showed enhanced expression of NMDA receptor subunit 2A and 2B expression in the prefrontal cortex and their abnormal aggressive behaviour can be selectively countered by administration of NMDAR antagonists [Bortolato and Godar, 2012]. This showed a
critical role of NMDA receptor in the pathogenesis of escalated aggression among MAOA knockout mice. Consistent with this, an NR1 subunit deficient mouse line shows reduced social investigation and lack of species-typical aggressive behaviour in a resident-intruder paradigm [Mohn et al., 1999; Duncan et al., 2004]. Therefore, interpretation of single gene knockout studies needs to be cautious and take into consideration downstream and compensatory changes in the context of the whole organism.

Two species of voles distinct in their social behaviours exist as a perfect model to study genes and aggression. Prairie and pine voles are highly social and monogamous, whereas meadow and montane voles are asocial and promiscuous [Insel and Shapiro, 1992; Young and Wang, 2004]. Prairie voles develop pair bonds between mates. Males display intense aggression toward female or male conspecific strangers in the resident-intruder paradigm but they maintain a high level of social affiliation with their familiar female partners [Aragona and Liu, 2006; Gobrogge et al., 2007]. Although similar in nonsocial behaviours, nonmonogamous vole species do not show partner preference or increased aggression towards stranger conspecifics [Insel et al., 1995]. Species comparisons show that polymorphisms in the arginine vasopressin (AVP) receptor gene, V1aR, were associated with distinct patterns of gene expression in the brain associated with differences in pair bonding and selective aggression of voles [Lim et al., 2004; Hammock et al., 2005; Ophir et al., 2008]. Genetic variations of V1aR and plasma levels of AVP were also associated with human social behaviours including aggression and partner relationships [Walum et al., 2008; Gouin et al., 2012; Luppino et al., 2014].

**Drosophila Models of Aggression**

Aggressive behaviour in the fruit fly, Drosophila melanogaster, has been observed since 1915 when first reported by Sturtevant [Sturtevant, 1915]. Males spread their wings and engage in antagonistic encounters when competing for mating females. Both offensive and defensive behaviours have been observed. Drosophila’s nervous system is simple but recapitulates a range of cellular and network properties relevant to humans. With modern genetic tools for drosophila, this model system has made significant contributions to our genetic understanding of aggressive behaviour. Similar approaches that we described for rodent models, such as artificial selection, QTL mapping and single gene manipulation, have been used in drosophila research. A detailed summary of these studies and the genetic, pheromonal regulation, neurobiological and genetic regulation of aggressive behaviour has been reviewed elsewhere [Dahanukar and Ray, 2011; Zwarts et al., 2012; Fernandez and Kravitz, 2013]. In this section we highlight several recent significant contributions.

Edwards et al. compared the transcriptomes of high vs. low aggression drosophila lines. They identified 1593 probe sets that were differentially regulated in these lines [Edwards et al., 2006]. Remarkably, out of 19 genes selected for behavioural validation using genomic manipulation in an isogenic background, 15 showed significant effects in altering aggressive behaviours after Bonferroni corrections. These genes are involved in diverse biological processes, including electron transport, catabolism, nervous system development and G-protein coupled receptor signaling. Seven were computationally predicted genes and none had been previously implicated in aggressive behaviour. Dierick and Greenspan also examined the gene expression between the high aggression and neutral
Among the significantly, differentially expressed genes, a cytochrome gene, Cyp6a20 that might be involved in pheromone degradation, was confirmed to directly regulate aggressive behaviour by using a mutant line and an odor-binding protein. Obp56a, showed the most robust reduction in expression in the aggressive line [Dierick and Greenspan, 2006].

High-throughput and automated behavioural assays were developed to measure drosophila social behaviour including aggression, enabling larger scale genetic correlations with the behaviour [Hoyer et al., 2008; Dankert et al., 2009]. Forty inbred lines were quantified for aggressive behaviour and genome-wide association screens for quantitative trait transcripts were performed on these lines [Edwards et al., 2006]. Two hundred sixty-six novel candidate genes associated with aggressive behaviour were identified. Nine genes were confirmed to show altered aggression from behavioural evaluation of 12 selected candidate genes [Edwards et al., 2006]. Furthermore, a network based co-expression analysis revealed functional modules of correlated transcripts that were associated with variations of aggressive behaviour. Table VI, lists the candidate genes for aggression implicated by drosophila studies. We also included the genes that were identified through the above describe expression analysis and were confirmed by behaviour changes on the mutant lines. Of note, none of these genes have been implicated in human aggression. More recently, collective efforts were made to generate 192 genome-sequenced inbred lines derived from a single Raleigh population. The drosophila melanogaster Genetic Reference Panel (DGRP) was constructed to share these inbred lines and their genetic data [Mackay et al., 2012]. DGRP provides powerful resources for mapping genotype-phenotype relationships. Taking the advantage of the DGRP resources and standardized quantitative behavioural assays, a GWAS study for aggressive behaviour was conducted. 74 common variants in 39 genes were reported as significant association candidates and one SNP in the intron of CG14869 (AdamTS-A) met the genome-wide significance threshold (2.61 x 10^-8) [Shorter et al., 2015]. Only one significant candidate gene association, 5-HT1A, had been previously implicated in aggression. Additionally, 22 genes harboring rare variants were significantly associated with aggressive behaviours and 10 passed Bonferroni corrections. None of these genes had been implicated in aggression previously [Shorter et al., 2015]. The same paper also described an extreme QTL GWA study of the advanced intercross populations (AIPs) derived from the most and least aggressive DGRP lines. This approach identified 746 SNPs in or near 355 genes with significant association, of which 22 passed Bonferroni corrections. The top genes included some in the serotonin, dopamine and glutamate pathways, consistent with the well-known roles of these genes in aggression. Due to the large number of genes with significant associations, these are not included in Table VI. See the original reference for the complete list of genes and variants [Shorter et al., 2015]. Surprisingly, this list of genes has almost no overlap with the GWA results from the original DGRP lines. Despite this non-overlap in genes and variants, two results were mapped and enriched onto a genetic interaction network inferred from an analysis of pairwise epistasis in the DGRP lines [Shorter et al., 2015]. This
### Table 5. Genes implicated in aggressive phenotype in knockout mice studies.

<table>
<thead>
<tr>
<th>Gene Names</th>
<th>Human Homolog</th>
<th>Aggression phenotype/domain</th>
<th>Studied for aggression in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVP receptor V1aR (Avpr1a)</td>
<td>AVPR1A</td>
<td>Social aggression was unaffected in KO mice(Wersinger, Caldwell et al. 2007)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>vasopressin 1b receptor (Avpr1b)</td>
<td>AVPR1B</td>
<td>Avpr1b gene knockout affected social memory, reduced inter-male aggression and maternal aggression(Toth, Tulogi et al. 2012, Scotti, Carlton et al. 2015)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>Corticotropin-releasing factor receptor type 1</td>
<td>CRHR1</td>
<td>Gene deficiency reduces maternal aggression(Gammie, Bethea et al. 2007)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>Corticotropin-releasing factor receptor type 2</td>
<td>CRHR2</td>
<td>KO mice showed increased aggression (Coste, Heard et al. 2006) and reduced maternal aggression(Gammie, Hasen et al. 2005)</td>
<td>No human studies</td>
</tr>
<tr>
<td>corticotropin releasing hormone binding protein</td>
<td>CRHBP</td>
<td>Gene Knockout specifically impaired maternal aggression(Gammie, Seasholtz et al. 2008)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Aromatase P450 (CYP19)</td>
<td>CYP19</td>
<td>KO male exhibited a complete loss of aggressive behavior (Toda, Saibara et al. 2001)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>estrogen receptor- (ER )</td>
<td>ESR1</td>
<td>Reduction of ER expression in preoptic neurons significantly increased aggression toward both sexual partners and male intruder(Ribeiro, Musatov et al. 2012)</td>
<td>Yes (Tables 2 and 4)</td>
</tr>
<tr>
<td>Estrogen receptor-beta(Esr2)</td>
<td>ESR2</td>
<td>Gene disruption elevated aggression levels(Nomura, Andersson et al. 2006)</td>
<td>No human studies</td>
</tr>
<tr>
<td>growth hormone releasing hormone(Ghrh)</td>
<td>GHRH</td>
<td>Gene knockout reduces aggressiveness(Sagazio, Shoreh et al. 2011)</td>
<td>No human studies</td>
</tr>
<tr>
<td>melanocortin-5 receptor (MC5R)</td>
<td>MC5R</td>
<td>MC5R deficiency disinhibits an aggression-suppressing pheromonal signal(Morgan, Thomas et al. 2004)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Steroidogenic factor 1 (SF1), or Nuclear Receptor Subfamily 5, Group A, Member 1 (NR5A1)</td>
<td>NR5A1</td>
<td>KO mice were significantly more aggressive (Grigurevic, Budelfeld et al. 2008)</td>
<td>No human studies</td>
</tr>
<tr>
<td>oxytocin (OT)</td>
<td>OT</td>
<td>KO mice showed reduced aggression and increased social investigation(Lazzani, Becker et al. 2013); enhanced offensive aggression and infanticidal behavior were observed in KO mice(Ragnauth, Devidze et al. 2005)</td>
<td>OT reduces reactive aggression in state anxious women(Campbell and Hausmann 2013)</td>
</tr>
<tr>
<td>Oxytocin receptor (Oxtr)</td>
<td>OXTR</td>
<td>Male Oxtr/- mice had elevated levels of aggression(Dhakar, Rich et al. 2012)</td>
<td>Yes (Tables 2 and 4)</td>
</tr>
<tr>
<td>Granulin (Grn), progranulin (Pgrn)</td>
<td>PGRN</td>
<td>Pgrn-deficient mice showed enhanced aggressiveness to intruders (Kayasuga, Chiba et al. 2007)</td>
<td>A missense mutation in PGRN gene was found in a patient of frontotemporal dementia with aggressiveness and abnormal sexual behavior(Rainero, Rubino et al. 2011)</td>
</tr>
<tr>
<td>Melanin-concentrating hormone (MCH)</td>
<td>PMCH</td>
<td>Mch Ko mice showed abnormal olfactory behaviors and male showed increased aggression(Adams, Domouzoglou et al. 2011)</td>
<td>No human studies</td>
</tr>
<tr>
<td>prostaglandin E receptor subtype EPi (Ptger1)</td>
<td>PTGER1</td>
<td>KO mice showed impulsive aggression (Matsuoka, Furuhashi et al. 2005)</td>
<td>No human studies</td>
</tr>
<tr>
<td>steroid-5-alpha-reductase, alpha polypeptide 1 (Srda1)</td>
<td>SRD5A1</td>
<td>KO mice is lack of testosterone induced aggression(Frye, Rhodes et al. 2002)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Thyroid Stimulating Hormone Receptor(Tshr)</td>
<td>TSHR</td>
<td>TSHR KO mice show ADHD phenotype with increased aggression (Mouri, Hoshino et al. 2014)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Urocortin 2 (Ucn2)</td>
<td>UCN2</td>
<td>Male UCN2 null mice showed reduced aggressiveness(Breu, Touma et al. 2012)</td>
<td>No human studies.</td>
</tr>
</tbody>
</table>
### Table 5. Genes implicated in aggressive phenotype in knockout mice studies. (continued)

<table>
<thead>
<tr>
<th>Gene Names</th>
<th>Human Homolog</th>
<th>Aggression phenotype/domain</th>
<th>Studied for aggression in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurochemical and Neurotransmitter systems</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholinesterase (AChE)</td>
<td>ACHE</td>
<td>AChE KO mice were lack of aggressive behavior (Duyssen, Stribley et al. 2002)</td>
<td>No human studies</td>
</tr>
<tr>
<td>adenosine receptor A1 (Adora1)</td>
<td>ADORA1</td>
<td>Mice lacking the adenosine A1 receptor are anxious and aggressive (Gimenez-Lloret, Fernandez-Teruel et al. 2002)</td>
<td>No human studies</td>
</tr>
<tr>
<td>adenosine A2a receptor</td>
<td>ADORA2A</td>
<td>KO male showed enhanced aggression towards intruder (Ledent, Vaugeois et al. 1997)</td>
<td>No human studies</td>
</tr>
<tr>
<td>adrenocortical alpha 2C (Adra2c)</td>
<td>ADRA2C</td>
<td>KO mice showed increased aggression (Scheinin, Sallinen et al. 2001)</td>
<td>No human studies</td>
</tr>
<tr>
<td>cannabinoid CB1 receptors (Cnr1)</td>
<td>CNR1</td>
<td>KO mice housed in groups showed higher levels of offensive aggression, and lack of isolation induced enhance in aggression (Rodriguez-Arias, Navarrete et al. 2013)</td>
<td>No human studies</td>
</tr>
<tr>
<td>dopamine beta-hydroxylase knockout (Dbh)</td>
<td>DBH</td>
<td>DBH KO mice showed absence of resident-intruder aggression (Marino, Bourdelat-Parks et al. 2005)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>Dopamine D2 receptor (Drd2)</td>
<td>DRD2</td>
<td>DRD2 long isofrom KO mice showed reduced aggression (Yukh, Sankoorikal et al. 2001)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>glutamic acid decarboxylase (GAD65)</td>
<td>GAD2</td>
<td>KO mice showed reduced intermale aggression (Stork, Ji et al. 2000)</td>
<td>No human studies</td>
</tr>
<tr>
<td>glutamate receptor, ionotropic, AMPA 3 (Gria3)</td>
<td>GRIA3</td>
<td>GluA3-deficient mice showed an increase in isolation-induced male aggression (Adamczyk, Mejias et al. 2012)</td>
<td>No human studies</td>
</tr>
<tr>
<td>glutamate delta-1 receptor (Grid1)</td>
<td>GRID1</td>
<td>KO mice showed robust aggression in the resident-intruder test (Yadav, Gupta et al. 2012)</td>
<td>No human studies</td>
</tr>
<tr>
<td>5-hydroxytryptamine (serotonin) receptor 1B (Htr1B)</td>
<td>HTR1B</td>
<td>KO mice showed increased aggression towards intruder (Bouwknec, Hizjen et al. 2001)</td>
<td>Yes (Tables 2 and 3)</td>
</tr>
<tr>
<td>monoamine oxidase A (Maoa)</td>
<td>MAOA</td>
<td>KO mice display enhanced aggression toward intruder mice (Scott, Bortolato et al. 2008), but showed reduction of defensive and fear-related behaviours (Goder, Bortolato et al. 2011).</td>
<td>Yes (Tables 2, 3 and 4)</td>
</tr>
<tr>
<td>membrane metalloendopeptidase (Mme)</td>
<td>MME</td>
<td>KO mice showed enhanced aggression to intruder (Fischer, Zernig et al. 2000)</td>
<td>No human studies</td>
</tr>
<tr>
<td>NPY1R, neuropeptide Y receptor Y1 (Npyy1)</td>
<td>NPY1R</td>
<td>Receptor deletion resulted in increase in territorial aggression (Karl, Lin et al. 2004)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Enkephalins (Enk), Proenkephalin (Penk)</td>
<td>PENK</td>
<td>ENK KO mice showed increased offensive aggression (Konig, Zimmer et al. 1996)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Solute Carrier Family 6 (Neurotransmitter Transporter), Member 1 (Slc6a1)</td>
<td>SLCA61</td>
<td>GABA transporter 1 KO mice showed reduced aggression (Liu, Liu et al. 2007)</td>
<td>Yes (Table 3)</td>
</tr>
<tr>
<td>solute carrier family 6 (neurotransmitter transporter), member 3 (dopamine transporter) (Slc6a3, Dat1)</td>
<td>SLC6A3/DAT1</td>
<td>DAT1 KO mice exhibited increased aggression (Rodriguez, Chu et al. 2004)</td>
<td>Yes (Tables 2 and 3)</td>
</tr>
<tr>
<td>solute carrier family 6 (5-HT transporter), member 4, Slc6a4</td>
<td>SLC6A4/5-HTT</td>
<td>5-HT transporter 5-HTT knockout mice showed reduced maternal aggression (Heiming, Monning et al. 2013)</td>
<td>Yes (Tables 2, 3 and 4)</td>
</tr>
<tr>
<td>tryptophan hydroxylase 2 (Tph2)</td>
<td>TPH2</td>
<td>Mice lacking Tph2 (and brain 5HT) show intense compulsive and impulsive behaviors to include extreme aggression (Angoa-Perez, Kane et al. 2012)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>Nerve System Development</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brain-derived neurotrophic factor (Bdnf)</td>
<td>BDNF</td>
<td>KO mice exhibited elevated conspecific aggression and social dominance (Ito, Chehab et al. 2011)</td>
<td>Yes (Tables 2, 3 and 4)</td>
</tr>
<tr>
<td>alpha-calcium/calmodulin-dependent protein kinase II (Camk2a)</td>
<td>CAMK2A</td>
<td>Camk2a overexpression increases offensive aggression (Hasegawa, Furuichi et al. 2009)</td>
<td>No human studies</td>
</tr>
<tr>
<td>calcium channel, voltage-dependent, N type, alpha 1B (Cacna1b)</td>
<td>CACNA1B</td>
<td>Gene KO enhanced aggressive behavior to the intruder (Kim, Jeon et al. 2009)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Gene Names</td>
<td>Human Homolog</td>
<td>Aggression phenotype/domain</td>
<td>Studied for aggression in humans</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------------</td>
<td>----------------------------------------------------------------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>calcium channel, voltage-dependent, beta 3 subunit(Cacnb3)</td>
<td>CACNB3</td>
<td>Null mice showed increased aggression (Murakami, Nakagawasai et al. 2007)</td>
<td>No human studies</td>
</tr>
<tr>
<td>cell adhesion molecule 1 (Cadm1)</td>
<td>CADM1</td>
<td>KO mice showed excessive aggression and anxiety (Tanabe, Fujita et al. 2013)</td>
<td>No human studies</td>
</tr>
<tr>
<td>CREB-regulated transcription coactivator 1 (Crtc1)</td>
<td>CRTC1</td>
<td>Crtc1(+/-) mice exhibit impulsive aggressiveness and many other behavioral abnormalities (Breuillaud, Rossetti et al. 2012)</td>
<td>No human studies</td>
</tr>
<tr>
<td>ENGRAILED 2 (EN2)</td>
<td>EN2</td>
<td>KO mice displayed reduced aggression (Cheh, Millonig et al. 2006)</td>
<td>No human studies</td>
</tr>
<tr>
<td>v-erb-b2 avian erythroblast leukemia viral oncogene homolog 4 (Erbb4) and 2 (Erbb2)</td>
<td>ERBB2 and ERBB4</td>
<td>ErbB2/B4-deficient mice show increased aggression (Barros, Calabrese et al. 2009)</td>
<td>No human studies</td>
</tr>
<tr>
<td>protein FEV (Fev) or plasmacytoma-expressed transcript 1 (Pett)</td>
<td>FEV (or PET1)</td>
<td>Pet-1 plays a critical role in 5-HT neuron development and is required for normal anxiety-like and aggressive behavior (Hendricks, Fyodorov et al. 2003)</td>
<td>No human studies</td>
</tr>
<tr>
<td>GDP Dissociation Inhibitor 1 (Gdi1)</td>
<td>GDI1</td>
<td>Gdi1-deficient mice show lowered aggression (D'Adamo, Welzl et al. 2002)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Neuronal Immediate Early Gene, 1 (Homeri)</td>
<td>HOMER1</td>
<td>Heterozygous mice showed increased aggression (Jaubert, Golub et al. 2007)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Densin-180, leucine rich repeat containing 7 (Lrrc7)</td>
<td>LRRC7</td>
<td>KO male showed enhanced aggression (Carlisle, Luong et al. 2011)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>limbic system-associated membrane protein (Lsamp)</td>
<td>LSAMP</td>
<td>KO mice showed reduced aggressiveness and reduced dominance (Innos, Philips et al. 2011)</td>
<td>No human studies</td>
</tr>
<tr>
<td>neural cell adhesion molecule (Ncam)</td>
<td>NCAM</td>
<td>NCAM deletion increased inter-male aggression and altered emotionality (Kohl, Riccio et al. 2013)</td>
<td>No human studies</td>
</tr>
<tr>
<td>methyl-CpG binding protein 2 (MeCP2)</td>
<td>MECP2</td>
<td>MeCP2 conditional knockout (CKO) mice were aggressive, hyperphagic, and obese (Fyffe, Neul et al. 2008).</td>
<td>A patient with Rett syndrome demonstrated episodes of uncontrolled aggression (Huppke, Maier et al. 2006)</td>
</tr>
<tr>
<td>neuregulin-1 (Nrg1)</td>
<td>NRG1</td>
<td>Mutant animals demonstrated increased aggressive following (O'Tuathaigh, O'Connor et al. 2008).</td>
<td>No human studies</td>
</tr>
<tr>
<td>Neuronal nitric oxide synthase (nNOS, Nos1)</td>
<td>NOS1</td>
<td>nNOS knockout mice were significantly more aggressive than wild type (Trainor, Workman et al. 2007)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>Taillless, nuclear receptor subfamily 2, group E, member 1 (Nr2e1)</td>
<td>NR2E1</td>
<td>Deletion of Taillless gene produced highly aggressive phenotype (Juarez, Valdovinos et al. 2013)</td>
<td>NR2E1 showed forebrain-specific expression and may be associated with bipolar disorder, schizophrenia, or aggressive disorders (Kumar, McChee et al. 2008)</td>
</tr>
<tr>
<td>neurexin 1 (Nrxn1)</td>
<td>NRXN1</td>
<td>Knockout increased intermale aggression (Grayton, Missler et al. 2013)</td>
<td>Gene mutations were found in autism and intellectual disabilities (Yangngam, Plong-On et al. 2014).</td>
</tr>
<tr>
<td>neuronal PAS domain protein 4 (Npas4)</td>
<td>NPAS4</td>
<td>Ko mice spend more time avoiding an unfamiliar male during a first encounter, showed higher social dominance than their WT littermates (Coutellier, Beraki et al. 2012).</td>
<td>No human studies</td>
</tr>
<tr>
<td>p21-activated kinase (Pak4, Pak5, and Pak6)</td>
<td>PAK4, PAK5, and PAK6</td>
<td>All the knockout genotypes were found to be less aggressive (Furnari, Jobes et al. 2013)</td>
<td>No human studies</td>
</tr>
<tr>
<td>ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 2 (St8sia2)</td>
<td>ST8SIA2</td>
<td>KO mice displayed both a decreased social motivation and an increased aggressive behavior (Calandreau, Marquez et al. 2010)</td>
<td>No human studies</td>
</tr>
</tbody>
</table>
Table 5. Genes implicated in aggressive phenotype in knockout mice studies. (continued)

<table>
<thead>
<tr>
<th>Gene Names</th>
<th>Human Homolog</th>
<th>Aggression phenotype/domain</th>
<th>Studied for aggression in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Olfactory and other sensory systems</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>alpha-1,3 galactosyltransferase gene (Ggta1)</td>
<td>A3GALT2</td>
<td>Increased aggression in KO mice(Sorensen, Dahl et al. 2008)</td>
<td>No human studies</td>
</tr>
<tr>
<td>acid-sensing ion channel 3 (Asic3)</td>
<td>ASIC3</td>
<td>Gene KO reduced aggressiveness(Wu, Lin et al. 2010)</td>
<td>No human studies</td>
</tr>
<tr>
<td>beta2-microglobulin (B2m)</td>
<td>B2M</td>
<td>B2M deficient mice show specific defect in inter-male aggression(Loconto, Papes et al. 2003)</td>
<td>No human studies</td>
</tr>
<tr>
<td>transient receptor potential cation channel, subfamily C, member 2 (Trpc2)</td>
<td>N/A</td>
<td>Trpc2 knockout mice is lack of male-male aggression(Miller 2014)</td>
<td>N/A</td>
</tr>
<tr>
<td>cyclic nucleotide-gated channel alpha2 (Cng2)</td>
<td>CNGA2</td>
<td>Knockout mice failed mate or fight(Mandiyan, Coats et al. 2005)</td>
<td>No human studies</td>
</tr>
<tr>
<td>mitogen-activated protein kinase 7 (Mapk7)</td>
<td>MAPK7</td>
<td>Conditional deletion of the Mapk7 gene in neural stem cells impairs several pheromone-mediated behaviors including aggression and mating in male mice(Zou, Storm et al. 2013)</td>
<td>No human studies</td>
</tr>
<tr>
<td>guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O (Gnao1)</td>
<td>GNAO1</td>
<td>G protein G(alpha)o is essential for vomeronasal function and aggressive behavior in mice. (Chamero, Katsoulidou et al. 2011)</td>
<td>No human studies</td>
</tr>
<tr>
<td>olfactory G-protein -subunit G8 (Gng8)</td>
<td>GNG8</td>
<td>Gene knockout reduced pheromone-mediated aggressiveness in both males and females, with other socio-sexual behaviours remaining unaltered(Montani, Tonelli et al. 2013)</td>
<td>No human studies</td>
</tr>
<tr>
<td>kin of IRRE like 3 (Kirrel3)</td>
<td>KIRREL3</td>
<td>Kirrel3(-/-) mice display a loss of male-male aggression(Prince, Brignall et al. 2013)</td>
<td>No human studies</td>
</tr>
<tr>
<td>prepronociceptin</td>
<td>PNOC</td>
<td>Group housed KO mice showed enhanced aggression under competitive conditions(Ouagazzal, Moreau et al. 2003)</td>
<td>No human studies</td>
</tr>
<tr>
<td>pituitary adenylate cyclase-activating polypeptide (Pacap)</td>
<td>PACAP</td>
<td>Social isolation induced aggressive behavior in KO mice but not in WT mice(Fishihama, Ago et al. 2010)</td>
<td>No human studies</td>
</tr>
<tr>
<td>pituitary adenylate cyclase-activating polypeptide (PACAP) type 1 receptor (PAC1)</td>
<td>PAC1</td>
<td>PAC1-deficient males displayed reduced aggression and increased mounting towards males(Nicot, Otto et al. 2004)</td>
<td>No human studies</td>
</tr>
<tr>
<td>potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3 (Kcnn3)</td>
<td>KCNN3</td>
<td>KO mice showed deficits in mating and aggressive behaviors(Kim, Ma et al. 2012)</td>
<td>No human studies</td>
</tr>
<tr>
<td>potassium inwardly-rectifying channel, subfamily J, member 3 (Kcnj3)</td>
<td>KCNJ3</td>
<td>KO mice showed deficits in mating and aggressive behaviors(Kim, Ma et al. 2012)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Vomeronasal Type-1 Receptor 1 (Vn1r1)</td>
<td>VNR1</td>
<td>Mice with deletion of a cluster of V1r genes display abnormal inter-male and maternal aggression(Del Punta, Leinders-Zufall et al. 2002)</td>
<td>No human studies</td>
</tr>
<tr>
<td><strong>Other unspecified genes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>early growth response 3 (Egr3)</td>
<td>EGR3</td>
<td>Gene KO increased offensive aggression towards the intruder(Gallitano-Mendel, Wozniak et al. 2008)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Glycogen synthase kinase-3 alpha (Gsk3a)</td>
<td>GSK3A</td>
<td>Mutant mice showed reduced aggression(Kaidanovich-Beilin, Lipina et al. 2009)</td>
<td>No human studies</td>
</tr>
<tr>
<td>TNF receptor type 1 and type 2 (Tnfrsf1a and Tnfrsf1b)</td>
<td>TNFRLF1A and TNFRLF1B</td>
<td>Combined deletion of two receptors resulted in a lack of aggressive behavior(Patel, Siegel et al. 2010)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Heat shock factor 1 (Hsf1)</td>
<td>HSF1</td>
<td>HSF1 deficiency increased aggression(Uchida, Hara et al. 2011)</td>
<td>No human studies</td>
</tr>
<tr>
<td>maternally imprinted/paternally expressed gene, Peg3</td>
<td>PEG3</td>
<td>KO animal showed higher maternal aggression(Champagne, Curley et al. 2009)</td>
<td>No human studies.</td>
</tr>
<tr>
<td>Prion protein (Prnp)</td>
<td>PRNP</td>
<td>Prnp knockout showed enhanced offensive aggression(Budefeld, Majer et al. 2014)</td>
<td>3’UTR polymorphism was associated with increased risk for delusions, anxiety, agitation/aggression(Flirski, Sieruta et al. 2012)</td>
</tr>
</tbody>
</table>
### Table 6. Drosophila Genes for Aggression

<table>
<thead>
<tr>
<th>Genes</th>
<th>Human Homolog</th>
<th>Methods in Fly</th>
<th>Phenotype</th>
<th>Fly Reference</th>
<th>Studied in Human aggression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neurochemical and neurotransmitter systems</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyramine-α-hydroxylase (TαH)</td>
<td>dopamine beta-hydroxylase (DBH)</td>
<td>Mutant line</td>
<td>Null animal show reduced Intermale aggression and maternal aggression</td>
<td>(Hoyer, Eckart et al. 2008)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>Tyrosine decarboxylase, neuronal (Tdc2)</td>
<td>N/A</td>
<td>Mutant line</td>
<td>Mutant fly showed reduced inter-male aggression</td>
<td>(Hoyer, Eckart et al. 2008)</td>
<td>N/A</td>
</tr>
<tr>
<td>Tryptophan hydroxylase (Trh)</td>
<td>tryptophan hydroxylase 1 (TPH1)</td>
<td>Overexpression</td>
<td>Overexpression increased aggression, escalated aggression</td>
<td>(Dierick and Greenspan 2007, Alekseyenko, Lee et al. 2010)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td>5-HT receptors (5-HT2a, 5-HT1A-like)</td>
<td>5-HT receptors (HTR2A, HTR1A)</td>
<td>Pharmacological manipulation</td>
<td>Activation of 5-HT2 receptors decreases overall aggression, activation of 5-HT1A-like receptors increases aggression. Different aspects of aggression was also affected by different receptor subtypes.</td>
<td>(Johnson, Becnel et al. 2009)</td>
<td>Yes (Tables 2, 3)</td>
</tr>
<tr>
<td>Dopa decarboxylase (Ddc),</td>
<td></td>
<td>Genetic inactivation of Ddc-neurons</td>
<td>Inactivation of Ddc-neurons eliminated mid- and high-level aggression</td>
<td>(Alekseyenko, Lee et al. 2010)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Odorant receptor 6y7d (Or6y7d);</td>
<td></td>
<td>Pharmacological activation</td>
<td>Pheromone cVA promotes aggression among males via activating Or6y7d expressing olfactory receptor neurons.</td>
<td>(Wang and Anderson 2010, Liu, Liang et al. 2011)</td>
<td>N/A</td>
</tr>
<tr>
<td>Odorant receptor 6y5a (Or6y5a)</td>
<td></td>
<td>Pharmacological activation</td>
<td>Pheromone cVA suppresses aggression via activating Or6y5a olfactory receptor neurons.</td>
<td>(Liu, Liang et al. 2011)</td>
<td>N/A</td>
</tr>
<tr>
<td>White, ATP-binding cassette (ABC) transporter</td>
<td>ATP-binding cassette sub-family G member 1 (ABCG1)</td>
<td>Mutation line</td>
<td>Mutant showed abnormal vision and impaired aggressive behavior such as Lunging behavior</td>
<td>(Hoyer, Eckart et al. 2008)</td>
<td>Yes (Table 2)</td>
</tr>
<tr>
<td><strong>Sensory (Olfactory and Vision) system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genes identified through expression analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome P450, 6a20 (Cyp6a20)</td>
<td>Gene expression and mutant line</td>
<td>Gene mutation or deficiency decreased aggression</td>
<td>(Dierick and Greenspan 2006, Robin, Daborn et al. 2007)</td>
<td>No human studies</td>
<td></td>
</tr>
<tr>
<td>Odor-binding protein (Obps6a)</td>
<td>N/A</td>
<td>Gene expression</td>
<td>Decreased gene expression in high aggressive lines.</td>
<td>(Dierick and Greenspan 2006)</td>
<td>N/A</td>
</tr>
<tr>
<td>Muscleblind (mbl)</td>
<td>muscleblind-like splicing regulator 3 (MBNL3)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>No human studies</td>
</tr>
</tbody>
</table>
Table 6. *Drosophila* Genes for Aggression (continued)

<table>
<thead>
<tr>
<th>Genes</th>
<th>Human Homolog</th>
<th>Methods in Fly</th>
<th>Phenotype</th>
<th>Fly Reference</th>
<th>Studied in Human aggression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG17154</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>N/A</td>
</tr>
<tr>
<td>CG5966</td>
<td>Pancreatic triacylglycerol lipase precursor (PNLIP)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>No human studies</td>
</tr>
<tr>
<td>CG30015</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>N/A</td>
</tr>
<tr>
<td>Darkener of apricot (Doa)</td>
<td>CDC-like kinase 2 (CLK2)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>No human studies</td>
</tr>
<tr>
<td>CG14478</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>N/A</td>
</tr>
<tr>
<td>CG12292, spichthyin (spict)</td>
<td>non-imprinted in Prader-Willi/Angelman syndrome 2 (NIPA2)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Tramtrack (ttk)</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>N/A</td>
</tr>
<tr>
<td>CG1623, Hebe</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>N/A</td>
</tr>
<tr>
<td>CG13512</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>N/A</td>
</tr>
<tr>
<td>SP71</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>N/A</td>
</tr>
<tr>
<td>longitudinals lacking (lola)</td>
<td>zinc finger protein with interaction domain (ZID)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>No human studies</td>
</tr>
<tr>
<td>scribbler</td>
<td>zinc finger protein 609 (ZNF609)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Male-specific RNA 87F</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>N/A</td>
</tr>
<tr>
<td>kismet</td>
<td>chromodomain helicase DNA binding protein 6,7,8,9 (CHD6, CHD7, CHD8, CHD9)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Rollmann et al. 2006)</td>
<td>No human studies</td>
</tr>
<tr>
<td>Genes</td>
<td>Human Homolog</td>
<td>Methods in Fly</td>
<td>Phenotype</td>
<td>Fly Reference</td>
<td>Studied in Human aggression</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>--------------------------------------</td>
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<td>----------------------------</td>
</tr>
<tr>
<td>CG11448 Rab interacting lysosomal protein-like 1 (RILPL1)</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Ayroles et al. 2009)</td>
<td>N/A</td>
</tr>
<tr>
<td>CG13760</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Ayroles et al. 2009)</td>
<td>N/A</td>
</tr>
<tr>
<td>CG2556</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Ayroles et al. 2009)</td>
<td>N/A</td>
</tr>
<tr>
<td>CG31038</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Ayroles et al. 2009)</td>
<td>N/A</td>
</tr>
<tr>
<td>CG32425</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Ayroles et al. 2009)</td>
<td>N/A</td>
</tr>
<tr>
<td>late bloomer, Tetraspanin 42Ek (Tsp42Ek)</td>
<td>N/A</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Ayroles et al. 2009)</td>
<td>N/A</td>
</tr>
<tr>
<td>Skuld (skd)</td>
<td>mediator complex subunit 13 (MED13)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed increased aggression score</td>
<td>(Edwards, Ayroles et al. 2009)</td>
<td>No Human Studies</td>
</tr>
<tr>
<td>GTPase-activating protein 1 (Gap1)</td>
<td>RAS p21 protein activator 3 (RASA3)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Ayroles et al. 2009)</td>
<td>No Human Studies</td>
</tr>
<tr>
<td>Schizo</td>
<td>ADP-ribosylation factor guanine nucleotide exchange factor 2 (ARFGEF2)</td>
<td>Gene expression and Mutant lines</td>
<td>Mutant showed decrease aggression score</td>
<td>(Edwards, Ayroles et al. 2009)</td>
<td>No Human Studies</td>
</tr>
</tbody>
</table>
observation supports the multifactorial nature of the genetic underpinnings for aggression and suggests that different aggression genes may converge on the same interconnected networks or pathways.

