Identifying intermediate phenotypes of ADHD and ASD
a cognitive-electrophysiological approach

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Identifying intermediate phenotypes of ADHD and ASD: a cognitive-electrophysiological approach

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Thesis submitted for the degree of Doctor of Philosophy

2012
The real voyage of discovery lies not in seeking new landscapes, but in having new eyes.

Marcel Proust
Abstract

The highly complex and heterogeneous nature of overlapping neurodevelopmental disorders is likely to increase misspecification in group allocation and hinder the identification of the mechanisms involved. One strategy to facilitate gene identification and to improve understanding of the pathophysiological mechanisms underlying clinical co-occurrence is the investigation of intermediate phenotypes that lie between genes and behaviour. In particular, a neurophysiological perspective which utilises the exquisite time resolution of electroencephalography (EEG) allows objective quantification of the underlying mechanisms. Attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) are complex childhood-onset disorders that are traditionally treated as separate research fields, in part due to current diagnostic systems precluding a co-diagnosis. The past decade, however, has witnessed an increase in the awareness of significant behavioural, cognitive and genetic overlap between these disorders.

The present thesis can be divided into two main objectives: (I) to examine the aetiological overlap between ADHD and quantitative EEG parameters, and (II) to identify shared and/or distinct cognitive-electrophysiological markers of ASD and ADHD and their common co-occurrence. This encompasses data collection in two samples: a community twin sample selected on the basis of consistently high or low ADHD symptoms, and a sample of clinically diagnosed children with ASD (n=19), ADHD (n=18), co-occurring ASD+ADHD (n=29) and typically developing controls (n=26).

In Part I, structural equation modeling was used to demonstrate heritability of and substantial shared genetic influences between selected quantitative EEG measures and ADHD, supporting these measures as candidate intermediate phenotypes of the disorder. In Part II, ASD and ADHD were directly compared on these measures and ERP indices of attention and social cognition. Across these analyses, while it was possible to dissociate ASD-only and ADHD-only on their basis of cognitive-electrophysiological parameters, ASD+ADHD largely showed the unique deficits of both disorders, thus supporting an additive co-occurrence. Disentangling phenotypic variation in gene-brain-behaviour relationships is likely to aid the identification of susceptibility genes and other causal mechanisms underlying the complex aetiology of ADHD and ASD. In addition, elucidating the basis of comorbidity can help to refine classification systems and enhance the assessment of complex cases for more specific treatment strategies.
Statement of work

This thesis focused on data from two studies. First, Chapters 2 and 3 used data from a subset of the Twins Early Development Study (TEDS), headed by Professor Robert Plomin, called the Neurophysiology of Activity and Attention in Twins (NEAAT) study, headed by Dr. Gráinne McLoughlin (funded by a National Institute for Health Research postdoctoral fellowship). Participants were selected using latent class analysis conducted by Dr. Fruhling Rijsdijk and recruited by a research assistant. As part of the testing team, I was trained in EEG data acquisition. I was responsible for cognitive and EEG data collection, under the supervision of Dr. McLoughlin. I was then in charge of formulating hypotheses and carrying out the EEG and model-fitting analyses on scalp measures of quantitative EEG, under the guidance of Dr. McLoughlin and Dr. Rijsdijk.

Secondly, Chapters 4 to 7 incorporated data from the Specificity of Electrophysiology in Neurodevelopmental Disorders (SEND) study (funded by the Waterloo Foundation (G686984) and the Steel Charitable Trust (G38575208), which consisted of a subset of participants from the Biomarkers of Neurodevelopmental Disorders (BioNeD) study, headed by Professor Patrick Bolton and Professor Philip Asherson (funded by the National Institute for Health Research Biomedical Research Centre). The majority of participants were previously recruited by the BioNeD research team who carried out screening and diagnostic assessments among a large cognitive battery. Subjects were recruited for the SEND study by a research assistant. In addition, 21 of 92 participants included in the SEND study were recruited by myself from local parent support groups and schools. Within the SEND study, I was in charge of participant selection, the selection of social cognitive tasks through collaboration with Birkbeck College and the Vita-Salute San Raffaele University, and carrying out the testing sessions. I had sole responsibility for managing the data, and analysing and interpreting the cognitive-electrophysiological data, under the guidance of my supervisors and collaborators.

The present thesis represents my own work.
List of publications and presentations relevant to this thesis

Publications
Sections of Chapter 1 are adapted from:


Chapter 3 is adapted from:


Chapter 6 is adapted from:


Chapter 7 is adapted from:


Presentations


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I have had help and support from many colleagues at the Institute of Psychiatry and beyond. I would particularly like to acknowledge Professor Robert Plomin for the opportunity to work with the TEDS sample, Dr Fruhling Rijsdijk for your guidance on twin analyses, Dr Jonna Kuntsi for your valuable advice, and ‘Team EEG’ – Caroline, Celeste, Glenn and Ruth – it has been great learning about and applying EEG with you, and I appreciate the proofreading and problem-solving sessions! I am grateful for the support from our collaborators who were extremely helpful in the analyses of the social cognitive tasks: Evelyne Mercure and Mark Johnson, and Marco Battaglia and Ele Bertoletti. A special thanks to Karen Ashwood, Bahare Azadi and Sally Cartwright for your hard work and advice on all aspects of the BioNeD project, and to the TEDS team, Sarah Lewis and Stuart Newman for your administrative and technical support.

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CHAPTER 1: The use of cognitive-electrophysiological parameters in the identification of intermediate phenotypes for ASD and ADHD

The opening chapter of this thesis will first introduce the nature of ADHD and ASD, providing an overview of the key findings regarding diagnosis, epidemiology, theories and underlying aetiology. I will then review the literature surrounding the current understanding of overlap between ADHD and ASD and its basis. Discussion will then shift to the potential of cognitive-electrophysiological markers to provide a marker of genetic risk for ADHD and ASD, which may further overlap or differentiate between ASD, ADHD and co-occurring ASD+ADHD. The chapter concludes by presenting the specific research questions of this thesis.

1.1 Overview of attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD)

1.1.1 Attention deficit hyperactivity disorder (ADHD)

1.1.1.1 Clinical manifestation

It is now 200 years since the first American medical description of ADHD-like behaviour was provided by Benjamin Rush: “One of them was addicted to every kind of mischief. Her wickedness had no intervals while awake, except when she was kept busy in some study or difficult employment. There is probably an original defective organization in those parts of the body which are occupied by the moral faculties of the mind” (Rush, 1812). In 1902, George Still described 43 children who presented with poor “moral control” and noted symptoms including aggressiveness, defiance, over emotionality and cruelty towards others (Still, 1902). Although the concept of ADHD has evolved, these observations were instrumental in determining the characteristics that are still valid today (Taylor, 2011).

Following these early insights, researchers have sought to refine the definition of ADHD to behavioural features that are necessary and sufficient to warrant a diagnosis. Various characteristics have been highlighted as the core feature of the disorder, with early proposals of ‘minimal brain dysfunction’ (Clements and Peters, 1962) and ‘hyperkinetic reaction of childhood’ (APA, 1968). The first formal diagnostic criteria promulgated in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (APA, 1980) were focused on
attentional problems identified as attention deficit disorder (ADD; with or without Hyperactivity). In the current DSM-IV (APA, 1994, 2000), the essential features of ADHD are patterns of inattention and/or hyperactivity-impulsivity (see Table 1-1 for current diagnostic criteria). These symptoms must have been present before age 7 and impairment from symptoms must be present in more than one setting (e.g. at home and at school/work), be chronic (present for at least 6 months), be developmentally inappropriate and maladaptive compared to peers of the same age and sex. There must be clear evidence of interference with social, academic or occupational functioning. In addition, the DSM-IV supports three subtypes of ADHD: (i) predominantly hyperactive-impulsive type, (ii) predominantly inattentive type and (iii) combined type. The two-dimensional structure of ADHD is supported by meta-analyses reporting reliably different associations with a wide range of correlates (Willcutt et al., in press) and distinct genetic inputs for inattention and hyperactivity (Nikolas and Burt, 2010). Twin studies reveal substantial genetic overlap between the dimensions but significant genetic heterogeneity (Greven et al., 2011, McLoughlin et al., 2011b, McLoughlin et al., 2007). The alternative diagnostic system using the current International Classification of Disease (ICD)-10 refers to the signs of ADHD as ‘hyperkinetic disorders’, which when combined with the presence of conduct disorder is ‘hyperkinetic conduct disorder’, characterized by “a repetitive and persistent pattern of dissocial, aggressive, or defiant conduct” (WHO, 2005). The item list in the ICD-10 is essentially the same as that outlined in Table 1-1, although the ICD does not split the diagnosis criteria into two sub-lists (inattention and hyperactivity-impulsivity), and a threshold of 3/5 symptoms of overactivity and 1/4 symptoms of impulsivity is required. Furthermore, the ICD-10 does not support formulation of subtypes within the disorder.
Table 1-1 Current diagnostic criteria for attention deficit hyperactivity disorder, taken from the text-revised edition of the DSM-IV (2000)

(A1) Inattention: six (or more) of the following symptoms persisting for at least 6 months to a degree that is maladaptive and inconsistent with developmental level:

- often fails to give close attention to details or makes careless mistakes in schoolwork, work, or other activities
- often has difficulty sustaining attention in tasks or play activities
- often does not seem to listen when spoken to directly
- often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not due to oppositional behaviour or failure to understand instructions)
- often has difficulty organising tasks and activities
- often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework)
- often loses things necessary for tasks or activities (e.g., toys, school assignments, pencils, books, or tools)
- is often easily distracted by extraneous stimuli
- is often forgetful in daily activities

(A2) Hyperactivity-impulsivity: six (or more) of the following symptoms persisting for at least 6 months to a degree that is maladaptive and inconsistent with developmental level:

Hyperactivity
- often fidgets with hands or feet or squirms in seat
- often leaves seat in classroom or in other situations in which remaining seated is expected
- often runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, may be limited to subjective feelings of restlessness)
- often has difficulty playing or engaging in leisure activities quietly
- is often "on the go" or often acts as if "driven by a motor"
- often talks excessively

Impulsivity
- often blurts out answers before questions have been completed
- often has difficulty awaiting turn
- often interrupts or intrudes on others (e.g., butts into conversations or games)

Other criteria for diagnosis:

(B) Some hyperactive-impulsive or inattentive symptoms that caused impairment were present before age 7 years.
(C) Some impairment from the symptoms is present in two or more settings (e.g., at school [or work] and at home).
(D) There must be clear evidence of clinically significant impairment in social, academic, or occupational functioning.
(E) The symptoms do not occur exclusively during the course of a Pervasive Developmental Disorder, Schizophrenia, or other Psychotic Disorder and are not better accounted for by another mental disorder (e.g., Mood Disorder, Anxiety Disorder, Dissociative Disorder, or Personality Disorder).
Chapter 1: Introduction

1.1.1.2 Epidemiology and development of ADHD

ADHD is considered one of the most common childhood disorders, with an estimated worldwide prevalence of 5% (Polanczyk et al., 2007). The prevalence of ADHD has continued to climb in the past decade (Boyle et al., 2011). Despite some controversy, there are limited prevalence differences by geographic location that would support ADHD as a Western disorder and rates are more likely to differ by the use of different diagnostic tools (Faraone et al., 2003). There are higher rates of ADHD in males compared to females, with ratios ranging from 1.5:1 to 12:1, which may reflect rater bias by parents, teachers and health professionals (Brassett-Harknett and Butler, 2007). The disorder frequently persists into adult life, with approximately 15% of children with ADHD retaining the diagnosis by the age of 25 years and a further 50% showing persistence of some symptoms giving rise to continued impairments (Faraone et al., 2006). Studies have shown that hyperactivity-impulsivity decreases throughout adolescence whereas inattentive symptoms are more persistent (Biederman et al., 2000). The continuity in ADHD symptoms can be attributed to genetic influences (Larsson et al., 2006), although distinct factors appear to contribute to child and persistent ADHD (Franke et al., 2009). During adolescence and into adulthood, ADHD is associated with high levels of comorbidity (Sobanski, 2006); the high probability of irritable aggression and conduct problems means this group of children are likely to go on to show antisocial problems, substance use disorders, unemployment, divorce and a range of interpersonal conflicts (Young et al., 2003). In a recent systematic review of 36 studies, ADHD was robustly related to reduced quality of life, including an impact on family activities, satisfaction with self and threats to achievement and academic achievement (Danckaerts et al., 2009).

1.1.1.3 Categorical versus dimensional approaches to ADHD

Diagnostic classification systems such as those outlined above provide a categorical classification. Most mental disorders are highly heterogeneous and do not have a discrete aetiology. The arbitrary cut-off points may limit the inclusion of less severe cases and shows little sensitivity to individual differences in symptom presentation. It is therefore proposed that mental disorders may better be described as quantitative dimensions (Brown and Barlow, 2005), which reduces the effect of clinical referral bias and increases power for genetic research (Neale et al., 1994). Quantitative genetic studies suggest that ADHD represents the extreme of one or more continuously distributed traits, rather than a distinct categorical disorder (Biederman et al., 1993, Chen et al., 2008, Goodman and Stevenson, 1989, Levy et al., 1997, McLoughlin et al., 2007). For example, high heritability estimates remain across definitions of ADHD as a continuum or disorder ranging from 0.75-0.91 (Levy et al., 1997). The conceptualisation of ADHD symptoms as continuous traits seems to better reflect the
underlying aetiological processes involved, in which risk factors for ADHD influence levels of ADHD symptoms throughout the population (Chen et al., 2008). Overall, quantitative genetic studies support the use of both categorical and quantitative trait locus (QTL) approaches in the investigation of genetic risk factors for ADHD (Plomin et al., 1994) and the underlying neurobiological processes involved (see Section 1.3).

1.1.1.4 Cognitive accounts of ADHD

A number of cognitive theories have been proposed to explain the symptoms observed in ADHD. These are outlined in brief, referring the reader to review papers or sections of this thesis that elaborate if necessary.

1.1.1.4.1 Executive function

Executive function refers to higher-order cognitive processes that are required for adaptive responses to novel situations, governed by the prefrontal cortex. Several theories propose executive function deficits in ADHD. An influential theory suggests that attention deficits are secondary consequences of a core deficit in response inhibition, referring to the ability to inhibit impulsive motor responses to sensory input (Barkley, 1997). This theory has received support from tasks such as the Stop task, in which subjects must respond to a stimulus but inhibit their response if a stop signal is presented (Nichols and Waschbusch, 2004). Nevertheless many tasks involve the execution of other cognitive processes, and subsequent research has shown that a single deficit is unlikely (Kuntsi et al., 2006a). A meta-analysis of studies exploring cognitive dysfunction in ADHD reports poor performance on several tasks, tapping into inhibition, vigilance, working memory and planning (Willcutt et al., 2005). Likewise cognitive-electrophysiological studies do not support a core deficit of response inhibition (Section 1.3.2.3.2).

1.1.1.4.2 State regulation

State regulation theories take into account the energetic factors of the individual (Sanders, 1983). Sergeant’s cognitive energetic model proposes that deficits are the result of the energetic state of the child (Sergeant, 2000). The model, presented in Figure 1-1, outlines three energetic pools that relate to the state and the allocation of energy to tasks. Activation and effort are particularly relevant to performance deficits in ADHD, and activation is suggested to be directly related to motor output. This theory has received support from reports of response variability in ADHD and improvement in task performance in conditions that optimise the child’s energetic state, such as faster event rate or incentives (Section 1.3.2.3.2).
1.1.1.4.3 Multiple pathways

An alternative account suggests that multiple psychological processes are involved in the presentation of ADHD, which can explain the heterogeneity of performance deficits. A number of studies have revealed impairments in reinforcement contingencies and reward processing in ADHD (Sagvolden et al., 1998, Scheres et al., 2001). It should be noted that such findings are not necessarily distinct from state regulation theories, in which individuals with ADHD are in need of stimulation and reinforcement. Expanding on such findings, the dual pathway model proposes that two routes lead to the deficits seen: (1) a motivational style mediated by delay aversion and (2) a core inhibitory dysfunction (Sonuga-Barke, 2002). The delay aversion hypothesis postulates that individuals with ADHD tend to choose small immediate rewards over larger delayed reward (Sonuga-Barke et al., 1992). This theory is supported by the dissociation between inhibition and delay aversion in ADHD (Solanto et al., 2001) and impairments in inhibition in ADHD irrespective of incentives (Shanahan et al., 2008), although electrophysiological markers of inhibition have been shown to normalise with motivational incentives in ADHD, suggesting that these pathways may interact (Groom et al., 2010). Also there are moderate associations between ADHD and performance in either domain, compared to a correct classification of 90% of children with ADHD when the domains are combined (Sonuga-Barke, 2003). More recently the notion of ‘hot’ (incentives and motivation, mediated by paralimbic orbitomedial and ventromedial frontolimbic brain regions) and ‘cool’ (attention, working memory, planning and inhibition mediated by lateral inferior and dorsolateral frontostriatal and frontoparietal networks) executive functions in ADHD has been proposed (Castellanos et al., 2006). Specifically, these authors propose that the inattentive subtype is associated with deficits in ‘cool’ functions and the hyperactive-impulsive subtype with ‘hot’ functions (Castellanos et al. 2006). Interdisciplinary research combining cognitive and brain function is likely to further elucidate whether these processes reflect the same underlying
construct or independent pathways in ADHD. These theories represent the conceptual change
that goes beyond a single cause of ADHD.

1.1.1.5 Genetic studies of ADHD

1.1.1.5.1 Family studies
Family studies investigate resemblance between parents, their offspring and siblings that live
together, that can be attributed to genetic or shared (i.e. family-wide) environmental factors,
referred to as familial influences. First-degree relatives (parents and siblings) who share 50% of
their genes provide greatest power in family studies. Siblings of probands with ADHD (the
affected individual) have a 4-5 fold risk of having the disorder compared to the general
population (Faraone et al., 2000, Willcutt, 2005), with 15-20% of mothers and 25-30% of
fathers reaching clinically significant levels of ADHD (Biederman et al., 1992). Taken together
these findings suggest familial transmission of ADHD. Family studies, however, are unable to
decompose genes and shared family environment.

1.1.1.6.2 Twin studies
Twin studies compare resemblance between identical monozygotic (MZ) twins that share
100% of their genes and non-identical dizygotic (DZ) twins that share 50% of their genes, like
other full siblings. If genetic factors are important, MZ twins would be more similar than DZ
twins. Twin studies are able to decompose familial variance into additive genetic effects and
shared environmental effects, with the remainder attributed to unique environmental effects
(see Section 2.3.6.5., Chapter 2 for further detail). Higher MZ concordance rates than DZ
concordance rates are reported in ADHD, providing evidence for genetic influences (Willcutt,
2005). As the concordance rates are below 1 this suggests environmental factors specific to
individuals, known as unique environmental influences, are also implicated. Meta-analyses
estimate the heritability of ADHD at 60-76% (Faraone et al., 2005, Willcutt, 2005, Wood et al.,
2010c), suggesting that genetic factors can explain a substantial proportion of the variance in
ADHD symptoms. However this estimate did not take into account varying sample sizes, and a
weighted meta-analysis estimates a broad-sense heritability at 70% (Burt, 2009). Most twin
studies show the most parsimonious fit to the data involves additive genetics combined with
unique environmental influences (including measurement error). However, shared
environmental influences (Greven et al., 2011, Wood et al., 2010c) and non-additive dominant
genetic effects (Burt et al., in press, Burt, 2009), such as gene-gene interactions or epistasis,
have also been implicated in the aetiology of ADHD on the basis of twin studies. Estimates are
similar when ADHD is defined by more extreme cut-offs (Larsson et al., 2012), which supports
the dimensional view of the disorder (Section 1.1.1.3). The stability of ADHD symptoms
between 2 and 7 years of age is also attributable to genetic influences (Kuntsi et al., 2005b, Price et al., 2005), while additional genetic influences and unique environmental effects emerge in adolescence (Larsson et al., 2006).

1.1.1.5.3 Molecular genetic studies

Several molecular genetic mechanisms are implicated in the aetiology of ADHD, which are selectively reviewed below, but generally show inconsistent results (Faraone et al., 2005). Findings from candidate gene studies typically implicate genetic variants involved in the regulation of dopamine and related neurotransmitter systems, predicted by the therapeutic effects of psychostimulant medications that increase the amount of synaptic dopamine (Swanson et al., 2007). The most consistent evidence of genetic associations with ADHD are for variants within or near the dopamine D4 and D5 receptor genes (DRD4 and DRD5) (Li et al., 2006), and a recent meta-analysis of dopamine system genes showed a significant association between ADHD and the short allele of DRD5, the TaqI A1 allele of dopamine receptor D2 (DRD2) and the 7-repeat allele of DRD4 (Wu et al., 2012). There are also numerous, yet inconsistent, reports of association with the 10-repeat allele of the dopamine transporter gene (DAT1) (Asherson et al., 2007, Brookes et al., 2008, Gizer et al., 2009, Li et al., 2006, Maher et al., 2002, Purper-Ouakil et al., 2005, Yang et al., 2007). Nevertheless, other studies report the 9-repeat allele as a risk factor for ADHD, suggesting genetic heterogeneity (Das and Mukhopadhyay, 2007). Other neurotransmitter systems are also likely to be involved. For example, serotonin is linked to poor impulse regulation (Lucki, 1998), low platelet and whole blood levels of serotonin have been reported in ADHD (Spivak et al., 1999) and several studies report association between ADHD and the serotonin transporter and serotonin 1B receptor genes (Banaschewski et al., 2010, Gizer et al., 2009). These genes also appear to exert their effects on continuous measures of ADHD in a population-based sample, supporting a QTL approach (Bidwell et al., 2011, Lasky-Su et al., 2008, Mill et al., 2005).

Recent genomewide association scans (GWAS) found no genetic variants that passed genomewide levels of significance, although there was evidence for association in a group analysis of 51 nominated candidate genes (Brookes et al., 2006b, Neale et al., 2008, Neale et al., 2010). A potential novel finding is association with the Cadherin gene (CDH13), which was implicated in more than one GWAS of ADHD associated with total ADHD symptom count and categorical diagnosis (Franke et al., 2009, Lasky-Su et al., 2008, Lesch et al., 2008) and lies within the only region that reached genome-wide significance in a meta-analysis of ADHD linkage studies (Zhou et al., 2008). This finding and other hints from GWAS indicate that genes...
involved in cell division, cell adhesion, neuronal migration and neuronal plasticity may also be implicated in ADHD (Franke et al., 2009).

Large chromosomal abnormalities also appear to increase risk for ADHD; for example, 40-45% individuals with the 22q11 deletion syndrome also have ADHD (Niklasson et al., 2009). Further to this, ADHD has recently been associated with copy number variation (CNV); de novo CNVs have been identified in 1.7% and inherited CNVs in 8% of children with ADHD (Lionel et al., 2011), and the most recent genome-wide analysis reports the rate of rare CNVs as 1.15 times higher in ADHD (Williams et al., 2012). Often these CNVs are identified at loci that have been implicated in other psychiatric disorders, namely schizophrenia and autism (Elia et al., 2010, Grayton et al., in press, Williams et al., 2010). See also Section 1.2.2.2.

1.1.1.6 Environmental studies of ADHD

Numerous psychosocial factors have been associated with ADHD, but represent non-specific predictors that show association with the majority of disorders (Biederman, 2005). Several prenatal factors are implicated in ADHD, and in particular reviews have highlighted the link between prenatal exposure to nicotine and risk of an ADHD diagnosis (Banerjee et al., 2007).

For example, Linnet et al. (2003) identify exposure to nicotine, alcohol, caffeine and psychosocial stress as potential intrauterine influences on ADHD, reporting the most significant associations for exposure to tobacco smoke in utero, with a general 3-fold increased risk (Linnet et al., 2003). Additionally, maternal smoking during pregnancy has withstood meta-analysis with an estimated odds ratio of 2.39 (Langley et al., 2005). This might be confounded by multi-collinearity across maternal lifestyle variables: smoking during pregnancy is more common in women with alcohol use disorder (Knopik et al., 2005). More recent studies demonstrate an increased likelihood of ADHD with paternal smoking in the absence of maternal smoking during pregnancy, thus removing a causal effect of intrauterine environment (Langley et al., 2012).

Accordingly, recent research has highlighted the role of environmental factors as specific triggers or modifiers. Gene-environmental correlations (exposure to a certain environmental factor which depends on the genetics of the individual or their parents) and interactions (genetic influences which exert their effect when the individual is exposed to an environmental risk) may also explain why some candidate genes and environmental factors have no main effect when considered alone (Moffitt et al., 2006, Plomin et al., 1977). For example, ADHD symptoms are associated with the development of drug dependence such as nicotine, due to its stimulant properties (Kollins et al., 2005) and thus maternal smoking may be a mechanism to alleviate inattentiveness (Lerman et al., 2001). In addition, using a novel comparison of
surrogate mothers and biological mothers who smoked during pregnancy, Thapar and colleagues demonstrated that offspring were more likely to develop ADHD if they were biologically related to the mother, supporting an inherited effect (Thapar et al., 2009). In terms of interaction with specific genetic variants, two studies have shown that children with the DAT1 10-repeat allele who were exposed to prenatal smoking have significantly higher hyperactive-impulsive symptom scores (Becker et al., 2008, Kahn et al., 2003). The association between DAT1 and ADHD is also greater if accompanied by exposure to maternal alcohol use during pregnancy (Brookes et al., 2006a). Nevertheless, these interactions are not consistently replicated and further investigation is required, for review see (Nigg et al., 2010).

1.1.2 Autism spectrum disorder (ASD)

1.1.2.1 Clinical manifestation

‘Infantile autism’ was first described by Leo Kanner in 1943 (Kanner, 1943), who observed several children who were characterised by an “inability to relate themselves in the ordinary way to people and situations” (p.242) and “an anxiously obsessive desire for the preservation of sameness” (p.245). In 1944, Hans Asperger identified a pattern of behaviour termed ‘autistic psychopathy’ including “a lack of empathy, little ability to form friendships, one-sided conversation, intense absorption in a special interest, and clumsy movements” (Asperger, 1944).

The first diagnostic criteria in the DSM-III (APA, 1980) reflected the more severe condition of autism with significant delay in language and cognitive skills. Today, autism spectrum disorders (ASDs) describe a range of conditions classified by the ICD-10 (WHO, 2006) and DSM-IV (APA, 2000) as neurodevelopmental disorders, encompassing a range of different diagnoses under the term pervasive developmental disorders (PDD). Within this less severe forms of autism are recognised, such as autism occurring without intellectual disability (known as high-functioning autism; HFA) and separate diagnostic categories. Table 1-2 provides a summary of the diagnostic features of autistic disorder, Asperger’s syndrome (AS) and pervasive developmental disorder-not otherwise specified (PDD-NOS) as defined by the DSM-IV-TR. Difficulties in social interaction, communication and restricted and repetitive behaviours and interests (RRBIs), known as the ‘triad of impairment’, are central to these formulations. Autism is diagnosed if the three core areas of impairment are observed before the age of three years, AS is defined as these same areas of impairment in the absence of language delay or general cognitive impairment, whereas a diagnosis of PDD-NOS would be made if the child shows atypical symptoms or do not meet full criteria for one domain. A diagnosis of HFA is similar to AS, although these children may have experienced language delay and a diagnosis is often
made at different stages of development. These distinctions are under consideration with the upcoming DSM revisions. While ICD-10 criteria for childhood autism are essentially the same and there are high rates of inter-rater reliability between the two diagnostic systems, there are differences in the criteria used. For example, the DSM-IV has a broad category of PDD-NOS, whereas the ICD-10 includes several corresponding diagnoses that do not meet diagnostic thresholds for autism, including atypical autism, other PDD and PDD, unspecified.

**Table 1-2:** Current diagnostic criteria for Pervasive Developmental Disorders (Autistic Disorder, Asperger’s disorder and Pervasive Developmental Disorder – Not Otherwise Specified), taken from the text-revised edition of the DSM-IV (2000)

<table>
<thead>
<tr>
<th>Autistic Disorder</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>(I) A total of six (or more) items from (A), (B), and (C), with at least two from (A), and one each from (B) and (C):</td>
<td></td>
</tr>
<tr>
<td><strong>A. Qualitative impairment in social interaction, as manifested by at least two of the following:</strong></td>
<td></td>
</tr>
<tr>
<td>i) marked impairment in the use of multiple nonverbal behaviours such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction</td>
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<tr>
<td>ii) failure to develop peer relationships appropriate to developmental level</td>
<td></td>
</tr>
<tr>
<td>iii) a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g. by a lack of showing, bringing, or pointing out objects of interest)</td>
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</tr>
<tr>
<td>iv) a lack of social or emotional reciprocity</td>
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</tr>
<tr>
<td><strong>B. Qualitative impairments in communication, as manifested by at least one of the following:</strong></td>
<td></td>
</tr>
<tr>
<td>i) delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)</td>
<td></td>
</tr>
<tr>
<td>ii) in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others</td>
<td></td>
</tr>
<tr>
<td>iii) stereotyped and repetitive use of language or idiosyncratic language</td>
<td></td>
</tr>
<tr>
<td>iv) lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level</td>
<td></td>
</tr>
<tr>
<td><strong>C. Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following:</strong></td>
<td></td>
</tr>
<tr>
<td>i) encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus</td>
<td></td>
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<tr>
<td>ii) apparently inflexible adherence to specific, non-functional routines or rituals</td>
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</tr>
<tr>
<td>iii) stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting, or complex whole-body movements)</td>
<td></td>
</tr>
</tbody>
</table>
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Table 1-2 (continued)

<table>
<thead>
<tr>
<th>iv) persistent preoccupation with parts of objects</th>
</tr>
</thead>
<tbody>
<tr>
<td>(II) Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years: A) social interaction, (B) language as used in social communication, or (C) symbolic or imaginative play.</td>
</tr>
<tr>
<td>III) The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder</td>
</tr>
</tbody>
</table>

Asperger's Disorder (AS)

See Sections A and C for Autistic Disorder, plus:

i) The disturbance causes clinically significant impairments in social, occupational, or other important areas of functioning.

ii) There is no clinically significant general delay in language (e.g. single words used by age 2 years, communicative phrases used by age 3 years)

iii) There is no clinically significant delay in cognitive development or in the development of age-appropriate self help skills, adaptive behaviour (other than in social interaction) and curiosity about the environment in childhood

iv) Criteria are not met for another specific Pervasive Developmental Disorder or Schizophrenia.

Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS)

This category should be used when there is a severe and pervasive impairment in the development of reciprocal social interaction or verbal and nonverbal communication skills, or when stereotyped behaviour, interests, and activities are present, but the criteria are not met for a specific Pervasive Developmental Disorder, Schizophrenia, Schizotypal Personality Disorder, or Avoidant Personality Disorder. For example, this category includes 'atypical autism' - presentations that do not meet the criteria for autistic disorder because of late age of onset, atypical symptomatology, or subthreshold symptomatology, or all of these.

1.1.2.2 Epidemiology of ASD

A recent systematic review of epidemiological surveys of ASD reports a median prevalence of 10/10,000 for autistic disorder and 62/10,000 for PDD overall in Europe (Elsabbagh et al., 2012a). In terms of worldwide prevalence, studies converge to show a median prevalence of 17/10,000 for autistic disorder and 62/10,000 for PDD, although the authors note considerable variability across countries (Elsabbagh et al., 2012a). Males consistently outnumber females in the vast majority of studies, with the ratio ranging from 1.33 to 16.0 for autistic disorder and 3.3 to 15.7 for PDD (Elsabbagh et al., 2012a). Diagnostic stability estimates range from 69-95% from 2 to 9 years of age (Charman et al., 2005, Lord et al., 2006). Despite the pervasiveness of autism symptoms, their presentation can change with development and sometimes decrease with age (Piven et al., 1996). In a follow up of children diagnosed at a mean age of 7 years, the majority of individuals at a mean age of 29 years were dependent on family or other support,
and were rated as having a ‘poor’ or ‘very poor’ outcome. Few lived independently, had close friends or permanent employment, potentially reflecting lower IQ (Howlin et al., 2004).

1.1.2.3 Categorical versus dimensional approaches to ASD
Similar to ADHD, the conceptualization of ASD has extended to a dimension through study of autistic-like traits (ALTs) in the general population. Community twin studies of autistic-like traits reveal high heritability (60-90%) using parent- and teacher-rated rating scale measures (Constantino and Todd, 2000, Robinson et al., 2012, Ronald et al., 2006a, Skuse et al., 2005) and more moderate heritability (36-57%) for self-report measures (Hoekstra et al., 2007, Ronald et al., 2008a). A modest effect of unique environment and, in some studies, modest shared environmental measures play a role in variation of ALTs (Constantino and Todd, 2003). Similar heritability estimates are reported for ALTs at the extreme and as a continuum (Lundstrom et al., 2012, Ronald et al., 2008a), and at the extreme there is greater clustering of the three impairments than throughout the continuum (Robinson et al., 2012, Ronald et al., 2006a, Ronald et al., 2006b, Ronald and Hoekstra, 2011). ALTs are also heightened in the relatives of ASD probands, termed the ‘Broader Autism Phentotype’ (Bailey et al., 1998, Bolton et al., 1994). Broader autism phenotype (BAP) rates in siblings compared to controls are manifested in an increased rate of less severe cognitive-communication abnormalities combined with social impairments or stereotyped behavior (Sucksmith et al., 2011). Taken together, these findings indicate that autism and ALTs lie on a continuum of impairment, whereby there is a combination of traits at the extreme. Such research has also tested the proposition that the autistic triad may be ‘fractionable’ at various levels of analysis, rather than explained by a single dimension (Happé and Ronald, 2008). This suggests that the underlying genes that are responsible for the impairments seen in ASD are also for common variation in social impairment.

1.1.2.4 Cognitive correlates of ASD
A number of cognitive theories have been proposed to explain the behavioural symptoms observed in ASD. These are outlined in brief, pointing the reader to review papers.

1.1.2.3.1 Theory of Mind
Lack of pretend play or difficulties in understanding non-literal language (e.g. irony) observed in ASD can be taken as a deficit in Theory of Mind (ToM), which refers to the ability to represent the mental states (beliefs, desires, intentions) of others, making it difficult for individuals with ASD to understand and predict other’s behaviour (Baron-Cohen et al., 1985). Support for this theory comes from findings using the false-belief task, involving a narrative in which a character has a belief incongruous with reality, which therefore requires the subject to
attribute a false belief. Several studies have shown worse performance in individuals with ASD, although older children do pass the task (Hamilton, 2009, Happé, 1995). However, the ToM account has had less success in explaining the non-social deficits of ASD.

1.1.2.3.2 Executive function

An influential theory of ASD suggests both social and non-social symptoms of ASD may arise from deficits in executive function (Pennington and Ozonoff, 1996). These authors suggest that many of the tests of ToM are confounded by executive function. Individuals with ASD show particular deficits in tasks of planning, mental flexibility and generativity (the ability to create novel ideas), with mixed findings for response inhibition and self-monitoring impairments (Hill, 2004). Communication difficulties may reflect a lack of cognitive flexibility, whereas RRBIs may reflect enhanced perseveration (see Hill 2004 for review). A limitation is the lack of specificity to ASD, as executive dysfunction is characteristic of ADHD (Section 1.3.2.3.2).

1.1.2.3.3 Weak central coherence

The weak central coherence account of autism refers to reduced processing of global information, meaning and context and a focus on local details and parts of objects (Frith, 1989). Individuals with autism perform well on tasks that require the detection of small details with a larger context at speed, such as the Embedded Figures Task (Happé and Frith, 2006). Weak central coherence is referred to as a cognitive style rather than a cognitive impairment, such that only in the presence of other deficits, such as ToM, would weak central coherence result in ASD impairment (Happé, 1999).

1.1.2.5 Genetic studies of ASD

1.1.2.5.1 Family studies

Family studies of ASD find between a moderate number of siblings of affected probands demonstrated ASD. Categorical estimates of sibling recurrence risk estimate a 22-fold risk of developing autism and 13-fold for AS and other PDDs, which are roughly twice as high if the mother was also diagnosed (Lauritsen et al., 2005). Recent studies suggest 10.9% siblings of children with ASD reach criteria for traditionally defined ASD (Constantino et al., 2010), that is approximately the rate shown in half-siblings (Constantino et al., in press), and 18.7% of infants with an older sibling proband go on to develop ASD (Ozonoff et al., 2011). In addition, as outlined above, high rates of subthreshold characteristics of ASD are found in siblings of affected individuals, suggesting different features of autism can be inherited in distinct patterns (Section 1.1.2.3).
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1.1.2.5.2 Twin studies

In terms of studies using strictly defined autism criteria, MZ concordance rates range between 36-96% and are higher than DZ concordance rates between 0-31% (Bailey et al., 1995, Folstein and Rutter, 1977, Ritvo et al., 1985, Rosenberg et al., 2009, Steffenburg et al., 1989). Heritability estimates range between 80-93% (Bailey et al., 1995, Lichtenstein et al., 2010). Such findings suggest autism is unlikely to be a single gene disorder, supported by the high MZ and low DZ concordance rates, which further indicate non-additive genetic effects such as possible gene-gene interaction. Moreover, as MZ concordance is not 100% unique environmental influences or stochastic processes are likely to play a role. More recently a lower estimate of 37% was reported, combined with 55% shared environmental effects (Hallmayer et al., 2011). The role of the shared environment is further questioned given the concordance rate for strict autism in DZ twins is not different from infant sibling recurrence rates (Geschwind, 2011). Differences between heritability estimates combined with propositions that previous estimates may be ‘too high’, might be explained by other genetic differences, such as copy number variation (see following section) and epigenetic mechanisms (Anderson, 2012).

1.1.2.5.3 Molecular genetic studies

About 10-20% of cases with autism have origins attributable to known genetic causes (Geschwind, 2011). Early linkage studies revealed the locus containing FMR1 implicated in the fragile X syndrome, MECP2 in Rett syndrome, and TSC1/TSC2 in tuberous sclerosis were associated with the diagnosis of autism in these individuals (see Geschwind 2011 for review). Each of these single gene disorders account for no more than 1% of ASD cases on average (Abrahams and Geschwind, 2008), and not all individuals with these diseases also display ASD symptomatology suggesting the role of other genetic or environmental risk factors.

Molecular genetic studies of the remaining ~90% of ‘idiopathic’ cases of autism with no clearly defined origin have had few successes in identifying the genes involved (Freitag et al., 2010). Several genome-wide linkage studies have been conducted, but findings are generally in several genomic locations indicating genetic heterogeneity (Freitag, 2006). A number of candidate genes have been associated with ASD, for which various reviews exist and more recently an extensive database (Abrahams and Geschwind, 2008, Freitag et al., 2010, Xu et al., 2012). Notable consistent findings that also bear relevance to this thesis include the Contactin Associated Protein 2 (CNTNAP2), which has been associated with executive function and the development of joint attention (Alarcon et al., 2008, Arking et al., 2008), and the engrailed 2 (EN2) gene, linked with the development of the midbrain and the cerebellum (Benayed et al.,
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2005, Brune et al., 2008, Wang et al., 2008). The serotonin transporter gene (SLC6A4/5-HTT) has received attention due to the role of serotonin in many psychological processes, including social behaviour; the short allele of the SHTT-LPR is a candidate gene for ASD (Freitag, 2006, Muhle et al., 2004), which decreases serotonin reuptake activity, also demonstrated in autism (Huang and Santangelo, 2008), although other findings are reported (Devlin et al., 2005). Similarly the oxytocin receptor gene (OXTR) has been implicated in social cognition and ASD (Jacob et al., 2007, Lerer et al., 2007). In addition, the first GWAS of ASD identified six genetic markers in a region that covers two Cadherin genes, CDH9 and CDH10, which have since been replicated (Ma et al., 2009, St Pourcain et al., 2010, Wang et al., 2009a). Other variants identified in GWAS have not been replicated (Devlin and Scherer, 2012), supporting the large genetic heterogeneity in ASD.

More recent studies highlight the role of rare mutations in ASD, and the high MZ concordance rate compared to low DZ concordance rate is indicative of de novo events. An estimated 1.19 fold increase in risk of carrying rare CNV is reported in individuals with ASD (Pinto et al., 2010), but these are often found in other diseases such as intellectual disability and schizophrenia (Grayton et al., in press, Guilmatre et al., 2009). See also Section 1.2.2.2. Analyses of biological networks or pathways implicated in ASD revealed the highest enrichment for rare and common variants in ASD are involved in synaptic and neuronal plasticity (Ben-David and Shifman, 2012), supporting the interplay of these (and other) mechanisms in the aetiology of ASD.

1.1.2.6 Environmental studies of ASD

A relative increase in interest toward the role of the environment in ASD has in part emerged due to realised rise in prevalence, such as the role of increased environmental exposure to neurotoxicants in the development of ASD and other neurodevelopmental disorders (Landrigan, 2010), and links with the MMR vaccine, which have not been supported (Nelson and Bauman, 2003). Nevertheless the effect of evolving diagnostic and assessment practices and other artifacts are not taken into account (Lawler et al., 2004). There have been a number of studies reporting the role of the prenatal environment on ASD risk. A review and subsequent meta-analysis converge to suggest advanced parental age at birth, along with reduced birth weight, order of birth, intrapartum hypoxia and duration of gestation are significant risk factors (Gardener et al., 2011, Kolevzon et al., 2007). In line with this, a number of studies report increased rates of subsequent ASD diagnosis in children born post-term and preterm (Movsas and Paneth, 2012), the latter of which itself is independently associated with cognitive impairment (Johnson et al., 2011). It is suggested that these early physical and
psychosocial factors play a role in disordered early brain development (Johnson et al., 2011), and preterm birth is associated with a number of disorders (see also Section 1.2.2.3). Similarly, a link between antidepressant use (SSRI) during pregnancy and increased risk of autism has been proposed that may further implicate serotonergic function or physiological changes operating directly on the developing brain, although further investigation is required due to the small number of participants exposed to SSRIs prenatally (Croen et al., 2011). These factors have not been identified conclusively to date, although it is likely that they impact interpretation of twin studies and therefore may act as mediators (Ronald and Hoekstra, 2011). For example, non-causal perinatal complications may result from causal genetic factors (pleitropic shared effects creating a gene-environment correlation), as supported by an association between obstetric complications and measures of autism severity, and a substantial correlation between obstetric adversity in probands and their unaffected siblings (Bolton et al., 1997).

1.1.3 Conclusions
According to the DSM-IV criteria outlined above, there is little overlap in the characteristics of ADHD and ASD, and the diagnosis of PDD continues to remain in the exclusionary criteria for ADHD (APA 2000). Nevertheless, drawing on the clinical characteristics and causes of the disorders it is possible to identify similarities. Both disorders are common (more so in ADHD), more prevalent in males than females, begin early in life (younger for ASD), show strong persistence over time, and range in severity supporting a continuum of impairment. Both are highly heritable and likely to reflect multigenetic inheritance, although multiplicative inheritance is indicated for ASD compared to additive gene action in ADHD. While specific genetic mechanisms have been associated with risk for ASD and for ADHD, findings are inconsistent due to the genetically heterogeneous nature of these disorders and complex interactions between genetic and environmental factors. More recent studies have examined the co-occurrence of these disorders and the aetiological basis of the overlap, discussed in the following section.

1.2 Comorbidity between ADHD and ASD and underlying aetiology

1.2.1 Co-occurrence of disorders and correlation in traits
In the study of comorbidity, it is firstly important whether the rate of comorbidity exceeds that expected of chance alone (Caron and Rutter, 1991). Epidemiological surveys can be used to calculate the expected rate (by multiplying the prevalence of each of the conditions i.e. ASD and ADHD). There are several methods of calculating this. Firstly, one can take the reported
prevalence of ASD and the reported prevalence of ADHD, albeit in separate studies. Epidemiological surveys presented above suggest an estimated world-wide prevalence of 5.3% in ADHD (Polanczyk et al., 2007), and 0.2% for autistic disorder and 0.6% for PDD (Elsabbagh et al. 2012a). The comorbidity of ADHD+autistic disorder expected by chance is 0.01%, and of ADHD+PDD by chance is 0.03%. As presented below, the number of individuals with clinically significant levels of ASD in ADHD (and vice versa) is much higher than the prevalence expected by chance and indeed that of ASD and ADHD alone, thus precluding a co-occurrence of the disorders purely by chance (Taurines et al., 2012).

A neater design would assess the prevalence of ASD, ADHD and ASD+ADHD within the same epidemiological survey. A design of this kind requires a large sample size. The most relevant study to this design is the British Child and Adolescent Mental Health Survey (BCAMHS). (Meltzer et al., 2000). The update to this was conducted in 2004 on 7,977 families drawn from child benefit records who completed interviews evaluating ICD-10 diagnoses (Green et al. 2005). The prevalence of hyperkinetic disorder was 1.5% across all children studied, with a greater prevalence in boys (2.6%) than girls (0.4%). The prevalence of ASD was 0.9% across all children, with a greater prevalence in boys (1.4%) than girls (0.3%). The prevalence of ASD+ADHD expected by chance would therefore be 0.01% across all children and 0.03% for boys only. While there was no data on comorbid ICD-10 diagnoses with ASD, across the 1999 and 2004 surveys, 2% of the children with ADHD had a comorbid ‘less common disorder’ (a category in which ASD has the greatest prevalence: tics disorder 0.0%, eating disorder 0.3% and mutism 0.1%) compared to 1% of those without ADHD and 1% in Great Britain as a whole. Moreover, 42% of the children with ASD had high parent ratings of hyperactivity, compared to 3% of those without ASD and 4% in Great Britain as a whole. In addition, again the prevalence of ASD+ADHD expected by chance is much lower than the rates reported below.

Numerous empirical studies that have directly investigated rates of ASD in ADHD and vice versa now exist using clinical samples. There are far more studies of ADHD symptoms in ASD cohorts, which may reflect the ASD diagnosis, in effect, subsuming any secondary diagnoses at first due to its higher level of severity. Higher rates of ADHD are found in younger children with ASD compared to adolescents (Ghaziuddin et al., 1998, Mukaddes and Fateh, 2010) and in PDD-NOS (Frazier et al., 2001, Hofvander et al., 2009, Yoshida and Uchiyama, 2004), although one study reports higher rates in autistic disorder (Lee and Ousley, 2006). Individuals with ASD tend to have combined type or predominantly-inattentive type of ADHD (Goldstein and Schwebach, 2004, Lee and Ousley, 2006, Leyfer et al., 2006, Sturm et al., 2004, Yoshida and Uchiyama, 2004), although one study reports higher rates for hyperactive-impulsive (Ponde et
In addition, significantly higher rates of the combined-type have been found in AS compared to HFA (Mukaddes et al., 2010). Significantly higher rates of ADHD are found in ASD compared to children with intellectual disability (Brereton et al., 2006), and PDD children are more likely to have ADHD in combination with tic disorders than ADHD alone (Gadow et al., 2004). Children with ADHD are most likely to show clinically significant impairments in social functioning and the least impairment in RRBIs (Clark et al., 1999, Kochhar et al., 2011, Santosh and Mijovic, 2004). In studies that include typically developing control subjects, subjects with ASD demonstrate significantly more ADHD symptoms (Abdallah et al., 2011, Hattori et al., 2006, Kochhar et al., 2011, Ooi et al., 2011), although no significant differences are demonstrated compared to clinical controls (Joshi et al., 2010). In direct comparisons across disorders, ASD subjects appear to display significantly elevated scores on ASD rating scales compared to ADHD, whereas similar scores are reported for ADHD rating scales across the disorders (Frazier et al., 2001, Goldstein and Schwebach, 2004, Hattori et al., 2006, Ooi et al., 2011). Associations between particular symptoms of each disorder reveal a significant correlation between hyperactivity-impulsivity and communication difficulties, and between inattentiveness and RRBIs (Sinzig et al., 2009).

Taken together, the findings suggest that between 7-83% of individuals with ASD meet diagnostic thresholds for ADHD, and between 9-86% of individuals with ADHD meet thresholds for ASD. Reasons for large variability in rates of overlap are likely to reflect differences in age, subtypes, gender and the presence of other psychiatric and medical disorders. For example, Sturm and colleagues included comorbid children (ASD+ADHD) in their assessment of ADHD symptoms in ASD individuals, which will bias findings (Sturm et al., 2004). In addition, rates vary on the basis of differing assessment tools, which might in turn alter inclusion in diagnostic groups (Kochhar et al., 2011).

Comorbidity estimates may also be inflated by less obvious similarities in the defining features of the disorders, such as inattention and hyperactivity in ASD. For example, in the Childhood Autism Rating Scale one criterion notes “persistent and forceful attempts are necessary to get the child’s attention at times”, compared to the Conners ADHD rating scale item “distractibility or attention-span problems”. Children with ASD have idiosyncratic attention-inattention patterns; they are able to attend to a stimulus that they find interesting but in more cognitively demanding activities impaired attention becomes more obvious, which causes confusion and often makes it difficult to reach DSM criteria (Clark et al., 1999, Dawson and Lewy, 1989). Similarly in terms of social impairment, criteria for ASD describe a difficulty mixing with other children, while in ADHD behaviours such as difficulty awaiting turn in games or activities or
disrupting others with talks or actions are defined. Despite this resemblance, social impairment in ASD is often thought to reflect a lack of interest/ability for social interaction, whereas children with ADHD are proposed to show impulsive disinhibited social approaches (Kennedy, 2002). This is supported by findings of peer or social rejection in ADHD (Gentschel and McLaughlin, 2000).

There is also possibility of an artifactual comorbidity as a result of Berkson’s bias; subjects with more than one disorder are likely to be part of a clinical sample, and those with more profound impairments are more likely to receive treatment (Banaschewski et al., 2007). It is therefore necessary to go beyond clinical data, although population data are much scarcer. One of the population studies available utilized a twin study and confirms clinical findings of greater rates of ASD in ADHD combined-type with the least for the hyperactive-impulsive subtype (Reiersen et al., 2007). A further study administered ADHD rating scales to ASD subjects that were selected on the basis of having a clinical diagnosis of ASD or a statement of special educational needs, revealing 28.1% of these children met criteria for concurrent ADHD (Simonoff et al., 2008). Likewise, in a sample of children with intellectual disability (ID) with and without autism, individuals with autism+ID were significantly more likely to meet diagnostic thresholds for ADHD or hyperkinetic disorder, and more likely to meet criteria for impulsivity and hyperactivity than inattention (Bradley and Isaacs, 2006).

The findings to date converge to suggest there is substantial clinical overlap, as well as overlap in the traits associated with the disorders, which has informed the debate of whether it is necessary and beneficial to maintain the distinction in diagnostic criteria. The most recent NICE guideline for ADHD in 2008 recommends that ADHD can be diagnosed in the presence of autism spectrum disorders, based on the strong evidence that they co-occur (Atkinson and Hollis, 2008). In addition, it is proposed in the upcoming fifth edition of the DSM, due to be released in 2013, that the exclusion criteria for PDD are removed from the ADHD criteria (www.dsm5.org). Beyond clinical implications, it is also likely that this overlap can provide clues to the aetiology of these highly complex disorders, such as the identification of pleiotropic genes (Section 1.2.2.2), shared early environmental triggers (Section 1.2.2.3) and/or common intermediate phenotypes (Section 1.3), which in turn provide insight into the basis of the overlap and whether it reflects a “true” comorbidity (Section 1.2.3).
1.2.2 Potential causal mechanisms of overlap between ASD and ADHD

1.2.2.1 Quantitative genetic studies

A number of quantitative genetic studies have examined the aetiology of the overlap between ASD and ADHD. These studies include family studies (sibling and parent) and twin studies. Firstly these studies confirm substantial overlap between ASD and ADHD traits. Correlations appear to be higher for teacher ratings (Kanne et al., 2009), suggesting some situational/context differences. Particularly in ASD children, there were no correlations across parent and teacher ratings between Child Behaviour Checklist ADHD scores and Social Reciprocity Scale (autistic trait) scores. Nevertheless similar correlations across parent and teacher ratings have been reported (0.51-0.54) suggesting this varies across assessment measures (Ronald et al., 2008b). Correlations also appear to vary by age with more modest estimates in infancy (Ronald et al., 2010a).

In terms of familial overlap in sibling studies, 43% of influence on ASD rating scales is attributable to scores on ADHD rating scales (Constantino et al. 2003) and ADHD siblings appear to show intermediate rates of ASD compared to controls, with a familial overlap of 56% in males (Mulligan et al., 2009). However another study found ASD symptoms were independent from ADHD traits in an ADHD sample, with no evidence for familial transmission (Nijmeijer et al., 2008). The authors suggest this may reflect the clinically referred sample compared to general population sample (Nijmeijer et al. 2008). In ASD samples, there are higher rates of ADHD symptoms in siblings of ASD probands (Kanne et al., 2009). Siblings show increased rates of ASD and ADHD; 15.6% have ASD-only, 9.4% have ADHD-only and 5.4% have comorbid ASD+ADHD, suggesting that ADHD and ASD show familial transmission and familial aggregation (Kanne et al., 2009). In a recent study on ADHD symptoms in parents of one or more child with ASD, 4% of mothers and fathers scored above threshold for inattention or hyperactivity-impulsivity on an ADHD rating scale, and within these parents scores on ASD and ADHD rating scales correlated at 0.28-0.48 (Van Steijn et al., in press). In addition, paternal inattention predicted daughter’s ASD symptoms whereas parental ASD symptoms did not predict offspring ADHD symptoms. The authors suggest that there is greater risk for ASD overlapping with ADHD, than ADHD overlapping with ASD (Van Steijn et al., in press). Twin studies suggest a moderate to substantial genetic correlation between ASD and ADHD traits (0.54-0.87), varying on the basis of age, informant, rating scale and gender (Lichtenstein et al., 2010, Lundstrom et al., 2011, Reiersen et al., 2008, Ronald et al., 2010a, Ronald et al., 2008a)
1.2.2.2 Molecular genetic studies

Based on the premise that there is genetic overlap between ADHD and ASD, various molecular genetic investigations have been reported to identify the specific variants that may underlie this. The following provides a brief review of the more pertinent findings in direct comparisons of the disorders. For a more detailed review, please see Rommelse and colleagues (Rommelse et al., 2009b).

In terms of candidate gene studies, the majority have focused on the dopaminergic system implicated in ADHD. The 10-repeat allele of DAT1 is associated with lower parent-rated hyperactive-impulsive symptoms compared to the 9-repeat allele in a sample of children with ASD (Gadow et al., 2008). This contrasts to findings in ADHD in which the 10-repeat allele is the risk variant, and therefore may reflect genetic heterogeneity reported in ADHD (Section 1.1.1.5). The 7-repeat allele of the DRD4 gene has been associated with a high score on the Social Reciprocity Scale, a measure of autistic features, in a sample of ADHD (Reiersen and Todorov, 2011), although the same risk variant is not associated with ASD (Grady et al., 2005).

In a larger study of ASD subjects, ADHD subjects and typically developing controls, the DRD3 gene reached the significant threshold for association with ASD (de Krom et al., 2009). There was, however, no shared genetic association between ASD and ADHD. In addition, the same risk allele has been associated with a specific type of RRBIs, insistence on sameness (Staal et al., 2012). In a comparison of ASD, ADHD and controls, there was reduced expression of DRD4 messenger ribonucleic acid (mRNA) in ASD and ADHD children, and DRD5 expression was reduced in ASD compared to ADHD and controls, although a large proportion (n=19/26) of these cases also had ADHD (Taurines et al., 2010a). Finally, in a study investigating the role of ASD candidate genes in ADHD, subjects with ASD could be differentiated from ADHD by SHTTLPR and polymorphisms on TPH2 and CNTNAP2 (Sizoo et al., 2010). Specifically, ADHD subjects showed a greater rate of the T-allele on the CNTNAP2 polymorphism compared to the ASD group, whereas carriers of the G-allele on TPH2 and the long allele of SHTTLPR predicted risk for ASD. The role of serotonergic dysfunction in these disorders has been reviewed in full (Sinzig and Lehmkuhl, 2007) and outlined in Sections 1.1.1.5 and 1.1.2.5.

A genome-wide linkage study on a large ADHD-sibling pair sample revealed suggestive linkage with scores on the Social Communication Questionnaire, a parental measure of ASD features, and 15q24 (communication scale), 16p13 (RRBI scale) and 18p11 (RRBI scale), and additionally on 7q36 (RRBI scale) and 12q24 (total scale) when scores on the Conners ADHD rating scale were included as a covariate, suggesting specific ASD effects (Nijmeijer et al., 2010a). The association with 15q, however, decreased which may suggest pleiotropic effects (Nijmeijer et
al. 2010). Taken together, GWAS studies suggest potential overlap in regions reaching significance in 16 SNPs. For example, the cadherin gene, CDH13, which has been implicated in more than one GWAS of ADHD (Section 1.1.1.5), also shows CNV that is potentially related to autism (Christian et al., 2008).

Finally, larger chromosomal alterations and CNV may play a more important role in the overlap between ASD and ADHD. Firstly, both ASD and ADHD are found at a higher rate in various disorders caused by single chromosomal abnormalities. A mutation of the FMR1 gene on the X-chromosome results in Fragile X syndrome, and has been associated with the clinical diagnosis of ASD and ADHD (Farzin et al., 2006). Deletions and duplications on chromosome 15q13.2-q13.3 result in a phenotype related to ASD and ADHD (Miller et al., 2009). In children with a deletion of 22q11, containing the catechol-O-methyltransferase (COMT) gene involved in the transmission of catecholamines including dopamine, 44% reached diagnostic criteria for at least one of the disorders (Niklasson et al. 2009). In addition CNV studies have revealed several potential lines of investigation. A study of patients with various neurodevelopmental disorders reports deletions and duplications at chromosome 16p13.11 region, associated with clinical features of autism (4 of 8 subjects), ADHD (2 of 8 subjects) and other behavioural/neurological abnormalities (Ramalingam et al., 2011). Duplications in this region have previously been associated with autism (Ullmann et al., 2007) and ADHD (Smalley et al., 2002, Williams et al., 2010). In addition, the gene encoding for Tuberous Sclerosis, TSC2, Is located near this region, which is associated with both ASD and ADHD symptoms (Smalley, 1998). A further study reports that in a sample of ADHD patients and their parents, the CNV-associated gene set was enriched for CNTNAP2, which has been implicated in ASD (Elia et al., 2010).

The findings converge to suggest there are potential effects of pleiotropic genes, particularly larger chromosomal abnormalities, which supports the proposition of similar pathogenic mechanisms underlying the co-occurrence of ASD and ADHD. Still there are some effects that appear to differentiate between the pure disorders. Nevertheless, high variability in the phenotypic presentations associated with candidate genes, CNVs and environmental exposure, suggests that it is unlikely that genetic variants exert specific phenotypic effects. In addition, the phenotypes of both disorders are further convoluted by the presence of other comorbid disorders. Further investigation of neurobiological mechanisms underlying dopamine and serotonin neurotransmission, or neuronal/synaptic plasticity, is required.

1.2.2.3 Environmental factors

Although genetic factors account for a substantial proportion of the overlap between ASD and ADHD, the genetic correlations are below 1, suggesting environmental factors may play a role.
In a study of ASD and ADHD traits in the longitudinal ALSPAC sample, various developmental trajectories were identified, showing that autistic symptoms were more stable over time, and that children with persistent hyperactive-impulsive traits (i.e. that persisted into adulthood) also showed persistent social-communication impairments, but not vice versa. These jointly modelled trajectories of persistent impairment were particularly associated with maternal smoking during pregnancy and maternal age below 20 years (St. Pourcain et al., 2011). A greater risk for ADHD (11.5% versus 2.9% in controls), with increased risk for predominantly inattentive subtype, and ASD (8% versus 0%) was reported in a sample of babies born preterm and assessed at 11 years of age (Johnson et al., 2010). In another study, symptoms of ASD in ADHD were not related to pre- or peri-natal factors (Kröger et al., 2011), which may be explained by stringent exclusion criteria in this study. In addition, prenatal maternal stress (such as parental divorce) was modestly associated with ASD and ADHD traits (Ronald et al., 2010b). Alternatively, these factors may act as mediators; in a sample of children with ADHD, while no main environmental effects were reported, an interaction between maternal smoking during pregnancy and the COMT val/val genotype on increased stereotyped behaviour and with the short allele on 5HTTLPR on increased problems in social interaction were demonstrated. In addition, the short/short genotype interacted with low birth weight to increase rigid behaviour (Nijmeijer et al., 2010b). However, it might be possible that the nature of social interaction problems in ADHD is due to environmental risk factors and different from the mainly genetically mediated social interaction problems in children with ASD, which should be explicitly tested (see also Santosh and Mijovic, 2004).