**Frustrative Non-Reward Reactions**

Frustrative non-reward aggression has been less well studied in animal models. Discontinuation or omission of scheduled reinforcement can effectively induce escalated levels of aggressive behaviour in fish [Vindas et al., 2012, 2014], birds [Azrin and Hutchinson, 1966; Cherek and Pickens, 1970], rodents [Stanford and Salmon, 1989; Miczek et al., 2001], pigs [Melotti et al., 2013], monkeys and humans [Barzman and Eliassen, 2014]. An operant procedure has been implemented in mice using sucrose as a reinforcer to examine extinction induced aggressive confrontation to intruder mice [Miczek et al., 2001]. Similar paradigms have been used to induce aggressive responses in other species. Studies have examined the roles of the nonadrenergic system [Stanford and Salmon, 1989], the 5-HT1B receptor [de Almeida and Miczek, 2002], neurosteroids and GABAA receptors [Miczek et al., 2003] in frustrative non-reward induced reactions in rodents and fish. Barzman et al found that the expression of TNF-related inflammatory cytokine genes was positively correlated with frustrative non-reward and aggressive behaviours in pediatric patients with bipolar disorder [Barzman and Eliassen, 2014]. However, no studies have examined the genes underlying frustrative nonreward aggression in animals.

**Summary of Animal Models of Aggression**

The face, construct and predictive validities for aggression models of various species have been extensively evaluated. Although evolutionarily conserved, many aggressive measurements in animal models are species-specific and should be cautiously translated to human behaviour. Nevertheless, animal models have facilitated our understanding of the neurobiological and molecular underpinning of normal and pathological aggressive behaviours. Although many classical pathways such as hormonal and neurotransmitter pathways have been largely replicated and confirmed in various animal and human studies, recent advances in genetic tools and network based analysis have suggested novel genetic mechanisms. This is not surprising, since previous candidate gene centered studies had already suggested a multifactorial genetic contribution with small and pleiotropic effects and complex epistatic relationships. Future directions are 1) to focus on developing network based analytic approaches to identify of causal genes and networks and to clarify the relationship of genes and networks with aggressive behaviour; and 2) to further delineate the speciesspecific and non-specific domains of aggressive behaviour as well as escalated/abnormal aggression, and to clarify the overlapping yet distinct causal genes and networks underlying these separable domains, particularly overlooked domains such as frustrative non-reward.

**Summary and conclusions**

In planning this review, we had set out to learn about the genetic underpinnings of the RDoC
constructs associated with aggression: frustrative non-reward, defensive aggression and offensive (or proactive) aggression. Although the constructs of defensive and offensive aggression have been widely used in the animal genetics literature, the human literature is mostly agnostic with regard to all the RDoC constructs. That said, many aggression phenotypes have been studied in human genetic paradigms and the insights from these studies are likely relevant to the RDoC constructs.

We know from twin studies that about half the variance in behaviour may be explained by genetic risk factors. This is true for both dimensional, trait-like, measures of aggression and categorical definitions of psychopathology. The non-shared environment seems to have a moderate influence with the effects of shared environment being unclear. Gene-environment interaction appears to play an important role but the details need to be worked out.

Human molecular genetic studies of aggression are in an early stage. The most promising candidates are in the dopaminergic and serotonergic systems along with hormonal regulators. Genome-wide association studies have not yet achieved genomewide significance, but current samples are too small to detect variants having the small effects one would expect for a complex disorder. These studies have implicated genes involved in neurodevelopmental processes and synaptic plasticity, not previously considered in candidate gene studies. This may indicate that aggressive behaviour does not only involve neurotransmitters or hormonal functions, but also molecules involved in establishing neuronal circuits, neuron-to-neuron connectivity and brain plasticity.

Future studies should improve the measurement of aggression by using a systematic method of measurement such as that proposed by the RDoC initiative, which differentiates defensive aggression, offensive aggression and frustrative non-reward [Sanislow et al., 2010]. Although the RDoC matrix provides some guidance about the measurement of frustrative non-reward in humans, it does not provide guidance for the measurement of offensive and defensive aggression, although relevant measures are well-developed in the animal literature. These measurement gaps suggest a role for the creation of reliable and valid measures of RDoC constructs for use in human aggression studies. Replication has been difficult for the field of psychiatric and behavioural genetics. Such problems will only be magnified for aggression if the field cannot come to a consensus about how aggression phenotypes should be measured.
References


Burt SA. 2013. Do etiological influences on aggression overlap with those on rule breaking? A meta-analysis. Psychol Med 43:1801–1812. DOI: 10.1017/S0033291712001894


Saliva Oxytocin, Cortisol, and Testosterone Levels in Adolescent Boys with Autism Spectrum Disorder, Oppositional Defiant Disorder/Conduct Disorder and Typically Developing Individuals

Published as:


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European Neuropsychopharmacology, Epub ahead of print
Abstract

The aim of the current study was to compare levels of oxytocin, cortisol, and testosterone in adolescents with either autism spectrum disorder (ASD), or oppositional defiant disorder (ODD)/conduct disorder (CD), and in typically developing individuals (TDI), and relate hormone levels to severity and subtype of aggression and callous-unemotional (CU) traits. Saliva concentrations of oxytocin, cortisol, and testosterone were assessed in 114 male participants (N=49 ASD, N=37 ODD/CD, N=28 TDI,) aged 12-19 years (M = 15.4 years, SD = 1.9). The ASD and the ODD/CD groups had significantly lower levels of oxytocin than the TDI group, and the ODD/CD group had significantly higher levels of testosterone than the ASD group. There were no group effects on cortisol levels. Group differences remained for oxytocin after correcting for the influence of CU traits, but were not significant after controlling for aggression. Results for testosterone became non-significant after correction for either CU traits or aggression. Across groups, higher levels of CU traits were related to higher levels of cortisol and testosterone, however, proactive and reactive aggression were unrelated to all three hormonal levels. The current findings show that, regardless of cognitive ability or comorbid disorders, the diagnostic groups (ASD, ODD/CD) differ from each other by their hormonal levels, with the ASD group characterized by relative low level of oxytocin, and the ODD/CD group by a relative low level of oxytocin and high level of testosterone. These group effects were partly driven by differences in CU traits between the groups.
Hormones play an important role in influencing reactions to our social and non-social environment (Heinrichs, Baumgartner, Kirschbaum, & Ehler, 2003; Jaeggi, Trumble, Kaplan, & Gurven, 2015; van Anders, Goldey, & Kuo, 2011). Three important hormones in this context are oxytocin, cortisol and testosterone. These hormones constitute active regulators of complex social cognition and affiliative behaviour (oxytocin) (Bakermans-Kranenburg & van, 2013; Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011; Ooi, Weng, Kossowsky, Gerger, & Sung, 2016), arousal and the stress system (cortisol) (Northover, Thapar, Langley, Fairchild, & van Goozen, 2016; Schechter, Brennan, Cunningham, Foster, & Whitmore, 2012), and arousal and aggression (testosterone) (Duke, Balzer, & Steinbeck, 2014).

Here, we assess the level of these three hormones in individuals with either autism spectrum disorder (ASD) or oppositional defiant disorder (ODD)/conduct disorder (CD) and in typically developing individuals (TDI) during adolescence. ASD are early onset neurodevelopmental disorders defined by core impairments in social interaction and verbal and nonverbal communication, stereotyped and restricted patterns of interest and activity and abnormal sensory processing according to DSM-5 criteria (American Psychiatric Association., 2013). ODD is characterized by angry and irritable mood, and argumentative, defiant and disobedient behavioral patterns. CD, is characterized by a pattern of aggressive, destructive, and/or deceitful behaviors that violate the rights of others according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria (APA, 2013). In this paper we will combine ODD and CD into one diagnostic group since both disorders are closely linked neurodevelopmental disorders of which ODD is either prodromal to CD or a subsyndromal form of CD (Biederman et al. 1996). Our interest for comparing these hormones in ASD and ODD/CD as two distinct disorders stems from the fact that both involve social/communication problems and deficits in empathy (Herpers, Klip, Rommelse, Greven, & Buitelaar, 2016). In individuals with ODD/CD these problems are associated with increased levels of aggression. Individuals with ASD also have elevated levels of aggressive behaviour when compared to TDI (Hill et al., 2014), although the aggressive behaviour is not a core symptom of ASD and is typically less severe in ASD than ODD/CD (APA, 2013).

Aggression is a heterogeneous and multifaceted construct, and a common way to subtype aggression is into reactive and proactive aggression (Smeets et al., 2017). Reactive aggression is known as an emotionally charged response to provocations or frustration and is also known as “impulsive”, “hot blooded” or “affective” aggression. Proactive aggression is defined as a conscious, goal-directed and planned act, used for personal gain or egocentric motives, also known as “premeditated”, “instrumental” or “cold-blooded” aggression (Smeets et al., 2017). Callous-unemotional (CU) traits are a category of interpersonal, affective, lifestyle and antisocial behaviours that are part of the broader clinical construct of psychopathy, marked by lack of remorse or guilt, callous-lack of empathy, lack of concern over performance, and shallow or deficient affect (Frick, Ray, Thornton, & Kahn, 2014). CU traits have been further proposed as personality traits that qualify the antisocial and aggressive behaviours of individuals with CD. CD with high CU traits has been in particular associated with reductions in specific forms of empathy, in particular responding to the fear, sadness, pain and happiness of others (Blair, 2013) and a worse outcome (Frick et al. 2014). As reviewed elsewhere, high CU traits are not unique to individuals with CD but may also observed in the general population and in individuals with other disorders as ASD.
or Attention-deficit Hyperactivity Disorder (ADHD); they may be conceptualized as cross-disorder traits (Herpers et al. 2012) that affect quality of life in ASD and other disorders (Herpers et al. 2016). Below we aim to provide a brief overview of the current literature on the three hormones: oxytocin, cortisol, and testosterone in ASD, ODD/CD, and typically developing individuals, and also in relation to empathy, aggression subtypes and CU traits.

Oxytocin
In TDI, higher basal oxytocin levels have been associated with lower generosity (i.e. less risk seeking behaviour) in preschool boys, regardless of whether the target of generosity was an in- or outgroup member (Fujii et al., 2016). This association was observed only under social conditions and thus may be reduced in completely anonymous situations. Intranasal oxytocin in adult TDI can improve empathy for victims of aggression by improving face recognition of neutral and angry, but not happy faces (Savaskan, Ehrhardt, Schulz, Walter, & Schachinger, 2008). Core deficits of ASD such as impairments in social interaction have also been associated with low oxytocin levels (Fieldman, Golan, Hirschler-Guttenberg, Ostfield-Etzion, & Zagoory-Sharon, 2014; Parker et al., 2014), given the results of intranasal administered oxytocin and/or measuring plasma oxytocin levels, but not on basal saliva levels. Whether oxytocin deficits are linked with aggressive behaviour in ASD is still unclear. Within ODD/CD some studies have linked lower oxytocin levels to aggressive behaviours (Fetissov et al., 2006; R. Lee, Ferris, Van de Kar, & Coccaro, 2009), suggesting that hypo-oxytocinergic function may account for aggressive behaviour (Fetissov et al., 2006). Also, molecular genetic studies reported significant associations between variants of oxytocin genes (i.e. single nucleotide polymorphisms rs6770632, rs1042778) and extreme persistent and pervasive aggressive behaviour in males (Beitchman et al., 2012; Malik, Zai, Abu, Nowrouzi, & Beitchman, 2012), and between the oxytocin rs237885 and rs2268493 A allele genotype and CU traits (Beitchman et al. 2012). A strong association has been observed between low basal saliva oxytocin levels and high levels of CU traits among male adolescents with conduct problems (Levy et al., 2015). The present study examines whether basal saliva oxytocin levels differ between individuals with either ASD or ODD/CD and TDI, and are associated with aggressive subtypes and CU traits.

Cortisol
A recent review reported diverse findings on basal cortisol levels in TDI compared to ASD individuals (Taylor & Corbett, 2014). For ODD/CD – but not ASD - higher basal cortisol levels were associated with aggression in preschoolers, lower levels in elementary school-aged children, and associations were non-significant in adolescents (Alink et al., 2008). With respect to ODD/CD, most studies found that specifically reactive aggression was related to higher levels of cortisol (Lopez, Vazquez, & Olson, 2004; van Bokhoven et al., 2005). Several studies demonstrated that CU traits were linked to lower basal cortisol levels (Loney, Butler, Lima, Counts, & Eckel, 2006; von Polier et al., 2013), but others found no significant association (e.g. Northover et al., 2016). In the current study, basal saliva cortisol level will be compared in individuals with either ASD or ODD/CD and related to aggression subtypes and CU traits.
Testosterone
Basal testosterone levels have been studied less frequently in relation to ASD and ODD/CD, in contrast to the relationship with aggression which is studied comprehensively. High levels of testosterone are thought to be related to aggressive behaviour in clinical samples (Gao, Glenn, Schug, Yang, & Raine, 2009). Recent individual studies and critical reviews focused on both TDI and clinical samples, report conflicting and equivocal (longitudinal) effects of endogenous testosterone levels in male adolescents (Archer, Graham-Kevan, & Davies, 2005; Bitsika et al., 2017; Duke et al., 2014). Studies reported diverse results on the so-called extreme-male-brain model (Baron-Cohen, 2002) in ASD, which suggests that core deficits of ASD can be attributed to being exposed to high prenatal testosterone levels. An explanation for the absence of aggression in some people with ASD and elevated levels of testosterone, is that they have a sufficient level of emotional empathy to inhibit any such tendencies (Wen & Wen, 2014), and as such testosterone may mediate social approach or avoidance behaviour (Kaldewaij, Koch, Volman, Toni, & Roelofs, 2017). Moreover, there is also evidence for a cortisol and testosterone interaction (Carre & Mehta, 2011). In delinquent males during resting conditions, high levels of testosterone have been related to aggressive behaviour, provided that cortisol levels are low (i.e. basal saliva levels 1 SD below the mean) (Popma et al., 2007; Terburg, Morgan, & van Honk, 2009). This was observed in covert (i.e. feeling angry without expressing openly) aggression only, suggesting a specific relation between hormones and types of aggression. Taken together, literature suggests limited relationships between higher testosterone levels and increased aggression in clinical samples only. In the current study, the role of basal salivary testosterone and the testosterone-cortisol (T/C) ratio is compared between individuals with either ASD or ODD/CD and TDI, and examined as a possible biological marker for type of aggression and/or CU traits.

To conclude, this is the first study designed to examine whether 1) individuals with either ASD or ODD/CD could be differentiated from TDI and each other by hormonal levels (i.e. oxytocin, cortisol, testosterone), 2) associations exist between hormone levels and type (i.e. reactive or proactive) of aggression, and 3) associations exist between hormone levels and self- or parent-rated CU traits. In both ASD and ODD/CD, attention deficit/hyperactivity disorder (ADHD) frequently co-occur (Matson, Rieske, & Williams, 2013; Matthys & Lochman, 2010), therefore we control for comorbid ADHD in our study. Both ASD and ODD/CD have been associated with impairments in various cognitive functions (Erskine et al., 2013; H. Lee, Kang, Kim, Kim, & Chung, 2011), therefore we control for cognitive ability in our study.

Experimental Procedures

Recruitment
Adolescents with ASD or ODD/CD were recruited through clinical institutes across the Netherlands, specialized in severe disruptive behaviour problems (Hoenderloo Group, Otto Gerhard Heldring Foundation, and Woodbrookers) or severe psychiatric problems (Karakter Child and Adolescent
Psychiatry) and through information leaflets that were sent to families via the Dutch federation of Autism (NVA). All participants who were recruited from clinical institutes, obtained a clinical ASD or ODD/CD diagnosis prior to study participation. Clinical diagnoses (ASD or ODD/CD) were established according to DSM-5 (APA, 2000) criteria by a multidisciplinary team based on information on developmental history and presenting clinical symptoms gathered by a certified child psychiatrist and/or child psychologist, and review of clinical and prior records (if available), including information available from school or other professional institutions involved with the child. Thus, a consensus diagnosis was assigned, which is seen as more reliable compared to structured interviews for assessing diagnostic categories (Leckman, Sholomkas, Thompson, Belanger, & Weissman, 1982). The TDI group was recruited via leaflets from a general community sample via city councils in the same geographical regions as the clinical groups.

Inclusion
All participants (TDI, ASD, ODD/CD) were recruited between April 2011 and March 2015 as part of a larger study on empathy (CU2-study). Both adolescents (if 12 years and older) and their legally appointed guardian provided written informed consent. Adolescents with a clinical ASD or ODD/CD diagnosis according to the criteria of the DSM-5 (American Psychiatric Association, 2013) and a total IQ ≥ 80, were included in this study. Adolescents were excluded if they fulfilled one or more of the following exclusion criteria (a) combined diagnosis of ASD and ODD/CD, (b) an estimated total IQ < 80); and/or (c) suffering from a condition which may affect neurological or cognitive functioning, such as schizophrenia, bipolar disorder, alcohol and/or drugs dependency, presence of tics, language disorders (e.g. dyslexia) and epilepsy. In- and exclusion criteria for TDI group were similar to the ASD and ODD/CD group, except for having a clinical psychiatric diagnosis. The use of non-psychotropic (5.5%) and antidepressant medication (2.3%) was allowed. If possible, psychotropic medication (i.e., stimulants, 21.8%; antipsychotics, 9.3%; atomoxetine, 2.3%) was stopped prior to testing. Stimulants were discontinued for at least 24 hours. Antipsychotics were discontinued for at least 72 hours. However, when discontinuation was thought to have severe deteriorating effects, medication was continued (ASD: n = 10, ODD/CD: n = 12). This study was approved by the Dutch Central Committee on Research involving Human Subjects, protocol number NL26773.000.09 (Centrale Commissie Mensgebonden Onderzoek; CCMO).

Pre-screening
The participants and their guardian were requested to complete pencil-and-paper questionnaires separately from each other at home or at the test location (a quiet room deprived of stimuli). In addition, all three groups were similarly screened for ASD using the parent Social Communication Questionnaire (SCQ) Rutter, Bailey, & Lords, 2003). This is a 40-item parent-report questionnaire that asks about characteristic autistic behaviour on a binary scale (yes/ no). The first item is a language screening question that is not included in the total score. Nineteen items rate current behaviour and 20 items rate behaviour when the child was 4-5 years old (Rutter et al., 2003). 81% Of the ASD group and 35% of the ODD/CD group had a SCQ score above 12. Typically developing adolescents were required to obtain non-clinical scores on the parent SCQ (i.e. raw scores of <12) in order to
be accepted in the current study. Based on English versions of the SCQ, sensitivity ranged between .85-.88 and specificity between .72 and .78 (Berument, Rutter, Lord, Pickles, & Bailey, 1999; Chandler et al., 2007; Charman et al., 2007). Cronbach’s Alpha for the total SCQ was .75 in the current study.

Participants
Initially, 432 subjects were approached to participate within the Netherlands, of whom n = 304 refused to participate, n = 6 ran away from home/institute, n = 18 did not meet inclusion criteria, and for n = 2 we were unable to obtain consent of legally appointed guardian. This resulted in a total of 114 male adolescents (n = 28 TDI, n = 49 with ASD, and n = 37 with either ODD or CD), aged between 12 and 19 years (M = 15.4 years, SD = 1.9), 81% of non-European origin, 100% boys. Main participant and demographic characteristics are summarized in Table 1.

Measures
DISC-IV: Adolescents and legal guardians who gave informed consent, were administered screening questionnaires and the DISC-IV interview to confirm the clinical diagnosis with a research diagnosis across all three groups (TDI, ASD, and ODD/CD). This information was used to classify the adolescents into the TDI, ASD, and ODD/CD groups. For all three groups, caretakers were asked to fill out a digital version of the National Institute of Mental Health Diagnostic Interview Schedule for Children (DISC-IV; (Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000), which is focused on the presence of axis-I disorders in terms of the DSM-IV-TR (APA, 2000). To assess relevant psychiatric comorbidities, parents were asked to fill out the following sections of the DISC-IV: ADHD, ODD, CD, Tic Disorder, misuse of Alcohol, Marihuana, and Other Drugs. In the TDI group, the absence of a clinical psychiatric diagnosis was assessed based on a DISC-IV parent interview.

WISC-III. Adolescents were required to have a minimum average estimated total full-scale intelligence quotient (FSIQ) IQ of ≥ 80. FSIQ was estimated using four subtests of the Dutch version Wechsler Intelligence Scale for Children (WISC-III): Picture Completion, Vocabulary, Similarities and Block Design (Wechsler, 1991). These selected WISC-III subtests are known to correlate between .90-.95 with the Full-scale IQ (Groth-Marnat, 1997). For adolescents older than 16 years, the Wechsler Adult Intelligence Scale (WAIS-III) was administered (Wechsler, 1997). When intelligence was assessed within a year prior to the inclusion, and either the WISC or WAIS was applied, we used the scores of that assessment. In case of a disharmonic intelligence profile, adolescents with a verbal intelligence scale ≥ 80 were included.

The Reactive Proactive aggression Questionnaire (RPQ) assesses the level of proactive and reactive aggression in adolescents (Raine et al., 2006). In the current study, the Dutch translation of the RPQ was used (Cima, Raine, Meesters, & Popma, 2013). This questionnaire consists of 23 propositions placed on a three-point scale ranging from 0 (‘never’) to 2 (‘often’). Proactive aggression is reported on 12 items (e.g., How many times have you bullied or threaten someone?), while reactive aggression is reported on 11 items (e.g., ‘How many times have you hit someone to defend yourself?’). Total scores are calculated by prorated summary scores of the 12 items related to proactive aggression, and the 11 items related to reactive aggression. Cronbach’s alpha was .91.
Table 1. Characteristics of the study population (N=114).

<table>
<thead>
<tr>
<th></th>
<th>Total group</th>
<th>TDI</th>
<th>ASD</th>
<th>ODD/CD</th>
<th>Contrasts</th>
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<tr>
<td></td>
<td>M ± SD</td>
<td>M</td>
<td>M ± SD</td>
<td>M ± SD</td>
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<tr>
<td>Age - years</td>
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<td>15.9</td>
<td>15.0 ± 2.1</td>
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<td>101.1 ± 13.8</td>
<td>105.9</td>
<td>103.4 ± 11.3</td>
<td>94.5 ± 6.8</td>
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<tr>
<td>CU traits total score</td>
<td></td>
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<td>ICU self-rated ***</td>
<td>26.8 ± 8.4</td>
<td>23.6</td>
<td>24.4 ± 7.3</td>
<td>31.2 ± 9.4</td>
<td>ODD/ CD &gt; ASD = TDI</td>
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<td>ICU parent-rated ***</td>
<td>28.9 ± 11.7</td>
<td>16.9</td>
<td>27.7 ± 8.3</td>
<td>37.9 ± 9.7</td>
<td>ODD/ CD &gt; ASD &gt; TDI</td>
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<td>RPQ</td>
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<td>RPQ total score ***</td>
<td>12.9 ± 8.2</td>
<td>7.6</td>
<td>11.1 ± 6.9</td>
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<td>9.2 ± 4.9</td>
<td>6.0</td>
<td>8.8 ± 5.1</td>
<td>11.6 ± 4.5</td>
<td>ODD/ CD &gt; ASD &gt; TDI</td>
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<td>RPQ proactive ***</td>
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<td>SCQ **</td>
<td>11.4 ± 7.1</td>
<td>3.9</td>
<td>15.5 ± 5.8</td>
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<td>TDI &lt; ASD &gt; ODD/ CD</td>
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<td>57</td>
<td></td>
<td>n.a.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ethnicity parents**

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
<th>Percentage</th>
<th>96</th>
<th>78.7</th>
<th>49</th>
<th>94.2</th>
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<tr>
<td>Both Caucasian</td>
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<td>78.7</td>
<td>26</td>
<td>92.9</td>
<td>49</td>
<td>94.2</td>
</tr>
<tr>
<td>Caucasian and other</td>
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<td>9.1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>5.8</td>
</tr>
<tr>
<td>Both are unknown</td>
<td>4</td>
<td>3.3</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>9.5</td>
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</tbody>
</table>

**Highest level of education parents**

<table>
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<tr>
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<th>Percentage</th>
<th>7</th>
<th>5.7</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower</td>
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<td></td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Middle</td>
<td>38</td>
<td>31.1</td>
<td>3</td>
<td>10.7</td>
<td>20</td>
<td>38.5</td>
</tr>
<tr>
<td>Higher</td>
<td>63</td>
<td>51.6</td>
<td>25</td>
<td>89.3</td>
<td>28</td>
<td>53.8</td>
</tr>
</tbody>
</table>

*FSIQ: Full-scale IQ; CU: Callous Unemotional traits based on the self-rated ICU: Inventory of Callousness Unemotional traits. RPQ: Reactive and Proactive Questionnaire. SCQ: Social Communication Questionnaire. TDI: Typical Developing Individuals; ASD: Autism Spectrum Disorder; ODD/CD: Oppositional Defiant Disorder / Conduct Disorder; n.a.: not assessed; n.s.: not significant; p-Value: * p < .05, ** p < .01, *** p < .001; Ethnicity parents A: data based on two parents; Highest level of education parents B: data based on family level.*
The Inventory of Callous-Unemotional traits (ICU) assesses CU traits in adolescents, divided into three subscales: uncaring (8 items), callousness (11 items) and unemotional (5 items) (Frick, Cornell, Barry, Bodin, & Dane, 2003). The ICU exists of 12 positively worded and 12 negatively worded items, rated on a four-point scale ranging from 0 (‘not at all true’) to 3 (‘definitely true’). An example of an item measuring the uncaring scale is ‘I am concerned about the feelings of others’. The item ‘I seem very cold and uncaring to others’, measures for example the callousness scale. An item which measures the unemotional scale is, for example, ‘I do not show my emotions to others’. Subscale scores are calculated by prorated summary scores of the item ratings. Subsequently, the total score can be calculated by summing the subscale scores. A higher score reflects higher levels of CU traits. The ICU was reported by both adolescents and their legal guardian. Cronbach’s alpha of the self-report was .78, compared to a Cronbach’s alpha .89 based on legal guardian/parent report.

### Table 2. Distribution of participants across the batches and basal saliva hormone levels after correction for age.

<table>
<thead>
<tr>
<th></th>
<th>B1 N</th>
<th>B2 N</th>
<th>B3 N</th>
<th>B4 N</th>
<th>Level of oxytocin</th>
<th>Level of cortisol</th>
<th>Level of testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDI(^A)</td>
<td>8</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>1=2=3</td>
<td>1=2=3</td>
<td>B3&gt;B1&amp;B2</td>
</tr>
<tr>
<td>ASD</td>
<td>14</td>
<td>24</td>
<td>1</td>
<td>10</td>
<td>1=2=3=4</td>
<td>B2&lt;B1</td>
<td>B1&gt;B3</td>
</tr>
<tr>
<td>ODD/CD</td>
<td>14</td>
<td>1</td>
<td>12</td>
<td>10</td>
<td>1=2=3=4</td>
<td>1=2=3=4</td>
<td>1=2=3=4</td>
</tr>
</tbody>
</table>

TDI: Typical Developing Individuals; ASD: Autism Spectrum Disorder; ODD/CD: Oppositional Defiant Disorder / Conduct Disorder; B1: Batch 1 analyzed on 30-08-2013; B2: Batch 2 analyzed on 17-12-2013; B3: Batch 3 analyzed on 10-05-2014; B4: Batch 4 analyzed on 21-10-2014. \(^A\) For the TDI group only: since batch 4 contains no samples, batches 1-3 are compared among the hormones.

### Hormone measures – oxytocin, cortisol, and testosterone

Hormones. Levels of oxytocin, cortisol, and testosterone were assessed in saliva without using oral stimulants for saliva production. Saliva collection is a reliable, simple, stress-free method, and is better suited to assessing hormone concentrations in adolescents than other methods, such as using plasma (Kirschbaum & Hellhammer, 1994; Daughters et al. 2015). Participants once sampled 6 ml saliva in a plastic clear test tubes provided by the lab. They were instructed to complete sampling at least one hour after eating, drinking, brushing their teeth or smoking. The saliva samples were directly stored at -20 degrees Celsius and subsequently assessed at the department of Laboratory Medicine, Radboud UMC, Nijmegen. The level of oxytocin was measured in saliva by a radio immuno assay (RIA) after solid phase extraction, as previously described (Althaus et al., 2016). Cortisol and testosterone were measured in saliva samples by an in-house developed RIA after a chromatographic paper separation and extraction (with correction for losses), as previously described (MacKenzie, Hoefnagels, Jansen, Benraad, & Kloppenborg, 1990; Swinkels, van Hoof, Ross, Smals, & Benraad, 1991). Cortisol is measured with an intra- and interprecision of 4.4% and 6.3% respectively. Testosterone is measured with an intra- and interprecision of 6.9% and 5.4% respectively. All assay concentrations were within the range of the standard linear concentration curve.
used for the calibration of each hormone. Oxytocin is measured with an intra- and interprecision of 6.4% and 7.0% respectively. Since cortisol inhibits testosterone, the T/C ratio was taken into account in the analyses. The ratio between testosterone and cortisol was calculated by dividing testosterone concentrations (nmol/L) by cortisol concentrations (nmol/L) so that higher values reflected higher testosterone to cortisol ratios. All participants (n=114) sampled saliva between 9 a.m. and 10 a.m. (average sample duration M = 9.33, SD = .83 minutes), except for a subgroup of adolescents due to practical reasons. Here saliva was sampled between 6:45 and 8:45 a.m. (n = 11), or between 10:00 and 12:30 a.m. (n = 11). From this subgroup the cortisol (M = 6.59, SD = 4.35), testosterone (M = .29, SD = .16) and oxytocin levels (M = 1.77, SD = 1.08) differed less than two standard deviations from the mean sample levels (cortisol: M = 4.18, SD = 2.19, testosterone: M = .19, SD = .13, oxytocin: M = 1.71, SD = .68). Their saliva samples were included in this study and sensitivity analyses were run with and without this subgroup. Few saliva samples (n = 6) could not be assessed due to technical problems. Hormones were assessed over four batches within one year (see Table 2).

**Statistical Analyses**

The hormonal measures were subjected to a Van der Waerden transformation to normalize the skewed measures (SPSS version 24) and measures were transformed into the same scale (z-scores). ANOVAS were conducted to examine whether hormonal values differed between the three groups, using group (TDI, ASD, and ODD/CD) as between-subject factor, and hormones (for oxytocin, cortisol, and testosterone) as the dependent variables. To examine whether hormonal values were related to aggression subtypes and CU traits across disorder, correlations were calculated between hormonal values (i.e. oxytocin, cortisol, and testosterone) and aggression subtypes assessed by the RPQ, and self- or parent-rated CU traits for the whole sample. Partial correlations were calculated corrected for IQ. For the TDI group some variables are a constant (i.e. medication use and ADHD comorbidity), therefore it was examined whether general medication use (yes/no discontinuation during test day) and ADHD comorbidity (yes/no) were related to hormonal levels in the clinical groups only. Furthermore, analyses were run without saliva sampled outside the 9-10 a.m. timeframe as between subject factors. As we conducted three ANOVAS (for the three hormones), a false discovery rate (FDR) multiple testing correction was used.

**Results**

**Descriptives**

See Table 1 for sample characteristics. All participants were male and the majority of the ODD/CD group was diagnosed having ODD (81.1%; see Table 1 for sample characteristics). Comorbid ADHD was found in ASD (44.2%) and ODD/CD group (66.7%). The ASD group had the highest percentage of medication use (63.5%), compared to the ODD/CD group (47.6%) and the TDI group (0%). Within both ASD and ODD/CD groups the most prescribed medication was stimulants (32.7% and 27% respectively) followed by antipsychotics (14.3% and 5.4% respectively). The three groups did not differ in age but did significantly differ in estimated full scale IQ (F (2, 110) = 14.38, p < .001), whereby
ODD/CD participants had lower IQ scores compared with both the ASD and the TDI groups. The three groups differed significantly on the RPQ total score (F (2, 102) = 19.52, p < .001), the ODD/CD participants had the highest scores, compared with both the ASD and the TDI groups (see also Table 1).

The three groups differed significantly on self-rated ICU-total score (F (2,110) = 9.99, p < .001), the ODD/CD had higher scores than both the ASD and the TDI groups. Similar results were found on parent-rated ICU-total score (F (2,109) = 47.82, p < .001), whereby the ODD/CD group had the highest score, the TDI group the lowest, and the ASD group scored between those two groups. The self- and parent-rated CU traits correlated r = .47 (p < .001). In the ASD group and the ODD/CD group, self-rated CU traits were lower than parent-rated traits (t (34) = -3.63, p < .001, r = .53 and t (48) = -2.4, p < .02, r = .33, respectively), whereas in the TDI group self-rated CU traits were higher than parent-rated traits (t (27) = 5.18, p < .001, r = .71). Participants were unevenly distributed among the four batches. Batch 1: both ASD and ODD/CD were equally represented (n=14 per group) and TDI the lowest (n=8). Batch 2: contained more ASD (n=24) compared to TDI (n=15) and ODD/CD (n=1). Batch 3: contained more ODD/CD (n=12) compared to TDI (n=5) and ASD (n=1). Batch 4: no samples of TDI were included (inclusion number was reached within previous batches) compared to ASD (n=10) and ODD/CD (n=10). Across batches, hormonal concentrations within each group (i.e. TDI, ASD, and ODD/CD) were the same for oxytocin, different for both cortisol (ASD only) and testosterone (all groups). Within ASD, cortisol levels were the lowest in batch two compared to batch one and four (batch three was excluded since it contained one sample only). For testosterone levels: the TDI group testosterone levels were the highest in batch three compared to one and two; the ASD group testosterone levels were the highest in batch one compared to batch four; within the ODD/CD groups testosterone levels were the highest in batch three compared to batch four.

**Hormonal differences between groups**

There was a significant correlation between cortisol and testosterone levels across groups
The univariate F-tests showed a significant main group effect for oxytocin ($F (2,100) = 5.39, FDR adjusted p = .006$). This effect was due to a significant difference between TDI ($M = .49, SD = .97$) and ASD ($M = -.22, SD = .90$) ($p = .002$); and TDI and ODD/CD groups ($M = -.12, SD = .94$) ($p = .011$). Thus, both the ASD and ODD/CD group had significantly lower levels of oxytocin than the TDI group, whereas the ASD and the ODD/CD groups did not significantly differ from each other. There was no main group effect for cortisol ($F (2,108) = 2.69, p = .073$) but the main group effect for testosterone was significant ($F (2,108) = 5.42, FDR adjusted p = .006$). This effect was attributable to a significant difference between the ASD ($M = -.26, SD = .99$) and the ODD/CD groups ($M = .42, SD = .99$) ($p = .001$). The ODD/CD group had significantly higher levels of testosterone than the ASD group. The TDI group did not significantly differ from the ASD group or the ODD/CD group.

### Relationship between aggression subtypes and CU traits and hormone levels

Next, we examined the correlations among the two (i.e. reactive or proactive aggression) subtypes of aggression, which was strong ($r = .63, p < .001$). Therefore, correlations were examined between total RPQ score and oxytocin, cortisol and testosterone levels over the whole sample and within groups.
Our results showed that all of these correlations were non-significant (p’s > .09) (see also Table 4).

We observed no significant correlations between self-rated CU traits and oxytocin, but found significant correlations between self-rated CU traits and cortisol (r = .38, p < .001) and CU traits and testosterone levels (r = .37, p < .001) across the three groups. The correlations for cortisol were significant within ODD/CD only (r = .42, p < .05) (see also Table 4). Parent-rated CU traits were unrelated to oxytocin, but were significantly correlated with cortisol (r = .32, p < .01) and testosterone levels (r = .27, p < .05) across the three groups. No significant correlations were reported within groups. The correlations between cortisol and either self- or parent-rated CU traits became non-significant after controlling for testosterone (p’s < .09).

**Group effects: driven by aggression subtypes and/or CU traits?**

The main group effect for oxytocin in the ANOVA became non-significant (p’s > .07) after adding two (i.e. reactive aggression or proactive aggression) subscales of the RPQ as covariates, although there were no main effects of RPQ (p’s > .09). Adding two subscales of the RPQ did not change the results of the group comparison of cortisol levels (i.e. remained non-significant), and there were no main effects of RPQ (p’s > .60). The main effect for testosterone became non-significant (FDR adjusted p’s > .02) after adding two subscales of the RPQ to the analyses, although there were no main effects of the RPQ (p’s > .08).

The main group effect for oxytocin remained significant (p = .003) after adding self-rated CU traits and became non-significant (p > .09) after adding parent-rated CU traits to the analyses,
there were no main effects of self-rated (p > .14) or parent-rated (p > .95) CU traits. When adding either self- or parent-rated CU traits to the analyses did not alter the group results for cortisol (i.e. remained non-significant), while there were still main effects of both self-rated (F (1,106) = 10.75, p = .001), and parent-rated CU traits (F (1,105) = 4.78, p = .031). This indicates that both higher self- and parent-rated CU traits are related to higher cortisol levels, independent of group effects. The main group effect for testosterone remained non-significant for either self- or parent-rated CU traits (FDR adjusted p’s > .02). In addition, there was only a significant main effect of self-rated CU traits (F (1,106) = 4.99, p = .016). This suggests that higher self-rated CU traits are related to higher testosterone level, independent of group effects.

**Sensitivity analyses**

We ran sensitivity analyses since the three groups significantly differed on estimated full IQ, ADHD comorbidity, and medication use. Correcting for IQ did not alter the results of the ANOVA’s on oxytocin (i.e. remained significant), cortisol (i.e. remained non-significant), and testosterone (i.e. remained significant). So no main effect of IQ with group was found on oxytocin, cortisol, or testosterone (p’s > .45). Correcting for either ADHD or medication use also did not alter the results of the main analyses on oxytocin, cortisol, and testosterone (i.e. hormonal levels between the ASD

**Table 5.** Means, standard deviations and range (minimum-maximum Z-scores) of the distribution of oxytocin, cortisol, testosterone, and cortisol-testosterone ratio for TDI, ASD, and ODD/CD groups.

<table>
<thead>
<tr>
<th></th>
<th>Total group</th>
<th>1. TDI</th>
<th>2. ASD</th>
<th>3. ODD/ CD</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (N)</td>
<td>SD (Min - Max)</td>
<td>M (N)</td>
<td>SD (Min - Max)</td>
<td>M (N)</td>
</tr>
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<td>zOxytocin</td>
<td>0.00 (103)</td>
<td>0.97 (-2.34 - 2.34)</td>
<td>0.49 (27)</td>
<td>0.97 (-2.34 - 2.34)</td>
<td>-0.22 (43)</td>
</tr>
<tr>
<td>zCortisol</td>
<td>0.00 (111)</td>
<td>0.96 (-1.87 - 2.37)</td>
<td>-0.02 (27)</td>
<td>0.75 (-1.35 - 2.10)</td>
<td>-0.19 (48)</td>
</tr>
<tr>
<td>zTestosterone</td>
<td>0.00 (111)</td>
<td>0.97 (-2.37 - 2.37)</td>
<td>-0.04 (28)</td>
<td>0.74 (-1.86 - 1.11)</td>
<td>-0.26 (49)</td>
</tr>
<tr>
<td>zTestosterone-cortisol ratio</td>
<td>0.00 (111)</td>
<td>0.97 (-2.37 - 2.37)</td>
<td>-0.06 (28)</td>
<td>0.88 (-1.93 - 1.47)</td>
<td>-0.08 (49)</td>
</tr>
</tbody>
</table>

Hormones are expressed in z-scores; 1.TDI: Typical Developing Individuals; 2.ASD: Autism Spectrum Disorder; 3.ODD/ CD: Oppositional Defiant Disorder / Conduct Disorder; min = minimum observed score; max = maximum observed score; pWC: p-value without correction; pRPQ: p-value corrected after adding either two subscales of the Reactive and Proactive Questionnaire (RPQ) to the analyses (results were similar including a correction for three subscales); pCU: p-value corrected after adding self- (SR) CU traits to the analyses (results were similar for parent-rated (PR) CU traits); p-value: *p < .05, **p < .01, ***p < .001.

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and ODD/CD groups remained non-significant). There were no main effects or interaction effects of comorbid ADHD with group (p’s > .11) or medication use with group (p’s > .14). Correction of the main analyses by excluding saliva samples sampled outside the timeframe of 9-10 a.m. resulted in the significant main group effect on oxytocin remaining; an altered main effect on cortisol (i.e. became significant, \( F(2,82) = 3.16, \) FDR adjusted \( p = .048 \)) only between ASD \( (M = -.41, SD = .54) \) and ODD/CD \( (M = .09, SD = .73) \); and an altered effect on testosterone (i.e. became non-significant). No significant group effects were reported on the testosterone-cortisol ratio. Results remained after including two subscales of the RPQ, self- or parent-rated CU traits to the analyses. Correcting for IQ, comorbid ADHD, or medication use did not alter the results, thus group effect remained non-significant. Results were not altered excluding the participants with CD from the ODD/CD group.

**Discussion**

The current study examined whether basal saliva levels of oxytocin, cortisol, and testosterone differ between male adolescents with ASD or ODD/CD and TDI, and are related to either severity or type of aggression and/or CU traits within and across disorder. Our main findings are that 1) ASD and ODD/CD were associated with significantly lower levels of oxytocin than TDI, 2) ODD/CD had significantly higher levels of testosterone than the ASD whereas ASD did not differ from TDI in testosterone levels, and 3) higher levels of CU traits were related to higher levels of cortisol and testosterone, There were no group effects on cortisol levels. Group differences remained for oxytocin after correcting for the influence of CU traits, but were not significant after controlling for aggression. Results for testosterone became non-significant after correction for either CU traits or aggression. Across groups, however, proactive and reactive aggression were unrelated to all three hormonal levels.

**Oxytocin in ASD and ODD/CD and relation with aggression and CU traits**

Our study was the first to compare two diagnostic groups (i.e. individuals with ASD and ODD/CD individuals) and a TDI group on basal saliva oxytocin levels. Our results are in line with the oxytocin deficit hypothesis of ASD (Fieldman et al., 2014; Parker et al., 2014) and, as a novel finding, show that oxytocin levels were also lower in individuals with ODD/CD compared to TDI. The latter result suggests that lower oxytocin levels may be a cross-disorder biomarker for deficient social processing including reduced social interaction, empathy, trust, and elevated social anxiety. In our sample, the ASD and in part the ODD/CD group had a SCQ score of above 12, which is an indication of deficits in social-communication skills. This in turn may increase the risk of misunderstandings, aggression, and potential conflict related to oxytocin. However, sensitivity analyses did not reveal significant correlations of the SCQ with each of the hormones. The predominant focus of the current literature on lower oxytocin levels in ASD is based on plasma levels, whereas our study assessed salivary oxytocin levels. A possible confounding effect worth considering, is whether the lower oxytocin levels in adolescents with ODD/CD are due to a long standing separation from their parent(s), as a consequence of being in a residential treatment setting (Levy et al., 2017). This would however not
explain lower oxytocin levels in adolescents with ASD, since they were not in residential treatment. While some studies have reported a significant relationship between higher oxytocin and reduction of risk seeking behaviour in TDI (Fujii et al., 2016) or between lower oxytocin and higher CU traits in ASD (Levy et al., 2015), our findings did not confirm an association between oxytocin levels and aggressive behaviour or CU traits in the dimensional analysis, which is in line with a recent meta-analysis (Rutigliano et al., 2016).

In the current study, we have used both self- and parent-reports of the ICU. Overall, the levels of CU traits reported in the current study were moderate to low. This might explain why we were unable to observe a significant relationship between oxytocin and CU traits regardless of disorder, while a recent study showed a strong association between low basal saliva oxytocin levels and high levels of CU traits (i.e. ICU score > 40) in male adolescents with conduct problems (Levy et al., 2015). Elevated oxytocin levels by means of intranasal administration may remedy social difficulties and improve social cognition for a brief period (Domes et al., 2013; Guastella et al., 2010). This warrants also further research into potential therapeutic use of oxytocin for ODD/CD by increasing social insight and decreasing aggressive behaviours towards others. Overall, our results contribute to the growing complexity in our understanding of the effects of oxytocin on social behaviour including individuals with psychiatric diagnoses.

Cortisol in ASD and ODD/CD and relation with aggression and CU traits

The three groups (i.e. TDI, ASD, and ODD/CD) did not differ in basal saliva cortisol levels. These results are in contrast with reports on lower baseline cortisol levels in adolescents with ASD (e.g. Alink et al., 2008; Taylor & Corbett, 2014), but are in line with other findings in ASD (e.g. Corbett et al., 2016). Firstly, the absence of group differences in cortisol levels is not surprising as the current study assessed basal cortisol levels without the administration of a challenge that would allow the assessment of cortisol reactivity. Secondly, the hypothalamic-pituitary-adrenal (HPA) axis, with cortisol as its end product, is a dynamic system that not only responds to psychological and physical stress, but also exhibits a marked diurnal rhythm (Kirschbaum & Hellhammer, 1994). Therefore assessing cortisol at one time point may be prone to interpretation problems, especially when there was no strict control for time of sample collection (Northover et al., 2016). However, sensitivity analyses revealed that the results were not influenced by basal saliva samples that were sampled outside the study protocol window of 9-10am. Cortisol levels were further uncorrelated with dimensional aggression scores.

Of note, we report, counterintuitively, a positive association between cortisol levels and CU traits. This is in contrast to studies reporting a negative association between basal cortisol levels and CU traits in for example ODD/CD participants (e.g. von Polier et al., 2013) or finding no significant association (e.g. Northover et al., 2016). However, the positive association in our study was driven by the link between cortisol and testosterone, and the positive association between CU traits and testosterone (i.e. correlations between cortisol and either self- or parent-rated CU traits became non-significant after controlling for testosterone).
Testosterone in ASD and ODD/CD and relation with aggression and CU traits

Results on testosterone showed that individuals with ODD/CD had significantly higher levels of testosterone than those with ASD. In both children and adults with clinical diagnoses - such as ASD and ODD/CD - problems have been related to increased prenatal and postnatal testosterone levels (Barzman et al., 2013; Golubchik, Mozes, Maayan, & Weizman, 2009; Montoya, Terburg, Bos, & van Honk, 2012; Wen & Wen, 2014). The testosterone levels in the TDI group did not differentiate from either the ASD or ODD/CD groups. Equally, the ODD/CD group differences with the ASD group became non-significant, when two subscales of the RPQ were included in the analyses. Likewise, they also became non-significant when including self-rated CU traits. Taken together, this suggests that group differences in testosterone levels between the ASD group and the ODD/CD group were attributable to different levels of aggression and self-rated CU traits. The non-significant differences in testosterone levels between the clinical groups after correction for aggression or CU traits is in line with research showing correlations between testosterone levels and measurements of aggression (Archer et al., 2005). The testosterone-aggression relationship is weak, however this may be explained by the possible mediating factor of cortisol (Mehta and Josephs, 2010). Although our results are not in line with the current literature, research showed that high testosterone levels in combination with low cortisol a) predicted physical aggression in prison inmates (Dabbs et al., 1991), and b) supported a significant relationship with aggressive behaviours (Honk et al., 2003). Another explanation is that aggressive behaviour is often more psychological instead of physical, and the underlying motives of aggressive behaviour are heterogeneous (i.e. premeditated versus reactive aggression) (Eisenegger et al., 2011). As such, testosterone may facilitate dominance instead of aggression (Eisenegger et al., 2010), and that testosterone would only increase aggression if that could increase one's social status. From this point of view, it follows that high testosterone could promote more prosocial behaviour, if that would improve social status. Another point may be the timing of the initial testosterone rise in puberty (onset) and the rate of testosterone change (tempo) may influence behaviour at this life stage (Duke et al., 2014). Though the adolescent groups were matched on age, assessments of puberty stage might have further illuminated the hormonal analyses.

Adolescents are usually less reliable in their report of their externalizing behaviour (Smith, Pelham, Gnagy, Molina, & Evans, 2000). This is supported by our findings, in which our clinical groups rated themselves as having lower CU traits compared to higher parent-rated CU traits. Interestingly, within the TDI group this pattern was reversed. One explanation could be that ASD and ODD/CD may have a different attitude towards their behaviour compared to their parents; such that the degree of conscientious in giving the correct information is different. For example, ASD and ODD/CD may see their behaviour as less severe compared to their parents. Regardless of source of informant in the current study, overall the levels of CU traits were moderate to low. Across groups, the lack of relationships between dimensional score of aggressive behaviour and each of the three hormonal levels may be masked by the presence of unmeasured co-occurring internalizing problems. The interaction between these exogenous factors (e.g. internalizing problems, trauma, and parental characteristics) may modify baseline hormone levels. For example, children with both CD and anxiety disorder are reported to have higher levels of salivary cortisol than children with CD without comorbid anxiety disorder (McBurnett et al., 1991). The vulnerability within an individual to express
more severe levels of aggressive behaviour could be based on different etiologies as suggested by Blair (2013), like perinatal factors, environmental factors in which context aggression is expressed (i.e. life-events, violence or neglect) or genetic vulnerability. Future studies should take into account both the externalizing as well as the internalizing problems of participants with ODD/CD.

Because many undesirable behaviours improve over time, despite continuing elevated hormone levels, it is possible that the speed of hormone change may be more important than the absolute hormone levels (Duke et al., 2014). Translated to our study, this may suggest that the degree of hormonal change of oxytocin, cortisol, and testosterone levels may be different across groups despite the development of persistent aggressive behaviour. Future studies should include a longitudinal design, to investigate the development of hormonal change and development of severity of aggression over time within the same individuals following stressful challenges. Promising new non-invasive methods to assess hormones may include hair cortisol, which allows for an estimate of long-term secretion over the course of several months (Stalder and Kirschbaum, 2012), and potentially fingernail cortisol, measuring periods of several weeks (Izawa et al., 2015).

To conclude, hormonal differences between both the clinical groups and the TDI group could also be seen in the light of its relation to contextual factors (e.g. being away from home in an institute), which may cause increased psychosocial stress, and perhaps not to the externalizing symptoms per se (Quinlan et al., 2017; Romero-Martinez & Moya-Albiol, 2016). Therefore, physiological measurements such as hormones, may be a reflection of a person’s state rather than a person’s trait (Weissenberger et al., 2017). In this light, physiological measurements cannot be seen as a biomarker, but rather seen as an internal instrument (i.e. a thermometer) of a person. Future research is warranted to investigate whether hormonal differences remain stable over time and could be defined as traits to describe individual’s characteristics.

Limitations

Our results need to be considered in the context of the study’s limitations. We included male adolescents in our study, thus we cannot extrapolate our findings to females with either ASD or ODD/CD. Hormone sampling did not occur at the same time of day across all participants due to practical reasons. However sampling stayed similar when excluding samples collected at another time. Testosterone was stored at -20°C, which has been shown to be less optimal to storing it at -80°C (Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004). However, all samples across all groups were processed in the same way so the assumption is made that any degradation of the hormones at -20°C may have changed absolute but not relative levels between the groups. One caveat is that our sample of TDI was smaller than the ASD and ODD/CD groups. This is because originally the current study was in part designed to sample ASD and ODD/CD groups to stratify by high and low CU traits which would result in equally participant numbers across CU straits. Both clinical groups contained a substantial number of participants using medication. However, antipsychotics and stimulant medications were stopped two days before and stimulants on the test day. Further, sensitivity analyses revealed results were not altered by the use of medication. As expected, our ASD and ODD/CD samples had also in part comorbid ADHD (Connor, Steeber, & McBurnett, 2010; Waschbusch, 2002). However, sensitivity analyses revealed that our results were not influenced by
the presence of comorbid ADHD. Future research is recommended to include ADHD severity instead of diagnosis only to explore the effect of ADHD severity on the three hormones.

**Conclusion**
The current findings show that 1) the diagnostic groups differed by their pattern of hormonal findings, with individuals with ASD characterized by relative low levels of oxytocin, and those with ODD/CD by a relative low level of oxytocin and high level of testosterone; and 2) these group effects were partly driven by differences in severity of aggression and/ or CU traits between the groups. Further research should explore whether these baseline hormonal factors can be used as stratification markers to predict response to treatment or course of these disorders.
### Table 6. Correlations between RPQ subscales across groups.

<table>
<thead>
<tr>
<th>Aggression Dimension</th>
<th>RPQ Total</th>
<th>RPQ proactive</th>
<th>RPQ1</th>
<th>RPQ2</th>
<th>RPQ3</th>
</tr>
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<tbody>
<tr>
<td>Based on two subscales</td>
<td>RPQ reactive</td>
<td>.88</td>
<td>.63</td>
<td>.63</td>
<td>.48</td>
</tr>
<tr>
<td></td>
<td>RPQ proactive</td>
<td>.92</td>
<td>1</td>
<td>.63</td>
<td>.87</td>
</tr>
<tr>
<td>Based on three subscales</td>
<td>RPQ 1</td>
<td>.88</td>
<td>1</td>
<td>.48</td>
<td>.63</td>
</tr>
<tr>
<td></td>
<td>RPQ 2</td>
<td>.77</td>
<td>1</td>
<td>.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RPQ 3</td>
<td>.88</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RPQ1: Reactive and Proactive Questionnaire - proactive subscale; RPQ2: reactive intern subscale; RPQ3: reactive extern subscale; RPQ total: Reactive and Proactive Questionnaire – total score; Significant correlations presented in bold p≤ .001
References


American Psychiatric Association (2013). Diagnostic and statistical manual of mental disorders (5th ed.).


Emotion valence detection in adolescents with oppositional defiant disorder / conduct disorder or autism spectrum disorder
Abstract

Oppositional defiant disorder, conduct disorder (ODD/CD), and autism spectrum disorder (ASD) share poor empathic functioning and have been associated with impaired emotional processing. However, no previous studies directly compared similarities and differences in these processes for the two disorders. A two-choice emotional valence detection task requiring differentiation between positive, negative and neutral IAPS pictures was administered to 52 adolescents (12-19 years old) with ODD/CD, 52 with ASD and 24 typically developing individuals (TDI). Callous-unemotional (CU) traits were assessed by self- and parent-report using the Inventory of Callous-Unemotional traits. Main findings were that adolescents with ODD/CD or ASD both performed poorer than TDI in terms of accuracy, yet only the TDI –not both clinical groups– had relatively most difficulty in discriminating between positive versus neutral pictures compared to neutral-negative or positive-negative contrasts. Poorer performance was related to a higher level of CU traits. The results of the current study suggest youth with ODD/CD or ASD have a diminished ability to detect emotional valence which is not limited to facial expressions and is related to a higher level of CU traits. More specifically, youth with ODD/CD or ASD seem to have a reduced processing of positive stimuli and/or lack a ‘positive perception bias’ present in TDI, that could either contribute to the symptoms and/or be a result of having the disorder and may contribute to the comorbidity of both disorders.
The detection of emotional valence—the intrinsic attractiveness/’goodness’ (positive valence) or averseness/’badness’ (negative valence) of a person, object, or situation provides crucial information for decision making [4]. Emotions with the same valence (e.g., anger and fear or pride and surprise) produce a similar influence on judgments and choices. The detection of negative valence activates the behavioural inhibition system (BIS), leading to a withdrawal from the person, object or situation [26]. Similarly, the detection of positive valence activates the behavioural activation system (BAS), leading to an approach toward the person, object, or situation [22]. In daily life, emotional valence detection is frequently active during the recognition of facial expressions [60], providing crucial information on whether the other person is willing to positively interact with you or not. However, it is similarly crucial to correctly identify the emotional valence of non-facial stimuli and situations, since a reduced ability to detect the negative emotional valence of situations will inappropriately activate a tendency to approach the object or situation, causing potentially dangerous situations such as getting into fights or other self-harming situations [7]. Similarly, a reduced ability to detect the positive emotional valence of objects or situations will falsely activate a tendency to withdraw from the object or situation, leading for instance to social shyness and self-isolation [10]. As such, the correct processing of emotional content is essential to fully understand functional and dysfunctional behaviour.

Emotional valence is processed in similar (i.e., limbic and prefrontal) brain areas across disorders [28]. In nearly all psychiatric disorders, an increased likelihood has been found for an altered and/or reduced detection of emotional valence [33]. Of specific interest in this domain are disruptive behaviour disorders—oppositional defiant disorder and conduct disorder (ODD/CD)—and autism spectrum disorder (ASD), since both disorders can be hypothesized to be characterized by a disbalance in the BIS/BAS [21, 45] as well as a pronounced reduction in empathy. This is in contrast with other disorders also characterized by a disbalance in the BIS/BAS system such as anxiety [31] or mood disorders [2]. Individuals with ODD/CD tend to approach people, objects, and/or situations that have a (clear) negative valence to people without ODD/CD more than controls (i.e., typically developing individuals and individuals with ADHD) [45]. A decreased emotional arousal has been found in individuals with CD when looking at emotionally evocative pictures, potentially explaining this phenomenon [55, 63]. In contrast, individuals with ASD tend to avoid people, (social) situations and sometimes objects that have a (clear) positive valence to individuals without ASD more than typically developing controls [44].

Notwithstanding the differences between both diagnostic categories, differentiation between both is sometimes difficult in everyday clinical practice. About a quarter of children with ASD show comorbid ODD/CD [35], and the incidence of ASD symptoms in ODD/CD is also clearly raised [1, 25]. Furthermore, both disorders frequently show comorbidity with ADHD (Hartman, Geurts, Franke, Buitelaar, & Rommelse, 2016; Van Steensel, Bogels, & de Bruin, 2013). This overlap shows that psychopathology does not exist in dichotomous entities (i.e., presence of absence of a disorder), and that liability for having one disorder may increase liability for having another (Blanco et al., 2015; Caspi et al., 2014). Therefore, it may be important to focus on a research classification system for mental disorders based upon dimensions of functional systems, neurobiology and observable behaviour, such as the Research Domain Criteria (RDoC) project. RDoC supports research to
explicate fundamental biobehavioural dimensions (such as emotional valence detection) that cut across current heterogeneous disorder categories (Cuthbert & Insel, 2013). Studying emotional valence detection may give more insight into the (non-)overlapping cognitive features of categorical disorders.