1.2.3 Conclusions and implications for models of psychiatric comorbidity

Above I reviewed empirical studies examining the overlap between ASD and ADHD, and the risk factors that may underlie this. The co-occurrence of disorders purely by chance is precluded by evidence from epidemiological studies, and it is unlikely that nosological issues (such as Berkson's bias) play a major role. Nevertheless, 'true' comorbidity raises several important issues. Determining the explanation for overlap between the conditions firstly entails examination of the extent to which risk factors are shared or correlate with ASD because of comorbid ADHD (or vice versa). Quantitative genetic studies appear to support a model in which both disorders share common underlying aetiological factors, as an increased incidence of ASD is observed in non-affected relatives of ADHD patients and vice versa. In addition, molecular genetic studies present preliminary evidence for potential shared (pleiotropic) genes underlying the co-occurrence of ASD and ADHD, and this familial/genetic overlap. In this conceptualisation, ASD and ADHD are differing expressions of the same underlying aetiology. Nevertheless, the different risk factor patterns presented support
different models of comorbidity between ASD and ADHD, and as such it is likely that in some individuals comorbidity is caused by overlapping genetic (e.g. CNV) or environmental (e.g. preterm birth) risk factors, whereas in others a combination of disorder-specific risk factors causes comorbidity (e.g. prenatal smoking for ADHD plus 5HTTLPR risk variant for ASD; Taurines et al. in press). Therefore, despite several studies reporting high rates of clinical and aetiological overlap between ASD and ADHD, the interaction between these conditions is still not well understood.

The use of the term ‘comorbidity’ to describe the current understanding of ASD+ADHD is consequently largely incorrect, as it is mostly unknown whether the presence of both diagnoses in one child reflects distinct clinical entities or refers to different manifestations of a single clinical entity (Maj, 2005). It is therefore necessary to stratify groups and compare underlying risk factors in the comorbid group to pure disorders (ASD-only vs. ADHD-only vs. ASD+ADHD). The next essential step that is taken in this thesis is to investigate whether associated features (e.g. intermediate phenotypes) differentiate between conditions, in order to determine whether the comorbid condition differs from the simple additive combination of the deficits or pathophysiology associated with ASD and ADHD when they occur alone (Banaschewski et al., 2007).

Several conceptualisations of comorbidity may apply (shown in Figure), which provide predictions for shared and/or distinct underlying pathophysiological mechanisms: (1) an ‘additive’ model, whereby the pure disorders can be differentiated from each other but when the comorbid condition is considered the manifestations converge, such that the unique features of the pure disorders are observed; (2) A ‘common aetiology’ model predicts that ASD, ADHD and ASD+ADHD would demonstrate the same pathophysiology; (3) a ‘symptomatic phenocopy’ model assumes that the comorbid condition is a symptomatic phenocopy of ADHD, such that it presents with the same behavioural manifestation, but the underlying pathophysiology is similar to ASD and not ADHD (or vice versa); (4) an ‘independent nosology’ model suggests that the three conditions are separate from each other, such that each displays its own unique deficits with no overlap in pathophysiological mechanisms (Banaschewski et al., 2007). There have been no studies showing whether the risk for ASD+ADHD is transferred separately from the risks of ASD or ADHD, but there are a handful of studies investigating cognitive/brain markers as intermediate phenotypes of the disorders. The potential role of these objective markers in evaluating the aetiology of ASD and ADHD and clinical implications for the comorbid condition are discussed in the following section.
Figure 1-2: Models of comorbidity between ASD and ADHD that can be assessed in this thesis

1.3 Candidate intermediate phenotypes of ASD and ADHD

1.3.1 Intermediate phenotypes: definitions and application

One approach to the investigation of pathophysiology is to examine intermediate phenotypes of the disorders. The search for intermediate phenotypes (or endophenotypes) of psychiatric disorders was largely conceived to address inconsistencies in molecular genetic research. Indeed, despite some advances in the identification of genetic mechanisms underlying the disorders and those that may account for the overlap between them, ADHD and ASD are both complex disorders that are likely to be affected by a combination of factors, such as genetic heterogeneity, poorly defined phenotypes (which itself implicates clinical overlap) and gene-
environment interactions. One approach to these problems is to gather the very large sample sizes needed for sufficient power to detect genes of very small effect. Yet there are complementary strategies that posit that molecular genetic research should not be restricted to the clinical phenotype alone, but should also investigate genetic factors that account for neurobiological processes that underlie the heterogeneity of ADHD, ASD and their co-occurrence, in line with the increased realisation that there are multiple routes to the same phenotype. Intermediate phenotypes are defined as neurobiological processes that mediate between genes and behaviour. Differing views exist on the required characteristics of endophenotypes (de Geus, 2002, Gottesman and Gould, 2003, Waldman, 2005, Walters and Owen, 2008), but converge to suggest that candidate markers must:

1) be associated with the clinical disorder;
2) be reliable, as reliability sets an upper limit on the estimates of heritability. Any deviations from perfect reliability will increase measurement error and therefore unique environmental influences (Kuntsi et al. 2005);
3) be heritable;
4) be stable over time and state-independent such that it manifests in an individual whether or not the disorder is active (this criterion has greatest relevance to fluctuating state-like conditions such as schizophrenia or major depression rather than the trait-like conditions of ADHD and ASD);
5) co-segregate with the disorder within families;
6) for disorders with complex inheritance patterns such as ADHD and ASD, found in non-affected family members at a higher rate than the general population;
7) share genetic influences with the disorder;
8) be associated with a candidate gene or region of a gene;
9) mediate genetic effects between phenotype and genotype rather than reflect pleiotropic influences

The latter two criteria highlight that the association should be theoretically meaningful, such that the endophenotype causes behaviour or is the result of a biologically plausible mechanism (Castellanos and Tannock, 2002). Notably, other authors propose that numerous candidate endophenotypes be associated with a given disorder, and that disorders that are genetically related also share endophenotypes, such as the co-occurrence of ASD and ADHD (Cannon and Keller, 2006).
Brain-based intermediate phenotypes that are well-grounded in neuroscience offer particular advantages over behaviour-based phenotypes (Castellanos & Tannock 2002). These endophenotypes are posited as more powerful in genetic analyses, because they might lie closer to the site of gene action, and therefore aid the identification of genetic mechanisms (Kendler and Neale, 2010). Accordingly, it is expected that a successful intermediate phenotype would be more highly heritable than the clinical phenotype itself, or that it is less genetically complex due to the involvement of fewer genes. Importantly, is likely to help elucidate the aetiological basis of the comorbidity by identifying shared and/or unique markers (Rommelse et al. 2011).

Beyond implications for elucidating the aetiology of the conditions, however, this approach is likely to improve understanding of the model of comorbidity between ASD and ADHD that is at play, based on the models outlined above. It is common for investigation of comorbidity to rely on epidemiological data and for empirical research to pay little attention to psychiatric classification issues, the findings of which may therefore reflect the correlates of the comorbid condition (Caron and Rutter, 1991). A paper by Robins & Guze proposes five steps for identifying valid psychiatric classifications: (1) clinical description, referring to symptoms and other demographic/precipitating characteristics (largely described in Section 1.1; (2) laboratory studies, including psychological and physiological tests, which are considered more precise and reliable; (3) delimitation from other disorders, to ensure the study of homogenous groups; (4) follow-up study, to assess differences in outcome and development of comorbid disorders; and (5) family studies, to investigate whether there is increased prevalence among the relatives of the original patients (Robins and Guze, 1970). The use of intermediate phenotypes in the context of this thesis bears relevance to points (2), (3) and (5), and supports their clinical relevance and application to classification and treatment strategies.

The next section aims to provide rationale for the use of selected cognitive-electrophysiological intermediate phenotypes in this thesis, as a tool to further the understanding of the co-occurrence of ASD and ADHD for both clinical and research avenues.

1.3.2 Candidate cognitive-electrophysiological intermediate phenotypes of ASD and ADHD
The search for candidate intermediate phenotypes can be made at several levels of analysis. The work presented in this thesis focuses on electrophysiological approaches using electroencephalography (EEG), which measures the ongoing electrical activity generated by underlying brain structures, recorded from electrodes placed on the scalp. Electrophysiological parameters are particularly ideal for intermediate-phenotype research in neurodevelopmental samples. Firstly, the supreme temporal resolution enables investigation of the stages of
impaired cognitive processing and abnormal state processes such as arousal or default mode network impairments. While other neuroimaging techniques excel at localizing processes involved in impairment, applications of EEG are able to elucidate the neural basis of cognitive correlates of these disorders within the millisecond range. This allows for the measurement of covert processes even in the absence of performance deficits (McLoughlin et al. 2005). In addition, the non-invasive nature of EEG acquisition that is less sensitive to movement artifact is preferable in developmental and psychiatric populations, who are likely to be more averse to lying in a scanner.

Furthermore, there are consistent findings across studies suggesting abnormal electrophysiological processes in ADHD (Kuntsi et al., 2006a, McLoughlin et al., 2005) and ASD (Jeste and Nelson, 2009), and evidence that some of the impaired processes are largely developmentally stable in ADHD (Albrecht et al., 2008, McLoughlin et al., 2009). More generally, many electrophysiological measures fulfil criteria for high reliability and heritability (Kuntsi et al., 2006a), and the non-invasive and cost-effective nature of EEG helps to generate the relatively large sample sizes required for molecular genetic studies. With these advantages in mind, the measurement of cognitive-electrophysiological parameters is beneficial in the identification of candidate intermediate phenotypes to elucidate the basis of overlap between ASD and ADHD.

Due to the current diagnostic criteria, ASD and ADHD are traditionally studied separately, and it is only recently that empirical research has started to address the similarities between them beyond behavioural co-occurrence. While there are several potential intermediate phenotypes, the following sections of this chapter will focus on three broad domains that will be directly investigated in this thesis, reviewing their association with ADHD and/or ASD, heritability, familial/genetic overlap and possible associations with candidate genes of the disorders (see Sections 1.1.1.5 and 1.1.2.5). In particular, these studies can be used to show that Models 6, 7 and 8 described above can more or less be ruled out, because the intermediate phenotypes of interest show heritability (thus does not mediate between environmental factors and the phenotype) and are largely observed in non-affected relative (thus are not a consequence of the disorder, and are not related to a different set of genes to the phenotype). A recent extensive review of cognitive and brain markers that are shared between ADHD and ASD reports executive functioning, response variability and social cognition as among the most promising shared endophenotypes, but also highlights other potential candidates which are beyond the scope of this thesis (Rommelse et al. 2011). As an additional note, while intermediate phenotype research in ADHD is relatively advanced, there
is limited direct research in ASD and as such I briefly refer either to studies in typical or other samples, or to cognitive processes known to relate to electrophysiological parameters, to highlight intermediate phenotype potential.

1.3.2.1 Disordered brain activity: EEG

1.3.2.1.1 Background

EEG power is traditionally quantified into certain frequency bands of functional interest (defined in Figure 1-3) and demonstrates high test-retest reliability (0.71-0.95), particularly for theta and delta frequency bands (Williams et al., 2005). Lower EEG frequencies, such as theta (4-8Hz), are associated with reduced arousal, whereas higher EEG frequencies, such as beta (12-20Hz) are associated with alertness. A relatively novel EEG investigation is the measurement of very low-frequency activity (VLF; <0.05Hz) that have a duration of 20 seconds for a single wave. VLF activity may be associated with the brain’s default-mode network (DMN) that is activated during rest and is characterised by slow fluctuations in hemodynamic signal (Raichle et al., 2001). Alternatively (but not necessarily exclusively), VLF activity is proposed to reflect cognitive resource allocation (Rösler et al., 1997), modulation of gross cortical excitability (Vanhatalo et al., 2004) or an index of conscious perception (He and Raichle, 2009). Infraslow (or VLF) EEG corresponds to regional correlations in the infraslow BOLD signal (He and Raichle, 2009) and might modulate higher frequency activity (Vanhatalo et al., 2004). Further research is required to clarify the precise relationship between the DMN and VLF activity before direct comparisons can be made, but current findings suggest VLF activity can be taken as a novel measure of arousal levels (see Chapter 2 for more detail).
Note: In quantitative EEG, recordings of brain electrical activity at the scalp are quantified in the frequency range of interest, which usually extends between 1 and 70 cycles per second (Hz). In ADHD research, this frequency range is traditionally separated into four frequency bands. Recently ADHD research has further extended to very low-frequency oscillations below 1.5Hz.

EEG can be measured during rest and task conditions, and therefore provides a measure of changes in arousal and activation that are likely to be pertinent to the study of performance deficits in neurodevelopmental disorders. While arousal refers to the current energetic state of the organism as a function of time, activation refers the task-related changes to arousal (VaezMousavi et al., 2007). For example, theta power is associated with underarousal, although the theta/beta power ratio has not been associated with skin conductance level (SCL; a measure of arousal) which has led to the proposition of theta power as a measure of activation (Barry et al., 2009). Therefore the different correlates of arousal and task-related activation have been investigated to examine what theta represents. Accordingly, anterior midline theta is thought to reflect cognitive processing and therefore shows increase in task conditions (Delorme et al., 2007, Min and Park, 2010). Likewise, typically a decrease in or desynchronisation of alpha power is observed from resting state to task-onset or with
increasing task demand, combined with an increase in theta power or synchronisation (Klimesch, 1999). A reduction in alpha power, in particular, is associated with increased SCL when opening the eyes (Barry et al., 2007), which itself is associated with low alpha power (Barry et al., 2009). Beta activity is thought to reflect cortical activation, which increases from rest to task activity (Jasper 1938). In addition, the suppression of alpha power from eyes-closed to an eyes-open condition is highly replicated particularly at posterior sites, whereas beta activity increases over frontal regions, which is associated with increased activation of higher-order processing (Barry et al., 2007).

Developmental changes are reported in EEG activity. Generally EEG studies indicate a predominance of slow-wave delta and theta in infancy (Benninger et al., 1984), which is thought to reflect brain immaturity (Hudspeth and Pribram, 1992). Alpha peak frequency increases from infancy to childhood (5-51 months) (Marshall et al., 2002). After 8 years of age, however, theta and alpha activity decrease (Matousek and Petersen, 1973). Higher frequency beta decreases with age (Matsouek & Petersen 1973). Each frequency band appears to develop at different times and with different topographies (Gasser et al., 1988).

1.3.2.1.2 Association with ADHD

Structural and functional imaging studies indicate widespread dysfunction and alteration in the ADHD brain, particularly implicating fronto-striatal networks, although the interplay of large-scale networks is of increasing importance (Castellanos & Praoul 2012). Associations between quantitative EEG parameters and ADHD are widely documented (Barry et al. 2003; Snyder & Hall 2006). In particular, individuals with ADHD typically exhibit increased theta activity and decreased beta activity, compared to controls (Bresnahan et al., 1999, Chabot and Serfontein, 1996, Clarke et al., 2001b, Janzen et al., 1995), although not all data is consistent with this finding suggesting heterogeneity in the EEG profile of ADHD and the potential presence of EEG-defined subtypes (Clarke et al., 2001d, Koehler et al., 2009). This is widely interpreted to support the presence of underarousal in ADHD due to the association of theta activity with drowsiness (Satterfield and Dawson, 1971). See Chapters 3 and 4 for further review.

Abnormal EEG activity during task conditions has also been reported in ADHD (Mann et al. 1992; Loo et al. 2009; Swartwood et al. 2003; Janzen et al. 1995), which may provide a neural correlate of poor performance (see Chapter 3 and Chapter 4). One hypothesis proposes that VLF activity intrudes on active processing where higher frequency oscillations are involved, due to a failure to effectively transition from default mode to processing mode. This has become known as the default-mode interference (DMI) hypothesis (Fox et al., 2005, Giambra, 1995, Sonuga-Barke and Castellanos, 2007). Several studies implicate the role of the DMN in ADHD
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(Castellanos et al., 2008, Fassbender et al., 2009, Tian et al., 2006, Uddin et al., 2008). Reduced VLF activity at rest and reduced rest-to-task VLF attenuation in adults is associated with a higher number of inattentive symptoms (Helps et al., 2008). The authors further demonstrated that adolescents with ADHD also showed reduced VLF activity at rest and reduced rest-task attenuation of VLF compared to controls (Helps et al., 2010). Such findings emphasise the potential importance of the link between arousal/activation levels as indexed by EEG and poor performance on cognitive tasks. See Chapters 2 and 3 for further review and investigation.

1.3.2.1.3 Association with ASD

Numerous brain structures have been implicated in ASD, including the cerebellum, brain stem, cerebral cortex, corpus callosum, limbic system and basal ganglia, and recent research has highlighted the potential central role of reduced neural connectivity in ASD (Stigler et al., 2011). While electrophysiological research is more limited in ASD compared to ADHD, there is evidence that impairments of arousal may be involved in the underlying neurodevelopmental mechanisms of autism (Dawson and Lewy, 1989, Hutt et al., 1964). In particular, relatively consistent reports of reduced alpha power has been taken as suggestive of hyperarousal (Chan et al., 2007), which in turn may affect response to social stimuli (Dalton et al., 2005). See Chapter 4 for further review. In addition, while individuals with autism also show altered DMN activity compared to controls (Kennedy and Courchesne, 2008, Kennedy et al., 2006), and decreased functional connectivity between DMN regions (Weng et al., 2010), there have been no studies investigating VLF activity in individuals with ASD.

1.3.2.1.4 Overlap between ASD and ADHD

In an investigation of ADHD children with and without autistic traits, the ADHD-only group displayed increased absolute power in all frequency bands, and relatively more theta and less delta power than typical controls (Clarke et al., 2011). Children with ADHD and autistic symptoms showed qualitative differences notably a generalized increase in relative beta activity (Clarke et al. 2010). There have, however, been no direct comparisons between ASD and ADHD. One of the aims of this thesis is to provide a systematic comparison of QEEG profiles in the disorders in both resting and task conditions (Chapter 4).

1.3.2.1.5 Heritability

It is well established that EEG parameters are largely determined by genetic factors. Higher twin concordance rates in the spectral characteristics of resting eyes-closed EEG have been reported for monozygotic (MZ) compared to dizygotic (DZ) pairs (Christian et al., 1996, Lykken et al., 1982, McGuire et al., 1998). The first large twin study of resting EEG found high heritability across all frequency bands (delta 76%, theta 89%, alpha 89%, beta 86%), with
heritability ranging from 55-90% in 5-year-old twins and from 70-90% in 16-year-old twins (Van Baal et al., 1996, Van Beijsterveldt et al., 1996). Meta-analysis estimated an average heritability of 79% for alpha power (Van Beijsterveldt and Van Baal, 2002). Frontal areas tend to exhibit more unique genetic influences for the individual frequency bands, compared to occipital sites where genetic influences are largely shared across frequency bands (Zietsch et al., 2007); highlighting the complexity of genetic influences on EEG across frequency bands and scalp locations additionally reported using bipolar electrode derivations (Tang et al., 2007). The specificity of genetic influences in frontal regions suggests that different neurobiological pathways may be responsible for different frequency bands of the EEG (Zietsch et al., 2007). These findings may link with studies indicating band specificity in ADHD (i.e. reduced theta, increased beta).

1.3.2.1.6 Familial/genetic overlap
EEG frequency bands were found to correlate between siblings in families with more than one member affected with ADHD. At rest siblings were more similar for lower frequency band theta (0.36-0.59) compared to the higher frequency bands (alpha: 0.42-0.49; beta1 (12-15Hz): 0.45-0.57; beta2 (16-20Hz): 0.28-0.52), suggesting reduced theta power observed at rest is familial (Loo et al. 2010). In contrast, for cognitive activation conditions (resting eyes open and completion of the continuous performance test (CPT) higher sibling correlations were reported for beta1 (0.45-0.61), which suggests familial influences underlie reduced beta power and lack of typical beta increase during cognitive activation conditions. In addition, highly significant parent-offspring correlations for alpha power were reported under resting eyes open (0.47-0.56) and CPT (0.46-0.50), similar to a previous preliminary study (Loo and Smalley, 2008). EEG further demonstrated familial clustering with ADHD subtypes and symptoms (Loo et al., 2010). In children increased theta was found in ADHD regardless of subtype, whereas in adults EEG, theta, alpha and beta varied according to ADHD subtype. Parents with the predominantly inattentive subtype displayed significantly elevated theta compared to parents with the combined subtype and unaffected parents, suggesting a potential link between ADHD that persists into adulthood, inattention and elevated theta. Some of the familial correlations for the EEG parameters are higher than expected for the action of genetic influences alone, suggesting the influence of the familial environment. However the selection of affected sibling pairs may inflate the familial correlations since they may reflect in part state effects (i.e. both having ADHD). A twin study of EEG power is required to confirm these parameters as ideal endophenotypes of ADHD (the aim of Chapter 3).
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Functional connectivity of the DMN is reported to be heritable with estimates at 0.42 (Glahn et al., 2010). However, as yet there is no information on the heritability of VLF EEG patterns or the extent to which shared genetic influences explain the phenotypic associations between ADHD and VLF activity, which is one of the aims of this thesis (Chapter 2).

There have, to date, been no studies investigating familial or genetic overlap between ASD and EEG frequency bands.

1.3.2.1.7 Genetic association studies

A recent review of the relationship between neurotransmitters and brain oscillations highlights the role of dopamine in brain oscillatory activity (Başar and Güntekin, 2008). Theta and beta2 (16-20Hz) have been associated with DRD4 (Loo et al., 2010): children with the 7-repeat allele (the risk allele associated with ADHD) had reduced beta2 power across all conditions (likely indicating reduced cortical activation) compared to children without the 7-repeat allele. Parents also demonstrated the same association between the 7-repeat allele and reduced beta2 power under resting eyes open and task conditions, but not under resting eyes closed condition (Loo et al., 2010), suggesting possible developmental effects. Lower levels of prefrontal cortex (PFC) dopamine levels, as indexed by the Val allele on the COMT Val158Met polymorphism, predicted increased power in the delta/theta range defined using independent components analysis (ICA; Mueller et al. 2011). The association between the dopamine transporter gene (SLC6A3/DAT1) and EEG patterns was investigated in a double-blind placebo-controlled methylphenidate (MPH) study in a small sample of 27 children with ADHD (Loo et al., 2003). Findings indicated poor performance on the CPT (increased reaction time variability and error rate) in children with two copies of the 10-repeat allele (risk allele associated with ADHD in children) compared to those with one or two copies of the 9-repeat allele. MPH treatment led to decreased theta activity and lower theta/beta ratio in children with the 10-repeat allele, whereas those with 9-repeat allele showed the opposite pattern. Genetic variation of DAT1 may therefore mediate medication-related changes on EEG, and response variability shown in the 10-repeat group might reflect underarousal. Such medication-related changes are reported elsewhere in the literature (Clarke et al., 2003, Loo et al., 2004), highlighting the potential for combining genetic and electrophysiological data when considering treatment response. Taken together, these findings suggest that variation in dopaminergic genes may mediate susceptibility to ADHD through effects on cortical activation.

Although there have been no direct studies of genetic association with VLF activity, fMRI studies suggest associations with the DMN. Reduced DMN deactivation in ADHD was normalized following administration of methylphenidate (Liddle et al. 2011), implicating the
role of dopaminergic neurotransmission in DMN function and associated attentional control (Mehta 2011). A recent preliminary study of typical children and adolescents further demonstrates that individuals carrying the short allele of SHTTLPR (a candidate gene of ASD) had reduced functional connectivity in DMN regions compared to those with one or more copies of the long allele (Wiggins et al., 2012). Nevertheless, it is not possible to draw direct comparisons between the DMN and VLF activity at this stage and further work is required.

1.3.2.2 Event-related potentials (ERPs): generation and nomenclature

The following two sections describe functional electrophysiological research that has been applied the event-related potential (ERP) technique. ERPs are fluctuations in voltage that are time-locked to the onset of a stimulus or response, reflecting the sum of synchronous activity of neuronal populations. Repeated occurrences of these single-trial ERPs are averaged together to create an averaged ERP waveform. This removes background ‘noise’ created from a source other than the event of interest and creates a replicable waveform that consistently reflects the event being processed. The resulting waveform consists of a series of characteristic peaks and troughs, where P and N refer to positive and negative peaks, respectively, and the number refers to the peak’s position or latency (e.g. P1, N1, P2, N2, P3) (Luck, 2005). A schematic diagram of ERPs elicited by visual stimuli, over different electrodes, is shown in Figure 1-4.

Figure 1-4: A schematic diagram of event-related potentials associated with engagement of attention to visual stimuli.

Note: In this diagram, the y axis shows negative amplitude going upwards.
1.3.2.3 Executive function and attention

1.3.2.3.1 Background
A task that is often used to assess different executive processes is the cued Go/No-Go task or the cued-Continuous Performance Task (CPT-AX or CPT-OX). The CPT-OX, when used in an ERP paradigm, measures attention and inhibition and additionally attentional orienting to a cue and motor response preparation that do not require an overt response. ERPs associated with these processes are the Go-P3 (enhanced positivity in parietal regions in response to the target) and the NoGo-N2 (an enhanced negativity at fronto-central locations in response to no-go stimuli and thought to reflect conflict monitoring (Nieuwenhuis et al., 2003), followed by the NoGo-P3 (enhanced positivity at fronto-central locations in response to no-go stimuli that is thought to reflect response inhibition (Donkers and van Boxtel, 2004). In addition, the fronto-central Cue-P2, centro-parietal Cue-P3 and contingent negative variation (CNV) occur in response to the cue stimulus and are thought to reflect attentional orienting to a cue and motor response preparation respectively (Van Leeuwen et al., 1998). The Go-P3 and NoGo-P3 demonstrate high reliability, with intra-class correlations for peak amplitudes of 0.85 and 0.92 respectively, over a period of 30 minutes (Fallgatter et al., 2001). Long-term reliability of topography over an average of 2.7 years found an intra-class correlation of 0.9 (Fallgatter et al., 2002).

1.3.2.3.2 Association with ADHD
An influential theory of ADHD posits deficits in executive dysfunction as a core deficit (Section 1.1.1.4.1), supported by associations with prefrontal brain regions and connected structures (e.g. Faraone & Biederman 1998). Poor behavioural performance on CPT or Go/No-Go tasks is frequently reported. Children with ADHD show decreased accuracy on both omission (non-response when required, indicative of sustained attention deficit) and commission errors (response when inhibition required, indicative of inhibitory deficit), and other constructs including working memory, planning, set shifting and processing speed (Willcutt et al., 2005). This supports a more generalized dysfunction or poor state regulation (see Section 1.1.1.4.2). This is further supported by a clear clinical characteristic of individuals with ADHD: frequent lapses of attention and moment-to-moment inconsistency in symptom expression. Response variability in ADHD reflects a greater proportion of slow responses, with fast responses on some trials leading to an overall inconsistent pattern (Leth-Steensen et al., 2000). One proposed measure of this is reaction-time variability (RTV). RTV has been found to best distinguish between ADHD cases and controls compared to mean and error measures (Castellanos et al., 2005, Klein et al., 2006). Faster-event rate (Sergeant et al., 2003, Van der
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Meere *et al.*, 1995), rewards (Scheres *et al.*, 2001, Slusarek *et al.*, 2001) or both (Kuntsi *et al.*, 2005a) can improve the performance of individuals with ADHD.

Numerous studies indicate that children and adults with ADHD exhibit altered electrophysiological correlates on tasks that require attention and inhibitory control (Barry *et al.*, 2003b, Kuntsi *et al.*, 2006a). In particular, individuals with ADHD demonstrate attenuation of the NoGo-P3 amplitude suggesting problems of inhibition in children (Brandeis *et al.*, 2002) and adults with ADHD (McLoughlin *et al.*, 2010); and reduced Cue-P3 and CNV activity indicating reduced response preparation in children (Banaschewski *et al.*, 2003, Banaschewski *et al.*, 2004, Van Leeuwen *et al.*, 1998) and adults with ADHD (McLoughlin *et al.*, 2010). Diminished N2 amplitudes are also found in ADHD, although these are mainly related to comorbidities (Wiersema *et al.*, 2006) or demonstrated in more demanding tasks, such as the Stop task (Albrecht *et al.*, 2005). In line with cognitive findings, motivational incentives appear to increase the amplitude of the P3 during a Go/No-Go task (Groom *et al.*, 2010), suggesting again that executive function deficits are not necessarily stable in ADHD. These components are investigated further in Chapter 5.

Similarly, although much less studied, children with ADHD show abnormal responses on auditory oddball tasks. In a typical auditory oddball tasks, the subject is required to respond to a target sound stimulus occurring 10% of the time, but are also presented with frequent stimuli (80%) and novel stimuli (10%). In these tasks, reduced N2 amplitude towards target and frequent stimuli and reduced P3 amplitude (Johnstone and Barry, 1996, Johnstone *et al.*, 2001, Kemner *et al.*, 1998, Satterfield *et al.*, 1990, Senderecka *et al.*, 2011) are associated with ADHD. A review of other ERP components of interest in ADHD can be found in Barry *et al.* (2003). Taken together, findings support multiple deficits rather than a single impairment at the neurophysiological level.

1.3.2.3.3 Association with ASD

ASD has been characterised by deficits in executive function (See Section 1.1.2.4.1), and is associated with damage to the prefrontal cortex (Bailey *et al.*, 1996). In an extensive review, participants with ASD demonstrate impairment in planning and flexibility/shifting attention, when compared to both age- and IQ-matched controls, with less consistent findings for an inhibitory deficit on Go/No-Go type tasks (Hill, 2004), which may be only be observed in certain tasks (Christ *et al.*, 2007). In addition, most studies of autism show intact sustained attention (Garretson *et al.*, 1990, Noterdaeme *et al.*, 2001, Pascualvaca *et al.*, 1998, Siegel Jr *et al.*, 1995). Increased RTV has been demonstrated in ASD (see next section). Interestingly,
however, this variability increases in faster event rates, further supporting hyperarousal in ASD (Raymaekers et al., 2004).

There is limited neurophysiological research on visual attention processes in ASD (Jeste and Nelson, 2009). In visual oddball tasks, abnormalities of the P3 and N2 components have been reported (Kemner et al., 1999, Sokhadze et al., 2009, Strandburg et al., 1993), although these findings have been inconsistent (Townsend et al., 2001, Verbaten et al., 1991) and null findings are also reported (Courchesne et al., 1989, Hoeksma et al., 2006, Pritchard et al., 1987, Tsai et al., 2011). On auditory oddball tasks or variants thereof, children with ASD typically show shorter and larger N1 amplitude (Ferri et al., 2003, Martineau et al., 1987, Oades et al., 1988), although findings are inconsistent (Bruneau et al., 1999), and an attenuated Go-P3 suggesting reduced allocation of attentional resources (Courchesne et al., 1984, Courchesne et al., 1989, Courchesne et al., 1985, Oades et al., 1988). A review of other attentional ERP components of interest in ASD can be found in Jeste & Nelson (2009).

1.3.2.3.4 Overlap between disorders

Studies that have directly compared ADHD and ASD dissociate the pure disorders on the basis of consistent inhibitory deficits in ADHD as indexed by increased errors in Go/NoGo and Stroop type tasks, and difficulties in flexibility and planning in ASD as indexed by impairments when required to shift attention or change strategy and poor performance on Tower of London tasks, respectively. Several findings however indicate this dissociation is not robust, and substantial overlap in inhibition, flexibility and planning across both disorders suggesting a common pathophysiological basis (see Rommelse et al. 2011 for review). In general, poor performance appears to be more severe and widespread in ASD than in ADHD (Geurts et al., 2004), although with apparent age-related improvement (Happé et al., 2006b). Conversely, however, comparisons of all three clinical groups suggest individuals with comorbid ASD+ADHD show similar inhibitory deficits to ADHD-only, with both groups more impaired than ASD-only (Bühler et al. 2011; Sinzig et al. 2008). In addition, only ASD+ADHD individuals show deficits in flexibility (Sinzig et al. 2008), suggesting previous findings of deficits in ASD may be due to the presence of comorbid ADHD symptoms. Another study, however, showed that ADHD-only appears to be associated with the most severe deficits on a Test of Variables of Attention (TOVA), while ASD+ADHD scored in the normal range (Nyden et al. 2010). In addition, in a sample of children with ASD, ADHD symptoms exacerbated deficits in executive function (Yerys et al., 2009). Likewise, within individuals diagnosed with ADHD, comorbid autistic traits are associated with poor executive functioning (Rommelse et al., 2009a).
In direct comparisons of sustained attention, studies using the continuous performance test (CPT) report no differences between ASD and ADHD (Riccio & Reynolds 2001; Swaab-Barneveld et al. 2000) and ASD+ADHD (Nyden et al. 2010). In a comparison of performance on a sustained attention to response task (SART), children with ADHD showed greater commission and omission errors suggesting deficits in sustained attention are more specific to ADHD (Johnson et al. 2007). The authors further report increased slow-frequency variability in reaction time in ADHD, suggestive of impaired arousal processes (Johnson et al. 2007). In terms of task factors influencing task performance, within the SEND sample used in this thesis (described in Chapter 4), increased response variability in ADHD shows greater improvement in fast-incentive conditions, which is not shown in ASD, indicating that ADHD are more sensitive to conditions that optimize performance compared to ASD (paper in preparation).

While not specifically related to the research questions of this thesis, a handful of ERP studies have examined executive function across the disorders. For example, early studies revealed attenuated P3 in auditory oddball tasks was specific to ADHD (Kemner et al. 1998). In addition, children with ADHD show attenuated error-related negativity (ERN) and error positivity (Pe) components compared to ASD and controls, and the latter normalized with MPH administration (Groen et al., 2008). Various studies exist of error monitoring in the pure disorders (Henderson et al., 2006, Tye et al., 2011, Vlamings et al., 2008). While these ERP studies suggest there may be some neurophysiological differentiation in executive function processes, there have been no direct ERP comparisons between ASD, ADHD and ASD+ADHD on covert attentional processes and inhibition (the aim of Chapter 5).

1.3.2.3.5 Heritability

In general, measures of executive function are moderately heritable (Doyle et al., 2005a), although it remains unclear whether the different processes share genetic variance (Polderman et al., 2009, Schachar et al., 2011, Wood et al., 2010a). Specifically for inhibitory control, 38% of lab-based measures and 58% of parent-ratings are attributable to genetic influences in infants of 24 months old (Gagne and Saudino, 2010) with similar estimates at 8 years of age (Schachar et al. 2011). Meta-analysis of child and adolescent data confirm average heritability around 60% for P3 amplitude and 51% for P3 latency (Van Beijsterveldt and Van Baal, 2002), although in paradigms that may elicit functionally distinct components (Dien et al., 2004). More recently comparable heritabilities using a Go/No-Go task were found in an adult twin sample of 60% in the NoGo-N2 component and 41% and 58% for Go-P3 and NoGo-P3 components (Anokhin et al., 2004). One study reports significant MZ correlations at frontal regions (0.67) but non-significant correlations at centro-parietal regions for visual P3...
amplitude (Bestelmeyer et al., 2009). The extent of genetic influences appears to be stable throughout development, with similar heritabilities reported in children, young adults and middle-aged adults (Smit et al., 2007). Longitudinal studies are however required to test whether this reflects the same genetic factors throughout development, or whether different sets of genes play a role at different developmental stages. One longitudinal study reported rate of change in P3 amplitude measured at 17, 20 and 23 was genetically influenced (Carlson and Iacono, 2006). Slow-cortical potentials such as the CNV demonstrate heritability between 30-43% (Smit et al., 2009). These ERP components therefore appear to index genetically influenced neural processes that are important for cognitive control.

1.3.2.3.6 Familial/genetic overlap

Deficits in neuropsychological performance are shown in relatives of ADHD probands. Specifically, unaffected siblings and co-twins of ADHD probands show significant impairment on executive function (including inhibition), processing speed and response variability (Bidwell et al., 2007, Slaats-Willemse et al., 2005) that is separate from IQ effects (Rommelse et al., 2008). Measures of inhibitory control in 24-month-old infants share moderate to substantial genetic influences with ADHD symptoms measured on the Child Behaviour Checklist, with a genetic correlation of 0.74 for parent-rated inhibition and 0.35 for observer-rated inhibition, using a laboratory measure (Gagne et al., 2011). There are, however, some inconsistent results (Doyle et al. 2005).

Family studies (Andreou et al., 2007, Frazier-Wood et al., 2012, Nigg et al., 2004, Uebel et al., 2010) and twin studies (Bidwell et al., 2007, Kuntsi and Stevenson, 2001) indicate substantial aetiological overlap between RTV and ADHD. This familial/genetic overlap is distinct from the effect of IQ (Wood et al., 2010a, Wood et al., 2010b) and commission errors (Kuntsi et al., 2010), and familial factors are associated with improvement in task performance in fast-incentive conditions (Andreou et al. 2007; Uebel et al. 2010). These findings have renewed interest in state regulation theories (see Section 1.1.1.4.2). There are only a few studies evaluating the familial association between ERP indices of executive function and ADHD. In one small study, similar P3 activity to increased conflict (P3a) was demonstrated when comparing siblings of ADHD probands and typical controls despite a significantly attenuated P3 in the ADHD probands, suggesting that in this study altered P3 showed no familial association with ADHD (Wild-Wall et al., 2009). However, other findings report impaired inhibitory control as indexed by the NoGo-P3 in parents of ADHD probands indicating a familial association with adult ADHD (McLoughlin et al., 2011a). In addition, attenuated Cue-P3 and CNV has been reported in non-affected siblings of ADHD probands compared to controls (Brandeis et al.,
2006) and attenuated Cue-P3 in parents of ADHD probands compared to controls (McLoughlin et al., 2011a), suggesting impaired attentional orienting and preparatory states might index familial risk for ADHD. A further twin study demonstrated modest phenotypic and genetic overlap between the Go-P3 in a visual oddball paradigm and externalising conditions associated with ADHD, including substance abuse disorders, conduct disorder and antisocial behaviour (Gilmore et al., 2010). This association is likely to be driven by genetic factors alone with an estimated genetic correlation of -0.22 (Hicks et al., 2007).

Executive function deficits in task performance are shown at a higher rate in relatives of ASD probands, as a sign of the broader autism phenotype (Sucksmith et al., 2011). Specifically, poorer performance on tasks tapping into cognitive flexibility is consistently found in non-affected siblings. Nevertheless, not all studies demonstrate executive function as a prominent feature of the BAP (Losh et al., 2009). Eye-tracking studies of 9-10 month infants “at-risk” for developing ASD (siblings of autism proband) demonstrate greater difficulty in disengaging attention from a central stimulus (Elsabbagh et al. 2009; Holmboe et al. 2010), which is likely to bear relevance to inability change strategy and shift set.

A recent study reports that autistic features in unaffected individuals are associated with executive function deficits in their sibling diagnosed with ADHD, using a construct of inhibition, cognitive flexibility, visuo-spatial working memory and verbal working memory (Rommelse et al., 2009a). This suggests that comorbid autistic traits share familial factors that give rise to neuropsychological dysfunction demonstrated in ADHD. Further studies of this kind, particularly those incorporating twin designs in ASD and ADHD samples, are required to fully determine the familial and genetic associations of these variables with the disorders.

1.3.2.3.7 Associations with specific genetic variants
A recent review linking executive function to specific genetic variants highlights associations with dopaminergic systems in ADHD (Kebir et al., 2009). One of the most consistent associations reported is between the 7-repeat allele of DRD4 and RTV, although some studies report association with increased RTV (Johnson et al., 2008, Swanson et al., 2000) and others with reduced RTV, suggesting a protective effect (Bellgrove et al., 2005, Manor et al., 2002). Response inhibition appears to be associated with the 10-repeat allele on DAT1, which itself was associated with ADHD symptoms within a population sample (Cornish et al., 2005), although inconsistent findings are reported. A recent study supports dissociation between dopaminergic genes, whereby DAT1 was associated with measures of impulsivity and DRD4 with measures of inattention (Gizer and Waldman, in press). In a recent linkage analysis, an aggregate of measures of intra-individual variability shared familial overlap with ADHD and
was associated with peaks at 17p13.3 (associated with ADHD), 12q24.3 (associated with ASD symptoms) and 13q22.2, suggesting variability may tap into loci conferring risk for both disorders (Frazier-Wood et al. 2012). In terms of genetic variants of interest in ASD, the heterozygous short/long allele on the 5HTT gene was associated with altered reward processing and delay aversion in ADHD patients, with a null effect for the DAT1 gene (Sonuga-Barke et al., 2011). These findings suggest different aspects of neuropsychological performance can be differentiated by genetic variants, highlighting the utility of the intermediate phenotype approach to reduce heterogeneity.

The P3 has been associated with specific genes involved in dopamine transmission. The A1 allele of the Taq1 polymorphism in the dopamine D2 receptor gene was associated with a reduction in P3 amplitude to rare targets in visual and auditory oddball tasks (Hill et al., 1998) and a longer parietal Go-P3 latency in a visual CPT task (Noble et al., 1994) in individuals “at-risk” for alcoholism, although negative findings were also reported in a sample of 134 young female controls (Lin et al., 2001). Similarly an association between the 7-repeat allele of DRD4 and reduced P3 amplitude to rare targets in an auditory oddball task has been demonstrated in young boys (Vogel et al., 2006) but not reported in young females (Tsai et al., 2003), suggesting a gender effect. Healthy individuals with the Val/Val genotype for the COMT gene showed increased Go-P3 amplitude and shorter Go-P3 latency compared to those bearing the Val/Met homozygote in a visual working memory task (Yue et al., 2009). An enhanced NoGo-P3 was reported in Val/Val homozygotes compared to those bearing the Met/Met genotype during a flanker task in a sample of 656 healthy students (Kramer et al., 2007), although was not reported in a sample of 187 consisting of individuals with schizophrenia, their relatives and healthy controls. In a study of event-related oscillations during a Go/No-Go task, carriers of the 7-repeat allele of DRD4 exhibited increased NoGo-related theta and reduced Go-related beta (Kramer et al., 2009). In addition, these components appear to be normalized by medication in ADHD; the amplitude of the N2 and P3 components increased following MPH administration during a Stop task (Pliszka et al., 2007) and a Go/No-Go task, independently of motivational incentives (Groom et al. 2010). These findings potentially suggest function and genetic variation of dopamine might be involved with altered regulation of the P3 response in ADHD. There is limited direct research linking executive function deficits in ASD to specific genetic variants.
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1.3.2.4 Social cognition

1.3.2.4.1 Background

Social cognition refers to the processing of information relevant to social situations. The face provides a wealth of socially relevant information, so to be able to detect and recognize faces is considered an important adaptation of social animals. In particular eye-contact is considered crucial for the development of successful social interaction, through providing information about the locus of someone’s attention, their mood and intentions (Baron-Cohen 1995). Direct gaze appears to modulate concurrent or subsequent cognitive and behaviour processing (Senju and Johnson, 2009). Another important cue that can be derived from the face is emotional expression, which plays an important role in nonverbal communication and adapting to the social environment. Sensitivity to emotional expressions is proposed as essential for ToM development (Harris et al., 1989).

ERPs have been widely used in the investigation of the timing of face processing. In particular, the N170 ERP component is proposed to be a “face-sensitive” component, supported by numerous findings (Eimer, 2011), notably the amplitude enhancement shown for faces compared to other non-face stimuli (Bentin et al., 1996). The N170 shows characteristic scalp topography over lateral posterior temporal sites, with amplitude more pronounced over the right hemisphere (Eimer, 2011). As the N170 is not modulated by familiarity (Bentin and Deouell, 2000) or emotion (Eimer and Holmes, 2002, Eimer et al., 2003), it is proposed that it reflects the perceptual encoding of faces.

Of interest to elucidating the function of the N170 is the face inversion effect (FIE), referring to the observation that individuals are slower and make more errors in processing inverted than upright faces, although they are equally good at processing other objects in both orientations (Yin, 1969). It is argued that upright faces are processed holistically whereas inverted faces are processed analytically by their features, and therefore are thought to disrupt face processing by limiting the configural information needed for accurate face recognition (Tanaka and Farah, 1993). The N170 is typically delayed and enhanced for inverted faces compared to upright faces (Bentin et al., 1996, Eimer, 2000a, Rossion et al., 2000, Sagiv and Bentin, 2001), and not modulated by the inversion of non-face stimuli in the right hemisphere (Rossion et al., 2002) or by monkey faces (de Haan et al., 2002), which has led to the proposition of its function as structural encoding of human faces. The amplitude enhancement may be explained by the additional recruitment of eye-sensitive cells when faces are inverted (Eimer 2011). Earlier studies suggested that the N170 is larger for eyes than faces (Bentin et al. 1996) leading to the view that this component may reflect gaze processing, although this particular response
Chapter 1: Introduction

decreases with age contrary to the FIE (Taylor et al. 2001). In addition, isolated eye regions and faces-without-eyes did not produce the N170 FIE, and from this it is argued that the FIE on the N170 is driven by the presence of the eyes (Itier et al., 2007). Alternatively, the amplitude enhancement may reflect the recruitment of additional object-sensitive processes (Rossion et al. 2000). Taken together, the N170 is thought to be involved in the configuration of face stimuli.

An additional component, the P1 recorded over occipital sites, has been measured in response to faces, reflecting an earlier stage of visual processing. The P1 is longer for objects compared to faces and for inverted faces compared to upright faces (Itier and Taylor, 2002, 2004b). In addition, shorter latency is observed for gaze cues congruent to target, compared to incongruent cues particularly in the right hemisphere (Schuller and Rossion, 2004). The specificity of the P1 response to faces is questionable, however, and therefore may reflect broad visual or sensory processing.

Familiarity and emotional expressions are hypothesised to be consciously processed at a later stage following identity processing (Munte et al., 1998). For example, the N400 ERP is proposed to reflect access to semantic memory to allow contextual and meaning evaluation, supported by modulation by familiarity and emotional expressions (Kutas and Federmeier, 2011, Munte et al., 1998, Olivares et al., 2003, Posamentier and Herve, 2003). Some studies, however, report global effects of emotion at shorter latencies as early as 90ms (Batty and Taylor, 2003, Eimer and Holmes, 2002), and a handful of studies show that the N170 can be modulated by emotion; studies report enhanced N170 amplitude to fearful faces (Batty and Taylor, 2003, Blau et al., 2007, Campanella et al., 2002), These more recent studies suggest that emotional face processing may occur rapidly and in parallel to visual or structural processing, although these effects may be paradigm-specific (Ashley et al., 2004, Eimer and Holmes, 2002, Holmes et al., 2003). The temporal resolution of ERPs can be used to inform models of face recognition like that of Bruce and Young (1976; Figure 1-5).
Figure 1-5: Bruce and Young’s (1986) face processing model.

Note: This model demonstrates that structural encoding and the detection of the emotional expression of faces represent two parallel and independent stages of processing, which may reflect reported dissociations between ERP components such as the N170 and N400.

1.3.2.4.2 Association with ADHD

The majority of research in ADHD has focused on the impulsivity, hyperactivity and inattentiveness associated with the disorder. More recently, research has begun to address the social impairment observed in individuals with ADHD and its underlying mechanisms (Uekermann et al., 2010). The majority of studies have assessed emotional impairment in ADHD (Dickstein and Castellanos, 2012), and therefore there is limited knowledge of basic face processing deficits. Although there have been no investigations of deficits in eye gaze direction processing or discrimination in ADHD, a recent investigation of ASD behaviours in children with ADHD identified poor eye gaze as measured on the Social Communication Questionnaire as significantly different from controls (Kochhar et al., 2011). One of the aims of this thesis is to characterise face and gaze processing in ADHD using the P1 and N170 ERP components (Chapter 6). Less accurate emotion recognition has been widely documented, globally across all emotions (Corbett and Glidden, 2000) and more specifically to angry and sad faces (Dickstein and Castellanos, 2012, Pelc et al., 2006). Such deficits have also been associated with executive function (Shin et al., 2008, Sinzig et al., 2008b), which has led to the proposition of executive and social impairment in ADHD that are interdependent (see also Section 1.1.4 on cognitive theories of ADHD). Children with ADHD appear to make more random errors when identifying emotions, which has been taken as suggestive of inattention or other general regulatory processes (Cadesky et al., 2000). If emotional deficits are the result of attention or executive function deficits it would be expected that they have no bias to one emotion, and preliminary analyses suggest more extensive scanpaths using eye-tracking in ADHD compared
Chapter 1: Introduction

to restricted paths in schizophrenia (Marsh et al., 2000, Marsh and Williams, 2006). A number of studies have now investigated ERP responses to emotional stimuli in ADHD that suggest particular deficits in processing positive emotion, reviewed in Chapter 7.

1.3.2.4.3 Association with ASD

Several studies have investigated the mechanisms underlying the social impairment characteristic of individuals with ASD, with the greatest emphasis on social cognition. Deficits in ToM are the basis of an influential theory of ASD (reviewed in Section 1.1.2.4.1). Many of the social impairments involve the ability to process information from faces, and based on the ability to measure ERP responses to faces in typically developing infants (Halit et al., 2003), alterations in ASD may provide an early index of abnormality (Elsabbagh and Johnson, 2010).

Behavioural studies demonstrate impairment in face discrimination and recognition in autism (Boucher et al., 1998, Gepner et al., 1996, Schultz, 2005, Wolf et al., 2008). Still, other studies report typical levels of face identification (Behrmann et al., 2006). There are numerous reports of atypical eye gaze processing in ASD and in particular with direct eye contact processing, which itself is included as a criterion in the clinical diagnosis (see Table 1-2). Children with ASD consistently demonstrate a reduced tendency to spontaneously follow another person’s eye gaze (Osterling and Dawson, 1994, Osterling et al., 2002), which is underpinned by perception of eye contact (Senju and Johnson, 2009). In addition, patients use different viewing strategies when looking at faces, such as reduced fixation on the eye region using eye tracking (Boraston et al., 2007, Pelphrey et al., 2002). Behavioural studies show that while typically developing children are more accurate and faster at detecting direct gaze than averted gaze, children with ASD show equal detection of direct and averted gaze (Senju et al., 2005a, Senju et al., 2008). ERP studies support abnormalities in face processing and gaze direction detection as indexed by the P1 and N170 components, reviewed in Chapter 6. These data show clear impairments in face and gaze processing in ASD at the behavioural and neural level.

In terms of behavioural studies of emotion recognition, while some studies report impairment in ASD, others show intact emotion processing (see Harms et al. 2010 for review), particularly in contextual processing of emotional cues (Da Fonseca et al., 2009). In a recent multi-modal investigation of emotion recognition, there was no gross deficit in the ASD group compared to controls, although they were impaired in recognising expressions of surprise (Jones et al., 2011). Seemingly a more consistent finding is problems with specific emotions; in particular individuals with ASD appear to display specific deficits in processing negative emotions such as sadness and fear (Ashwin et al., 2006, Boraston et al., 2007, Corden et al., 2008, Pelphrey et al., 2002, Wallace et al., 2008). The deficit in perceiving fearful facial expressions may arise
from a failure to fixate on the eyes (Corden et al. 2008; Pelphrey et al. 2002), particularly as deficits in gaze processing are consistently demonstrated (see above), highlighting the significant interplay between these deficits. Electrophysiological studies suggest neural abnormalities underlie impaired emotional processing, reviewed in Chapter 7. The majority of studies, however, have not used the full range of emotions or not analysed ERPs as a function of emotion modulation, which is improved upon in this thesis.

1.3.2.4.4 Overlap between ASD and ADHD

In direct comparisons similar performance on emotion recognition tasks has been reported in ASD and ADHD (Buitelaar et al., 1999, Fine et al., 2008), although some studies demonstrate poorer performance in ASD (Downs and Smith, 2004). A comparison of emotional face recognition revealed greater impairment in ASD+ADHD and ADHD-only compared with ASD-only and typically developing controls (Sinzig et al., 2008b). Specifically, children with ADHD-only showed more difficulties in recognition of photographs of eye-pairs depicting joy, and ASD+ADHD performed worse on eye-pairs depicting surprise, compared to controls (Sinzig et al., 2008b). Lack of consistency may reflect the selection of different emotions (e.g. simple versus complex) and type of stimuli (static versus video vignettes). There have been no ERP studies evaluating the overlap.

1.3.2.4.5 Heritability

There are limited studies on the heritability of social cognition and its neural correlates. ToM ability generally shows modest heritability (7-12%) with the remainder attributed to shared environmental effects (22-48%) and unique environmental effects (45-66%) (Hughes et al., 2005, Ronald et al., 2006c). However there is large overlap between ToM and verbal ability that can be attributed to genetic influences, or a combination of genetic influences and shared environmental effects (Hughes et al. 2005; Ronald et al. 2006). Genetically sensitive studies of face processing report substantial genetic influences. High MZ concordance rates that are more than twice DZ concordance rates (0.70 versus 0.30) are reported for face recognition abilities, and they appear specific from other cognitive abilities (Wilmer et al., 2010). Similar findings are reported in an independent study on tasks of face recognition ability, the face inversion effect and the composite-face effect (Zhu et al., 2010). This large genetic component suggests a small role of the environment and experience, although further studies across the lifespan are required (McKone and Palermo, 2010). A recent study extends this work to show high heritability of ERP components elicited in response to emotional face expressions; 55-64% for the N240 (resembling the N170) and 42-62% for the P300 component (Anokhin et al., 2010). In addition, in a lexical decision task, the N400 component showed heritability between
9-30% for primed words and 13-41% for unprimed (incongruent) words that typically elicit greater N400 amplitude than primed words (Almasy et al., 2001), although no studies have explored heritability within an emotion task.

1.3.2.4.6 Familial and genetic overlap

No studies to date have investigated social cognitive measures in relatives of individuals with ADHD. A recent review shows higher rates of impairments in social interaction, such as ToM deficits in infants “at-risk” for autism, and atypical face and gaze processing in older relatives of ASD probands suggesting presence of the BAP, outlined in Section 1.1.2.3 (Sucksmith et al., 2011). For example, relatives with the BAP show reduced processing of information from the eye regions in comparison to the mouth region of the face (Adolphs et al., 2008). In addition a number of social cognition tasks involving emotion processing and attribution of emotion, parents of autism probands have particular difficulties with the identification and processing of negative emotion. Further, it appears a subset of relatives with social difficulties or social “aloofness” struggle on social cognition tasks more (Adolphs et al., 2008, Losh et al., 2009). In terms of ERP studies, parents of children with ASD do not show the typical latency shift for faces compared to objects, and fail to show right hemisphere lateralisation of the N170 (Dawson et al., 2005a). Infant siblings “at-risk” for ASD show a slower P400 response to faces and faster N290 response to objects (McCleery et al., 2009). A recent study investigated electrophysiological components of face processing in infant siblings of autistic probands, and reports prolonged latency of the P400 component in response to direct gaze, but no difference in the earlier N290 components (Elsabbagh et al., 2009a). Furthermore, these ERP atypicalities were later found to predict subsequent ASD diagnosis (Elsabbagh et al. 2012). In the first imaging study investigating this question, unaffected adolescent siblings of ASD probands presented with happy and neutral faces during fMRI recording displayed significantly reduced activity in several brain regions associated with face processing and empathy compared to controls, but not compared to ASD probands (Spencer et al., 2011). Siblings showed no difference in activation when processing fearful expressions compared to controls or ASD (Spencer et al. 2011). Finally, a twin study reports substantial shared genetic influences between emotion attribution ability and autistic traits measured on the CAST (71%), which was independent of the association with psychopathic tendencies (Jones et al., 2009).

1.3.2.4.7 Associations with specific genetic variants

In terms of social cognition at the behavioural level, greater ToM ability has been associated with the 4-repeat allele of the DRD4 gene, but not with DAT1 or COMT (Lackner et al., 2012). In addition, several studies link oxytocin to social cognition (Ross and Young, 2009). An
interesting study associated variation in the OXTR susceptibility gene of ASD to social cognition in a subset of subjects with ADHD (measured using the Social and Communication Disorders Checklist), despite not being associated with ADHD symptoms per se (Park et al., 2010). There have been no direct genetic associations with gaze processing, although a number of disorders with known genetic origin show deficits in processing gaze. For example, adolescents with Fragile-X disorder demonstrate increased brain activation toward direct gaze (Watson et al., 2008). In addition, oxytocin has been shown to increase gaze to the eye region of the face (Guastella et al., 2008), which may implicate genetic variants. Finally, there are several lines of investigation for the genetics of emotion processing. A number of studies using the emotional expression processing task used in this thesis (Chapter 7) have revealed genetic associations. Specifically, shorter latency of the N170 was associated with having one or more copies of the met allele of the COMT gene in typically developing children (Battaglia et al., 2007). In addition, an independent association in the same sample was revealed between reduced N400 amplitude in response to angry facial expressions in carriers of 1 or more copies of the short allele of the 5HTTLPR polymorphism compared to subjects homozygous for the long allele (Battaglia et al., 2005). This supports the use of this task in the identification of shared or distinct endophenotypes in ASD and ADHD. In addition, dopaminergic and serotonergic systems appear to modulate emotion processing in different ways. In a study of the Nc and Pb ERP components (Nelson and De Haan, 1996) in 7-month old infants, carriers of the met allele of the COMT showed enhanced processing of fearful facial expressions at central and parietal electrodes. Conversely, carriers of the long allele of 5-HTTLPR demonstrated a negativity in response to happy faces, whereas carriers of the short allele showed a positivity to the display of happy facial expressions (Grossmann et al., 2011). Dopaminergic contributions are further supported by modulation of emotion processing by MPH administration in ADHD patients (Williams et al. 2008).

1.3.3 Conclusions

This section has outlined three domains of impairment in ASD and ADHD - EEG-indexed arousal, and ERP components of executive function and social cognition - which each show association with both disorders, and in some cases their co-occurrence, evidence of heritability, shared familial/genetic influences and some links with specific genetic variants, although often unreplicated. Overall this suggests these are key markers of potential genetic risk for ASD and ADHD and tools to elucidate the basis of co-occurring ASD and ADHD. The disorders appear to display comparable neuropsychological performance on a variety of tasks encompassing EF and ToM, and disordered brain activity measured using EEG. In addition, certain dissociations can be noted; for example, there is stronger evidence for an inhibitory
deficit in ADHD and a problem in contextual processing of emotional stimuli, whereas in ASD, findings suggest clearer deficits in cognitive flexibility and face processing in general. Similarly, while hypoarousal is posited as a key feature of ADHD and its associated symptoms, some theorists have likened ASD impairments to mechanisms associated with hyperarousal. Of the studies available, findings generally suggest ASD+ADHD are an additive co-occurrence, although some studies do point toward non-additive effects and therefore the formulation of a distinct subtype. Such comparable neuropsychological task performance may, however, be associated with different underlying pathophysiological mechanisms (or vice versa). In addition, inconsistencies in the literature may reflect misspecification in group allocation, such as the inclusion of comorbid autistic traits in ADHD studies. With concurrent recording of event-related potentials (ERPs), the present thesis aims to capture fast-occurring covert processes even in the absence of an overt response, and as such provide a direct neural measurement of the correlates and precursors of this poor performance across domains for each of the three conditions.

1.4 Overall conclusions and aims of thesis

This chapter outlined key findings in ADHD and ASD, in relation to the clinical profile and their genetic and environmental underpinnings. Clinical and population-based studies indicate substantial clinical and behavioural overlap between the disorders, which can be at least partly attributed to shared genetic influences. Progress towards understanding the mechanisms underlying this overlap is limited and very little is known to date about pathophysiological mechanisms that might be shared or unique to the disorders. The complexity of the aetiology of ADHD and ASD necessitates the understanding the cognitive and neurophysiological mechanisms that might mediate genetic effects on behaviour. Candidate cognitive and electrophysiological markers appear inconsistent both within and between disorders, which is likely to reflect large phenotypic and genetic heterogeneity. An investigation of these impairments in twin designs that are able to partition aetiological variance is a clear next step. Beyond this, direct comparisons between disorders at the neurophysiological level, particularly with the inclusion of a comorbid ASD+ADHD group is likely to delineate phenotypic heterogeneity, and provide a basis for further investigation of these domains as candidate shared intermediate phenotypes.

Based on these observations and propositions, this thesis has two main aims:

Part I
Chapter 1: Introduction

The aim of Part I was to investigate the genetic overlap between ADHD symptoms and quantitative EEG measures known to be associated with ADHD in a community twin sample, in order to evaluate their potential as intermediate phenotypes.

- Very low-frequency activity is first investigated as a novel measure of arousal in ADHD during a task condition (Chapter 2).
- Elevated theta power is consistently associated with ADHD, and the aim of Chapter 3 was to apply quantitative genetic analysis to ascertain its genetic overlap with ADHD during resting and task conditions.

It was expected that VLF power and theta power are associated with ADHD and poor performance on the task, due to their putative relationship with cognitive processing, show heritability and share genetic influences with ADHD symptoms.