It may be further hypothesized that an altered/reduced ability to detect emotional valence in ODD/CD and ASD may be related to a reduced ability to understand, feel and show empathy found in both disorders [39]. Empathy is the capacity to recognize, understand and share the emotional states of others [18]. Empathy may be dissected into three domains, that is, emotional empathy, cognitive empathy and motor empathy [8]. Emotional empathy refers to experiencing emotions consistent with and in response to those of others. [9]. Cognitive empathy refers to reduced abilities to identify and describe how others may perceive situations [5], and to automatically track others' mental states [29]. Motor empathy refers to automatically and unconsciously mirroring the facial expressions of another person, known as facial mimicry [9]. In CD, deficits in emotional empathy have been related to callous-unemotional (CU) traits [23]. CU traits (‘limited prosocial emotions’) have been adopted as a specifier to CD in the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5), and are defined as a set of personality traits comprising lack of empathy, lack of remorse or guilt, shallow affect and being unconcerned about performance. Indeed, conduct problems and high CU traits have been found to be related to deficits in emotional empathy, whereas ASD was found to be related to cognitive empathy deficits [34, 53]. However, recent studies have shown that CU traits might be best seen as cross-disorder construct with increased prevalence not only in CD, but in ODD and ASD as well [30, 42]. As such, an altered emotional valence detection may underlie the increased prevalence of CU traits across disorders and may help understand the comorbidity rates between both disorders.

Therefore, the current study set out to examine the overlapping and specific features of altered emotional valence detection in individuals with ODD, CD or ASD and whether this is related to the severity of CU traits in both clinical groups. A two-choice emotional valence detection task was administered in adolescents with ODD/CD, or ASD, and in typically developing individuals (TDI). In each trial, two pictures with emotional valences were contrasted (neutral-positive, neutral-negative, negative-positive). As the literature on this topic is sparse, we did not formulate a hypothesis how the different diagnostic groups would perform, and whether a moderating role of CU traits could be detected.

**Method**

**Participants**
Initially, 166 male subjects were approached to participate, of whom 38 dropped out because of: refusal to participate (n = 28), not meeting inclusion criteria (n = 8), or not able to obtain consent of legally appointed guardian (n = 2). This resulted in a total sample of 128 male participants (n = 52 patients with ODD or CD (ODD/CD), n = 52 patients with ASD, and n = 24 TDI group). Participants
with ODD or CD were grouped together, since both disorders are closely linked neurodevelopmental disorders of which ODD is either prodromal to CD (Biederman et al., 1996; Burke, Hipwell, & Loeber, 2010) or a subsyndromal form of CD (Biederman et al., 1996). All participants aged between 12 and 19 years (M = 15.3 years, SD = 1.9), 81% were of Caucasian origin. They were recruited between April 2011 and September 2014 as part of a larger study on empathy (CU2-study). Participants were recruited through clinical institutes in the Netherlands, specialized in severe disruptive behaviour problems (De Hoenderloo Group, Ottho Gerhard Heldring Foundation, and Woodbrookers) or severe psychiatric problems (Karakter, Child and Adolescent Psychiatry) and through information leaflets that were sent to families via the Dutch federation of Autism (NVA). Adolescents were excluded if they fulfilled one or more of the exclusion criteria (a) a combined diagnosis of ODD/CD and ASD, (b) an estimated total IQ < 80, and/or (c) suffering from a condition which may affect neurological or cognitive functioning, such as schizophrenia, bipolar disorder, alcohol and/or drugs dependency, presence of tics, language disorders (e.g., dyslexia) and epilepsy. The TDI group was recruited from a general community sample via city councils in the same geographical regions as the clinical groups. In- and exclusion criteria for the TDI group were similar to the ODD/CD and the ASD groups, except for having a clinical psychiatric diagnosis.

Diagnoses in the ODD/CD and the ASD groups were established according to DSM-IV-TR [3] criteria by a multidisciplinary team based on information gathered by a child psychiatrist, a child psychologist, and a review of clinical and prior records (if available), including information available from school or other professional institutions involved with the child. Thus, a consensus diagnosis was assigned, which is seen as more reliable compared to structured interviews for assessing diagnostic categories [41]. In the TDI group, the absence of a clinical psychiatric diagnosis was assessed based on parent report. For all three groups, legal guardians were asked to fill out a digital version [58] of the National Institute of Mental Health Diagnostic Interview Schedule for Children (DISC-IV; [54]). Legal guardians were asked to fill out the following sections: Attention-Deficit/Hyperactivity Disorder, ODD, CD, Tic Disorder, Alcohol, Marihuana, Other Drugs, in order to control for possible psychiatric comorbidities. Because DSM-IV-TR diagnoses were automatically generated, an experienced child and adolescent psychiatrist (PH) and psychologist (MB) evaluated diagnostic findings of the computerized DISC. The use of non-psychotropic (5.5%) and antidepressant medication (2.3%) was allowed. If possible, psychotropic medication (i.e., stimulants, 21.8%, antipsychotics, 9.3%, atomoxetine, 2.3%) was stopped prior to testing. Stimulants were discontinued for at least 24 hours. Antipsychotics were discontinued for at least 72 hours. However, when discontinuation was thought to have severe deteriorating effects, medication was continued (ODD/CD: n = 12, ASD: n = 10). Participants were required to have a minimum average estimated total full-scale intelligence quotient (FSIQ) of ≥ 80. FSIQ was estimated using four subtests of the Dutch version Wechsler Intelligence Scale for Children (WISC-III): Similarities, Vocabulary, Block Design and Picture Completion [65]. These selected WISC-III subtests are known to correlate between .90-.95 with the Full-scale IQ [27]. For children older than 16 years, the Wechsler Adult Intelligence Scale (WAIS-III) was administered [66]. When intelligence was assessed within a year prior to the inclusion, and either the WISC or WAIS was applied, we used the scores of that assessment.

This study was approved by the Dutch Central Committee on Research involving Human Subjects, protocol number NL26773.000.09 (Centrale Commissie Mensgebonden Onderzoek;
CCMO). Both parents and the adolescents (if 12 years and older) signed the informed consent.

Measures
A two-choice emotional valence detection task was used with a high load on set shifting abilities. This task has previously shown to be associated with differential reaction times in adults, being grouped as forensic psychiatric patients, psychopaths, and normal controls [12], indicating differentiating properties across different diagnostic groups of this task. The task required participants to pay attention to stimuli with a predefined affective load presented in the centre of the screen, and to respond as rapidly as possible by pressing the space bar for target stimuli (hits), while withholding the response to non-target stimuli. Stimuli consisted of pictures from the International Affective Picture System (IAPS) database which were validated for their valence [40], and grouped into three categories (positive and negative valence, and neutral; for examples see Figure 1). In each block, two valences were contrasted (neutral-positive, neutral-negative, negative-positive), to investigate whether changes in false positive reactions and in reaction time are related to specific (combinations of) emotions. Only two emotional valences were presented per block. Each valence-combination was presented twice in two different blocks varying the valence of target stimuli (50%) versus non-target stimuli (50%) to investigate whether changes in false positive reactions and in reaction time are related to stimulus order (i.e., the order in which specific combinations of emotions are presented. Thus, valence contrast was manipulated (with three emotional valences) and stimulus order (e.g., neutral stimuli as target stimuli and positive as non-target stimuli in block 1 and the reverse in block 2). This resulted in 6 experimental blocks (neutral-positive, positive-neutral; neutral-negative, negative-neutral; negative-positive, positive-negative), each with 32 trials. Each single block lasted approximately 45 seconds in duration, with a fixed 500 ms stimulus presentation and response window, followed by a 900 ms inter-stimulus interval. A short practice session of 10 trials with the same presentation rate preceded each block to learn the distinction between the two affective categories being used in each block. The practice block was automatically repeated once if the participant did not reach the 80% accuracy cut-off the first time. Thereafter, the experimental block

Figure 1. Examples from the emotional valence detection task

On the left a positive stimulus, in the middle a neutral stimulus, on the right a negative stimulus
was started. Outcome variables were the percentage of hits, the mean reaction time per block and the signal to noise ratio $d'$ (percentage hits – percentage false positives).

The test battery was shown on a Dell Latitude D530 laptop with a Windows Vista operating system. The task was presented using the MINDS-software program (version 1.2.7) [11], which is a digital test manager used to present a test battery. Participants were positioned in front of a laptop about 60 cm from a 15-inch screen with a resolution of 1024x768 pixels and a refresh rate of 60 Hz. Images were presented in the centre of the display with neutral grey background. Images were 15.5 x 20.5 cm in size, covering a visual angle of 15 degrees vertically and 20 degrees horizontally. Finishing the total task took about 15 minutes.

CU traits were assessed by self- and parent-report on the Inventory of Callous-Unemotional traits (ICU), Dutch translation [51]. The ICU contains 24 items, which are rated on a 4-point Likert scale ranging from 0 = does not apply at all to 3 = applies very well. Internal consistency of the Dutch ICU was shown to be good [19, 51]. In the current study Cronbach’s alpha was high (.80 for ICU-SR, .90 for ICU-PR). Concurrent validity between the ICU and psychopathy scales is acceptable ($r = .45-.68$ between ICU and Antisocial Process Screening Device [38], and Childhood Psychopathy Scale [51].

ASD symptoms were assessed by administering the Social Communication Questionnaire (SCQ) [52]. This is a 40-item parent-report questionnaire that asks about characteristic autistic symptoms. Each item is rated as either Yes or No. Nineteen items rate current behaviour and 20 items rate behaviour when the child was 4-5 years old. Cut-off score is $\geq 15$. Sensitivity was found to range between .85-.88, Specificity was found to range between .72 and .78 in English-language versions [6, 13, 14]. In this study, Cronbach’s alpha for the total SCQ was .75. For analyses, the current behaviour items were used.

Procedures
A short telephone screening and, subsequently, screening questionnaires were used to verify if families could participate. Those families were invited to visit one of the participating clinics. Testing of the participants took place in a quiet room at their clinical institute. The task described here was part of a broader neuropsychological assessment battery used in the ‘psychopathology and the lack of empathy’ (CU2) project. Youths completed the battery in approximately two hours and the order of the task administration was counterbalanced. Participants were motivated with small breaks and received a financial compensation (vouchers of € 20.00) after test administration.

Data analyses
In order to examine the group differences regarding emotional valence detection and set-shifting capacities, repeated measure ANOVAs were conducted with group as between subjects factor (3 levels: TDI, ODD/CD, ASD), valence-contrast as within subjects factor (three levels: neutral-positive, neutral-negative, positive-negative) and response-set as within subjects factor (two levels: original set vs. reversed set). Analyses were separately run for percentage hits, reaction time of hits, and $d'$ (signal to noise ratio, calculated as percentage hits – percentage false positives). Analyses were
Table 1. Characteristics of the study population (N = 128)

<table>
<thead>
<tr>
<th></th>
<th>Total group</th>
<th>TDI</th>
<th>ODD/CD</th>
<th>ASD</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M ±SD</td>
<td>M ±SD</td>
<td>M ±SD</td>
<td>M ±SD</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>15.3 ± 1.9</td>
<td>15.9 ± 1.8</td>
<td>15.5 ± 1.7</td>
<td>14.9 ± 2.0</td>
<td>ns</td>
</tr>
<tr>
<td>FSIQ***</td>
<td>99.4 ± 11.1</td>
<td>106.1 ± 8.1</td>
<td>92.7 ± 8.9</td>
<td>102.7 ±11.1</td>
<td>ODD/CD &lt; ASD = TDI</td>
</tr>
<tr>
<td>VIQ***</td>
<td>100.1 ± 14.0</td>
<td>108.3 ± 9.7</td>
<td>90.5 ± 12.5</td>
<td>103.8 ± 12.7</td>
<td>ODD/CD &lt; ASD = TDI</td>
</tr>
<tr>
<td>PIQ</td>
<td>99.6 ± 14.1</td>
<td>103.7 ± 14.1</td>
<td>95.1 ± 12.6</td>
<td>101.3 ± 14.5</td>
<td>ns</td>
</tr>
<tr>
<td>ICU-SR total score**</td>
<td>26.9 ± 9.0</td>
<td>23.2 ± 6.3</td>
<td>30.7 ± 10.0</td>
<td>24.8 ± 7.5</td>
<td>ODD/CD &gt; ASD = TDI</td>
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<tr>
<td>ICU-PR total score***</td>
<td>29.4 ± 11.9</td>
<td>16.0 ± 7.0</td>
<td>38.9 ± 9.4</td>
<td>28.0 ± 8.4</td>
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<td>SCQ total score***</td>
<td>11.7 ± 7.1</td>
<td>3.1 ± 2.4</td>
<td>11.9 ± 6.1</td>
<td>15.5 ± 5.7</td>
<td>ASD &gt; ODD/CD &gt; TDI</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N %</th>
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<th>n %</th>
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<td>0</td>
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<tr>
<td>Dutch Association for Autism</td>
<td>8</td>
<td>6.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Comorbidity**        |             |             |             |             |           |
| ADHD                 | 53 | 41.4 | 0 | 0 | 31 | 59.6 | 22 | 42.3 |
| None                 | 64 | 50.0 | 24 | 100 | 12 | 23.1 | 29 | 55.8 |
| Missing              | 11 | 8.6 | 0 | 0 | 9 | 17.3 | 1 | 1.9 |

| Medication*         |             |             |             |             |           |
| Yes                  | 51 | 39.7 | 0 | 0 | 18 | 34.6 | 33 | 63.5 |
| No                   | 70 | 54.7 | 23 | 95.8 | 27 | 51.9 | 19 | 36.5 |
| Missing              | 7 | 5.5 | 1 | 4.2 | 7 | 13.5 | 0 | 0 |

| Ethnicity parents (n = 258)*** |             |             |             |             |           |
| Caucasian             | 207 | 80.9 | 46 | 95.8 | 60 | 57.7 | 101 | 97.1 |
| African               | 19 | 7.4 | 0 | 0 | 19 | 18.3 | 0 | 0 |
| Unknown               | 25 | 9.4 | 2 | 4.2 | 21 | 20.2 | 1 | 1.0 |
| Missing               | 6 | 2.3 | 0 | 0 | 4 | 3.8 | 2 | 1.9 |

| Highest level of education parents* |             |             |             |             |           |
| Lower                 | 8 | 6.3 | 0 | 0 | 5 | 9.6 | 3 | 5.8 |
| Middle                | 36 | 28.1 | 3 | 12.5 | 14 | 26.9 | 19 | 36.5 |
| Higher                | 60 | 46.9 | 21 | 87.5 | 10 | 19.2 | 29 | 55.8 |
| Missing               | 24 | 18.8 | 0 | 0 | 23 | 44.2 | 1 | 1.9 |

---

1 If possible, psychotropic medication was stopped prior to testing. For 12 youths with ODD/CD and 9 youths with ASD discontinuation of medication was not possible. p-value after Bonferroni correction: * p < .05, ** p < .01, *** p < .001
2 Highest level of education parents: lower = primary education / preparatory lower-level vocational education; middle = preparatory middle-level vocational education; higher = higher-level vocational education / preparatory university education. TDI = typically developing individual; ODD/CD = oppositional defiant disorder / conduct disorder; ASD = autism spectrum disorder; ICU-SR = Inventory of Callous Unemotional traits – self-report; ICU-PR = Inventory of Callous Unemotional traits – parent-report; SCQ = Social Communication Questionnaire. M = mean; SD = standard deviation; ns = not statistically significant (p > .05)
run with and without IQ as covariate, and medication and comorbidity (i.e., ADHD present or not present) as between subjects factor. Covariates were entered separately to examine the unique effects on the results. Discrepancies in results were reported. Across and within groups, Pearson’s correlations between CU and ASD symptoms (SCQ current items) and task performance were examined. Correlational analyses between CU traits and task performance were run within groups. All analyses were carried out in SPSS version 24. Power-analysis indicated a sample size of N = 102 was needed to achieve 95% power to detect main and interaction-effects of d ≥ .25 using the proposed repeated measure ANOVA with three groups. Missing data was 0% for the emotional valence detection task performance (N = 128) and ICU self-report questionnaire (N = 128), and 7.8% for the parent rated SCQ and ICU (N = 118). Missing data were not replaced.

Results

See Table 1 for sample characteristics. The majority (84.6%) of the ODD/CD group was diagnosed with having ODD, 15.4% with CD. Comorbid ADHD was found in 59.6% of the ODD/CD group and in 42.3% of the ASD group. In the clinical groups, 34.4% used psychotropic medication. Stimulants were the mostly prescribed medication type (21.8%), followed by antipsychotics (9.3%) and non-psychotropic medication (5.5%), with stimulants being significantly more prescribed in the ASD group, compared to the ODD/CD group (p < .05). For 12 youths with ODD/CD and 10 youths with ASD discontinuation of medication was not possible.

The three groups did not differ in age but did significantly differ in IQ (F (2, 120) = 19.65, p < .001), whereby TDI (M = 106.1, SD = 8.1) and ASD (M = 102.7, SD = 11.1) had a higher mean IQ compared to ODD/CD (M = 92.7, SD = 8.9). The ODD/CD group showed significantly higher self-reported CU-scores than both the TDI and the ASD groups, whereas the TDI and the ASD groups did not differ from each other (F (2, 125) = 9.04, p < .01). The ODD/CD group scored significantly higher on parent reported CU-traits than the ASD group, and the ASD group scored significantly higher than the TDI group (F (2, 115) = 57.06, p < .001). For SCQ scores, the ASD group scored significantly higher than the ODD/CD group, and the ODD/CD group scored significantly higher than the TDI group (F (2, 110) = 44.46, p < .001).

Task manipulation effects

Main effects of response-set were found for percentage hits, reaction time hits and d’, in which performance on the second (i.e., reversed set) was made with slightly slower RTs, percentage hits and lower d’, suggesting the reversed response-set taxed set shifting abilities. However, since there were no two-way (diagnosis by response-set) or three-way (diagnosis by response-set by valence-contrast) effects on any of the outcome variables, for simplification further analyses were carried out with data collapsed for blocks contrasting the same valences. Main effects of valence-contrast were found on all parameters, in which discriminating between positive and neutral pictures appeared more difficult compared to negative versus positive or negative versus neutral pictures, as reflected by fewer hits, longer RTs for hits and a lower d’. No differences were found for the ability to discriminate between negative versus positive and negative versus neutral. Please see Table 2 for results of the repeated measure ANOVAs.
Table 2. Descriptives of average scores per valence-contrast, response-set, and diagnosis; results of the repeated measures ANOVAs

<table>
<thead>
<tr>
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<th>ASD</th>
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<td>473.8 (129.6)</td>
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<td>15.1; &lt; .001; .20</td>
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<td>NEU-POS</td>
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<td>ns</td>
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<td>438.5 (150.4)</td>
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<td>NEG-NEG</td>
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<td>375.0 (202.6)</td>
<td>434.9 (134.6)</td>
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<td><strong>d’ (percentage hits – percentage false positives)</strong></td>
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<td>Response-set</td>
<td>6.35; .01; .05</td>
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<td>72.2 (21.0)</td>
<td>67.2 (23.1)</td>
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<td>Diagnosis * valence-contrast * response-set</td>
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Note. This two-choice emotional valence detection task required participants to pay attention to stimuli with a predefined affective load presented in the centre of the screen, and to respond as rapidly as possible by pressing the space bar for target stimuli, while withholding the response to non-target stimuli. The task consists of six blocks presented in random order, each with 32 trials. Stimuli consisted of pictures from the IAPS database, and grouped into three categories: positive, neutral and negative valence. Because of randomization, type of valence-contrast did not relate to block order. Repeated measure ANOVAs were conducted with group as between subjects factor (3 levels: TDI, ODD/CD, ASD), valence-contrast as within subjects factor (three levels: neutral-positive; neutral-negative; positive-negative) and response-set as within subjects factor (two levels: original set vs reversed set). TDI = typically developing individual; ODD/CD = oppositional defiant disorder / conduct disorder; ASD = autism spectrum disorder; M = mean; SD = standard deviation; ns = not statistically significant (p > .05).
Table 3. Descriptives of average scores per block number and diagnosis; results of the repeated measures ANOVAs

<table>
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<th>TDI</th>
<th>ODD/CD</th>
<th>ASD</th>
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<td>65.2 (22.9)</td>
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</table>

1 Because of randomization, block number did not relate to valence-contrast. ² Repeated measure ANOVAs were conducted with group as between subjects factor (3 levels: TDI, ODD/CD, ASD) and block number as within subjects factor (6 levels: 1-6). TDI = typically developing individual; ODD/CD = oppositional defiant disorder / conduct disorder; ASD = autism spectrum disorder; M = mean; SD = standard deviation; ns = not statistically significant (p > .05)
Diagnostic effects
A main effect of diagnosis was found for percentage of hits (Table 2), with adolescents with ODD/CD or ASD performing poorer than TDI (both p's =.01), with no difference between adolescents with ODD/CD or ASD (p = .95) (Figure 2). Also, a trend main effect of diagnosis on d' was present, with overall a poorer signal-to-noise ratio in adolescents with ODD/CD or ASD compared to TDI (p =.02 and .05, respectively), with no difference between adolescents with ODD/CD or ASD (p = .63). However, a trend-significant diagnosis * block order effect was found, with post-hoc tests showing that performance decreased linearly in the TDI group (F (1, 23) = 6.57, p = .017), but remained more stable in both clinical groups (ODD/CD: F (1, 51) = 2.95, p = .09; ASD: F (1, 51) = 0.51, p = .63). Furthermore, a diagnosis by valence-contrast was found for hit RT. Post-hoc tests showed that valence-contrast had no significant effect on RT in the ASD group (F (2,50) = 2.00, p = .15), whereas it did in the TDI group (F (2,22) = 5.25, p = .01) and the ODD/CD group (F (2,50) = 4.58, p = .02). The TDI group was slower in discriminating between positive versus neutral pictures than in the other two conditions (both p's < .01) that did not differ from each other. In contrast, the ODD/CD group was faster in discriminating between positive versus negative pictures than in the other two conditions (p's < .03) that did not differ from each other (Figure 3). Covarying for ASD symptoms did change the p-values but not the overall pattern of findings.

Figure 2. Percentage hits (mean and standard error) by diagnosis

Moderating role of CU traits
Parent rated CU traits and ASD symptoms (SCQ current items) correlated significantly (r = .31, p < .001). Self-rated CU traits did not correlate with parent rated ASD symptoms (r = .04, p = .69). Self and parent-rated CU traits correlated significantly (r = .50, p < .001). Significant small correlations were found between ASD symptoms and slower reaction times in 3 out of 6 blocks (NEG-POS: r
= .21, p = .025; NEU-NEG: r = .22, p = .016; NEG-NEU: r = .25, p = .006), but not for measures of accuracy. Parent rated CU traits correlated with reduced accuracy on 2 out of 6 blocks (fewer hits: NEU-POS: r = -.21, p = .024; NEU-NEG: r = -.25, p = .006) and lower d’ scores (NEU-POS: r = -.25, p = .008; NEU-NEG: r = -.18, p = .048). Parent rated CU traits did not correlate with measures of speed.

Figure 3. Reaction time (mean and standard error) in milliseconds to discriminate two pictures with distinct valence by diagnosis

Similarly, self-rated CU traits did not correlate with measures of speed, but did correlate with poorer performance in terms of accuracy on 1 out of 6 blocks (NEU-POS: fewer hits r = -.30, p = .001; lower d’ r = -.30, p < .001). To examine the moderating role of CU traits, CU traits (parent and self-rated separately) were added to the main model as main effect as well as in a two-way interaction with diagnosis. No (trend) significant two-way interaction effect were found between diagnosis and CU traits on task performance, suggesting no support for a moderating role of CU traits in explaining the group differences in task performance. Running correlational analyses within groups, within the control group and ASD, no significant correlations were found between task performance measures and ASD or CU symptoms. Within the ODD/CD group, significant correlations were found between ASD symptoms and percentage false positives for discriminating between positive and neutral and between positive and negative pictures, with higher ASD symptom levels relating to a lower percentage of hits (r=-.33, -.31, respectively).

Sensitivity analyses
Adding IQ, age or comorbid ADHD did not alter the results. Taking into account medication use, the main effect of diagnosis and the diagnosis * block interaction on d’ in the analyses on performance
across time independent of valence-contrast became non-significant. Post-hoc analyses indicated no significant differences between the group with ODD ($n = 44$) and the group with CD ($n = 8$). On all parameters, estimated marginal means were quite similar for both groups, although slightly better in the CD group (Percentage hits: ODD $M = 89.1$, CD $M = 91.0$; reaction time hits: ODD $M = 491.6$, CD $M = 478.0$).

**Discussion**

This study aimed to investigate the overlapping and specific features of altered emotional valence detection in individuals with either ODD/CD or ASD and whether this is related to the severity of CU traits in both clinical groups. A two-choice emotional valence detection task was administered in 128 adolescents using validated pictures from the IAPS database [40]. Main findings were that adolescents with ODD/CD or ASD both performed poorer than TDI in terms of accuracy, yet only the TDI group—not both clinical groups—had relatively most difficulty in discriminating between positive versus neutral pictures compared to neutral-negative or positive-negative contrasts. Poorer performance was related to a higher level of CU traits. The results of the current study suggest youth with ODD/CD or ASD have a diminished ability to detect emotional valence which is not limited to facial expressions and is related to a higher level of CU traits. More specifically, youth with ODD/CD or ASD seem to have a reduced processing of positive stimuli and/or lack a ‘positive perception bias’ present in TDI, that could either contribute to the symptoms and/or be a result of having the disorder, and may contribute to the comorbidity of both disorders. The continuous switching of response-set affected the performance of TDI more so than that of both clinical groups, albeit TDI had more room for deterioration in performance compared to both clinical groups.

To the best of our knowledge, this is the first study to investigate and directly compare emotional valence detection capacities in youths with ODD/CD and those with ASD. Overlapping results for both groups were found for accuracy: both groups had more difficulty in detecting the emotional valence of the stimuli presented compared to the TDI group. This is in line with previous studies showing that emotion recognition deficits are found in both disorders [9, 44, 55]. Furthermore, in neither group, the valence contrast (i.e., discriminating between positive, negative and neutral valences) strongly affected performance, which was in striking contrast with the TDI. The TDI group showed clear difficulty in discriminating between positive and neutral valences in comparison to discriminating between negative and neutral or positive valences. It seems that the TDI group spent more time processing the stimuli given that positive vs. neutral was the most difficult differentiation based on the accuracy data. This may reflect a reduced processing of positive stimuli in those with ODD/CD or ASD compared to TDI. However, it may also tentatively be argued that TDI have a ‘positive perception bias’ in which they have more difficulty in differentiating between neutral and positive valences, compared to both clinical groups. This seems in line with previous studies [57, 59, 61] and our data suggest that this positive perception bias is absent in both clinical groups. The absence of a positive perception bias in both clinical groups may also suggest a more negative perception bias in both clinical groups, but it would then be expected that both clinical groups had
relatively more difficulty in discriminating between neutral and negative pictures, which was not the case. The current results therefore support the idea that the absence of a positive perception bias may either increase the risk of developing the social interaction problems as present in ASD and ODD/CD and/or may be the result of having social interaction problems.

In this study, we expected differential impairment in emotional valence detection between the clinical groups. However, differences between the ODD/CD group and the ASD group were small and nonsignificant. There are several possible explanations for this negative finding. It may be that both groups had difficulty in distinguishing emotional valence because we used pictures without differentiating emotions specifically. Research regarding emotional face recognition has shown that emotion recognition in ODD/CD seems to be impaired for negative emotions only (Bons et al., 2013). For youths with ODD/CD this impairment may be related to fearful, and to a lesser extent, sad emotion only (Herpers, Scheepers, Bons, Buitelaar, & Rommelse, 2014), while emotion recognition in ASD seems to be impaired across all emotions (Bons et al., 2013). Another explanation may be that we used static pictures and not dynamic. Similar research in ODD/CD has focused on the use of dynamic pictures (Bons et al., 2013). However, as youths with CP show other responses other responses to real-life situations than to hypothetical situations (Herpers, Scheepers, Bons, Buitelaar, & Rommelse, 2014), one could infer that recognizing the emotional valence of static pictures may be more difficult to youths with ODD/CD than dynamic pictures. Youths with ASD seem to be less sensitive to static pictures than to dynamic pictures, whereas whether there is such a correlation for youths with CD is unknown (Bons et al., 2013). A further explanation could be that the duration of stimulus presentation was too short, as ASD youths seem to need more time for a correct reaction (Oberman, Winkielman, & Ramachandran, 2009; Schwenck et al., 2012). Yet, stimulus duration of 500 ms (i.e., >160 ms), as presented in our study, should suffice for correct identification of emotional valence (Oberman et al., 2009). A last possible explanation to mention here is that research shows that youth with conduct problems may experience emotional valence differently from other youth. Negative emotion, especially aggression, may be related to approach, whereas anxiety may be related to withdrawal (see also Van Honk and Schutter (2006), and (Blair, 1995). Furthermore, negative emotion may be amusing, especially to youth with conduct problems and high CU traits (De Wied et al., 2012). However, these possible explanations need further investigation.

Diminished ability to detect emotional valence was related to a higher level of CU traits, although we found no support for a moderating role of CU traits in explaining the group differences in task performance. In youth with conduct problems, high CU traits have been found to be related to a subjective pleasant judgement to negative images, compared to neutral and positive pictures [43], but see [47, 56] possibly explaining more difficulty discriminating between negative, neutral and positive pictures in the current study. Furthermore, in comparison to youth with conduct problems and low CU traits and normal controls, youth with conduct problems and high CU traits were found to show decreased distraction from distressing pictures [32, 36, 37], pinpointing to a decreased activation of the BIS in youth with high CU traits. The latter appears particularly so for children with high CU traits that have no major environmental factor (i.e., maltreatment) explaining the presence of CU traits, showing a smaller acoustic startle response when viewing negative IAPS pictures [17]. Our data and that of previous studies may suggest a diminished ability to detect emotional valence
is related to an increased level of CU traits. As such, our findings are in line with a recent study in which an emotional Go/No-go task was applied, reporting that the difficulties that children with combined CU traits and oppositional conduct problems have in processing emotions are more of an emotional rather than an attentional nature [20].

Self-reported CU traits were found to be related to poorer detection of positive pictures, while parent-reported CU traits were related to poorer detection of positive and negative pictures. As already mentioned, it may be that ODD/CD youth with high CU traits may experience negative pictures in a positive way (De Wied et al., 2012), and therefore find difficulty in discriminating valence. However, poor detection of positive pictures is more difficult to explain. It may be that ODD/CD youth with high CU traits may experience negative pictures in a positive way (De Wied et al., 2012), and thus find difficulty in discriminating valence. One could argue that either neutral or positive pictures are misinterpreted also, as youth with conduct problems and high CU traits have problems to correctly identify emotion when expressed facially, vocally and through bodily postures (see also (Herpers et al., 2014). As such, our finding could implicate that youth with conduct problems and high CU traits have difficulty in identifying emotional valence in a more general way. However, to clarify the underpinnings of this difficulty further research would be needed.

Deviant performance on this emotional valence detection task could have been caused by executive functioning (EF) difficulties, since task performance not only required emotional valence recognition, but also behavioural inhibition, learning new rules and unlearning old rules and switching of response set. Surprisingly, when data were analysed with performance indices measured across blocks when continuous demands were placed on switching-abilities between response-rules, working memory and inhibitory control, no major group differences emerged. If anything, the TDI group showed more difficulty with maintaining the high accuracy level over time than both clinical groups did (who had poorer performance already from the first block onwards). These findings suggest that previously reported weaknesses in executive functions [15, 24, 29, 48] and to a lesser extent in individuals with ODD/CD [46], did not explain the current results of diminished ability to detect emotional valence of objects, situations or people by youth with ODD/CD or ASD.

Despite its strengths, such as the direct comparison of an ODD/CD group and an ASD group, and its focus on an emotional valence detection task, our study also showed limitations. The emotional valence detection task tapped into multiple processes (behavioural inhibition, attentional bias, recognition of emotional valence, working memory and reversal learning), making it difficult to precisely pinpoint why subjects with ODD/CD or ASD had difficulty with the task. However, when analyses were repeated explicitly examining effects of continuous switching of response-set on performance, both clinical groups did not perform worse than the TDI group, making it unlikely that these broader EF processes strongly influenced the overall poorer performance of both clinical groups in terms of valence detection. Both clinical groups contained a substantial amount of participants using medication. However, antipsychotics were stopped two days before and stimulants on the test day, making it unlikely that medication strongly influenced results. However, correcting for medication use only influenced results for d’, but not for the other measures. Furthermore, when studying disorders such as ODD, CD, or ASD, it is important to take comorbidity into account, as both disorders are known to be highly comorbid with attention-deficit/hyperactivity disorder (ADHD)
ADHD is known to be related to significant impairment on EF tasks [67] as well as emotional valence detection [50]. However, sensitivity analyses revealed results were not influenced by the presence of a comorbid diagnosis of ADHD. Another limitation that should be noted is that we do not know if the findings are unique to ODD/CD and ASD, or whether they reflect a deficit that cuts across those with psychopathology relative to TDI. An additional clinical group without core deficits in empathy could further add to the significance of our findings.

In conclusion, the results of the current study suggest youth with ODD/CD or ASD have a diminished ability to detect emotional valence which is not limited to facial expressions and is related to a higher level of CU traits. More specifically, youth with ODD/CD or ASD showed marked difficulty in the distinction between neutral and positive pictures, compared to TDI. We hypothesized that this may be due to reduced processing of positive stimuli, or to a lack of ‘positive perception bias’ which seems to be present in TDI. Both tendencies could either contribute to the symptoms and/or be a result of having the disorder, but may contribute to the comorbidity of both disorders.
References


Emotional face recognition in male adolescents with autism spectrum disorder or disruptive behavior disorder: an eye-tracking study

Published as:
*Both authors contributed equally to this work

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European Child & Adolescent Psychiatry, ePub ahead of print
Abstract

Autism Spectrum Disorder (ASD), Oppositional Defiant Disorder (ODD), and Conduct Disorder (CD) are often associated with emotion recognition difficulties. This is the first eye-tracking study to examine emotional face recognition (i.e., gazing behavior) in a direct comparison of male adolescents with Autism Spectrum Disorder or Oppositional Defiant Disorder/Conduct Disorder, and typically developing (TD) individuals. We also investigate the role of psychopathic traits, callous–unemotional (CU) traits, and subtypes of aggressive behavior in emotional face recognition. A total of 122 male adolescents (N=50 ASD, N=44 ODD/CD, and N=28 TD) aged 12–19 years (M=15.4 years, SD=1.9) were included in the current study for the eye-tracking experiment. Participants were presented with neutral and emotional faces using a Tobii 1750 eye-tracking monitor to record gaze behavior. Our main dependent eye-tracking variables were: (1) fixation duration to the eyes of a face and (2) time to the first fixation to the eyes. Since distributions of eye-tracking variables were not completely Gaussian, non-parametric tests were chosen to investigate gaze behavior across the diagnostic groups with Autism Spectrum Disorder, Oppositional Defiant Disorder/Conduct Disorder, and Typically Developing individuals. Furthermore, we used Spearman correlations to investigate the links with psychopathy, callous, and unemotional traits and subtypes of aggression as assessed by questionnaires. The relative total fixation duration to the eyes was decreased in both the Autism Spectrum Disorder group and the Oppositional Defiant Disorder/Conduct Disorder group for several emotional expressions. In both the Autism Spectrum Disorder and the Oppositional Defiant Disorder/Conduct Disorder group, increased time to first fixation on the eyes of fearful faces only was nominally significant. The time to first fixation on the eyes was nominally correlated with psychopathic traits and proactive aggression. The current findings do not support strong claims for differential cross-disorder eye-gazing deficits and for a role of shared underlying psychopathic traits, callous–unemotional traits, and aggression sub-types. Our data provide valuable and novel insights into gaze timing distributions when looking at the eyes of a fearful face.
When communicating with others, non-verbal communication modalities such as body movements, hand gestures, and facial expressions yield essential information, in addition to verbal communication. Decoding facial expressions is one of the most efficient ways for understanding others’ emotions and feelings. Individuals with psychiatric disorders as Autism Spectrum Disorder (ASD), Oppositional Defiant Disorder (ODD), and Conduct Disorder (CD) exhibit deficits in regulating emotions and problems inhibiting aggressive tendencies [45, 46]. This may in turn explain dysfunctions in interpreting emotions of facial expressions. ASD are early onset neurodevelopmental disorders defined by core impairments in social interaction and verbal and non-verbal communication, stereotyped and restricted patterns of interest and activity, and abnormal sensory processing according to DSM-5 criteria [1]. ODD is characterized by angry and irritable mood, and argumentative, Defiant, and disobedient behavioral patterns. CD is characterized by a pattern of aggressive, destructive, and/or deceitful behaviors that violate the rights of others according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria (APA, [1]. In this paper, we will combine ODD and CD into one diagnostic group, since both disorders are closely linked neurodevelopmental disorders of which ODD is either prodromal to CD or a subsyndromal form of CD [9]. The rationale for comparing these two distinct diagnostic cohorts (ASD versus ODD/CD) is that both involve social/communication problems and deficits in empathy (related to cognitive and emotional empathy, respectively).

In the latest version of the DSM-5, callous–unemotional (CU) traits were added as a specifier for a more severe form of CD labeled as having ‘limited prosocial emotions’ [1]. This form of CD is particularly associated with reductions in empathy when responding to fear, sadness, pain, and happiness of others [12]. ASD has also been associated with dysfunctional empathic functioning [1, 44, 73] and with increased levels of CU traits [55]. However, commonly deployed diagnostic questionnaires for ASD lack specificity to probe for CU traits, the relationship remains elusive. Nevertheless, empathy regulation is defined by two different constructs, namely (1) cognitive empathy (i.e., the ability to understand another’s feelings) and (2) emotional empathy (i.e., the experience of emotion, elicited by an emotional stimulus) [27].

Individuals with ASD often appear to have cognitive empathy deficits, but demonstrate average levels of emotional empathy [27, 49, 76]. In contrast, those with behavioral disorders (CD and ODD) show the opposite pattern (e.g., [16, 13]). Looking at facial emotion recognition from a behavioral perspective, no significant differences were detected when comparing ASD adolescent individuals to CD and TD individuals [51].

Eye tracking in ASD reports inconsistent findings regarding gazing at emotional faces. For an extensive meta-analysis and a summary of the reported differences during development, see [21, 41, 64]. The variation in reported results may partly be due to the variability in the methods utilised to study eye gazing in emotion recognition paradigms. Studies differ on their use of table-mounted remote eye-tracking devices or head-mounted ones. They also differ on their use of static and dynamic facial stimuli and the core characteristics of the faces (e.g., gender, intensity of emotions, and the appearance of the faces). In addition, many methodological issues cannot be properly controlled for which introduces additional heterogeneity. Studies differ in sampling frequencies of eye-tracking devices, the selection strategy of areas of interest, fixation classification filters, and the informed use of parametric or non-parametric statistical tests. Some of the earlier eye-tracking
studies in adults and adolescents with ASD reported that less attention was paid to the eyes and other core features of faces [68] or focused more on the mouth and less on the eyes [53, 60]. Other studies confirmed that adults with ASD gazed less at the eye region while exploring a face [25, 43]. In contrast, more recent studies have not observed significant differences between individuals with ASD and typically developing youth in eyegazing behavior [78, 82, 83]. More broadly speaking, gazing at the eyes can facilitate more accurate and faster responding to several emotions like fear, surprise, and disgust [6] and thus enable better social interaction. Numerous experimental studies have found strong evidence for reduced accuracy in identifying negative emotions in individuals with ASD [4, 7, 25, 48, 84], although there is no consensus in the field.

Overall, insufficient gazing to the eyes can lead to impaired emotional recognition which may influence disruptive behaviors and increase social anxiety in individuals with ASD [25].

Antisocial behavior is also associated with poor recognition and processing of fearful faces [56]. Recent studies confirm impaired recognition of multiple emotions (anger, fear, and happiness) in adolescents with CD relative to TD individuals [35, 36, 80]. Furthermore, children with greater behavioral problems (as indexed through the Psychopathy Screening device) also showed poorer recognition of angry, sad, and fearful facial expressions [10]. Those children and adolescents with both CD and high CU traits showed more pervasive impairments in emotional recognition than those with low CU traits [33, 36]. Recently, the first well-powered eye-tracking study on a large cohort of male and female adolescents with CD has been published. Martin-Key et al. [57] used an emotion recognition task with both static and dynamic morphed faces. They found that male adolescents fixated less on the eyes when viewing fearful and sad expressions. Although the differences were considered small, the authors suggest that behaviorally detected emotion recognition deficits were not mediated by abnormal fixation patterns [57].

ASD symptoms may moderate the relationship between CU traits and aspects of emotional empathy [70]. Pijper et al. [70] suggest that CU traits are inversely related to empathic sadness at low levels of ASD symptoms, while others document it only for higher levels of ASD symptoms [65]. Psychopathic traits seem to predict lower numbers of fixations and fixation durations to the eye region in fearful faces in TD male adolescents [29]. Individuals with ASD also have elevated levels of aggressive behavior compared to TD individuals [47], although aggression is not a core symptom of ASD and is typically less severe in ASD than ODD/CD [3]. For CD and ODD, both proactive and reactive aggression are considered hallmarks of the disorder [17], and the relation of subtype of aggression and eye-tracking patterns of emotional face processing is unclear. From a broader perspective, it seems that many concepts (i.e., psychopathic traits, CU traits, and subtypes of aggression) in different disorders (i.e., ASD, ODD, and CD) seem to be interlinked and associated with each other, while actual direct links remain elusive and a direct comparison is missing.

In summary, eye-tracking data in the literature related to emotional face processing are inconsistent in ASD and studies have not been properly replicated in large well-phenotyped psychiatric cohorts for CD and ODD. These relationships still remain elusive and the field suffers from inconsistency in approach to data collection and analyses and using fairly small sample sizes [41]. Our relatively large cohort (total N=122; ASD =52, ODD/CD =42, TD =28) consisting of male adolescents enables us to examine the common and unique eye-tracking patterns of emotional face
processing in individuals with either ASD, ODD, or CD, in comparison with TD, and explore the possible modulatory role of CU traits, psychopathic traits and subtypes of aggression. We hypothesize that high CU traits, high psychopathic traits, and heightened proactive and reactive aggression will be associated with less time spent to the eye region for negative emotions (e.g., sadness, fear, and anger) in both male adolescents with ASD or CD/ODD. Furthermore, we hypothesize that both male adolescents with ASD and ODD/CD will show similar differences on the time to first fixation to the eye region of an emotional face.

**Methods**

**Recruitment of participants**

Initially, 423 individuals were approached to participate in a larger study on empathy (CU2 study). Individuals with an ODD/CD diagnosis were approached via institutes specialized in severe juvenile psychiatric problems (Karakter, Child and Adolescent Psychiatry) or severe disruptive behavior problems (De Hoenderloo Group, Otto Gerhard Heldring Foundation, and Woodbrookers). Individuals with ASD were recruited via information leaflets that were sent to families by the Dutch federation of Autism (NVA). The typically developing individuals’ control groups were recruited via leaflets that were sent to a community sample. These individuals were selected on the basis of their geographical location. The recruitment period lasted from April 2011 to September 2014. Of those approached, 265 did not respond or were not interested to participate. Of the 158 that were interested in participation, 18 did not meet the inclusion criteria (see below for more information). Two participants did not obtain consent from a legally appointed guardian and 6 participants were not able to participate due to their personal situation. In total, 132 were included for the broader CU2 study. Of the 132 participants, 6 participants did not undergo the extensive eye-tracking battery. Of the 126 participants, 4 participants had to be excluded based on exclusion criteria for eye-tracking data quality. Thus, all the presented data are from the 122 participants (50 with ASD, 44 with ODD or CD and 28 TD individuals). All participants were male adolescents [age range (12–19 years old, mean age=15.26 years, SD=1.9)]. Main participant and demographic characteristics are summarized in Table 1. The difference between the number of participants initially approached and the final inclusion in this eye-tracking study is considerable large. In many cases, participants with ODD/CD were not interested in participating in an extensive clinical study. Many had behavioral problems and were often not in a position to participate. There were restrictions to leave closed institutions or their personal situation did not allow participation. Here, one can think of the occurrence of violent and/or oppositional incidents, escape attempts, and (temporary) dysfunctional relationships with their caregivers.

**Inclusion and exclusion criteria for participation**

All participants who were recruited from clinical institutes obtained a clinical ASD or ODD/CD diagnosis prior to the study. Clinical diagnoses (ODD/CD and ASD) were established according to the DSM-IV-TR criteria [5] by a multidisciplinary team (experienced psychiatrist and psychologist). In
**Table 1. Characteristics of the study population (N=122)**

<table>
<thead>
<tr>
<th></th>
<th>Total group</th>
<th>TD</th>
<th>ODD/CD</th>
<th>ASD</th>
<th>Contrasts</th>
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<tbody>
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<td></td>
<td>M ± SD</td>
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<tr>
<td><strong>Age (years)</strong></td>
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<td>15.9 ± 1.8</td>
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<td><strong>FSIQ</strong></td>
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<td>106.3 ± 9.5</td>
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<td>103.4 ± 11.1</td>
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<td><strong>VIQ</strong></td>
<td>101.9 ± 13.6</td>
<td>108.5 ± 12.9</td>
<td>92.1 ± 11.1</td>
<td>104.8 ± 12.0</td>
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<td><strong>PIQ</strong></td>
<td>101.1 ± 14.0</td>
<td>105.4 ± 15.1</td>
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<td>ICU self-rated</td>
<td>26.8 ± 8.8</td>
<td>23.6 ± 6.3</td>
<td>31.0 ± 10.0</td>
<td>24.9 ± 7.6</td>
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<td>ICU parent-rated</td>
<td>28.8 ± 11.3</td>
<td>17.0 ± 7.1</td>
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<td>28.1 ± 8.3</td>
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<td><strong>YPI self-rated</strong></td>
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<td>Total score</td>
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<td>82.21 ± 17.1</td>
<td>106.87 ± 23.2</td>
<td>88.45 ± 20.6</td>
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<td>CU subscale</td>
<td>27.22 ± 6.7</td>
<td>23.75 ± 5.3</td>
<td>30.80 ± 6.9</td>
<td>26.09 ± 5.8</td>
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<td><strong>RPQ self-rated</strong></td>
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<td>Total score</td>
<td>13.46 ± 8.4</td>
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<td>19.30 ± 8.4</td>
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<td>Reactive</td>
<td>9.54 ± 5.2</td>
<td>6.00 ± 3.3</td>
<td>12.30 ± 4.8</td>
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</tr>
<tr>
<td>Both Caucasian</td>
<td>96</td>
<td>78.7</td>
<td>26</td>
<td>92.9</td>
<td>21</td>
<td>50</td>
<td>49</td>
<td>94.2</td>
<td>ODD/CD &lt; ASD = TD***</td>
</tr>
<tr>
<td>Caucasian and other</td>
<td>11</td>
<td>9.1</td>
<td>2</td>
<td>7.1</td>
<td>6</td>
<td>14.2</td>
<td>3</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Both are unknown</td>
<td>4</td>
<td>3.3</td>
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<td>4</td>
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<td>Highest level of education parents A %</td>
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<td></td>
<td></td>
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<tr>
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<td>0</td>
<td>4</td>
<td>9.5</td>
<td>3</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>38</td>
<td>31.1</td>
<td>3</td>
<td>10.7</td>
<td>15</td>
<td>35.7</td>
<td>20</td>
<td>38.5</td>
<td>ODD/CD &lt; ASD &lt; TD*</td>
</tr>
<tr>
<td>Higher</td>
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<td>25</td>
<td>89.3</td>
<td>10</td>
<td>23.8</td>
<td>28</td>
<td>53.8</td>
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</tr>
</tbody>
</table>

n.s.: not significant, *p<.05; **p<.01***p<.001 FSIQ: Full-scale IQ; ICU: Callous Unemotional traits based on the Inventory of Callousness Unemotional traits. YPI: youth psychopathic traits inventory. RPQ: Reactive and Proactive Questionnaire. SCQ: Social Communication Questionnaire. TD: Typical Developing Individuals; ASD: Autism Spectrum Disorder; ODD/CD: Oppositional Defiant Disorder/Conduct Disorder; n.a.: not assessed; n.s.: not significant; p-Value: * p < .05, ** p < .01, *** p < .001; Ethnicity parents A: data based on two parents; Highest level of education parents B: data based on family level.
a large proportion of our ASD participants, the clinical diagnoses were confirmed by clinical scores on the ‘golden standard’ of the ADOS and ADI, although this was not a fixed criterion for inclusion in this study. They both gathered information and reviewed (prior) clinical records and information provided by schools and other agencies involved in the care of the adolescent. This workflow ensured that the proper clinical diagnosis was confirmed, before individuals were included in the current study. This is a robust and more reliable approach compared to only using structured interviews for the allocation of individuals to clinical groups [54]. For all the three groups, caretakers (i.e., biological parents or legal guardians) were asked to fill out a digital version of the National Institute of Mental Health Diagnostic Interview Schedule for Children (DISC-IV; [77]). Parents and/or caregivers had to complete the following sections of the DISC-IV: Attention-Deficit/Hyperactivity Disorder, ODD, CD, Tic Disorder, alcohol, marihuana, and other drug use. The social communication questionnaire (SCQ) was used as an instrument to assess ASD characteristics across the three groups (ASD, ODD/CD, and TD). For the typically developing group, the absence of a clinical psychiatric diagnosis was assessed based on the DISC-IV parent interview [77]. The outcomes of the DISC-IV and the SCQ were evaluated by an experienced child and adolescent psychiatrist (PH) and psychologist (MJB). We excluded participants who fulfilled one or more of the exclusion criteria (a) a combined diagnosis of ASD and CD/ ODD, (b) an estimated total IQ<80); and/or (c) suffering from a condition which may affect neurological or cognitive functioning, such as schizophrenia, bipolar disorder, alcohol and/or drugs dependency, language disorder (e.g., dyslexia), epilepsy, and the presence of tics. The TD individuals were not allowed to have a clinically established psychiatric diagnosis to participate. The other inand exclusion criteria were the same as for the clinical groups. Participants with a diagnosis of ODD or CD from the CU2 project were grouped together in this study, because both disorders are on a spectrum of behavior problems and aggressive tendencies. In addition, the ODD/CD group included only a few CD participants to be handled as a stand-alone group.

Medication use
The use of non-psychotropic and anti-depressant medication was allowed for the inclusion in the study. If possible, psychotropic medication (i.e., antipsychotics, stimulants, and atomoxetine) was stopped prior to testing. Stimulants were discontinued for at least 24 h prior to participation and antipsychotics for at least 72 h. Only in cases, when a health care professional judged the discontinuation to have potential severe detrimental effects, the medication was not stopped. In total, 9 participants with ODD/CD and 8 participants with ASD were still on medication during the testing days.

Cognitive assessments
Participants were required to have a minimum average estimated total full-scale intelligence quotient (FSIQ) IQ of≥80. The FSIQ was estimated using four subtests of the Dutch version of the Wechsler Intelligence Scale for Children (WISC-III): Similarities, Block Design Picture Completion, and Vocabulary [85]. These WISC-III subtests are known to be highly correlated (r=0.90–0.95) with full-scale IQ [40]. For the participants that were 16 years or older, the Wechsler Adult Intelligence Scale III (WAIS-III) was administered [86].
Procedures
A short telephone screening and, subsequently, screening questionnaires were used to verify if families could participate. Those families were invited to visit one of the participating clinics. Testing of the participants took place in a quiet room at the test location. Experimenters used stimulus deprived rooms to limit the influence of distraction. Participants were given short breaks and received a financial compensation (vouchers of € 20.00) for this test administration. Ethical approval

Ethical approval
This study was approved by the Dutch Central Committee on Research involving Human Subjects, protocol number NL26773.000.09 (Centrale Commissie Mensgebonden Onderzoek; CCMO). Both adolescents (if 12 years of age and older) and their legally appointed guardian provided written informed consent.

Description of clinical measures

Social communication questionnaire (SCQ)
The social communication questionnaire is a 40-item parent-report questionnaire that investigates ASD characteristics on a binary scale (yes/no). The questionnaire contains 19 items on current behavior and 20 items on the period when the child was 4–5 years old [75]. A cut-off score of >10 was used as a positive screening outcome on ASD characteristics. TD participants could only be included when they did not have a clinical score on the parent-rated SCQ (i.e., raw scores of <10). In calculating the total score, the first item was excluded, because it only probed for sufficient language ability. The English version of the SCQ has a sensitivity ranging between 0.85 and 0.88 and a specificity between 0.72 and 0.78 [8, 19, 20]. The Cronbach’s alpha for the total SCQ score was 0.75 in the final sample.

Inventory of Callous-Unemotional traits (ICU)
The Inventory of Callous–Unemotional traits (ICU) assesses CU traits in adolescents, divided into three subscales: uncaring, callousness, and unemotional [37]. We used the official Dutch translated version of this questionnaire. Internal consistency of the Dutch ICU was shown to be good [34, 74]. The ICU exists of 12 positively framed items and 12 negatively framed items. Items are rated on a 4-point scale ranging from 0 (‘not at all true’) to 3 (‘definitely true’). The uncaring scale consists of 8 items, the callousness subscale of 11 items, and the unemotional subscale of 5 items. An example of an item on the uncaring scale is ‘I am concerned about the feelings of others’. An example item for the callousness scale is: ‘I seem very cold and uncaring to others’. Finally, an example of the unemotional scale: ‘I do not show my emotions to others’. Subscale scores are calculated by summing the individual item scores. The reverse and ‘opposite’ framing of sentences is taken into account in the scoring. Subsequently, the total score is calculated by summing up the subscale scores. A higher total score reflects a higher levels of CU traits. We administrated both the parent version (legal guardian) and the self-rated version of the ICU. For the final sample, the Cronbach’s alpha for the self-report was 0.78 and the Cronbach’s alpha for the parent report was 0.90.
Youth Psychopathic Trait Inventory (YPI)
The youth psychopathic traits inventory (YPI) is a 50-item self-report questionnaire [2]. It has been designed to assess core psychopathic personality traits for adolescents of 12 years of age and older. It reflects 3 dimensions of psychopathy: the grandiose manipulative, callous–unemotional, and impulsive–irresponsible [24]. Higher YPI total scores reflect the presence of high psychopathic traits. Internal consistency has been reported as 0.94 Cronbach’s alpha for the total score of the YPI, 0.82 for the grandiose–manipulative subscale, 0.64 for callous–unemotional subscale, and 0.76 for impulsive–irresponsible subscale.

Reactive and Proactive Aggression questionnaire (RPQ)
The Reactive and Proactive Aggression Questionnaire (RPQ) was developed by Raine et al. [71]. In the current study, the Dutch translation of the well-validated 23-item RPQ was used which is designed to probe for reactive and proactive aggression in children and adolescents from the age of 8 years of age and older [22]. The reactive subscale has 11 items. Example questions include: ‘He/she gets mad or hit others when they tease him/her’ and ‘He/she damages things when he/she is mad’. The proactive subscale has 12 items. Example questions for this subscale are: ‘He/she damages or breaks things for fun’ and ‘He/she threatens and bullies other kids’. The questions of the RPQ do not reference to a certain time period in the past or current behavior. Participants just have to report how often they have engaged in particular behaviors. The total score of the RPQ is calculated by summing all items together. The Cronbach’s alpha for the RPQ was 0.91 in our final sample.

General study protocol
Participants and their legal guardians that gave informed consent were screened using the DISC-IV interview device [77]. The information of the DISC-IV was combined with the clinical diagnosis information to allocate participants into the different groups. The participants and their legal guardians were asked to fill out questionnaires (paper and pencil) separately from each other. This could either be at home or at the test location. For the test location, experimenters used a stimulus deprived quiet room. The influence of external noise and distraction was limited. For completing the questionnaires at home, the participants and their legal guardians were asked to sit in a quiet room with as few external distractions as possible.

Task design
We used an emotional recognition task that consisted of 60 trials with static images of emotional and neutral faces. Each trial always had the same structure: ‘fixation cross (1 s)–facial stimulus (6 s)–question—gray screen (3 s)’. The rationale behind the presentation of the gray screen was twofold. First, we wanted to avoid the confound of pupil response to the differences in light intensity of the facial stimuli (presented on a black background) and the questions (presented on a white background). Second, the use of the gray screen countered potential ‘wash over effects’ of gazing at emotional faces and neutral faces and vice versa. Trials with emotional faces and neutral faces were interleaved. The whole task consisted of two sessions of 30 trials that was interrupted by a short break. Both the sessions had a different order of the presentation of the emotional and neutral
faces. All used faces were balanced on gender, ethnicity, and in the adult age range. A set of face stimuli were selected from the online NimStim of Facial Expressions set (available to the scientific community at http://www.macbrain.org/resources.htm) [81]. The faces differed on the intensity of portrayed emotion from high to low. Both the types of emotion and the portrayed intensity have been previously validated [42]. The Dutch question asked to the participants was presented on the screen and can be translated as ‘What kind of emotion did you see?’. The participants always had five answer options: neutral, happy, sad, angry, and fear. The order of the answers on the screen was balanced over the trials.

**Figure 1.** Percentage of total fixation duration on the eyes of fearful faces

![Box plot showing percentage of total fixation duration on the eyes of fearful faces for TD, ASD, and ODD/CD groups.](image)

**Data pre-processing**
We exported the fixation data from Tobii studio 2.2.08 and used Matlab 2016B [58] to pre-process the eye-tracking data. We used stringent data exclusion and inclusion criteria. Trials were excluded if there was no fixation data for 25% or more of the trial duration. At least 1.5 s of the 6 s trial duration had to contain valid eye-tracking data. To overcome and counter potential artifacts, we did not take the first 100 ms of the trial into account for the time to first fixation. In the first 100 ms, it is hard to disentangle ‘real fixations’ from potential measurement artifacts or limitations of the used apparatus with sampling rate of 50. Moreover, we excluded participants in which 50% or less of the trials were valid. Applying these criteria led to the exclusion of 4 participants (2 participants with ASD and 2 with ODD/CD).

**Normality of distributions**
We investigated the distributions of all our eye-tracking output variables and checked for violations of normality. We used skewness and kurtosis to establish normality values (see supplementary Table 1...
for more information). For all three groups and all the eye-tracking variables, normality could not be completely assumed. This led to the choice to use non-parametric statistics such as the Kruskal–Wallis tests [18], non-parametric Mann–Whitney post hoc tests, and Spearman correlations. The Spearman correlations are rank order free and resistant to violations of normality assumptions. In this way, we could ensure that eye-tracking variables in milliseconds would still have biological plausible meaning.

### Statistical analysis

We applied a non-parametric trial-based approach to investigate gazing behavior on the AIO (eyes, mouth, and rest of the image) of emotional and neutral faces [18]. For all our three main eye-tracking variables (total fixation duration, time to first fixation, and percentage total fixation), we used Kruskal–Wallis one-way ANOVA tests (two-tailed, significance level $\alpha = 0.05$) to test for group differences. For the variable ‘time to first fixation’ on the eye AIO, we investigated the relative distribution (percentagewise) for all three groups (ASD, ODD/CD, and TD) for values in time bins of 50 ms. To overcome and counter potential artifacts, we did not take the first 100 ms of the trial into account for the time to first fixation. In the first 100 ms, it is hard to disentangle ‘real fixations’ from potential measurement limitations of the used apparatus. We ran five tests separately for all the different emotions (anger, sad, fear, and happiness) and neutral faces (see Fig. 1). We applied Bonferroni corrections for multiple testing (two-tailed, significance level, $\alpha = 0.01$). We performed Mann–Whitney post hoc tests to examine the specific directionality of effects between the groups. The same rationale was followed for all of our eye-tracking variables. For the investigation of behavioral results of the emotion recognition task, we looked at the percentages of correct answers per group and tested for group differences via t tests after z score transformations. Furthermore, we investigated

<table>
<thead>
<tr>
<th>Total fixation duration</th>
<th>N</th>
<th>Degrees of freedom</th>
<th>Chi-Square</th>
<th>Significance</th>
<th>Contrasts post hoc test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anger</td>
<td>774</td>
<td>2</td>
<td>51.5</td>
<td>$p = .003$</td>
<td>TD &gt; ODD/CD***</td>
</tr>
<tr>
<td>Fear</td>
<td>835</td>
<td>2</td>
<td>15.1</td>
<td>$p &lt; .01$</td>
<td>TD &gt; ODD/CD***</td>
</tr>
<tr>
<td>Sad</td>
<td>816</td>
<td>2</td>
<td>0.6</td>
<td>$p = .74$</td>
<td>n.s.</td>
</tr>
<tr>
<td>Happy</td>
<td>835</td>
<td>2</td>
<td>15.2</td>
<td>$p = .001$</td>
<td>n.s.</td>
</tr>
<tr>
<td>Neutral</td>
<td>2866</td>
<td>2</td>
<td>31.2</td>
<td>$p &lt; .001$</td>
<td>TD &gt; ASD*** TD &gt; ODD/CD ***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time to first fixation</th>
<th>N</th>
<th>Degrees of freedom</th>
<th>Chi-Square</th>
<th>Significance</th>
<th>Contrasts post hoc test</th>
</tr>
</thead>
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<tr>
<td>Fear</td>
<td>248</td>
<td>2</td>
<td>6.11</td>
<td>$p = .047$</td>
<td>TD &gt; ASD = ODD/CD*</td>
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<tr>
<td>Anger</td>
<td>222</td>
<td>2</td>
<td>4.04</td>
<td>$p = .1$</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sad</td>
<td>234</td>
<td>2</td>
<td>2.47</td>
<td>$p = .5$</td>
<td>n.s.</td>
</tr>
<tr>
<td>Happy</td>
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<td>2</td>
<td>5.79</td>
<td>$p = .055$</td>
<td>n.s.</td>
</tr>
<tr>
<td>Neutral</td>
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<td>2</td>
<td>3.25</td>
<td>$p = .19$</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s.: Not significant, *=p<.05; **=p<.01***=p<.001

TD: typically developing individuals; ASD: autism spectrum disorder; ODD: oppositional defiant disorder; CD: conduct disorder
the correlation (Spearman test, two-tailed, significance level $\alpha=0.05$) between eye-gazing pattern for emotional faces with CU traits (total score of ICU, and YPI CU subscale scores), psychopathic traits (YPI total score), and severity of aggression (RPQ scores for proactive and reactive aggression).