Part II

The aim of Part II was to investigate whether the neurophysiological processes implicated in ADHD are found in ASD and vice versa, and to elucidate the basis of co-occurring ASD+ADHD, using candidate intermediate phenotypes identified for ADHD and/or ASD, over four domains of impairment:

- Arousal levels as indexed by EEG measured during rest and at three stages during a cognitive activation condition (CPT-OX), in order to examine their role in cognitive performance and sustained attention across conditions (Chapter 4).
- The Cue-P3, NoGo-P3, CNV and Go-NoGo-N2 difference measured during a cued-CPT-OX, to index potential impairment in attention and inhibition in ASD and ASD+ADHD that are typically shown in ADHD (Chapter 5).
- The P1 and N170 components measured during passive viewing of upright and inverted faces, with direct or averted gaze, to assess the nature of the FIE and gaze direction detection in ADHD and ASD+ADHD typically impaired in ASD (Chapter 6).
- The N170 and N400 components measured during passive viewing of emotional faces with neutral, angry, disgusted, fearful and happy expressions, to examine the precise temporal correlates of emotion processing deficits across the three conditions (Chapter 7).

The main prediction was that ADHD-only and ASD-only could be dissociated at the neural level on the basis of these EEG parameters, whereas the comorbid ASD+ADHD would demonstrate the deficits of both disorders.
2.1 Overview

Data presented in this thesis is drawn from two studies: the Neurophysiology of Activity and Attention in Twins (NEAAT) study (Part I) and the Specificity of Electrophysiology in Neurodevelopmental Disorders (SEND) study (Part II). The aim of this chapter is to provide background information on the study methodology used in each study, including an overview of participant selection and recruitment procedures, questionnaire and interview measures, data acquisition, data cleaning and the general analysis strategy. Further details specific to individual studies is given in the following chapters.

2.2 NEAAT study overview

2.2.1 Funding and ethical approval

The NEAAT study was conducted at the MRC Social, Genetic and Developmental Psychiatry Centre at the Institute of Psychiatry, supported by a National Institute for Mental Health Research fellowship to Dr Gráinne McLoughlin. Participating families gave their written informed consent and the study was approved by King’s College London Psychiatry, Nursing and Midwifery Reseach Ethics Sub-Committee (PNM/08/09-89).

2.2.2 Participant selection and recruitment

The sample was selected from the Twins’ Early Development Study (TEDS), a birth cohort study of all twins born in England and Wales between 1994 and 1996 (Trouton et al., 2002). Zygosity was determined using a zygosity questionnaire that has been shown to have 95% accuracy (Price et al., 2000); For cases where zygosity was unclear from this questionnaire, DNA testing was conducted. The TEDS sample is representative of the general population in terms of parental education, ethnicity and employment status (Kovas et al., 2007).

Twin pairs were selected for the NEAAT study based on an analysis of symptom development over time using the program MPLUS. Latent Class Analyses (LCA) on longitudinal data can be used to identify subgroups in the sample that show different developmental trajectories e.g. consistently high or low (Connell and Frye, 2006). These analyses were used here to optimally select either concordant high or low ADHD twin pairs for the EEG study. LCA were applied on 3
time points of data at age 8, 12 and 14 for measures of (i) hyperactivity-impulsivity; (ii) inattention and (iii) ADHD, the log-transformed total score from a DSM-IV measure of ADHD (Conners et al., 1998a), as calculated on boys only, with medical cases excluded. The LCA were conducted on individual data, with the COMPLEX analysis option in Mplus (Muthén and Muthén, 1998-2007) to account for the non-independence of observations and with missing data managed through Full Information Maximum Likelihood.

The analyses typically involve fitting a series of models, starting with one and moving to multiple-class models. The most parsimonious number of classes can be selected by means of a number of fit indices as well as usefulness (interpretation) of classes and previous findings in the literature. In this case, for the purpose of selection, for all 3 scales a three-class model was opted where consistently high, low and middle class of individuals were clearly identified. The output takes the form of both a posterior probability belonging to each class and an assigned ‘class or trajectory membership’ based on the highest probability. We then selected twin pairs where both twins were from the consistently high class of combined hyperactivity-impulsivity and inattention symptom scores (concordant for ADHD); twin pairs who were both from the consistently low class of combined hyperactivity-impulsivity and inattention symptom scores (control pairs) and pairs where one twin was from the high class and the co-twin was from the low class (discordant pairs). This identified sub-groups of individuals who have been stably high or stably low at ages 8, 12 and 14, using the 18 DSM-IV ADHD items from the Long Version of the Conners’ Parent Rating Scale (Conners et al., 1998a). This ensured that the selected twin pairs were consistently concordant or discordant for high levels of ADHD symptoms (corresponding to a clinical diagnosis) or unaffected controls who had consistently low ADHD symptoms. Participant selection is presented graphically in Figure 2-1.
Chapter 2: General methodology

Figure 2-1: Participant selection and recruitment flow-chart for the NEAAT study

Abbreviations: DZ: dizygotic; MZ: monozygotic.
2.2.3 Sample demographics

The final Neurophysiological Study of Activity and Attention in Twins (NEAAT) sample consisted of 67 male twin pairs in groups of 22 pairs concordant for high levels of ADHD symptoms (MZ: 11; DZ: 11), 8 pairs discordant for ADHD symptoms (MZ: 2; DZ: 6) and 37 control pairs concordant for low levels of ADHD symptoms (MZ: 21; DZ: 16). The sample is sufficient to detect with 80% power a genetic component of 60% for electrophysiological indices and a genetic correlation of 0.40 between ADHD and electrophysiological indices, assuming a heritability of 60% for ADHD. Demographic characteristics of the NEAAT sample are given in Table 2-1.

Within the twins scoring high for ADHD, there were lower parent- and teacher-rated ADHD symptom scores in DZ twins compared to MZ twins, particularly for teacher ratings. Several twin studies in ADHD report lower concordance rates for DZ twins compared to MZ twins, which is thought to reflect contrast effects. These can be attributed to either competitive sibling interaction (whereby the behaviour of one twin influences the behaviour of the co-twin) or a form of rater bias, typically that raters emphasise behavioural differences in DZ twins. In most studies, parents rate a child with high ADHD symptoms higher and a child with low ADHD symptoms lower, suggesting that true differences in behaviour are unlikely (Simonoff et al. 1998). Large rater contrast effects therefore result in the underidentification (or overidentification) of true cases, and may subsequently artificially increase concordances between MZ pairs compared to DZ pairs. Nevertheless, previous work in the TEDS sample has suggested minimal contrast effects using the Conners’ rating scale at age 8 (McLoughlin et al. 2007) and age 12 (Greven et al. 2012), and only parent ratings were utilised. While we can be fairly confident these biases did not affect the selection procedure, we acknowledge that it may affect the estimates calculated and result in an overestimation of the genetic overlap between ADHD and EEG measures. An additional issue is that these findings suggest the ADHD phenotype depends on who rates the child, although greater bias is usually shown for parent ratings (Hartman et al. 2007). As teachers are proposed as less likely to exaggerate differences between twins due to having many children with whom to compare the twins, lower teacher-rated DZ scores may reflect different behaviours in the twins, particularly in a setting that requires different demands. This is supported by unique genetic influences on parent- and teacher-rated ADHD (e.g. McLoughlin et al. 2007). Further work could investigate whether twins have the same or different teachers.
Chapter 2: General methodology

Table 2-1: Raw scores and mean comparisons of demographic characteristics of the NEAAT sample adjusted for genetic relatedness

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<th>DZ Control (n=38)</th>
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<td>13.82</td>
<td>-2.29</td>
<td>.04</td>
<td>DZ Control (n=21,23)</td>
<td>103.51</td>
<td>12.93</td>
<td></td>
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<tr>
<td>Parent Conners (T-score)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DSM-IV symptom subscale: Inattention</td>
<td>MZ Control (n=44)</td>
<td>43.03</td>
<td>3.45</td>
<td>12.60</td>
<td>&lt;.001</td>
<td>DZ Control (n=38)</td>
<td>42.18</td>
<td>2.49</td>
<td></td>
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<tr>
<td></td>
<td>MZ ADHD (n=24)</td>
<td>57.13</td>
<td>8.41</td>
<td></td>
<td></td>
<td>DZ ADHD (n=28)</td>
<td>54.18</td>
<td>9.10</td>
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<tr>
<td>DSM-IV symptom subscale: Hyperactivity/Impulsivity</td>
<td>MZ Control (n=44)</td>
<td>44.73</td>
<td>2.72</td>
<td>10.74</td>
<td>&lt;.001</td>
<td>DZ Control (n=38)</td>
<td>43.86</td>
<td>1.44</td>
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<tr>
<td></td>
<td>MZ ADHD (n=24)</td>
<td>62.21</td>
<td>11.61</td>
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<td>DZ ADHD (n=28)</td>
<td>59.50</td>
<td>12.84</td>
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<td>Teacher Conners (T-score)</td>
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</tr>
<tr>
<td>DSM-IV symptom subscale: Inattention</td>
<td>MZ Control (n=25)</td>
<td>45.92</td>
<td>6.76</td>
<td>5.57</td>
<td>&lt;.001</td>
<td>DZ Control (n=23)</td>
<td>46.61</td>
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<tr>
<td></td>
<td>MZ ADHD (n=11)</td>
<td>64.55</td>
<td>10.97</td>
<td></td>
<td></td>
<td>DZ ADHD (n=15)</td>
<td>51.87</td>
<td>11.35</td>
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</tr>
<tr>
<td>DSM-IV symptom subscale: Hyperactivity/Impulsivity</td>
<td>MZ Control (n=25)</td>
<td>48.00</td>
<td>8.62</td>
<td>1.86</td>
<td>.07</td>
<td>DZ Control (n=23)</td>
<td>46.48</td>
<td>5.08</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>MZ ADHD (n=11)</td>
<td>57.36</td>
<td>9.11</td>
<td></td>
<td></td>
<td>DZ ADHD (n=15)</td>
<td>49.27</td>
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</tbody>
</table>

2.2.4 Additional measures

2.2.4.1 General cognitive ability

General cognitive ability was assessed at age 14 as part of ongoing TEDS web-based data collection. The twins were tested on the WISC-III-PI vocabulary multiple choice subtests
Chapter 2: General methodology

(Wechsler, 1992) and Raven’s standard and advanced progressive matrices (Raven et al., 1996). Missing scores (Table 2-1) were imputed from multiple IQ subtest scores across ages 7, 12 and 14 using the ICE command in Stata version 10 statistical software (Stata Corp, College Station, Texas) for each outcome variable. A g score was created with equal weights for the two tests by summing their standardized scores within the NEAAT sample. Further information about g as measured in TEDS can be found elsewhere (Haworth et al., 2010). Measures of g and IQ correlate highly and both provide an index of general intelligence (Jensen, 1998) and as such IQ can be calculated from standardised g using the formula IQ=(g*15)+100.

2.2.4.2 Conners’ rating scale

In addition to using the Conners’ rating scale to select twin pairs, we also collected Conners’ rating scales as part of the study (Table 2-1). The Conners’ rating scale uses a quantitative Likert response scale, which allows testing of DSM-IV ADHD symptoms from a quantitative perspective. During the testing session, parents completed the long version of the Parent Conners’ rating scale that consists of 80 items (Conners et al., 1998a). Here I report the hyperactive-impulsive and inattentive DSM-IV symptoms subscales that each include nine items. The parent indicated on a four-point scale how well each attribute described the child: not true at all (0), just a little true (1), pretty much true (2), very much true (3). Items include, for example, “is always ‘on the go’ or acts as if driven by a motor” and “has difficulty sustaining attention in tasks or play activities”. Teachers were contacted following completion of the testing session to complete the long version of the Teacher Conners’ rating scale, consisting of 59 items (Conners et al., 1998b). Approximately 46-61% of teacher data was collected. Raw scores were converted to T-scores, which are standardized scores with a mean of 50 and standard deviation of 10. T-scores were used for comparative and interpretive value. A T score of 65 or greater is viewed as clinically significant.

2.2.5 Testing procedure

The assessments detailed above were part of a larger test battery within the NEAAT study, for which twin pairs were assessed at the MRC SGDP Centre over 5-6 hours, including a refreshment break. Twin 1 and Twin 2 were counterbalanced according to birth order. Following EEG preparation (approximately 30 minutes), each participant completed 12 minutes of resting EEG (Chapter 4), the flankered CPT-OX (Chapters 3 and 4), an Eriksen Flanker task and the Fast task, with a duration of 60 minutes. Presentation of the tasks was
ordered in the same way for each group to control for effects of practice and fatigue. These
tasks are described in full in the relevant individual chapters (the reader is directed to Tye
et al. 2011 for a description of ERPs relevant to the Flanker task and Kuntsi et al. (2005) for
a description of the Fast task, which are not reported upon in this thesis). While the first
twin completed the EEG recording, the second twin and the parent completed
questionnaires/interviews (not relevant to this thesis), and following a refreshment break
and blood collection, the protocols were completed on the remaining twin. In addition, at
regular points throughout the testing session, the participant was required to give saliva
samples to measure changes in cortisol. Families were compensated for their time and
reasonable travel costs were reimbursed. Families later received a newsletter detailing
results from the study.

In Part I, I investigate two quantitative EEG parameters that are consistently altered in
ADHD and based on theoretical proposals are likely to further our understanding of arousal
processes and their impact on behaviour. Restricting the selection of parameters for
analysis is primarily due to limited power in the twin design employed, such that it is
difficult to model multiple variables together. In addition, it is likely that other EEG and ERP
measures are correlated, and according to twin modelling assumptions should therefore be
combined to ensure homoscedascity. Calculating combinations of measures would reduce
our ability to interpret the findings according to previous literature and theories. Finally, on
a more practical note, analysis of EEG/ERP data on the NEAAT project based on the tasks
outlined above was delegated to different researchers, with my interest in scalp QEEG
measures.

2.3 SEND study overview

2.3.1 Funding and ethical approval

The SEND study was conducted at the MRC Social, Genetic and Developmental Psychiatry
Centre at the Institute of Psychiatry. The overarching BioNeD study was funded by the
National Institute for Health Research Biomedical Research Centre, and the SEND EEG
component was funded by the Waterloo Foundation (G686984) and the Steel Charitable
Trust (G38575208). The study protocol was approved by the Wandsworth Research Ethics
Committee (REC reference: 08/H0903/161). Research and Developmental approval was
obtained on behalf of Lambeth, Southwark, Croydon and Lewisham to allow recruitment
Chapter 2: General methodology

from Primary Care Trusts (Reference: RDLDL454). Written parental consent was given before the experiment began.

2.3.2 Inclusion and exclusion criteria

The participants were required to be male, have an IQ>70, normal or corrected-to-normal vision, speak English as the main language (to ensure participants understood task instructions) and not to be taking any medication except for stimulants, which had to be interrupted 48h prior to the experiment. Exclusion criteria included specific medical disorders, history of traumatic brain injury, a diagnosis of epilepsy and other comorbid psychiatric disorders, such as major mood disorder, severe OCD, severe generalized anxiety disorder, Tourette’s/tics disorder, conduct disorder and genetic disorders, not including oppositional defiant disorder (ODD). The latter was included as studies indicate that ADHD associated with conduct disorder (CD) may be a distinct subtype (Faraone et al., 1995), and is a separate condition in the ICD-10 (see Section 1.1.1), which does not appear to be the case for ODD (Faraone et al. 1995).

2.3.3 Recruitment of clinical cases

The majority of the patients were recruited through Biomedical Research Council coordinators working in neurodevelopmental clinics, who identified individuals with a clinical diagnosis made according to ICD-10 or DSM-IV criteria (autism, Aspergers syndrome, ADHD combined type/hyperkinetic disorder) and who also met the inclusion/exclusion criteria outlined above. The BioNeD team (Bahare Azadi, Karen Ashwood, Sally Cartwright) sent an information pack to those who indicated willingness to participate, including a consent form and screening questionnaires that were to be sent back if they wanted to participate. Additional participants were recruited through special educational needs units and specialist schools, via the National Autistic Society (NAS) website and an email advert to local ASD parent support groups, using the same procedure described above. The overall response rate for the BioNeD study was 46%.

All participants then underwent systematic clinical assessment during the study (summarized in Figure 2-2). Cases were initially evaluated with Conners’ Parent Rating Scale short form (Conners, 2008) and Social Communication Questionnaire (SCQ; (Rutter et al., 2003). To ensure that all participants met clinical diagnostic criteria the Autism Diagnostic Interview–Revised (ADI–R) and the Autism Diagnostic Observation Schedule (ADOS-G) were used to diagnose cases of ASD (Lord et al., 1997, Lord et al., 2000). Cases of ADHD were diagnosed using Parent Account of Childhood Symptoms (PACS; (Taylor et al., 1986) and
Conners’. Co-morbid ASD+ADHD cases met full diagnostic criteria for ASD and full diagnostic criteria for ADHD using the ADI−R, ADOS (n=27), PACS and Conners’. In addition, the ADI−R was conducted for ADHD participants who scored above threshold on the SCQ, and the PACS for ASD participants who scored above threshold on the Conners’. If participants scored above threshold on these diagnostic interviews they were reallocated into the ASD+ADHD group (ADHD n=3, ASD n=16). One participant clinically diagnosed as ASD+ADHD did not reach ADHD diagnosis as defined by the PACS interview (Inattention: 1; Hyperactivity/Impulsivity: 1) and therefore was reallocated into the ASD-only group. Two additional measures were administered to aid in group classification and in-depth assessment where diagnostic classification was unclear: the Strengths and Difficulties Questionnaire (SDQ) and Development and Wellbeing Assessment (DAWBA). In addition, additional clinic records and where possible educational records were collected. An experienced clinical academic (PB) reviewed the available data and decided on the ‘best estimate’ diagnosis using this multi-measure multi-informant approach, with greater weight given to clinical diagnosis, followed by ADI−R, PACS and DAWBA. On the basis of this review 5 cases were excluded (ASD=2; ADHD=3) as well as 2 controls who met criteria for ADHD on the PACS interview. These participants are not included in the demographic characteristics or analyses described in Part II of this thesis. Further details on measures and criteria used can be found in Section 2.3.6. The recruitment and group allocation procedures are graphically presented in Figure 2-2.
Chapter 2: General methodology

Recruitment of clinical cases through BRC co-ordinators in neurodevelopmental clinics and contact with parent support groups

Families consent to take part in BioNeD study

Participants selected for SEND study

Families contacted for SEND study

Screening questionnaires

Families tested for SEND study

Diagnostic measures

Review for research diagnosis

Excluded on diagnostic basis*

Reallocated on diagnostic basis

Excluded for poor data**

Final SEND sample used in further analyses

Inclusion criteria
6-17; male; normal vision; English; IQ>70; no medication

Exclusion criteria
Medical and psychiatric disorders; brain injury

ASD
n = 53

ASD+ADHD
n = 22

ADHD
n = 48

Aged between 8-13 years
Minimal missing data

ASD
n = 44

ASD+ADHD
n = 19

ADHD
n = 33

SCQ

Conners

ASD
n = 39

ASD+ADHD
n = 12

ADHD
n = 26

ADI-R

ADOS-G

PACS

DAWBA

SDQ

Teachers

n=2

n=0

n=3

n=16

n=1

n=3

n=2

n=1

n=2

SCQ ≥ 15
Positive ADI-R² and/or ADOS-G b
Conners ≤ 60 or PACS ≤ 6

SCQ ≥ 15
Positive ADI-R a and/or ADOS-G b
Conners ≥ 60
PACS ≥ 6

Conners ≥ 60 and PACS ≥ 6
SCQ ≤ 15 or negative ADI-R² and/or ADOS-G b

Families contacted for SEND study

Participants selected for SEND study

ASD
n = 53

ASD+ADHD
n = 22

ADHD
n = 48

Families consent to take part in BioNeD study

Recruitment of clinical cases through BRC co-ordinators in neurodevelopmental clinics and contact with parent support groups

Inclusion criteria
6-17; male; normal vision; English; IQ>70; no medication

Exclusion criteria
Medical and psychiatric disorders; brain injury

Participants selected for SEND study

Families contacted for SEND study

Screening questionnaires

Families tested for SEND study

Diagnostic measures

Review for research diagnosis

Excluded on diagnostic basis*

Reallocated on diagnostic basis

Excluded for poor data**

Final SEND sample used in further analyses

SCQ ≥ 15
Positive ADI-R² and/or ADOS-G b
Conners ≤ 60 or PACS ≤ 6

SCQ ≥ 15
Positive ADI-R a and/or ADOS-G b
Conners ≥ 60
PACS ≥ 6

Conners ≥ 60 and PACS ≥ 6
SCQ ≤ 15 or negative ADI-R² and/or ADOS-G b
Abbreviations: TD, typically developing controls; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid ASD and ADHD; ADI-R, Autism Diagnostic Interview-Revised; ADOS-G, Autism Diagnostic Observation Schedule; Conners, Conners 3rd Edition Parent Rating Scale Short Form; PACS, Parental Account of Childhood Symptoms.

a ADI-R cut-off: Impairment of Social Interaction = 10; Impairment of Communication = 8; Stereotyped Behaviour = 3; participants may fall one point below the threshold in one of the behavioural domains according to modified criteria

b ADOS-G cut-off: Social Affect and RRBI = 7

*Excluded on diagnostic basis following review of all available evidence

** Refusal to complete EEG acquisition or extreme artifact (slow drift and EMG throughout recording). 1 ADHD was excluded due to administration of medication prior to the testing session, without the examiner’s knowledge.

Only participants with a clinical diagnosis combined subtype ADHD were recruited and tested, although during the diagnostic review stage some participants did not meet criteria for combined type. Within the final SEND sample, the proportions of subtypes within the ADHD-only group (n=18) were 9 for combined subtype (50%), 6 for the inattentive subtype (33%) and 3 for the hyperactive-impulsive subtype (17%). In the comorbid ASD+ADHD group (n=29), there were 13 participants in the combined subtype (41%), 14 participants in the inattentive subtype (48%) and 2 in the hyperactive-impulsive subtype (7%). I have also noted that differing subtype distributions may affect the results, when discussing heterogeneity within the sample. Within the final SEND sample, 6 participants with ADHD were on medication (1 Equasym, 3 Concerta, 1 Equasym and Concerta, 1 unspecified) and 6 participants with ASD+ADHD were on medication (5 Concerta, 1 dexamphetamine). All participants on psychoactive medication were asked to refrain from taking medication for a minimum of 48 hours before testing. One ADHD participant completed the task battery although later questioning revealed medication had been taken before the testing session, and thus was removed from analysis.

2.3.4 Recruitment of typically developing controls
The TD group consisted of children recruited through distribution of information sheets at local schools, adverts in local online forums, and personal contacts. Individual were asked to contact the BioNeD/SEND team if they were interested in taking part. Children were not
included if they had any psychiatric diagnosis or had received mental health treatment/psychiatric medication, and were assessed with the Strengths and Difficulties Questionnaire (SDQ), SCQ and Conners’ questionnaires. Eleven TD participants scored above threshold on the Conners’. Further assessment of 9 of these children with the PACS interview confirmed that these children did not reach a diagnosis of ADHD and thus were retained in the study. Although not reaching the diagnostic threshold, four of these participants had a high score of above 5 on one or both domains of the PACS interview. In addition it was not possible to conduct the PACS interview on the remaining two participants who scored above threshold on the Conners. We therefore also applied a more stringent approach to control selection (excluding these 2 participants who were not assessed on the PACS and those scoring 5 or above on either domain of the PACS interview), which did not affect any of the results reported in Chapters 4 to 7. I also conducted univariate analysis of variance (ANOVA) that confirmed these children did not differ from controls on any performance or EEG/ERP parameter reported in Chapters 5 to 8. In addition, the retention of these control subjects in comparison of the 4 groups works against any hypotheses predicting changes in results and thus the inclusion of these participants only strengthens the findings.

### 2.3.5 Sample demographics

The final sample consisted of 19 male participants with ASD, 18 males with ADHD, 29 males with both ASD and ADHD, and 26 typically developing male controls (TD) aged between 8 and 13 years of age took part in the study. Table 2-2 summarizes the clinical and demographic characteristics of the sample. Statistical power calculations (using the software G*Power), with alpha set at 0.05 indicated that these numbers will have good power to detect the large effect sizes (Cohen’s d = 0.7-0.99) expected for electrophysiological measures, giving moderate to excellent power to assessing case-control differences (70-92%). Expected effect sizes were determined from previously published findings relating to the hypotheses under investigation.
Table 2-2: Clinical and Demographic Characteristics of the SEND sample

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>TD (n = 26)</th>
<th>ASD (n = 19)</th>
<th>ADHD (n = 18)</th>
<th>ASD+ADHD (n = 29)</th>
<th>F</th>
<th>p</th>
<th>Post-hoc</th>
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<tbody>
<tr>
<td>Mean</td>
<td>Age</td>
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<td>10.48</td>
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<td>1.79</td>
<td>1.70</td>
<td>1.91</td>
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</tr>
<tr>
<td>Mean</td>
<td>Right-handed (%)</td>
<td>23</td>
<td>88%</td>
<td>18</td>
<td>95%</td>
<td>17</td>
<td>94%</td>
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<tr>
<td>SD</td>
<td>x² = 6.79</td>
<td>.079</td>
<td>n.s.d.</td>
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<tr>
<td>Mean</td>
<td>Verbal IQ</td>
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<td>113.79</td>
<td>105.94</td>
<td>2.48</td>
<td>.066</td>
<td>n.s.d.</td>
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<tr>
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<td>14.40</td>
<td>23.87</td>
<td>18.47</td>
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</tr>
<tr>
<td>Mean</td>
<td>Performance IQ</td>
<td>115.73</td>
<td>111.05</td>
<td>101.67</td>
<td>106.72</td>
<td>4.86</td>
<td>.004</td>
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<tr>
<td>SD</td>
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<td>13.89</td>
<td>13.31</td>
<td>11.60</td>
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<td></td>
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<tr>
<td>Mean</td>
<td>Full-scale IQ</td>
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<td>109.72</td>
<td>5.31</td>
<td>.002</td>
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<td>SD</td>
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<td>13.42</td>
<td>15.73</td>
<td>14.23</td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>SCQ</td>
<td>3.88</td>
<td>20.11</td>
<td>10.89</td>
<td>24.79</td>
<td>81.12</td>
<td>&lt;.001</td>
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<tr>
<td>SD</td>
<td></td>
<td>3.54</td>
<td>6.42</td>
<td>5.36</td>
<td>5.71</td>
<td></td>
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<tr>
<td>Mean</td>
<td>Conners DSM- Inattentive</td>
<td>56.08</td>
<td>67.11</td>
<td>83.94</td>
<td>80.21</td>
<td>29.85</td>
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<td>7.41</td>
<td>11.59</td>
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<tr>
<td>Mean</td>
<td>Conners DSM-Hyperactive</td>
<td>58.88</td>
<td>66.11</td>
<td>87.89</td>
<td>84.00</td>
<td>32.76</td>
<td>&lt;.001</td>
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<tr>
<td>SD</td>
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<td>17.02</td>
<td>12.99</td>
<td>3.25</td>
<td>7.63</td>
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</tr>
</tbody>
</table>

*Note: Post-hoc analyses conducted using Sidak correction for multiple testing.*

*Abbreviations: TD, typically developing controls; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid ASD and ADHD; Conners, Conners Third Edition Parent Rating Scale Short Form; DSM, Diagnostic and Statistical Manual of Mental Health Disorders; IQ, intelligence quotient; SCQ, Social Communication Questionnaire. n.s.d. = non-significant difference*
2.3.6 Questionnaire and interview measures

2.3.6.1 The Wechsler Abbreviated Scale of Intelligence (WASI)
All children completed the WASI measure (Wechsler, 1999), comprising of two measures of verbal ability (Vocabulary and Similarity tasks) and two measures of performance ability (the Block Design and Matrix Reasoning). The full scale IQ estimate is used in analyses, which is adjusted for age.

2.3.6.2 Social Communication Questionnaire (SCQ)
The SCQ (Rutter et al. 2003) includes 40 items assessing social difficulties, communication problems and RRBIs currently and at age 4-5 years. Statements are scored according to whether certain difficulties have been observed (yes=1, no=0) with a cut-off point of 15 for a probable ASD. The SCQ incorporates item relevant to the ADI-R (described below). ADHD-only required a score below 12 on the SCQ (typical cut-off=15).

2.3.6.3 Conners’ Parent Rating Scale
The Conners’ is described in Section 2.3.2.2, Chapter 2. The Conners’ used in the BioNeD study was the 3rd edition of the short version, which comprises 43 items (Conners, 2008). These items can be used to calculate T-scores for Inattention, Hyperactivity/Impulsivity, Learning Problems, Executive Functions, Aggression and Peer Relations, as well as positive/negative impressions. In the SEND study, a classification of ADHD required a T score of 60 or above on either the Inattention or Hyperactivity/Impulsivity scale, and ASD-only required a T-score below 59 on the Conners’ rating scale.

2.3.6.4 Autism Diagnostic Interview-R (ADI-R)
The ADI-R is a gold-standard diagnostic tool for the assessment of ASD (Le Couteur et al. 2003; Lord et al. 1994). It is a semi-structured interview of 111 questions conducted by a trained reliable researcher over 2-3 hours. It measures current behaviours and also autistic-like symptoms earlier in childhood, to give a lifetime differential diagnosis of ASD. Most items are scored from 0 (if a child shows no impairment in that domain) to 3 (if the child shows severe impairment, often with a strong impact on family life). The algorithms used for scoring provide different totals and clinical cut-offs for social interaction difficulties (10), communication problems (8) and RRBIs (3). In the BioNeD study, participants may fall one point below the threshold in only one of the behavioural domains according to modified criteria (IMGSAC, 1998).
2.3.6.5 Autism Diagnostic Observation Schedule – Generic (ADOS)

The ADOS is a second gold-standard tool for diagnosing ASD during childhood, using observational activity-based assessment over 40-50 minutes (Lord et al. 2000). The BioNeD study used module 3 of the ADOS, designed to be used with children who are verbally fluent. This consists of 13 different structured activities to assess autistic-like behavior in social and non-social situations. Based on these activities, the child is given a score (0-2 or 0-3), with a higher score reflecting greater difficulties. Scores were transformed according to protocol and used to create a diagnostic algorithm score (Gotham et al., 2009, Gotham et al., 2007), which is derived by adding the Social Affect (social interaction and communication items) and RRBI subtotals. In order to meet ASD diagnosis, participants were required to meet or exceed a threshold of seven items.

2.3.6.6 Parental Account of Childhood Symptoms (PACS)

The PACS (Taylor et al. 1986) is a semi-structured interview used to assess symptoms of ADHD. Parents were asked to describe their child’s behavior in various settings, including unstructured (e.g. playing alone), semi-structured (e.g. meal times) and structured (e.g. homework). Inattention and hyperactivity/impulsivity behaviours were rated on a 4-point scale on severity and frequency, and entered into a computerized algorithm. This generated symptom scores out of a total of 9 for each scale, and in order to meet a diagnosis of ADHD participants were required to meet or exceed a cut-off of 6 or above in either domain.

2.3.6.7 Strengths and Difficulties Questionnaire (SDQ)

Parents completed the SDQ (Goodman, 1997), which comprises five subscales each consisting of five items to assess emotional symptoms, conduct problems, hyperactivity/inattention, peer problems and prosocial behaviour, this assessing both negative and positive behaviour. Parents rate these 25 items as not true (0), somewhat true (1), or certainly true (2). Of relevance in the BioNeD study was the hyperactivity score (cut-off: 7-10), the prosocial behaviour score (cut-off: 0-4) and the total difficulties score (cut-off 20-40). The SDQ has been shown to discriminate between ASD and ADHD; higher scores on the hyperactivity/inattention scale are shown in ADHD compared to lower scores (and therefore greater difficulty) on the prosocial scale in ASD (Iizuka et al., 2010).

2.3.6.8 Development and Well Being Assessment (DAWBA)

Parents completed the DAWBA diagnostic interview, in order to assess childhood psychiatric symptoms (Goodman et al., 2000). A computerised version of the DAWBA was completed online in the participant’s own time, or a member of the BioNeD guided them through the assessment over the phone. The DAWBA provides diagnostic algorithms to determine whether
an individual meets ICD or DSM criteria and parental descriptions of past and current behaviour.

2.3.7 Testing procedure

The assessments detailed above were part of a larger test battery within the BioNeD (and when necessary SEND) study, for which participants attended one or two sessions lasting approximately three hours each, conducted either at the participant’s home or the MRC SGDP Centre. Participants were invited to return for the SEND EEG component at the MRC SGDP Centre, which consisted of an EEG assessment and when necessary IQ assessment (Section 2.3.6.1). Following EEG preparation during which the participant watched a cartoon (approximately 30 minutes), each participant completed 12 minutes of resting EEG, the flankered CPT-OX (Chapter 6; McLoughlin et al. 2010), a face and gaze processing task (Chapter 7; Farroni et al. 2002), an emotional expressions task (Chapter 8; Battaglia et al. 2005) and the Fast task (Kuntsi et al. 2005), with a duration of 70 minutes. A short (10 minutes) break was given halfway through the testing session if required. Presentation of the tasks was ordered in the same way for each group to control for effects of practice and fatigue. These tasks are described in full in the relevant individual chapters (the reader is directed to Kuntsi et al. (2005) for a description of the Fast task, which is not reported in this thesis). Families were compensated for their time and reasonable travel costs were reimbursed. As part of the larger study, clinic and family reports were produced detailing the results of standardized assessments. A family newsletter is in preparation reporting the overall group results of the cognitive and electrophysiological assessments.

2.4 Assessment of cognitive function using electroencephalography (EEG)

The main method used to address the research questions outlined in Section 1.4, was cognitive-electrophysiology, involving Fast-Fourier Transform (FFT) frequency analysis on the ongoing electroencephalogram (EEG) and extraction of event-related potentials (ERPs) from the EEG (see Sections 1.3.2.1.1 and 1.3.2.2). Data acquisition is detailed below, with individual analyses outlined in the individual chapters.

2.4.1 Data acquisition

For EEG data acquisition protocols outlined above (Section 2.2.5 and Section 2.3.7), subjects were seated on a height-adjustable chair in a video-monitored testing cubicle. The CPT and Flanker task were administered using the Presentation software package, and the Fast task,
face and gaze processing task and emotion processing task were administered using the C++ programming software.

EEG was recorded using a 62 channel electrode cap (extended 10-20 montage). Each active electrode with Ag/AgCl sensors integrates noise substraction circuits and a display of individual impedance. The reference electrode was positioned at FCz. Prior to EEG data acquisition the participant was asked to wash their hair the day before and refrain from using any product in the the hair. A circumference measurement was taken at home to allow the set-up of the system prior to their visit. The electrode cap was placed on the participant’s head and a measurement was taken between the nasion and inion, and between the preauricular points, to ensure the vertex electrode (Cz) was in the centre. A chest strap was secured, which provides reduced likelihood of electrode displacement compared to a chin strap. Impedances were reduced using application of electrolyte gel through a blunt sterilised needle, to reduce the likelihood of skin potentials and improve recording. Vertical and horizontal electrooculograms (EOGs) were simultaneously recorded from electrodes above and below the left eye and at the outer canthi. Participants were asked to minimise eye movements and remain as still as possible during the recording. Using the Brain Vision Recorder (Brain Products, Munich, Germany), EEG was continually monitored online and when necessary electrodes were adjusted for improved recording. Direct Current corrections were calculated at set points during task completion and manually entered when necessary, to correct for continuous change in conductance across the scalp-electrode junction that needs to be ‘zeroed’ during recording. The amplifier used in all studies was the BrainAmp DC (direct current) with a lowpass filter of 70Hz. The amplifier increases the signal for analysis, by amplifying the difference between the active-ground voltage and reference-ground voltage, subtracting any noise that is present in the ground. The signal was digitized at a 500Hz sampling rate, stored and analyzed offline. Following acquisition, the participant was given the option of washing their hair, and the cap and individual electrodes were thoroughly cleaned.

2.4.2 General preprocessing strategy

Data were analysed in Brain Vision Analyzer (2.0) (Brain Products, Munich, Germany). The signal was downsampled to 256Hz and re-referenced offline to the average reference, by subtracting the mean of all electrodes from each electrode. The data was filtered to remove unwanted frequencies, typically by removing frequencies below 0.1 Hz and above 30Hz which are not of focus and contain low-frequency artifact and electrical noise at 50Hz (excluding Chapter 3 in which I was only interested in very low frequencies between 0.02-4 Hz). The data were subsequently manually inspected to identify bad channels due to poor scalp contact or
Chapter 2: Methodology

High/fluctuating impedance. In addition, artifacts in the continuous data were manually marked as bad prior to ICA to ensure noise was not included in the calculation. Ocular artifacts (eye blinks and saccades) were removed from the data using biased infomax Independent Component Analysis (ICA (Jung et al., 2000)). The extracted independent components were manually inspected and ocular artefacts were removed by back-projection of all but those components. Channels that were removed were re-inserted with topographic interpolation using spherical splines, which utilises the similar voltage from neighbouring channels due to electrical volume conduction. Semi-automatic artifact detection over specified maximum-minimum values was subsequently performed to increase signal-to-noise ratio, which is reduced by noise caused by external electrical equipment, muscle activity and slow drift (e.g. sweating). For the majority of analyses, the max-min was set to remove artifacts greater than 200µV. In Chapter 3, however, this criteria was reduced to 120µV following thorough exploratory analysis, revealing the best signal-to-noise ratio using this criteria, likely because any deflections exceeding this criteria are likely to reflect artifact outside the range of very low-frequency activity. Following these steps, continuous data were segmented in time according to the research question, averaged across all segments/trials of interest in order to extract signals from background noise, and either subjected to FFT analysis or ERP ‘peak-picking’ analysis. Further details are noted in the individual chapters.

2.5 General analysis strategy

2.5.1 Data cleaning
Data were first examined for extreme outliers. Within the NEAAT sample outliers were defined as ±3 standard deviations from the mean and in the SEND sample outliers were defined as ±3.5 standard deviations from the mean. The discrepancy in definitions is the result of thorough exploratory analysis of the data in both samples, leading to a decision to increase the threshold in the SEND sample, based on their clinical status and younger age, it was deemed that removing outliers below this threshold reduced the sample sizes and removed ‘true’ clinical variance.

Subsequently, the normality of the data was examined using the sktest command in Stata. If data were non-normal, they were transformed. All data were skewed in the NEAAT sample, and so in order to provide a normal distribution for a more successful ordinal cut and to fulfil twin model assumptions data were log-transformed using the optimised minimal skew (through the Inskew0 command in Stata). In the SEND sample, where possible skewed data were entered into non-parametric analyses (that do not hold the normality assumption) or
transformed, using a command based on the ladder and gladder commands in Stata (further detail in relevant chapters).

2.5.2 Covariates

Age and general intelligence (IQ) were considered as covariates on the basis of correlations with the EEG/ERP parameter of interest or a priori hypotheses that they would have an effect.

In the NEAAT study, age and IQ were regressed out (as is uniformly done prior to twin modelling) of the EEG data using Stata due to significant associations with both VLF activity and theta power. Although general cognitive ability is confounded by the presence of psychiatric disorder, given that age and IQ are highly predictive of cognitive performance, regressing out these effects before twin modelling ensures straightforward interpretation; any reported associations between VLF activity and ADHD are independent of general cognitive ability and neurodevelopmental changes, and prevents these influences inflating the effect of shared environment.

Similarly, within the SEND sample, correlations between IQ and age and each of the dependent variables were calculated due to differences in IQ between groups and potential developmental effects on these parameters. When these correlations were significant (noted in the individual chapters), analyses of covariance (ANCOVAs) were conducted, or for correlations age-corrected residuals were calculated. If IQ and/or age were significant as covariates in the ANCOVA they were retained in the analysis. In addition, there were a-priori reasons to control for age in Chapters 7 and 8, which examined face processing ERP components known to change substantially with development (Taylor et al. 2004).

2.5.3 Statistical analysis

2.5.3.1 Twin modeling (Part I)

2.5.3.1.1 Preparation of Data Prior to Model Fitting

Simultaneous analyses of dichotomous and continuous data could not be performed in the Mx program (see below) so both ADHD, which was scored as a dichotomous attribute, and the VLF measure, which was scored as a continuous variable, were modelled as threshold traits. Accordingly, each VLF measure was ordinalised into 5 equal classes in terms of proportions, which should capture most of the information in the continuous data (scored 0-5). The number of classes was selected on the basis of visual inspection and correlations between continuous data and ordinal data above r=.95. The Mx software for structural equation modelling (Neale et al., 2003) was then used to estimate polychoric correlations and genetic model parameters
using maximum likelihood statistics, while correcting for the selected nature of the sample. This is a widely used statistical tool for the analysis of twin data through the interpretation of matrix algebra in the form of a script. With regard to the figures described in the following sections, each path in the diagram can be specified within a matrix. Once all paths have been specified, Mx provides estimates and confidence intervals for each parameter, using an iterative process beginning at a specified starting value for each parameter (Neale et al., 2003).

2.5.3.1.2 Twin correlations
Twin correlations between EEG activity and ADHD were estimated by fitting a constrained correlational model to the observed MZ and DZ data to produce (i) one overall within-twin across-trait correlation regardless of zygosity, i.e. between ADHD and EEG, (ii) the MZ and (iii) the DZ cross-twin within-trait correlation for EEG, and (iv) the MZ and (v) DZ cross-twin cross-trait correlation, by comparing one twin’s ADHD score and the co-twin’s EEG score. A significant cross-twin cross-trait correlation would indicate shared familial factors, and using the MZ: DZ ratio of the DZ cross-twin cross-trait correlation correlations, these aetiological influences can be inferred as genetic or environmental in origin. For example, if the DZ cross-twin cross-trait correlation were greater in MZ twins than DZ twins, this would imply that genetic factors contribute to the phenotypic association.

The MZ and DZ cross-twin correlations were fixed according to the point estimates for ADHD derived from the heritability estimate of a meta-analysis (rMZ= 0.76, rDZ= 0.38; (Faraone et al., 2005), due to the uncertain ascertainment process for twins concordant and discordant for ADHD symptoms (see Section 2.5.3.1.4; (Toulopoulou et al., 2007)).

2.5.3.1.3 Genetic model fitting
A more sophisticated approach to the analysis of twin data is the use of structural equation models that model correlations between variables within individuals and across twins using relationships between observed and latent variables (Rijsdijk and Sham, 2002). Twin model-fitting can be used to estimate (1) the heritability of EEG activity and (2) genetic and environmental correlations of ADHD with EEG activity, through examination of the differences in correlations between MZ (who share 100% of their genes) and DZ (who share 50% of their genes) twin pairs. If genetic influences are an important source of genetic variation, the correlations will correspond to the pattern of genetic relatedness. The variance of EEG activity can be decomposed into additive genetic (A; the sum of effects of the individual alleles at all loci that influence the trait), shared environmental (C; influences that make family members similar) or non-additive (D) effects refer to interactions between alleles at the same (dominance) or different locus (epistasis), and unique environmental (E; influences that make
family members different) components. Of note, C and D influences cannot be estimated in the same twin model, due to confounding effects on the pattern of twin data. In structural equation modeling the correlation between additive genetic factors influencing each twin is set at 1.0 for an MZ pair and 0.5 for a DZ pair. In an ACE model, the correlation between the shared environmental influences is set at 1.0 for each twin pair. In an ADE model, the correlation between the genetic dominance influences on each twin is set to 1.0 for MZ pairs and 0.25 for DZ pairs. There is no correlation between the nonshared environmental components as, by definition, these are independent for each twin. As MZ pairs share the same genes and rearing environment, any difference between them must be due to nonshared environment. This is illustrated in Figure 2-3 (for one variable).

**Figure 2-3:** Path diagrams of a univariate (one variable) ACE model (left) and ADE model (right).

*Note:* The observed value refers to the trait being measured, separately for Twin 1 and Twin 2 (grey rectangles). The variance of this variable is decomposed into three unobserved latent factors (coloured circles, in this case ACE or ADE).

*Abbreviations:* A: additive genetic effects; C: shared environmental effects; D: non-additive genetic effects; E: unique environmental effects

Bivariate twin models further allow the investigation of a relationship between two variables (e.g. ADHD and EEG) using cross-twin cross-trait correlation ratios (e.g. ADHD in twin 1 and EEG in twin 2; see Section 2.3.6.4). Using these correlations within a bivariate twin model allows us to calculate an estimate of the extent to which the effect is genetically or environmentally mediated. Additionally, the extent to which the phenotypic correlation between two variables is due to genetic or environmental factors can also be estimated. The Cholesky decomposition is commonly used in bivariate analysis, although this model is dependent on the order of variables. Accordingly, the mathematically equivalent correlated factors solution is often used, illustrated below in Figure 2-4 (Loehlin, 1996). This calculates the
extent to which each variable is influenced by latent factors A, C and E, and estimates the correlations between A, C and E across variables.

**Figure 2-4:** The correlated factors solution

*Abbreviations:* A: additive genetic effects; C: shared environmental effects; D: non-additive genetic effects; E: unique environmental effects

The parameter estimates from the model can be used to estimate the correlation between the genetic factors for ADHD and EEG activity ($r_g$), which is an index of shared genetic effects between these parameters, and similarly for correlations of unique environmental factors ($r_e$). For example, a genetic correlation of 1 would imply that the same genetic factors influence both variables, whereas a correlation of zero would imply that the variables were genetically independent. As the $r_g$ and $r_e$ correlations do not take into account the heritability of either trait, it is possible for a large genetic correlation to actually explain a very small portion of the observed covariation between these two traits. Combining the information from the $r_g$ and $r_e$ with the heritabilities of each trait, we can establish the genetic ($r_{ph-a}$) and unique environmental ($r_{ph-e}$) contributions to the total phenotypic correlation ($r_{ph}$) between ADHD and EEG activity (Toulopoulou *et al.*, 2007). See Figure 2-5 for the model described in this thesis.

2.5.3.1.4 Selected samples design

The above describes techniques that are typically used to investigate continuous measures in population-based samples. However, the twin model-fitting analysis described in this thesis examines EEG parameters within a sample where ADHD is categorically defined (high ADHD...
versus low ADHD). Accordingly, liability-threshold models were used for both ADHD and EEG variables (Falconer and Mackay, 1996). The liability-threshold models for the dichotomized ADHD phenotype (affected versus non-affected) assume that risk is normally distributed on a continuum and that the disorder occurs only when a certain threshold is exceeded (Neale and Kendler, 1995). Both affected and unaffected individuals were assumed to be part of the same distribution of liability to the disorder, with each individual being either below or above the threshold (see also Section 1.1.1.3).

In addition, because the data are from twins selected for high or low ADHD symptoms scores, rather than a random sample, the heritability of ADHD cannot be estimated. Selected samples are more efficient and can be more powerful when studying low prevalence disorders (Neale et al., 1994), but model fitting analyses will usually require an ascertainment correction. Since selection is based on ADHD and blind to EEG values, the required ascertainment correction will depend only on the model for ADHD. The model parameters for ADHD were fixed to constant values supported by a meta-analysis of 20 studies of ADHD (model 1: $h^2=0.76$, $c^2=0$, $e^2=0.24$; Faraone et al., 2005); univariate twin modelling of DSM-IV based ADHD scores on the Conners’ scores at age 8 in the TEDS sample (model 2: $h^2=0.89$, $c^2=0$, $e^2=0.11$ (Ronald et al., 2008b); and at age 12 in the TEDS sample (model 3: $h^2=0.73$, $c^2=0.13$, $e^2=0.14$; C. Greven, unpublished data). In these models, the variance components for EEG activity, as well as their relationship with ADHD, are free parameters to be estimated from the data (Figure 2-3). The different parameter estimates for ADHD from the different models had no effect on the variance components for EEG activity and thus only those of the first model are reported. This method has been applied successfully in a number of studies (Hall et al., 2007a, Hall et al., 2007b, Rijsdijk et al., 2005, Toulopoulou et al., 2007). In addition, the ADHD prevalence rate was fixed to a lifetime risk of 5% as indicated in epidemiological studies (Polanczyk et al., 2007). The applied ACE bivariate correlated factors liability-threshold model used in Part I of this thesis is illustrated in Figure 2-5.
Figure 2-5: Constrained correlated factors bivariate model for ADHD and EEG activity

Note: Circles represent latent additive genetic (A), shared environmental (C) and non-shared environmental (E) factors. a1, a2, e1, e2, c2 represent genetic and environmental path estimates. rg and re represent genetic and environmental correlations between ADHD and VLF activity. Parameters for ADHD (heritability and unique environmental estimates) are fixed values according to meta-analysis (Faraone et al., 2005).

2.5.3.2 Specificity analyses (Part II)

2.5.3.2.1 Analysis of variance (ANOVA)
All analyses described in Part II were conducted in SPSS 15 (SPSS, Chicago, Ill). Statistical analyses were performed on the EEG and ERP data using analysis of variance (ANOVA) models. The within-subjects factor was the EEG/ERP parameter/s of interest. In order to evaluate the utility of this method to dissociate clinical groups and elucidate the basis of comorbidity, the between-subjects factor was defined in two ways: (1) a comparison of 4 groups of ASD-only, ADHD-only, ASD+ADHD and TD to assess differences between pure and comorbid groups; (2) 2x2 comparisons with ADHD (ADHD/ASD+ADHD) and ASD (ASD/ASD+ADHD) to examine the interaction between the disorders. A non-significant interaction between the disorders is compatible with an additive model.
Across these analyses, diagnostic tests included checking for equality of error variances (Levene’s test), equality of covariance matrices (Box’s test) and sphericity (Mauchly’s test). If the assumption of sphericity was violated Greenhouse-Geisser corrected degrees of freedom were used and are reported to the nearest degree of freedom. Where data were skewed and could not be successfully transformed or entered into non-parametric analysis (Chapters 7 and 8), multivariate ANOVA is relatively robust to small violations of non-normality, as long as the skew is not caused by univariate or multivariate extreme outliers (Tabachnick and Fidell, 2001). By removing extreme outliers at the start of the analysis procedure it was possible to employ multivariate approaches to overcome any potential problems. No more than three outliers were removed for each variable, thus reduced power was not an issue.

2.5.3.2.2 Post-hoc tests
Sidak correction is a modification of the Bonferroni technique (using t-tests with a modified alpha correction whereby each contrast is tested at the \(1 - (1-\alpha)^{1/k}\) where k=number of comparisons), which has less impact on statistical power due to, for example, small sample sizes (Keppel and Wickens, 2004, Šidák, 1967). I used Tamhane correction that caters for unequal variance (Field, 2011).

2.5.3.2.3 Effect size
Due to the relatively small sample, effect sizes (Cohen’s d) are presented for case control differences in each EEG/ERP measure. These are interpreted according to Cohen (1988) where 0.2 is indicative of a small effect, 0.5 a medium and 0.8 a large effect size (Cohen, 1988). Other than group effects only significant effects are reported in the results sections for ease of interpretation. The results of partial eta-squared calculations (conducted in SPSS) demonstrated no moderate or large effect sizes for other potential outcomes of task manipulations (<.06; Cohen, 1988), suggesting null findings were not a result of limited power.

2.5.3.2.4 Dimensional analyses
In order to limit the number of comparisons, Spearman’s correlations were run between EEG/ERP parameters, symptom scores and performance measures where significant group differences were found, with age partialled out where appropriate. Due to a highly skewed distribution on the Conners, and moderate correlations between scores on the SCQ and Conners rating scales (SCQ-Conners Inattention: \(r=.41, p<.001\); SCQ-Conners Hyperactivity-Impulsivity: \(r=-.46, p<.001\)), rating-scale-corrected residuals (i.e. for analysis on the SCQ the Conners score was controlled, for analysis on the Conners the SCQ score was controlled) were entered into the correlation analysis, in order to control for the effect of measures that are correlated with both ASD and ADHD. For symptom scores, correlations that were significant
across groups were taken forward for hierarchical multiple regression, in order to ascertain the relative contribution of each measure in predicting the EEG measure while controlling for the other. A hierarchical method allows the specification of a fixed order of entry for variables in order to control for the effects of covariates or to test the effects of certain predictors independent of the influence of others. For each EEG variable, the first regression model entered age in the first block ensuring it is controlled for (if it was a significant confounder), SCQ scores in the second block and Conners scores in the third block. Subsequently, a second regression model was applied entering Conners scores in the second block and SCQ scores in the third block. For performance measures, correlational analyses were carried out across both groups, and repeated for the case and control groups separately to identify any differences in the relationship between variables which may have been associated with clinical status. Significant differences in the strength of the association (i.e., size of the correlations) by group were tested using a Fisher z transformation. Throughout Chapters 5 to 8, dimensional analyses often use calculated difference scores based on where the effects were seen. For ease of interpretation, these have been calculated to indicate abnormality as a negative correlation with the effect of interest.

A significance level of p<0.05 (two-tailed) was adopted throughout the analyses described in this thesis, with trends (p<.1) also reported. Further individual details on statistical analysis are described in the individual chapters.
Part I: Aetiological overlap between ADHD symptoms and EEG measures

PART I

Investigating the aetiological overlap between

ADHD symptoms and quantitative EEG measures
3.1 Summary

ADHD is a common and highly heritable neurodevelopmental disorder with a complex aetiology. The identification of candidate intermediate phenotypes that are both heritable and genetically linked to ADHD may facilitate the detection of susceptibility genes and elucidate aetiological pathways. Very low-frequency (VLF; <0.5Hz) EEG activity represents a promising indicator of risk for ADHD, but it is currently unclear whether it is heritable or genetically linked to the disorder. Direct-current (DC)-EEG was recorded during a cognitive activation condition in 30 monozygotic and dizygotic adolescent twin pairs concordant or discordant for high ADHD symptom scores, and 37 monozygotic and dizygotic matched-control twin pairs with low ADHD symptom scores. Structural equation modelling was used to quantify the genetic and environmental contributions to the phenotypic covariance between ADHD and VLF activity. High ADHD symptoms were significantly associated with reduced VLF power during cognitive activation, which suggests reduced synchronisation of widespread neuronal activity. VLF power demonstrated modest heritability (0.31) and the genetic correlation (-0.80) indicated a substantial degree of overlap in genetic influences on ADHD and VLF activity. VLF activity is a potential candidate intermediate phenotype of ADHD, which warrants further investigation of the neurobiological and genetic basis of VLF activity.

3.2 Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most prevalent neurodevelopmental disorders and is characterised by impairing levels of inattentive, impulsive and hyperactive symptoms. ADHD persists beyond childhood in around 65% of cases and is associated with high levels of clinical, psychosocial and economic burden (Faraone et al., 2006, Kendall et al., 2008). Family and twin studies suggest ADHD is under substantial genetic influence, with an average heritability estimate of 0.76 (Faraone et al., 2005). Candidate gene association studies support the role of genetic factors; however these studies have shown inconsistency and non-replication of findings, which suggests a complex genetic inheritance with a small risk conferred by individual genetic variants (Faraone et al., 2005). See Chapter 1, Section 1.1.1 for further detail.
One strategy to facilitate the detection of susceptibility genes and elucidate aetiological pathways is the identification of neurobiological processes that underlie the disorder and potentially mediate between genes and behaviour (Gottesman and Gould, 2003). In order to be useful for genetic research, these intermediate phenotypes must be associated with the disorder, be heritable and share genetic effects with the disorder (see Section 1.3.1). Several candidate endophenotypes have been reported for ADHD (Kuntsi et al., 2006a, McLoughlin et al., 2005). Electrophysiological abnormalities as measured by electroencephalography (EEG), in particular, are among the most promising indicators of increased genetic risk for ADHD with consistent associations and moderate to high heritability (Section 1.3).

Investigations of brain function in ADHD have recently extended to include very low-frequency activity (VLF; <.05 Hz) that can be measured using direct-current (DC)-coupled EEG recordings. Spontaneous VLF fluctuations synchronise activity across functionally specific but widespread distributed neural networks, demonstrating a high degree of coherence within these circuits (Balduzzi et al., 2008, Buszaki and Draguhn, 2004, Fransson, 2005, Vanhatalo et al., 2004). It has also been suggested that VLF activity represents a reflection of the brain’s default-mode network (DMN) that is typically characterised by a low-frequency BOLD signal (Fox et al., 2005, Sonuga-Barke and Castellanos, 2007). Specifically, based on findings from fMRI studies, VLF fluctuations are posited to reflect toggling between two anti-correlated brain networks: a task-negative network that is active during wakeful resting states and characterised by these slow oscillations, and the task-positive network that activates during goal-oriented activity (Fox et al., 2005, Sonuga-Barke and Castellanos, 2007). In addition, EEG studies have reported that VLF activity modulates the activity of higher frequency bands, suggesting regulation of gross cortical excitability (Monto et al., 2008, Vanhatalo et al., 2004). During an active task condition, slow cortical activity can also be generated in response to stimuli, and as such reflect event-related rather than spontaneous activity. These time-locked slow cortical potentials, such as the contingent negative variation (CNV), have been posited as an index of conscious perception (He and Raichle, 2009), and are important to consider in the investigation of VLF activity during task conditions (see also Chapter 6 for further discussion of the CNV).

Abnormalities in VLF activity are associated with several neuropathological disorders (Broyd et al., 2009). Adults with attentional problems demonstrated reduced spontaneous VLF power and reduced rest-to-task VLF attenuation (Helps et al., 2009, Helps et al., 2008), which was replicated in a sample of adolescents with ADHD (Helps et al., 2010). In addition, reduced rest-to-task VLF attenuation has been associated with poor task performance (Helps et al., 2010) and similar slow fluctuations in task performance (Helps et al., 2009, Monto et al., 2008),
which may underlie the deficits in task performance exhibited in ADHD (Castellanos et al., 2005). This is supportive of the default-mode interference (DMI) hypothesis, which proposes that VLF activity usually exhibited at rest persists during cognitive activation in ADHD producing periodic lapses of attention (Fox et al., 2005, Sonuga-Barke and Castellanos, 2007). Measurement of VLF activity during a cognitive task allows investigation of this brain-behaviour association and its persistence during activation states that are altered in ADHD (Sergeant, 2000).

Twin research conducted on frequency bands above 1Hz suggests that EEG is highly heritable (Smit et al., 2005b). A preliminary study of affected sibling pairs with ADHD indicated high sibling correlations of 0.53 to 0.76 during a cognitive activation condition (Loo and Smalley, 2008) though smaller estimates (0.22 to 0.61) have been reported in a larger study of multiplex families with ADHD (Loo et al., 2010). These studies have used family designs that are unable to discriminate between genetic and environmental influences. The twin design however allows separation of these effects by utilising the different levels of genetic relatedness between monozygotic (MZ; 100%) and dizygotic (DZ; 50%) twin pairs (Neale and Cardon, 1992).

No twin study on VLF activity or its genetic overlap with ADHD has been conducted to date. This study aims to evaluate VLF activity as a potential intermediate phenotype of ADHD by 1) estimating the heritability of VLF activity 2) quantifying the strength of the phenotypic relationship of VLF activity with ADHD symptoms and 3) examining the genetic and environmental overlap with ADHD symptoms. We measured VLF activity during a cognition activation condition (cued continuous performance test (CPT-OX); (Doehnert et al., 2010, McLoughlin et al., 2010, Valko et al., 2009) in adolescent MZ and DZ twin pairs (12-15 years old) concordant and discordant for high and low ADHD symptom scores. Structural equation modelling was applied enabling separation of the phenotypic covariance between these two parameters (i.e. ADHD and VLF activity) into genetic and environmental components (Toulopoulou et al., 2007). Significant heritability and genetic overlap between ADHD and VLF activity would support this measure as a candidate endophenotype for the disorder, reflecting mediating processes on ADHD (Gottesman and Gould, 2003) or pleiotropic effects of genes (Kovas and Plomin, 2006).
Chapter 3: Genetic overlap between ADHD symptoms and VLF EEG activity

3.3 Methods

3.3.1 Sample
The Neurophysiological Study of Activity and Attention in Twins (NEAAT) subset of the TEDS sample used in this study consisted of 67 male twin pairs in groups of 22 pairs concordant for high levels of ADHD symptoms (MZ: 11; DZ: 11), 8 pairs discordant for ADHD symptoms (MZ: 2; DZ: 6) and 37 control pairs concordant for low levels of ADHD symptoms (MZ: 21; DZ: 16). The sample is described in full in Section 2.2.1 and demographic characteristics are shown in Table 2-1. Twin pairs were selected based on an analysis of symptom development over time using the program MPLUS. This selection process is described in Section 2.2.1, Chapter 2.