**Results**

**Descriptive results**

See Table 1 for sample characteristics. The three groups did not differ in age, but significantly differ in estimated full-scale IQ (FSQ). A post hoc test revealed that the FSQ was lowest for the ODD/CD

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**Figure 2.** Time to first fixation in milliseconds on the eyes of fearful faces.

![Figure 2](image)

**Figure 3.** Distributions of the time to first fixation on the eyes of fearful faces for 100-1000 milliseconds. Timebins are 50 milliseconds each.

![Figure 3](image)

*TD: typically developing individuals; ASD: autism spectrum disorder; ODD: oppositional defiant disorder; CD: conduct disorder*
group, and highest in the TD group, while the ASD group scored in between of the two. There was no significant difference between the ASD group and the TD group. The three groups significantly differed on SCQ, post hoc test revealed that the ASD group scored higher than the ODD/CD group. Regarding self-rated CU traits, the ODD/CD group showed significantly higher CU scores than both the TD and ASD groups, whereas the TD and ASD groups did not differ from each other. Regarding the parent-rated CU traits, the ODD/CD group scored significantly higher than the ASD group, and the ASD group scored significantly higher than the TD group. The three groups differed significantly from each other on aggressive behavior (RPQ total score). A post hoc test showed that the ODD/CD group had significantly higher scores on the RPQ total score then both the ASD and the TD groups. The ASD group did not differ from the TD group. Regarding reactive aggression, the ODD/CD group had significantly higher scores than both the ASD and TD groups. The ASD group and the TD group did not differ from each other. Regarding proactive aggression, the ODD/CD group had significantly higher scores than both the ASD and TD groups. The ASD group and the TD group did not differ from each other (Table 2).

Eye-tracking results and behavioral results
We found a main group effect for relative total fixation time to the eye region for fearful (Kruskal–Wallis one-way ANOVA, $\chi^2 (df=2, N=835)=15.1, p<0.01$) (presented in Fig. 2), angry $\chi^2 (df=2, N=774) =$
179

When correcting for multiple comparisons via Bonferroni correction (p=0.05/5=0.01), these main effects remained significant. Mann–Whitney post hoc tests revealed that the TD group had significantly more fixations to the eye region than the participants with ASD or ODD/CD for fearful, angry, happy, and neutral faces, whereas the ASD and ODD/CD groups did not differ from each other. There was no main group effect for sad faces [χ² (df=2, N=819)=0.6, p=0.7]. We found a main group effect for the time to first fixation towards the eye region for fearful faces [Kruskal–Wallis one-way ANOVA, χ² (df=2, N=248)=6.11, p=0.046] (Fig. 3). When correcting for multiple comparisons via Bonferroni (0.05/5=0.01), this main group effect did not survive. We performed Mann–Whitney post hoc tests to investigate the directionality of this nominal significant main effect. That revealed that both groups with ASD or ODD/CD took significantly longer time to first fixate on the eyes of a fearful face, compared to TD participants. We did not find any main group effects on time to first fixation to the eye AOI for sad, angry, happy, and neutral faces. The behavioral results for the emotion recognition task are presented in Table 3. We looked at the percentages of correct answers and the group differences via t tests via normalized z scores. We found significant differences between the ODD/CD group and the TD group for the happy faces (p<0.005) and sad faces (p=0.01). We also found differences between the ODD/CD group and the ASD group for neutral faces (p<0.05), sad faces (p=0.03), and fearful faces (p=0.02). These are all nominal significant, since only the result for happy faces survives Bonferroni correction (0.05/5=0.01).

Correlations eye-tracking variables and behavioural traits

Only in the ODD/CD group, we found a nominal significantly negative Spearman correlation between the time to first fixation at the eyes of fearful faces and psychopathic traits (r = 0.35, p = 0.02) (Fig. 4). When correcting for multiple comparison via Bonferroni correction (p=0.05/5=0.01), the Spearman correlation did not survive this correction. In addition, proactive aggression was also negatively correlated (r=− 0.33, p=0.04) with time to first fixation to the eyes of fearful faces in the ODD/CD group. When correcting for multiple comparison via Bonferroni correction (p = (0.05/5) = 0.01), this correlation also did not survive (Fig. 5). For the other three emotions; happiness, sadness, anger, and neutral faces, the psychopathic traits, CU traits, and aggressive tendencies did not correlate with any of the eye-tracking variables on any of the AOIs.

Table 3. Behavioral results emotion recognition task*

<table>
<thead>
<tr>
<th>Emotions</th>
<th>TD</th>
<th>ODD/CD</th>
<th>ASD</th>
<th>Contrast</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral</td>
<td>69.3</td>
<td>66.1</td>
<td>63.2</td>
<td>ODD/CD – TD*</td>
<td>p = 0.046</td>
</tr>
<tr>
<td>Angry</td>
<td>46.6</td>
<td>45.5</td>
<td>41.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Happy</td>
<td>96.1</td>
<td>91.7</td>
<td>90.3</td>
<td>ODD/CD – TD***</td>
<td>p = 0.005</td>
</tr>
<tr>
<td>Sad</td>
<td>59.9</td>
<td>52.1</td>
<td>46.1</td>
<td>ODD/CD – TD*</td>
<td>p = 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ODD/CD – ASD*</td>
<td>p = 0.03</td>
</tr>
<tr>
<td>Fearful</td>
<td>84.0</td>
<td>80.6</td>
<td>75.2</td>
<td>ODD/CD – ASD*</td>
<td>p = 0.02</td>
</tr>
</tbody>
</table>

*Depicted are the percentages correctly recognized emotional faces The effects are bases on t-tests, normalized with z-transformation. n.s.: Not significant, *=p<.05; **=p<.01; ***=p<.001.
TD: typically developing individuals; ASD: autism spectrum disorder; ODD: oppositional defiant disorder; CD: conduct disorder.
Control-analyses
To check if our effects were not driven by the known significant differences in intelligence, ADHD comorbidity and medication use between groups, we undertook additional analyses. We regressed out full-scale intelligence (FSQ) from the model. The Spearman correlation between the total YPI score and the time to first fixation on the eyes was not significant anymore (r = 0.26, p=0.14). For the ASD group, the correlation was significant (r = 0.31, p = 0.04), but did not survive Bonferroni correction (p = 0.05/5 = 0.01). The Spearman correlation between proactive aggression and time to first fixation on the eyes of fearful faces was not significant anymore (r = 0.18, p = 0.3). Concluding, the regression of fullscale intelligence scores from the model did not change the directions of the effect and all Spearman correlation still did not survive correction for multiple comparison. Furthermore, we ran analyses to control for the effects of ADHD comorbidity and medication use (for details, see supplementary Tables 2, 3, 4, and 5). In the sample, 50 (28 subjects with ODD/CD and 22 subjects with ASD) of the 122 subjects had a comorbid ADHD diagnosis, and in the case of 9 participants, information was missing. For both control analyses, we excluded those participants with either ADHD (or missing information) or on medication (or missing information). The directions of the effects in both control analyses did not differ from the effects in the main analysis for total fixation duration, relative total fixation duration, and time to first fixation. Moreover, we ran control analysis for the Spearman correlation with behavioral traits (psychopathic traits and proactive aggression). In this case, we only selected participants without ADHD or that were not using medication (or missing information). The direction of the effects between the time to first fixation on the eyes of fearful faces in the ODD/CD group and psychopathic traits (YPI total score) and proactive aggression (RPQ proactive aggression subscale) did not change (see supplementary Table 5 for these results).

Discussion
This study aimed to investigate common cross disorder and unique disorder-specific patterns of eye gaze during emotional face processing by a head-to-head comparison of male adolescents with either ASD, or ODD/CD, compared to TD for eye-tracking measures (1) time to first fixation on an AIO, (2) total fixation duration to an AOI, and (3) percentage of total fixation duration on an AOI relative to the rest. We also examined the modulating role of CU and psychopathic traits, and aggression subtypes. We chose not to include subjects with a combined diagnosis of both ASD and ODD/CD to facilitate a clear crossdisorder comparison. In this way, we are not looking at the combined comorbid group (with a diagnosis of both ASD and ODD/CD) and cannot compare synergistic effects arising from the comorbidity of these disorders. Our results showed that (1) participants with either ASD or ODD/CD both did fixate proportionally and significantly less on the eye region of emotional faces (with sadness excepted) and neutral faces, compared to TD. (2) participants with either ASD or ODD/CD both took longer time to first fixation on the eye region in fearful faces, but not in faces with the other emotions/emotional expressions (i.e., neutral, anger, sadness, and happiness). However, this effect did not survive multiple comparison correction. (3) When looking at the relationship between eye gazing and CU traits, psychopathic traits, and aggression, we found a seemingly opposing effect. Higher scores for psychopathic traits and of proactive aggression within the ODD/CD group were nominally significant associated with shorter time to first fixation at the eye
region for fearful faces compared to the TD group. All three groups were paying more attention to the eye region compared to the mouth region and other parts of the face. Since these effects did not survive multiple comparison and regressing out full-scale intelligence scores did not change this, we did not find solid evidence for the hypothesized relationships.

Some studies have indicated that excessive attention to the mouth region may be adaptive for ASD children with well-developed language skills [72]. More recent work falsifies the gaze aversion to the eyes in infants [59]. These findings are not confirmed in our high-functioning adolescent male population with ASD. The differential results can be explained by differences in methodology across laboratories and also the high heterogeneity in gazing behavior for individuals with ASD. The different age ranges of samples and their intelligence profiles may also partly explain differences in findings [41].

Earlier studies reported poorer recognition of emotional facial expressions in individuals with CD [36, 87] and also abnormally low amygdala activations to fearful or angry emotional faces in individuals with CD, particularly those with high CU traits [50, 67]. We observed both in the ODD/CD and ASD groups proportionally less gazing at the eye region of emotional and neutral faces. This suggests that less gazing at the eye region of emotional faces might still be a cross-disorder trait that is not unique to ASD, but shared with other disorders like ODD/CD, which is in line with findings that emotion recognition problems characterize a wide range of child psychiatric disorders, varying from ASD, ADHD, and CD to mood and anxiety disorders and eating disorders and schizophrenia [23].

The novelty of our study is that we are providing insight into differences of eye-gazing behavior on fearful faces between clinical groups that are well-phenotyped and look at the links with psychopathic traits, CU traits, and aggression. We found that the time to first fixation is delayed for the ODD/CD group for the time to first fixating on the eyes of fearful faces. A delayed first fixation to fearful eyes might lead to slower processing and delayed evaluating of the fearful state of the other person. Small distortions in synchrony of emotional communication between individuals due to delayed processing of emotional information may already disrupt social interactions and predispose to inadequate and even harmful behavior [14, 53].

There was a nominally significant negative correlation in our ODD/CD group between the time to first fixation to the eyes of fearful faces and psychopathic personality traits (YPI). This effect did not survive multiple comparison correction. This dimensional effect concerning higher psychopathic traits is opposite the group effect of eye gazing in our ODD/CD participants that gaze later to the eyes of fearful faces. The absence of a relationship between psychopathic/ CU traits and gaze fixation in both the ASD group and the TD group might be due to the smaller variance in psychopathy and CU scores (for details, see Table 1) in these groups. It might be that a selection bias led to the oversampling of participants lower than average on psychopathic traits, CU traits, aggression for those that score average or high might be less willing to be subjected to testing in a clinical research setting.

In general, a modulating role of psychopathic traits is consistent with findings in functional MRI studies, where amygdala activation to fearful or angry faces is low in the presence of high psychopathic and high in their absence [50, 67]. Klapwijk et al. [51] also found decreased amygdala responses in both adolescents with ASD and individuals with CD and high CU traits. We also found a possible association with the severity of in particular proactive aggression and time to first fixation on the eyes of fearful faces. Children as well as adolescents and adults with ODD/CD and high levels
of psychopathy/CU traits are more likely to have high levels of proactive aggression [26, 39]. Our data does seem to suggest a potential link between ODD/CD, high psychopathic traits, proactive aggression, and impaired fear processing.

Although we document similar patterns of abnormal gaze behavior to emotional faces in ASD and ODD/CD, the underlying mechanism might be disorder specific. There are three theories trying to explain abnormal emotional face processing in ASD. First, gazing at faces and eyes in particular may lead to increased (negatively valence) emotional responses in individuals with ASD and even found to be aversive [31]. Looking at the mouth is then just a byproduct of avoiding gazing at the eyes. Second, another theory poses that individuals with ASD cannot “read the language of the eyes”, i.e., they do not understand visual information from the eyes which may be linked to problems in using a Theory of Mind [52]. The failure to use information from the eye region in combination with an ability to use visual information from the mouth for speech related processing is driving the deficit of excess fixation on the mouth and diminished fixation on the eyes. Third, another explanation is that individuals with ASD are suffering from impaired social orientation and that the “most social” part of the face, the eye region lacks saliency and does not arouse sufficient intrinsic interest to be looked at [41]. Unfortunately, our paradigm and our results not allow us to differentiate between these potential explanations.

Impaired affective responses and emotional processing in CD has been addressed by three main theories [32]. The attention to the eyes hypothesis proposes that emotion processing deficits in CD/psychopathy arise from a lack of spontaneous attention to the eye region [30, 28] which negatively affect the processing of all emotional expressions. The distress-specific hypothesis states that individuals with CD/psychopathy fail to effectively process in particular others’ expressions of distress (fear and sadness). As a result, their antisocial actions are not inhibited by aversive feelings of remorse and guilt, resulting in callous behavior and shallow affect [15, 11]. Finally, the enhanced selective attention hypothesis [63, 61, 62] states that the enhanced ability to focus on a task and to ignore goal irrelevant stimuli underlies affective deficits. This superior selective attention can enhance the top–down ability to suppress emotional information that is irrelevant to one’s goals, for example, another person’s distress if the psychopath wants to steal their money. Since the gaze pattern with proportionally less attention to the eye region was observed for all emotions except for sadness, our results are mostly in line with the attention to the eye hypothesis or the enhanced ability to focus hypothesis.

Our groups did not differ with respect to the total fixation duration on the eyes while processing sad faces. Other studies have shown that emotional recognition deficits for sadness are present in people with ODD/CD [79, 87]. These discrepant findings may be due to differences between studies in sample selection and characteristics. The Woodworth and Waschbusch [87] sample consisted of both male and female children with high levels of CU traits, which is quite different from our male adolescent sample. The Stevens et al.’s [79] sample did not use a formal clinical diagnosis of ODD or CD and selected participants on the basis of a score of 25 or higher on the psychopathy screening device [38].

Despite its strengths, such as the direct comparison of a well-powered ASD and ODD/CD group and its focus of gaze behavior by means of eye-tracking measures which ruled out the influence of social desired expected answers of questionnaires, our study also showed limitations. We were not able to control the gaze duration to the fixation cross prior to the faces that were portrayed on the screen. As half of our trials contained neutral faces, we did not have enough trials per emotion to look
into the effects of gender, ethnicity, and the intensity effects of the emotions portrayed on the faces. The stimuli used in this study were selected from a validated database of emotional expressions: including stimuli with facial characteristics such as wrinkles and facial hair. Facial characteristics can be seen as a factor that may influence the study outcomes. In contrast to studies that use morphed faces, our facial stimuli are closer to emotional faces in the real world. On the other hand, this might potentially revert the attention of the participants and confound the outcome. Both diagnostic groups also contained a substantial amount of participants with comorbid ADHD and/or using medication. Although antipsychotics (where possible) were stopped 2 days before, and stimulants on the test day, we cannot rule out possible medication effects. However, sensitivity analyses revealed that results were not influenced by the presence of a comorbid diagnosis of ADHD or by medication use.

Implications
Considering the consequences of aggression, there is a need for a better understanding of underlying causes and maintaining factors. The current study contributes to the enhancement of this understanding by revealing (1) two cross-disorder traits for ASD and ODD/CD; (2) disorder-specific traits for ODD/CD with proactive aggression as a potential factor. Future research is warranted to examine possible other crossdisorder traits (e.g., biological and genetic) and/or disorder-specific traits; and (3) adding to knowledge and understanding in fractioning empathy to emotional stimuli by means of eye-gazing processing as a part of the MATRICS project (http://matrics-project.eu/). MATRICS examines the neural, genetic, and molecular factors involved in the pathogenesis of aggression/antisocial behavior and that in relation with callous–unemotional traits.

Moreover, as the current treatments, which mainly involve skill training, are not suitable or developed to alter implicit characteristics, other methods are needed to improve the efficacy of aggression treatment, and techniques like virtual reality seem to be promising [66, 69]. Clinical implications are mainly optimization of psychological interventions by therapists requiring eye-gazing information. A future study would definitely also benefit from the presentation of both static and dynamic faces as stimuli and comparing outcomes.

Conclusions
To conclude, we reported that male adolescents with ASD or ODD/CD looked less at the eyes in fearful, angry, happy, or neutral emotional expressions. They also took nominal significantly more time to first fixate on the eyes of fearful faces compared to TD. Those male adolescents with ODD/CD that exhibit faster first fixations on the eyes of fearful faces had nominal significant higher scores on psychopathic traits. Nevertheless, we did not find strong evidence that survived multiple comparisons to support that in ASD and ODD/CD higher scores on CU traits, psychopathy, and aggression were related to eye gazing on the eyes of fearful faces. Our data do provide valuable and new insight into the gaze behavior distributions of ODD/CD and ASD groups when looking at the eyes of emotional faces.
**Supplementary table 1. Normality indices of eye tracking variables**

<table>
<thead>
<tr>
<th>Fixation duration</th>
<th>All subjects - skewness</th>
<th>All subjects - kurtosis</th>
<th>ASD group - skewness</th>
<th>ASD group - kurtosis</th>
<th>ODD/CD group - skewness</th>
<th>ODD/CD group - kurtosis</th>
<th>TD group - skewness</th>
<th>TD group - Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fear</td>
<td>-1.34</td>
<td>5.79</td>
<td>-2.40</td>
<td>10.56</td>
<td>-0.52</td>
<td>2.74</td>
<td>-0.63</td>
<td>2.37</td>
</tr>
<tr>
<td>Sad</td>
<td>-0.95</td>
<td>4.42</td>
<td>-0.69</td>
<td>3.58</td>
<td>-1.10</td>
<td>4.83</td>
<td>-0.72</td>
<td>2.34</td>
</tr>
<tr>
<td>Happy</td>
<td>-1.30</td>
<td>5.66</td>
<td>-1.99</td>
<td>9.24</td>
<td>-0.82</td>
<td>3.15</td>
<td>-0.73</td>
<td>2.90</td>
</tr>
<tr>
<td>Neutral</td>
<td>-0.05</td>
<td>2.57</td>
<td>-0.07</td>
<td>3.07</td>
<td>0.10</td>
<td>2.51</td>
<td>-0.22</td>
<td>2.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time to first fixation</th>
<th>Fear</th>
<th>Anger</th>
<th>Sad</th>
<th>Happy</th>
<th>Neutral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixation duration</td>
<td>2.96</td>
<td>3.35</td>
<td>3.29</td>
<td>2.80</td>
<td>3.50</td>
</tr>
<tr>
<td>N</td>
<td>458</td>
<td>461</td>
<td>467</td>
<td>467</td>
<td>1575</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chi-Square</td>
<td>14.83</td>
<td>12.75</td>
<td>12.7</td>
<td>8.15</td>
<td>14.35</td>
</tr>
<tr>
<td>Significance</td>
<td>p = .001</td>
<td>p &lt; .001</td>
<td>p &lt; .001</td>
<td>p = .01</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Contrasts Post hoc tests</td>
<td>TD &gt; ODD/CD**</td>
<td>TD &gt; ODD/CD*</td>
<td>TD &gt; ODD/CD**</td>
<td>TD &gt; ODD/CD**</td>
<td>TD &gt; ODD/CD*</td>
</tr>
</tbody>
</table>

**Supplementary table 2. Control analysis ADHD comorbidity**

<table>
<thead>
<tr>
<th>Total fixation duration</th>
<th>N</th>
<th>Degrees of freedom</th>
<th>Chi-Square</th>
<th>Significance</th>
<th>Contrasts Post hoc tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anger</td>
<td>458</td>
<td>2</td>
<td>14.83</td>
<td>p = .001</td>
<td>TD &gt; ODD/CD**</td>
</tr>
<tr>
<td>Fear</td>
<td>461</td>
<td>2</td>
<td>12.10</td>
<td>p &lt; .001</td>
<td>TD &gt; ODD/CD*</td>
</tr>
<tr>
<td>Happy</td>
<td>467</td>
<td>2</td>
<td>13.48</td>
<td>p = .01</td>
<td>TD &gt; ODD/CD**</td>
</tr>
<tr>
<td>Neutral</td>
<td>1575</td>
<td>2</td>
<td>15.10</td>
<td>p &lt; .001</td>
<td>TD &gt; ODD/CD**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time to first fixation</th>
<th>Fear</th>
<th>Anger</th>
<th>Sad</th>
<th>Happy</th>
<th>Neutral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixation duration</td>
<td>2.96</td>
<td>3.35</td>
<td>3.29</td>
<td>2.80</td>
<td>3.50</td>
</tr>
<tr>
<td>N</td>
<td>458</td>
<td>461</td>
<td>467</td>
<td>467</td>
<td>1575</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chi-Square</td>
<td>6.07</td>
<td>p = .04</td>
<td>TD = ODD/CD (n.s)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.s.: Not significant, * = p<.05; ** = p<.01; ***= p<.001

**Supplementary table 3. ADHD-comorbidity control analysis for the correlation with YPI and RPQ**

<table>
<thead>
<tr>
<th>Correlation time to first fixation - YPI</th>
<th>Group</th>
<th>Spearman (R)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fear</td>
<td>TD</td>
<td>-.17</td>
<td>p = .42</td>
</tr>
<tr>
<td>Fear</td>
<td>ASD</td>
<td>.04</td>
<td>p = .84</td>
</tr>
<tr>
<td>Fear</td>
<td>ODD/CD</td>
<td>-.27</td>
<td>p = .45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Correlation time to first fixation - RPQ proactive subscale</th>
<th>Group</th>
<th>Spearman (R)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fear</td>
<td>TD</td>
<td>-.08</td>
<td>p = .76</td>
</tr>
<tr>
<td>Fear</td>
<td>ASD</td>
<td>-.10</td>
<td>p = .63</td>
</tr>
<tr>
<td>Fear</td>
<td>ODD/CD</td>
<td>-.63</td>
<td>p = .08</td>
</tr>
</tbody>
</table>

* = p < .05, ** = p < .01, *** = p < .001

TD: typically developing individuals; ASD: autism spectrum disorder; ODD: oppositional defiant disorder; CD: conduct disorder
**Supplementary table 4. Control analysis for medication use during testing days**

<table>
<thead>
<tr>
<th>Total fixation duration</th>
<th>N</th>
<th>Degrees freedom</th>
<th>Chi-Square</th>
<th>Significance</th>
<th>Contrasts post hoc tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anger</td>
<td>476</td>
<td>2</td>
<td>5.50</td>
<td>p = .06</td>
<td>TD = ASD (n.s.) TD &gt; ODD/CD*</td>
</tr>
<tr>
<td>Fear</td>
<td>487</td>
<td>2</td>
<td>6.76</td>
<td>p = .03</td>
<td>TD = ASD (n.s.) TD &gt; ODD/CD*</td>
</tr>
<tr>
<td>Happy</td>
<td>484</td>
<td>2</td>
<td>6.45</td>
<td>p = .04</td>
<td>TD = ASD (n.s.) TD &gt; ODD/CD*</td>
</tr>
<tr>
<td>Neutral</td>
<td>1653</td>
<td>2</td>
<td>8.27</td>
<td>p = .02</td>
<td>TD &gt; ASD* TD &gt; ODD/CD**</td>
</tr>
</tbody>
</table>

**Time to first fixation**

| Fear | 120 | 2 | 6.07 | p = .04 | TD < ASD* TD = ODD/CD (n.s.) |

n.s.: Not significant, * = p < .05, ** = p < .01, *** = p < .001
TD: typically developing individuals; ASD: autism spectrum disorder; ODD: oppositional defiant disorder; CD: conduct disorder

**Supplementary table 5. Control analysis medication use during testing days**

<table>
<thead>
<tr>
<th>Group</th>
<th>Correlation (Rs)</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to first fixation – YPI - total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fear</td>
<td>TD</td>
<td>-.18</td>
</tr>
<tr>
<td>Fear</td>
<td>ASD</td>
<td>-.02</td>
</tr>
<tr>
<td>Fear</td>
<td>ODD/CD</td>
<td>-.22</td>
</tr>
<tr>
<td>Correlation time to first fixation RPQ - proactive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fear</td>
<td>TD</td>
<td>-.07</td>
</tr>
<tr>
<td>Fear</td>
<td>ASD</td>
<td>-.15</td>
</tr>
<tr>
<td>Fear</td>
<td>ODD/CD</td>
<td>-.41</td>
</tr>
</tbody>
</table>

TD: typically developing individuals; ASD: autism spectrum disorder; ODD: oppositional defiant disorder; CD: conduct disorder
References


7

General discussion
As part of a larger project on empathy in aggressive male adolescents (i.e. CU2 project), the purpose of this thesis was to examine proactive and reactive subtypes of aggression, aggression with or without CU traits, and their genetic, (neuro)endocrine, (neuro)cognitive, and behavioural underpinnings as transdiagnostic markers. The main question in this thesis was whether there is evidence for across-disorder (i.e. transdiagnostic) or disorder specific markers regarding aggressive behaviour and/or CU traits in a study that compares male adolescents with the clinical disorders ODD/CD or ASD and typically developing individuals (TDI). This trans-diagnostic approach may improve our understanding of the biological substrate of aggression, and contribute to the improved understanding of the aetiological and clinical heterogeneity of aggression and inform cross-disorder interventions in clinical practice. In addition, this thesis aimed to provide an overview of the efficacy of non-pharmacological treatments for conduct disorder problems.

First, a summary of the main results per chapter will be presented, followed by a discussion and integration of the individual chapters in the context of the literature. Second, a critical reflection on the strengths and limitations of this thesis will be given, along with future directions emerging from this research. To conclude, implications for clinical practice and directions for future research will be provided, and a brief conclusion will be given.

**Summary of the main results**

The efficacy of non-pharmacological interventions in children and adolescents with a clinical CD diagnosis and/or clinical CD problems (including ODD problems) and who have an IQ of minimally 80 is described in the systematic review and meta-analysis in chapter 2. Although we specifically searched databases for non-pharmacological treatments other than psychological treatments, all identified studies used psychological (behavioural) treatments. Results from 17 randomised-controlled trial studies (of which only three included a blinded observer to rate conduct problems) showed that psychological treatments have a small effect in reducing parent-, teacher- and observer-rated CD problems in children and adolescents with clinical CD problems/diagnosis. Overall effect size based on parent-reports was 0.37 (95% CI = 0.27–0.47), based on teacher-reports 0.21 (95% CI = 0.12–0.49), and based on observer-reports 0.26 (95% CI = 0.06–0.47). In the studies included in this review, CD problem outcomes were mainly scored with the CBCL and the ECBI questionnaires. Most studies were of very poor to fair quality and reported treatment effects with small effect sizes. Comorbidity, gender, age, number of sessions, duration, intervention type, setting, medication use or dropout percentage did not influence the effect of treatment. Future studies need to provide detailed information about randomisation and blinding arrangements, to triangulate parent reported measures. Furthermore, there is insufficient evidence to support any one psychological treatment over another in this patient group (including parenting programmes).

In chapter 3 a systematic review was conducted to examine the evidence for genetic underpinnings of aggression and to determine to what degree prior studies have examined aggression phenotypes that fit neatly, or at all, into the RDoC framework. This review focused on three types of genetic studies: 40 twin studies, 277 molecular genetic studies of aggression in humans, 34 genetic studies of aggression in drosophila, and 86 studies on aggressive phenotypes in mice. Around 50% of the variance in aggressive behaviour in humans was explained by genetic influences. Non-shared
<table>
<thead>
<tr>
<th>Chapter and aim</th>
<th>Methods</th>
<th>Key-findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chapter 2</strong></td>
<td>Meta-analysis and systematic review</td>
<td>Psychological treatments for children/adolescents with CD and CD-related problems are modestly effective, as rated by parents (effect size (ES)=0.37 (95% CI = 0.27–0.47)), teachers (ES=0.21 (95% CI = 0.12–0.39)), and blinded observers (0.26 (95% CI = 0.16–0.37)), but not by children; however, the ES varied according to the instrument used. The largest ES (2.07) was found on Eyberg child behaviour inventory - intensity / problem behaviour scale (parent-rated). There is not enough evidence to support one type of psychological treatment over another. The overall quality of the studies was poor, and many studies failed to provide important details, such as subtypes of aggression and presence of CU traits, information that is needed to further optimize psychological treatment strategies.</td>
</tr>
<tr>
<td><strong>Chapter 3</strong></td>
<td>Systematic review</td>
<td>Based on twin studies we know that about half of the variance in aggressive behaviour may be explained by genetic risk factors. This is true for both dimensional, trait-like, measures of aggression and categorical definitions of psychopathology. The non-shared environment seems to have a moderate influence, whereas effects of shared environment were found to be small or absent. Human molecular genetic studies of aggression are in an early stage. The most promising candidates are in the dopaminergic and serotonergic systems along with hormonal regulators. The strongest molecular evidence for a genetic basis of aggression comes from animal models comparing aggressive and non-aggressive strains or documenting the effects of gene knockouts.</td>
</tr>
<tr>
<td><strong>Chapter 4</strong></td>
<td>Empirical paper on salivary hormone levels (ANCOVA’s)</td>
<td>Regardless of cognitive ability or comorbid disorders, the diagnostic groups (ASD, ODD/CD) differ from each other by their hormonal levels, with the ASD group characterized by relative low levels of oxytocin and testosterone, and the ODD/CD group by a relative low level of oxytocin and high level of testosterone. These group effects were partly driven by differences in CU traits between the groups. The severity of CU traits was related to both higher cortisol and testosterone levels, but not to oxytocin levels. The positive association between cortisol and CU traits in our study was driven by the link between cortisol and testosterone, and by the positive association between CU traits and testosterone (i.e. correlations between cortisol and CU traits became non-significant after controlling for testosterone). Indicating that testosterone is the driving factor in the association.</td>
</tr>
<tr>
<td><strong>Chapter 5</strong></td>
<td>Empirical paper of the emotional Go/No-go task (Repeated measure ANOVA’s)</td>
<td>Adolescents with ODD/CD or ASD have a diminished ability to detect emotional valence which is not limited to facial expressions and is related to a higher level of CU traits. More specifically, adolescents with ODD/CD or ASD seem to have a reduced processing of positive stimuli and/or lack a ‘positive perception bias’ present in TDI. This could either contribute to the symptoms and/or be a result of having the disorder, and may contribute to the comorbidity of both disorders.</td>
</tr>
<tr>
<td><strong>Chapter 6</strong></td>
<td>Empirical paper employing eye tracking (Kruskal Wallis One-way ANOVA’s)</td>
<td>The relative total fixation duration to the eyes was decreased in both ASD and ODD/CD for several emotional expressions (with sadness excepted) and neutral expression. In both ASD and ODD/CD, increased time to first fixation on the eyes of fearful faces was only nominally significant, but this effect did not survive multiple comparison correction. The time to first fixation on the eyes also correlated nominally with psychopathic traits and proactive aggression. Results suggest that specifically eye gazing on fearful eyes might be related to proactive aggression and psychopathic traits.</td>
</tr>
</tbody>
</table>
environmental influences were moderate. With regard to shared environments, studies implicated small or no influences: About half of the reviewed studies report no influence of shared environment while other studies indicate estimates between 0.15 and 0.35. As human molecular genetic studies of aggression are in an early stage, the strongest molecular evidence comes from animal models comparing aggressive and non-aggressive rodent strains or documenting the effect of loss or gain of candidate gene functions. Many constructs or operationalisations of aggression in animal models appear to be context-specific and should be cautiously translated to human behaviour.

In chapter 4 the hormones oxytocin, cortisol, and testosterone were examined in relation to (severe) aggressive behaviour and/or empathy in a total of 114 subjects with either ODD/CD (n=37), or ASD (n=49), or without a psychiatric disorder (TDI, n=28). Both the ASD and the ODD/CD groups had significantly lower levels of oxytocin than the TDI group, and the ODD/CD group had significantly higher levels of testosterone than the ASD group. There were no group effects on cortisol levels. Group differences remained for oxytocin after correcting for the influence of CU traits, but became non-significant when controlling for aggression subtypes as measured by the reactive and proactive aggression questionnaire. Results for testosterone became non-significant after correction for either CU traits or aggression. Across groups, higher levels of CU traits were related to higher levels of cortisol and testosterone, however, proactive and reactive aggression were not correlated with all three hormonal levels. These findings suggest that the severity of CU traits is related to both higher cortisol and testosterone levels, but not to oxytocin levels. The positive association in our study was driven by the link between cortisol and testosterone, and the positive association between CU traits and testosterone (i.e. correlations between cortisol and either self- or parent-rated CU traits became non-significant after controlling for testosterone).

In chapter 5 emotion processing was investigated during a neuropsychological (emotional Go/No-go) task. In this study, a total of 128 subjects participated, with either ODD/CD (n=52) or ASD (n=52), or without a psychiatric disorder (TDI, n=24). Results showed no group differences regarding the rate of false positive reactions. In addition, the ASD group was slower than the TDI group and the ODD/CD group on two out of three valence discriminations (negative-neutral and negative-positive). In contrast, reaction time in the ODD/CD group did not differ significantly from the TDI group on any of the three valence discriminations. The TDI group showed significant difficulty in contrasting positive with neutral pictures compared to negative-neutral or negative-positive pictures, whereas this effect was absent in both the ASD and the ODD/CD groups. CU traits, comorbidity with ADHD, and medication use were not related to task performance. Adolescents with ODD/CD performed in the range of the TDI group, whereas the ASD group was clearly slower than the TDI group regardless of type of emotion. Both the ASD and the ODD/CD groups differed from the TDI group who presented a ‘positive perception bias’ by being rather slow in differentiating positive from neutral valence. The results suggest that adolescents with ODD/CD or ASD seem to have a reduced processing of positive stimuli and/or lack a ‘positive perception bias’ present in TDI, that could either contribute to the symptoms and/or be a result of having the disorder, and may contribute to the comorbidity of both disorders.

In chapter 6 the common (cross-disorder) and unique (disorder specific) emotional face processing deficits, as reflected in the time to first fixation and fixation duration were examined in a
total of 122 male participants, consisting of individuals with either ODD/CD (n=44) or ASD (n=50), and TDI (n=28). Furthermore, the relationship of psychopathic traits (including CU traits) and aggressive behaviour with eye-tracking measures was examined. Results showed that total fixation duration on the eyes in emotional and neutral expressions (except for sadness) was significantly lower in both the ASD and the ODD/CD groups, compared to TDI. Time to first fixation on the eyes of fearful faces was significantly longer in both the ASD and the ODD/CD groups compared to TDI. For the other emotional or neutral faces, no significant effect was found. On the other hand, psychopathic traits and severity of particularly proactive aggression were significantly negatively correlated with both total fixation duration and also time to first fixation to the eyes of fearful faces in the ODD/CD group.

Integration and discussion of the results

In the search of a better understanding of aggressive behaviour with or without CU traits, an integrated model of aggression, was provided in chapter 1, which included different levels: 1) genetic, (neuro)endocrine, and neural level (i.e. brain mechanisms), 2) cognitive and affective level, 3) behavioural level, and 4) environmental influences. Certain aspects (i.e. genetics, (neuro)endocrine, task performance, and eye tracking) were included given the scope of the current thesis. As mentioned in the general introduction, it is recommended to include in future research also other possible factors such as environmental risk factors and brain regions related to information processing, and look into interactions between all these mechanisms. This will provide additional insights in the search of a better understanding of the origins, the maintenance, and development of persistent aggressive behaviour. Results from this thesis have shown, in part, a relationship between different trans-diagnostic markers and aggressive behaviour and/or CU traits, which deliver some suggestions on how to improve and/or develop psychological treatments. This will be discussed more in detail below.

To start with, the results (i.e. the small effect sizes of psychological interventions) as provided in chapter 2, may lead to two conclusions that are not necessarily opposing. The first is that CD problems are persistent and rather refractory to treatment. The second is that there is much room for improvement of psychological interventions for CD problems, to make them more effective (see below). Individual studies showed great variety of treatment efficacy in reducing conduct problems, depending on which questionnaire(s) (e.g. behavioural outcomes) was (were) chosen. While this allows generalizability of treatment effects across a range of CD problems, future studies should use a more homogeneous set of outcome measures to improve comparability across studies. Alongside the questionnaire(s) of treatment outcome, it is important to investigate possible moderators (i.e. gender, age, number of sessions, duration, intervention type, setting, medication use or dropout percentage) of treatment effect, since this information is needed to further optimize psychological treatment strategies. In our meta-analysis we did not find support for these moderators influencing treatment efficacy, but this may be linked to the overall poor quality of the studies. In addition, many
studies failed to provide important details, such as subtypes of aggression and presence of CU traits, which may have influenced the results.

Findings from our meta-analysis and recent reviews (e.g. Baumel, Pawar, Mathur, Kane, & Correll, 2017) raise questions on how to improve current or develop new treatments, since in the literature a recurrent conclusion is that overall results show small to moderate effect sizes of psychological interventions. Instead of delivering interventions in a standardized way, one of these directions could be the focus on subtypes of aggression and/or CU traits, as research confirms the need for direct and specific treatment approaches (Fung, 2017). Improving insights of understanding subtypes of aggression with or without CU traits may help to create more homogeneous treatment groups for different treatments, or even more desirable, preventive interventions. For example, targeting reducing proactive and reactive aggression there are effective treatments (such as the multimodal intervention Aggression Replacement Training [ART] (Brännström, Kaunitz, Andershed, South, & Smedslund, 2016) which can be used in the clinical practice, although they do not appear effective in reducing CU traits (see also Smeets, PhD Thesis “subtyping aggression and predicting cognitive behavioural treatment response in adolescents - what works for whom?”, 2017). The need for the targeting of cases with aggression and CU traits is high (given the high social, criminal, and economic consequences): one possibility is additional parent-management training or empathy training. CU traits are accompanied with other severe clinical behavioural problems such as reactive and proactive aggression, and some have suggested that deficits in decision making behaviour (reflected in decreased striatal and ventromedial prefrontal cortex responsiveness) (Blair, 2013) may underlie this. For example, to these brain regions, research shows that adolescents with ODD/CD (comorbid with ADHD) and adolescents with psychopathic traits have deficits in decision making, resulting in choosing significantly smaller amounts of immediate reward than waiting for a larger delayed award (Matthys et al. 2013; White et al. 2014). In line with this, a recent study shows that reduced amygdala-ventromedial prefrontal cortex activity during high provocation in the ultimatum game task (social fairness game) predicts the level of reactive aggression in youths with ODD/CD (White et al., 2016). Cognitive biases, such as weak executive skills, may inhibit adaptation to environmental stress and increase risk for aggression. Psychological treatments may benefit from an individualized approach that depends on the weaknesses and strengths of the (neuro)cognitive characteristics of the child and the adolescent (Matthys, Vanderschuren, Schutter, & Lochman, 2012).

Psychological treatments targeting aggression problems show that children and adolescents may develop more knowledge of impulse control and alternative behavioural responses (Matthys et al., 2012). It is thought that the acquirement of knowledge makes the cognitive system more flexible as it now has more representations of the environment and more ways to achieve a particular behavioural outcome (Lovden, Backman, Lindenberger, Schaefer, & Schmiedek, 2010). It has been suggested that children and adolescents with ODD/CD problems are the ones with the least knowledge and insights into their own behaviour and have limited alternative behavioural response options. Perhaps focusing on improving this understanding may also contribute to gaining control over one’s impulses. As such, programmes such as the U.S. based Mendota Juvenile Treatment Center Program (MJTC) are demonstrating significant treatment response in CD youth. The development of skills and knowledge about control over aggressive impulses might be at risk by the presence of rather
persistent cognitive distortions. As these distortions are often used to protect a positive self-image, to neutralize feelings of blame and guilt, and to trivialize aggressive behaviour (Barriga, Hawkins, & Camelia, 2008; Helmond, Overbeek, & Brugman, 2014). As a result, disproportionate aggression may be strengthened and/or maintained. In addition, despite the stable character of CU traits (Fanti, Collings, Andershed, & Sikki, 2017; Scheepers et al, 2010) research on psychological treatment (e.g. socio-emotional, cognitive processing, multimodal or behavioural therapy) showed that high levels of CU traits can decrease over time (varying per individual) (Muratori et al., 2015; Wilkinson et al., 2015). This suggest that children and adolescents with elevated CU traits are not “untreatable” and that these children can show reductions in both their CU traits and their antisocial behaviour (Wilkinson, Waller, & Viding, 2016), although they typically begin treatment with poorer premorbid functioning and can still end with higher levels of aggressive behaviour (Wilkinson et al., 2016). The improvement in functioning in those with high CU traits coupled to antisocial behaviour is evident in intervention programmes such as the MJCT. Additional single and/or a combination of different treatments (e.g. parent management training, modulation of arousal level, and/or empathic skills training) could target these CU traits in youth further. More specifically, based on the social learning model of Bandura, adolescents appear to learn to express and share their emotions by observing their peers doing so. This group setting improves their understanding of other people’s emotions and empathy level, which seems to be more effective than individual treatment (Muratori et al., 2015). In addition, a study of Masi et al. (2016) shows that medication added to psychosocial treatments could reduce aggression, but not CU traits. This is highlighted further by the clinical observations that psychostimulants such as methylphenidate that reduce aggression do not necessarily improve social behaviour per se. Thus adding medication is not of additional value when aiming to reduce levels of CU traits. Overall, this highlights the need for clinical practice to investigate whether high levels of CU traits are present in individual with aggression because additional or different treatments may be needed. In order to direct specific treatments, better understanding of the possible biological mechanisms involved in aggressive behaviour and CU traits is likely to be helpful. Stratification by the use of biomarkers may help to identify biologically more homogenous subgroups, to which treatment could be focused and accommodated. These biomarkers can be stratified for example on genetic, (neuro)endocrine, (neuro)cognitive, and behavioural levels in order to examine subtypes of aggression and the role of CU traits. Alongside behavioural questionnaires to measure CU traits, also physiological measurements such as baseline heart rate and its variability to stressors could be a biological correlate of CU traits (Ortiz & Raine, 2004; Stadler et al., 2008; Van Hulle, Corley, Zahn-Waxler, Kagan, & Hewitt, 2000). Research show that children and adolescents with CD and CU traits have a lower magnitude of heart rate change (de Wied, van Boxtel, Matthys, & Meeus, 2012). In individuals who manifest restrained, controlled aggression are less likely to be affectively unstable; their aggression is instrumental, in the sense that it is normally used to achieve a goal beyond harming a victim (van Goozen, Fairchild, Snoek, & Harold, 2007). The level of arousal of individuals engaging in this form of aggression is thought to be low, as evidenced by their low baseline heart rate and skin conductance levels, although it is not known whether their arousal levels are also low during the commission of violent or antisocial acts themselves.

Overall, the results in the current thesis in combination with current research advocates the
need for more detailed psychological treatments based on better defined homogeneous groups (e.g. based on aggression subtypes and/or biological factors), and targeted specifically on the presence/absence of high CU traits.

Biological factors such as genetic factors might play a role in the development or maintenance of aggressive behaviour, and it is less likely to be explained by a few candidate genes (e.g. MAOA, 5HTTLPR, COMT, DRD4), but rather by a complex interaction between multiple genes (Vassos, Collier, & Fazel, 2014). Results from chapter 3 showed that there is a lot of research on different genes in animal research, but not all of their findings are investigated or confirmed in human research. Overall, there is a risk of bias arises in the cited studies, given that the power to detect shared environmental influences is often low in biometric analyses of twin data and these studies assume that the environmental effects are free of influence by genetic effects (Burt, 2013). First results from genome-wide association of human studies are emerging (Tielbeek et al., 2017). Molecular genetic findings may also be sex-specific (see also see also van Donkelaar, PhD Thesis “Genetic and neurobiological mechanisms underlying aggression subtypes”, 2018). Molecular genetics offers no immediate solution to the problem of aetiological heterogeneity.

Regarding the RDoC approach, research confirm links between aggression genes/environmental risk factors and brain regions relevant to aggressive behaviour (Fonagy & Luyten, 2017). However, this does not provide an answer to the question whether the studied brain regions are mediating or moderating between genes and behaviour in ODD/CD in particular. This illustrates the complex map of biological pathways from gene to behaviour via the brain. Although research in ADHD is more advanced regarding risk genes, life stressors, and ADHD symptoms (van der Meer, Hartman, et al., 2017; van der Meer et al., 2016; van der Meer, Hoekstra, et al., 2017; van der Meer et al., 2015), this work may provide a template that might also be used for aggression and antisocial behavioural research. Future research is warranted to investigate the possible causal influence of brain regions involved in aggression and CU traits on the expression of genes and their epigenetic markers coding for the influence of the environment throughout life.

Based on the results from the study in the hormonal concentrations (chapter 4) we can conclude that there are group effects on both testosterone (both disorder-specific and across disorders) and oxytocin (between clinical and non-clinical individuals (i.e. ODD/CD and ASD showed lower oxytocin levels compared to TDI)). Thus at (neuro)endocrine level, the type of hormone is important to differentiate across disorders (i.e. based on oxytocin concentrations) or between disorders (i.e. based on testosterone concentrations); these effects were driven by the dimensional (i.e. partly driven by CU traits) findings. Further research is warranted to replicate our findings, and should explore whether these baseline hormonal factors can be used as stratification markers to predict response to treatment or course of ODD/CD and ASD or map on other independent variables. The design of this study does not create the option to investigate whether the differences in hormonal concentrations between and across groups are a result of a person’s trait or a reflection of a person’s inner state. This question could be answered by conducting for example an experimental intranasal oxytocin study, by which hormonal concentrations could be observed both in the short and long term
and how this may improve empathy which in turn may reduce aggressive behaviour (see also clinical implications). The observational design of the CU2 project does preclude to draw conclusions about the causality of the association between hormonal concentrations and aggression and/or CU traits. It is of note there is an overall developmental model on CU traits, as this was formerly described as being stable across the development, although this view has recently been disputed (Wilkinson, Waller, & Viding, 2016). Alongside this finding, recent results suggest that CU personality attributions present in toddlers and preschoolers are different compared to older children (Fonagy & Luyten, 2017). More specific, CU attributions in toddlers and preschoolers do not include a distinguishing feature such as a lack of emotional responsiveness. This suggests not only to take into account a developmental approach, but also a person-based approach (i.e. how are the CU personality attributions presented within a person) in research on CU traits. Currently, several EU-funded research consortia such as AGGRESSOTYPE, MATRICS, and FemNAT-CD are collecting data towards this goal in children and adolescents with CD.

Multi-informant ratings (self- and parent-rated questionnaires) in the CU2 project are a strength of this thesis. Results in chapter 4-6 indicate that the link between CU traits and outcome measures is rater-specific. Regarding the (neuro)endocrinological level: cortisol concentrations were related to either higher self- or parent-rated CU traits, and testosterone concentration were only related with self-rated CU traits. Regarding the (neuro)cognitive level: no rater specified links of CU traits were reported with poorer performance in terms of accuracy within the emotional valence study. Within the eye-tracking study, eye-tracking parameters were regardless rater not at all linked with CU traits but more on a general level with psychopathic traits. The latter is in line with previous research on the associations between CU traits and general and maladaptive personality traits indicating that CU traits tend to be located in different parts of the personality space (Decuyper, De Bolle, De Fruyt, & De Clercq, 2011; Decuyper, De Clercq, De Bolle, & De Fruyt, 2009; Roose, Bjittebier, Decoene, Claes, & Frick, 2010). This underscores the multifaceted nature of psychopathic traits. Assessment of CU traits solely based on self-reports potentially leads to bias due to lack of self-knowledge, deception, and impression management. Individuals scoring high on maladaptive personality traits are even more likely than other people to have distorted views of themselves (Oltmanns & Carlson, 2013). Indeed, some groups believe that collecting both self- and parent-rated version of the Youth Psychopathic Trait Inventory (YPI) and the Inventory of Callous Unemotional Traits (ICU) may offer improved insight into CU traits than either alone (Fanti et al., 2017). The DSM recommends that several informants are necessary to diagnose the specifier “with limited prosocial emotions”, thus including multiple sources may lead to assess CU traits in a more complete and accurate way. Given the additional resources needed in both research and clinical samples to collect data from different informants, it is important to determine whether distinct informants add incremental and valid information in the assessment of CU traits (Decuyper, De Caluwe, De Clercq, & De Fruyt, 2014).

Results from chapter 5 does not provide evidence to differentiate based on clinical disorders, since the clinical groups had diminished ability to process positive stimuli compared to the TDI group. These findings are in line with a recent study in which an emotional Go/No-go task was applied, reporting that the difficulties that children with combined CU traits and oppositional conduct
problems have in processing emotions are more of an emotional rather than an attentional nature (Ezpeleta et al., 2017). In addition, both clinical groups had decreased total fixation duration for several emotional expressions compared to the TDI group (see also chapter 6). Overall, the results suggest that individuals with both ODD/CD and ASD have diminished emotion processing at the neural level. Furthermore, it is not clear whether task performance of individuals with ODD/CD and ASD is explained by their indifference or lack of motivation to deliver an optimal performance.

Not being able to recognise and process emotions may have detrimental effects for treatment adherence and outcome. For example, children and adolescents may need more time and guidance in understanding and/or practicing in what they learn in treatment. Thus the treatment focus should be targeted on individual’s characteristics and include targeted treatment on homogeneous groups. In addition, as suggested above, treatments focusing on targeting for example amygdala or insular cortex, may involve fMRI neurofeedback, transcranial magnetic brain stimulation or transcranial direct current stimulation (Dambacher et al., 2015). The latter two are promising techniques that may be able to further clarify the role of the prefrontal, temporal, and insular cortices in mediating aggressive behaviour.

The tests administered in the two neurocognitive studies in the current thesis (chapter 5 and 6) did not address issues of learning from reinforcement and punishments. Within this perspective, it is also advised to include more targeted tests such as the reinforcement approach to treatment. As this approach is focused on rearranging environmental contingencies so that withholding from aggressive behaviour is more rewarding than expressing it. Within the clinical practice, this approach can be used in functional analysis of aggressive behaviour with or without CU traits. This analysis focuses on the functional relations between the different aspects of the aggressive behaviour and environmental variables (e.g. Burger, 1994). A functional analysis seeks to determine how aggressive behaviour has been learnt and maintained by means of positive and negative reinforcements (e.g. more attention from parents or friends, avoiding aversive social situations), and prosocial behaviour has been discouraged through punishment (Kendall, Slavenburg, van Bilsen, 2013).

In this thesis, we also looked at the demographic factors such as IQ, socio-economic status, and smoking, but found no relationship with mechanisms (i.e. (neuro)endocrine and (neuro) cognitive) of aggressive behaviour. This suggests that the investigated mechanisms in this thesis were not influenced by these demographic variables. An exception is the association between medication use and signal to noise ratio (d’ scores, calculated as percentage hits – percentage false positives on the affective GoNoGo task) signal to noise ratio (i.e. calculated as percentage hits – percentage false positives), indicating that individuals of both clinical groups being treated with medication had lower d’ scores than adolescents without medication (see also chapter 5).

Strengths, limitations, and future directions

Strengths of this thesis were as follows: a systematic review was conducted to provide an extensive overview of psychological treatments and treatment efficacy targeting conduct problems in both children and adolescents. Moreover, an extensive overview was provided of quantitative and
molecular genetic studies of aggressive behaviour in both humans and animals. Another strength was the use of different methodological approaches such as multi-informant (i.e. self-rated and parent-rated), objective measures and potential biomarkers rather than just subjective reports (e.g. which ruled out the influence of social desired expected answers) in a direct comparison of an ODD/CD group and an ASD group. Despite its strengths our results need to be considered in the context of the study's limitations.

**Quality of existing studies**

In addition to the limitations in the studies based on the CU2 project, there are also limitations to the two review chapters (i.e. chapters 2 and 3). Overall, reviews are always limited by the quality of existing studies. To allow better evaluation of the quality of studies, future randomized controlled trials should provide detailed information on their methods of randomization and blinding and on the fate of all trial participants (including dropouts). Many constructs or operationalizations of aggression in animal models appear to be species-specific and should be cautiously translated to human behaviour.

**Role of CU traits**

Further, our sample of TDI was smaller than the ASD and ODD/CD groups. This is because originally the CU2 project was in part designed to sample ASD and ODD/CD groups to stratify by high and low CU traits. Until recently, there was no clear cut-off score for the inventory of callous-unemotional traits (ICU) questionnaire used in the current thesis. Docherty et al., (2017) conducted a large study in adolescents in the community as well as detention facilities and provided cut-off scores (based on predicted probabilities of > .5) as follows: for youth report ICU, 28; for parent report ICU, 30; for teacher report ICU, 33; for the model with all three types of report, the cut-off for youth was 40, for parents 30, and for teacher 36. However, in practice, in the CU2 project there were not enough participants with high CU traits to implement such a stratification, as the resulting high-scoring group would have been too small. Furthermore, recent taxometric research showed that the latent structure of CU traits is best described as dimensional rather than categorical (Herpers, Klip, Rommelse, Greven, & Buitelaar, 2016). A common problem in research in psychiatric disorders is that individuals with the more severe clinical manifestations are less likely to participate in research. That also happened in our CU2 project, where many adolescents with rather severe acute behavioural problems (i.e. occurrence of violent and/or oppositional incidents, escape attempts and severely dysfunctional relationships with their caregivers) were considered as too “instable” or “dangerous” to participate. As a result, their institute did not allow them to participate in research projects, and/or their parents/caregivers were unwilling to provide informed consent. In addition, these youngsters were also themselves less likely to give informed consent. Not being able to include severe cases of ODD/CD in the CU2 project may have led to selection bias and an incomplete sample where possible subgroups (e.g. high on CU traits) have been left out. As a consequence, our results may be generalized to ODD/CD with moderate CU traits in particular.

Within clinical disorders there is much heterogeneity too (e.g. severity of CU traits within CD), although this was not investigated within the current thesis. Participants with a diagnosis of...
ODD or CD from the CU2 project were grouped together in this thesis, because both disorders are on a spectrum of behaviour problems and aggressive tendencies. In addition, the ODD/CD group included only a few CD participants to be handled as a stand-alone group. Therefore, outcomes of the chapters in this thesis included mainly ODD participants, which limits the ability to make disorder-specific conclusions. In order to control for the possible influence of CD, post hoc analyses were run with and without CD participants, and results tended to be similar.

Role of medication
Both clinical groups in the CU2 project contained a substantial number of participants using medication. However, antipsychotics were stopped, where possible, two days before and stimulants on the test day. Further, possible confounding effects of medication were controlled for or examined statistically where possible, making it unlikely that medication strongly influenced results.

Early- or late-onset CD
Whether participants had early- or late-onset CD was not included in the current study, although we intended to collect this information. However, we were unable to retrieve this relevant information on the onset of CD, as it was often not provided in the case files. Furthermore, onset of CD could often not be objectively stated by participants and/or their parents/caregivers for various reasons. For example, it happened that information provided by adolescents with CD was in contradiction with information provided by their parents/caregivers. In a majority of cases the biological parents were not available for answering our questions and legal guardians were not always informed about the onset of problems.

Environmental factors
We did not include contextual information (e.g. neglect or traumatic life events), while this information is important regarding the expression and aetiology of aggression. For example, a history of aggressive behaviour, may contribute to the probability that developmental changes in hormones will influence behaviour (Susman, Granger, Murowchick, Ponirakis, & Worrall, 1996). Future studies should take care to collect this data particularly in light of future efforts to examine epigenetic markers of CD.

Measurements
The studies in the current thesis found that a small percentage of variance of aggression was explained by both categorical (i.e. small group differences) and dimensional aspects (i.e. subtypes of aggression, and/or CU traits), regarding molecular genetics and (neuro)endocrine factors, (neuro)cognitive task performance, and eye tracking parameters. The amount of variance explained, highlights the need for future research. Future directions may include 1) to focus on developing neural and (epi)genetic network-based analytic approaches to identify causal genes and networks and to clarify the relationship of genes and networks with aggressive behaviour; and 2) to further delineate the species specific and non-specific domains of aggressive behaviour as well as escalated or abnormal aggression, and to clarify the overlapping yet distinct causal genes and networks underlying these separable domains, particularly overlooked domains such as frustrative non-reward.
In addition, future research should also pay attention to the assessment of aggressive behaviour with several objective as well as subjective instruments, such as observations, questionnaires by different informants (e.g. parent-, teacher-, and self-reports), neurocognitive measurements and also using fMRI assessment (brain studies) to also take into account brain mechanisms in relation to for example reinforcement learning as the inability for those with high CU traits to learn from punishment may be an important point of intervention for CU trait moderation in the future.

**Demographics**

The CU2 project included only male adolescents, thus we cannot extrapolate our findings to females with either ASD or ODD/CD. Future research should take into account both females and males. Recent data suggest that there may be sex-specific biological factors underpinning aggression (Tielbeek et al., 2017).

Whether the findings in this thesis are unique to ODD/CD and ASD or whether they reflect results that also apply to those with other forms of psychopathology remains unclear. An additional clinical group without core deficits in empathy and/or emotion regulation, such as attention deficit hyperactivity disorder (ADHD) not comorbid to either ASD or ODD/CD (Graziano & Garcia, 2016), could further add to the significance of our findings.

**Genetics**

The complex nature of ODD/CD and ASD necessitates large multi-study collaborations and big-data efforts to include transdiagnostic markers which may in turn clarify heterogeneity, comorbidity and inform cross-disorder interventions in the clinical practice. Therefore, especially when conducting research on a challenging study population, such as ODD/CD, large international collaborations are useful that combine research questions, data, and expertise in order to conduct research in larger study samples and of higher quality. A cognomic approach (i.e. a better understanding of links between cognition and the brain at a molecular level) in analysis is still largely missing from the literature, potentially due to the lack of sufficiently large imaging samples (see also see also van Donkelaar, PhD Thesis “Genetic and neurobiological mechanisms underlying aggression subtypes”, 2018). Thus, adding a cognomics approach to future research projects could enlighten the model of aggression as presented in chapter 1. In addition, it is unclear whether outcomes in pathways, such as neural pathways, is a consequence of chronic disease or long-term treatment rather than being related to disease aetiology. Therefore, cognomics of disease-related genes should also be performed in typically developing subjects.

**Hormones**

While basal hormonal concentrations in human studies including ASD and ODD/CD are relatively understudied, there is extensive literature on intranasal oxytocin trials in clinical populations samples including for example ASD (Bakermans-Kranenburg & van, 2013; Dadds et al., 2014; Weisman, Zagoory-Sharon, & Fieldman, 2014). Intranasal oxytocin may have therapeutic benefits, as Domes et al. (2007) showed that oxytocin has a modulating effect on amygdala activity as a response on emotional faces. Elevating oxytocin levels by means of intranasal administration may remedy social
difficulties and improve social cognition for a brief period (Domes et al., 2013; Guastella et al., 2010), although previous reviews and individual studies showed effective but temporary results of intranasal oxytocin administration in improvement of social functioning. Recent research showed that in ASD in particular, efficacy for improvement of social impairment was equivocal, partially due to mixed methodological designs, dosing regimens, and outcome measures (DeMayo, Song, Hickie, & Guastella, 2017). Furthermore, it remains unclear what the long-term effects are of intranasal oxytocin administration and/or the effects of multiple administrations over a period of time. This also warrants further research, in which promoting affiliation and empathy of intranasal oxytocin will need to be tested in selected cohorts of high-aggressive patient groups such as ODD/CD with high CU traits.

Cognition and neurophysiology
The studies in this thesis were part of the larger CU2 project which includes additional assessments of various aspects of empathy, of which not all could be examined in the scope of this thesis. The CU2 project examined in a cross-sectional design whether different aspects of empathy were differentially affected across reactive and proactive aggression. Three different ways to measure different components of empathy were conducted: 1) mimicry by means of electromyogram of the facial musculator (motoric), 2) physiological measures such as sympathetic nervous system response of heart rate and skin conductance (which represents the “fight/flight” emotional response) and 3) (neuro)psychological tasks, such as theory of mind task (cognition). Identification of these components and whether these components are cross-disorder or disorder specific provides additional insights to the current models of aggression and this could deliver ideas on how to improve and/or develop effective treatments. Future research on treatment could also explore (neuro) psychological tasks such as theory of mind task (i.e. cognitive empathy) and use this information in the development and evaluation of a personalized treatments for both aggression subtypes such as reactive and proactive aggression. Cognitive empathy was also a part of the CU2 project and this data is currently being preprocessed to be analyzed.

Future research is warranted to explore different aspects of empathy, taking into account physiological measures such as autonomic response of heart rate and skin conductance (markers of arousal) as objective instruments to provide information about an adolescent’s inner state (van Dooren, de Vries, & Janssen, 2012). This may in turn help children and adolescents with conduct problems to become aware of physical tension in their body and emotions, and take actions to reduce or control their emotions like going to a less stressful room to calm down which may prevent escalating aggressive and antisocial behaviour.

Suggestions for treatment
It is suggested that psychological treatments lead to change in associated brain functioning, although this needs to be confirmed in research yet. As stated above, future research could include brain imaging technologies (e.g. TMS) to enhance these psychological treatments. While TMS can enhance or disturb brain activity in a specific region by means of electromagnetic induction, tDCS induces low electric currents into brain tissue to either decrease or increase the excitability of the
stimulated areas (Dambacher et al., 2015). While the use of biofeedback of arousal to normalize physiological deviance as in neuro-feedback treatment of ADHD has shown to be effective (Chapin & Russell-Chapin, 2014; Caria, Sitaram, & Birbaumer, 2012; Larsen, 2012), it has not been followed by controlled biofeedback trials for paediatric aggression. Thus, future research is warranted to investigate whether arousal modulation in young children at high risk for CD and with CD (low arousal and high CU traits), and real-time fMRI neurofeedback in adolescents with CD are effective in reducing aggression.

Taken from a different perspective on ‘traditional’ face-to-face contact in treatment, quite recently, researchers have examined the efficacy of digital oriented treatment programmes in reducing disruptive behaviour (Baumel et al., 2017), which includes the potential for increased accessibility for participants. Results showed that digitally assisted parent training programmes are effective in reducing aggressive behaviour across a range of therapy formats (e.g. with and without therapist support) applied in real-world settings. Thus, future research could focus on digital oriented treatment targeting homogeneous subgroups of aggression and/or CU traits in order to increase treatment efficacy in reducing aggression.