3.3.2 Task and stimuli
The cued-continuous performance test (CPT-OX; flanker version; (Doehnert et al., 2010, Doehnert et al., 2008, McLoughlin et al., 2010, McLoughlin et al., 2011a, Valko et al., 2009) consists of a black letter array formed of a centre letter flanked on each side by distractor letters, presented in four identical blocks of 100 letter arrays each. Subjects were instructed to ignore the distractor letters and attend only to the centre letter. There were 11 different centre letters (O, X, H, B, C, D, E, F, G, J and L) subtending approximately 0.5 degrees. Target centre letters ‘X’ and ‘O’ were flanked by the incompatible letter ‘O’ or ‘X’ and distractor letters were flanked by either ‘X’ or ‘O’. The 80 cues (XOX) initiated 40 cue-target (XOX-OXO) and 40 cue non-target sequences (XOX-XDX). In 40 cases, a distractor-X letter array (OXO) was not preceded by the cue and had to be ignored, as well as any other irrelevant letters. Subjects were instructed to respond only to cue-target sequences (XOX-OXO) by pressing a button as quickly as possible with the index finger of their preferred hand. The task was practised and comprehension ascertained based on correct performance prior to task onset. The letter arrays were presented briefly (150ms) every 1.65s in a pseudo-random sequence at the centre of a computer monitor at the viewing distance of 120cm. Duration of the task was 11 minutes.
3.3.3 Scoring overt performance

Performance measures in the cued CPT task included target reaction time (MRT, mean latency of responding in ms after target onset), within-subject variability in reaction times (SD-RT), and the coefficient of reaction time variability, which is an estimate of variability that controls for differences in individual reaction time (CV, SD-RT/MRT). Previous work has shown high phenotypic correlations between SD-RT and MRT, which also appear to load onto a single familial factor (Andreou et al., 2007, Kuntsi et al., 2010). MRT and SD-RT were calculated across correctly answered target trials. Hits were characterized by target-OXOs that were detected between 200 and 1500 ms after stimulus onset. False alarms were responses to letters other than target-OXO. Errors were broken down into subcategories (omission errors, total commission errors, and O-not-X commission errors).

3.3.4 EEG recording and analysis

See Section 2.4.2, Chapter 2, for details of EEG data acquisition and general preprocessing. Data were analysed in Brain Vision Analyzer (2.0). The signal was re-referenced offline to the average reference and downsampled to 256Hz. We applied 0.02-4Hz (12dB/Oct) Butterworth filters in order to capture the VLF power and exclude higher frequencies and the artefact associated with them. Continuous EEG was segmented into 50 second epochs. Segments with artifacts exceeding 120µV peak-to-peak in any channel were rejected. A DC-Detrend command was executed to remove linear drifts from the data. Fast-Fourier Transform analysis was performed on the data from each of the electrodes for each participant. 50-second Hanning windows were used to capture VLF power as slow as 0.02Hz and power was calculated. The current study focused on the frequency band 0.02-0.2Hz that has been implicated previously in ADHD (Helps et al., 2010). In order to allow comparison with other studies investigating familial effects on higher frequency bands in ADHD (Loo et al., 2010), VLF power was
compared across frontal (Fz, F3, F4), central (Cz, C3, C4), parietal (Pz, P3, P4) and, in addition, occipital (Oz, O1, O2) scalp electrode locations. The contingent negative variation (CNV), a slow cortical potential, is elicited after the cue stimulus in the CPT-OX, and was therefore analysed in order to investigate potential effects of event-related VLF activity. Please see Section 6.3.3, Chapter 6, for a full description of the CNV.

3.3.5 Statistical analysis

3.3.5.1 Data preparation
Two participants were excluded from analysis due to excessive artifact (DZ control) and extreme commission errors (n=37) indicative of insufficient task engagement (MZ ADHD). For the analysis of performance data, the effects of age and general intelligence were regressed out of the data using Stata (see Section 2.5.3.2). The effects of age and general intelligence were regressed out of the data using Stata due to significant associations with both ADHD (Table 2-1 and Section 2.5.2) and VLF activity (age $r = -0.12$ to $-0.19$; $g r = -0.26$ to $-0.33$).

3.3.5.2 Data analysis
The comparisons of mean values were analyzed by means of a regression command in Stata that allows for non-independent observations (e.g. twin pairs) by using a robust cluster command to estimate standard errors. The association between the EEG activity and ADHD symptom scores, and EEG activity and performance measures, was investigated using Pearson’s product moment correlation coefficient on transformed residuals. Performance measures for correlation analysis were selected on the basis of trends toward case-control differences.

Twin correlations and twin model fitting were computed using the Mx program (see Section 2.5.3.2). In order to correct for the selected sample, model parameters for ADHD were fixed to constant values supported by a meta-analysis of 20 studies of ADHD (model 1: $h^2=0.76$, $c^2=0$, $e^2=0.24$; Faraone et al., 2005).

3.4 Results

3.4.1 Results from the regression and correlation analyses
Regression analyses indicated no significant differences between ADHD and control twins for cognitive performance measures (Table 3-1). VLF power was highest in central regions and was significantly reduced for central and parietal locations in the ADHD group, with the strongest association with central locations (Table 3-2). Therefore, to reduce the total number of
variables and subsequent multiple testing bias. VLF power at central regions was used in correlations between symptom scores and performance measures, and in twin modelling analyses in order to minimise heteroscedascity. Significant associations between VLF power and symptom scores were found in the control sample only (Table 3-3), suggesting increased symptoms of inattention and hyperactivity/impulsivity are associated with increased VLF power in typically developing adolescents. Reduced VLF activity was significantly associated with increased response variability in the ADHD group only (Table 3-3). All other associations were non-significant. I also conducted correlation analysis with Sidak correction to assess the association between VLF power and higher frequency bands, which revealed a positive correlation between VLF power and delta power (1.5-3.5Hz; r=0.42, p<.05), with no further significant associations. In addition, there was no significant association between VLF power and the event-related CNV (r=0.22, p>.05), although without multiple testing correction this association was significant.
Table 3-1: Summary statistics of raw scores and mean comparisons adjusted for genetic relatedness for task performance measures in the NEAAT sample

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MRT (msec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n=44)</td>
<td>411.36</td>
<td>60.04</td>
<td>1.27</td>
<td>.21</td>
</tr>
<tr>
<td>DZ Control (n=38)</td>
<td>379.18</td>
<td>47.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n=23)</td>
<td>441.30</td>
<td>75.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZ ADHD (n=28)</td>
<td>407.93</td>
<td>56.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SD-RT (msec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n=44)</td>
<td>95.27</td>
<td>42.46</td>
<td>1.88</td>
<td>.06</td>
</tr>
<tr>
<td>DZ Control (n=38)</td>
<td>77.08</td>
<td>30.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n=23)</td>
<td>128.09</td>
<td>64.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZ ADHD (n=28)</td>
<td>102.00</td>
<td>38.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CV (SD-RT/MRT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n=44)</td>
<td>0.23</td>
<td>0.08</td>
<td>1.95</td>
<td>.06</td>
</tr>
<tr>
<td>DZ Control (n=38)</td>
<td>0.20</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n=23)</td>
<td>0.28</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZ ADHD (n=28)</td>
<td>0.25</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Commission errors (n)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n=44)</td>
<td>2.64</td>
<td>2.64</td>
<td>1.21</td>
<td>.22</td>
</tr>
<tr>
<td>DZ Control (n=38)</td>
<td>3.79</td>
<td>3.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n=23)</td>
<td>4.78</td>
<td>4.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZ ADHD (n=28)</td>
<td>3.79</td>
<td>4.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>O-not-X commission errors (n)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n=44)</td>
<td>1.91</td>
<td>2.63</td>
<td>-0.69</td>
<td>.49</td>
</tr>
<tr>
<td>DZ Control (n=38)</td>
<td>2.32</td>
<td>2.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n=24)</td>
<td>2.74</td>
<td>3.40</td>
<td></td>
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<tr>
<td>DZ ADHD (n=28)</td>
<td>2.39</td>
<td>3.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Omission errors (n)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n=44)</td>
<td>0.68</td>
<td>0.86</td>
<td>1.43</td>
<td>.16</td>
</tr>
<tr>
<td>DZ Control (n=38)</td>
<td>0.92</td>
<td>1.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n=22)</td>
<td>1.87</td>
<td>1.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZ ADHD (n=28)</td>
<td>1.82</td>
<td>2.58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Statistical analysis based on age- and IQ-regressed scores. Abbreviations: MRT: mean reaction time in milliseconds; SD-RT: within-subject variability in RTs in milliseconds; CV: coefficient of variation (SD-RT/MRT); MZ: monozygotic; DZ: dizygotic.
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| Table 3-2: Summary statistics and mean comparisons adjusted for genetic relatedness for VLF power (in µV²) |
|--------------------------------------------------|------------------|------------------|--|------------------|
| | Mean | SD | t | p |
| VLF frontal | | | | |
| MZ Control (n=43) | 4.36 | .36 | -1.88 | .07 |
| DZ Control (n=37) | 4.36 | .40 | | |
| MZ ADHD (n=21) | 4.10 | .45 | | |
| DZ ADHD (n=27) | 4.28 | .48 | | |
| VLF central | | | | |
| MZ Control (n=44) | 5.29 | .18 | -3.03 | .003 |
| DZ Control (n=37) | 5.28 | .19 | | |
| MZ ADHD (n=20) | 5.17 | .14 | | |
| DZ ADHD (n=26) | 5.18 | .17 | | |
| VLF parietal | | | | |
| MZ Control (n=44) | 4.92 | .27 | -2.25 | .03 |
| DZ Control (n=37) | 4.87 | .23 | | |
| MZ ADHD (n=21) | 4.79 | .33 | | |
| DZ ADHD (n=27) | 4.74 | .27 | | |
| VLF occipital | | | | |
| MZ Control (n=44) | 4.34 | .46 | 1.99 | .05 |
| DZ Control (n=38) | 4.28 | .37 | | |
| MZ ADHD (n=21) | 4.20 | .51 | | |
| DZ ADHD (n=26) | 4.10 | .38 | | |

Note: Scores and analysis based on transformed age- and IQ-regressed scores with extreme outliers removed.

Abbreviations: VLF: very low frequency EEG power; MZ: monozygotic; DZ: dizygotic.
Table 3-3: Phenotypic correlations between VLF power at central scalp locations, symptoms of ADHD and task performance measures

<table>
<thead>
<tr>
<th></th>
<th>Whole sample</th>
<th>All controls</th>
<th>All ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MZ</td>
<td>DZ</td>
<td>MZ</td>
</tr>
<tr>
<td>Inattention</td>
<td>0.05</td>
<td>0.41**</td>
<td>0.19</td>
</tr>
<tr>
<td>Hyperactivity/Impulsivity</td>
<td>0.07</td>
<td>0.44**</td>
<td>0.34**</td>
</tr>
<tr>
<td>SD-RT</td>
<td>-0.02</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>CV</td>
<td>-0.02</td>
<td>0.08</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Note: Pearson’s product moment correlation on transformed age and IQ-regressed scores. ADHD symptom scores based on the Long Version of the Parent Conners’ Rating Scale (Conners et al., 1998a) collected on the day of testing.

Abbreviations: ADHD, attention deficit hyperactivity disorder; CV: coefficient of variation (SD-RT/MRT); DZ: dizygotic; MRT: mean reaction time in milliseconds; SD-RT: within-subject variability in RTs in milliseconds; MZ: monozygotic; **p<.01, *p<.05, †p<.1

3.4.2 Results from the twin modelling analyses

The MZ cross-twin within-trait correlation for VLF power (r=0.37; 95%CI, 0.002 to 0.64) was greater than the DZ cross-twin within trait correlation for VLF power (r=0.21; 95%CI, -0.21 to 0.56) which suggests that genetic effects contribute to VLF power. As the MZ correlation for VLF power deviated from 1, this suggests that unique environmental influences (which include measurement error) contribute substantially to VLF power. Further, the DZ cross-twin within-trait correlation for VLF power was slightly more than half the MZ correlations, suggesting the presence of shared environmental effects. The MZ cross-twin cross-trait correlation between ADHD and VLF power (r= -0.42; 95%CI, -0.65 to -0.09) was greater than the DZ cross-twin cross-trait correlation (r=-0.14; 95%CI, -0.39 to 0.13), suggesting genetic effects contribute to the association between reduced VLF power and ADHD. The DZ cross-trait cross-twin correlation is less than half the MZ correlation, suggesting that non-additive genetic dominance effects may contribute to this overlap.

Structural equation modelling indicated that genetic factors accounted modestly for the total variation in VLF power (h²=0.31; 95%CI, 0.01 to 0.64). Shared environment did not significantly explain individual differences in VLF power (c²=0.06; 95%CI, 0 to 0.44), whereas unique environmental effects (incorporating also measurement error) accounted for a moderate part of the variance in VLF power (e²=0.63; 95%CI, 0.38 to 0.92). The extent to which ADHD and VLF
activity share the same genetic and unique environmental effects is given by the correlations $r_g$ and $r_e$, respectively. Significant genetic correlations ($r_g$=-0.80; 95%CI, -1.00 to -0.15) indicated substantial genetic overlap between ADHD and reduced VLF power. Unique environmental effects did not have a significant overlap with VLF power ($r_e$=0.41; 95%CI, -0.61 to 0.96). The negative phenotypic correlation ($r_{ph}$) suggested that increased liability to ADHD was associated with reduced VLF power (-0.23; 95%CI, -0.44 to -0.01). Due to the moderate to high heritabilities and moderate genetic correlation, the phenotypic correlation between ADHD and VLF power appears to be largely attributable to genetics ($r_{ph-a}$= -0.39; 95%CI, -0.60 to -0.08). Unique environmental contributions to phenotypic variance were non-significant ($r_{ph-e}$= 0.16; 95%CI, -0.24 to 0.42).

### 3.5 Discussion

This study aimed to evaluate very low-frequency (VLF) neuronal activity as an intermediate phenotype of ADHD in a sample of adolescent monozygotic and dizygotic twin pairs concordant and discordant for ADHD symptoms. Genetic analyses showed that VLF activity demonstrates modest heritability, with no evidence of significant shared environmental effects. Structural equation modelling revealed a significant phenotypic association between high ADHD symptom scores during adolescence and reduced VLF activity during cognitive activation. Genetic factors were the main source of this association and unique environmental factors were not significant. This is the first study to support VLF activity as an electrophysiological marker of genetic risk in ADHD.

Heritability of VLF activity during a cognitive activation condition is consistent with reports of high heritability (Smit et al., 2005b) and high sibling similarity in an ADHD sample (Loo et al., 2010) in higher frequency bands. This suggests that VLF activity has a genetic basis combined with a moderate contribution from unique environmental effects. The substantial genetic correlation between ADHD and VLF activity indicates that they are substantially influenced by the same genes, supporting VLF activity as a putative intermediate phenotype of the disorder. This finding is an important step in understanding the neurobiological pathways involved in the disorder and potentially to facilitate in the detection of susceptibility genes. For example, findings suggest a catecholaminergic deficiency underlies abnormal VLF oscillations, which is also widely reported in ADHD (Castellanos et al., 2005). Indeed methylphenidate, which is used as a treatment for ADHD, blocks pre-synaptic dopamine transporter transmission and has been found to modulate slow oscillations in subcortical structures (Ruskin et al., 2001). Causal tests of mediation are necessary to identify if these electrophysiological markers mediate...
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aetiological effects on ADHD, rather than pleiotropic (or epiphenomenal) processes (Walters and Owen, 2008).

As noted in Section 1.3.1, in order to fulfil criteria as a candidate intermediate phenotype the marker in question should be heritable, and some researchers advocate the marker to be more heritable than the disorder in question. In this chapter, VLF power shows a relatively low heritability compared to other EEG parameters and ADHD. Reliability sets an upper limit on the estimates of heritability; any deviations from perfect reliability will increase measurement error and therefore unique environmental influences (Kuntsi et al., 2006c). Studies of test-retest reliability are therefore required to investigate the stability of both phenotypic associations and genetic influences on VLF activity (de Geus, 2002). A low heritability estimate may alternatively suggest that genes do not affect variation in the measure, and as such there is limited use in linking genes to the measure (Cannon & Keller 2006). This calls into question the validity of VLF considering the high heritability of ADHD. Still, such markers may be useful for the identification and development of behavioural interventions (Cannon & Keller 2006). While the low heritability therefore throws doubt on the use of VLF as a candidate intermediate phenotype for genetic studies, it nevertheless shows that it has a biological basis and importantly shares substantial genetic influences with the disorder in question, which is essentially an important step beyond heritability. Future work should investigate other potential indices of VLF activity, and also explore the heritability of other EEG markers of ADHD such as increased theta power (see Chapter 4).

The phenotypic association between ADHD and VLF activity is consistent with previous studies reporting reduced VLF power at rest in ADHD (Helps et al., 2010, Helps et al., 2008). In addition, previous studies report an association between increased rest-to-task VLF attenuation and a higher number of inattention symptoms in typical adults (Helps et al., 2009) and a clinical ADHD group (Helps et al., 2010). In the present study, higher VLF activity was associated with increased levels of inattention and hyperactivity/impulsivity in the control group. Such discrepancies between control and ADHD participants may reflect neuropathological differences between groups, and also the robustness of the longitudinal method employed for group selection, compared to investigating symptom scores at the single time-point of data collection. In addition, it has been proposed that two attentional states can be defined that predict the relationship between the DMN and task performance: increased DMN activity associated with a less error-prone state versus a more effortful mode of processing characterised by reduced DMN activity (Esterman et al., in press). This may explain the differing correlations between VLF activity and ADHD symptom scores (controls
versus ADHD respectively) although the association between the DMN and VLF activity needs to be clarified (see below).

Overall, the findings for both groups support spontaneous VLF activity, typically associated with resting periods as exhibited by slow fluctuations of the BOLD signal, as present during cognitive activation, suggesting it represents a continuous process that is present during both resting and goal-oriented periods (Fransson, 2006). VLF activity are proposed to be an index of information integration or functional connectivity through synchronisation over widely distributed neuronal networks (Biswal et al., 2005, He and Raichle, 2009). The association between ADHD and reduced VLF power in this study may therefore reflect reduced synchronisation of these widespread circuits. As VLF activity is proposed to relate to the DMN, one of several widely distributed networks as defined by fMRI (Sonuga-Barke and Castellanos, 2007), these findings suggest that individuals with ADHD demonstrate reduced DMN synchronisation during cognitive activation, or abnormal toggling between the anti-correlated introspective task-negative and extroceptive task-positive networks producing impairment to the functions they each serve (Fox et al., 2005, Fransson, 2005, Sonuga-Barke and Castellanos, 2007). A restricted length of resting state and excessive artefact associated with VLF recording during resting state limited the reliability of the data for use in this study. The focus on VLF power during the task condition limits the interpretation of the findings in relation to default-mode (VLF) interference. Importantly, therefore, future work should investigate VLF power at rest and change from rest-to-task using prolonged resting conditions.

The measurement of VLF activity during this cognitive task may include both spontaneous VLF activity and event-related VLF slow cortical potentials time-locked to the cue stimulus (the CNV). Although in this particular study there is limited evidence for a relationship between spontaneous VLF activity and event-related VLF activity, it is still important that future work considers this relationship, particularly as without correction for multiple testing there is a small yet significant correlation between these measures. Along with the significant correlation between VLF activity and delta activity, such phenotypic overlap may suggest that the genetic contributions reported may be shared across frequency bands. In accordance, VLF phase has been shown to correlate with the magnitude of higher frequency bands suggesting a modulation of gross cortical excitability (Monto et al., 2008, Vanhatalo et al., 2004). Further work investigating phase locking with higher frequency bands as a measure of synchronisation will increase our understanding of the mechanisms underlying this overlap, particularly when used in genetically sensitive designs.
Advances in EEG source localisation techniques are likely to provide further insight into the function of VLF activity. Recent work using sLORETA identified differential localisation of rest-task VLF attenuation dependent on ADHD status, in the absence of case-control differences in rest-task attenuation itself (Broyd et al., 2011). This highlights potential topographical and source differences between control and clinical populations. In accordance with this, in the current study we identified group differences in VLF activity at central and parietal scalp locations only. Nevertheless, trends toward reduced VLF power were observed across the scalp regions investigated. Localisation differences may reflect the measure of VLF activity used (i.e. a comparison of VLF activity between rest and task, as opposed to VLF activity during the task itself) and task-specific demands that particular brain processes/regions subserve. Examination of changes over time and space in VLF activity in well-validated and robust EEG/ERP paradigms is required, in order to further understanding of the relationship between the DMN and VLF activity.

For measures of task performance, phenotypic associations with ADHD were non-significant. This is contrary to findings reporting slower and more variable reaction-times in ADHD (Klein et al., 2006, Kuntsi et al., 2010) and increased omission errors (Willcutt et al., 2005). The lack of a group difference in the performance data is likely to be due to the use of the CPT-OX with flankers which was not specifically designed to optimally measure cognitive performance. Another possible reason for our lack of cognitive performance findings is our use of a population sample, rather than a clinical sample, albeit one selected for low and high ADHD symptom scores. One study reported increased RT variability in a population sample using ADHD symptom scores (similar to the study design here), but the data was collected at a younger age, in a larger sample and using task conditions that maximise RT variability, such as a slow event rate and lack of rewards (Kuntsi et al., 2009). The greater association between ADHD and neurophysiological markers compared to task performance suggests EEG is a more sensitive index of genetic loading for ADHD than the cognitive performance measures, at least as derived from the task used in this study.

According to the default-mode interference hypothesis, a failure to fully attenuate VLF activity in cognitive activation conditions contributes to attentional lapses associated with ADHD (Sonuga-Barke and Castellanos, 2007). VLF activity was not associated with task performance measures in this study across the whole sample, but when collapsing the sample by ADHD status and zygosity, significant associations were found between reduced VLF power and increased response variability in the ADHD group only. This suggests reduced synchronisation during a task is associated with poor performance in ADHD, and is in accordance with previous
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Studies that report associations between rest-task attenuation and increased errors and variability (Helps et al., 2010). Further work incorporating analysis of trial-to-trial changes in the synchronicity between task performance and VLF activity is required (Castellanos et al., 2005, Helps et al., 2009) and linking this to optimal measures of RT variability using spectral analyses will help to clarify this association.

Certain limitations must be taken into consideration. Firstly the sample size is relatively small for a twin study, such that although the estimates are modest to large, the confidence intervals around these estimates are also large introducing an overlap in the confidence intervals for MZ and DZ twins. This might suggest that the contribution from genetics is not significant; nevertheless combining the significant MZ correlations with the significant mean correlation differences suggests a familial nature that is likely to be of a genetic basis based on the fixed parameters used. This limited statistical power also restricted the estimation of shared environmental (as suggested by cross-twin within-trait correlations for VLF power) and non-additive genetic influences (as suggested by cross-twin cross-trait correlations between ADHD and VLF power). It is acknowledged that such influences may have contributed to the estimates of heritability and genetic overlap between ADHD and VLF activity in our study, although the genetic contribution presented is not overestimated. The substantial contribution of unique environmental influences on VLF activity may reflect error or instability in the measure. Reliability sets an upper limit on the estimates of heritability; any deviations from perfect reliability will increase measurement error and therefore unique environmental influences (Kuntsi et al., 2006c). Studies of test-retest reliability are required to investigate the stability of both phenotypic associations and genetic influences on VLF activity (de Geus, 2002). In addition, the imputation of a relatively substantial proportion of general cognitive ability as a potential confounding influence may introduce further error arising from predicted scores, although the use of a robust multiple-imputation method is likely to have reduced this error variance. As we do not know whether the underlying causes of altered VLF activity are shared or disorder-specific, particularly as abnormalities are reported in several other neuropathological disorders such as autism and schizophrenia (Broyd et al., 2009), it is important that future work directly compares VLF activity profiles across disorders.

In conclusion, applying genetic model fitting for the first time to VLF neuronal activity in ADHD, we have demonstrated modest heritability of VLF activity and substantial genetic overlap between ADHD and VLF activity. This supports the relevance of this novel measure of physiological arousal to our understanding of ADHD and indicates that lower VLF activity during cognitive activation is a potential intermediate phenotype for ADHD. This provides a
Chapter 3: Genetic overlap between ADHD symptoms and VLF EEG activity

basis for investigation of specific genes that influence both ADHD and VLF activity, and warrants the investigation of the underlying mechanisms of VLF activity, in order to provide clues to the pathophysiological underpinnings of ADHD.
4.1 Summary

One of the most consistent and robust neural abnormalities in attention deficit hyperactivity disorder (ADHD) is increased power in the theta band (4-8 Hz), particularly during resting state. The present study used a twin design to estimate the extent of genetic overlap between increased theta power and risk for ADHD in order to validate theta power as an intermediate phenotype of ADHD. EEG was measured during resting and cognitive activation conditions in 30 monozygotic and dizygotic adolescent twin pairs concordant or discordant for high ADHD symptom scores, and 37 monozygotic and dizygotic control twin pairs with low ADHD symptom scores. Structural equation modelling was used to estimate the heritability of theta power and partition the genetic and environmental contributions to overlap between ADHD and theta power. Significant phenotypic correlations between ADHD symptoms and elevated theta power at rest and an increase in task-related change in theta power from resting to cognitive activation were found. Theta power demonstrated moderate to high heritability estimates (resting: 0.77; task-related change: 0.44) and substantial genetic correlations with ADHD (resting: 0.35; task-related change: 0.68) suggesting shared genetic influences. Increased theta power is therefore a potentially valid endophenotype for ADHD, which warrants further investigation of the biological and genetic mechanisms that underlie the genetic relationship.

4.2 Background

Attention deficit hyperactivity disorder (ADHD) is a common childhood-onset neurodevelopmental disorder characterized by deficits in attention and/or hyperactivity and impulsivity (APA, 2000). While family and twin studies demonstrate increased rates of ADHD in closely related family members and high heritability, the findings from molecular genetic studies have yielded small effect sizes (Faraone et al., 2005). This might be explained by the highly complex and heterogenous nature of ADHD, such that a number of different underlying cognitive and neural mechanisms, and multiple genes of small effect result in the behavioural profile (Asherson and Consortium, 2004, Kuntsi et al., 2006b). It is now widely documented that continuously distributed traits underlie the categorical diagnosis of complex disorders, such as ADHD, that may provide more power to identify quantitative trait loci (Chen et al.,
Chapter 4: Genetic overlap between ADHD symptoms and theta power

2008, Levy et al., 1997, Wood and Neale, 2010). Therefore there is increasing interest in identifying quantitative markers of disorders that are associated with the normal variation of these traits. As such, one approach to refine the ADHD phenotype is the identification of quantitative cognitive or neurobiological processes that increase risk for the disorder and mediate between genes and behavior (Castellanos and Tannock, 2002, Doyle et al., 2005b). In order to be viable, the main criteria state that the candidate trait must have good metric properties, be associated with the disorder, heritable, and share genetic influences with the disorder (see Section 1.3.1, Chapter 1 for further detail).

EEG is an index of brain function through measurement of electrical activity at the scalp generated by the underlying brain structures, which can index neural activity both when an individual is engaged in a task or at rest (McLoughlin et al., 2005). Lower EEG frequencies, such as theta (4-8Hz), are associated with reduced arousal, whereas higher EEG frequencies, such as beta (12-20Hz) are associated with alertness. In addition, EEG power represents a continuous measure with no floor or ceiling effects, and lower frequency bands, in particular, show high temporal stability (Smit et al., 2005b) and test-retest reliability (Williams et al., 2005) and therefore represent ideal intermediate phenotypes due to their psychometric properties.

While associations between ADHD and other EEG frequency bands are more variable (see Chapter 5 for further detail), the most consistent EEG abnormality in ADHD is increased low-frequency activity, predominantly high levels of absolute and relative theta in frontal and central regions during resting and cortical activation (Barry et al., 2003a, El-Sayed et al., 2002, Mann et al., 1992, Monastra et al., 2001, Monastra et al., 1999, Quintana et al., 2007, Shi et al., in press, Snyder and Hall, 2006, Snyder et al., 2008). Elevated theta persists into adolescence and adulthood (Bresnahan et al., 1999, Bresnahan and Barry, 2002, Koehler et al., 2009), and sensitivity and specificity rates support theta power as a potential diagnostic tool for ADHD (Quintana et al., 2007, Snyder and Hall, 2006, Snyder et al., 2008). For example, elevated theta is not found in oppositional defiant disorder (ODD), mood disorder, anxiety or conduct disorder, demonstrating specificity for ADHD (Matsuura et al., 1993, Rabiner, 2001). Interestingly, while Ogrim et al. (2012) found no ADHD-control differences in EEG power overall in 7-16 year olds, theta correlated with inattention and executive problems and negatively correlated with hyperactivity/impulsivity on parent rating scales, such that this association goes beyond diagnostic boundaries (Ogrim et al., in press). Taken together, these findings have led to the proposition that theta-indexed cortical underarousal is a robust marker of ADHD.
Chapter 4: Genetic overlap between ADHD symptoms and theta power

Individuals with ADHD demonstrate a wide range of cognitive deficits, which appear to go beyond a single ‘core’ deficit, such as response inhibition (Kuntsi et al., 2001, Willcutt et al., 2005). This supports a more generalized dysfunction or poor state regulation (see Section 1.1.1.4.1), which is further supported by a clear clinical characteristic of individuals with ADHD: frequent lapses of attention and moment-to-moment inconsistency in symptom expression. Response variability in ADHD reflects a greater proportion of slow responses, with fast responses on some trials leading to an overall inconsistent pattern (Leth-Steensen et al., 2000). One proposed measure of this is reaction-time variability (RTV). RTV has been found to best distinguish between ADHD cases and controls compared to mean and error measures (Castellanos et al., 2005, Klein et al., 2006). Associations between these cognitive markers and theta power support hypothesised effects of cortical underarousal on poor task performance, and theoretical proposals of a failure to optimize energetic state in ADHD (Andreou et al., 2007, Kuntsi et al., 2010, Sergeant, 2005). For example, elevated theta has been associated with increased response variability across scalp regions (Loo and Smalley, 2008), oddball accuracy in frontal regions (Hermens et al., 2005), and increased relative theta to several indices of visual attention during an integrated visual-auditory (IVA) CPT in ADHD children (Shi et al., in press).

Vast changes are typically seen in EEG activity from resting to cognitive activation (Barry et al., 2007, Klimesch, 1999, VaezMousavi et al., 2007). The most robust change is a decrease in alpha power or alpha desynchronisation observed from resting state to task-onset or with increasing task demand, combined with an increase in theta power or theta synchronization (Klimesch, 1999). Indeed while theta power as an index of arousal (e.g. at rest) is associated with underarousal, frontal midline attentional theta is thought to reflect cognitive processing (Schacter, 1977) and shows increase or phasic correlation in response to various stimuli (Delorme et al., 2007, Makeig et al., 2002, Onton et al., 2005). It therefore appears necessary to investigate potentially altered task-related changes in EEG in ADHD, or activation versus arousal measures, and it’s relationship to task performance. For example, individuals with ADHD require greater activation (reduced alpha power) over the course of cognitive activation conditions compared to typical controls, proposed as a mechanism to sustain their attention (Loo et al. 2009). It can be hypothesised that task-related activation changes in theta power are present in ADHD and underlie altered task performance. Therefore, findings converge to suggest that theta power is a particularly promising neural marker of the cognitive deficits associated with ADHD.
Accumulating evidence indicates that EEG is highly heritable and associated with specific genetic variants (see Section 1.3.2, Chapter 1). Specifically for theta, an early study of adolescent twin pairs demonstrated heritability of 89% (Van Beijsterveldt et al., 1996). More recently, heritability of theta power has been estimated at a mean of 0.85 in young adults, with a range of 0.82-0.90 across scalp regions (Smit et al., 2005a). In a multivariate analysis of EEG frequency bands, theta heritability was highest in occipital regions (0.85) compared to frontal regions (0.64) (Zietsch et al., 2007).

High heritability combined with consistent associations with ADHD provides rationale for investigation of theta power as an endophenotype for ADHD. Studies to date, however, are limited. A preliminary study of affected sibling pairs with ADHD indicated sibling correlations for absolute theta power were highest in frontal regions during resting (0.57) that increased for a cognitive activation condition (0.69; Loo & Smalley, 2008), supported by, albeit lower, correlations in a larger study of multiplex families with ADHD (resting: 0.25; CPT: 0.30; Loo et al., 2010). Family studies that use mean comparison analyses are not able to partition familial variance into genetic and environmental contributions and the amount of familial overlap is not quantified. Using structural equation modelling in twin samples, however, it is possible to explore whether this association is due to shared genetic influences using the different levels of genetic relatedness between monozygotic (MZ) and dizygotic (DZ) twin pairs (Neale and Cardon, 1992).

The present study aimed to overcome these limitations and validate the use of theta power as a marker for molecular genetic studies of ADHD by demonstrating heritability and genetic overlap with the disorder. Theta power was measured at rest and during a cognitive activation condition used in previous studies (cued CPT-OX; see Chapter 2) in a twin sample selected for consistently high or low ADHD symptoms (see Chapter 2). Based on previous findings, I expected (a) theta power to be moderately to highly heritable, (b) increased theta during resting and altered task-related theta power to be associated with higher ADHD symptom scores, and (c) genetic overlap between these EEG parameters and ADHD symptoms, to support theta power at rest (arousal) and change in theta power from rest-to-task (activation) as candidate endophenotypes for the disorder. In addition, based on previous findings between poor performance and arousal, I predicted an association between task performance, symptom scores and EEG-indexed activation levels (VaezMousavi et al., 2007).
4.3 Methods

4.3.1 Sample
The Neurophysiological Study of Activity and Attention in Twins (NEAAT) subset of the TEDS sample used in this study consisted of 67 male twin pairs in groups of 22 pairs concordant for high levels of ADHD symptoms (MZ: 11; DZ: 11), 8 pairs discordant for ADHD symptoms (MZ: 2; DZ: 6) and 37 control pairs concordant for low levels of ADHD symptoms (MZ: 21; DZ: 16). The sample is also described in full in Section 2.2.1 and demographic characteristics are shown in Table 2-1. Twin pairs were selected based on an analysis of symptom development over time using the program MPLUS. This selection process is described in Section 2.2.1, Chapter 2.

4.3.2 Task and stimuli
EEG recording consisted of a resting condition (eyes open) and a task condition, the cued continuous performance test (CPT-OX; see Section 2.3.2). Resting EEG was collected for three minutes at the beginning of the testing session. The eyes open (as opposed to eyes closed) condition was used in our data analysis as evidence suggests that it is a more suitable baseline for task-activation comparisons (Barry et al., 2005, Loo and Smalley, 2008, VaezMousavi et al., 2007). The CPT-OX was administered as previous family studies of ADHD use this as the cognitive activation condition (Loo and Smalley, 2008, Loo et al., 2010), and the CPT typically shows case-control differences between ADHD and typically developing controls (e.g. Banaschewski et al. 2003). Performance measures in the cued CPT-OX are described in Section 3.3.3 and questionnaire measures used in Section 2.3.4.

4.3.3 EEG recording and analysis

4.3.3.1 EEG recording and pre-processing
See Section 2.4.1 and 2.4.2, Chapter 2, for details of EEG data acquisition and general preprocessing. Data were analysed in Brain Vision Analyzer (2.0). The signal was re-referenced offline to the average reference and downsampled to 256Hz. We applied 0.1–30 Hz (24dB/Oct) Butterworth filters. Ocular artefacts were removed from the data using ICA (ICA). Continuous EEG was segmented into 2s epochs. Segments with artefacts exceeding 200μV peak-to-peak in any channel were rejected.

4.3.3.2 EEG frequency analysis
Fast-Fourier Transform analysis was performed on the data from each of the electrodes for each participant. Two second Hanning windows were used, and absolute power was calculated for the following frequency bands: theta (4-8Hz), alpha (8-12Hz), beta1 (12-16Hz) and beta2
To reduce the number of comparisons, and in order to allow comparison with other studies investigating familial effects in ADHD (Loo et al., 2010), electrodes were grouped by region in the following way: frontal (F1, F2, F3, F4, F5, F6, F7, F8 and Fz), central (C1, C2, C3, C4, C5, C6 and Cz) and parietal (P3, P4, P7, P8 and Pz) scalp electrode locations. Change in EEG from off-to-on task conditions was calculated by subtracting values for theta power during resting from task conditions.

4.3.4 Statistical analysis

4.3.4.1 Data preparation

Two participants were excluded from analysis of the resting due to excessive artifacts (1 DZ ADHD and 1 DZ control; slow drift throughout/removal of >10 electrodes) and an additional participant from the CPT-OX due to extreme commission errors \((n = 37)\) indicative of insufficient task engagement (MZ ADHD).

Preparation of EEG data prior to model fitting was the same as that outlined in Section 2.5.3.2. Specifically for these data, there were significant associations between EEG activity and age \((r=-0.18\) to \(-0.30)\) and IQ \((r=-0.09\) to \(-0.19)\), and therefore the effects of age and general intelligence were regressed out of the data. Each EEG parameter had skewed distributions as defined by sktest in Stata (Stata Corp, College Station, Texas) and therefore were log-transformed (optimized minimal skew through the lnskew0 command in Stata). In addition, each EEG measure was ordinalised into 7 equal classes in terms of proportions, which should capture most of the information in the continuous data. The number of classes was selected on the basis of visual inspection and correlations between continuous data and ordinal data above \(r=0.95\).

4.3.4.2 Data analysis

The comparisons of mean values were analyzed by means of a regression command in Stata that allows for non-independent observations (e.g. twin pairs) by using a robust cluster command to estimate standard errors. The association between the EEG activity and ADHD symptom scores, and EEG activity and performance measures, was investigated using Pearson’s product moment correlation coefficient on transformed residuals. Performance measures for correlation analysis were selected on the basis of trends toward case-control differences.

Twin correlations and twin model fitting were computed using the Mx program (see Section 2.5.3.1, Chapter 2). In order to correct for the selected sample, model parameters for ADHD
were fixed to constant values supported by a meta-analysis of 20 studies of ADHD (model 1: $h^2=0.76$, $c^2=0$, $e^2=0.24$; (Faraone et al., 2005), as described in full in Section 2.5.3.1.

4.4 Results

4.4.1 Results from the regression and correlation analyses

Regression analyses indicated no significant differences between ADHD and control twins for cognitive performance measures (see Table 3-1). There were no significant differences between groups for theta power during the cognitive activation condition (Table 4-1). Theta power at rest was highest in frontal regions and was significantly increased in frontal locations in the ADHD group compared to the control group (Table 4-1). There were also trends towards increased theta power in central and parietal regions in the ADHD group. Subjects with high ADHD symptom scores also demonstrated a greater increase in theta power from resting to cognitive activation in central scalp locations, compared to the control group (Table 4-1). The significant case-control differences were subsequently used in correlation and twin modeling analyses.

Results from correlation analyses are shown in Table 4-2. There was a significant association between increased theta power at rest and increased hyperactivity/impulsivity symptom scores across the whole sample. Task-related change in theta was negatively correlated with inattention and hyperactivity/impulsivity scores in the control sample only. There was a trend toward an association between increased theta power and SD-RT across the whole sample, and a trend toward an association between increased theta power and CV in the control group only. For task-related theta changes, the control group showed positive correlations between increased theta change and increased SD-RT. Conversely, subjects with high ADHD symptoms, particularly MZ twins, showed negative correlations between increased theta change and increased SD-RT and CV. All other associations were non-significant.
### Table 4-1: Summary statistics and mean comparisons adjusted for genetic relatedness for theta power (in $\mu V^2$)

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<td>.16</td>
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<td><strong>Theta central difference</strong></td>
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<tr>
<td>MZ Control</td>
<td>2.37</td>
<td>.32</td>
<td>4.71</td>
<td>&lt;.00</td>
<td>DZ Control</td>
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<tr>
<td>MZ ADHD</td>
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<td></td>
<td>DZ ADHD</td>
<td>2.53</td>
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<tr>
<td>MZ Control</td>
<td>1.77</td>
<td>.05</td>
<td>-0.84</td>
<td>.402</td>
<td>DZ Control</td>
<td>1.79</td>
<td>.06</td>
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<tr>
<td>MZ ADHD</td>
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<td>.09</td>
<td></td>
<td></td>
<td>DZ ADHD</td>
<td>1.75</td>
<td>.14</td>
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</table>

*Note: Scores and analysis based on transformed age- and IQ-regressed scores. Abbreviations: ADHD: attention deficit hyperactivity disorder; CPT, continuous performance test; MZ: monozygotic; DZ: dizygotic*
Table 4-2: Correlations between theta power, ADHD symptoms and task performance

<table>
<thead>
<tr>
<th></th>
<th>Whole sample</th>
<th>All controls</th>
<th>MZ</th>
<th>DZ</th>
<th>All ADHD</th>
<th>MZ</th>
<th>DZ</th>
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</thead>
<tbody>
<tr>
<td><strong>Frontal theta power at</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>resting</td>
<td>Inattention</td>
<td>0.15</td>
<td>0.04</td>
<td>0.12</td>
<td>-0.08</td>
<td>-0.08</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Hyperactivity/Impulsivity</td>
<td>0.26**</td>
<td>0.15</td>
<td>0.22</td>
<td>0.01</td>
<td><strong>0.10</strong></td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>SD-RT</td>
<td>0.15†</td>
<td>0.18</td>
<td>0.22</td>
<td>0.16</td>
<td>-0.01</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>CV</td>
<td>0.14</td>
<td>0.20†</td>
<td>0.22</td>
<td>0.20</td>
<td>-0.05</td>
<td>-0.06</td>
</tr>
<tr>
<td><strong>Central theta power difference from rest-to-task</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Inattention</td>
<td>0.03</td>
<td>-0.35**</td>
<td>-0.29†</td>
<td>-0.47**</td>
<td>-0.20</td>
<td>-0.45*</td>
<td>-0.05</td>
</tr>
<tr>
<td>Hyperactivity/Impulsivity</td>
<td>0.08</td>
<td>-0.30**</td>
<td>-0.24</td>
<td>-0.48**</td>
<td>-0.06</td>
<td>0.01</td>
<td>-0.24</td>
</tr>
<tr>
<td>SD-RT</td>
<td>0.13</td>
<td>0.25*</td>
<td>0.32*</td>
<td>0.15</td>
<td>-0.23</td>
<td>-0.48*</td>
<td>-0.04</td>
</tr>
<tr>
<td>CV</td>
<td>0.10</td>
<td>0.22†</td>
<td>0.24</td>
<td>0.18</td>
<td><strong>-0.25</strong></td>
<td>-0.50*</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Note: Pearson’s product moment correlation on transformed age and IQ-regressed scores. ADHD symptom scores based on the Long Version of the Parent Conners’ Rating Scale collected on the day of testing.*

*Abbreviations: ADHD: attention deficit hyperactivity disorder; CV, coefficient of variation; DZ: dizygotic; MZ: monozygotic; SD-RT: within-subject variability in RTs in milliseconds. **p<.01, *p<.05, †p<.1*
4.4.2 Results from the twin modelling analyses

4.4.2.1 Resting theta power

The MZ cross-twin within-trait correlation for theta power \( (r = 0.79; 95\% \, \text{CI}, \, 0.60 \, \text{to} \, 0.89) \) was greater than the DZ cross-twin within-trait correlation for theta power \( (r = 0.13; 95\% \, \text{CI}, \, -0.23 \, \text{to} \, 0.46) \) which suggests that genetic effects contribute to theta power. As the MZ correlation for theta power deviated from 1, this suggests that unique environmental influences, including measurement error, contribute to theta power. Furthermore, the DZ cross-twin within-trait correlation for theta power was less than half the MZ correlations, suggesting that nonadditive genetic dominance effects may contribute to this. The MZ cross-twin cross-trait correlation between ADHD and theta power \( (r = 0.26; 95\% \, \text{CI}, \, 0.01 \, \text{to} \, 0.49) \) was greater than the DZ cross-twin cross-trait correlation \( (r = 0.13; 95\% \, \text{CI}, \, -0.13 \, \text{to} \, 0.39) \), suggesting that genetic effects contribute to the association between increased theta power and ADHD.

Structural equation modelling indicated that genetic factors accounted substantially for the total variation in theta power during resting \( (h^2 = 0.77; \, 95\% \, \text{CI}, \, 0.43 \, \text{to} \, 0.89) \). Shared environment did not significantly explain individual differences in theta power \( (c^2 = 0.00; \, 95\% \, \text{CI}, \, 0 \, \text{to} \, 0.27) \), whereas unique environmental effects (incorporating also measurement error) accounted for a modest part of the variance in theta power \( (e^2 = 0.23; \, 95\% \, \text{CI}, \, 0.11 \, \text{to} \, 0.45) \).

The extent to which ADHD and theta activity share the same genetic and unique environmental effects is given by the correlations \( r_g \) and \( r_e \), respectively. Significant genetic correlations \( (r_g = 0.35; \, 95\% \, \text{CI}, \, 0.04 \, \text{to} \, 0.68) \) indicated moderate genetic overlap between ADHD and elevated theta power during resting state. Unique environmental effects did not have a significant overlap with theta power at rest \( (r_e = -0.28; \, 95\% \, \text{CI}, \, -0.90 \, \text{to} \, 0.65) \). The phenotypic correlation \( (r_{ph}) \) suggested that increased liability to ADHD was associated with increased theta power \( (0.21; \, 95\% \, \text{CI}, \, 0.01 \, \text{to} \, 0.39) \). Due to the high heritability and a moderate genetic correlation, the phenotypic correlation between ADHD and theta power appears to be largely attributable to genetics \( (r_{ph-a} = 0.27; \, 95\% \, \text{CI}, \, 0.03 \, \text{to} \, 0.48) \). Unique environmental contributions to phenotypic variance were non-significant \( (r_{ph-e} = -0.07; \, 95\% \, \text{CI}, \, -0.25 \, \text{to} \, 0.17) \).
4.4.2.2 Change in theta power from resting to cognitive activation

The MZ cross-twin within-trait correlation for theta power \(r = 0.56; 95\% \text{ CI}, 0.31 \text{ to } 0.74\) was greater than the DZ cross-twin within-trait correlation for theta power \(r = 0.19; 95\% \text{ CI}, -0.27 \text{ to } 0.56\) which suggests that genetic effects contribute to theta power. As the MZ correlation for theta power deviated from 1, this suggests that unique environmental influences (and possibly measurement error) contribute to theta power from rest-to-task. The MZ cross-twin cross-trait correlation between ADHD and theta power \(r = 0.63; 95\% \text{ CI}, 0.38 \text{ to } 0.76\) was greater than the DZ cross-twin cross-trait correlation \(r = -0.11; 95\% \text{ CI}, -0.34 \text{ to } 0.14\), suggesting that genetic effects contribute to the association between increased theta power and ADHD. The DZ cross-twin within-trait correlation for task-related change in theta power was less than half the MZ correlations both within-trait and cross-trait, suggesting that nonadditive genetic dominance effects may contribute to these effects.

Structural equation modelling indicated that genetic factors accounted modestly for the total variation in task-related change in theta power \(h^2 = 0.46; 95\% \text{ CI}, 0.02 \text{ to } 0.76\). Shared environment did not significantly explain individual differences in theta power \(c^2 = 0.13; 95\% \text{ CI}, 0 \text{ to } 0.57\), whereas unique environmental effects (incorporating also measurement error) accounted for a moderate part of the variance \(e^2 = 0.41; 95\% \text{ CI}, 0.11 \text{ to } 0.45\).

Significant genetic correlations \(r_g = 0.68; 95\% \text{ CI}, 0.17 \text{ to } 1.00\) indicated substantial genetic overlap between ADHD and increased task-related change in theta power. Unique environmental effects did not have a significant overlap with change in theta power \(r_e = -0.19; 95\% \text{ CI}, -0.99 \text{ to } 0.76\). The phenotypic correlation suggested that increased liability to ADHD was associated with increased theta power from resting to cognitive activation \(r_{ph} = 0.34; 95\% \text{ CI}, 0.09 \text{ to } 0.53\). The phenotypic correlation between ADHD and task-related change in theta power appears to be largely attributable to genetics \(r_{ph-a} = 0.40; 95\% \text{ CI}, 0.11 \text{ to } 0.68\). Unique environmental contributions to phenotypic variance were nonsignificant \(r_{ph-e} = -0.06; 95\% \text{ CI}, -0.38 \text{ to } 0.27\).

4.5 Discussion

In this study, I investigated the association between ADHD and theta EEG power as the most consistent neural marker of the disorder, and its potential as an endophenotype in a selected sample of twins concordant and discordant for ADHD symptoms. Genetic analyses showed that theta power was highly heritable at rest, and task-related theta change was
moderately heritable. Adolescents with consistently high ADHD symptom scores displayed elevated theta power at rest and increased task-related change in theta power from resting to task-onset. Genetic factors were the main source of these effects, and shared genetic influences between theta power and ADHD were moderate to large. These findings converge to support theta power as an electrophysiological marker of genetic risk in ADHD.

Substantial heritability of frontal theta power at rest is consistent with previous reports in twin samples (Smit et al., 2005a, Van Beijsterveldt et al., Zietsch et al., 2007). The estimate reported here is slightly reduced, which may reflect differences in age (adult versus adolescent), sample (typically developing participants versus selection on the basis of ADHD symptoms) and EEG recording procedures, but also suggests the role of unique environmental effects. In addition, moderate heritability was shown for task-related theta change, suggesting tonic changes in theta activity have a genetic underpinning. As this is a novel finding, replication is required in future studies and the reliability of this effect should be examined (Kuntsi et al., 2005a). There was no evidence of shared environmental effects, in line with previous research (Smit et al., 2005a), which suggests that environmental factors that make family members similar are not the same as those that influence theta power. Heritability of theta power was greater when measured during resting state compared to task-related change in theta power. This may reflect the two different constructs these measurements index: arousal during rest and activation from rest-to-task. Heritability of theta at rest is similar to previous work. Heritability of theta change from rest-to-task may firstly reflect reduced reliability of the measure (i.e. increased ‘noise’ in the marker) which sets an upper limit on the heritability estimate (see also Chapter 3 with regard to low heritability of VLF activity. Future work should investigate the reliability of these activation measures, and these propositions may also explain why there is high heritability for a compound measure such as scalp-recorded theta at rest (arousal) compared to a task- and topography-specific activation measure (Barry et al. 2007).

Alternatively reduced heritability may genuinely reflect less genetic influence or greater environmental influence on this measure that could have implications for behavioural intervention.

The phenotypic associations are largely consistent with previous studies of EEG in ADHD, showing elevated theta in the high ADHD group that was also associated with increased hyperactivity/impulsivity scores across all subjects (Barry et al., 2003a, Monastra et al., 2001, Monastra et al., 1999, Shi et al., in press, Snyder and Hall, 2006, Snyder et al., 2008).
Although findings were only significant for frontal regions (in line with previous research), there were trends toward elevated theta in central and parietal regions, suggesting a broad effect of arousal (VaezMousavi et al., 2007). As theta activity is proposed to be an index of underarousal, this may reflect suboptimal energetic state at rest in ADHD which is not sufficiently stimulating or rewarding for the subject (Borger and Van der Meere, 2000, Kuntsi et al., 2005a, Scheres et al., 2001, Sergeant, 2000, Slusarek et al., 2001, Van der Meere et al., 1995), and the action of a top-down attention control network that regulates the arousal level (Sergeant et al., 2003).

In addition, while all participants demonstrate an increase in theta from rest-to-task, individuals with high ADHD symptom scores displayed a larger increase in task-related theta change, and this increase was associated with lower inattention and hyperactivity/impulsivity symptoms. Combining this with reports of associations between theta increase during tasks and cognitive performance (Klimesch, 1999, Schacter, 1977), this may be an index of preserved cognitive function and reflect a compensatory mechanism to increase neural resources and perform at the typical level, particularly as group differences in task performance are minimal (see Chapter 2). Theta activity has been associated with the amplitude of visual evoked potentials (Başar et al., 1998), and has been found to spike when inhibitory processing is required (Kirmizi-Alsan et al., 2006). This may therefore reflect the higher demand for inhibition during the CPT-OX. As theta is related to large distributed networks (von Stein and Sarnthein, 2000), the integration of higher order processes may therefore rely on these top-down effects of theta increase (Min and Park, 2010, Sauseng et al., 2008). This should be investigated explicitly in ADHD samples in order to verify these propositions, such as the investigation of pre-stimulus theta as a measure of cognitive readiness (Min and Park, 2010). It must be noted that these studies refer to attentional localised theta, typically fronto-central, which is likely therefore to display different properties to broad theta measured at rest, and as such the sources or components need to be investigated to before firm conclusions are made about the implications of this finding.

The lack of significant group differences for altered theta power during completion of the CPT-OX may suggest the task was not sufficiently difficult for the ADHD participants, and theta power should be examined in tasks that provide an optimal measure of underarousal and task performance in ADHD (Andreou et al., 2007, Kuntsi et al., 2005a), or through investigation of changes in EEG power over the course of the task (Loo et al., 2009) see
Correlations between theta power and response variability suggest a biological correlate of cognitive performance measures that are consistently altered in ADHD (Klein et al., 2006, Loo and Smalley, 2008). Stronger associations between theta power and response variability during the task were found for task-activation theta change compared to theta power at rest, suggesting this may represent a stronger neural marker of cognitive function (Barry et al., 2005, VaezMousavi et al., 2007). It would be interesting to investigate the genetic architecture of this correlation, although as performance on the task did not differ significantly between cases and controls it was not taken forward for twin modeling (see Table 3-1, Chapter 3). It is often the case that differential associations between brain and performance measures are found when analysed separately by group (Hermens et al., 2005). In particular the associations between brain activity from resting to cognitive activation and performance differ by group: the high ADHD group show an association between increased theta activity and decreased response variability, compared to increased response variability in controls, which may suggest altered relationship between neural and cognitive markers and abnormal task-related activity in ADHD (see also Chapter 3 for discussion of differing relationships between neural and cognitive markers).

Importantly, significant genetic correlations between ADHD and theta power at rest, and more substantially for task-related theta change, suggest there is overlap in their genetic influences. Establishing a shared genetic variance between ADHD and increased theta power validates its use as a valid candidate endophenotype (Gottesman and Gould, 2003). Future work should aim to identify specific genes that influence ADHD and theta power. For example, dopaminergic alterations are consistently reported in ADHD and have been linked with EEG power (see Chapter 1 for review). More specifically, associations with theta power may vary on the basis of specific genetic variants that are themselves associated with ADHD (Gizer et al., 2009, Li et al., 2006); in a sample of multiplex families of ADHD, individuals with the DRD4 7-repeat allele had increased frontal theta power compared to individuals with no copies of the 7-repeat allele (Loo et al., 2010). In addition, methylphenidate treatment led to decreased theta activity and lower theta/beta ratio in children with the 10-repeat allele of DAT1, whereas those with DAT1-9R showed the
opposite pattern (Loo et al., 2003). Therefore, this finding warrants the continued investigation of biological mechanisms and genes underlying theta activity. The integration of these approaches is likely to provide substantial insight into the pathophysiology of ADHD, the implications of which are further discussed in Chapter 9.

While these findings are promising, they should be interpreted with caution due to the relatively small sample size. The lack of group differences in the cognitive activation condition appears to be due to large standard deviations and therefore increased variance. This may reflect differences within the group scoring high for ADHD symptoms and the heterogeneous nature of the disorder that appears to give rise to EEG-defined subtypes (Clarke et al., 2001b, d). This limited power also limits the detection of shared environmental and non-additive genetic effects that, according to the twin correlations, may represent a proportion of the variance in theta power. In addition, some studies study some differentiation by subtype (Clarke et al., 1998, 2001c) and elevated theta power is reported in ADHD with autistic traits (Clarke et al., 2011). Therefore, these and other electroencephalographic abnormalities are not necessarily homogenous within and specific to ADHD, which should be explicitly examined (the aim of Chapter 4). While we have focused on a consistent neural marker of ADHD, other parameters in EEG, as well as event-related potentials (ERPs), may identify ADHD with greater accuracy, either alone or in combination.

In conclusion, this study demonstrated that elevated theta power at rest and from rest-to-task are associated with ADHD symptoms, are heritable and share genetic influences with the disorder, thereby validating theta as a potential intermediate phenotype of ADHD. These findings may facilitate the detection of susceptibility genes through the use of a quantitative marker for increased power in molecular genetic research. Moreover, these findings provide improved understanding of underlying pathophysiology through convergence with biological mechanisms, which support the use of biological markers, such as theta power, as complementary tools in the assessment and treatment of ADHD (see Chapter 8). This warrants the investigation of potential causal relationships and the biological underpinnings of the genetic interplay between ADHD and theta power.
PART II

Investigating the specificity of cognitive-electrophysiological parameters to ASD, ADHD and ASD+ADHD

In Part I, I presented evidence supporting EEG parameters that are consistently altered in ADHD as candidate intermediate phenotypes of ADHD (reduced VLF power and increased theta power). In order to be useful in clinical practice, and to direct molecular genetic research in the search for either specific or pleiotropic genes, it is important to identify whether these and other potential intermediate phenotypes are specific to ADHD as a pure disorder and when presenting with comorbidities. As reviewed in Chapter 1, there are high rates of clinical, behavioural and genetic overlap between ADHD and ASD. In particular, both children with ASD and ADHD demonstrate consistently disordered and atypical brain activity, yet there are limited direct comparisons of the two disorders. In addition, it is unclear whether children with comorbid ASD+ADHD demonstrate similar or distinct impairments compared to the pure disorders. The second part of this thesis will therefore directly compare the three conditions across four domains that are associated with impairment in one or more disorders.
CHAPTER 5: Evidence for specific QEEG profiles in children with ASD, ADHD and ASD+ADHD

5.1 Summary

Attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) demonstrate substantial overlap. Both disorders display consistently altered brain activity, but there are limited direct comparisons across pure and comorbid disorders. EEG provides an ideal neuroscientific technique to compare pathophysiology and provide insight into the role of cortical activation and arousal in cognitive processing across disorders. Quantitative EEGs were measured in resting and task conditions (cued CPT-OX) in 8-13 year old males with ASD (n=19), ADHD (n=18), ASD+ADHD (n=29) and typically developing (TD) controls (n=26). During resting, subjects with ADHD (ADHD/ASD+ADHD) demonstrated increased relative theta power, whereas subjects with ASD (ASD/ASD+ADHD) demonstrated increased absolute and relative delta power, combined with reduced absolute and relative alpha power. Subjects with ADHD showed reduced cortical activation at the beginning of the task (reduced beta) and required greater cortical arousal (reduced alpha) toward the end of the task, while subjects with ASD displayed greater cortical arousal (reduced alpha) from the beginning of the task. The findings suggest sub-optimal arousal in children with ADHD at rest, who subsequently require a greater level of cortical activation to sustain attention to task. In contrast, children with ASD demonstrate hyperarousal and alternative mechanisms to meet the demands of the task. Children with comorbid ASD+ADHD present as an additive co-occurrence with the unique deficits of both disorders. The identification of specific QEEG profiles is likely to aid the assessment of comorbid conditions and provide insight into the pathophysiological basis of behavioural deficits.

5.2 Introduction

Atypical patterns of brain structure and function are consistently demonstrated in both ASD (Jeste and Nelson, 2009, Stigler et al., 2011) and ADHD (Barry et al., 2003a, Konrad and Eickhoff, 2010). An ideal method to investigate the temporal dynamics of brain activity in developmental psychiatric populations is through non-invasive measurement of electroencephalography (EEG) activity on the scalp. EEG gives a direct measure of brain
activity and provides information on the overall arousal level of the individual (McLoughlin et al., 2005). This is a particularly useful method for ADHD and ASD, for which theoretical proposals implicate hypo- and/or hyper-arousal as key mechanisms underlying abnormal cognition and behavior (see Sections 1.1.4 and 1.2.4, Chapter 1, for a review of cognitive theories). Given the considerable overlap in cognitive and neural dysfunction across the ADHD and ASD (Section 1.3, Chapter 1), it is important to directly compare the quantitative EEG profile across these disorders that may relate to differing behaviour. In addition, in order to elucidate the basis of the comorbid condition (ASD+ADHD), it is necessary to compare ASD and ADHD to individuals presenting with symptoms of both disorders (Banaschewski et al., 2007).

Children and adolescents with ADHD consistently demonstrate abnormal patterns of EEG activity. Along with elevated absolute and relative power in the theta band, which in addition shares genetic influences with ADHD (see Chapter 3), children and adults with ADHD often show significantly increased delta power (Bresnahan et al., 1999, Bresnahan and Barry, 2002, Clarke et al., 1998), reduced beta power that appears to normalize with age (Bresnahan et al., 1999, Bresnahan and Barry, 2002, Callaway et al., 1983, Clarke et al., 1998, 2001a, c, Lazzaro et al., 1998, Mann et al., 1992) and increased theta/beta ratio (Mann et al., 1992, Shi et al., in press, Snyder and Hall, 2006). Correlations between EEG parameters and symptom scores also suggest that elevated theta and reduced beta transcend diagnostic boundaries (Ogrim et al., in press). Due to the association between theta and underarousal, findings of elevated theta support theoretical proposals of suboptimal energetic state in ADHD (Sergeant, 2000). There are, nevertheless, inconsistencies in the findings; a subset of individuals with ADHD appear to display excessive beta (Clarke et al., 2001d), and there are reports of reduced alpha power or “alpha slowing” in ADHD (Callaway et al., 1983, Clarke et al., 2001c), although this is less consistent with other studies reporting increased power in children (Swartwood et al., 2003) and adults (Koehler et al., 2009) with ADHD. There are further inconsistencies as some studies report no ADHD-control differences in theta, beta and theta/beta ratios (Ogrim et al., in press), which suggests large heterogeneity in the EEG profile of these individuals. Importantly, studies indicate that EEG parameters share familial and genetic influences with ADHD (Loo and Smalley, 2008, Loo et al., 2010, Tye et al., 2011), which suggests that EEG may provide an ideal marker of genetic risk for the disorder. In addition, high sensitivity and specificity rates suggest EEG markers could be used to identify individuals with ADHD (Snyder and Hall, 2006, Snyder et al., 2008). In order to be useful as
a diagnostic tool and to direct molecular genetic research, however, it is important to know whether these parameters are altered in other overlapping neurodevelopmental disorders.

Although less studied and less consistent, individuals with ASD exhibit abnormalities in their EEG profile, particularly pathophysiology in cerebral organization and connectivity, as indexed by EEG and MEG (Rippon et al., 2007). Indeed, while the majority of more recent research on ASD focuses on cognitive function, it was suggested from the earliest description of autism that individuals may have abnormalities in more basic physiological functions, such as arousal, observed in a lack of responsiveness to external stimuli. While some advocate reduced arousal in ASD (Des Lauriers and Carlson, 1969), the majority of cardiovascular and EEG studies support hyperarousal (Hutt et al., 1964). Typically, the transition from resting wakefulness to sleep shows a pattern of reduced alpha, whereas the transition from an alert state to resting wakefulness is accompanied with an increase in alpha (Klimesch, 1999). These patterns appear to be altered in ASD. For example, increased levels of alpha power in individuals with ASD compared to controls have been reported during sleep (Ogawa et al., 1982). A recent larger study of 90 control and 66 ASD children reports less relative alpha and more relative delta in the ASD group (Chan et al., 2007), supporting previous findings of a pattern of increased slow-wave/reduced alpha in ASD (Cantor et al., 1986, Harrison et al., 1998). Reduced alpha power shows the best autism-control distinction, with a sensitivity of 91% and specificity of 73% (Chan et al., 2007, Sheikhani et al., 2007). Cortical hyperarousal in ASD is also supported by a high proportion of fast beta activity (Coben et al., 2008, Rossi et al., 1995). Findings are, however, inconsistent; some studies report increased alpha (Daoust et al., 2004), reduced beta and increased theta (Daoust et al., 2004, Murias et al., 2007), suggestive of underarousal, and reduced EEG power over all frequency bands (Dawson et al., 1995), compared to controls. Differences in EEG measurement condition, age and ability and the large heterogeneity of the samples are likely to contribute to these mixed findings.

Both ASD and ADHD are associated with prominent executive dysfunction (see Section 1.3.2.3, Chapter 1, for a review of the literature). In particular, both disorders demonstrate impairments in maintaining attention over longer periods of time (Rommelse et al., 2011). In direct comparisons, studies using the continuous performance test (CPT) report similar deficits in inaccuracy, impulsivity, mean reaction time and perceptual sensitivity between ASD and ADHD (Riccio and Reynolds, 2001, Swaab-Barneveld et al., 2000) although normal performance has been reported in ASD+ADHD (Nyden et al., 2010). In a comparison of
performance on the sustained attention to response task (SART), children with ADHD showed greater commission and omission errors suggesting deficits in sustained attention are more specific to ADHD (Johnson et al., 2007a). The authors further report increased slow-frequency variability and fast-frequency variability in reaction time in ADHD compared to ASD and controls, suggestive of impaired arousal processes and top-down processes (Johnson et al. 2007a). In agreement with findings of reduced arousal in ADHD, time-on-task effects suggest that individuals with ADHD demonstrate a sharper decrease in vigilance over time (Swaab-Barneveld et al. 2000) and increase in reaction time and reaction time variability (Johnson et al., 2007b). Further, while controls display a gradual increase in alpha power, adults with ADHD appear to maintain the same level of power during completion of the CPT (Loo et al., 2009) or display reduced power during reading (Swartwood et al. 2003), indicating that a greater level of activation is required to sustain their attention. Additional studies showed that increased slow cortical activity and decreased fast cortical activity is most noticeable during task conditions in ADHD, evidencing potential task-specificity of case-control differences (El-Sayed et al., 2002, Janzen et al., 1995, Mann et al., 1992). Further comparison of changes in EEG activity during cognitive activation is likely to provide further insight into the relationship between neural and cognitive markers (Barry et al., 2005, VaezMousavi et al., 2007), and whether sustained attention deficits in ASD and ADHD are underpinned by the same or different pathophysiological processes.

Although there have been no direct comparisons between ADHD and ASD on EEG data, an investigation of ADHD children with and without autistic traits found that the ADHD-only group displayed increased absolute power in all frequency bands, and relatively more theta and less delta than controls (Clarke et al., 2011). Children with ADHD and autistic symptoms showed qualitative differences notably a generalized increase in relative beta activity (Clarke et al. 2011). While this study provides some insight into potential differences between pure and more complex cases of ADHD, a direct comparison across all three conditions is required, using both resting and cognitive activation conditions.

The current study was designed to extend this previous research, by examining whether QEEG profiles can be used to differentiate children with systematically diagnosed ASD and ADHD, and elucidating the QEEG profile of children with comorbid ASD+ADHD. Based on the most consistent findings in previous research including our own (see Chapter 3), I expected (1) subjects with ADHD-only to display elevated theta power; (2) subjects with
ASD-only to display reduced alpha power; (3) subjects with comorbid ASD+ADHD to present as an additive condition with the unique deficits of both disorders (high theta/low alpha). In addition, I expected (4) altered time-on-task effects to be most pronounced in the ADHD groups, due to the greater association between ADHD and deficits in sustained attention. Finally, I aimed to examine whether quantitative trait measures of the two disorders independently predict EEG parameters of interest.

5.3 Methods

5.3.1 Sample

Nineteen male participants with ASD, 18 with ADHD, 29 with ASD and ADHD, and 26 typically developing controls (TD) between 8 and 13 years of age took part in the study. Participants underwent systematic clinical assessment to confirm research diagnoses. See Section 2.2, Chapter 2, for full description of participant recruitment and assessment.

5.3.2 Task and stimuli

EEG recording consisted of a resting condition (eyes open) and a task condition, the cued continuous performance test (CPT-OX; see Chapter 3). The CPT-OX was administered as previous studies use this as the cognitive activation condition (Loo and Smalley, 2008, Loo et al., 2010) and in comparisons of ASD and ADHD (Nyden et al. 2010; Riccio and Reynolds, 2001; Swaab-Barneveld et al. 2000). In addition using these conditions allows a direct comparison with findings presented in Chapter 4. Cognitive performance was assessed using the measures described in Section 3.3.3 (Chapter 3): MRT, SD-RT, CV, omission errors, commission errors and O-not-X commission errors).

5.3.3 EEG recording and analysis

5.3.3.1 EEG recording and pre-processing

See Section 2.4.1 and 2.4.2, Chapter 2, for details of EEG data acquisition and general preprocessing. See Section 4.3.3.1 (Chapter 4) for specific details of preprocessing of these data.

5.3.3.3 EEG analysis

See Section 4.3.3.3 (Chapter 4) for details on EEG analysis.
5.3.4 Statistical analysis

5.3.4.1 Statistical analysis for group differences

The general analysis strategy is described in Section 2.5.3.2 (Chapter 2). For behavioural performance data, errors and SD-RT were non-normally distributed and were therefore successfully transformed using the square-root transformation (as indicated by the ladder command in Stata). In the EEG analyses, one TD, one ADHD and one ASD+ADHD were removed from the resting condition, and one TD was removed from both resting and the CPT condition, due to excessive artifact (slow drift throughout/removal of >10 electrodes). Age was included as a covariate due to reported developmental effects (Section 1.3.2.1), and was a significant covariate in all measures of absolute and relative resting state EEG measures. IQ was not significant as a covariate for any EEG parameter.

Firstly, groups were compared in resting EEG for each frequency band of interest (based on previous findings in ASD and ADHD; delta, 0.5-3.5 Hz; theta, 4-8 Hz; alpha, 8-12Hz; beta, 12-20 Hz) on absolute and relative powers. Relative power is represented by the percentage of power in a given frequency band compared with the total power across all frequency bands. Relative beta power, for example, is equal to (absolute beta power/[absolute delta power + absolute theta power + absolute alpha power + absolute beta power]). EEG data for absolute and relative power in both conditions were skewed (indicated by sktest in Stata; Stata Corp, College Station, Texas) and were therefore log transformed. Location (frontal, central, parietal) was used as a within-subject variable and diagnostic status as a between-subjects variable in a repeated measures ANOVA with age as a covariate.

Secondly, I calculated absolute and relative activity for three equal-sized blocks of time (approximately 4 minutes each) over the course of the task condition (cued CPT-OX) as a measure of changes in arousal. Groups were compared in the task condition over time on separate repeated measures ANOVAs with age as a covariate (when significant), with a focus on alpha (8-12 Hz) and beta power (12-20 Hz) due to their association with cortical activation (see Section 1.3.2.1.1). For analyses of EEG activity over time in the task condition all absolute and relative EEG data were skewed and log transformed. Time-on-task (beginning Time-1, middle Time-2, end Time-3) and location (frontal, central, parietal) were used as within-subject variables and diagnostic status as a between-subjects variable in a repeated measures ANOVA.
Post-hoc comparisons for location and group used Sidak correction to adjust confidence intervals for multiple comparisons. For post-hoc comparisons of interaction effects between group and location, the significance level was Bonferroni corrected (adjusted alpha level of .017). The investigation of EEG changes over time-on-task, planned contrasts between each of the three time points were examined based on noted differences between these time points in previous literature (Loo et al. 2009).

5.3.4.2 Dimensional analyses
Details for analyses investigating the association between symptom scores and EEG parameters are given in Section 2.5.3.2 (Chapter 2).

5.4 Results

5.4.1 Task performance
Task performance is shown by group in Table 5-1. IQ had no significant effects on performance measures (all p>0.5). Age was a significant covariate for MRT, SD-RT and CV and omission errors and therefore was retained as a covariate in these analyses. There was a significant effect of group on the number of omission errors \[F (3, 80) =2.89, p=.04\]. Post-hoc analyses revealed significantly higher number of errors made by comorbid ASD+ADHD subjects compared to TD (p=.04, d=0.79), with no other significant group effects (p>.1). Significant main effects of group were found on CV \[F (3, 80) =3.58, p=.02\] and post-hoc analyses revealed that the ADHD-only group had more variable reaction times than the TD group (p=.02, d=0.99) with no other significant group effects (p>.1). No significant main group effects were found for MRT, SD-RT, commission errors or O-not-X commission errors (all p>.1).

When combined, subjects with ADHD (ADHD-only and ASD+ADHD) showed increased RT variability as measured by SD-RT \[F (1, 80) =4.31, p =.04, d=0.59\], and CV \[F (1, 82) =6.06, p=.02, d=0.53\] and increased omission errors \[F (1, 80)= 6.54, p =.01, d=0.72\], compared to subjects with no ADHD (ASD-only and TD). Comparisons of subjects with ASD (ASD-only and ASD+ADHD) and without ASD (ADHD-only and TD) revealed no significant differences on any performance measures (all p>.05) and no evidence of moderate effect sizes (d>0.45). The interaction between ADHD and ASD was non-significant for SD-RT \[F (1, 80) =0.55, p =.46\] and omission errors \[F (1, 80) =0.38, p =.54\], suggesting additive effects of ASD and ADHD. There was, however, a significant interaction between ASD and ADHD for CV \[F (1, 80) =4.30, p =.04\].
Chapter 5: Specific QEEG profiles in children with ASD, ADHD and ASD+ADHD

Table 5-1: Descriptive data for behavioural performance on the CPT-OX in the SEND sample by group

<table>
<thead>
<tr>
<th></th>
<th>TD (n = 25)</th>
<th>ASD (n = 19)</th>
<th>ADHD (n = 16)</th>
<th>ASD+ADHD (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>MRT</td>
<td>485.88</td>
<td>114.15</td>
<td>443.68</td>
<td>69.79</td>
</tr>
<tr>
<td>SD-RT</td>
<td>137.24</td>
<td>57.31</td>
<td>139.37</td>
<td>34.56</td>
</tr>
<tr>
<td>CV</td>
<td>0.28</td>
<td>0.07</td>
<td>0.31</td>
<td>0.05</td>
</tr>
<tr>
<td>Total commissions</td>
<td>3.96</td>
<td>3.78</td>
<td>4.54</td>
<td>4.30</td>
</tr>
<tr>
<td>O-not-X commissions</td>
<td>2.09</td>
<td>2.68</td>
<td>2.23</td>
<td>2.72</td>
</tr>
<tr>
<td>Omissions</td>
<td>2.08</td>
<td>2.41</td>
<td>3.16</td>
<td>3.35</td>
</tr>
</tbody>
</table>

*Abbreviations*: TD, typically developing controls; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid ASD and ADHD; MRT, mean reaction time; SD-RT, within-subject variability in RTs in milliseconds; CV: coefficient of variation (SD-RT/MRT).

5.4.2 Resting condition

Descriptive statistics are shown in Table 5-2 (absolute power) and Table 5-3 (relative power) along with a summary of significant effects (Table 5-4) for ease of interpretation. Power maps for absolute power are shown in Figure 5-1 and for relative power in Figure 5-2. The statistics for these and other effects are first detailed below.

5.4.2.1 Absolute power in resting condition

5.4.2.1.1 Delta power

Across the sample, there was a main effect of age on absolute delta power \(F (1, 83) =16.43, \ p<.001\), indicating that absolute power decreases with age \(r=-.36, \ p=.001\). A main effect of location on absolute delta power was shown \(F (2, 140) =7.11, \ p=.002\), and post-hoc analyses revealed significant higher delta power in frontal compared to central locations \(p<.001\), and parietal locations \(p<.001, \ d=0.44\) and greater power in central compared to parietal locations \(p<.001\).

There was a trend toward a main effect of group on delta power \(F (3, 83) =2.19, \ p=.10\). When grouping by ADHD diagnosis, a significant main effect of group emerged \(F (1, 83) =5.99, \ p=.02, \ d=0.54\), indicating that subjects with ADHD (ADHD/ASD+ADHD) had reduced
delta compared to subjects without ADHD (TD/ASD). There was no group effect when combined by by ASD symptoms, suggesting that this effect is specific to ADHD \[F (1, 85) =1.41, p=.24, d=0.16\]. The interaction term between ASD and ADHD was non-significant, suggesting an additive effect of the disorders on delta power \[F (1, 83) =0.00, p=.97\]. No interaction between group and location or age and location across these analyses was observed (all \(p>.09\)).

5.4.2.1.2 Theta power

Generally, there was a main effect of age on theta power \[F (1, 82) =29.98, p<.001\], indicating that theta power decreases with age \((r=-.52, p<.001)\). A main effect of location on absolute theta power emerged \[F (2, 134) =5.65, p=.01\]. Post-hoc analyses showed that there was significantly reduced theta power in central compared to frontal \((p=.003)\) and parietal locations \((p=.001)\). There was no significant difference between frontal and parietal locations \((p=.77)\).

There was no main effect of group on absolute theta power \[F (3, 83) =0.35, p=.79\] or interaction between group and location \[F (5, 134) =1.14, p=.34\]. When combining by the presence or absence of ASD or ADHD diagnosis there was no change to group findings. No interaction between ASD and ADHD was observed (all \(p>.09\)).

5.4.2.1.3 Alpha power

There was no main effect of age on absolute alpha power \[F (1, 82) =0.05, p=.82\], although a significant interaction between age and location was shown \[F (2, 166) =3.67, p=.03\]. Contrasts revealed a significant difference between frontal and parietal locations \[F (1, 83) =7.53, p=.01\] with a trend toward a difference between frontal and central regions \[F (1, 83) =3.97, p=.05\]. There was no significant difference between central and parietal regions \[F (1, 83) =0.22, p=.64\]. After Bonferroni correction, this interaction indicated that with increasing age frontal alpha power increases compared to central alpha power \((r=.52, p<.01)\).

A main effect of group on alpha power was observed \[F (3, 83) =3.29, p=.03\]. Post-hoc analyses revealed a trend toward reduced alpha power in ASD+ADHD compared to TD \((p=.05, d=0.73)\). This effect remained significant when combining by the presence of ASD diagnosis \[F (1, 85) =9.67, p=.003, d=0.67\], and was not shown when combining by the presence of ADHD diagnosis \[F (1, 85) =1.01, p=.62, d=0.11\]. There was also a trend toward an interaction between location and group \[F (6, 164) =1.97, p=.07\]. When combining by
the presence of ASD, the statistic increased [F (2, 170) =5.05, p=.01], indicating that subjects with ASD (ASD/ASD+ADHD) showed decreased alpha power in central compared to frontal locations, subjects without ASD (TD/ADHD) demonstrated increased alpha power (p=.01, d=0.56). In addition, whereas subjects with ASD demonstrated a reduced increase in alpha power from frontal to parietal locations (p=.01, d=0.46). This interaction with location was not shown when combining by ADHD symptoms [F (2, 166) =0.62, p=.54], indicating a specific effect of ASD. In addition, there was no evidence for an interaction between ASD and ADHD for the main effect of alpha power [F (1, 83) =0.09, p=.71] nor for the interaction between group and location [F (2,166) =0.32, p=.73], suggesting these effects are additive on the comorbid condition.

5.4.2.1.4 Beta power
There was a main effect of location on absolute beta power across the sample [F (2, 134) =15.71, p<.001]. Post-hoc analyses revealed a significantly reduced beta power in central compared to frontal (p<.001, d=1.04) and parietal locations (p=.001), but no significant difference between frontal and parietal regions in beta power (p=.61). Although there was no main effect of age [F (1, 83) =2.92, p=.09], an interaction between age and location emerged [F (2, 166) =7.16, p=.002]. Contrasts between the three locations by age revealed significant differences between frontal and central locations [F (1, 83) =9.17, p=.003], frontal and parietal locations [F (1, 83) =7.95, p=.01], but not between central and parietal regions [F (1, 83) =0.24, p=.63]. Correlations indicated that with increasing age, frontal beta power reduces compared to central (r=-.34, p<.01) and parietal power (r=-.30, p=.01).

There was no main effect of group [F (3, 83) =0.13, p=.94] or group by location interaction on absolute beta power [F (5, 134) =0.70, p=.62]. When grouping by the presence, absence or interaction of ASD and ADHD diagnosis there were no changes to results.
Table 5-2: Mean (SD) absolute EEG power (in µV²) during resting condition

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta frontal</td>
<td>11.75 (6.17)</td>
<td>11.06 (4.55)</td>
<td>10.05 (7.89)</td>
<td>11.81 (8.50)</td>
</tr>
<tr>
<td>Delta central</td>
<td>6.25 (2.58)</td>
<td>6.80 (3.28)</td>
<td>5.28 (2.80)</td>
<td>5.85 (3.62)</td>
</tr>
<tr>
<td>Delta parietal</td>
<td>9.33 (3.75)</td>
<td>8.73 (3.09)</td>
<td>8.28 (3.12)</td>
<td>9.06 (5.24)</td>
</tr>
<tr>
<td>Theta frontal</td>
<td>2.43 (0.88)</td>
<td>2.22 (0.81)</td>
<td>2.47 (0.96)</td>
<td>2.58 (1.68)</td>
</tr>
<tr>
<td>Theta central</td>
<td>1.84 (0.52)</td>
<td>1.69 (0.62)</td>
<td>2.07 (1.34)</td>
<td>1.84 (0.93)</td>
</tr>
<tr>
<td>Theta parietal</td>
<td>2.47 (0.74)</td>
<td>2.13 (0.79)</td>
<td>3.02 (1.94)</td>
<td>2.45 (1.11)</td>
</tr>
<tr>
<td>Alpha frontal</td>
<td>1.46 (0.55)</td>
<td>1.14 (0.49)</td>
<td>1.32 (0.45)</td>
<td>1.25 (0.73)</td>
</tr>
<tr>
<td>Alpha central</td>
<td>1.88 (1.11)</td>
<td>1.35 (1.37)</td>
<td>1.45 (0.67)</td>
<td>1.11 (0.65)</td>
</tr>
<tr>
<td>Alpha parietal</td>
<td>2.51 (1.48)</td>
<td>1.75 (0.99)</td>
<td>2.27 (1.18)</td>
<td>1.64 (0.97)</td>
</tr>
<tr>
<td>Beta frontal</td>
<td>0.64 (0.59)</td>
<td>0.49 (0.21)</td>
<td>0.50 (0.26)</td>
<td>0.56 (0.34)</td>
</tr>
<tr>
<td>Beta central</td>
<td>0.36 (0.23)</td>
<td>0.33 (0.17)</td>
<td>0.32 (0.19)</td>
<td>0.31 (0.15)</td>
</tr>
<tr>
<td>Beta parietal</td>
<td>0.53 (0.31)</td>
<td>0.49 (0.23)</td>
<td>0.50 (0.22)</td>
<td>0.50 (0.25)</td>
</tr>
</tbody>
</table>

Note: Mean (SD) before log transformation and age adjustment.

Abbreviations: TD, typically developing control; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid autism spectrum disorder and attention deficit hyperactivity disorder.
### Chapter 5: Specific QEEG profiles in children with ASD, ADHD and ASD+ADHD

#### Figure 5-1: Maps of absolute EEG power in the resting condition for each frequency band and group

<table>
<thead>
<tr>
<th>Frequency Band</th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute delta</td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
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<tr>
<td>Absolute theta</td>
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<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
</tr>
<tr>
<td>Absolute alpha</td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
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<tr>
<td>Absolute beta</td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
<td><img src="image" alt="Map" /></td>
</tr>
</tbody>
</table>

**Note:** Black represents TD, red represents ASD-only; blue represents ADHD-only; green represents ASD+ADHD.
5.4.2.2 Relative power in resting condition

5.4.2.2.1 Delta power

There was no main effect of age on relative delta power \([F (1, 83) = 0.57, p=.45]\), but there was an interaction between age and location \([F (2, 166) =4.34, p=.02]\). Contrasts revealed significant differences between frontal and parietal locations \([F (1, 83) =6.08, p=.02]\) and between frontal and central locations \([F (1, 83) =7.12, p=.01]\), but not between central and parietal locations \([F (1, 83) =0.40, p=.53]\). Correlations revealed that with increasing age there is a reduced decrease in frontal delta power compared to parietal power \((r=.23, p=.03)\) with a trend between frontal and central locations \((r=.20, p=.06)\).

There was a main effect of group on delta power \([F (3, 83) =4.18, p=.01]\). Post-hoc analyses revealed significantly greater relative delta power in ASD-only compared to ADHD-only \((p=.01, d=1.08)\). This group difference was also shown when combining by the presence of ASD \([F (1, 83) =11.17, p=.001, d=0.71]\), and a trend for subjects with ADHD to have reduced relative delta power was shown when groups were combined by ADHD diagnosis \([F (1, 85) =1.25, p=.08, d=0.38]\). There was also an interaction between group and location for relative delta power \([F (6, 166) =2.98, p=.01]\). Contrasts revealed significant differences between frontal and central locations \([F (3, 83) =4.74, p=.004]\), indicating greater delta power in frontal locations compared to central locations in ASD-only subjects compared to TD subjects \((p=.01, d=0.84)\) and ADHD-only subjects \((p=.02, d=0.94)\). This interaction statistic increased when combining by the presence of ASD symptoms \([F (2, 166) =7.34, p=.001]\); frontal versus central \([F (1, 85) =11.48, p<.01, d=0.92]\); frontal versus parietal \([F (1, 85) =7.80, p=.01, d=0.57]\), and was not shown when combining by ADHD symptoms \([F (2, 170) =0.11, p=.89]\). In addition, there was no evidence for an interaction between ASD and ADHD for the main effect of relative delta power \([F (1, 83) =0.00, p=.95]\) nor for the interaction between group and location \([F (2, 166) =1.59, p=.21]\), suggesting these effects are additive on the comorbid condition.

5.4.2.2.2 Theta power

There was a main effect of location on relative theta power \([F (2, 155) =6.92, p=.002]\). Post-hoc comparisons revealed significantly greater relative theta power in central compared to frontal \((p<.001)\) and parietal locations \((p<.001)\), and greater power in parietal compared to frontal locations \((p<.001)\). There was also a main effect of age \([F (1, 83) =4.50, p=.04]\), indicating with increasing age, relative theta power decreases \((r=-.27, p=.01)\). An interaction between age and location on relative theta power also emerged \([F (2, 154)\]
3.79, p=.03]. Contrasts revealed a significant difference between frontal and parietal locations [F (1, 83) =5.97, p=.02] and between central and parietal locations [F (1, 83) =5.44, p=.02]. Correlations indicated that with increasing age there was decreased parietal power compared to frontal (r=.26, p=.01) and central power (r=.19, p=.07).

A main effect of group emerged when the groups were combined by ADHD diagnosis [F (1, 83) =5.08, p=.03, d=0.48], indicating greater relative theta power in subjects with ADHD. This was not shown when combining by the presence of ASD diagnosis, suggesting elevated theta power is specific to ADHD [F (1, 83) =1.07, p=.31, d=0.24]. There was no interaction between ASD and ADHD, suggesting additive effects of relative theta power [F (1, 83) =0.51, p=.48]. There was no interaction between group and location [F (6, 154) =0.51, p=.48].

5.4.2.2.3 Alpha power
There was a main effect of age on relative alpha power [F (1, 83) =10.18, p=.002], indicating an increase in relative alpha power with increasing age (r=.27, p=.012). There was a main effect of group [F (3, 83) =4.21, p=.01]. Post-hoc tests revealed significant differences between ASD-only and TD (p=.042, d=0.99) and ASD-only and ADHD-only (p=.02, d=1.08). When combining by the presence of ASD diagnosis there was also a main effect of group [F (1, 83) =12.14, p=.001, d=0.79], which was not shown when combining by ADHD diagnosis [F (1, 83) =1.47, p=.23, d=0.28]. There was a trend toward an interaction between group and location [F (6, 166) =1.97, p=.07], which became significant when combining by the presence of ASD diagnosis [F (2, 170) =4.27, p=.02]. Contrasts revealed a significant difference between frontal and central locations [F (1, 85) =8.63, p=.004, d=0.57], indicating increased in relative alpha power in frontal compared to central locations in subjects without ASD (TD/ADHD) compared to subjects with ASD symptoms (ASD/ASD+ADHD). This interaction between group and location was not shown when combining by ADHD [F (2, 166) =0.44, p=.65]. Moreover, there were no significant interactions between ASD and ADHD for the main effect of relative alpha power [F (1, 83) =0.23, p=.63] nor for the interaction between group and location [F (2,166) 1.22, p=.30], suggesting these effects are additive on the comorbid condition.

5.4.2.2.4 Beta power
There was no main effect of group on relative beta power [F (3, 83) =0.53, p=.66] or interaction between group and location [F (5, 128) =0.97, p=.44]. There was however a main effect of location [F (2, 128) =4.51, p=.02]. Post-hoc analyses revealed significantly
greater relative beta power in central locations compared to parietal locations (p<.001). There were no significant differences between relative beta power in frontal and central (d=0.07) or parietal locations (d=0.09). There was an interaction between age and location [F (2, 128) =4.08, p=.03]. Contrasts revealed significant differences between frontal and central locations [F (1, 83) =7.01, p=.01], indicating increased beta power in central locations compared to frontal locations with increasing age (r=-.29, p=.01).

Table 5-3: Mean (SD) relative EEG power (in µV²) during resting condition

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta frontal</td>
<td>0.70 (0.08)</td>
<td>0.74 (0.08)</td>
<td>0.67 (0.08)</td>
<td>0.71 (0.67)</td>
</tr>
<tr>
<td>Delta central</td>
<td>0.59 (0.11)</td>
<td>0.67 (0.10)</td>
<td>0.57 (0.08)</td>
<td>0.63 (0.08)</td>
</tr>
<tr>
<td>Delta parietal</td>
<td>0.62 (0.10)</td>
<td>0.67 (0.08)</td>
<td>0.59 (0.09)</td>
<td>0.65 (0.07)</td>
</tr>
<tr>
<td>Theta frontal</td>
<td>0.16 (0.03)</td>
<td>0.15 (0.04)</td>
<td>0.19 (0.05)</td>
<td>0.17 (0.05)</td>
</tr>
<tr>
<td>Theta central</td>
<td>0.18 (0.04)</td>
<td>0.17 (0.04)</td>
<td>0.22 (0.05)</td>
<td>0.21 (0.05)</td>
</tr>
<tr>
<td>Theta parietal</td>
<td>0.17 (0.04)</td>
<td>0.16 (0.04)</td>
<td>0.20 (0.06)</td>
<td>0.19 (0.05)</td>
</tr>
<tr>
<td>Alpha frontal</td>
<td>0.10 (0.05)</td>
<td>0.08 (0.03)</td>
<td>0.11 (0.04)</td>
<td>0.08 (0.03)</td>
</tr>
<tr>
<td>Alpha central</td>
<td>0.19 (0.09)</td>
<td>0.12 (0.08)</td>
<td>0.17 (0.07)</td>
<td>0.13 (0.06)</td>
</tr>
<tr>
<td>Alpha parietal</td>
<td>0.17 (0.09)</td>
<td>0.13 (0.05)</td>
<td>0.17 (0.07)</td>
<td>0.12 (0.04)</td>
</tr>
<tr>
<td>Beta frontal</td>
<td>0.04 (0.03)</td>
<td>0.03 (0.01)</td>
<td>0.04 (0.02)</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>Beta central</td>
<td>0.03 (0.02)</td>
<td>0.03 (0.01)</td>
<td>0.04 (0.02)</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>Beta parietal</td>
<td>0.04 (0.02)</td>
<td>0.04 (0.01)</td>
<td>0.04 (0.01)</td>
<td>0.04 (0.01)</td>
</tr>
</tbody>
</table>

Note: Mean (SD) values are shown before log transformation and age adjustment. Abbreviations: TD, typically developing control; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid autism spectrum disorder and attention deficit hyperactivity disorder.
### Figure 5-2: Maps of relative EEG power in the resting condition for each frequency band and group

<table>
<thead>
<tr>
<th></th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Relative delta</strong></td>
<td><img src="image1" alt="Map" /></td>
<td><img src="image2" alt="Map" /></td>
<td><img src="image3" alt="Map" /></td>
<td><img src="image4" alt="Map" /></td>
</tr>
<tr>
<td><strong>Relative theta</strong></td>
<td><img src="image5" alt="Map" /></td>
<td><img src="image6" alt="Map" /></td>
<td><img src="image7" alt="Map" /></td>
<td><img src="image8" alt="Map" /></td>
</tr>
<tr>
<td><strong>Relative alpha</strong></td>
<td><img src="image9" alt="Map" /></td>
<td><img src="image10" alt="Map" /></td>
<td><img src="image11" alt="Map" /></td>
<td><img src="image12" alt="Map" /></td>
</tr>
<tr>
<td><strong>Relative beta</strong></td>
<td><img src="image13" alt="Map" /></td>
<td><img src="image14" alt="Map" /></td>
<td><img src="image15" alt="Map" /></td>
<td><img src="image16" alt="Map" /></td>
</tr>
</tbody>
</table>

**Note:** Black represents TD, red represents ASD-only; blue represents ADHD-only; green represents ASD+ADHD.
Table 5-4: Summary of main group effects with effect sizes (Cohen’s d) in EEG power during the resting condition; TD versus clinical group unless stated

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>ASD (n=19)</th>
<th>ADHD (n=17)</th>
<th>ASD+ADHD (n=28)</th>
<th>ASD/ADHD (n=47)</th>
<th>ADHD/ASD+ADHD (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute delta</td>
<td>0.27</td>
<td>0.51</td>
<td>0.27</td>
<td>0.16</td>
<td>0.54*</td>
</tr>
<tr>
<td>Absolute alpha</td>
<td>0.74</td>
<td>0.21</td>
<td>0.73†</td>
<td>0.67**</td>
<td>0.11</td>
</tr>
<tr>
<td>Relative delta</td>
<td>(ADHD)1.08**</td>
<td>0.41</td>
<td>0.13</td>
<td>0.71**</td>
<td>0.38†</td>
</tr>
<tr>
<td>Relative theta</td>
<td>0.08</td>
<td>0.71</td>
<td>0.27</td>
<td>0.24</td>
<td>0.48*</td>
</tr>
<tr>
<td>Relative alpha</td>
<td>0.99*</td>
<td>0.15</td>
<td>0.52</td>
<td>0.79**</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Note: Medium effect sizes in italics (d=0.5), large effect sizes in bold (d=0.8). ** p<.01; *p<.05; †p<.1

Abbreviations: ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid ASD and ADHD;

5.4.3 Cognitive activation condition: patterns of cortical activation during sustained attention task across time

Descriptive statistics are shown in Table 5-5 (absolute power) and Table 5-6 (relative power). Graphs for illustration of group effects are shown in Figure 5-3. Power maps for absolute power are shown in Figure 5-4 and for relative power in Figure 5-5. The statistics for these and other effects are first detailed below.

5.4.3.1 Absolute power in cognitive activation condition

5.4.3.1.1 Alpha power

Generally, there was a main effect of location on alpha power in the CPT-OX [F (2, 348) =6.73, p<.01]. Post-hoc analyses revealed significantly reduced power in central locations compared to frontal (p=.01) and parietal locations (p<.01). There was a significant interaction between time-on-task and location [F (4, 348) =9.51, p<.001]. Contrasts revealed significant differences between time-1 and time-2; alpha power in frontal locations decreased from time-1 to time-2, whereas central [F (1, 87) =25.60, p<.001] and parietal [F (1, 87) =26.40, p<.001] alpha power increased from time-1 to time-2, suggesting...
that with time-on-task, alpha power decreases in frontal locations but increases in centro-parietal locations.

There was no main effect of group \([F (3, 87) =0.28, p=.84]\), but when combining by the presence of ADHD diagnosis, a significant three-way interaction between group, time and location emerged \([F (4, 306) =3.18, p=.02]\). Contrasts revealed significant differences between time-2 and time-3 comparing parietal to frontal \([F (1, 87) =9.59, p=.01, d=0.61]\) and central locations \([F (1, 87) =8.83, p=.01, d=0.55]\). Specifically, subjects with no ADHD (TD/ASD) showed increased alpha power from time-2 to time-3 in frontal and central locations, whereas subjects with ADHD (ADHD/ASD+ADHD) showed reduced change in alpha power between time-2 and time-3 in frontal and central locations. Similarly, in parietal locations, subjects with ADHD demonstrated similar levels of alpha power between time-2 and time-3, whereas subjects without ADHD showed reduced alpha power. This three-way interaction was not shown when combining by the presence of ASD diagnosis \([F (4, 306) =2.01, p=.10]\) and there was no interaction between ASD, ADHD, time and location suggesting additive effects \([F (3, 306) =0.60, p=.64]\).

5.4.3.1.2 Beta power

There was a main effect of time-on-task on beta power \([F (2, 344) =3.31, p=.04]\). Post-hoc analyses indicated significantly lower beta power at time-2 compared to time-1 \((p=.01)\). There was a main effect of location on beta power \([F (2, 344) =9.97, p<.001]\). Post-hoc analyses revealed significantly reduced power at central locations compared to frontal locations \((p<.001)\). A Pearson correlation between absolute alpha and beta power revealed a moderate positive association \((r=.45, p<.001)\).

There was no main effect of group \([F (1, 86) =0.46, p=.71]\), but there was a three-way interaction between group, time and location \([F (4, 344) =1.89, p=.04]\). When combined by the presence of ADHD diagnosis, this interaction term increased \([F (4, 300) =4.07, p=.01]\). Contrasts revealed significant differences by group between time-1 and time-2 when comparing frontal and central locations \([F (1, 86) =8.35, p=.01 d=0.57]\), indicating that while both groups demonstrate decreased beta power between time-1 and time-2, greater reduction is shown at frontal locations for ADHD subjects, compared to a greater reduction at central locations in subjects without ADHD. In addition, significant group differences emerged between time-1 and time-3 when comparing frontal and parietal locations \([F (1, 86) =9.50, p=.003, d=0.59]\) and frontal and central locations \([F (1, 86) =7.04, p=.01, d=0.46]\), indicating reduced beta power from beginning to end of task in subjects with ADHD.
(ADHD/ASD+ADHD) compared to subjects without ADHD (TD/ASD), particularly in frontal regions. This interaction was not shown when combining by the presence of ASD diagnosis \[F (4, 300) =1.34, p=.26\] nor was there an interaction between ASD and ADHD on absolute beta power over the task \[F (4, 344) =0.64, p=.62\], suggesting an additive effect.

5.4.3.2 Relative power in cognitive activation condition

5.4.3.2.1 Alpha power

Across the sample, there was a main effect of time-on-task on relative alpha power \[F (2, 344) =5.55, p=.01\]. Post-hoc analyses revealed significantly greater alpha power at time-1 compared to time-2 \(p=.01\) and time-3 \(p=.02\). There was a main effect of location \[F (2, 344) =8.64, p<.001\]. Post-hoc comparisons revealed significantly greater power in parietal compared to frontal \(p=.02\) and central locations \(p<.001\). There was an interaction between time and location \[F (4, 344) 7.02, p<.001\]. Contrasts revealed significant differences between time-1 and time-2 whereby central \[F (1, 86) =14.97, p<.001\] and parietal \[F (1, 86) =17.29, p<.001\] locations have increased alpha power over time compared to frontal locations.

There was no main effect of group \[F (3, 86) =0.23, p=.87\], but an interaction between time-on-task, location and group emerged \[F (12, 344) =2.39, p=.01\]. Contrasts revealed significant group differences between time-1 and time-2 when comparing frontal and parietal locations \[F (3, 86) =4.98, p=.003\]. Post-hoc analyses on the difference score showed enhancement in alpha power from time-1 to time-2 at parietal regions in TD compared to ASD-only \(p=.02, d=1.03\) and ASD+ADHD \(p=.009, d=0.82\), which was not shown in frontal regions. The specificity of this three-way interaction to subjects with ASD was supported by combining subjects with and without ASD diagnosis \[F (4, 305) 4.46, p=.002\], which was reduced when combining subjects by the presence of ADHD diagnosis \[F (4, 352) =2.09, p=.09\]. In addition, there was no evidence for an interaction between ASD and ADHD on this effect \[F (4, 305) =0.70, p=.58\], suggesting an additive effect.

5.4.3.2.2 Beta power

Across the whole sample, there was a main effect of time on relative beta power \[F (2, 344) =7.43, p=.001\]. Post-hoc analyses indicated reduced beta power at time-2 compared to both time-1 \(p=.001\) and time-3 \(p=.02\). There was a main effect of location \[F (2, 344) =5.88, p=.003\]. Post-hoc analyses revealed significantly greater power in frontal regions
compared to central regions (p=.003). There was an alpha level interaction between time and location [F (4, 344) =2.75, p=.04], which did not survive Bonferroni correction.

There was no main effect of group [F (3, 86) =.95, p=.42], but when combining by the presence of ADHD symptoms, a significant three-way interaction between group, time and location emerged [F (4, 301) =3.38, p=.01]. Contrasts revealed significant differences by group between time-1 and time-2 when comparing frontal and parietal locations [F (1, 88) =7.83, p=.01, d=0.59], indicating that at frontal regions subjects with ADHD (ADHD/ASD+ADHD) show greater reduction of beta power compared to subjects without ADHD (TD/ASD), who show reduced power at parietal locations. This interaction was not shown when combining by the presence of ASD diagnosis [F (4, 301) =0.53, p=.69] nor was there an interaction between ASD and ADHD [F (4, 301) =0.64, p=.61], suggesting the comorbid disorder represents an additive condition.

A Pearson correlation between relative alpha and beta power revealed a modest positive association (r=.26, p=.01).

Figure 5-3: Group effects on change in EEG power over time on the CPT-OX task.

Note: Based on log-transformed and age-adjusted scores. Figure is shown on the following page.
Chapter 5: Specific QEEG profiles in children with ASD, ADHD and ASD+ADHD

<table>
<thead>
<tr>
<th>No ADHD (TD/ASD)</th>
<th>ADHD (ADHD/ASD+ADHD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolue alpha power (μV)</td>
<td>Time-1</td>
</tr>
<tr>
<td></td>
<td>Time-2</td>
</tr>
<tr>
<td></td>
<td>Time-3</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>No ASD (TD/ADHD)</td>
<td>No ASD (TD/ADHD)</td>
</tr>
<tr>
<td>Relative beta power (μV)</td>
<td>Time-1</td>
</tr>
<tr>
<td></td>
<td>Time-2</td>
</tr>
<tr>
<td></td>
<td>Time-3</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD (ASD/ASD+ADHD)</td>
<td>ASD (ASD/ASD+ADHD)</td>
</tr>
<tr>
<td>Relative alpha power (μV)</td>
<td>Time-1</td>
</tr>
<tr>
<td></td>
<td>Time-2</td>
</tr>
<tr>
<td></td>
<td>Time-3</td>
</tr>
</tbody>
</table>
Table 5-5: Mean (SD) absolute EEG power (in µV^2) during cognitive activation condition (CPT-OX) by group

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta frontal</td>
<td>9.58 (2.42)</td>
<td>10.36 (4.37)</td>
<td>10.48 (6.47)</td>
<td>11.82 (9.46)</td>
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<tr>
<td>Delta central</td>
<td>6.53 (1.97)</td>
<td>8.22 (5.25)</td>
<td>6.58 (3.22)</td>
<td>6.42 (3.22)</td>
</tr>
<tr>
<td>Delta parietal</td>
<td>9.79 (3.42)</td>
<td>10.24 (4.89)</td>
<td>10.65 (5.25)</td>
<td>9.57 (4.59)</td>
</tr>
<tr>
<td>Theta frontal</td>
<td>2.67 (0.90)</td>
<td>2.43 (1.01)</td>
<td>2.95 (1.52)</td>
<td>2.58 (1.71)</td>
</tr>
<tr>
<td>Theta central</td>
<td>2.24 (0.75)</td>
<td>1.92 (0.66)</td>
<td>2.30 (1.19)</td>
<td>1.99 (1.10)</td>
</tr>
<tr>
<td>Theta parietal</td>
<td>3.31 (1.22)</td>
<td>2.78 (1.42)</td>
<td>3.70 (2.15)</td>
<td>3.01 (2.19)</td>
</tr>
<tr>
<td>Alpha frontal</td>
<td>1.46 (0.70)</td>
<td>1.21 (0.67)</td>
<td>1.33 (0.64)</td>
<td>1.10 (0.48)</td>
</tr>
<tr>
<td>Alpha central</td>
<td>1.77 (0.96)</td>
<td>1.21 (0.93)</td>
<td>1.50 (0.71)</td>
<td>1.06 (0.56)</td>
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<tr>
<td>Alpha parietal</td>
<td>3.02 (1.97)</td>
<td>2.30 (1.87)</td>
<td>2.81 (2.02)</td>
<td>1.96 (1.14)</td>
</tr>
<tr>
<td>Beta frontal</td>
<td>0.63 (0.55)</td>
<td>0.50 (0.24)</td>
<td>0.50 (0.29)</td>
<td>0.43 (0.19)</td>
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<tr>
<td>Beta central</td>
<td>0.37 (0.22)</td>
<td>0.33 (0.16)</td>
<td>0.34 (0.20)</td>
<td>0.28 (0.14)</td>
</tr>
<tr>
<td>Beta parietal</td>
<td>0.54 (0.29)</td>
<td>0.49 (0.27)</td>
<td>0.53 (0.25)</td>
<td>0.46 (0.24)</td>
</tr>
</tbody>
</table>

Note: Mean (SD) before log transformation and age adjustment.

Abbreviations: TD, typically developing control; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid autism spectrum disorder and attention deficit hyperactivity disorder
**Figure 5-4:** Maps of absolute EEG power during the cognitive activation (CPT-OX) condition for each frequency band and group

*Note:* Black represents TD, red represents ASD-only; blue represents ADHD-only; green represents ASD+ADHD.
### Table 5-6: Mean (SD) relative EEG power (in $\mu V^2$) during cognitive activation condition (CPT-OX) by group

<table>
<thead>
<tr>
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<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta frontal</td>
<td>0.67 (0.07)</td>
<td>0.71 (0.08)</td>
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<td>0.72 (0.08)</td>
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<tr>
<td>Delta central</td>
<td>0.60 (0.09)</td>
<td>0.68 (0.10)</td>
<td>0.60 (0.10)</td>
<td>0.66 (0.08)</td>
</tr>
<tr>
<td>Delta parietal</td>
<td>0.59 (0.09)</td>
<td>0.65 (0.11)</td>
<td>0.59 (0.10)</td>
<td>0.64 (0.07)</td>
</tr>
<tr>
<td>Theta frontal</td>
<td>0.19 (0.05)</td>
<td>0.17 (0.05)</td>
<td>0.20 (0.08)</td>
<td>0.17 (0.06)</td>
</tr>
<tr>
<td>Theta central</td>
<td>0.22 (0.05)</td>
<td>0.18 (0.05)</td>
<td>0.21 (0.06)</td>
<td>0.20 (0.05)</td>
</tr>
<tr>
<td>Theta parietal</td>
<td>0.20 (0.01)</td>
<td>0.18 (0.01)</td>
<td>0.21 (0.01)</td>
<td>0.20 (0.01)</td>
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<tr>
<td>Alpha frontal</td>
<td>0.10 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.10 (0.01)</td>
<td>0.08 (0.01)</td>
</tr>
<tr>
<td>Alpha central</td>
<td>0.16 (0.01)</td>
<td>0.11 (0.02)</td>
<td>0.16 (0.02)</td>
<td>0.11 (0.02)</td>
</tr>
<tr>
<td>Alpha parietal</td>
<td>0.18 (0.02)</td>
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<td>Beta frontal</td>
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<td>0.04 (0.01)</td>
<td>0.04 (0.01)</td>
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<tr>
<td>Beta central</td>
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<td>0.03 (0.00)</td>
<td>0.03 (0.00)</td>
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</tr>
<tr>
<td>Beta parietal</td>
<td>0.03 (0.00)</td>
<td>0.03 (0.00)</td>
<td>0.03 (0.00)</td>
<td>0.03 (0.00)</td>
</tr>
</tbody>
</table>

**Note:** Mean (SD) before log transformation and age adjustment.

**Abbreviations:** TD, typically developing control; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid autism spectrum disorder and attention deficit hyperactivity disorder
Figure 5-5: Maps of relative EEG power during the cognitive activation condition (CPT-OX) for each frequency band and group.

Note: Black represents TD, red represents ASD-only; blue represents ADHD-only; green represents ASD+ADHD.
5.4.4 Dimensional analyses between EEG parameters, symptom scores and task performance measures

5.4.4.1 Associations between EEG parameters and symptom scores
In order to examine whether EEG parameters are related to quantitative trait measures of the disorders, I conducted correlations between parameters that showed differences between diagnostic groups and parent-rated symptom scores (see Table 5-7). Across the whole sample, an increased number of autism symptoms were related to increased relative delta ($r=.22$, $p=.04$), reduced absolute alpha ($r=-.29$, $p=.01$) and relative alpha ($r=-.21$, $p<.05$) and altered time-on-task effects for relative alpha ($r=-.22$, $p=.04$), which were not significantly correlated with ADHD symptoms (all $p>.05$). Conversely, a higher number of ADHD symptoms were related to time-on-task effects between time-2 and time-3 for absolute alpha (Hyperactivity-Impulsivity: $r=.26$, $p=.01$), between time-1 and time-3 for absolute beta (Inattention: $r=.27$, $p=.01$; Hyperactivity-Impulsivity: $r=.25$, $p=.02$) and relative beta (Inattention: $r=.29$, $p=.01$; Hyperactivity-Impulsivity: $r=.28$, $p=.01$), which were not significantly associated with ASD symptoms (all $p>.05$). EEG parameters which were significantly correlated across both groups with the SCQ and Conners scores, respectively, were then taken forward for inclusion in multiple linear regression analyses with hierarchical entry, to determine the relative contributions of ASD and ADHD symptoms measures on these different EEG parameters. These models confirmed the above correlations supporting the SCQ or the Conners as predictors of specific EEG measures; for each parameter that was associated with one rating scale and not the other, the regression model showed a significant increase $R^2$ indicating enhanced prediction of the EEG parameter, which remained after controlling for the potential effect of age and the confounding rating scale.
Table 5- 7: Correlations between EEG parameters of interest and symptom scores across the whole sample

<table>
<thead>
<tr>
<th>EEG parameter</th>
<th>Effect</th>
<th>Electrode</th>
<th>SCQ</th>
<th>Conners Inattention</th>
<th>Conners Hyp/Imp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>Absolute delta</td>
<td>Group All</td>
<td>-.07</td>
<td>.05</td>
<td>-.06</td>
</tr>
<tr>
<td></td>
<td>Absolute alpha</td>
<td>Group All</td>
<td>-.29*</td>
<td>.01</td>
<td>-.01</td>
</tr>
<tr>
<td></td>
<td>Group x location</td>
<td>Frontal-central</td>
<td>-.19†</td>
<td>.12</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frontal-parietal</td>
<td>-.19†</td>
<td>-.02</td>
<td>-.02</td>
</tr>
<tr>
<td></td>
<td>Relative delta</td>
<td>Group All</td>
<td>.22*</td>
<td>-.08</td>
<td>-.07</td>
</tr>
<tr>
<td></td>
<td>Group x location</td>
<td>Frontal-central</td>
<td>-.16</td>
<td>-.09</td>
<td>-.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frontal-parietal</td>
<td>-.21†</td>
<td>-.11</td>
<td>-.03</td>
</tr>
<tr>
<td></td>
<td>Relative theta</td>
<td>Group All</td>
<td>-.07</td>
<td>.21†</td>
<td>.15</td>
</tr>
<tr>
<td></td>
<td>Relative alpha</td>
<td>Group All</td>
<td>-.21*</td>
<td>-.05</td>
<td>-.03</td>
</tr>
<tr>
<td></td>
<td>Group x location</td>
<td>Frontal-parietal</td>
<td>-.19†</td>
<td>.03</td>
<td>.03</td>
</tr>
<tr>
<td>Time-on-task</td>
<td>Absolute alpha</td>
<td>Time-2 to Time-3</td>
<td>Frontal-parietal</td>
<td>-.15</td>
<td>.15</td>
</tr>
<tr>
<td></td>
<td>Time-2 to Time-3</td>
<td>Central-parietal</td>
<td>.209</td>
<td>.14</td>
<td>.09</td>
</tr>
<tr>
<td></td>
<td>Relative alpha</td>
<td>Time-1 to Time-2</td>
<td>Frontal-parietal</td>
<td>-.22*</td>
<td>-.06</td>
</tr>
<tr>
<td></td>
<td>Time-1 to Time-3</td>
<td>Frontal-central</td>
<td>-.01</td>
<td>.27**</td>
<td>.25**</td>
</tr>
<tr>
<td></td>
<td>Time-1 to Time-3</td>
<td>Frontal-parietal</td>
<td>-.14</td>
<td>.20†</td>
<td>.14</td>
</tr>
<tr>
<td></td>
<td>Relative beta</td>
<td>Time-1 to Time-2</td>
<td>Frontal-parietal</td>
<td>-.11</td>
<td>.29**</td>
</tr>
</tbody>
</table>

*Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; ASD+ADHD, comorbid autism spectrum disorder and attention deficit hyperactivity disorder; Conners, Conners Third Edition Parent Rating Scale Short Form; Hyp/Imp: Hyperactivity/Impulsivity; SCQ, Social Communication Questionnaire; TD, typically developing control; **p < .01. *p < .05 †p<.10.
5.4.4.2 Correlations between EEG parameters and performance measures

For associations between task performance and cortical activation measures (EEG during the CPT-OX), across the whole sample there were significant associations between reduced relative beta power and increased reaction-time variability in performance (SD-RT: $r=-.24$, $p=.02$; CV: $r=-.23$, $p=.03$). However, differential patterns of correlations emerged according to diagnostic group suggesting that cortical activation patterns are associated with different cognitive processes among ADHD and control groups (see Table 5-8). Fisher’s transformations revealed a number of significant group differences in associations. In particular, in TD reduced absolute alpha and beta power were correlated with increased errors ($r=-.27$ to -.39) and increased reaction-time variability (CV: $r=-.29$ to -.31), whereas ASD+ADHD demonstrated reduced errors ($r=.22$ to .44) and reduced reaction-time variability (CV: $r=.29$ to .33). See Table 5-8 on the following page.
Chapter 5: Specific QEEG profiles in children with ASD, ADHD and ASD+ADHD

**Table 5-8:** Correlations between EEG parameters of interest during the cognitive activation condition and task performance measures by group

<table>
<thead>
<tr>
<th>EEG parameter</th>
<th>Group</th>
<th>Omission errors</th>
<th>SD-RT</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absolute alpha</strong></td>
<td>All</td>
<td>.06</td>
<td>-.03</td>
<td>-.07</td>
</tr>
<tr>
<td></td>
<td>TD</td>
<td>-.27</td>
<td>-.09</td>
<td>-.29</td>
</tr>
<tr>
<td></td>
<td>ASD</td>
<td>-.14</td>
<td>-.16</td>
<td>-.21</td>
</tr>
<tr>
<td></td>
<td>ADHD</td>
<td>.26</td>
<td>.09</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td>ASD+ADHD</td>
<td><strong>.44</strong></td>
<td>.20</td>
<td><strong>.33</strong></td>
</tr>
<tr>
<td><strong>Absolute beta</strong></td>
<td>All</td>
<td>.08</td>
<td>-.10</td>
<td>-.16</td>
</tr>
<tr>
<td></td>
<td>TD</td>
<td><strong>-.39</strong> †</td>
<td>-.18</td>
<td>-.31</td>
</tr>
<tr>
<td></td>
<td>ASD</td>
<td>.06</td>
<td>-.13</td>
<td>-.16</td>
</tr>
<tr>
<td></td>
<td>ADHD</td>
<td><strong>.40</strong> †</td>
<td>-.18</td>
<td>-.39</td>
</tr>
<tr>
<td></td>
<td>ASD+ADHD</td>
<td>.31</td>
<td>.11</td>
<td>.29</td>
</tr>
<tr>
<td><strong>Relative alpha</strong></td>
<td>All</td>
<td>.02</td>
<td>-.14</td>
<td>-.13</td>
</tr>
<tr>
<td></td>
<td>TD</td>
<td>-.18</td>
<td>-.21</td>
<td>-.32</td>
</tr>
<tr>
<td></td>
<td>ASD</td>
<td>-.10</td>
<td>-.12</td>
<td>-.11</td>
</tr>
<tr>
<td></td>
<td>ADHD</td>
<td>-.05</td>
<td>.00</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>ASD+ADHD</td>
<td><strong>.42</strong> †</td>
<td>-.15</td>
<td>-.01</td>
</tr>
<tr>
<td><strong>Relative beta</strong></td>
<td>All</td>
<td>.02</td>
<td>-.24*</td>
<td>-.23*</td>
</tr>
<tr>
<td></td>
<td>TD</td>
<td>-.30</td>
<td>-.31</td>
<td>-.32</td>
</tr>
<tr>
<td></td>
<td>ASD</td>
<td><strong>.19</strong></td>
<td>-.02</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>ADHD</td>
<td>.09</td>
<td>-.29</td>
<td>-.37</td>
</tr>
<tr>
<td></td>
<td>ASD+ADHD</td>
<td><strong>.22</strong></td>
<td>.27</td>
<td>-.10</td>
</tr>
</tbody>
</table>

*Note:* Correlations with cell borders denote significantly different correlations for case versus TD groups; single border $p < .10$, double border $p < .05$, triple border $p < .01$.

**Abbreviations:** TD, typically developing control; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid autism spectrum disorder and attention deficit hyperactivity disorder; CV, coefficient of variation; SD-RT, within-subject variability in reaction time.

**Significance Levels:** $**p < .01, *p < .05, †p < .10.$
5.5 Discussion

The current study provides a detailed examination of EEG-indexed cortical arousal during resting and cognitive activation conditions in children with ASD, ADHD and comorbid ASD+ADHD, compared to typically developing controls. The findings appear to dissociate ASD and ADHD on the basis of different QEEG profiles. Specifically for our hypotheses, (1) during resting children with ASD show increased relative delta and decreased absolute and relative alpha, whereas (2) children with ADHD demonstrated elevated relative theta. The results demonstrate that (3) children with ASD+ADHD are an additive co-occurrence of both disorders, because they display the QEEG characteristics of both. In addition, (4) the ADHD groups showed altered patterns of EEG activation over the course of the task, which was itself aberrantly related to cognitive performance measures. These effects shall now be discussed in further detail.

5.5.1 Resting EEG

Measurement of cortical EEG resting state activity revealed that both groups of children with ASD display elevated delta power and attenuated alpha power, particularly in central scalp locations. This is consistent with previous findings (Cantor et al. 1986; Dawson et al. 1995; Chan et al. 2007; Harrison et al. 1998), and supports altered delta/alpha power as important neurophysiological markers in ASD. Given that reduced alpha power is associated with increased cortical arousal (Klimesch et al. 1999) and inversely related to the fMRI BOLD response (Laufs et al., 2006), this supports models of hyperarousal in ASD. This may suggest that individuals with ASD may need an environment with minimal stimuli in order to obtain sufficient relaxation. As these findings appear to be observed across the scalp, this also supports widespread effects of arousal on the individual (Barry et al., 2005), and may reflect the structural and functional neural abnormalities observed in ASD (Stigler et al., 2011). In addition, the finding of altered delta and alpha power in ASD may suggest that previous findings in the ADHD literature of reduced alpha (Callaway et al. 1983; Clarke et al. 2001c) or inconsistent findings for alpha (Loo et al. 2009; Koehler et al. 2009; Ogrim et al. 2012) may be due to misspecification in group allocation such that mild ASD was present, which supports the rigorous assessment of comorbid conditions when conducting investigations into underlying mechanisms.

Conversely, children with ADHD demonstrated elevated relative theta power, consistent with the literature (Chapter 4). One study reported increased theta in ADHD subjects with ASD symptoms compared to ADHD-only, although these subjects were older and not formally
diagnosed with ASD as in the present sample (Clarke et al., 2011). Combined with results presented in Chapter 4, this finding suggests that theta power is an ideal intermediate phenotype of ADHD that shares genetic overlap and is specific to the disorder, regardless of comorbidity. It is proposed that cortical underarousal reflects suboptimal state regulation and the interplay between top-down attentional control systems that govern overall levels of arousal (Sergeant, 2000), highlighting conceptual advances of ADHD that go beyond a single cognitive deficit (Castellanos et al., 2006, Sonuga-Barke, 2002). Interestingly, children with ADHD also demonstrated reduced absolute delta power compared to ASD, particularly in frontal regions. This is contrary to some findings (Clarke et al., 1998). This might be explained by findings of increased relative delta power in posterior regions found in ADHD (Clarke et al., 1998) and minimal brain dysfunction (Matousek et al., 1984), such that this effect is one of topographical differences. Alternatively this may reflect clinical heterogeneity in previous literature. For example, the inattentive subtype of ADHD display less delta power (Clarke et al., 1998, Kuperman et al., 1996) and children with ADHD and no autistic traits show less relative delta across the scalp compared to controls (Clarke et al., 2011).