Current research projects

The findings of the current thesis fit within the current model of aggression as presented in the general introduction and add in part to a large international research project which examines other aspects of the aggression model. This international research is also known as: aggression sub-typing for improved insight and treatment innovation in paediatric psychiatric disorders (AGGRESSOTYPE). This project employs highly innovative approaches in humans and animal models and maximize the project's output by optimally balancing the use of large, existing data sets with new data acquisition. Through this, a knowledge chain from molecule to behaviour will be built, investigating known and novel genes, gene-networks and their epigenetic interactions, and mapping their mode of action from the molecular via the cellular to the brain-circuit level. Based on innovative bio-informatics multimodal data integration, this interdisciplinary research will lead to novel, accurate algorithms for reliable aggression prediction, which will be validated in existing longitudinal studies in children and tested for their predictive value in adult outcome. In addition to this approach towards prevention, promising non-pharmacological biofeedback will be tested for personalised treatment and prevention of overt aggression. The neural, genetic, and molecular basis for this biofeedback will be investigated in another project known as: multidisciplinary approaches to translational research in conduct syndromes (MATRICS). The overarching goal of the MATRICS project is to test the key hypothesis that different aggression phenotypes result from differential impairment of arousal mechanisms which in turn dysregulates three basic neural functions: regulation of control mechanisms of aggression, emotional value rating of others, and empathy and moral decision making. Thus, MATRICS proposes to deconstruct current classifications of aggression phenotypes into these three neural systems that are coupled with hypo- and hyper-arousal mechanisms. To study these functions, same psychological tasks in both animal aggression models and human disruptive behaviour disorders samples are conducted, concurrent with the assessment of neural, neurochemical, genetic, autonomic nervous system and endocrinological markers.
Clinical implications

Insights of similarities and/or differences in mechanisms across disorders may be helpful to improve current or develop new psychological treatments, since the current efficacy of these interventions is low. This is important for researchers, clinicians, and justice officials to be able to determine which treatment to advice for children, adolescents and their parents in order to reduce aggression. First, results from chapter 1 in this thesis show that, in order to prevent future aggression problems, there is a need for prevention and intervention programmes for children and adolescents, which should already be given already in (elementary) schools. These programmes should focus on aspects such as development of coping strategies on how to deal with aggression like self-control, empathic skills, expression of emotions and reward sensitivity. In order to develop programmes that are effective in preventing aggression and antisocial behaviour, researchers should take into account the role of false-positives and false-negatives along in early detection screening tools, there is a need for effective screening tools. It is important to consider false-positive issues in the context of individual studies by taking the specific features of individual study designs into account and by considering both factors that enhance and factors that reduce the risk of obtaining and reporting false-positive findings. Such a balanced view could help prevent an unnecessary devaluation of non-pharmacological treatments and pave the way for a more productive discussion on how to make reliable and innovative scientific discoveries in this field. Second, alongside these preventive strategies, it is important for child mental health professionals to be aware of environmental risk factors influencing development, especially those relating to violence (e.g. dysfunctional family life, harsh and inconsistent parenting, abuse and neglect). These environmental risk factors could be reduced by the focus on improving parental guidance in raising their child. Finally, nearly all reported findings in chapter 4-6 were dependent on aggressive behaviour and/or CU traits in both ASD and ODD/CD participants, which suggests a cross-disorder role of aggression and CU traits. Importantly, this speaks in favour of a cross-disorder approach to treatment where (neuro)endocrine or (neuro)cognitive problems are the primary focus instead of a diagnosis, which could be an indication to form more homogeneous treatment groups.

Conclusion

The multiplicity of factors associated (i.e. environmental, genetics, (neuro)endocrine, cognitive, and behavioural factors) with the emergence, development and maintenance of aggressive behaviour suggests that aggression is a complex behaviour involving many different processes. Additionally, these factors may all be associated with each other, which makes unravelling the underlying mechanisms even more complicated. Insights of different mechanisms may help to identify more homogeneous subgroups for development and or improvement of customized medical and/or psychological treatments. Current research on psychological treatments in particular, show a modest effect on reducing (severe) aggressive behaviour, which may in part be a result of the heterogeneity of both across and within clinical disorders. This issue could be tackled by increased insights of biomarkers which may help to identify more homogeneous subgroups. As human molecular genetic studies of aggression are in an early stage, the strongest molecular evidence comes from animal models.
comparing aggressive and non-aggressive strains or documenting the effect of gene knockouts. Furthermore, individuals with either ODD/CD or ASD, or without a clinical disorder partially differed on (neuro)endocrine and (neuro)cognitive factors. More specific, hormonal concentrations, emotion processing, time to first fixation and fixation duration were different between disorders. However, we do not know if these findings are unique to ODD/CD and ASD or whether they reflect a deficit that cuts across those with psychopathology relative to TDI. An additional clinical group without core deficits in empathy could further add to the significance of our findings. The work in this thesis contributes to enhancement of understanding and knowledge of trans-diagnostic markers based on scientific research focused on both a diagnostic and a dimensional approach. In the longer term, current and future research results will contribute to reduce the impact of adaptive aggression on society.
References


Appendices
Nederlandse samenvatting


Problemen gerelateerd aan agressie bij jonge mensen vallen onder de noemer agressieve gedragsstoornis, waaronder oppositionele-opstandige stoornis (oppositional defiant disorder; ODD) en gedragsstoornis (conduct disorder; CD) te brengen zijn. De termen CD en ODD zijn afkomstig uit de DSM (Diagnostic and Statistical Manual of Mental Disorders) een van de meest gebruikte internationale classificatiesystemen in de geestelijke gezondheidszorg. Aan CD en/of ODD wordt voldaan wanneer het negatieve gedrag ernstig is, vaker voorkomt dan gemiddeld, daarnaast al langere tijd aanwezig is en niet wordt veroorzaakt door omstandigheden. Beide stoornissen zijn zeer complex, zowel klinisch als op etiologisch vlak. Meer kennis is nodig over genetische, biologische, (neuro)cognitieve en gedragsmatige kenmerken van agressie in ODD en/of CD. In 2013 werd een nieuwe versie van de DSM gepubliceerd waarbij extra kenmerken zijn toegevoegd als onderscheid voor een ernstigere vorm van CD met beperkte sociale emoties. Deze kenmerken worden in het Engels callous unemotional (CU) traits genoemd, vrij vertaald als kille en emotieloze kenmerken. CU traits worden gekenmerkt door symptomen als gebrek aan empathie, kilheid/oppervlakkigheid, gebrek aan spijt of schuldgevoel (American Psychiatric Association [APA], 2013). Deze vorm van CD is geassocieerd met vermindering van specifieke vormen van empathie, met name in reactie op angst, verdriet, pijn en geluk van anderen (Blair, 2013). Verminderde empathische functies is ook een kenmerk van autisme spectrum stoornissen (autism spectrum disorder; ASD) (Herpers, Klip, Rommelse, Greven, & Buitelaar, 2016; APA, 2013; Rogers, Viding, Blair, Frith, & Happe, 2006). Mensen met ASD hebben ook verhoogd agressief gedrag in vergelijking met controle deelnemers (Hill et al., 2014), hoewel agressie geen kern symptoom is van ASD en agressie in ASD over het algemeen minder ernstig is in vergelijking met ODD/CD (APA, 2013).

Tot op heden hebben we een beperkt begrip van de gedragsmatige, (neuro)cognitieve en biologische processen die ten grondslag liggen aan CU traits/beperkte empathie en agressief gedrag in ODD/CD en ASD. CU traits en agressie en hun onderliggende processen kunnen dienen als transdiagnostische markers die een brug vormen tussen stoornissen en biologische grondslag van gedrag (Rodriguez-Seijas, Eaton, & Krueger, 2015; Walkup, Mathews, & Green, 2017). Deze transdiagnostische markers tussen de klinische groepen en biologische basis voor gedrag zou heterogeniteit en comorbiditeit kunnen verklaren en suggesties aanleveren voor stoornis-overschrijdende interventies in de klinische praktijk. Dit proefschrift onderzoekt de proactieve en reactieve subtypen van agressie, agressie met of zonder CU traits en hun genetische, (neuro)endocrine, (neuro)cognitieve en gedragsmatige onderbouwing als transdiagnostische markers door middel van een studie van mannelijke adolescenten met ODD/CD of ASD in vergelijking met adolescenten zonder psychiatrische stoornis. Dit proefschrift is ook gericht op het leveren van een overzicht van de effectiviteit van psychologische behandelingen.
In hoofdstuk 2 bevinden zich de resultaten van een literatuurstudie (meta-analyse) naar 17 studies gericht op psychologische behandelingen van kinderen en adolescenten met een klinische diagnose CD en/of CD problemen in het klinische gebied (inclusief ODD problemen) en een minimaal IQ van 80. Hoewel we specifiek in databases gezocht hebben naar non-medicamenteuze behandelingen anders dan psychologische behandelingen, hebben alle geïdentificeerde studies gebruik gemaakt van psychologische (gedragsmatige) behandelingen. Resultaten van 17 gerandomiseerd-gecontroleerde studies (onder wie slechts drie studies met geblindeerde observeerders voor het scoren van conduct problemen) lieten zien dat psychologische behandelingen een klein effect hebben op het verminderen conduct problemen in kinderen en adolescenten met klinische CD problemen/diagnosen op basis van rapportages door ouder(s) (Cohen’s $d = .37$, 95% CI = 0.27–0.47), leerkracht (Cohen’s $d = .21$, 95% CI = 0.12–0.49) en observeerder (Cohen’s $d = .26$, 95% CI = 0.06–0.47).

De studies geïncludeerd in deze literatuurstudie hebben CD problemen voornamelijk gescroond door middel van de CBCL en de ECBI vragenlijsten. De meeste geïncludeerde studies waren van erg lage tot redelijke kwaliteit en rapporteerden kleine behandeleffecten. Comorbiditeit, geslacht, leeftijd, aantal sessies, duur, interventie type, setting, medicatie gebruik of dropout percentages hadden geen invloed op het effect van de behandeling. Het is belangrijk dat toekomstige onderzoeken details geven rondom randomizatie en regelingen rondom geblindeerdheid, rapportages door ouder(s). Op basis van deze literatuurstudie is er onvoldoende bewijs om een specifieke psychologische behandeling aan te wijzen als meest effectieve in vergelijking met de andere psychologische behandelingen binnen de klinische groep van CD en/of CD problemen (inclusief behandelingen gericht op ouders).

In hoofdstuk 3 was een systematische review uitgevoerd om te onderzoeken of er bewijs is voor genetische onderbouwing van agressie en om te bepalen in welke mate voorgaande studies fenotyperen van agressie hebben onderzocht dat nauw aansluit, of helemaal niet, in het kader van RDoC. Het huidige review richt zich op drie typen genetica onderzoeken: 40 tweelingen onderzoeken, 277 moleculaire genetische onderzoeken van agressie in mensen, 34 genetische onderzoeken van agressie in fruitvliegjes en 86 onderzoeken naar fenotype van agressie in muizen. Rond 50% van de variantie van agressief gedrag in mensen was verklaard door genetische invloeden. Niet-geeldeelde omgevinginvloeden was matig. Gedeelde omgevinginvloeden lieten een klein of geen effect zien: ongeveer de helft van de gereviewde onderzoeken rapporteerden geen invloed van een gedeelde omgeving, terwijl andere onderzoeken een schatting aangeven tussen 0.15 en 0.35. Op mens georiënteerde moleculaire genetische onderzoeken van agressie zijn in een vroege fase; sterk moleculaire bewijs komt van dier modellen. Hierin worden agressieve en niet-agressieve knaagdier stammen vergeleken op het potentiële effect van verlies of toename van genen. Veel constructen of operationalisaties van agressie in diermodellen blijken context specifiek te zijn en men dient voorzichtig te zijn met deze bevindingen te vertalen naar menselijk gedrag.

In hoofdstuk 4 werden de hormonen oxytocine, cortisol en testosteron onderzocht in relatie tot (ernstig) agressief gedrag en/of empatie bij 114 deelnemers met ofwel ODD/CD (n = 37) of ASD (n = 49), of zonder een psychiatrische stoornis (controle, n = 28). Zowel de ASS- als de ODD/CD-groepen hadden significant lagere niveaus van oxytocine dan de controle groep; de ODD/CD-groep
had significant hogere niveau’s van testosteron dan de ASD-groep. Er waren geen groepeffecten op cortisolspiegels. Groepsverschillen bleven voor oxytocine na correctie voor de invloed van CU traits, maar werden niet significant bij het controleren op agressie subtypen zoals gemeten door de reactieve en proactieve agressievragenlijst. Resultaten voor testosteron werden niet-significant na correctie voor beide CU traits of agressie subtypen. Over de groepen heen genomen, waren hogere niveaus van CU traits gerelateerd aan hogere cortisol- en testosteronspiegels, maar proactieve en reactieve agressie waren niet gecorreleerd met alle drie de hormonale niveaus. Deze bevindingen suggereren dat de ernst van de CU traits gerelateerd is aan zowel hogere cortisol- als testosteronniveaus, maar niet aan oxytocinespiegels. De positieve associatie in onze studie was te danken aan het verband tussen cortisol en testosteron en de positieve associatie tussen CU traits en testosteron (dat wil zeggen: correlaties tussen cortisol en CU traits met zelf- of ouder rapportages werden niet significant na correctie voor testosteron).

In hoofdstuk 5 werd de emotieverwerking onderzocht tijdens een neuropsychologische taak (emotionele Go/No-Go). In deze studie namen in totaal 128 proefpersonen deel, met ofwel ODD/CD (n = 52) of ASD (n = 52), of zonder een psychiatrische stoornis (controle, n = 24). De resultaten toonden geen groepsverschillen met betrekking tot het aantal fout-positieve reacties. Bovendien was de ASD-groep langzamer dan de controle groep en de ODD/CD-groep op twee van de drie valentie-discriminaties (negatief-neutraal en negatief-positief). Daarentegen verschilde de reactietijd in de ODD/CD-groep niet significant van de controle groep op een van de drie valentie-onderscheidingen. De controle groep vertoonde aanzienlijke moeilijkheden bij het contrasteren van positieve en neutrale beelden in vergelijking met negatief-neutrale of negatief-positieve beelden, terwijl dit effect afwezig was in zowel de ASD- als de ODD/CD-groepen. CU traits, comorbiditeit met ADHD en medicijngebruik waren niet gerelateerd aan taakprestaties. Adolescenten met ODD/CD uitgevoerd in het bereik van de controle groep, terwijl de ASD-groep duidelijk langzamer was dan de controle groep, ongeacht het type emotie. Zowel de ASD- als de ODD/CD-groepen verschillen van de controle groep die een ‘positieve perceptie-vooroordeel’ vertoonde door vrij langzaam te zijn in het differentiëren van positieve en neutrale valentie. De resultaten suggereren dat adolescenten met ODD/CD of ASS een verminderde verwerking van positieve stimuli lijken te hebben en/of een ‘positieve perceptieafwijking’, wat aanwezig is in controle adolescenten, die ofwel kan bijdragen aan de symptomen en/of een gevolg kan zijn van het hebben van de aandoening en kan bijdragen aan de comorbiditeit van beide aandoeningen.

In hoofdstuk 6 werden de algemene (cross-disorder) en unieke (stoornis specifieke) emotionele gezichten verwerking, zoals weerspiegeld in de tijd tot de eerste fixatie en fixatieduur, onderzocht bij in totaal 122 mannelijke deelnemers, bestaande uit jongeren met ofwel ODD/CD (n = 44) of ASD (n = 50) en controle (n = 28). Verder werd de relatie van psychopathische kenmerken (inclusief CU traits) en agressief gedrag met eye-tracking-maatregelen onderzocht. De resultaten toonden aan dat de totale fixatieduur van de ogen in emotionele en neutrale expressies (behalve voor verdriet) significant lager was in zowel de ASS- als de ODD/CD-groepen, vergeleken met controle groep. Tijd tot eerste fixatie op de ogen van angstige gezichten was significant langer in zowel de ASS- als de ODD/CD-
groepen in vergelijking met controle groep. Voor de andere emotionele of neutrale gezichten werd geen significant effect gevonden. Aan de andere kant waren psychopathische kenmerken en de ernst van bijzonder proactieve agressie significant negatief gecorreleerd met zowel de totale fixatieduur als ook de tijd tot de eerste fixatie aan de ogen van angstige gezichten in de ODD/CD-groep.

De klinische praktijk

Inzichten van overeenkomsten en/of verschillen in mechanismen van verschillende psychopathologie kunnen nuttig zijn om de huidige of nieuwe psychologische behandelingen te verbeteren, omdat de huidige effectiviteit van deze interventies laag is. Dit is belangrijk voor onderzoekers, clinici en gerechtspersoneel om te kunnen bepalen welke behandeling moet worden aanbevolen voor kinderen, adolescenten en hun ouders om agressie te verminderen.

Ten eerste laten de resultaten van hoofdstuk 1 in dit proefschrift zien dat, om toekomstige agressieproblemen te voorkomen, er behoefte is aan preventie- en interventieprogramma's voor kinderen en adolescenten, die al op (basis-) scholen zouden moeten worden gegeven. Deze programma's zouden zich moeten richten op aspecten zoals het ontwikkelen van copingstrategieën over hoe om te gaan met agressie zoals zelfbeheersing, empathische vaardigheden, uitdrukking van emoties en beloningsgevoeligheid. Om programma's te ontwikkelen die effectief zijn in het voorkomen van agressie en antisociaal gedrag, is er behoefte aan effectieve screeninginstrumenten, waarbij rekening moet worden gehouden met de rol van o.a. vals positiven in deze instrumenten.

Het is belangrijk om valspositieve kwesties in de context van individuele onderzoeken te overwegen door rekening te houden met de specifieke kenmerken van individuele onderzoeksontwerpen en door rekening te houden met zowel factoren die verbeteren als factoren die het risico van het verkrijgen en melden van fout-positieve bevindingen verminderen. Een dergelijk evenwichtig beeld kan een onnodige devaluatie van niet-medicamenteuze behandelingen helpen voorkomen en de weg banen voor een productiewere discussie over hoe betrouwbare en innovatieve wetenschappelijke ontdekkingen op dit gebied kunnen worden gedaan.

Ten tweede is het naast deze preventiestrategieën belangrijk voor professionals in de kindergeneeskunde om zich bewust te zijn van omgevingsrisicofactoren die van invloed zijn op de ontwikkeling (bijvoorbeeld disfunctioneel gezinsleven, streng en inconsistent ouderschap, misbruik en verwaarlozing). Deze omgevingsrisicofactoren kunnen worden beperkt door de focus te leggen op het verbeteren van ouderlijke begeleiding bij het opvoeden van hun kind.

Ten slotte waren bijna alle gemelde bevindingen in hoofdstuk 4-6 afhankelijk van agressief gedrag en/of CU traits bij zowel ASD- als ODD/CD-deelnemers, wat duidt op een rol van agressie en CU traits in de stoornis. Dit is een aanwijzing voor het vormen van meer homogene behandelingsgroepen, waarbij de (neuro) endocriene of (neuro)cognitieve problemen centraal staan, in plaats van een diagnose.
Conclusie

De veelheid van factoren (milieu, genetica, (neuro) endocriene, cognitieve en gedragsfactoren) die verband houden met de opkomst, ontwikkeling en instandhouding van agressief gedrag suggereert dat agressie een complex gedrag is dat vele verschillende processen omvat. Bovendien kunnen deze factoren ook allemaal weer verband houden met elkaar, waardoor het ontrafelen van de onderliggende mechanismen nog ingewikkelder wordt. Inzichten in de verschillende mechanismen kunnen helpen om meer homogene subgroepen te identificeren. Dat is belangrijk voor de ontwikkeling en verbetering van aangepaste medische en psychologische behandelingen. Huidig onderzoek naar psychologische behandelingen toont een bescheiden effect op het verminderen van (ernstig) agressief gedrag bij kinderen en adolescenten met een psychiatrische stoornis, wat deels het gevolg kan zijn van de heterogeniteit van de klinische stoornissen. Dit probleem kan worden aangepakt door biomarkers te vinden voor meer homogene subgroepen. Omdat humane moleculaire genetische studies van agressie zich in een vroeg stadium bevinden, is het sterkste moleculaire bewijs afkomstig van diermodellen die agressieve en niet-agressieve stammen vergelijken. Onderzoek uit dit proefschrift laat zien dat mannelijke adolescenten met psychiatrische stoornissen ASD, ODD/CD verschillen in hormonale concentraties, emotieverwerking, en de manier waarop ze kijken naar een emotioneel gezicht. Toch is onbekend of deze bevindingen uniek zijn voor deze psychiatrische stoornissen of dat ze een tekort weerspiegelen dat aanwezig is bij mensen met psychopathologie ten opzichte van mensen zonder psychopathologie. Een extra klinische groep zonder beperkingen op het gebied van empathie kan het belang van deze bevindingen verder vergroten. Het werk in dit proefschrift draagt bij aan het begrip en de kennis van trans-diagnostische markers op zowel een diagnostische als een dimensionale benadering. Op de langere termijn zullen huidige en toekomstige onderzoeksresultaten bijdragen aan de vermindering van adaptieve agressie op de samenleving.
Referenties


Curriculum Vitae

Mireille Huvenaars-Bakker was born on the 14th of January 1985 in Wageningen, The Netherlands and grew up in neighboring village Wekerom. After completing secondary education at Het Streek in Ede (2003), she moved to Nijmegen to study Clinical and Developmental Psychology at Radboud University Nijmegen. During both her Bachelor’s and Master’s degree she coached children with an Autism Spectrum Disorder and/or Attention Deficit Hyperactivity Disorder both on an individual as well as group level. She spent six months in Curaçao for a clinical internship. After completing her Master’s, she worked as a research assistant coordinating the data collection and ethical approval for several international studies at Radboud University Medical Center. She also spent seven months at University of South Australia, Adelaide, to investigate blood flow velocity in very young children by using transcranial doppler technology. Back in The Netherlands, she came back to Radboud University Medical Center to start her PhD research working closely with Karakter Child and Adolescent Psychiatry in Nijmegen. Her PhD-project focused on genetic, hormonal, and cognitive factors of aggression in male adolescents. The results of this project are described in this thesis and were also presented at several international conferences (e.g. AACAP, EFCAP, ISRA, ECP). Additionally, she was involved in several side-projects, such as the supervision of bachelor- and masterstudents, internships, and assisting in tutoring courses in developmental psychopathology, neuropsychology, psychology in the clinical practice and gave lectures regarding emotion at the Radboud University Nijmegen. Importantly, she always combined her research with clinical work. She strongly believes the collaboration between research and practice is imperative for the development of these two fields. Currently, she is a healthcare psychologist at the Karakter Child and Adolescent Psychiatry and a co-developer at Learning & Resilience focused on empathy, compassion and resilience. In the future, she aspires to bring her research skills to practice-driven research, and finally to combine this with clinical work.
List of publications


Dankwoord

Met de nodige naïviteit begon ik eind 2014 aan mijn promotietraject en euforisch las ik in december 2017 de verlossende woorden: mijn manuscript was goedgekeurd! De afgelopen vier jaren zijn een enorm leerzaam traject geweest. Gedurende mijn promotietraject heb ik veel geleerd over wetenschap beoefenen, mezelf, om te gaan met voor- en tegenslagen, en de wereld van wetenschap. Ik ben heel blij dat ik al die tijd omringd was door geweldige en getalenteerde mensen die met passie naast mij hebben gewerkt om dit proefschrift te realiseren. Ik wil graag de volgende mensen daarvoor bedanken.

Allereerst wil ik mijn promotor, Jan, bedanken voor de mogelijkheid om van je te leren. Ik heb enorm veel bewondering voor je kennis en dat je naast alle ballen in de lucht houden ook nog tijd had om samen een rondje te hardlopen rondom het Colloseum tijdens een van de vele projectmeetings. Corina, thank you very much for your guidance during my project, I feel very lucky to have had you on the team. Jeffrey, thank you for your unconditional faith in me as a researcher.

Ik heb de eer gehad om met fijne mede-collega onderzoekers aan de slag te gaan met CU2 data en daarbij wil ik in het bijzonder Nanda, Danielle, Pierre, Evita, Carsten, Natalia en Kim bedanken. Dankzij jullie kennis, brainstormsessies en gezelligheid hebben we een paar prachtige papers neergezet! Met veel plezier kijk ik terug op de vele buitenlandse projectmeetings waar ik samen met team Nijmegen: Marjolein, Jill en Shahrazad naar toe ging. Dank jullie wel voor het samen ontdekken van de fantastische plekken waar we heen gingen en te leren van elkaars expertise.

Graag wil ik de geliefde onderzoeks-collega’s van Karakter (a.k.a. (ex-)kippetjes) en Psychiatrie van het Radboudumc in het zonnetje zetten: Kirsten, Jennifer, Loes, Mirjam, Yvette, Leonie, Saskia, Daphne, Anoek, Jolanda, Andrieke, Iris, Yvonne, Annemieke, Nienke, Manon, Margreet, Francesca, Karlijn, Desiree, Zita, Danique: Dank jullie wel voor de gezellige researchmeetings, het delen van PhD-euvels, ik-heb-nu-koffie-nodig-momentjes, even een frisse neus halen in park Brakkestein, groepsfoto in de badkuip en congres avonturen. Mijn promotietraject was onvergetelijk dankzij jullie!

Er zijn altijd mensen op de achtergrond die je eigenlijk niet ziet of hoort, maar een onmisbare rol hebben bij het uitvoeren van een project zoals dat van mij. Dankjewel Nadine voor het plannen van afspraken en je hulp. In het bijzonder wil ik ook graag de Otho Gerard Heldering Stichting, De Hoenderloo groep en de Woodbrookers bedanken voor het mogelijk maken om deelnemers te werven. Aansluitend wil ik graag ook alle deelnemers van het CU2 project bedanken voor hun inzet. De werving van de deelnemers bleek een hele klus te zijn (weglopers, ouders die met moeite op te sporen waren, test spullen sjouwen, deelnemers motiveren) die ik nooit had kunnen klaren zonder de hulp van de fantastische stigiaires: Rogier, Annelore, Anne, Suzanne, Fieke, Marleen, Carlijn, Seyma, Bernadette, Katinka, Marlies en Linda. Samen met jullie gingen we als een speer en hebben we met zijn allen de dataverzameling kunnen afsluiten, yes we did it!
Lieve Silvia, Wendy en Rik. Wat een fantastisch avontuur was het toendertijd om samen met jullie het studentenleven te ontdekken en wat zijn jullie mij dierbaar. Afzonderlijk van elkaar zijn jullie van onschatbare waarde geweest tijdens mijn promotietraject, bedankt voor de ontelbare koffiemomentjes, samen het metro-krantje lezen, onvooraardelijke steun, schrijfdates en vele dansjes.

Lieve dr. Frau Stone en dr. Herr. N., wie had dat gedacht dat terwijl we bleu op studiereis ronddoolde in Budapest en elkaar leerde kennen, we alledrie een promotietraject zouden aangaan. Ik kijk graag terug op onze gesprekken in vloeiend Duits en onze wetenschappelijke discussies over het leven van een PhD’r en de lol die we samen hierover hebben. Een memorabele date is natuurlijk die ene waarbij jullie Andreas hebben geïntroduceerd in mijn leven, waarvoor eeuwig dank :)

Gedurende mijn studenten- en PhD-tijd heb ik verschillende mensen leren kennen die elk een bijzonder plekje in mijn hart hebben gekregen. Stefanie, je fantastische nuchtere kijk op het leven, onze potjes schaak bij de ijssalon en wandelingen zijn mij erg dierbaar. Suus, dankjewel voor wie je bent en je tip dat Kerstliedjes ook prima al in september kunnen worden geluisterd. Lieve Yvonne, ooit als huisgenootjes begonnen in het Tirol’r huis, wat is jouw humor en beide-benen-op-de-grond mentaliteit toch goud waard. Shaha, thank you for just being you, your strength is an example to me, I don’t know (yet ;) how you manage it all. Lieve ‘Old School Friends’: Yuli, Anoeshka, Mariske, Heleen, Roos, Lieke, Tamara. Werkelijk over alles kunnen we het met elkaar hebben, lachbuien, huilen, feitjes opduiken over grootste milieuvervuiling, Kerstkaart filmpjes maken, wat zijn jullie toch een heerlijke vriendengroep! Dank jullie allemaal voor het meeleven. meedenken en de nodige afleiding en ontspanning te vinden in de afgelopen jaren, op naar nog vele jaren samen! Lieve Ricky, Yvonne en Wilka -De Dushi’s- wat hebben we toch een bijzondere vriendschap. Onze herinneringen aan Curaçao en weekendjes samen weg zijn mij erg dierbaar. Bedankt voor jullie nuchtere blik, onvooraardelijke steun en verfrissende Amstel Brights! Wekelijks blaren kweken in de boulderhal van Nijmegen, bedankt voor de aanmoedigingen, delen van frustraties en meelevens: Kevin, Dirk, Joost, Tobias, Pieter, Dennis (a.k.a. de klimplantjes). Bijzonder dierbare herinneringen heb ik aan Lowlands en vooral met de mensen waarmee ik daar naar toe ben gegaan en heb leren kennen: lieve Miriam, Teun, Miel, Ilonka, Wouter, George, Loes, Alex, Linda, Frank, Bart, Laura. Jullie hilarische acties en steun tijdens en buiten onze festival dates zorgden voor de nodige ontspanning en relativering. Wat zijn jullie toch een fantastische groep!

Lieve familie Huvenaars en van der Heijden, en in het bijzonder Willeke, Mart, Karlijn, Matthijs, Thomas, Marleen, Pim, Siem, Mees. Wat een heerlijke grote en warme familie zijn jullie, bedankt voor jullie steun en voor wie jullie zijn.

Lieve Alexandra, Han, Anna, Eva, Ans, Han, Michel en Anneloes - de Bosz’jes-, eindelijk is mijn ‘scriptie’; klaar. Jullie hebben een bijzondere plek in mijn hart. Bedankt voor wie jullie zijn, de aandacht die jullie hebben voor de ander en de fijne bbq’s in de achtertuin.

Across the other side of the world there is also family who have a special place in my heart: Patty,
Ben, Oliver, Bronte, Roger, Louise, Nick, Tim, Bernadette, Louise, Sam, Harry - the Dutton’s- you mean the world to me. I am touched by the love and care I feel during my visits to Australia and back in Holland when we Skype or email. I feel blessed that I am part of your family too.

En deze laatste regels van dit dankwoord, de ere-plaats, zijn natuurlijk voor jou: lieve Andreas. De afgelopen jaren hebben we veel moeten trotseren, dat heeft ons samen alleen maar sterker gemaakt. Jij bent het allerbeste wat mij is overkomen. Je humor, bemoedigende woorden, vele kopjes thee cappuccino’s, de spreekwoordelijke schop onder mijn kont en heerlijke voedzame maaltijden voor mijn hersencellen hebben ervoor gezorgd dat we hier samen zijn.

“I tell you life is sweet, in spite of the misery. There’s so much more, be grateful” - Natalie Merchant
Donders Graduate School for Cognitive Neuroscience

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