5.5.2 Task-related EEG

There were no overall group differences in absolute or relative alpha and beta power during the task, which suggests resting state EEG is likely to provide a more optimal condition for discriminating QEEG profiles of ASD and ADHD. This may suggest that altered EEG parameters reflect the overall arousal states of the children rather than specific cognitive dysfunction. However, an examination of changes in cortical activity over the course of the task revealed interesting group differences. From the beginning to the middle of the task, group differences are apparent in beta power, which typically increases during cortical activation (Ray and Cole, 1985). In contrast, children with ADHD display reduced beta as the task progresses, in line with previous studies that document reduced beta during cognitive tasks (Mann et al., 1992). Between the middle and the end of the task, however, children with ADHD maintained a similar level of absolute alpha compared to ASD and controls who displayed elevated alpha power (in frontal and central regions). This suggests that typically developing children (and ASD) show a maintained level of cortical activation (beta) that subsequently allows them to reduce their level of cortical activation (enhanced alpha; Loo et al. 2009). Conversely children with ADHD show reduced (beta) activation or alertness during the first part of the task, while in the latter part of the task must maintain or enhance their level of cortical activation (reduced alpha) in order to meet the demands of the task, as supported by a modest association between alpha and beta during the task. This extends and supports previous
electrophysiological (Loo et al., 2009, Swartwood et al., 2003) and neuropsychological findings (Swaab-Barneveld et al., 2000). In addition, alpha synchronization has been associated with successful inhibitory processes (Klimesch et al., 2007), and thus these findings may reflect weak inhibitory processing shown in these children (see Chapter 6). It should be noted that between the beginning and the middle of the task, typically developing children showed enhanced relative alpha power, whereas children with ASD maintained the same level. This may therefore reflect an alternative mechanism that can be observed from the beginning of the task to comply with the demands of the experimental situation and enhance task performance, as supported by typical task performance and differential associations between neural and behavioural markers (see below). Alternatively, the consistently reduced alpha power in ASD may reflect the persistence of hyperaroused states into the task condition. Accordingly, it has been suggested that hyper-arousal may lead to over-selective attention (Easterbrook, 1959), which may explain intact task performance (see below) and difficulties in shifting focus in ASD (Chapter 5). The interaction of these effects with topographical location necessitates an examination of the potential sources of these differences beyond scalp recording (Michels et al. 2010), particularly as group differences emerge in frontal regions often implicated in ADHD. These topographical differences may indicate distinct, task-related activation processes. Arousal should result in global changes, due to its proposed relationship with thalamus, so these topographical specificities suggest the presence of activation differences (Barry et al. 2004).

### 5.5.3 Association with task performance

Task performance was impaired in both ADHD groups, shown by increased response variability and reduced accuracy, supporting previous findings (Klein et al., 2006, Kuntsi et al., 2010, Willcutt et al., 2005), and suggesting increased variability is specific to ADHD (Johnson et al., 2007a). The lack of further group differences in performance is likely to be due to the use of the flanker version of the CPT-OX which was not specifically designed to optimally measure task performance. The larger effect sizes for neurophysiological markers in comparison to task performance highlight the greater sensitivity of EEG measures to detect differences between diagnostic groups.

A number of associations were reported between EEG parameters and task performance. This is in line with several studies demonstrating associations between EEG-indexed arousal and task performance measures in ADHD (see Chapter 3), suggesting task performance reflects cortical arousal levels supporting cognitive-energetic models (Sergeant 2000). Interestingly,
these associations differ by diagnostic group. In particular, the comorbid ASD+ADHD (and less so the ADHD-only group) showed significantly different associations compared to controls; enhanced alpha and beta power was associated with more omission errors in children with comorbid ASD+ADHD, with the opposite pattern in typically developing controls, suggesting an altered role of arousal on task performance. The use of these alternative mechanisms may therefore require increased cortical activation to sustain attention over a long period of time (Loo et al., 2009).

In addition, differential associations between task performance and arousal levels during the task lend support to an ‘inverted U’ hypothesis of arousal, as it suggests a non-linear relationship between arousal and function. These theories propose that arousal affects learning or performance through interaction with other factors (such as drugs, neurotransmitters or a comorbidity), and that optimal performance is achieved at an intermediate level of arousal (Baldi and Bucherelli, 2005) (Hardy and Parfitt, 1991). For example, while in typically developing controls increased beta power is associated with reduced errors on the task, in children with ADHD reduced beta power was associated with reduced errors. Perhaps then these impairments are better viewed as a continuum of reactivity (Mayes, 2000). In addition, differing relationships between brain activity and task performance may reflect the activation of differing brain regions (Esterman et al., in press; see Chapter 3). These propositions may explain inconsistent sustained attention deficits reported in previous studies in ASD (Johnson et al., 2007a, Nyden et al., 2010, Riccio and Reynolds, 2001). Previous work suggests that children with ASD have worse performance on auditory sustained attention tasks compared to visual tasks, with the opposite pattern shown for ADHD (Corbett and Constantine, 2006), and on tasks in which the onset of stimuli is unpredictable (Johnson et al., 2007a). Accordingly, future work should address deficits in different modalities, and the potential EEG activation patterns underlying this poor performance.

5.5.4 Implications

The findings therefore converge to suggest a dissociation between ASD and ADHD on the basis of QEEG profiles, whereby children with ASD display a high-delta/low-alpha pattern and children with ADHD display elevated theta. In addition, while both groups demonstrate an increase in cognitive activation during the task, this can be observed at differing stages over the course of the task. Notably, these associations appear to go beyond diagnostic boundaries, as shown by specific correlations with symptom scores and as independent predictors in hierarchical regression, which indicates these quantitative markers may be a marker of disease...
Considerations of the clinical implications of the findings are discussed in Chapter 8. Importantly, the comorbid group displays the unique deficits of both disorders, suggestive of an additive co-occurrence rather than a qualitatively distinct nosological entity. For the majority of impairments during the resting condition, however, it appears that the comorbid group demonstrate reduced abnormalities in comparison to the pure cases according to effect sizes compared to the control group. I tentatively suggest that this is due to the comorbid condition being a product between the pure ASD and pure ADHD cases, whereby extreme arousal levels are in effect cancelled out through an additive occurrence. A particularly clear example is relative delta power, where ASD+ADHD show a small effect size most likely because children with pure ADHD demonstrate reduced delta whereas children with pure ASD show increased delta. Moreover the interaction statistic for these findings further supports an additive interpretation (although based on a small sample this statistic cannot be taken as affirmative). One of the explanations of this observation is that QEEG profiles in ASD and in ADHD are not dependent on or exacerbated by having the comorbidity, but rather QEEG profiles in the comorbid group are the product of both conditions. Nevertheless it is important that further work uncovers these differing relationships, and whether an additive interpretation is indeed most valid.

5.5.5 Limitations
Several limitations must be taken into consideration. Firstly, although I was interested in all frequency bands reported as they have been implicated in previous ADHD and/or ASD research, it may be the case that these results would not withstand more stringent criteria for multiple testing correction. This highlights the limited power in this small sample that restricts firm conclusions. Further examination of EEG-defined subtypes in ADHD and ASD (Clarke et al., 2001b) are required in a larger sample, which may explain the lack of further findings in ADHD, such as reduced beta at rest. In addition, further insight might be gained with the use of individualized frequency bands and an examination of cortical activity across multiple electrode sites, particularly considering the topographical differences shown. Moreover, activity measured during the CPT-OX task may have incorporated task-specific activations, which are explicitly measured in the following chapter (Chapter 6). Similarly, as resting EEG power reported here was collected at the beginning of the testing session it may reflect the potential anxious state of the child in a new clinical environment, and as such analysis of resting EEG at the end of the task battery would be informative.
5.5.6 Conclusion

In conclusion, the results of the present study extend largely consistent previous studies of QEEG profiles ASD and ADHD by identifying distinct QEEG profiles, while demonstrating that children with ASD and ADHD display the unique deficits of both disorders. Such findings are likely to show clinical value by providing a quantitative biomarker to complement diagnostic interviews. Examination of EEG activity across baseline and cognitive activation conditions is therefore able to elucidate the basis of these overlapping neurodevelopmental disorders and the potential biological mechanisms that underlie this comorbidity. While examination of these frequency bands has furthered understanding of the differing role of arousal and activation in ASD and ADHD, the next three chapters utilise the excellent temporal resolution of EEG to directly explore cognitive dysfunction, using event-related potentials (ERPs).
CHAPTER 6: Attention and inhibition in children with ASD, ADHD and comorbid ASD+ADHD: an event-related potential study

6.1 Summary

Deficits in executive function are characteristic of both ASD and ADHD, but these impairments have not been directly compared across pure and comorbid subjects using ERPs. Behavioural parameters and ERPs were recorded during a flankered cued-continuous performance task (CPT-OX) administered to 8-13 year old males with ASD (n=19), ADHD (n=18), comorbid ASD+ADHD (n=29) and typically developing (TD) controls (n=26). Preparatory processing (CNV) and attentional orienting (Cue-P3) at cues, response execution at targets (Go-P3), inhibitory processing at non-targets (NoGo-P3) and conflict monitoring between target and non-target trials (Go-N2 vs NoGo-N2) were examined. Categorical diagnoses and quantitative trait measures indicated that subjects with ADHD symptoms (ADHD and ASD+ADHD) made more omission errors and exhibited increased reaction time variability and reduced amplitude of the Cue-P3 and NoGo-P3, compared to TD subjects. Subjects with ASD symptoms (ASD and ASD+ADHD) demonstrated reduced N2 enhancement from Go to NoGo trials compared to TD subjects. Subjects with ASD-only displayed enhanced CNV amplitude compared to ASD+ADHD and TD subjects. These findings indicate that children with ADHD show specific deficits in attentional orienting and inhibitory control, while children with ASD show specific abnormalities in conflict monitoring and response preparation. Children with comorbid ASD+ADHD present as an additive co-occurrence with deficits of both disorders, although non-additive effects are suggested for response preparation. Measuring ERPs that index attention and inhibition is useful in disentangling cognitive markers of ASD and ADHD and elucidating the basis of co-occurring ASD+ADHD to guide clinical assessment.
6.2 Introduction

Attention deficit hyperactivity disorder (ADHD) is a common childhood disorder characterised by developmentally inappropriate and impairing levels of inattentiveness, hyperactivity and impulsivity (see Section 1.1.1, Chapter 1). Although current diagnostic criteria preclude a co-diagnosis, high rates of behavioural and genetic overlap with autism spectrum disorder (ASD) have been documented (see Section 1.2, Chapter 1 for extensive review), a disorder characterised by abnormalities in social interaction, communication and restricted/repetitive behaviour. It is under discussion whether this common co-occurrence reflects an additive comorbidity as supported in the upcoming DSM-V, rather than a separate condition with distinct impairments (Rhee et al., 2008, Taurines et al., 2010b). One approach to distinguish between the different comorbidity models is to evaluate underlying pathophysiological mechanisms associated with ASD and ADHD in the comorbid ADHD+ASD group (Banaschewski et al. 2007).

One domain of shared cognitive impairment is executive function (Rommelse et al., 2011) and ASD symptoms appears to share familial influences with EF deficits within an ADHD sample (Rommelse et al., 2009a). While there is evidence for substantial overlap (Geurts et al., 2004, Goldberg et al., 2005, Nyden et al., 1999, Ozonoff et al., 2004, Verte et al., 2006), other studies suggest the disorders can be dissociated on the basis of, for example, intact inhibitory control and more severe impairments in cognitive flexibility and planning in ASD (Geurts et al., 2004, Happé et al., 2006a, Ozonoff and Jensen, 1999, Verte et al., 2006). There are limited studies of comorbid ASD+ADHD; deficits in inhibition similar to ADHD have been reported, while other studies demonstrate reduced flexibility deficits compared to ASD (Bühler et al., Sinzig et al., 2008a) and conversely relatively intact performance on a continuous performance test (CPT) compared to ADHD, suggesting these impairments are not necessarily an additive effect of the pure disorders (Nyden et al., 2010).

Evidence for an inhibitory deficit in ADHD is supported by event-related potential (ERP) studies that allow measurement of distinct temporal stages in overt and covert cognitive processing (McLoughlin et al., 2005). Several studies have demonstrated, using a cued-CPT (CPT-OX) task, a reduced fronto-central NoGo-P3 component in response to nontarget stimuli as an index of response inhibition (Banaschewski et al., 2004, Doehnert et al., 2010, Fallgatter et al., 2004, Valko et al., 2009). Importantly, however, this deficit is typically preceded by attenuated electrophysiological responses to cues, as indexed by the parietal Cue-P3 and the subsequent...
central contingent negative variation (CNV; Albrecht et al., 2005, Banaschewski et al., 2003, Banaschewski et al., 2004, Brandeis et al., 2002, Doehnert et al., 2010, Valko et al., 2009, Van Leeuwen et al., 1998). Notably, the Cue-P3 and the CNV index covert processing since these deficits occur without concurrent responses or performance errors and predict subsequent performance (Banaschewski et al., 2003, Banaschewski et al., 2004, Van Leeuwen et al., 1998).

In addition, N2 enhancement from Go to NoGo trials, an index of conflict monitoring (Yeung and Cohen, 2006), is reduced in ADHD during the Stop Task (Pliszka et al., 2000), although case-control differences are not reported in the CPT-OX (Banaschewski et al., 2004, Fallgatter et al., 2004, Overtoom et al., 1998) unless ADHD has co-occurring oppositional defiant disorder (Overtoom et al., 1998). These impairments are seen in ADHD during childhood, adolescence (Doehnert et al., 2010, Spronk et al., 2008, Valko et al., 2009) and adulthood (McLoughlin et al., 2010). They also share familial influences with ADHD in children (Brandeis et al., 2006) and adults (McLoughlin et al., 2011a), suggesting that they are a marker of genetic risk in ADHD (Tye et al., 2011).

Despite consistent evidence for attention deficits in ASD (Sanders et al., 2008), there is limited neurophysiological research on these processes (Jeste and Nelson, 2009). In visual oddball tasks, longer latencies of the P3 and N2 (Sokhadze et al., 2009) and larger amplitude of the P3 to target stimuli have been reported in subjects with ASD (Kemner et al., 1999, Strandburg et al., 1993), although inconsistent (Townsend et al., 2001, Verbeaten et al., 1991) and null findings are also reported (Courchesne et al., 1989, Hoeksma et al., 2006, Pritchard et al., 1987, Tsai et al., 2011). Longer P3 to incongruent (invalidly cued) trials has been shown, which was taken as suggestive of attentional orienting deficits affecting stimulus classification (Tsai et al., 2011). Nevertheless no ERP studies have directly measured attentional orienting and inhibitory control in ASD, and no study has directly compared the two disorders with comorbid ASD+ADHD cases, on these attention processes at the neurophysiological level.

The aim of this study was therefore to investigate whether ERP abnormalities associated with ADHD are also found in ASD and comorbid ASD+ADHD, in a direct comparison of children following in-depth diagnostic assessments that minimises misspecification in group allocation. This analysis was exploratory due to the lack of previous studies investigating these constructs, and thus limited hypotheses are made. Based on previous literature, I hypothesised: (1) children with ADHD would demonstrated reduced inhibitory processing; (2) children with ASD would show reduced conflict monitoring (due to it’s close association with shifting strategy).
and (3) children with ASD+ADHD would show both of these deficits, but would not necessarily present as an additive co-occurrence, based on the limited studies of all three conditions.

6.3 Methods

6.3.1 Sample

Nineteen male participants with ASD, 18 with ADHD, 29 with ASD and ADHD, and 26 typically developing controls (TD) took part in the study. The age range was 8-13 years, and participants were age-matched at the group level. See Section 2.5.3.4, Chapter 2 for a full description of participant recruitment and assessment.

6.3.2 Tasks and stimuli

The cued-CPT (flanker version) was administered, as described in Section 3.3.2 (Chapter 3). Cognitive performance was assessed using the measures described in Section 3.3.3 (Chapter 3): MRT, SD-RT, CV, omission errors, commission errors and O-not-X commission errors.

6.3.3 Electrophysiological recording and analysis

6.3.3.1 EEG recording and preprocessing

See Section 2.4.1 and 2.4.2, Chapter 2, for details of EEG data acquisition and general preprocessing.

6.3.3.2 ERP analysis

Baseline correction was performed using a 200msec prestimulus reference period. Stimulus-locked epochs (−200-1000 ms peristimulus window) were averaged for the following trial types: cue (trials to letter XOX); go (trials to OXOs preceded by XOX); no-go (trials to random target letters e.g., ODO following XOX). Averages contained at least 19 segments (Table 6-1), only included trials with correct responses (Go) or correctly rejected trials (NoGo, Cue), and were free from residual artifacts.

ERP amplitudes were restricted to leads for which effects were expected to be largest, based on previous studies and confirmed with visual inspection (Banaschewski et al., 2003, Banaschewski et al., 2004, Jonkman, 2006, Valko et al., 2009). The P3 was calculated as the mean amplitude in a 400-700msec latency window, because the activity within this time window occurred over a long period making it difficult to identify one peak, as has been done in previous similar studies (Groom et al.). The Cue-P3 and Go-P3 were measured at Pz, and the NoGo-P3 was measured at Cz, Cpz and Pz due to increased anteriorisation with increasing age.
(Jonkman, 2006, Valko et al., 2009). This is supported by our topographical maps (Figure 6-3). The N2 was scored as the maximal negative peak at Fz between 170-400msec. The CNV was calculated as the mean area at Cz between 1300-1650msec.

Table 6-1: Mean (SD) number of segments in each ERP average per stimulus and group during the CPT-OX

<table>
<thead>
<tr>
<th>Stimulus type</th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cue, mean (SD)</td>
<td>68.92 (4.49)</td>
<td>65.89 (6.62)</td>
<td>68.19 (4.84)</td>
<td>68.52 (5.28)</td>
</tr>
<tr>
<td>Go, mean (SD)</td>
<td>34.36 (4.33)</td>
<td>32.63 (3.66)</td>
<td>30.70 (5.47)</td>
<td>30.30 (5.17)</td>
</tr>
<tr>
<td>NoGo, mean (SD)</td>
<td>30.40 (4.43)</td>
<td>29.00 (3.64)</td>
<td>30.44 (2.73)</td>
<td>30.08 (4.36)</td>
</tr>
</tbody>
</table>

Note: Number of segments entered into univariate ANOVA to check for group differences, with group as between-subjects variable (TD, ASD, ADHD, ASD+ADHD)

Abbreviations: TD, typically developing controls; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid ASD and ADHD; ERP, event-related potential.

6.3.4 Statistical analysis

The general analysis strategy is described in Section 2.5.1, 2.5.2 and 2.5.3.2 (Chapter 2). 6 children were excluded from performance and ERP analysis on the basis of extreme omission errors (>70%, upper 5% of sample) indicating a lack of attention to task and/or poor understanding of task instructions that limited the number of segments for reliable ERP analysis (ADHD n=2; ASD+ADHD n=4). 1 TD subject was removed from analysis due to technical difficulties during recording and two additional ASD+ADHD subjects were removed from the Go condition due to insufficient segments. One outlier was removed from the CNV parameter (±3.5 SD).

6.3.4.1 Statistical analysis for group differences

The NoGo-P3 at the three scalp locations was entered into a MANOVA with Tamhane correction that caters for unequal variance. A repeated measures ANOVA was used to assess conflict processing by comparing peak amplitude of the N2 component in Go and NoGo trials. For other ERP measures (Cue-P3, Go-P3, CNV) the groups were compared for differences using separate univariate ANOVAs. Sidak correction was used to correct for multiple testing, unless otherwise stated. In order to evaluate the utility of this method to dissociate clinical groups and elucidate the basis of comorbidity, the between-subjects factor was defined in two ways, as described in Section 2.5.3.2, Chapter 2.
Correlations between IQ and age and each of the dependent variables were calculated due to differences in IQ between groups and potential developmental effects on these parameters. When these correlations were significant, analyses of covariance (ANCOVAs) or partial correlations were conducted to analyze whether group differences were influenced by IQ and/or age.

6.3.4.2 Dimensional analyses
Dimensional analyses followed the same analysis strategy described in Section 2.5.3.2, Chapter 2. Differences between groups in correlations between symptom scores and performance measures were examined using Fisher’s transformations.

6.4 Results

6.4.1 CPT performance
The CPT performance data are summarized by group in Table 5-1, Chapter 5 and discussed in Section 5.4.1, Chapter 5. Briefly, subjects with ADHD diagnosis (ADHD/ASD+ADHD) demonstrated a higher number of omission errors and greater response variability, as indexed by the standard deviation of reaction time and coefficient of variation.

6.4.2 ERP parameters
The ERP data is summarised by group in Table 6-2 and grand average ERP maps are shown in Figures 6-1 and 6-2. Age and IQ were not significant as covariates for any of the ERP measures (all p>0.5).

Table 6-2: Mean amplitude (in μV) for the ERP components in the CPT-OX by group

| Diagnosis | | | | |
## 6.4.2.1 Cue-P3

ERP grand averages and maps of all four groups are illustrated in Figure 6-2. A significant main effect of group on amplitude of the Cue-P3 emerged \[F (3, 81) = 4.79, p = .004\]. Post-hoc analyses indicated attenuated Cue-P3 in ASD+ADHD \(p = .002, d = 1.16\) compared to the TD group. Subjects with ADHD (ADHD/ASD+ADHD) \[F (1, 81) = 7.81, p = .01, d = 0.68\] were driving the attenuation of the Cue-P3 with a trend for subjects with ASD (ASD/ASD+ADHD) \[F (1, 81) = 3.71, p = .06\]. The interaction between ADHD and ASD was non-significant suggesting additive effects on the Cue-P3 \[F (1, 81) = 0.52, p = .48\].

## 6.4.2.2 Go-P3

There was no main effect of group on the amplitude of the Go-P3 \(F (3, 79) = 1.91, p = .13\). When combined by the presence of ADHD diagnosis there was a trend toward attenuated Go-P3 amplitude \[F (1, 79) = 3.68, p = .06\], which was not shown when combined by ASD diagnosis \[F (1, 79) = 1.14, p = .29\]. There was no significant interaction between ASD and ADHD \[F (1, 79) = 0.10 p = .75\].

## 6.4.2.3 NoGo-P3

ERP grand averages and maps of all four groups are illustrated in Figure 6-3. A significant multivariate effect on group emerged for the NoGo-P3 \[F (9, 237) = 2.19, p = .02; Pillai’s
trace=.23]. Univariate testing indicated a significant effect of group on CPz only using a Bonferroni adjusted alpha level of .017 \( [F (3, 79) =4.68, p=.01] \). Post-hoc analyses revealed the ASD+ADHD group displayed significantly attenuated NoGo-P3 at CPz compared to TD \( (p=.006, d=1.04) \) with a trend compared to ASD-only subjects \( (p=.08, d=0.86) \). When combined, subjects with ADHD (ADHD/ ASD+ADHD) had significantly attenuated NoGo-P3 at each scalp location: Cz \( [F (1, 79) =5.15, p=.02, d=.053] \); CPz \( [F (1, 79) =12.82, p=.001, d=0.79] \); and Pz \( [F (1, 79) =5.88, p=.03, d=0.52] \) and when the NoGo-P3 was grouped across all scalp locations \( [F (3, 77) =4.35, p=.02] \) compared to subjects without ADHD (TD/ASD). There was no effect of the presence of ASD (ASD/ASD+ADHD; all \( p>.05 \)). There was no significant interaction between ASD and ADHD suggesting additive effects \( [F (3, 77) =1.08, p=.36] \).

### 6.4.2.4 N2

A main effect of condition emerged showing enhanced amplitude in the NoGo condition \( [F (1, 79) =7.66, p=.01] \). A main effect of group on N2 amplitude across both conditions emerged \( [F (3, 79) =5.83, p =.001] \), with post-hoc analyses revealing the N2 was significantly attenuated in the ASD+ADHD group compared to the TD group \( (p=.001, d=1.12) \) and the ASD-only group \( (p=.040, d=.086) \). When grouped according to diagnosis, both ADHD diagnosis (ADHD/ASD+ADHD; \( F (1, 79) =11.25, p=.001, d=0.73 \)) and a trend for ASD diagnosis (ASD/ASD+ADHD; \( F (1, 79) =3.55, p=.06, d=0.46 \)) were associated with reduced N2 amplitude.

A significant interaction between group and condition emerged \( [F (3, 79) =3.69, p=.02] \). Post-hoc analyses revealed that subjects with ASD-only demonstrated significantly reduced N2 amplitude enhancement in the NoGo condition \( (p=.02, d=0.98) \), with a trend emerging for ASD+ADHD subjects \( (p=.08, d=0.73) \); see Figure 6-1). ASD diagnosis (ASD/ASD+ADHD) appear to account for this deficit \( [F (1, 79) =4.51, p=.04, d=0.53] \) and there was no effect of the presence of ADHD diagnosis (ADHD/ASD+ADHD; \( [F (1, 79) =1.27, p=.26, d=0.30 \)). There was a trend towards an interaction between ASD and ADHD and condition, suggesting possible non-additive effects on N2 enhancement in this task \( [F (1, 79) =3.89, p=.05] \).
Chapter 6: Neurophysiological correlates of attention in ASD, ADHD and ASD+ADHD

Figure 6-1: Group differences on N2 enhancement from Go to NoGo trials

Abbreviations: TD, typically developing controls; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid ASD and ADHD
Note: Black represents TD, red represents ASD-only; blue represents ADHD-only; green represents comorbid ASD+ADHD.

6.4.2.5 CNV

ERP grand averages and maps of all four groups are illustrated in Figure 6-2. Analyses indicated a significant main effect of group on amplitude of the CNV \[ F (3, 81) = 4.32, p = .01 \]. Post-hoc analyses revealed that subjects with ASD-only had significantly greater amplitude compared to the TD group \( p = .03, d = .087 \) and the ASD+ADHD group \( p = .01, d = 1.00 \). When combining the groups by the presence of ASD (ASD/ASD+ADHD) or ADHD (ADHD/ASD+ADHD) diagnosis there were no significant group differences, suggesting an enhanced CNV is specific to ASD-only. The interaction term between ASD and ADHD was significant suggesting non-additive effects \[ F (1, 81) = 10.44, p = .002 \]. In order to examine the potential contribution of developmental effects to this finding, we ran the analyses excluding the youngest third of participants (age<9.82), revealing a trend level effect of group on CNV amplitude \[ F (3, 54) = 2.37, p = .08 \], with no significant post-hoc differences between groups.
Figure 6-2: Grand mean ERPs to Cue stimuli for each group and isocontour maps derived for the grand-average in the 400-700ms window for Cue-P3 and 1300-1650ms window for CNV for each group, plus t-maps for the group comparison.

Note: Black represents TD, red represents ASD-only; blue represents ADHD-only; green represents comorbid ASD+ADHD.
Figure 6- 3: Grand mean ERPs to NoGo stimuli for each group and isocontour maps derived for the grand-average in the 400-700ms window for NoGo-P3 for each group, plus t-maps for the group comparison.

Note: Black represents TD, red represents ASD-only; blue represents ADHD-only; green represents ASD+ADHD.
6.4.3 Dimensional analyses between ERP parameters, symptom scores and performance measures

6.4.3.1 Associations between ERP parameters and symptom scores

In order to examine whether ERP parameters are related to quantitative trait measures of the disorders, I conducted correlations between parameters that showed differences between diagnostic groups and parent-rated symptom scores (see Table 6-3). Across the whole sample, the Cue-P3 amplitude was significantly associated with ADHD symptoms (Inattention: $r=-.23$, $p=.04$; Hyperactivity-Impulsivity: $r=-.26$, $p=.02$) and not ASD symptoms ($r=-.16$, $p=.19$). Attenuated NoGo-P3 was associated with ADHD symptom scores (Inattention: $r=-.26$, $p=.02$; Hyperactivity-Impulsivity: $r=-.29$, $p=.01$), but not ASD (SCQ: $r=-.05$, $p=.68$). The N2 amplitude enhancement from Go to NoGo trials was correlated with autism symptom scores (SCQ: $r=.23$, $p=.04$) but not ADHD symptom scores (Inattention: $r=-.00$, $p=.99$; Hyperactivity: $r=.12$, $p=.29$).

ERP parameters which were significantly correlated across all groups with the SCQ and Conners scores, respectively, were then taken forward for inclusion in multiple linear regression analyses with hierarchical entry, to determine the relative contributions of ASD and ADHD symptoms measures on these different ERP parameters. These models largely confirmed the above correlations supporting the SCQ or the Conners as predictors of specific ERP measures; for each parameter that was associated with one rating scale and not the other, the regression model showed a significant increase $R^2$ indicating enhanced prediction of the ERP parameter, which remained after controlling for the potential effect of the confounding rating scale. However, the NoGo-P3 associations were reduced and no longer significantly correlated with the Conners rating scale ($p>.05$). For this reason, I also ran the correlations separately by group for the NoGo-P3, which revealed a significant difference between the ASD and TD group. In the ASD group, the Conners inattention subscale was differentially correlated with the NoGo-P3 compared to the TD group, indicating greater inattention symptom scores were associated with enhanced NoGo-P3 amplitude.

6.4.3.3 Correlations between ERP parameters and performance measures

Noted correlations between ERP parameters and performance measures across the whole sample and by group are shown in Table 6-3. Attenuated Cue-P3 was correlated with poor task performance (SD-RT: $r=-.26$, $p=.01$; CV: $r=-.28$, $p=.01$; omission errors: $r=-.32$, $p=.003$) and attenuated NoGo-P3 ($r=.44$, $p<.001$). The amplitude of the NoGo-P3 was associated with response variability (SD-RT: $r=-.28$, $p=.01$; CV: $r=-.23$, $p=.03$). Reduced N2 amplitude
enhancement was associated with increased response variability on the task (CV: \( r = -0.25 \), \( p = 0.02 \)). The amplitude of the CNV was associated with increased response variability on the task (SD-RT: \( r = 0.22 \), \( p < 0.05 \)).

A few differential patterns of correlations emerged according to diagnostic group suggesting that ERP indices of attention are associated with different cognitive processes among case and control groups (see Table 5-3). Fisher’s transformations only revealed several significant group differences in correlation. There appeared to be differing relationship between inattentive symptoms and ERP components in the clinical conditions compared to controls. Participants with ADHD (both ADHD and ASD+ADHD) demonstrated a relationship between poor performance and reduced N2 amplitude enhancement from Go to NoGo trials. Participants with ASD-only showed a negative correlation between CNV amplitude and CV, suggesting enhanced amplitude of the CNV is associated with increased variability.

Table 6-3: Correlations between ERP parameters, symptom scores and performance measures across the whole sample, followed by each group separately

<table>
<thead>
<tr>
<th></th>
<th>Cue-P3</th>
<th>NoGo-P3</th>
<th>Go-N2 – NoGo-N2</th>
<th>CNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERP parameters</td>
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<tr>
<td>Cue-P3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NoGo-P3</td>
<td>.44**</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Go-N2 – NoGo-N2</td>
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<td>.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CNV</td>
<td>-.08</td>
<td>-.20†</td>
<td>-.07</td>
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<tr>
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<tr>
<td>symptom scores</td>
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<td></td>
</tr>
<tr>
<td>SCQ</td>
<td>-.16</td>
<td>-.04</td>
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<td>.02</td>
</tr>
<tr>
<td>Conners DSM-Inattentive</td>
<td>-.23*</td>
<td>-.26*</td>
<td>-.00</td>
<td>-.10</td>
</tr>
<tr>
<td>Conners DSM-Hyperactive</td>
<td>-.26*</td>
<td>-.29**</td>
<td>-.12</td>
<td>.12</td>
</tr>
<tr>
<td>Performance measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omission errors</td>
<td>-.32**</td>
<td>-.10</td>
<td>-.13</td>
<td>-.02</td>
</tr>
<tr>
<td>SD-RT</td>
<td>-.27*</td>
<td>-.21†</td>
<td>-.17</td>
<td>.22*</td>
</tr>
<tr>
<td>CV</td>
<td>-.28*</td>
<td>-.24*</td>
<td>-.25*</td>
<td>.12</td>
</tr>
</tbody>
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Table 6-3 (continued)

<table>
<thead>
<tr>
<th>TD</th>
<th>Cue-P3</th>
<th>NoGo-P3</th>
<th>Go-N2 – NoGo-N2</th>
<th>CNV</th>
</tr>
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<tr>
<td>ERP parameters</td>
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<td></td>
<td></td>
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<tr>
<td>NoGo-P3</td>
<td>.65**</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Go-N2 – NoGo-N2</td>
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<td>.34†</td>
<td>-</td>
<td>-</td>
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<tr>
<td>CNV</td>
<td>-.11</td>
<td>-.38†</td>
<td>-.18</td>
<td>-</td>
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<tr>
<td>Parent-rated</td>
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<td>.01</td>
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<td>-.10</td>
</tr>
<tr>
<td>Conners DSM</td>
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<td>-.28</td>
<td>-.45*</td>
<td>.28</td>
</tr>
<tr>
<td>Conners DSM-Hyperactive</td>
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<td>-.30</td>
<td>.17</td>
</tr>
<tr>
<td>Performance</td>
<td></td>
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<td></td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Omission errors</td>
<td>-.28</td>
<td>-.01</td>
<td>.03</td>
<td>-.07</td>
</tr>
<tr>
<td>SD-RT</td>
<td>-.20</td>
<td>-.15</td>
<td>.16</td>
<td>.37†</td>
</tr>
<tr>
<td>CV</td>
<td>-.30</td>
<td>-.25</td>
<td>.09</td>
<td>.30</td>
</tr>
</tbody>
</table>

| ASD               |        |         |                 |      |
| ERP parameters    |        |         |                 |      |
| NoGo-P3           | .01    | -       | -               | -    |
| Go-N2 – NoGo-N2   | .11    | -.16    | -               | -    |
| CNV               | -.41†  | -.52*   | .07             | -    |
| Parent-rated      |        |         |                 |      |
| symptom scores    |        |         |                 |      |
| SCQ               | .04    | -.12    | -.14            | .20  |
| Conners DSM      | .16    | -.03    | .08             | -.31 |
| Conners DSM-Hyperactive | .03 | -.03 | .08 | .17 |
| Performance       |        |         |                 |      |
| measures          |        |         |                 |      |
| Omission errors   | .13    | .08     | .02             | -.20 |
| SD-RT             | -.27   | .15     | -.12            | .03  |
| CV                | -.20   | .31     | -.23            | -.28 |
Note: Correlations with cell borders denote significantly different correlations for case versus TD groups; single border \( p < .10 \), double border \( p < .05 \), triple border \( p < .01 \).

**Abbreviations**: Conners, Conners Third Edition Parent Rating Scale Short Form; CNV, contingent negative variation; CV: coefficient of variation (SD-RT/MRT); SCQ, Social Communication Questionnaire; SD-RT, within-subject variability in RTs in milliseconds. ** \( p < .01 \) * \( p < .05 \) † \( p < .10 \)
6.5 Discussion

This novel study investigated overlap in neural correlates of attention and inhibition in children, following detailed assessments of ASD and/or ADHD to ensure minimal diagnostic blurring and using an ERP paradigm that has been used in previous studies of ADHD. Findings from group differences for diagnostic status and correlations between quantitative trait measures converge to suggest both shared and unique deficits across the two disorders. For the majority of findings, children with comorbid ASD+ADHD display deficits of both disorders suggestive of an additive co-occurrence, rather than a separate condition with a distinct pattern of deficits.

6.5.1 Main findings

The attenuation of the NoGo-P3 in both ADHD groups supports previous studies that indicate abnormal inhibitory processing in children with ADHD (Banaschewski et al., 2004, Doehnert et al., 2010, Fallgatter et al., 2004, Valko et al., 2009). Notably, these deficits were specific to ADHD and intact in ASD, which is in line with previous cognitive research (Bühler et al., Happé et al., 2006b). In addition, both ADHD groups displayed reduced amplitude of the Cue-P3, in agreement with previous studies (Banaschewski et al., 2003, Banaschewski et al., 2004, Doehnert et al., 2010, Van Leeuwen et al., 1998). The non-significant effect for ASD-only and ASD-related symptoms implies attentional orienting impairments are specific to ADHD, which may suggest reported deficits in attentional orienting in ASD are due to undetected ADHD symptoms (Sanders et al., 2008, Tsai et al., 2011). Given that the Cue-P3 also correlated with poor task performance associated with ADHD (see Chapter 4) and the NoGo-P3 as an index of response inhibition (McLoughlin et al., 2010), this finding may indicate less effective recruitment of cognitive resources to process subsequent stimuli, and suggests behavioural impairments are temporally or causally preceded by neuronal deficits in covert processes (Spronk et al., 2008).

Attenuated N2 amplitude across both conditions was found in children with ASD+ADHD, suggesting this group display the most severe deficits on this component. Reduced or absent N2 amplitude enhancement from Go to NoGo trials was only found in the ASD groups. This complements previous work in ASD reporting problems in shifting from one response to another (Hill, 2004, Sanders et al., 2008). The intact N2 amplitude enhancement in ADHD is in line with previous studies using the CPT-OX that did not find case-control differences (Banaschewski et al., 2004, Fallgatter et al., 2004, Overtoom et al., 1998). Differences have been found in other more demanding tasks, such as the Stop task (Albrecht et al., 2005) and
the Eriksen flanker task (Albrecht et al., 2008, McLoughlin et al., 2009), which may suggest that reduced N2 in ADHD is task-dependent. These findings suggest impaired conflict monitoring is related to ASD symptoms and as children with ASD-only appear to present with the greatest deficits, investigation of a continuum of ASD severity increasing with impairment is warranted in this domain.

Children with ASD-only had enhanced CNV compared to TD and ASD+ADHD children. This may indicate that children with ASD+ADHD have impaired preparatory processes compared to ASD-only children, and further that ASD-only children allocate more cognitive resources to prepare for the upcoming stimulus compared to ASD+ADHD and TD children. This is supported by associations between the CNV and the NoGo-P3, suggestive of a compensatory strategy or alternative mechanism to strengthen typical inhibitory control that is present in the ASD-only group (O’Hearn et al., 2008), and the presence of disorder-specific associations with the NoGo-P3 and coefficient of variation. This finding also points toward syndrome-specific abnormalities in pure and complex cases of ASD. The lack of significant abnormality in ADHD-only subjects is inconsistent with previous work (Banaschewski et al., 2004, Valko et al., 2009) although not all have reported differences (Van Leeuwen et al., 1998). This may be due to developmental changes in the CNV (Klein and Feige, 2005); studies of ADHD show an enhanced late CNV in occipital regions at 5-7 years of age (Spronk et al., 2008) with a trend reported at 6-12 years (Hennighausen et al., 2000). These findings may therefore be due to varying stages of development in the sample, as supported by additional analyses revealing limited group effects when excluding younger participants.

In support of previous research using the same task, there was limited evidence of impaired response execution processes as indexed by the Go-P3 (Banaschewski et al., 2004, Van Leeuwen et al., 1998). As these impairments in target detection are more apparent in slow conditions (Wiersema et al., 2006), this might suggest these deficits in ADHD are related to suboptimal arousal, and further that they can be reduced by valid cues as used in the present task (McLoughlin et al., 2010), which may also explain typical Go-P3 amplitude in ASD contrary to some previous work (Townsend et al., 2001).

6.5.2 Implications

Taken together, the findings indicate deficits in response preparation and conflict monitoring in ASD, which is in line with theoretical accounts that propose children with ASD have deficits in the ability to flexibly shift to different cognitive demands (Hill, 2004) and disengage attention ("sticky attention"; e.g. (Holmboe et al., 2010)). Children with ADHD display deficits
in attentional orienting, inhibitory processing and behavioural performance, suggestive of specific deficits compared to ASD as well as widespread attentional dyscontrol. According to cognitive energetic models suboptimal arousal may be the basis of these varied deficits, due to problems in cognitive resource allocation to activation and arousal systems (Sergeant, 2000). The findings provide insight into the pathophysiological basis of the comorbidity between ADHD and ASD and are generally compatible with an additive co-occurrence demonstrating deficits of both disorders, supporting findings from twin studies (Ronald et al., 2008b). Because the deficits are most apparent in the comorbid group for inhibitory processing and attentional orienting, the specificity of ADHD correlates may be dependent on the presence or absence of comorbid disorders. Accordingly, in a sample of children with ASD, ADHD symptoms exacerbated deficits in executive function (Yerys et al. 2009). Nevertheless, the significant interaction between ASD and ADHD on CNV amplitude as well as on response variability as indexed by the CV suggests non-additive effects and the conceptualization of the comorbid condition as a distinct condition. The highly heterogeneous nature of all clinical groups are nevertheless likely to give rise to various models of comorbidity across domains and tasks (Rhee et al., 2008, Taurines et al., 2010b). The next step is to explore a domain which is consistently altered in ASD in order to clarify these interpretations (Chapter 6).

6.5.3 Limitations

Certain limitations should be taken into consideration. The relatively small sample size poses difficulties in the interpretation of the data due to low power and may lead to ambiguous conclusions. For example, differing correlations between symptom scores and ERP parameters by group are based on small group sizes The presence of subthreshold symptoms of other disorders associated with impaired inhibitory processing, such as conduct disorder, may influence group results (Banaschewski et al., 2004). As findings of EF deficits across ADHD and ASD is somewhat dependent on the task used (Sergeant et al., 2002), it may be the case that inhibitory deficits are displayed in ASD in different tasks or age groups. Similarly, it may be argued that this task taps into response selection processes and interference control rather than response inhibition per se, which should be explicitly tested using alternative tasks. In addition, the exclusion of participants due to poor performance and subsequent insufficient trials may reflect task difficulty and potential floor effects. While we have proposed a specific inhibitory deficit in ADHD, these processes do not necessarily have distinct neuroanatomical or biological underpinnings, and as such further work investigating the mechanisms underlying these neurophysiological responses is required.
6.5.4 Conclusion

This is the first study to directly investigate shared and distinct ERP correlates of attention in children with ASD and/or ADHD using the same test paradigm across groups. The findings support specific neuronal deficits in attention for ASD and ADHD, and suggest an additive model of ASD+ADHD. This complements and extends cognitive findings and suggests previous inconsistent findings in the literature may be due to misspecification in group allocation. Along with accumulating evidence of co-occurring ADHD and ASD, this supports the adoption of a broader view of psychopathology when assessing underlying pathophysiology. Efforts to further define these disorders may help refine classification systems and enhance the assessment of these complex cases for more specific treatment strategies (Banaschewski and Brandeis, 2007). Disentangling behavioural variation in executive function is also likely to aid the identification of shared or disorder-specific susceptibility genes and other causal mechanisms underlying the complex aetiology of ASD and ADHD.
CHAPTER 7: Neurophysiological responses to faces and gaze direction differentiate children with ASD, ADHD and ASD+ADHD

7.1 Summary

In Chapter 6, I investigated ERP indices of executive function that are consistently altered in ADHD, and consequently the next step is to investigate a domain which is consistently altered in ASD. Both children with autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) demonstrate social impairment that may be underpinned by neural abnormalities. Despite substantial overlap between ASD and ADHD, ERP markers of face and gaze processing have not been directly investigated in ADHD or compared across disorders. Systematically assessed groups of ASD (n=19), ADHD (n=18), comorbid ASD+ADHD (n=29) and typically developing (TD) controls (n=26) were presented with upright or inverted faces with direct or averted gaze, with concurrent electrophysiological recording of the P1 and N170 event-related potentials (ERPs). While the N170 was predominant in the right hemisphere in TD and ADHD, children with ASD (ASD/ASD+ADHD) showed a bilateral distribution. In addition, TD and ADHD children displayed shorter P1 latency and enhanced midline-N170 amplitude to averted gaze compared to direct gaze, whereas children with ASD showed shorter P1 latency to direct gaze and no sensitivity to gaze direction on the midline-N170. In contrast, while TD and ASD children exhibited delayed and enhanced responses to inverted faces compared to upright faces, children with ADHD (ADHD/ASD+ADHD) exhibited a reduced face inversion effect on P1 latency. These findings suggest children with ASD have specific impairments in processing gaze direction and abnormalities in neural specialisation, whereas children with ADHD have specific abnormalities in configural processing which may be exacerbated by visual attention deficits. Across these impairments children with comorbid ASD+ADHD present as a hybrid with deficits of both disorders. The identification of unique neural markers of social cognition of ASD and ADHD is likely to improve the understanding of the basis of co-occurring ASD+ADHD to inform clinical assessment and treatment strategies.

7.2 Introduction

Autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) are both common and severely impairing neurodevelopmental disorders with childhood onset.
Although current diagnostic criteria preclude a co-diagnosis, high rates of co-occurrence have been documented and evidence is accumulating for substantial clinical, neuropsychological and genetic overlap (see Section 1.2, Chapter 1 for review). In particular, while social difficulties are a core impairment in ASD, children with ADHD also frequently exhibit social difficulties comparable to those shown in ASD (Clark et al., 1999, Greene et al., 1996, Landau et al., 1998, Luteijn et al., 2000, Mulligan et al., 2009, Santosh and Mijovic, 2004). It is under discussion whether this common co-occurrence reflects a true comorbidity, such that comorbid ASD+ADHD shares common risk factors with the pure disorders, or is a distinct condition with qualitatively different impairments to both pure disorders. In order to elucidate this issue it is important to concurrently assess the nature of co-occurring ASD+ADHD when investigating the underlying mechanisms of social difficulties in ASD and ADHD.

The ability to process faces is considered fundamental to typical development of social abilities (Dawson et al., 2005b). In particular, gaze direction detection is linked to Theory of Mind (ToM) abilities, successful face and emotion recognition and orienting of social attention, which has led to the proposition of a specialised neural mechanism (Baron-Cohen, 1995, Emery, 2000, Itier and Batty, 2009). Event-related potentials (ERPs) provide the excellent temporal resolution necessary to investigate different temporal stages of information processing when attending to face stimuli. In typical individuals the right-lateralised tempor-occipital N170 ERP component appears to be particularly sensitive to face stimuli (see Section 1.3.3.1, Chapter 1 for review). The N170, along with the preceding occipital P1 component, are affected by disruptions of the configuration of facial features, as they show longer latency and larger amplitude to inverted faces compared to upright faces (“face inversion effect”) (Bentin et al., 1996, de Haan et al., 2002, Itier and Taylor, 2002, 2004a, Rossion et al., 1999, Rossion and Gauthier, 2002, Rossion et al., 2000). While the N170 is also sensitive to eyes alone (Bentin et al., 1996), most studies of children and adults do not find modulation of the N170 by gaze direction (Grice et al., 2005, Klucharev and Sams, 2004, Schweinberger et al., 2007, Taylor et al., 2001) although see (Watanabe et al., 2002). Behavioural studies suggest that an upright face is required for enhanced processing of direct gaze (Senju et al., 2005a). While one study found no modulation by gaze when presented in inverted faces on a component peaking at 240ms in 4 month old infants (proposed to be the infant precursor to the adult N170) (de Haan et al., 2002, Farroni et al., 2004), further investigation of the N170 is required to confirm the reliance of gaze perception on configural processing and overlap in the neural mechanisms subserving these processes.
While typically developing infants as young as 3 months old show a preference for face like-stimuli (Hood et al., 1998), individuals with ASD look less at human faces and this is evident in children as young as 6-12 months old (Maestro et al., 2002, Osterling and Dawson, 1994). Studies also report impairments in face perception and recognition in individuals with ASD (see Section 1.3.2.4.3). ERP studies suggest abnormal responses to face stimuli, notably a reduction or absence of the face inversion effect, on P1 and N170 amplitude in individuals with autism (Batty et al., 2011, Dawson et al., 2002, Dawson et al., 2005b, Hileman et al., 2011, Mc Cleery et al., 2009, McPartland et al., 2004, McPartland et al., 2011, O'Connor et al., 2005, 2007, Webb et al., 2006, Webb et al., 2009). The lack of sensitivity to face inversion has been used to support theories of ‘weak central coherence’ in ASD, referring to a cognitive bias toward local detail (Happé, 1999), which could be associated with a reliance on features to process faces and/or an impairment in configural face processing.

Children with autism also demonstrate atypical patterns of gaze processing using eye-tracking, such as less fixation on or saccades away from the eyes (Dalton et al., 2005, Itier and Batty, 2009, Klin et al., 2002b, Pelphrey et al., 2002, Spezio et al., 2007). ERP studies support abnormalities in gaze direction detection. Firstly, using passively viewed front-view face stimuli, a larger N170 over midline channels to direct than averted gaze was observed in young children with autism with no such effect for age-matched controls (Grice et al., 2005), similar to that shown in 4-month-old typically developing infants (Farroni et al., 2004). In older children explicitly processing gaze direction in laterally averted faces, the N170 was enhanced by direct gaze in controls but remained uninfluenced by gaze in autism (Senju et al., 2005b). Abnormal ERP responses to gaze direction are also observed in infant siblings of children with ASD (Elsabbagh et al., 2012b, Elsabbagh et al., 2009a) and are predictive of subsequent ASD diagnosis (Elsabbagh et al., 2012b). In addition, while the N170 is larger in the right hemiscalep compared to the left hemiscalep in typically developing individuals, individuals with autism show an atypical bilateral scalp distribution (Carver and Dawson, 2002, Mc Cleery et al., 2009, McPartland et al., 2004, Senju et al., 2005b) suggestive of abnormal cortical specialisation for faces (Dawson et al., 2005b). As abnormalities in these face-sensitive ERPs have been associated with impaired social skills (Hileman et al., 2011), it is likely that these abnormalities reflect the social and communication deficits observed in autism.

There is limited knowledge regarding basic face processing in ADHD at the neural level and particularly how this compares to deficits reported in ASD. While there is consistent evidence for impaired emotion perception and recognition in ADHD (see Chapter 7), it is unclear
whether emotion deficits are accompanied by or temporally/causally preceded by abnormalities in structural face processing and gaze direction detection. One study reported poor performance in adults with ADHD in recognising human versus animal faces (Rapport et al., 2002). Studies of ERP responses to emotional face expressions report reduced P1 amplitude, increased N170 amplitude and reduced P300 amplitude in temporal regions to neutral faces compared to controls (Williams et al., 2008). A recent ERP study reports deficits in N170 modulation to emotional face stimuli that were not accompanied by impairments in basic face processing, as supported by an enhanced N170 response to face stimuli compared to word stimuli (Ibanez et al., 2011). Importantly no study has assessed P1 and N170 responses to face stimuli in individuals with ASD+ADHD. As social difficulties in ADHD are associated with greater impairment (Nijmeijer et al., 2008), a closer investigation of the neural correlates of face processing in ADHD and their overlap with ASD is required.

The aim of this study was to investigate whether the ERP abnormalities in face and gaze processing associated with ASD are also found in ADHD and comorbid ASD+ADHD. We presented upright and inverted faces with direct and averted gaze in an experimental design previously used (Farroni et al., 2004, Grice et al., 2005) to cases of ASD, ADHD, ASD+ADHD and typically developing children that were systematically assessed to ensure minimal misspecification in group allocation. We hypothesized that the clinical groups would not show the typical amplitude enhancement in the right hemiscalp or sensitivity to face orientation and gaze direction as indexed by the P1 and N170 ERPs. We expected the ASD and comorbid group to show a more profound impairment in face and gaze processing compared to ADHD children, and the magnitude of the deficits to be associated with the number of autism symptoms reported by parents. In addition, as reported by previous studies suggesting overlap between face and gaze processing, modulation of neural responses by gaze were expected in upright faces only, particularly in the TD group who have been shown to rely on configural processing.

7.3 Methods

7.3.1 Sample

Nineteen male participants with ASD, 18 with ADHD, 29 with co-occurring ASD and ADHD, and 26 typically developing controls (TD) took part in the study. The age range was 8-13 years, and participants were age-matched at the group level. See Section 2.5.3.4, Chapter 2 for a full description of participant recruitment and assessment.
7.3.2 Task and stimuli

The stimuli were colour images of three female faces with direct or averted gaze (looking right or left; see Figure 6-1). These images were presented either in upright or inverted orientation on a grey background. Faces subtended 15.8° x 10.2° from a viewing distance of 90cm. Each trial began with the presentation of a fixation stimulus that had a variable inter-trial interval of 800 and 1200msec to reduce stimulus repetition effects and ensure the child could not predict the onset of the face stimulus. The fixation stimuli consisted of various static cartoon images. Face stimuli were presented for 500msec followed by a 500msec interval without visual stimulus, and were aligned vertically so that the eyes appeared at the same height as the fixation stimuli, in order to orient attention towards the eyes. 360 trials were presented in four blocks of 80 trials with randomised presentation. Participants were asked to count the appearances of flags among the fixation stimuli, in order to stimulate the child’s participation and attention. The approximate number of flags was used as a benchmark for attention to task and participants were also continually monitored by video recording.

![Figure 7-1: Examples of experimental stimuli showing direct and averted gaze in upright and inverted faces.](image)

*Note*: Stimuli were presented individually.
7.3.3 Electrophysiological recording and analysis

7.3.3.1 EEG recording and preprocessing

See Section 2.4.1 and 2.4.2, Chapter 2, for details of EEG data acquisition and general preprocessing.

7.3.3.2 ERP analysis

Baseline correction was performed using a 200msec prestimulus reference period. Stimulus-locked epochs (−200 to 700 msec peristimulus window), which retains comparability with face-elicited components investigated in Chapter 6. Segments were averaged for the following trial types: upright orientation/direct gaze; upright orientation/averted gaze; inverted orientation/direct gaze; and inverted orientation/averted gaze. Averages were computed for each participant in each experimental condition on a minimum of 55 trials per stimulus (see Table 7-1). Based on visual inspection of the grand average and congruent with the topography of the components and the literature (Batty et al., 2011, Churches et al., 2010, Eimer, 2011, O’Connor et al., 2005), the N170 was scored as the maximal negative peak at P7 and P8 and the midline-N170 at Pz, in a 150-290ms latency window, and the P1 was scored as the maximal positive peak in a 100-200ms latency window at O1 and O2.

Table 7-1: Mean (SD) number of segments in each ERP average per stimulus and group during the face and gaze processing task

<table>
<thead>
<tr>
<th>Stimulus type</th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upright, direct</td>
<td>72.13 (4.33)</td>
<td>70.33 (4.43)</td>
<td>70.18 (5.58)</td>
<td>70.83 (3.58)</td>
</tr>
<tr>
<td>Upright, averted</td>
<td>72.50 (4.66)</td>
<td>70.22 (5.07)</td>
<td>69.47 (6.14)</td>
<td>70.28 (4.81)</td>
</tr>
<tr>
<td>Inverted, direct</td>
<td>70.96 (5.20)</td>
<td>68.28 (4.66)</td>
<td>69.12 (5.59)</td>
<td>70.93 (4.94)</td>
</tr>
<tr>
<td>Inverted, averted</td>
<td>72.50 (3.85)</td>
<td>69.56 (4.78)</td>
<td>70.12 (5.88)</td>
<td>70.48 (5.47)</td>
</tr>
</tbody>
</table>

Abbreviations: TD, typically developing controls; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid ASD and ADHD.

7.3.4 Statistical analyses

The general analysis strategy is described in Section 2.5.1, 2.5.2 and 2.5.3.2 (Chapter 2). One ASD subject was removed due to extreme outlier scores (±3.5 SD) on the P1 in all conditions. One TD subject was removed from analysis due to insufficient segments for reliable ERP measurement. One ADHD and one TD subject were removed from analysis due to poor attention to task shown in video recording.
7.3.4.1 Statistical analysis for group differences

A repeated-measures ANOVA was conducted on each ERP parameter (P1 amplitude, P1 latency, N170 amplitude, N170 latency, N170 midline amplitude, N170 midline latency) with orientation (upright/inverted), gaze (direct/averted) and, for P1 and occipito-temporal N170 hemisphere (left/right), as the within-subjects factors, and group as the between-subjects factor. In order to evaluate the utility of this method to dissociate clinical groups and elucidate the basis of comorbidity, the between-subjects factor was defined in two ways, as described in Chapter 2, Section 2.5.3.2. Post-hoc analyses were carried out when necessary using Tamhane correction that caters for unequal variances.

IQ was not a significant covariate on any analyses and therefore was removed. Age was not a significant predictor of P1 amplitude, P1 latency, midline-N170 amplitude or midline-N170 latency and therefore was removed as a covariate. However, due to reported developmental changes on the P1 and N170 components (Taylor et al., 2004), any changes to reported significant findings when age was included as a covariate are noted (main effects on P1 amplitude and latency). For ease of interpretation, only significant effects are reported in the results section. The results of partial eta-squared calculations (conducted in SPSS) demonstrated no moderate effect sizes for other parameters (partial eta<.06), suggesting null findings were not a result of limited power.

7.3.4.2 Dimensional analyses

Dimensional analyses followed the same analysis strategy described in Section 2.5.3.2, Chapter 2.

7.4 Results

7.4.1 P1 amplitude

Descriptive statistics are shown in Table 7-2. Grand average ERPs and topographical maps are shown in Figure 7-5. There was a main effect of gaze on P1 amplitude \([F (1, 84) =4.49, p=.04, d=0.07]\), indicating greater amplitude for averted gaze compared to direct gaze, that did not remain when controlling for age \([F (1, 84) =.01, p=.93]\). There was also a main effect of hemisphere (left versus right) on P1 amplitude \([F (1, 84) =7.77, p=.01, d=0.23]\): greater amplitude was shown in the right hemisphere compared to the left hemisphere in all groups across all conditions, which did not remain when controlling for age \([F (1, 84) =.122, p=.73]\).
There was no main effect of group on P1 amplitude \( [F(3, 84) = .12, p = .95] \), and no significant interactions with orientation \( [F(3, 84) = 1.47, p = .23] \), gaze \( [F(3, 84) = 0.96, p = .42] \) or hemisphere \( [F(3, 84) = 0.46, p = .71] \), nor were effect sizes beyond small observed for these contrasts (all \( d < .20 \)). No group effects emerged when combining the groups by the presence of ASD \( [F(1, 86) = 0.03, p = .87] \) or ADHD diagnosis \( [F(1, 86) = 0.17, p = .68] \), indicating that P1 amplitude effects described below are characteristic of all groups. There was no interaction between ASD and ADHD on these parameters (all \( p > .05 \)).

### 7.4.2 P1 latency

Across all groups there was a main effect of orientation of faces on the latency of the P1 \( [F(1, 84) = 24.79, p < .001, d = 0.46] \): latency was longer for inverted faces compared to upright faces, which did not remain when age was controlled for \( [F(1, 84) = 1.09, p = .30] \). There was no main effect of group on P1 latency \( [F(3, 84) = .95, p = .42] \), and no significant interactions with orientation \( [F(3, 84) = 1.52, p = .22] \) or gaze \( [F(3, 84) = 1.46, p = .23] \). There was, however, a significant interaction between group and hemisphere \( [F(3, 84) = 4.51, p = .01] \). Post-hoc analyses revealed that TD children showed longer latency in the RH whereas the ASD+ADHD group showed longer latency in the LH (\( p = .02, d = 0.94 \)) with a similar non significant tendency in the ASD-only (\( p = .08, d = 0.88 \)), but a non-significant effect for ADHD-only (\( p = .18, d = 0.72 \); Figure 7-2).

![Figure 7-2](image)

**Figure 7-2**: The interaction between hemisphere and diagnostic group on P1 latency (± 1SE)

**Abbreviations**: TD, typically developing controls; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid ASD and ADHD; LH, left hemisphere; RH, right hemisphere

Interesting group differences emerged when combining the groups by the presence or absence of ADHD or ASD diagnosis. The interaction between group and hemisphere remained when grouping subjects by the presence or absence of ASD (ASD/ASD+ADHD: \( F(1, 84) = 6.36, p = .01 \),...
d=0.60) with a trend for ADHD (ADHD/ASD+ADHD: F (1, 84) =3.10, p=.08, d=0.46). There was no significant interaction between ASD and ADHD [F (1, 84) =2.34, p=.13].

When combined by the presence or absence of ASD, a three-way interaction between ASD, gaze and orientation emerged [F (1, 84) =6.68, p=.01, d=0.50]. Planned contrasts revealed that in upright faces, there was a significant interaction between group and gaze: TD and ADHD children showed longer latency to direct than averted gaze, whereas children with ASD and ASD+ADHD showed shorter latency to direct than averted gaze (p=.026 d=0.48; Figure 6-3). In inverted faces, however, there were no significant differences between groups (all p<.05), supporting a gaze effect specific to upright faces (Figure 7-3). This interaction was not shown when combining the groups by ADHD [F (1, 84) =1.72, p=.19], nor was there a significant interaction between ASD and ADHD [F (1, 84) =0.21, p=.65].

Figure 7-3: The interaction between gaze direction and ASD diagnosis on P1 latency in upright faces (± 1 SE).
Abbreviations: No ASD = TD and ADHD; ASD = ASD and ASD+ADHD

When combining the groups by the presence or absence of ADHD diagnosis, an interaction between group and orientation emerged [F (1, 84) =6.03, p=.02, d=0.49] indicating a reduced effect of face inversion in children with ADHD (Figure 6-4) that was not present when combining by ASD diagnosis [F (1, 84) =1.26, p=.26, d=0.13]. There was no interaction between ASD and ADHD [F (1, 84) =0.20, p=.66], suggesting additive effects.
Figure 7-4: The interaction between face orientation and ADHD diagnosis on P1 latency across upright and inverted faces (±1SE).

Abbreviations: No ADHD = TD and ASD; ADHD = ADHD and ASD+ADHD
Table 7-2: Mean (SD) amplitude (in μV) and latency (in msec) for the P1 component on each stimulus by group

<table>
<thead>
<tr>
<th>Stimulus</th>
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<th>ASD</th>
<th>ADHD</th>
<th>ASD+ ADHD</th>
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</thead>
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<td>13.32</td>
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<tr>
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<td>(11.08)</td>
<td>(7.36)</td>
<td>(7.83)</td>
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<td>O2</td>
<td>149.02</td>
<td>142.25</td>
<td>145.11</td>
<td>140.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(9.51)</td>
<td>(14.79)</td>
<td>(12.46)</td>
<td>(11.81)</td>
</tr>
</tbody>
</table>

Abbreviations: ASD: autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid autism spectrum disorder and attention deficit hyperactivity disorder.
Chapter 7: Neurophysiological correlates of face and eye gaze processing in ASD, ADHD and ASD+ADHD

Figure 7-5: Grand mean P1 ERPs to face stimuli for each group and isocontour maps derived for the grand-average at peak latency for each group.

Note: Black represents upright-direct, red represents upright-averted; blue represents inverted-direct; green represents inverted-averted. Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; ASD+ADHD, comorbid attention deficit hyperactivity disorder and autism spectrum disorder.
7.4.3 N170 amplitude

Descriptive statistics are shown in Table 7-3. Grand average ERPs and topographical maps are shown in Figures 7-6. Across the whole sample there was a main effect of orientation on the amplitude of the N170 \( [F(1, 83) =10.51, \ p=.002] \) with increased amplitude for inverted compared to upright faces. An orientation by age interaction \( [F(1, 83) =12.18, \ p=.001] \) indicated that enhanced amplitude of the N170 to inverted faces increases with development \( (r=-.30, \ p=.004) \). An interaction between orientation and gaze was found \( [F(1, 83) =5.34, \ p<.05] \). Posthoc analyses revealed enhanced N170 amplitude for averted gaze compared to direct gaze in upright faces only. There was a three-way interaction between orientation, gaze and age \( [F(3, 83) =4.97, \ p=.029] \), indicating that the gaze effect in upright faces only is shown with increasing age \( (r=.23, \ p=.030) \).

Although no main effect of group emerged on N170 amplitude \( [F(1, 83) =.59, \ p=.63] \), an interaction between group and hemisphere was found \( [F(3, 83) =4.09, \ p=.01] \). Post-hoc analyses revealed enhanced N170 in the left hemisphere for ASD+ADHD subjects compared to enhanced amplitude in the right hemisphere for TD \( (p=.01, \ d=0.94) \) with a trend compared to ADHD \( (p=.07, \ d=0.78) \). When combining the groups by the presence or absence of ASD or ADHD, this interaction appeared to be driven by ASD diagnosis \( [F(1, 83) =8.23, \ p=.01, \ d=0.71] \) and not by ADHD diagnosis \( [F(1, 83) =1.60, \ p=.21, \ d=0.38] \). There was no interaction between ASD and ADHD on this measure \( [F(1, 83) =0.01, \ p=.93] \).

There was also a trend towards an interaction between ASD and orientation with a small effect size \( [F(1, 83) =3.38, \ p=.07, \ d=0.24] \), indicating greater N170 amplitude enhancement to inverted faces compared to upright faces in TD/ADHD, compared to participants with an ASD diagnosis (ASD/ASD+ADHD). An effect of orientation was not found when combining by the presence of an ADHD diagnosis \( [F(1, 83) =2.74, \ p=.10, \ d=0.16] \), nor was there an interaction between ASD and ADHD on orientation \( [F(1, 83) =0.00, \ p=.96] \). There was no interaction between group and gaze \( F(2, 83) =1.14, \ p=.34] \).

7.4.4 N170 latency

Across the whole sample there was a main effect of age \( [F(1, 83) =19.92, \ p<.001] \) showing decreased latency of the N170 with increasing age \( (r=-.43, \ p<.001) \). There was a significant interaction between gaze direction and age \( [F(1, 83) =4.01, \ p=.049] \) indicating longer latency of the N170 to averted gaze is shown only in older subjects \( (r=.21, \ p=.050) \).
Although there was no main effect of group overall on N170 latency [F (3, 83) =1.88, p=.14], when combined by ASD diagnosis there was a trend toward a main effect of group on N170 latency [F (1, 83) =3.68, p=.059, d=0.23], indicating slightly longer N170 latency in participants with ASD/ASD+ADHD compared to TD/ADHD. In addition, a weaker trend toward a main effect of ADHD diagnosis [F (1, 83) 3.07, p=.084, d=0.13], suggested shorter N170 latency in ADHD and ASD+ADHD when combined. There was no interaction between ASD and ADHD diagnosis suggesting an additive effect of the conditions [F (1, 83) 0.08, p=.778]. There was no interaction between group and orientation [F (3, 83) 2.06, p=.112], nor between group and gaze [F (3, 83) 1.33, p=.27] on N170 latency.

**Table 7-3:** Mean amplitude (in μV) and latency (in msec) for the N170 component for each stimulus by group

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Electrode</th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amplitude</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upright, direct</td>
<td>P7</td>
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<td>-2.06</td>
<td>-1.95</td>
<td>-3.79</td>
</tr>
<tr>
<td></td>
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<td>(4.64)</td>
<td>(4.28)</td>
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<tr>
<td></td>
<td>P8</td>
<td>-5.43</td>
<td>-2.33</td>
<td>-4.61</td>
<td>-2.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.40)</td>
<td>(6.81)</td>
<td>(5.86)</td>
<td>(4.58)</td>
</tr>
<tr>
<td></td>
<td>Pz</td>
<td>-3.78</td>
<td>-3.30</td>
<td>-3.15</td>
<td>-3.40</td>
</tr>
<tr>
<td></td>
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<td>(2.88)</td>
<td>(4.10)</td>
<td>(3.52)</td>
<td>(3.32)</td>
</tr>
<tr>
<td>Upright, averted</td>
<td>P7</td>
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<td>-3.33</td>
<td>-2.52</td>
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</tr>
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<td></td>
<td></td>
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<td>(3.70)</td>
<td>(4.24)</td>
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<td>-3.17</td>
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<td>(7.27)</td>
<td>(4.54)</td>
<td>(4.70)</td>
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<tr>
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<td>Pz</td>
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<td>-3.81</td>
<td>-3.12</td>
</tr>
<tr>
<td></td>
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<td>(2.60)</td>
<td>(3.67)</td>
<td>(3.12)</td>
<td>(3.03)</td>
</tr>
<tr>
<td>Inverted, direct</td>
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<td>-2.18</td>
<td>-2.18</td>
<td>-3.40</td>
<td>-4.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.06)</td>
<td>(4.73)</td>
<td>(4.50)</td>
<td>(3.44)</td>
</tr>
<tr>
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<td>(7.37)</td>
<td>(4.88)</td>
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<tr>
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<td>-3.77</td>
<td>-1.64</td>
<td>-3.07</td>
</tr>
<tr>
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<td></td>
<td>(3.76)</td>
<td>(3.39)</td>
<td>(3.08)</td>
<td>(3.62)</td>
</tr>
<tr>
<td>Inverted, averted</td>
<td>P7</td>
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<td>-1.82</td>
<td>-3.75</td>
<td>-3.47</td>
</tr>
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<td>(3.67)</td>
<td>(3.75)</td>
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<tr>
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<td>-5.55</td>
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<td>(4.87)</td>
<td>(7.06)</td>
<td>(3.87)</td>
<td>(3.92)</td>
</tr>
<tr>
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<td>Pz</td>
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<td>-3.48</td>
<td>-3.27</td>
<td>-3.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.10)</td>
<td>(3.60)</td>
<td>(3.44)</td>
<td>(3.12)</td>
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### Table 7-3 (continued)

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<th>Latency</th>
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<th>P8</th>
<th>Pz</th>
</tr>
</thead>
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<tr>
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<td>209.15 (19.62)</td>
<td>209.47 (17.61)</td>
<td>191.73 (22.95)</td>
</tr>
<tr>
<td></td>
<td>217.01 (18.96)</td>
<td>209.20 (18.67)</td>
<td>203.99 (17.34)</td>
</tr>
<tr>
<td></td>
<td>210.71 (23.82)</td>
<td>205.88 (10.85)</td>
<td>203.36 (17.16)</td>
</tr>
<tr>
<td></td>
<td>214.44 (14.67)</td>
<td>207.57 (15.04)</td>
<td>190.19 (24.08)</td>
</tr>
<tr>
<td><strong>Upright, averted</strong></td>
<td>208.90 (19.87)</td>
<td>208.42 (16.36)</td>
<td>197.92 (20.08)</td>
</tr>
<tr>
<td></td>
<td>214.41 (14.94)</td>
<td>210.50 (17.35)</td>
<td>197.59 (19.41)</td>
</tr>
<tr>
<td></td>
<td>211.86 (15.44)</td>
<td>201.75 (12.07)</td>
<td>200.02 (15.00)</td>
</tr>
<tr>
<td></td>
<td>213.97 (18.25)</td>
<td>209.39 (12.80)</td>
<td>190.60 (20.34)</td>
</tr>
<tr>
<td><strong>Inverted, direct</strong></td>
<td>206.71 (15.28)</td>
<td>205.73 (11.21)</td>
<td>198.57 (29.50)</td>
</tr>
<tr>
<td></td>
<td>200.96 (27.56)</td>
<td>202.26 (19.58)</td>
<td>196.18 (29.58)</td>
</tr>
<tr>
<td></td>
<td>200.83 (14.42)</td>
<td>198.99 (11.00)</td>
<td>209.56 (25.24)</td>
</tr>
<tr>
<td></td>
<td>206.36 (12.79)</td>
<td>206.49 (13.28)</td>
<td>195.31 (31.92)</td>
</tr>
<tr>
<td><strong>Inverted, averted</strong></td>
<td>209.31 (16.92)</td>
<td>209.23 (16.97)</td>
<td>201.99 (26.41)</td>
</tr>
<tr>
<td></td>
<td>211.70 (16.88)</td>
<td>207.03 (20.91)</td>
<td>198.35 (15.71)</td>
</tr>
<tr>
<td></td>
<td>201.29 (11.56)</td>
<td>196.81 (14.23)</td>
<td>203.47 (17.54)</td>
</tr>
<tr>
<td></td>
<td>207.57 (11.05)</td>
<td>207.23 (13.64)</td>
<td>197.40 (26.67)</td>
</tr>
</tbody>
</table>
Figure 7-6: Grand mean N170 ERPs to face stimuli for each group and isocontour maps derived for the grand-average at peak latency for each group.

Note: Black represents upright-direct, red represents upright-averted; blue represents inverted-direct; green represents inverted-averted. Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; ASD+ADHD, comorbid attention deficit hyperactivity disorder and autism spectrum disorder; TD, typically developing control.
**7.4.5 Midline N170 amplitude**

Descriptive statistics are shown in Table 6-3. Grand average ERPs and topographical maps are shown in Figure 6-7. There was no main effect of group on midline-N170 amplitude \[F (1, 83) =0.88, p=.46\], nor an interaction between group and orientation \[F (3, 83) =1.68, p=.18\]. A main effect of gaze was shown across the whole sample \[F (1, 84) =5.82, p=.02\], revealing enhanced amplitude to averted gaze compared to direct gaze. There was a trend towards an interaction between group and gaze \[F (3, 83) =2.30, p=.08\], and when combined by the presence of ASD diagnosis, there was a significant interaction between group and gaze \[F (1, 84) =4.24, p=.04, d=0.44\], indicating a reduced gaze effect in children with ASD (ASD/ASD+ADHD) compared to those without an ASD diagnosis (TD/ADHD). This effect was not shown when combining by the presence of ADHD diagnosis \[F (1, 84) =0.46, p=.50, d=0.04\]. In addition, there was no significant interaction between ASD and ADHD, suggesting an additive effect of the disorders \[F (1, 84) =2.46, p=.12\].

There was a trend toward age being significant as a covariate \[F (1, 84) p=.043\]. Therefore the analyses were run with and without age, revealing that the main effect of gaze was not present when controlling for age \[F (1, 83) =0.39, p=.53\]. Group effects on midline-N170 amplitude remained the same regardless of age. No other effects of task manipulation were significant (all \(p>.05\)).

**7.4.6 Midline N170 latency**

There was no significant main effect of group on the midline-N170 latency \[F (1, 84) =1.17, p=.33\], nor an interaction between group and orientation \[F (1, 84) =1.59, p=.20\] and gaze \[F (3, 84) =1.45, p=.24\]. There was, however, a trend toward a main effect of orientation across the whole sample \[F (1, 84) =3.41, p=.07\], indicating increased latency to inverted faces compared to upright faces. All other effects were non-significant (all \(p>.10\)).
Chapter 7: Neurophysiological correlates of face and eye gaze processing in ASD, ADHD and ASD+ADHD

Figure 7-7: Grand mean midline-N170 ERPs at Pz to face stimuli for each group and isocontour maps derived for the grand-average at peak latency for each group.

*Note:* Black represents upright-direct, red represents inverted-direct; blue represents upright-averted; green represents inverted-averted.

*Abbreviations:* ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; ASD+ADHD, comorbid attention deficit hyperactivity disorder and autism spectrum disorder.

### 7.4.7 Associations between ERP parameters and symptom scores

An examination of the association between P1 and N170 components revealed no significant correlation overall (r=.10, p=.34), although when examined in each group separately, P1 amplitude was related to N170 amplitude in TD (r=.38, p=.07), but not ASD-only (r=.19, p=.46), ADHD-only (r=.03, p=.91) or ASD+ADHD (r=-.06, p=.76).

In order to examine whether ERP parameters are related to quantitative trait measures of the disorders, I conducted correlations between parameters that showed differences between diagnostic groups and parent-rated symptom scores (see Table 6-3). Across the whole sample, the interaction between group and hemisphere on N170 amplitude showed a significant association with parent-rated symptom scores on the SCQ (r=.31, p=.004) and not on the
Conners (Inattention: $r=-.01$, $p=.91$; Hyperactivity-Impulsivity: $r=-.01$, $p=.93$). All other correlations were non-significant.

Table 7-4: Correlations between ERP parameters and symptom scores

<table>
<thead>
<tr>
<th>ERP</th>
<th>Effect</th>
<th>SCQ</th>
<th>Conners Inattention</th>
<th>Conners Hyperactivity/Impulsivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 latency</td>
<td>Hemisphere</td>
<td>-.16</td>
<td>.02</td>
<td>.01</td>
</tr>
<tr>
<td>P1 latency</td>
<td>Gaze</td>
<td>.09</td>
<td>.01</td>
<td>.03</td>
</tr>
<tr>
<td>P1 latency</td>
<td>Orientation</td>
<td>-.03</td>
<td>-.08</td>
<td>-.09</td>
</tr>
<tr>
<td>N170 amplitude</td>
<td>Gaze</td>
<td>.09</td>
<td>-.02</td>
<td>-.17</td>
</tr>
<tr>
<td>N170 amplitude</td>
<td>Hemisphere</td>
<td>.31**</td>
<td>.01</td>
<td>.01</td>
</tr>
</tbody>
</table>

Abbreviations: Conners, Conners Third Edition Parent Rating Scale Short Form; ERP, event-related potential; SCQ, Social Communication Questionnaire. ** $p<.01$

ERP parameters which were significantly correlated across both groups with the SCQ and Conners scores, respectively, were then taken forward for inclusion in multiple linear regression analyses with hierarchical entry, to determine the relative contributions of ASD and ADHD symptom measures on these different ERP parameters. These models confirmed the above correlation supporting the SCQ as predictors of reduced lateralisation of N170 amplitude; the regression model showed a significant increase $R^2$ indicating enhanced prediction of the ERP parameter, which remained after controlling for the potential effect of age and the Conners rating scale.

7.5 Discussion

This study compared ERP markers of face and gaze processing in cases of ASD, ADHD and ASD+ADHD that were individually assessed using screening questionnaires and diagnostic interviews. Disorders were associated with distinct impairments: children with ASD symptoms showed abnormalities in hemispheric distribution of ERP components and processing of gaze direction, whereas children with ADHD exhibited similar responses to upright and inverted faces, indicating a reduced effect of face inversion. Children with ASD+ADHD present as an additive condition with the unique deficits of both conditions.
7.5.1 Face inversion effects

Across all groups, inverted faces elicited delayed P1 (when age is not controlled) and enhanced N170 components, consistent with previous findings (Bentin et al., 1996, Taylor et al., 2004). This might reflect disruption of configural processing both in early sensory components associated with low-level visual processes (Itier et al. 2007) and later components associated with higher-level category recognition. However, while TD and ASD groups showed delayed P1 latency to inverted faces compared to upright faces, both ADHD groups displayed similar P1 latency across upright and inverted faces. This suggests children with ADHD may not process faces configurally and employ an alternative strategy, although evidence for a featural processing style in ADHD is limited (Booth et al., 2003). The P1 can also reflect early modulation of top-down attention (Taylor, 2002), which is supported by it’s modulation by inversion itself (Doi et al., 2007). Thus an alternative explanation is that typically developing children expect an upright face, whereas in ADHD these predictive/attentive mechanisms do not operate. As group differences on the inversion effect are shown on the early visual P1 component, deficits in the ADHD group may be sensory in nature, although importantly this would not due to visual properties of the stimuli that were identical across conditions. Future work should measure responses to a control stimulus with no social value (e.g. a building) incorporated into the design, to examine whether this response is specific to faces or simply a response to an inverted or incongruous stimulus, reflecting these predictive/attentive deficits.

A reduced inversion effect has previously been demonstrated in adults with ASD on the N170 (McPartland et al., 2004, Webb et al., 2009), although most recent behavioural studies have shown an intact inversion effect in ASD (Gross, 2008, Jemel et al., 2006, Lahaie et al., 2006, Teunisse and de Gelder, 2003). Our results indicate that previous findings may be due to underlying comorbid ADHD and/or core symptoms of ADHD. Still, the trend toward a reduced inversion effect on the N170 in the ASD groups may suggest limited power to detect this effect on this component. Further research should explicitly test the face inversion effect in ADHD and it’s associations with poorer face discrimination abilities, emotional impairment, attention or other symptoms associated with the disorder, in order to examine it’s basis.

7.5.2 Gaze effects

Several ERP parameters were modulated by gaze direction. Firstly, across all groups averted gaze produced larger P1 (when age was not controlled) and delayed N170 components, suggesting slower and enhanced processing for averted gaze. In addition, the modulation of gaze processing by face orientation on P1 and N170 components suggests gaze direction
detection was diminished in inverted faces (Farroni et al., 2004, Itier et al., 2007, Senju et al., 2005a). This supports theories of configural/holistic processing of the face and featural processing of the eyes occurring in parallel (Bentin et al., 1996, Itier et al., 2007). As there was no difference by group on the N170 for the interaction between face orientation and gaze direction, this suggests that gaze direction is mediated by configural processing in all groups. The lack of an interaction between orientation and gaze at midline regions suggests topographical differences in this effect that should be explored.

Secondly, noteworthy group differences on P1 and N170 responses to gaze direction emerged suggesting specific abnormalities in gaze processing in ASD. In upright faces, participants with ASD symptoms (ASD and ASD+ADHD) had delayed latency to averted gaze compared to delayed P1 latency to direct gaze in controls. These group differences show a sensitivity to gaze direction in both groups that varied as to which gaze direction they were sensitive to, corresponding to recent fMRI work (Pitskel et al., 2011) and suggests ASD children recruit distinct mechanisms for processing gaze at early sensory processing stages. In addition, enhanced N170 at midline scalp locations to averted gaze shown in TD and ADHD children was absent in children with ASD symptoms. This midline-specific effect is consistent with previous research (Grice et al., 2005), and indicates equivalent processing of direct and averted gaze. Enhanced processing of averted gaze, however, is contradictory to some previous findings suggesting faster detection of or enhanced N170 response to direct gaze in typical development, although task differences (behavioural vs. ERP; passive vs. active; front view vs. lateral view; static vs. dynamic gaze shifts (Conty et al., 2007, Senju et al., 2005b) and developmental effects (Elsabbagh et al., 2009a, Watanabe et al., 2002) are likely to underlie these discrepancies. Because averted gaze can be used to infer the locus of attention of others, these findings may signify the increasing importance of averted gaze with development, which corresponds with behavioural failure to detect direct gaze (Klin et al., 2002b, Pelphrey et al., 2002, Senju et al., 2005a). Alternatively, this might also imply the use of a different strategy, for example using low level information such as the position of the pupil/iris rather than social cues (Ristic et al., 2005) or deficits in visual attention or perception, supported by consistent sensory processing deficits in ASD (Leekam et al., 2007). Nevertheless, studies support occipito-temporal negative components as modulated specifically by gaze direction as compared to changes in non-facial visual stimuli (Puce et al., 2000).
7.5.3 **Hemispheric effects**
Enhanced amplitude of the N170 component in the right hemisphere in control and ADHD children was not shown in ASD groups. Converging with previous work, this suggests reduced or altered neural specialisation of face and gaze processing is specific to children with ASD symptoms (Carver and Dawson, 2002, McCleery et al., 2009, McPartland et al., 2004, Senju et al., 2005b) supported by structural imaging studies (Pierce et al., 2001, Schultz et al., 2000). In addition, this association transcended diagnostic grouping, as supported by correlations with parent-rated symptom scores of ASD. Bilateral scalp activity in response to faces is also shown in younger children, suggesting this effect may indicate delayed development in ASD (Mercure et al., 2009). It is likely, therefore, that individuals with ASD employ alternative face processing strategies that are perhaps more akin to object processing and associated with immature development (Behrmann et al., 2006).

7.5.4 **Limitations**
The relatively small sample size limits power and the ability to make firm conclusions. This could explain the lack of overall amplitude differences between the groups as reported by previous studies, although this may be explained by group differences in the strength of underlying dipole sources previously described (Wong et al. 2008). The findings reported are dependent on task type. For example, children with ASD can discriminate gaze direction when they are cued or told explicitly to pay attention to gaze (Lopez et al., 2004, Ristic et al., 2005) and therefore the passive nature of the present task may exacerbate the impairment. While participants who did not attend to the task sufficiently were excluded from analysis, it is possible that findings are affected by group differences in face scanning patterns and reduced foveation (Dalton et al., 2005, Elsabbagh et al., 2009b). In addition, findings vary depending on autistic severity and therefore studies investigating neural correlates of social cognition should be extended to low-functioning individuals.

7.5.5 **Implications**
The findings revealed that ASD and ADHD can be dissociated on the basis of ERP abnormalities in the first stages of face processing. As inversion effects are an index of structural face processing, this suggests basic face processing impairments in ADHD that may be a consequence of differences in perceptual processing style and/or visual attention deficits. In contrast, specific abnormalities shown in gaze processing in ASD are likely to be more relevant to the characteristic social deficits of autism and theories suggesting a lack of interest for the human face from early in development (Jemel et al., 2006), which may then in turn lead to
ToM deficits and problems in social interactions (Baron-Cohen, 1995, Dawson et al., 2005b). The comorbid ASD+ADHD group demonstrated unique deficits of both disorders, shown by both reduced face inversion effects, altered gaze processing and topographical differences suggestive of a hybrid condition. It is possible, therefore, that different subgroups within the autism spectrum have a difficulty with configural processing and these subgroups can be defined by comorbid symptoms of other disorders. As the comorbid group was most severely impaired on hemispheric differences, the sensitivity of ERP correlates of social cognition to ASD may be dependent on the presence of comorbid conditions. An additive model is supported by findings in the previous chapter of this thesis reporting specific neural deficits of inhibition and attentional orienting in ADHD and ASD+ADHD that were not shown in ASD-only (Chapter 6). These findings converge to suggest the disorders can be dissociated on the basis of inhibitory deficits and gaze processing impairments at the neural level, which is supported by previous behavioural investigations of inhibition and ToM (Ames and White, 2011, Bühler et al., 2011). Further research on the behavioural correlates of these effects in both disorders, particularly ADHD, is warranted. In addition, it is necessary to examine a domain in which behavioural difficulties are consistently seen in both disorders, which may reveal similar impairments at the neural level across the disorders rather than a dissociation (the aim of Chapter 8).

7.5.6 Conclusion

This study reports novel distinct impairments in face and gaze processing in children with ASD, ADHD and comorbid ASD+ADHD and extends electrophysiological studies of face and gaze processing. A better understanding of the nature of the comorbid condition is likely to aid in enhanced assessment and treatment of these complex cases, such as early interventions that improve social attention (see Chapter 9). This supports the adoption of a broader view of psychiatric disorders when examining underlying mechanisms, and warrants systematic assessment of clinical cases to ensure minimal misspecification in group allocation in future research.
8.1 Summary

Children with autism spectrum disorder (ASD) demonstrate characteristic deficits in processing facial information, particularly emotional expressions. There are high rates of clinical and behavioural overlap between ASD and attention deficit hyperactivity disorder (ADHD), and emotional impairment is also shown in ADHD. Emotional impairment in the two disorders, however, has not been directly compared using event-related potentials (ERPs) that are able to measure distinct temporal stages in emotional processing. The N170 (an index of structural encoding) and N400 (an index of contextual and meaning processing) ERP components were measured during passive presentation of face stimuli with different emotional expressions (neutral, anger, fear, disgust, joy) to groups of ASD (n=19), ADHD (n=18), comorbid ASD+ADHD (n=29) and typically developing (TD) controls (n=26). Subjects with ASD symptoms (ASD and ASD+ADHD) displayed reduced N170 amplitude across all stimuli compared to children without ASD symptoms. Conversely, subjects with ADHD symptoms (ADHD and ASD+ADHD) demonstrated reduced modulation of N400 amplitude at parietal sites by happy expressions compared to children with ASD and fearful expressions compared to subjects without ADHD symptoms. These findings indicate that while children with ASD demonstrate deficits at structural encoding stages of face processing (indexed by the N170), children with ADHD show impairments in contextual processing of emotion (indexed by the N400), which suggests a dissociation between disorders on the basis of distinct temporal and functional stages of emotion processing. The comorbid ASD+ADHD group display the unique deficits of both disorders in early and late emotion processing, supporting the comorbid disorder as an additive condition rather than a separate disorder with distinct impairments. This supports the use of objective neural measurement of emotional function to delineate pathophysiological mechanisms and guide clinical assessment.
8.2 Introduction

Autism spectrum disorder (ASD) is a childhood disorder characterised by social and emotional impairments that are apparent from 1 year of age, and are thought to be associated with theory-of-mind (ToM) impairments and emotional problems (Baron-Cohen, 1995, Baron-Cohen et al., 1993). The human face is one of the most emotionally salient stimuli, and inference of emotional cues from the face is considered crucial for the development of complex social behaviours such as ToM (Baron-Cohen et al., 1993). Many of the social impairments in ASD are likely to involve the ability to process socio-emotional signals including information from faces, as there is consistent evidence of deficits in face processing among these subjects (Dawson et al., 2005b). For example, reduced attention to faces and decreased eye contact with others are symptomatic of the disorder (see Table 1-2, Chapter 1) and individuals with ASD show abnormalities in the detection of gaze direction using both eye-tracking (Klin et al., 2002a, Pelphrey et al., 2002) and electrophysiological correlates, as demonstrated in Chapter 6 (Grice et al., 2005, Senju et al., 2005b). Particular deficits in face processing are reported in the expression and recognition of emotional expressions in ASD (Harms et al., 2010), with the greatest deficits shown for negative expressions (Boraston et al., 2007, Humphreys et al., 2007).

Until recently, ASD and attention deficit hyperactivity disorder (ADHD) have been considered as independent disorders. Recent findings suggest, however, that there is substantial clinical and behavioural co-occurrence between the disorders that can be largely accounted for by overlapping genetic influences (see Section 1.2, Chapter 1). Although the majority of research in ADHD has focused on cognitive correlates of the core symptoms of inattention, hyperactivity and impulsivity, there is accumulating evidence to suggest that cognitive impairment in ADHD is accompanied by emotional impairment (Castellanos et al., 2006, Uekermann et al., 2010), particularly as deficits in theory-of-mind (ToM), social skills and empathy have been reported (Uekermann et al., 2010). A recent review of face processing in ADHD reports consistent deficits in recognising and identifying emotional facial expressions, and understanding their contextual meaning (Dickstein and Castellanos, 2012), which suggests an impairment in emotional processing in addition to impairment in face processing. While some studies suggest a generalised deficit (Corbett and Glidden, 2000, Singh et al., 1998), others suggest specific deficits in recognising disgust and fear (Boakes et al., 2007), and anger and sadness (Pelc et al., 2006). Performance on emotion tasks is associated with more errors on a continuous performance test (Shin et al., 2008), a Go/No-Go test of inhibition and a sustained attention
task (Sinzig et al., 2008b), and problems in social interaction in ADHD are often, therefore, attributed to attentional difficulties rather than core social cognitive impairment (Perner et al., 2002, Uekermann et al., 2010). In line with this, a link between the frontostriatal network, which is implicated in ADHD, and brain regions suberving social cognition has been suggested based on the documented role of the prefrontal cortex in social cognition (Sonuga-Barke, 2003, Uekermann et al., 2010).

Direct comparisons of ToM abilities in ASD and ADHD show impaired performance in both ASD and ADHD (Buitelaar et al., 1999, Nyden et al., 2010), although intact performance has been reported in ADHD (Ames and White, 2011, Bühler et al., 2011, Charman et al., 2001, Dyck et al., 2001). In direct comparisons similar performance on emotion recognition tasks has been reported in ASD and ADHD (Buitelaar et al., 1999, Fine et al., 2008), although some studies demonstrate poorer performance in ASD (Downs and Smith, 2004). A comparison of emotional face recognition revealed greater impairment in ASD+ADHD and ADHD-only compared with ASD-only and typically developing controls (Sinzig et al., 2008b). Specifically, children with ADHD-only showed more difficulties in recognition of photographs of eye-pairs depicting joy, and ASD+ADHD performed worse on eye-pairs depicting surprise, compared to controls (Sinzig et al., 2008b). Given that individuals with ADHD may demonstrate greater impairment at the behavioural level, efforts to further the understanding of the neurobiological mechanisms underlying these deficits and their overlap with those demonstrated in ASD are justified. In particular, the classification of emotional responses by the two orthogonal dimensions of “motivational salience” is likely to be informative for the comparison of psychiatric disorders: arousal, referring to the intensity of the stimulus and amount of resources allocated to process emotion; and valence, either positive (i.e. rewarding) or negative (i.e. aversive) (Dickstein and Castellanos, 2012, Lang et al., 1998).

Emotional facial expressions elicit robust neural changes that can be measured using event-related potentials (ERPs) (Eimer and Holmes, 2002). The visuo-social N170 component is thought to be sensitive to faces as it is predominantly evoked by faces compared with objects or words (Bentin et al., 1996). It is generally thought to be unaffected by changes in emotional expression (Eimer and Holmes, 2002, Munte et al., 1998), although inversion of faces elicits a larger and delayed N170 (see Chapter 6). These and other observations have led to the proposition that the N170 reflects structural encoding of faces, occurring prior to a comparison of these structural descriptions with representations stored in semantic memory (Bentin and Deouell, 2000, Eimer and Holmes, 2002, Eimer et al., 2003, Holmes et al., 2005). The later
centro-parietal N400 component is proposed to be associated with the latter, reflecting access to semantic memory to allow contextual and meaning evaluation, supported by modulation by familiarity and emotional expressions (Kutas and Federmeier, 2011, Munte et al., 1998, Olivares et al., 2003, Posamentier and Herve, 2003, Schweinberger and Burton, 2003). The temporal differentiation of the ERP components supports a stage-like model of face processing (Bruce and Young, 1986, Eimer, 2000b). More recent studies, however, challenge this view and show modulation of the N170 by emotional expressions, as shown by larger amplitude and longer latency, which suggests emotion-sensitive processes are related and may occur in parallel to visual processes (Batty and Taylor, 2003, Blau et al., 2007, Caharel et al., 2005, Campanella et al., 2002, Pizzagalli et al., 2002).

Abnormal ERP responses to emotional face expressions have been reported in ASD. For studies measuring electrophysiological responses to emotional face stimuli overall, children with autism demonstrate delayed P1 and N170 and smaller P1 amplitude to emotional faces compared to age-matched controls (Hileman et al. 2011). In one study, when matched on verbal ability, only P1 amplitude remained affected in autism suggesting perceptive abnormalities (Batty et al. 2011). Conversely, another study reported delayed P1 and N170 and smaller N170 was found only in adults but not children with ASD (O'Connor et al., 2005). Similarly, typical scalp-recorded ERPs have been reported in children with ASD in response to both explicit and implicit processing of emotional faces (Wong et al., 2008). Analysis of underlying dipole sources using Brain Electrical Source Analysis (BESA), however, revealed weaker and slower responses in frontal, fusiform and visual cortices and increased responses in parietal somatosensory cortices which may relate to greater more effortful processing of emotional expressions in ASD (Wong et al., 2008). In contrast, in a study of children with ASD passively viewing emotional faces showed delayed N300 response to fearful faces and failed to show larger negative slow wave to fearful versus neutral expressions compared to age-matched controls (Dawson et al., 2004). Inconsistencies may therefore reflect age differences, task differences and overall analysis (i.e. main effect of looking at an emotional face versus modulation by emotion).

ERP studies of adults with ADHD show reduced early posterior negativity (EPN) to non-face positive stimuli (Herrmann et al., 2009b), and reduced modulation of the N170 component particularly for positive emotion, which is in turn associated with deficits in recognising emotional expressions from eye-pairs and various aspects of executive function, including working memory (Ibanez et al., 2011). The specific deficit with positive emotion has been
interpreted as reflecting abnormalities in reward processing (Ibanez et al., 2011, Sonuga-Barke, 2002). Adolescents with ADHD show reduced P120 response at occipital sites to anger and fear which correlated with emotion recognition deficits, and subsequent enhanced N170 and reduced and delayed P300 responses (Williams et al., 2008). Notably, also, these reductions were normalised by stimulant medications, suggesting emotional impairments respond to treatment (Williams et al., 2008).

Findings to date converge to suggest both individuals with ASD and ADHD demonstrate behavioural and neural abnormalities in response to emotional face expressions at both early and later stages of processing. The two disorders, however, have not been directly compared and there is no ERP study on emotion processing in children presenting with symptoms of both disorders. The aim of the present study was, therefore, to compare children with comprehensively assessed diagnoses of ASD, ADHD and comorbid ASD+ADHD to ensure minimal misspecification in group allocation. While the previous chapters have focused on a domain which is consistently altered in one of the disorders, emotional impairment is characteristic of both. Examination of this domain is likely to further understanding of the specific deficits and in turn reveal more about the model of comorbidity between ASD and ADHD (Section 1.2). Based on previous results, we expected (1) the N170 and N400 components to be modulated by emotional face expressions; (2) both ASD and ADHD groups to show abnormal neural responses to emotional face expressions at both early and late stages of processing; (3) children with ASD to show specific deficits in processing negative emotional expressions, whereas children with ADHD to show deficits processing both positive and negative emotional expressions; and (4) children with comorbid ASD+ADHD to show deficits of both disorders. I also aimed to examine the association between dimensional measures of ASD and ADHD and these ERP parameters.

8.3 Methods

8.3.1 Sample

Nineteen male participants with ASD, 18 with ADHD, 29 with ASD and ADHD, and 26 typically developing controls (TD) between 8 and 13 years of age took part in the study. Participants underwent systematic clinical assessment to confirm research diagnoses. See Section 2.2 (Chapter 2) for full description of participant recruitment and assessment.
8.3.2 Task and stimuli

The stimuli were 6 black-and-white pictures of a boy and a girl standardised for size, contrast and luminosity and presented in an oval aperture that occluded sex-specific features (see Figure 2). The faces were of other children of similar age to the present sample (8-9 years). These images have been used in previous studies investigating behavioural emotional expression discrimination (Battaglia et al., 2004) and ERP studies of the associations between specific genetic variants and the N170 (Battaglia et al., 2007) and N400 (Battaglia et al., 2005). The boy pictures were drawn from a collection of Linda Camras, PhD, de Paul University, Chicago (Camras and Allison, 1985). The girl pictures were selected under the direction of Camras from a pool of pictures that had been developed by showing model prototypical pictures as a reference (Ekman and Friesen, 1976). In accordance with Camras’ standard-validation procedure, the girl pictures were correctly classified in more than 80% of evaluations by 20 undergraduates of both sexes, and overall a rate of 72% correct identification across stimuli was reported (Battaglia et al., 2004). Five of the basic emotional expressions were selected for presentation, based on group differences reported in previous research (disgust, fear, anger, joy and neutral).

On each trial subjects were first presented with a child’s face (total time on screen, 1300 milliseconds), which they were instructed to watch carefully until a blue circle appeared superimposed around the centre of the picture. As soon as a blue circle appeared (700 milliseconds after the appearance of the stimulus), they had to click a mouse. Thus, the ERPs relevant to this study were all generated before the motor task, which was merely set up to stimulate subject’s participation and attention. The monitor screen remained dark between trials for periods that varied randomly from 1200 to 1600 milliseconds. The stimuli were presented to all the children in a fixed sequence that alternated male and female pictures and that avoided close repetition of the same expression. Each of the 10 stimuli was presented 20 times to ensure sufficient ERP acquisition (total, 200 presentations in a complete session). Every child was exposed to a pre-experiment trial of 6 pictures not belonging to the same set used for the experiment to make sure she or he understood the procedure well. Stimuli were presented in two blocks of 100 trials. Participants were continually monitored to ensure attention to task. The task was administered as part of a larger test battery (other tasks not presented here) that had a duration of 65 minutes. Presentation of the tasks was ordered in the same way for each group to control for effects of practice and fatigue.
8.3.3 Electrophysiological recording and analysis

8.3.3.1 EEG recording and preprocessing
See Section 2.4.1 and 2.4.2, Chapter 2, for details of EEG data acquisition and general preprocessing.

8.3.3.2 ERP analysis
Baseline correction was performed using a 200msec prestimulus reference period. Stimulus-locked epochs (~200 to 700 msec peristimulus window) were averaged for the following trial types: neutral, disgust, fear, anger, joy. Baseline corrected. All trials were inspected visually. Based on visual inspection of the grand average and congruent with the literature (see Chapter 7), the N170 was scored as the maximal negative peak at the P7 (left hemisphere) and
P8 (right hemisphere) electrodes, and the N400 was defined as the maximal negative peak in a 300-500 ms latency window with respect to the preceding maximal positive peak (P300) in order to obtain a more robust measure of this component, measured at both channel Cz and channel Pz (Battaglia et al. 2005). Averages were computed for each participant in each experimental condition on a minimum of 30 trials (see Table 8-1).

Table 8-1: Mean (SD) number of segments in each ERP average per stimulus and group during the emotional expressions task

<table>
<thead>
<tr>
<th>Stimulus type</th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral</td>
<td>36.81 (2.42)</td>
<td>36.59 (2.12)</td>
<td>36.56 (2.04)</td>
<td>36.41 (1.85)</td>
</tr>
<tr>
<td>Angry</td>
<td>36.15 (2.09)</td>
<td>36.88 (1.12)</td>
<td>36.39 (1.85)</td>
<td>36.67 (1.85)</td>
</tr>
<tr>
<td>Fearful</td>
<td>36.88 (1.58)</td>
<td>36.47 (1.94)</td>
<td>35.94 (2.26)</td>
<td>35.93 (2.04)</td>
</tr>
<tr>
<td>Disgusted</td>
<td>36.65 (2.26)</td>
<td>36.59 (2.18)</td>
<td>35.82 (1.88)</td>
<td>36.74 (2.35)</td>
</tr>
<tr>
<td>Happy</td>
<td>35.58 (1.92)</td>
<td>35.41 (1.91)</td>
<td>34.67 (2.00)</td>
<td>35.15 (2.23)</td>
</tr>
</tbody>
</table>

Abbreviations: TD, typically developing controls; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; ASD+ADHD, comorbid ASD and ADHD; ERP, event-related potential.

8.3.4 Statistical analyses

The general analysis strategy is described in Section 2.5.1, 2.5.2 and 2.5.3.2 (Chapter 2). Data from one ASD+ADHD participant is not included due to technical issues with the recording. One extreme outlier was removed from each individually assessed ERP parameter (±3.5 SD from the mean; ASD+ADHD). Due to poor attention to task and excessive movement shown in video recording, 1 ASD and 1 ASD+ADHD were removed from all analyses.

8.3.4.1 Statistical analysis for group differences

A repeated-measures ANOVA was conducted on each ERP parameter of interest (N170 amplitude, N170 latency, N400 amplitude, N400 latency). Emotion (neutral, anger, fear, disgust and joy) and, for N170 hemisphere (left/right) and for N400, electrode (Cz/Pz) were included as the within-subjects factors. Diagnostic group was entered as the between-subjects factor, described in Section 2.5.3.2, Chapter 2. Post-hoc comparisons for location and group used Tamhane correction that caters for unequal variance, to adjust confidence intervals for multiple comparisons.

Where the effect of emotion was significant, planned contrasts were performed: (1) between neutral and emotional faces, indexing ‘arousal’ and (2) between negative (anger, fear, disgust) and positive (happy) faces, indexing ‘valence’ (Lang et al. 1998).
Age was included as a covariate in analyses of the N170, due to substantial developmental changes in this component over the age range in the current sample (Taylor et al. 2004). For other parameters (N400), correlations between IQ and age and the dependent variables were calculated. When these correlations were significant, analyses of covariance (ANCOVAs) were conducted to assess the role of these potentially confounding variables.

8.3.4.2 Dimensional analyses

Dimensional analyses followed the same analysis strategy described in Section 2.5.3.2 (Chapter 2).

8.4 Results

8.4.1 N170 amplitude

Mean peak amplitudes and latencies of the N170 for each group are shown in Table 8-2. Grand average ERPs and topographical maps are shown in Figure 8-2. Across the whole sample, a main effect of age was shown on N170 amplitude \[F (1, 82) =11.98, p=.001\], indicating that amplitude increases with age \((r=.36, p=.001)\). There was a trend toward a main effect of hemisphere (left versus right) \[F (1, 82) =2.72, p=.10\], indicating enhanced amplitude in the right hemisphere. There was no main effect of emotion on N170 amplitude \[F (4, 79) =1.08, p=.38\]. In order to examine the potential contribution of developmental effects to this null finding, the analyses were run on the youngest third of participants (age<9.52), revealing no effect of emotion on N170 amplitude \[F (4, 22) =0.35, p=.84\], whereas in the eldest third of participants (age>11.75), there was a main effect of emotion \[F (4, 21) =3.02, p=.04\]. Planned contrasts revealed significantly reduced amplitude of the N170 to neutral faces, compared to happy \[F (1, 24) =12.53, p=.01\] and a trend for angry faces \[F (1, 24) =3.06, p=.09\].

There was a main effect of group \[F (3, 82) =3.70, p=.02\], and posthoc tests revealed subjects with ASD had significantly reduced N170 amplitude compared to TD subjects \(p=.009, d=1.03\). When combining the groups by the presence or absence of ASD diagnosis, there was a main effect of group \[F (1, 84) =5.09, p=.03, d=0.48\], indicating that children with ASD (ASD/ASD+ADHD) show reduced amplitude across all stimuli compared to children without ASD (TD/ADHD). There were no effects when combining by the presence or absence of ADHD \[F (1, 84) =0.29, p=.59, d=0.12\]. In addition, there was no interaction between ASD and ADHD, suggesting an additive effect on the comorbid condition \[F (4, 83) =2.96, p=.11\]. In addition, a trend toward an interaction between ASD and emotion emerged \[F (4, 81) =2.42, p=.06\],
although planned contrasts revealed no significant group differences. There was no interaction between group and hemisphere [F (3, 82) =0.74, p=.53].

7.4.2 N170 latency

Across the whole sample, there was a main effect of age [F (1, 82) =8.94, p=.004), indicating that. N170 latency decreases with increasing age (r=-.32, p=.002). There was no main effect of hemisphere on N170 latency [F (1, 82) =0.16, p=.69]. There was a trend toward a main effect of emotion on N170 latency [F (4, 79) =2.39, p=.06]. Planned contrasts revealed significantly longer latency to neutral faces compared to happy faces [F (1, 82) =5.07, p=.03]. Similar to above, in order to assess potential developmental effects, N170 latency was examined in different age groups. In the youngest third of participants, there was no modulation of N170 latency by emotion [F (4, 23) =1.84, p=.16], whereas in the eldest third there was a trend towards a main effect of emotion [F (4, 21) =2.36, p=.09]. Planned contrasts on the eldest third revealed significantly longer latency to neutral faces compared to happy faces [F (1, 24) =5.93, p=.02].

There was no main effect of group on N170 latency [F (3, 82) =0.05, p=.98] and no interaction between group and emotion [F (12, 243) =1.06, p=.39] or hemisphere on N170 latency [F (3, 82) =0.10, p=.83].
**Table 8-2**: Mean (SD) amplitude (in μV) and latency (in msec) for the N170 component on each emotional expression stimulus by group

<table>
<thead>
<tr>
<th>N170</th>
<th>Stimulus</th>
<th>Electrode</th>
<th>TD</th>
<th>ASD</th>
<th>ADHD</th>
<th>ASD+ADHD</th>
</tr>
</thead>
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<td>-4.78</td>
<td>-6.66</td>
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<td></td>
<td></td>
<td>(28.07)</td>
<td>(24.02)</td>
<td>(23.32)</td>
<td>(20.43)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P8</td>
<td>217.25</td>
<td>211.40</td>
<td>213.76</td>
<td>217.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(19.58)</td>
<td>(19.48)</td>
<td>(18.60)</td>
<td>(18.15)</td>
</tr>
<tr>
<td></td>
<td>Happy</td>
<td>P7</td>
<td>203.38</td>
<td>202.21</td>
<td>206.81</td>
<td>206.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(27.09)</td>
<td>(22.46)</td>
<td>(16.71)</td>
<td>(24.62)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P8</td>
<td>208.38</td>
<td>204.39</td>
<td>205.30</td>
<td>202.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(14.57)</td>
<td>(16.74)</td>
<td>(20.24)</td>
<td>(20.04)</td>
</tr>
</tbody>
</table>

**Abbreviations**: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; ASD+ADHD, comorbid attention deficit hyperactivity disorder and autism spectrum disorder; TD, typically developing control
Figure 8-2: Grand mean N170 ERPs at P7 and P8 to emotional face stimuli for each group and isocontour maps derived for the grand-average of all expressions at peak latency for each group.

Note: Black represents neutral, red represents anger; blue represents fear; green represents disgust; pink represents joy.

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; ASD+ADHD, comorbid attention deficit hyperactivity disorder and autism spectrum disorder; TD, typically developing control
8.4.3 N400 amplitude

Mean peak amplitudes and latencies of the N400 for each group are shown in Table 8-3. Grand average ERPs and topographical maps are shown in Figure 8-4.

Across the whole sample, age was not significant as a covariate on the N400 \( F(4, 78) =0.58, p=.68 \) and therefore was dropped from the analysis. There was no main effect of emotion \( F(4, 80) =0.21, p=.93 \), but there was an interaction between emotion and electrode \( F(4, 80) =3.08, p=.02 \). Planned contrasts revealed significant differences between happy and angry faces \( F(1, 83) =11.10, p=.001 \), with a trend for fearful \( F(1, 83) =2.96, p=.09 \) and disgusted faces \( F(1, 83) =3.14, p=.08 \). These patterns showed that at Cz, happy facial expressions elicited reduced amplitude compared to negative facial expressions, whereas at Pz the opposite pattern emerged. In addition, there was a significant difference between neutral and angry faces \( F(1, 83) =4.08, p=.05 \), indicating enhanced amplitude for angry faces at Cz, with the opposite pattern at Pz.

There was no main effect of group \( F(3, 83) =0.36, p=.79 \), interaction between group and emotion \( F(12,246) =0.32, p=.99 \) or interaction between group and electrode (Cz/Pz) \( F(3, 83) =2.03, p=.12 \). When combining the groups by the presence or absence of ADHD diagnosis, an interaction between group and electrode emerged \( F(1, 85) =5.64, p=.02, d=0.51 \), indicating in subjects without ADHD (TD/ASD) there was greater amplitude at Cz compared to Pz, while subjects with ADHD (ADHD/ASD+ADHD) showed greater amplitude at Pz. This interaction was not shown when combining by ASD diagnosis \( F(1, 83) =0.25, p=.62, d=0.01 \), nor was there an interaction between ASD and ADHD on this effect, suggesting additive effects \( F(1, 83) =0.27, p=.60 \).

There was a trend for a three-way interaction between group, electrode and emotion \( F(12, 246) =1.65, p=.08 \). Planned comparisons revealed a significant difference between neutral and happy faces \( F(3, 83) =3.48, p=.020 \). Posthoc comparisons on this contrast indicated a significant difference between ASD and ADHD participants \( p=.02, d=0.91 \), showing that while subjects with ASD-only show reduced amplitude to happy faces compared to neutral faces at Cz, compared to enhanced amplitude to happy faces at Pz, children with ADHD-only show reduced amplitude for happy faces across electrodes.

When combined by ADHD diagnosis, there was also a trend towards an interaction between group, emotion and electrode \( F(4, 82) =2.08, p=.08 \). Planned contrasts revealed neutral faces elicited a trend towards altered N400 amplitude in ADHD compared to fearful faces \( F(1, 85) \).
=3.41, p=.05, d=0.42]: at Pz, subjects without ADHD (TD/ASD-only) showed enhanced amplitude for fearful versus neutral faces, which was not shown in subjects with an ADHD diagnosis (ADHD/ASD+ADHD). There were no group effects when combining by the presence or absence of ASD diagnosis [F (4, 82) =1.90, p=.12, d=0.15], and there was no interaction between ASD and ADHD [F (4, 80) =0.32, p=.87], suggesting an additive effect.

8.4.4 N400 latency

Across the whole sample, age was not significant as a covariate [F (1, 82) =0.13, p=.72] and therefore was removed from the analysis. There was a main effect of electrode [F (1, 83) =32.05, p<.001], indicating longer latency at Pz compared to Cz.

There was no main effect of group [F (3, 83) =1.29, p=.28], nor between group and hemisphere [F (3, 83) =0.19, p=.91] on N400 latency. There was a trend towards an interaction between group and emotion [F (12, 246) =1.63, p=.08] and planned contrasts revealed a significant difference between happy and fearful expressions [F (3, 83) =2.74, p=.05]. Posthoc analyses showed that subjects with ASD-only demonstrated longer latency for fearful faces compared to happy faces, whereas the opposite pattern was shown in ADHD (p=.032, d=0.89). There was no effect when combined by ADHD diagnosis [F (4, 82) =1.33, p=.27] or ASD diagnosis [F (4, 82) =1.90, p=.12] and no interaction between ASD and ADHD on this effect [F (4, 80) =1.18, p=.33].
### Table 8-3: Mean (SD) amplitude (in μV) and latency (in msec) for the N400 component on each emotional expression stimulus by group

<table>
<thead>
<tr>
<th>N170</th>
<th>Stimulus</th>
<th>Electrode</th>
<th>TD Mean (SD)</th>
<th>ASD Mean (SD)</th>
<th>ADHD Mean (SD)</th>
<th>ASD+ADHD Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cz</td>
<td>6.76 (2.58)</td>
<td>6.83 (3.47)</td>
<td>5.65 (3.24)</td>
<td>6.28 (3.55)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>6.82 (3.38)</td>
<td>5.07 (3.26)</td>
<td>7.70 (5.04)</td>
<td>7.24 (4.39)</td>
</tr>
<tr>
<td></td>
<td>Angry</td>
<td>Cz</td>
<td>7.02 (2.11)</td>
<td>6.62 (3.11)</td>
<td>6.98 (3.20)</td>
<td>6.44 (3.17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>6.34 (2.81)</td>
<td>5.62 (2.27)</td>
<td>6.84 (4.10)</td>
<td>6.86 (3.16)</td>
</tr>
<tr>
<td></td>
<td>Fearful</td>
<td>Cz</td>
<td>6.54 (2.81)</td>
<td>5.95 (3.65)</td>
<td>6.39 (3.85)</td>
<td>6.56 (3.53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>6.39 (3.48)</td>
<td>5.58 (3.02)</td>
<td>7.15 (5.27)</td>
<td>7.00 (4.16)</td>
</tr>
<tr>
<td></td>
<td>Disgusted</td>
<td>Cz</td>
<td>7.18 (2.99)</td>
<td>6.79 (3.10)</td>
<td>5.67 (3.54)</td>
<td>5.93 (3.58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>6.53 (3.97)</td>
<td>5.21 (2.20)</td>
<td>7.28 (4.47)</td>
<td>7.16 (2.99)</td>
</tr>
<tr>
<td></td>
<td>Happy</td>
<td>Cz</td>
<td>6.94 (3.06)</td>
<td>6.20 (3.06)</td>
<td>5.90 (3.23)</td>
<td>5.86 (3.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>7.13 (4.19)</td>
<td>6.29 (2.79)</td>
<td>6.60 (4.21)</td>
<td>7.85 (3.58)</td>
</tr>
<tr>
<td>Latency</td>
<td>Neutral</td>
<td>Cz</td>
<td>400.84 (37.16)</td>
<td>386.50 (47.91)</td>
<td>388.10 (35.96)</td>
<td>390.63 (44.72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>406.40 (36.67)</td>
<td>402.78 (49.50)</td>
<td>396.14 (43.10)</td>
<td>403.55 (35.32)</td>
</tr>
<tr>
<td></td>
<td>Angry</td>
<td>Cz</td>
<td>402.79 (36.83)</td>
<td>376.30 (41.12)</td>
<td>392.46 (38.25)</td>
<td>394.98 (31.91)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>412.41 (35.44)</td>
<td>387.59 (51.34)</td>
<td>409.93 (44.48)</td>
<td>408.20 (34.76)</td>
</tr>
<tr>
<td></td>
<td>Fearful</td>
<td>Cz</td>
<td>399.64 (37.05)</td>
<td>411.24 (44.70)</td>
<td>385.11 (30.26)</td>
<td>395.58 (38.98)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>406.55 (31.81)</td>
<td>417.32 (38.13)</td>
<td>393.61 (45.44)</td>
<td>416.77 (39.21)</td>
</tr>
<tr>
<td></td>
<td>Disgusted</td>
<td>Cz</td>
<td>390.02 (34.49)</td>
<td>365.23 (35.00)</td>
<td>376.84 (31.25)</td>
<td>393.18 (37.12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>407.75 (36.14)</td>
<td>394.97 (39.42)</td>
<td>394.53 (31.31)</td>
<td>416.32 (40.70)</td>
</tr>
<tr>
<td></td>
<td>Happy</td>
<td>Cz</td>
<td>295.22 (32.27)</td>
<td>296.66 (36.78)</td>
<td>292.74 (29.57)</td>
<td>296.12 (28.85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pz</td>
<td>315.26 (31.08)</td>
<td>320.31 (32.60)</td>
<td>319.16 (43.16)</td>
<td>311.45 (32.35)</td>
</tr>
</tbody>
</table>

**Note:** N400 amplitude is calculated as peak-to-peak amplitude from the preceding P300 component, thus a more positive amplitude indicates enhanced amplitude.

**Abbreviations:** ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; ASD+ADHD, comorbid attention deficit hyperactivity disorder and autism spectrum disorder; TD, typically developing control.
Figure 8- 3: Grand mean N400 ERPs at Cz and Pz to emotional face stimuli for each group and isocontour maps derived for the grand-average of all emotional expressions at peak latency for each group.

Note: Black represents neutral, red represents anger; blue represents fear; green represents disgust; pink represents joy.

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; ASD+ADHD, comorbid attention deficit hyperactivity disorder and autism spectrum disorder; TD, typically developing control
8.4.5 Dimensional analysis between symptom scores and ERP parameters

Over all stimuli, there were no significant correlations between N170 and N400 amplitudes for TD (r=-.32, p=.12) and ASD (r=-.30, p=.24) but moderate associations in ADHD (r=-.45, p=.07) and ASD+ADHD groups (r=-.47, p=.02).

Table 8-4 shows correlations between ERP parameters that showed significant group differences and symptom scores. There was a trend for topographical differences on the N400 to be related to hyperactive-impulsive scores (r=-.19, p=.09). The modulation of the N400 by electrode on joyful versus neutral expressions was related to symptom scores on the SCQ (r=.35, p=.001), with opposite correlations to scores on the Conners (inattention: r=-.35, p=.001; Hyperactivity/Impulsivity: r=-.20, p=.06). Similar patterns were shown for the modulation of the N400 by neutral versus fearful expressions (SCQ: r=.18, p=.10; Inattention: r=-.28, p=.01; Hyperactivity/Impulsivity: r=-.13, p=.24).

ERP parameters which were significantly correlated across both groups with the SCQ and Conners scores, respectively, were then taken forward for inclusion in multiple linear regression analyses with hierarchical entry, which confirmed the above correlations supporting the SCQ or the Conners as predictors of specific EEG measures. For neutral versus happy effects on the N400 component, the regression model showed a significant increase in $R^2$ when the Conners and the SCQ were entered into the model, including when controlling for the effect of the other rating scale. For neutral versus fearful effects on the N400 component, there was a significant increase in $R^2$ for Conners scores only, which remained when controlling for the effect of SCQ scores. Scores on the SCQ did not significantly predict this effect.

**Table 8-4:** Correlations between ERP parameters and symptom scores

<table>
<thead>
<tr>
<th>ERP</th>
<th>Effect</th>
<th>SCQ</th>
<th>Conners Inattention</th>
<th>Conners Hyp/Imp</th>
</tr>
</thead>
<tbody>
<tr>
<td>N170 amplitude</td>
<td>Overall</td>
<td>-.15</td>
<td>.10</td>
<td>.06</td>
</tr>
<tr>
<td>N400 amplitude</td>
<td>Electrode</td>
<td>-.03</td>
<td>-.10</td>
<td>-.19†</td>
</tr>
<tr>
<td></td>
<td>Neutral-joy</td>
<td>.35**</td>
<td>-.35**</td>
<td>-.20†</td>
</tr>
<tr>
<td></td>
<td>Neutral-fear</td>
<td>.18†</td>
<td>-.28*</td>
<td>-.13</td>
</tr>
</tbody>
</table>

Abbreviations: ERP, event-related potential; Conners, Conners Parent Rating Scale; SCQ, Social Communication Questionnaire * p<.05 ** p<.01 † p<.1
8.5 Discussion

This study provides new evidence to show abnormalities in emotional face processing in ASD and ADHD that can be differentiated on the basis of early (N170) and late (N400) ERP components. Children with comorbid ASD+ADHD displayed deficits at both temporal stages of face processing, suggesting an additive condition.

8.5.1 N170 effects

There was no modulation of N170 amplitude or latency by emotional expression across the whole sample. However, it is likely that these null findings reflect developmental effects, as emotional sensitivity on the N170 is seen to emerge with increasing age, with greater effects shown at 14-15 years of age (Batty and Taylor, 2006). This is supported by emotional modulation of the N170 in the older participants in this sample (and in addition when age is removed as a covariate). Findings in the older age group were consistent with previous reports. Specifically, the latency of the N170 component was modulated by emotion in all groups: happy faces evoked shorter latency compared to all other expressions, supporting previous studies (Batty and Taylor, 2003). It has been suggested that this latency difference between positive and negative faces is because negative emotions require additional subcortical processing prior to cortical processing (Adolphs, 2002, Batty and Taylor, 2003, Leppänen and Hietanen, 2004). In addition, although consistent with previous studies suggesting modulation of N170 amplitude by emotion (Batty and Taylor, 2003, Blau et al., 2007, Campanella et al., 2002, Eger et al., 2003, Leppänen et al., 2007), there was reduced N170 amplitude for all emotional expressions compared to neutral faces, particularly for anger and happiness. This may suggest emotional (particularly threat-related) faces are processed in a more efficient involuntary manner compared to neutral faces, particularly as studies suggest emotional faces do not need to be attended to in order to be processed (Vuilleumier and Schwartz, 2001). This supports the nonconscious nature of the N170 (Williams et al., 2004) and potential adaptive mechanisms that may underlie this (Ohman and Soares, 1993). The increased amplitude for neutral faces shows an effect of intensity or arousal for neutral versus emotional (negative and positive) faces on the N170, similar to previous findings (Utama et al., 2009). This supports the view of the N170 as a non-specific generalised response to emotional stimuli of any valence (Breiter et al., 1996), associated with encoding of structural facial cues rather than emotional significance (Ashley et al., 2004, Vuilleumier and Pourtois, 2007). Although some studies report no modulation of the N170 by emotion, modulation is more likely in passive tasks as in the present study, when the stimuli appear as incidental to the
primary task (Pizzagalli et al., 1999, Williams et al., 2004), and likewise, inconsistencies in findings are likely to reflect paradigm differences. The findings do however generally suggest that emotional processing begins at an early stage of visual processing in late childhood/early adolescence (Batty and Taylor, 2003).

Group differences based on ASD emerged on N170 amplitude regardless of these developmental differences; children with ASD (ASD and ASD+ADHD) displayed reduced N170 amplitude across all face stimuli irrespective of emotion compared to TD and ADHD, suggesting a defect in attention does not impair a neurobiological correlate of face processing. This is consistent with previous work (Hileman et al., 2011, O’Connor et al., 2005) and suggests abnormalities in allocation of resources to process faces, and a likely deficit in structural encoding as indexed by the N170 (see Section 1.3.3.1, Chapter 1). The N170 amplitude reduction for emotional expressions may therefore be driven by the ASD groups and this may reflect hypoarousal to emotional expressions in ASD (Dawson et al., 2005b, Grelotti et al., 2002). While a trend toward an interaction between ASD diagnosis and emotion was observed, there were no significant differences between specific emotions. Some previous work reports deficits in processing of negative emotion, particularly fear, although indexed by emotion recognition in behavioural tasks (Ashwin et al., 2006) and reduced modulation of the N300 component by fear in younger children (Dawson et al., 2004). The N170 is altered by the presence or absence of eyes within a face (Schyns et al., 2003) and as individuals with ASD display atypical scanning patterns and ERP responses to eye gaze, reported in Chapter 6, any deficits in may reflect these gaze processing deficits (Akechi et al., 2011). As the N170 is thought to reflect detection and/or structural encoding rather than the recognition of faces (Schweinberger and Burton, 2003), our findings suggest there is a general disadvantage in processing faces in ASD, rather than a specific deficit in processing emotion (Dawson et al., 2004).

8.5.2 N400 effects

The amplitude of the N400 component was enhanced for emotional expressions, supporting previous findings (Posamentier and Herve, 2003). This modulation appeared to index a valence effect, because larger differences were reported between positive (happy) and negative (anger/fear/disgust) compared to between neutral and emotional expressions (Lang et al., 1998, Luo et al.). Happy facial expressions are processed differently to all other facial expressions (Adolphs et al., 1996) and are the most easily recognised emotion together with surprise (Battaglia et al., 2009, Kirita and Endo, 1995) This may indicate that negative
emotional expressions are more semantically complex than expressions of joy and therefore require additional cortical involvement or interaction between sensory and top-down processes (Adolphs, 2002, Taddei et al.). As these effects could be differentiated by topographical location, the neuroanatomical basis of these differences should be investigated using source localisation techniques, such as LORETA.

Children with ADHD (ADHD and ASD+ADHD) demonstrated altered modulation of happy and fearful faces versus neutral faces compared to children without ADHD. Abnormal N400 modulation by happy faces is in line with previous work reporting particular deficits toward positive stimuli (Becker et al., 1993, Conzelmann et al., 2009, Herrmann et al., 2009b, Ibanez et al., 2011). This might reflect impairments in reward processing (Scheres et al., 2007) and abnormalities in the motivational system as reflected by delay aversion (Sonuga-Barke et al., 2008). As aberrant processing of happy expressions was shown in ADHD in comparison to ASD children, this suggests there may be different reward processing abnormalities in these diagnostic groups (see Section 1.3.2.3.4, Chapter 1). Reduced modulation of fearful faces supports consistent deficits in recognising threat-related (anger, fear) expressions in ADHD (Cadesky et al., 2000, Pelc et al., 2006, Singh et al., 1998). The group differences in emotion modulation were influenced by topographical location, indicating group differences in modulation emerged at Pz.

In addition, children with ADHD demonstrated topographical differences in N400 amplitude. This is particularly important because the N400 is thought to reflect activity from a distributed comprehension system (Kutas and Federmeier, 2011), specifically a cortico-amygdala pathway (Williams et al., 2004), and it may be the case that individuals with ADHD utilize alternative neural mechanisms to process emotion. For example, typical activation of the amygdala but enhanced activation of the frontal and posterior cingulate cortex to angry (threat-related) faces is observed in ADHD (Marsh et al., 2008) that may reflect such differential activation. Conversely, both fearful and happy expressions have been associated with amygdala activation (Breiter et al., 1996, Morris et al., 1996, Scott et al., 1997), suggesting the potential involvement of the amygdala abnormality in ADHD as supported by recent MRI findings (Frodl et al., 2010). Overall, these findings suggest children with ADHD, regardless of comorbidity with ASD, have unique deficits in evaluation of the meaning and context of emotion (Da Fonseca et al., 2009, Shin et al., 2008, Sinzig et al., 2008b), and that deficits shown in emotion recognition in ASD may be at least partly due to ADHD symptoms. This supports multiple pathway models of ADHD that suggest deficits beyond a sole deficit in executive function.
Chapter 8: Neurophysiological correlates of emotion processing in ASD, ADHD and ASD+ADHD

(Christie and Proal, 2012, Castellanos et al., 2006), and warrants further investigation of emotional impairments and their associations with attentional impairment, particularly as social cognition impacts on several areas of functioning (Nijmeijer et al., 2008).

8.5.3 Implications

Taken as a whole, while both the N170 (age—dependent) and N400 were modulated by emotion, the components temporally and functionally dissociated ASD and ADHD on the basis of different ERP abnormalities; deficits in structural encoding were observed in ASD (indexed by the N170 ERP component) compared to abnormalities in contextual processing in ADHD (as indexed by the N400 ERP component), which may in turn impair social functioning in the disorders. This dissociation is further supported by examination using a dimensional view through parental questionnaires’ scores; data reveal that the effects on N400 amplitude can be predicted by ADHD symptom scores. The difference between emotional and neutral emotions’ activation bears a negative correlation to ASD symptoms during early visual encoding of faces (as indexed by the N170), while the correlation evolves into positive in the later phase of evaluating the significance of face expression (as indexed by the N400). The opposite was true for ADHD symptoms as rated by the Conners’ (most clearly the inattention items) parental scales. The intact emotion comprehension and conscious integration with semantic memory as indexed by N400 in ASD may reflect a compensatory mechanism allowing intact affective processing, such as the use of explicit cognitive processes rather than automatic emotion processing (Harms et al., 2010), which may be reflected in the shorter N400 latency to fearful faces and greater N400 amplitude at parietal sites to happy faces observed in this group, and/or differences in age and ability of the subjects (Dyck et al., 2006, O’Connor et al., 2005, Rump et al., 2009). Conversely, the intact N170 combined with impaired N400 in ADHD may signify an emotional deficit rather than a deficit processing emotion from faces. These propositions are in line with conclusions from the previous Chapter 7, suggesting abnormalities on the N170 component in children with ASD that are not observed in ADHD, and that aberrant processing of these stimuli in ADHD may not be due to problems processing faces per se. These findings also suggest mismatched temporal stages in emotion encoding, as supported by stage-like models of phased face processing (Bruce and Young, 1986).

Importantly, while the pure disorders can be dissociated, the comorbid ASD+ADHD group appear to display the unique deficits of both disorders, shown by reduced N170 amplitude combined with altered modulation of the N400 by fearful and happy expressions (at differing topographical locations), which supports an additive model of comorbidity. This supports and...
adds to the findings presented in previous chapters, by showing a similar pattern of comorbidity in a domain which has been associated with both ASD and ADHD, and for which we might therefore expect some overlapping abnormalities where instead dissociations are still observed. It will be necessary to compare these altered ERP components to behavioural or neural measures of ToM, attention and inhibition in order to fully understand the basis of altered social-cognitive processing, particularly as processing of facial emotion is associated with executive function in ADHD (Ibanez et al., 2011, Pessoa, 2008, Shin et al., 2008, Sinzig et al., 2008b).

**8.5.4 Limitations**

Certain limitations should be taken into consideration. The relatively small sample size may underlie small or trend-level effects and therefore limits firm conclusions. There is large variability in emotion processing depending on the intellectual ability of ASD subjects; many studies report intact emotion recognition in high-functioning individuals, which may explain why the N400 was intact in ASD (Adolphs et al., 2001). Although IQ was not significant as a covariate, this warrants the investigation of ERP correlates of emotion processing in a range of abilities. The lack of concurrent eye-tracking means we cannot rule out the effect of different face scanning patterns, as suggested by previous findings (Pelphrey et al., 2002). In addition, neural abnormalities during emotion processing are displayed in other disorders, such as anxiety and depression (Kring and Werner, 2004), and therefore subthreshold symptoms may affect the specificity of these effects.

**8.5.5 Conclusion**

This work demonstrates that abnormalities in early (N170) and late (N400) electrophysiological responses to emotional face expressions differentiate between children with ASD and ADHD, respectively. The ASD+ADHD group present as an additive co-occurrence with the unique deficits of both disorders, which warrants the assessment of ADHD symptoms in ASD and vice versa in the examination of neural and aetiological mechanisms underlying the disorders. This highlights the advantages of ERP markers to disentangle emotional impairments of these disorders and provide insight on the pathophysiological basis of comorbid conditions. Such efforts to further our understanding of the brain-behaviour relationships underlying emotional impairment in ASD and ADHD are likely to yield scientific and clinical benefits.
CHAPTER 9: Overall conclusions and future directions

The overarching aim of this thesis has been to identify candidate intermediate phenotypes of ADHD, ASD and ASD+ADHD, using quantitative genetic and cognitive-electrophysiological approaches. The following sections will re-examine the research questions proposed in Chapter 1 and summarise the main findings. Interpretations and implications of these findings will be discussed with regards to cognitive accounts of the disorders, underlying genetic and biological mechanisms and models of comorbidity with the consideration of avenues for further research throughout discussion. The limitations of this work will then be considered, concluding with implications for clinical diagnosis and intervention.

9.1 Summary of major findings

The first section (Part I) used a population twin sample to quantify the extent of phenotypic and genetic overlap between ADHD symptoms and EEG markers previously associated with ADHD. Participants were selected using latent class analysis to identify subgroups based on developmental trajectories at ages 8, 12 and 14. Twin pairs (MZ and DZ) were identified with consistently high or low ADHD symptom scores (inattention and hyperactivity/impulsivity) over development, which were subsequently allocated into concordant ADHD, discordant ADHD and concordant control twin pair groups. Structural equation modeling using bivariate liability threshold models allowed the decomposition of variance in the EEG parameters into genetic and environmental components.

The first study (Chapter 3) investigated a novel measure of arousal, very low frequency (VLF) activity (0.02-0.2Hz) and revealed modest heritability (0.31) for this measure, which suggests a large contribution from unique environmental and substantial overlap between genetic influences for reduced VLF power and ADHD symptoms (0.80). In addition, reduced VLF power was associated with increased response variability in the high-ADHD group only.

The second study (Chapter 4) evaluated theta power as a candidate intermediate phenotype using the same sample and twin analysis. Theta power (0.4-0.8Hz) was elevated in frontal regions in adolescents with high ADHD symptom scores and showed substantial heritability (0.77). In addition, adolescents with high ADHD symptoms demonstrated an increase in theta power from resting to task conditions and this increase showed modest heritability (0.44). The
genetic correlations indicated moderate shared influences between ADHD and theta power at rest (0.35) and from rest-to-task (0.68). There were also associations between theta power and task performance that differed by group; a positive correlation between response variability and theta change from rest-to-task was demonstrated in the low ADHD group, compared to a negative correlation in the high ADHD group.

We can conclude from Part I that reduced VLF power and elevated theta power show phenotypic association with ADHD, moderate to high heritability and shared genetic influences with the disorder, fulfilling criteria as intermediate phenotypes, and further show an altered relationship between behavioural performance and neural activity in adolescents with high ADHD symptom scores compared to low ADHD symptom scores.

The overall aim of the second part of this thesis was to investigate the specificity of candidate electrophysiological intermediate phenotypes to ADHD and ASD, and to elucidate the basis of co-occurring ASD+ADHD, using a theory-driven task battery consisting of variables that have been previously associated with ASD and/or ADHD. The comparison of 18 children with ADHD-only, 19 children with ASD-only, 29 children with comorbid ASD+ADHD and 26 typically developing controls that were subjected to rigorous diagnostic assessment, enabled the investigation of the pathophysiological processes that may mediate between behavioural and genetic overlap.

Chapter 5 drew upon work conducted in the first part of this thesis, to provide a detailed investigation of the QEEG profiles of children with ASD, ADHD and ASD+ADHD. The results identified a similar pattern to that previously reported in the pure disorders. Specifically, children with ADHD (ADHD/ASD+ADHD) demonstrated elevated theta power at rest, whereas children with ASD (ASD/ASD+ADHD) displayed increased delta power and reduced alpha power. These findings support the differing role of arousal in the two disorders. During the task, children with ADHD (ADHD/ASD+ADHD) demonstrated reduced cortical activation (reduced beta) and subsequently required a greater level of cortical activation (reduced alpha) to sustain their attention, which was not shown in ASD. These children also displayed altered associations with task performance measures, suggesting an alternative mechanism was employed to sustain attention over time.

Chapter 6 investigated ERP indices of attention and inhibition. The results identified a similar pattern of altered cognitive-electrophysiological processing to that previously reported in ADHD of weaker orienting of attention at cues and inhibitory processing. We extended these
previous findings by demonstrating that the Cue-P3 and NoGo-P3 appear to be specific to ADHD (ADHD/ASD+ADHD) compared to ASD. In contrast, children with ASD (ASD/ASD+ADHD) exhibited reduced conflict monitoring and children with ASD-only showed stronger cognitive preparation processes compared to control and comorbid ASD+ADHD children. Further, there were significant associations between these parameters and task performance that differed by group, suggesting alternative mechanisms employed to perform successfully on the task.

The specificity of electrophysiological correlates of social cognition to ASD was investigated in Chapter 7, using ERP indices of faces and eye gaze processing. In line with previous findings, children with ASD (ASD/ASD+ADHD) displayed altered modulation of visual processing by gaze direction as indexed by the P1 component, and absence of modulation by gaze direction on the N170 component, a proposed index of structural encoding of faces. In addition, these children showed reduced neural specialization of face and gaze processing. These abnormalities were not shown in children with ADHD. However, children with ADHD (ADHD/ASD+ADHD) did demonstrate an absence of the face inversion effect on the P1 component, an index of visual processing.

The final chapter reports dissociation between ASD and ADHD on the basis of temporal stages of emotional face processing (Chapter 8). Specifically, children with ASD (ASD/ASD+ADHD) displayed reduced N170 amplitude across all emotional expressions, and in particular attenuated amplitude for fearful expressions compared to neutral expressions. In contrast, children with ADHD (ASD/ASD+ADHD) demonstrated reduced modulation of the N400 component to happy and fearful facial expressions, differing on the basis of topographical location. These findings indicate deficits in structural encoding of emotion in ASD and contextual processing of emotion in ADHD.

Notably, across the studies in Part II of this thesis, an examination of these parameters using a dimensional view revealed that relationships between EEG/ERP parameters can be predicted by trait indices of impaired social communication and attention (SCQ and Conners respectively). Importantly, the comorbid ASD+ADHD group largely display the unique deficits of both disorders, which supports an additive model of comorbidity.

Taken together, research included in this thesis is the first to examine genetic and environmental overlap between cognitive-electrophysiological parameters and ADHD within a twin sample, and to investigate shared and distinct cognitive-electrophysiological profiles in
pure ASD, pure ADHD and comorbid ASD+ADHD cases. Theoretical and clinical implications of these findings will now be discussed.

9.2 ADHD overview in relation to these findings

9.2.1 Cognitive accounts

Findings reported in Part I of this thesis support the role of physiological arousal processes as markers of genetic risk for ADHD (Tye et al., 2011). While cognitive research has focused on executive function impairments in ADHD, the most ubiquitous finding in ADHD is variability in reaction time, and increased RTV and CV was observed in our population twin sample (at a trend level) and clinical ADHD sample, which was not shown in ASD that replicates previous findings (Johnson et al., 2007a). Importantly, increased RTV was associated with EEG-indices of arousal and activation. Taken together, these findings are supportive of state regulation models that propose performance deficits in ADHD are the result of a sub-optimal energetic state rather than a stable cognitive deficit (Sergeant, 2000). Alternatively, these findings could reflect the persistence of default-mode activity into task state resulting in interference with cognitive processes required for goal-directed task performance (Sonuga-Barke and Castellanos, 2007). Future work should investigate whether these associations are altered in the presence of task factors that have been shown to optimize arousal levels, such as a faster event rate and/or rewards (see Section 1.3.2.3.2). In addition, the findings highlight the importance of task-related change in EEG power, which showed greater genetic overlap with ADHD symptoms and was specific to ADHD compared to ASD, suggesting the measurement of arousal processes in conditions that require sustained attention is important for understanding the gene-brain-behaviour pathways implicated. In particular, these findings suggest the adoption of an alternative strategy to compete with the demands of the task.

While these measures of arousal show important genetic associations with ADHD, functional investigations of cognition using ERPs further revealed reduced inhibitory processing accompanied by reduced attentional orienting at cues. This supports previous work and suggests that response inhibition is unlikely to be the core cognitive deficit in ADHD, but rather a part of widespread executive dysfunction (Banaschewski et al., 2003, Banaschewski et al., 2004, Willcutt et al., 2005). In addition, ERP indices of social cognition indicated an attenuated face inversion effect in ADHD on the early P1 component, and altered contextual processing of fearful and happy emotional face expressions, lending support to emotional dysregulation in ADHD. These findings bring into play the potential contribution of ToM deficits and altered
face processing strategies to social problems observed in ADHD. The combination of these impairments lends support to multiple deficit accounts of ADHD described in Chapter 1, that propose ‘hot’ motivational executive processes and ‘cool’ attentional executive processes (Castellanos et al., 2006, Sonuga-Barke, 2002). In addition, it is likely that these processes interact with each other (Pessoa, 2008, 2009); for example, reduced modulation by face inversion may reflect the allocation of top-down cognitive resources. Further research should examine these associations within the same task, and work that is able to identify causal direction between these deficits will increase our understanding of their interplay. This work is important for identifying candidates for molecular genetic investigations and providing clues to underlying neural mechanisms, discussed in the following section.

9.2.2 Potential genetic and biological underpinnings

As reviewed in Chapter 1, ADHD is highly heritable and several studies report inconsistent associations with common and rare genetic variants. The identification of biological markers that mediate between genes and behaviour is one strategy to facilitate the identification of susceptibility genes, and further our understanding of biological pathways involved in the pathophysiology of ADHD (see Section 1.3, Chapter 1). On this basis, the present thesis extended previous work conducted in singleton and family studies of ADHD, by quantifying the phenotypic correlation between reduced VLF power and elevated theta power with ADHD trait measures, and obtaining moderate to strong heritability estimates in line with previous work. Importantly, moderate to substantial shared genetic influences between ADHD symptoms and these measures were reported. In addition, the findings from Chapters 3 and 4 converge to suggest elevated theta power is a heritable trait that shares genetic influences with ADHD and is specific to ADHD compared to ASD. The next step is to identify genetic variants that are associated with these parameters. In Chapter 1, I outlined several candidate gene associations with EEG/ERP parameters particularly those involved in dopaminergic neurotransmission. For example, children with the 7-repeat allele of DRD4, the risk allele associated with ADHD, show increased frontal theta power (Loo et al., 2010). In addition, children with the 10-allele of DAT1 show decreased theta activity with MPH treatment (Loo et al., 2003). Similarly, the role of dopamine has been implicated in DMN activity (Liddle et al., 2011, Mehta, 2011). Combining our findings of increased response variability and reduced inhibitory processing in ADHD and reported associations between dopaminergic systems and executive function both at the behavioural performance and neural level may implicate this as gene-brain-behaviour pathways that are specific to ADHD. Serotonergic dysfunction appears to be involved with emotion processing; specifically reduced modulation of the N400 component to negative
expressions (observed in ADHD in this thesis) is associated with the short allele of 5HTTLPR (Battaglia et al., 2005), and the 5-HTTLPR has been associated with neural responses to happy expressions in infants (Grossmann et al., 2011).

Theoretical models have further implications for the potential brain regions and functions associated with the electrophysiological abnormalities reported. As has been stressed in the relevant empirical chapter, the biological mechanisms underlying VLF activity are in need of exploration. A source localization study using sLORETA identified overlap between regions involved in the DMN and the deactivation of VLF power from rest-to-task, specifically medial prefrontal regions, posterior cingulate cortex and temporal regions (Broyd et al., 2011). Importantly, these sources varied by group; participants with ADHD did not deactivate medial prefrontal regions, which may indicate a disconnection between anterior and posterior regions of the DMN (Broyd et al., 2011, Castellanos et al., 2008). Converging evidence suggests the VLF activity is an index of widespread synchronization (Biswal et al., 2005, He and Raichle, 2009), which has implications for current models of ADHD.

The presence of multiple deficits in this thesis is supported by recent structural and functional imaging findings, in particular those implicating resting state functional networks (such as the DMN). Typically ADHD is associated with a prefrontal-striatal—cerebellar and fronto-parietal model that governs ‘cool’ executive functions such as response inhibition (Bush et al., 2005, Dickstein et al., 2006). Recently this is proposed to extend to other networks (Castellanos and Proal, 2012). For example, an impairment of the motivational system that governs reward processing, comprised of the ventral striatum and amygdala, is altered in ADHD populations (Scheres et al., 2007, Ströhle et al., 2008). The N400 is proposed to be a temporal correlate of cortico-amygdala activity (Williams et al., 2004), which may reflect altered contextual processing of face expressions reported here, particularly as there is no difference in amygdala activity in response to fearful and happy expressions (Morris et al., 1996). Moreover, specific deficits shown for ADHD in this thesis may support specific brain abnormalities. For example, a recent direct comparison of ADHD and ASD using fMRI revealed more severe dysfunction of the left dorsolateral prefrontal cortex in ADHD during sustained attention (Christakou et al., in press). Further imaging work using a varied task battery will improve our understanding of the brain regions involved in these executive and emotional dysfunctions, and their interaction.

Finally, an interesting approach that may provide a platform for the integration of these varied deficits is the study of local and global brain connectivity, and the concept of “small-world networks”. In ADHD an increase in local connections compared to reduced global connectivity
during resting-state result in an inefficient brain (Wang et al., 2009b). These theories may further explain the presence of multiple deficits in ADHD. For example, it is proposed that the amygdala is one of the most highly connected regions of the brain, with projections to prefrontal areas, which may underpin the relationship between executive function and emotion (Pessoa, 2009). It is likely therefore that reported deficits arise from abnormalities in these dynamic interactions between brain networks.

9.3 ASD overview in relation to these findings

9.3.1 Cognitive accounts

Reduced alpha power in the ASD groups is indicative of hyperarousal in this population. Over-arousal theories suggest that children with ASD have heightened arousal in response to certain stimulation. This over-arousal is proposed to lead to blocking of neural sensory pathways, which leads to avoidance of ‘novelty’ and subsequent stereotypic behaviours and withdrawal from the social world, which is inherently unpredictable (Gomot and Wicker, 2011, Hutt et al., 1964). Nevertheless, increased slow-wave delta power may suggest hypoarousal. For example, in DesLauriers and Carlson’s theory children with autism have a suppressed limbic system which prevents normal environmental input (Des Lauriers and Carlson, 1969). These theories have implications for social and non-social impairments in ASD, as detailed below. These findings do suggest altered neuronal activity in the unpredictable setting of ‘rest’ in ASD, measured at the beginning of the testing session.

Diminished neural responses associated with response selection conflict (Donkers and van Boxtel, 2004) support the presence of deficits in flexibly shifting attention with changing task demands. In addition, the CNV component has been associated with planning in typically developing adults, with amplitude increasing with more difficult problems (Byrd et al., 2011), which further supports abnormalities in planning in ASD. The novel findings presented in this thesis therefore support the presence of executive dysfunction in ASD at the neural level, particularly in cognitive flexibility and planning (Hill, 2004) and suggest attentional deficits go beyond social relevance. Future work should investigate the associations between these deficits and others in ASD, and how they relate to and explain the triad of impairments (Happé and Ronald, 2008).

Findings on the social cognition tasks revealed that children with ASD recruit distinct mechanisms for processing gaze at early sensory processing temporal stages, an absence of
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modulation by gaze at the structural encoding stage and, in a separate task, attenuated electrophysiological responses to emotional facial expressions, in particular fear at an early temporal stage of emotional processing. Interestingly, social cognition abnormalities were largely shown on the N170 component, proposed to be face-sensitive (Bentin et al., 1996). These findings converge to suggest a general difficulty in structural processing of faces rather than emotion (although difficult to dissociate). The findings also support a stage-like model of face processing, as only in ASD were effects of emotion observed on the earlier N170 component (Bruce and Young, 1986). This general difficulty with processing faces in ASD may be attributable to impairment in social motivation (Dawson et al., 2005b). Faces may not be intrinsically emotionally salient leading to less attention to faces (Schultz, 2005) and eyes (Dalton et al., 2005). A lack of interest for the human face from early in development (Jemel et al., 2006) and therefore distortions in sensory input in infancy (‘social deficit’) could disrupt the development of more complex cognitive abilities (Tanguay and Edwards, 1982). This may then in turn lead to ToM deficits and problems in social interactions (Baron-Cohen, 1995). Conversely difficulties in face processing may be the consequence of a perceptual difficulty (Behrmann et al., 2006, Dakin and Frith, 2005). These perceptual alterations (e.g. weak central coherence) are thought to be separate from executive function, social behavior and theory of mind (Happé and Frith, 2006). It may therefore be that individuals with ASD employ alternative strategies to process faces that are more akin to object processing. In this way abnormal electrophysiological differences could be a marker of early abnormal sensory processing rather than the primary deficit. However, these explanations need not be mutually exclusive and both might contribute. Likewise, the presence of hyperarousal may affect cognitive flexibility and social cognition. For example, it is proposed that direct gaze is aversive and more arousing than nonsocial stimuli to individuals with ASD, due a low aversion threshold and low range of optimal arousal in autism (Dawson and Lewy, 1989). This emphasizes the importance of a developmental viewpoint, and as such longitudinal investigations are required to evaluate the validity of these arguments (Nation and Penny, 2008).

In addition, the association between quantitative trait measures of ASD and altered cognitive-electrophysiological parameters suggests that these vary with autistic severity and supports the dimensional view of autism as the extreme end of a continuously distributed trait.

9.3.2 Potential genetic and biological underpinnings

The identification of cognitive-electrophysiological markers that appear to be specific to ASD is likely to assist molecular genetic research. For example, in Chapter 1 I noted that the EN2 gene
has been associated with the cerebellar structure (see below for potential link with cognitive findings), and various candidate genes for ASD are associated with social cognition. A recent review highlights the role of the serotonin transporter gene in emotion regulation and social cognition (Canli and Lesch, 2007), which itself is consistently associated with ASD. The short allele of the 5HTTLPR polymorphism has been associated with fearful face expressions in particular and reduced sensitivity and shorter N170 latency with the val/val allele compared to increased sensitivity for carriers with the met allele of the COMT gene (Drabant et al., 2006, Grossmann et al., 2011, Herrmann et al., 2009a). In addition, oxytocin reduces response to emotional expression in the amygdala (Domes et al., 2007), enhances direct eye gaze, and is associated with the ability to identify and remember faces (Guastella et al., 2008, Savaskan et al., 2008), which may implicate alterations of this system in ASD.

In addition, it points toward specific brain structures and functions that may be altered. For example, in terms of executive function deficits, conflict monitoring has been linked with the anterior cingulate cortex (van Veen and Carter, 2002), hypoactivation of which has been associated with ASD during change detection (Gomot et al., 2006). In addition, in a direct comparison of ADHD and ASD using fMRI during a sustained attention task, individuals with ASD showed enhanced cerebellar activation (Christakou et al., in press). Interestingly, the cerebellum has been associated with the CNV due to its relation with timing abilities (Meck et al., 2008). In line with this, individuals with ASD appear to perform similarly to or outperform controls on tasks involving timing reproduction (Wallace and Happé, 2008). This is a clear example of how intermediate phenotype research can provide insight into the potential pathophysiological mechanisms underlying observable behaviour.

The N170 was altered specifically in ASD, the source of which has been localized to brain regions that are activated in response to faces, such as the fusiform gyrus (Rossion et al., 1999) and posterior superior temporal sulcus (Itier & Taylor 2004), which has shown hypoactivation in ASD (Schultz, 2005). Reduced processing of fearful facial expressions implicates amygdala dysfunction in ASD, with which bidirectional connections exist with the fusiform gyrus (Herrington et al., 2011), which appear to be altered in ASD (Kleinhans et al., 2008). In line with our findings of reduced sensitivity to emotional face expressions, adults with autism have reduced or absent activation of the FFA during explicit emotion processing, and the left amygdala and cerebellum during implicit emotion processing (Critchley et al., 2000), and use alternative individual specific brain regions (Pierce et al., 2001).
Finally, and similarly to ADHD, there has been a vast increase in interest in connectivity models of ASD, which show an alteration of transmission of information within the brain (Vissers et al., 2011). Reduced connectivity is shown children with autism as young as 1-3 years old (Dinstein et al., 2011) and altered complexity in infant siblings of ASD probands (Bosl et al., 2011). This is a future step for brain research in ASD using EEG connectivity measures (Bosl et al., 2011, Smit et al., 2008) and measurement of VLF activity (see Chapter 3), and may provide a neurobiological framework for the varied cognitive deficits observed.

9.4 Implications for models of comorbidity - revisited

9.4.1 Models of comorbidity
Above I have summarized the implications for the single disorders, but importantly the findings support several double dissociations between ASD and ADHD based on the profile of their cognitive-electrophysiological correlates, with the major findings summarized below in Figure 9-1. This suggests the presence of unique brain-behaviour pathways in the disorders that may aid in the identification of genetic and biological underpinnings, such as those outlined above, and may inform previous limited and inconsistent associations between intermediate phenotype and gene (e.g. Kebir et al., 2009). Taken together, the findings from Part II suggest that in their pure forms the disorders can be dissociated using cognitive-electrophysiology, but when the comorbid condition is considered the manifestations converge at the pathophysiological level.
As reviewed in Chapter 1, the majority of quantitative genetic studies suggest the behavioural overlap between ASD and ADHD is largely attributable to genetic influences. Beyond this, however, examining the profile of correlates in the comorbid ASD+ADHD group relative to the pure disorders points toward the mode of aetiology between the two disorders. The majority of findings suggest an additive model of comorbidity (see Figure 9-1), as the comorbid disorder displays the unique deficits of both disorders. A proportion of the impairments are most severe in the comorbid group compared to the pure conditions, suggesting observed deficits in pure groups may depend upon the presence of the comorbid condition (Banaschewski et al. 2007). These findings are in line with additional intermediate phenotype criteria: that numerous candidate intermediate phenotypes are associated with a given disorder, and that disorders that are genetically related (observed for ASD+ADHD) also share intermediate phenotypes (Cannon & Keller 2006). This suggests that the comparison of these overlapping conditions may aid in the selection of intermediate phenotypes for subsequent genetic analyses. Likewise, specific genetic or environmental influences on one disorder can be investigated as potential risk factors for the other disorder (Rhee et al., 2008).

**Figure 9-1:** Dissociations between ASD and ADHD in this thesis and evidence for an additive model of comorbidity.

*Note:* Deficits or abnormalities indicated from findings in this thesis are shown in bold text.
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It is important to note that dissociated cognitive-electrophysiological processes may rely partly on the same neuronal circuits (such as amygdala function and serotonergic transmission in emotion processing), and similar abnormalities may rely on differing neuronal structures in pure, comorbid and control groups. To date, there have been no structural or functional imaging studies of comorbid ASD+ADHD children in comparison to the pure disorders, and it is also important that future work investigates the sources or components underlying these ERP/EEG markers that may reveal further insight. In addition, the coefficient of variation (an index of response variability), the CNV component (an ERP index of response preparation) and N170 latency to angry faces showed evidence for non-additive effects. Closer inspection reveals CV in the comorbid group was more similar to ASD-only, suggesting this may be an ADHD-specific effect. Comorbid children showed reduced amplitude of the CNV and overall descriptive results suggest this group is a distinct condition with a unique impairment or abnormality in this domain. These findings are in line with the comorbid condition as a separate nosological entity, and therefore several models of comorbidity are likely to apply. Furthermore, it is possible that an additive interpretation can be the result of the comorbid condition as less impaired than the pure groups (or vice versa). An example of this was presented in Chapter 5 for relative delta power, whereby children with pure ADHD demonstrate reduced delta whereas children with pure ASD demonstrate increased delta, yielding a small effect in ASD+ADHD as the potential result of additive impairments. It is important therefore to look at the pattern of findings in detail to uncover the potential mechanism of the effect.

9.4.2 Aetiology

Elucidating the model of comorbidity using intermediate phenotypes that may lie between genes and behaviour holds implications for its aetiological basis and the potential role of pleiotropic genes (where one gene influences multiple phenotypic traits). The following figure illustrates various models describing the potential interplay between intermediate phenotypes, (pleiotropic) susceptibility genes and ASD, ADHD and ASD+ADHD (Figure 9-2).
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Model 1a: Endophenotype mediates between pleiotropic genes and both ASD and ADHD

Model 1b: Endophenotype mediates between genes and comorbid ASD+ADHD

Model 2: ASD and ADHD are different manifestations of the same overarching disorder

Model 3: Endophenotype is related to a different set of genes for ASD and ADHD

Model 4a: Endophenotype is an epiphenomenon related to similar genes as ASD and ADHD

Model 4b: Endophenotype is an epiphenomenon related to separate genes for ASD and ADHD

Model 5: Endophenotype is artificial and explained by a third, unknown/unmeasured variable

Model 6: Endophenotype mediates between environmental factors and ASD and ADHD

Model 7: Endophenotype is a consequence of the disease or state

Model 8: Endophenotype is related to a different set of genes than ASD and ADHD
Briefly Models 1a and 1b refer to an additive model, the difference being that the comorbid group also displays unique deficits in 1b, in which pleiotropic genes are likely to play a role. In model 2 ADHD is a less impaired subtype of ASD, in which pleiotropic genetic factors may be a subset of those involved in ASD, or shared with ASD+ADHD. In model 4a, although the intermediate phenotype may not mediate between gene and behaviour but can still be useful for identification of pleiotropic genes. Similarly, although the relationship between gene and behaviour is explained by an unknown variable in Model 5, indirect genetic effects could be identified. The following models, however, limit the detection of pleiotropic genes as such, whereby the intermediate phenotype is related to different aetiologies for ASD and ADHD (model 3) epiphenomenon that does not mediate between gene and behaviour (Model 4b), mediates between environmental factors and the phenotype (Model 6), is a consequence of the disease (Model 7) or is related to different genes than the phenotype (Model 8; see Rommelse et al. 2011 for further detail).

Based on the findings in this thesis, it can be tentatively suggested that model 1 is at play, as the comorbid group show similar impairments to the pure groups. In this model, there are certain intermediate phenotypes that are unique to the pure disorders (ASD-only and ADHD-only) and intermediate phenotypes that mediate between susceptibility genes and both ASD and ADHD. Nevertheless, the presence of altered preparatory processes in ASD+ADHD suggests that this abnormality may mediate uniquely between susceptibility genes and ASD+ADHD, or alternatively that the intermediate phenotype does not mediate between genes and behavior (among other models; see Rommelse et al. 2011). In addition, these models do not visually support a double dissociation of ASD and ADHD which is suggested in this thesis at the neurophysiological level.

In order to elucidate the aetiological basis of the comorbidity, the next fundamental step, therefore, is to conduct a study within a genetically sensitive design to establish whether these impairments are familial/genetic within or across disorders (Neale and Kendler, 1995). It would be possible to test a number of hypotheses. It would be interesting to replicate previous
research supporting the broader autism phenotype and the equivalent of this in ADHD, by examining whether cognitive/brain markers identified as specific in this thesis are shown to a lesser extent in their siblings. Further to this statistical analyses could be performed to quantify the familiality of the measures (i.e. it would be hypothesised that these individuals are significantly different from controls but not from their proband siblings). In addition, this design can further our understanding of the comorbid condition in a number of ways. Firstly, we can see whether cognitive/brain markers associated with both ASD and ADHD are found in the siblings of comorbid children. Secondly, it is possible to conduct a cross-sibling cross-trait analysis whereby a correlation is calculated between the cognitive/brain marker in the proband and the ASD or ADHD traits in the sibling (or vice versa; see Rommelse et al. 2009 for a description of this design). This will reveal more about the familial (and based on twin studies most likely genetic) overlap between ASD and ADHD, and importantly between intermediate phenotypes of ASD (e.g. reduced alpha power) and ADHD symptoms, and vice versa, beyond phenotypic correlations.

Further analyses are required to examine whether the intermediate phenotype actually mediates between genes and behavior (see Section 1.3.1) and subsequently to conduct molecular genetic analyses. Following, this it will become possible to falsify certain models of this interplay between the disorders (see Rommelse et al. 2011 for further detail). Doing so will also provide insight into whether comorbidity between ASD and ADHD is due to the disorders sharing the same risk factor/s, or that the risk factor/s themselves are correlated (Taurines et al. 2012; Caron & Rutter, 1991), particularly as at the brain level the disorders appear dissociated.

These propositions have implications for the concept of a general genes hypothesis for developmental disorders, in which the same genes are responsible for one deficit in a disorder influence other deficits in that disorder, quantitative trait measures of the disorder, and other disorders (Plomin and Kovas, 2005). In addition, it is likely that these ‘generalist genes’ affect multiple brain structures and functions, which each affect one or multiple cognitive processes (Kovas and Plomin, 2006). In line with this, some authors propose to examine comorbid conditions as a general risk factor; using a sample of multigenerational families ascertained for high levels of comorbid conditions, Jain et al. (2007) reported that assuming a broader phenotype that incorporated ADHD plus ODD, CD, alcohol abuse/dependence and nicotine dependence provided additional power in linkage analysis and association with previously reported and novel candidate genes. The authors conclude from this that refining the ADHD
An additional consideration is differing developmental trajectories and compensatory processes in the examination of aetiology. For example, we propose that the enhanced CNV in children with ASD-only may reflect a compensatory strategy considering their successful task performance, and development may bolster compensatory strategies but not necessarily typical processing. Likewise, in ADHD longitudinal studies report a normalization of the NoGo-P3 with age indicating maturational delay, whereas the Cue-P3 appears to be attenuated throughout adolescence, suggesting a developmental deviation model (Doehnert et al., 2010), although case-control differences were not shown in adults (Doehnert et al., 2012). One hypothesis put forward in recent years is that ADHD is associated with enduring subcortical dysfunction, but recovery through development is through improvements in executive (cortical) control (Halperin et al., 2008). Moreover, there were differences in the association between arousal levels and task performance across the groups. It is proposed that the interaction between these develops to inhibit over-stimulation and facilitate interplay between cortical systems. Over time the individual is better able to tolerate more intense and varied stimulation, and alter activation on the basis of the relative valence of the environment, and this development in turn is shaped by early experiences (Mayes, 2000). Investigation of developmental trajectories will reveal more about the potentially differing relationship between arousal and function in these groups of children. As ASD is generally diagnosed and observable earlier in life, studies of the temporal ordering of these disorders (i.e. early manifestations of the same disorder or the presence of one disorder increasing risk for another; Caron & Rutter, 1991) and their associated pathophysiology are required to inform our understanding, as reciprocal relationships are likely to change over time (Angold et al., 1999). Such studies will ultimately allow the formation of more homogenous groups based on emergence and persistence of key behavior and brain markers, and provide insight into whether the intermediate phenotype mediates between gene and behavior, or is an epiphenomenon (Rommelse et al., 2011).

Beyond this, the findings also demonstrate the utility of a dimensional approach when assessing underlying aetiology of ASD+ADHD. Categorical diagnoses, although useful for clinical practice, may underemphasise valuable clinical information and possible genetic differences (Brown and Barlow, 2005). The dimensional approach reflects the variation in behavior and
intermediate phenotypes, and future work examining the aetiology of these conditions should incorporate both measures concurrently (see also Section 9.5.4).

9.5 Limitations and future considerations

Limitations that were specific to the design or analyses of each study were discussed at the end of each empirical chapter. Here I focus on limitations and reflections that are more general and recurred throughout the thesis.

9.5.1 Task recording and analysis

The analysis performed in this thesis used basic analysis techniques, the Fast-Fourier Transform and the ERP method. More advanced techniques in tasks of varying difficulty are likely to reveal more about the overlap in pathophysiological mechanisms. For example, several findings were dependent on topographical location, and therefore further work examining the sources of the scalp-recorded activity would be informative. It should also be noted that Chapter 3 reports elevated absolute theta power is associated with ADHD symptoms, whereas elevated relative theta power is associated with clinical ADHD in Chapter 4. This is in line with studies that have suggested that relative power is more sensitive in ADHD populations (Barry et al., 2003a), and future work should incorporate both measures.

Resting state EEG was analysed at the beginning of the testing session in Chapters 4 and 5, following the novel experience of preparation for EEG data acquisition. Children may therefore be more anxious and less relaxed, which could be reflected in the findings. It is important that future work analyses resting state EEG after task performance, towards the end of the testing session and compares the findings. In addition, this analysis will provide further information on changes in arousal and activation in the disorders, especially if combined with psychophysiological measures such as skin conductance response (Vaez-Mousavi et al. 2007).

There were no task performance parameters for the social cognition tasks, although participants responded to target stimuli either by counting the number of occurrences of certain stimuli or with a mouse-click to ensure they were attending to the stimuli, which has been used in several previous studies. The lack of behavioural data, however, renders it difficult to make firm conclusions regarding the social nature of the tasks and the ERP components they evoke, and interpretation is based instead on previous empirical work. For example, a behavioural measure may provide further insight on the nature of the reduced face inversion effect in ADHD. The identification of aberrant covert processing of social stimuli
supports the use of ERP measures, but ideally concurrent behavioural performance should be measured. Moreover, additional cognitive tasks could be administered, such as emotion recognition or a measure of social functioning/aptitude beyond the SCQ, would be useful to assess the overt social cognitive ability of the individuals and to correlate with neural measures.

9.5.2 Sample characteristics

9.5.2.1 Population versus clinical sample
In the first part of this thesis ratings based on parent-rated ADHD rating scales in a population sample were used to investigate the aetiological overlap between ADHD symptoms and EEG parameters. The second part of this study used a clinical sample of ADHD (and ASD and ASD+ADHD), and benefited from the use of gold-standard diagnostic tools: the ADI-R, ADOS and PACS. I have drawn comparisons between these samples that were carried out using different measurements at similar ages and thus these two samples investigate the ADHD phenotype at different levels. However, several lines of research suggest that ADHD can be conceptualized as the extreme end of a continuous distribution (Chen et al., 2008, Levy et al., 1997). A notable observation is that our findings for theta power are largely consistent across a population and a clinical sample (measured using the same task and EEG recording/analysis), and EEG and ERP measures showed association with quantitative trait measures of ADHD and ASD, which supports these propositions. This goes beyond previous findings in clinical samples by considering the wide variation in behaviour, and offers secondary support for the dissociation of ASD and ADHD. Future work should investigate aetiological overlap with the executive function and social cognition measures in a twin sample. Although dimensional approaches may help to quantify risk, it does not directly lend itself to identifying distinct aetiologies and unique genotypes, so categorical and dimensional approaches should be used as complementary approaches to psychiatric genetics.

9.5.2.2 Strengths and limitations of the clinical sample
A further conclusion from these findings is the importance of strictly defined groups when conducting investigations into the underlying aetiology of these complex psychiatric disorders. It is clear that misspecification in group allocation further ‘blurs’ diagnostic boundaries and therefore increases phenotypic heterogeneity, which can lead to inconsistent findings and therefore ambiguous conclusions. The rigorous assessments and stringent criteria employed in the recruitment of the BioNeD sample reduce the likelihood of misspecification in group allocation and other confounding factors. Many previous cognitive-electrophysiological studies
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of ASD and ADHD either do not include a comparison group of the other disorder or do not form a separate comorbid group. Not only does this introduce ‘blurring’ of diagnostic groups, but limits any conclusions being made about the nature of deficits in the comorbid group. Thus the participants included in the second part of this thesis can be considered as a ‘pure’ sample.

As described in Chapter 4 and Appendix B, the BioNeD team experienced challenges in acquiring this sample which resulted in a large discrepancy between clinical diagnosis and research diagnosis, and the possibility that these individuals are unrepresentative of individuals with ASD and ADHD seen at the clinic and incomparable with research studies of ASD and ADHD. The stringent exclusion criteria also resulted in a small sample size that may limit firm conclusions. It may be possible that with a larger sample greater overlap between the pure disorders is observed by removing Type II error. In addition, as is the case with clinic-based recruitment, there is the possibility of bias in families that agreed to take part and therefore reduced generalisability of the findings.

Efforts to limit the heterogeneity of the sample were taken in including males only. For the SEND subset of BioNeD I also chose to limit the age range between 8 and 13 years (compared to 7-16 years in the full BioNeD study) to reduce age effects, and the four groups were age-matched at group-level. The inclusion of age as a covariate in Chapter 6 and Chapter 7 was due to theoretical considerations and findings were upheld. Despite the wider range in IQ scores, there was no evidence of associations between these measures and cognitive-electrophysiological parameters. In the case of the emotion task, this is quite surprising; for example, some children with autism tend to pass emotion recognition tasks when matched by verbal IQ to the control group (Loveland et al., 1997). Nevertheless, the passive nature of the task removes reliance on intellectual (particularly verbal) ability, and retaining variance in IQ in analyses ensures meaningful group differences are not removed. For example, there is a moderate degree of genetic overlap between IQ and ADHD (Kuntsi et al., 2004). In addition, the IQ scores are higher than typical reports, which may reflect the highly selected nature of the sample. Potential similarities in the questionnaires, and the moderate correlation between these ADHD and autism trait questionnaires were controlled for in all dimensional analyses, ensuring minimal confounding effects.

9.5.2.3 Clinical heterogeneity

An important consideration is the phenotypic heterogeneity of the disorders. This thesis investigated cognitive-electrophysiology in the combined subtype of ADHD. In Part I, twin pairs were selected on the basis of consistently high or low ADHD symptom scores across both
inattentive and hyperactive-impulsive domains. In Part II, only participants that had been clinically diagnosed with the combined type were included. However, following research diagnostic assessments a proportion of these cases did not meet criteria and therefore may have presented with predominantly inattentive subtype (33%) or predominantly hyperactive-impulsive subtype (17%), although the small sample size did not facilitate further delineation of the sample. The use of subtypes may improve correspondence between phenotype and genotype; analyses have indicated that the subtypes show only partial genetic overlap (Greven et al., 2011, McLoughlin et al., 2011b, McLoughlin et al., 2007), and these subtypes appear to display some unique EEG characteristics (Clarke et al., 1998). Likewise, we were unable to decompose our ASD sample into different subtypes (e.g. HFA, AS, PDD-NOS) due to the small sample size. Such heterogeneity may have influenced the results. For example, differences in emotion processing are reported between the subtypes (see Harms et al. 2010 for a review). In addition, as outlined in Chapter 1, different rates of ASD are found in different ADHD subtypes and vice versa. It may therefore be possible that different subtypes exist within and across disorders, such as a subtype of ADHD combined with atypical autism (Mandy et al., 2011), and these subtypes are associated with differing cognitive-electrophysiological correlates.

Importantly, such within-group heterogeneity may decrease the detection of case-control differences (increasing Type II errors. For example, ADHD subtypes appear to display some unique EEG characteristics, as individuals with the inattentive subtype appear closer to the typical QEEG profile (Clarke et al., 1998). The lack of ADHD-control differences in beta activity in this thesis may reflect heterogeneity, and differences in the distribution of ADHD subtypes in the research diagnostic groups of ADHD and ASD+ADHD may enhance differences between these groups. Similarly, recent work suggests that difficulties in emotion processing in autism may be attributable to comorbid alexithymic symptoms, referring to impairments in recognising and describing emotions (Cook et al., in press). This could explain a lack of impairment to specific emotions in ASD. This clinical heterogeneity could be seen in our control participants as a proportion demonstrated high ADHD symptom scores. While tests were conducted to ensure that the control participants that scored highly for ADHD symptoms did not differ on EEG/ERP parameters of interest from other control participants, there is still a possibility that the inclusion of these participants prevents the detection of true differences, and makes interpretations between TD and ASD participants particularly problematic. Investigation of these sources of heterogeneity would be an important direction for future research, although the use of categorical and dimensional measures of the disorders may have partially addressed this question.
It is also important to realize the frequency of comorbidity with other disorders. Comorbid ASD+ADHD is likely to be associated with a higher frequency of other comorbid psychiatric conditions, and high rates of ASD and ADHD are seen in several other disorders (Joshi et al., 2010, Taurines et al., 2010b). Increased autistic traits in children with ADHD have been associated with the presence of other comorbid disorders such as ODD and CD (Mulligan et al., 2009), which themselves are associated with reduced inhibitory processing in the CPT-OX (Banaschewski et al., 2004). Similarly, one study reports as many as 95% of children with ASD also have 3 or more other comorbid disorders and 74% have 5 or more comorbid disorders (Joshi et al., 2010) and a particularly frequent co-occurrence is anxiety disorder (de Bruin et al., 2007, Joshi et al., 2010, Simonoff et al., 2008). An important step for elucidating the role of social cognition in ADHD and how it may differ from ASD would be to assess anxiety levels or internalizing traits across disorders. Symptoms of anxiety and mood are strongly linked to emotional processing impairments (Etkin and Wager, 2007, Leppänen, 2006), and many individuals with comorbid ASD+ADHD are likely to have an emotional disorder as well (Gjevik et al., 2010). In addition, in the same emotion task used in Chapter 7, shyness (which can predate social anxiety disorder) was associated with smaller N400 amplitude to anger and neutral expressions (Battaglia et al., 2005). Taken together, these factors make the delineation of the phenotype more difficult. Other comorbid psychiatric disorders were an exclusion criterion, but there may be sub-threshold symptoms that affect neural correlates and beyond this, the potential presence of similarities in cognitive-electrophysiological parameters in these psychiatric groups requires investigation. It is likely that drawing upon trait, cognitive, neurophysiological and genetic measures will help to identify more homogenous groups.

9.5.3 Twin analyses

Chapters 2 and 3 analyzed data from the NEAAT twin sample, which relies on the basic assumptions of the twin design. The main assumption of the twin study is the ‘equal environments assumption’, which presumes that both MZ and DZ twin pairs share the same environments, and as such greater concordance rates for MZ compared to DZ twins would indicate greater genetic influences on the trait of interest. However, if MZ twin pairs experience more similarity in their environments, such as a similar peer group, this could inflate the heritability estimate (Neale and Cardon, 1992). The analyses also assume absence of gene-environment correlation or interaction and assortative mating, which refers to the greater likelihood of individuals mating when they have similar genotypes and/or genotypes. The main limitation of the twin studies reported in this thesis relate to statistical power. Only two MZ twin pairs that were discordant for ADHD symptoms were identified. The relatively
small sample size limited the detection of non-additive genetic and shared environmental influences, as noted in the empirical chapters. Nevertheless, the selected sample design increases power, since studying relatively rare disorders in population-based twin samples will result in the majority of twin pairs being concordant for low scores or unaffected.

**9.5.4 Intermediate phenotypes**

Cognitive-electrophysiological measures that share genetic influences with ADHD may reflect the multiple outcomes of the genes involved, rather than necessarily representing processes that mediate between genes and ADHD behaviours. Tests of mediation versus pleiotropy (or ‘liability-index’) can be used to specifically infer the causal role of a neurobiological process. One other approach would be to test for co-variation of ADHD and neurobiological measures during the treatment response (Kendler and Neale, 2010); however, it is not yet clear whether factors such as course or treatment response are more closely related to ADHD per se or to a particular endophenotype. In addition, the identification of moderate to large genetic correlations between ADHD symptoms and electrophysiological parameters does not necessarily represent common genes influencing these two traits. For example, the relationship between gene and behaviour might be explained by an unknown/unmeasured variable other than the intermediate phenotype (Rommelse et al., 2011).

The validity and reliability of several measures is not certain, and therefore it is difficult to conclude they are more objective and therefore less complex than the behavior itself for genetic applications (Bearden and Freimer, 2006, Flint and Munafo, 2007). For example, non-shared environmental influences on VLF activity were high which may indicate the presence of measurement error. As such, substantial sample sizes may still be required to detect susceptibility genes. Accordingly, one of the next steps with this work is to investigate associations with genetic variants. Another potential direction is the use of multiple correlated intermediate phenotypes (such as that shown in the correlations in Chapter 5; see Section 5.4.3) in univariate or multivariate designs that will improve the reliability of these measures (Kendler and Neale, 2010, Rommelse et al., 2009a). Twin studies can help reduce data by identifying parameters that are highly genetically correlated despite a low phenotypic correlation, in order to be used in multivariate intermediate phenotype analyses (Wood and Neale, 2010). In addition, the influence of gene-environmental interplay should not be underestimated, considering its role in both ASD and ADHD.

Despite these shortcomings, the intermediate phenotype approach is likely to further our understanding of the pathophysiology underlying complex psychiatric disorders, by providing...
further insight into shared and distinct risk factors, and subsequently more specific treatment strategies, further discussed in the following final section.

9.6 Translational implications

9.6.1 Diagnosis and assessment

Beyond theoretical and research implications, this thesis presents notable clinical implications. There are two broad findings that offer implications for clinical practice: 1) ADHD and ASD can be dissociated at the neural level and 2) comorbid ASD+ADHD shows the unique impairments of the pure disorders. This provides an improvement in knowledge regarding the classification of these disorders, as it suggests that the definitions of ADHD and ASD are suitable (Robins and Guze, 1970). The finding that ASD+ADHD presents with the unique deficits of both disorders suggests it cannot be assumed that the correlates and aetiology of ASD are the same regardless of the presence or absence of ADHD, and vice versa (Caron and Rutter, 1991).

Secondly, it supports the importance and prevalence of co-occurring ASD and ADHD. In the SEND subset of the BioNeD sample, 47% (16/34) of participants clinically diagnosed with ASD met research diagnostic criteria for ADHD and 14% (3/21) of ADHD met criteria for ASD and were reallocated to the comorbid group. Of the children clinically diagnosed with ASD+ADHD, 91% (10/11) retained their diagnosis following research assessments. These observations alone support the inclusion of a comorbid diagnosis of ASD and ADHD in the upcoming DSM-V, which is further strengthened by findings across arousal, executive function and social cognition domains that demonstrate the comorbid condition shares the unique deficits of the pure disorders. The awareness of comorbid ASD+ADHD is particularly important, as several studies demonstrate greater impairment in children with both disorders (Goldstein and Schwebach, 2004, Joshi et al., 2010), greater use of medication and medication in combination with psychotherapy (Frazier et al., 2001), and higher rates of medical and psychiatric comorbidity in children (Holtmann et al., 2007, Joshi et al., 2010). In addition, children with ASD and co-occurring ADHD increase public health expenditure significantly, based on US medical insurance data (Peacock et al., 2012). The studies presented suggest the comorbid condition is not artefactual, and warrants the fostering of cross-talk between clinicians and researchers. Furthering our understanding of the brain basis of this overlap can help to resolve some of the controversies apparent over diagnosis, treatment and comorbidity in these disorders.

Beyond these more conceptual nosological issues, these findings may aid in the diagnosis and assessment of these complex cases. For example, findings of emotional processing dysfunction
that is specific to ADHD highlights the importance of studying social cognition in ADHD, which may provide answers for the social dysfunction often observed in these individuals. In addition, there is potential for formation of diagnostic subgroups based on their *endophenotype* status (e.g. reduced alpha power) rather than *phenotype* status (i.e. ASD or ADHD). It is possible that different subtypes exist within and across disorders and these subtypes are associated with differing cognitive-electrophysiological correlates. Identification of endophenotypic subtypes is likely to reduce heterogeneity and therefore the identification of subtypes with certain genetic/pathophysiological profiles. Subgroups could be formed on the presence or absence of: 1) phenotypic qualities, such as alexithymia; 2) performance-based markers, such as response variability; and/or 3) biological-based markers, such as reduced alpha activity. For example, previous work has demonstrated a subgroup of children with ADHD display increased beta activity, rather than the more consistent and theoretically hypothesised finding of reduced beta activity (Clarke *et al.* 2001). In particular, these pathophysiological markers are of interest in the Research Domain Criteria (RDoC) project, the aim of which is for mental disorder classification systems to be informed by genomics and neuroscience and better aligned with scientific findings such as those emerging from this thesis (Insel *et al.*, 2010). While a biological or brain-based diagnostic tool may be a long way off, these assessments are likely to complement the diagnosis and target treatment plans for these more homogenous clinical subgroups, discussed in the following section.

### 9.6.2 Treatment possibilities

Treating a combination of ASD and ADHD is likely to be more challenging than treating the single disorders, with potential differences in developmental trajectories and prognosis (Achenbach, 1995). There are several levels of treatment that can be applied: pharmacological, brain-based and cognitive/behavior-based.

A review of treatments for autism concluded that early behavioural interventions were the most useful (Howlin *et al.*, 2009). Conversely, the most effective treatment for ADHD is stimulant medication which is consistently shown to improve core symptoms more than non-pharmacological treatment (Meijer, 2009 #1260, Makrygianni and Reed, 2010). In addition, methylphenidate (MPH) appears to normalize neural correlates of ADHD reported in this thesis. In a Go/No-Go task, MPH enhanced the amplitude of the N2 and P3 components in adolescents with ADHD (Groom *et al.* 2010). Interestingly, motivational incentives also increase the amplitude of these components, an effect that appears additive to that of MPH (Groom *et al.* 2010). Similarly, MPH administration in ADHD enhances the amplitude of the
N170 component during emotional face processing (Williams et al. 2008) and in our own work, VLF activity has been shown to normalize with MPH medication in adults with ADHD (Cooper et al., in prep). Examining treatment effects on ERP markers of ASD and ASD+ADHD are required, particularly as children with ASD+ADHD appear to respond well to stimulant medication (Santosh et al., 2006). Similarly, in another study MPH reduced ADHD symptom scores on the Conners by 50% while not affecting ASD symptom scores (Handen et al., 2000). The NICE guidelines for ADHD propose that the administration of treatment for these cases should be assessed with care, due to the increased risk of medical problems and in some cases lack of communication skills, which would require the assistance of a carer. In addition, little is known about the effect of these medications on restricted and repetitive behaviours and interests (Atkinson & Hollis, 2008). As such further randomized control trials in autism are required to assess and tailor individual requirements.

More recently, the use of drug-free treatments has been advocated. For example, neurofeedback (NF) uses operant conditioning to train patients to enhance poorly regulated EEG patterns. Several studies document the efficacy of NF for ADHD. For example, a decrease in theta activity is reported that was associated with improvements in ADHD symptom scores (Gevensleben et al., 2009). A meta-analysis concluded that NF treatment for ADHD can be considered at the top level as “Efficacious and Specific”, shown through large improvements in inattention and impulsivity, and to a lesser extent hyperactivity (Arns et al., 2009). The stability of these improvements has been demonstrated even 2 years after the initial treatment (Gani et al., 2008). Based on the success of NF for individuals with ADHD, clinicians are evaluating it as a viable treatment for ASD. In the largest controlled group study to date, NF gave an 89% success rate based on parental judgement of outcome and reduced ASD symptoms by 40%, which itself was associated with reduced hyperconnectivity in the brain (Coben and Padolsky, 2007). Moreover, improvements in executive function and social cognition were maintained over a 12-month period (Kouijzer et al., 2009a, Kouijzer et al., 2009b). Nevertheless a recent review judged the treatment as not effective in ASD, whereby the effects seen relate to an improvement in comorbid ADHD symptoms (Holtmann et al., 2011). The findings in this thesis may provide more precise training, and it is likely that applying individual-specific NF to comorbid individuals will improve symptoms of both disorders.

It may also be helpful to utilize cognitive findings. For example, the observation that children with ADHD demonstrate reduced contextual processing of emotional face expressions that is related to executive dysfunction may support the use of cognitive-behavioural therapy that
enhances emotional processing from a young age, such as development of the ability to stop, look and anticipate consequences (Marsh & Williams, 2006; Uekermann et al. 2010). Poor social skills in ADHD impact on several areas of functioning and persist into adulthood at which point ADHD is associated with comorbid substance abuse disorders anxiety. Likewise, theories propose that autism symptoms develop due to a ‘social deficit’ (see above), with roots in developmental theory; for example, the interactive specialization theory argues that brain development involves specialisation of regions such that they are initially broadly tuned, and becomes increasingly fine-tuned to certain stimuli, through determination of selectivity to certain stimuli based on exposure (Johnson, 2001). Accordingly, for both disorders, ‘the earlier the better’ is the common suggestion for effective treatment strategies. A recent novel study that applied the eye-tracking technique to train attentional control in typical infants used gaze-target contingency to reinforce task performance, and discovered improvement in various aspects of executive function, including disengaging attention (a feature characteristic of ASD), that persisted three days after the final training session and were significantly improved compared to a control group who merely observed videos (Wass et al., 2011). The next step is to apply this technique in populations “at-risk” for developing ASD or ADHD, such as those with an older sibling diagnosed with the disorder. This methodology has been applied to ASD, and recent findings show, for example, that reduced sensitivity to gaze direction as measured by ERPs is predictive of subsequent ASD diagnosis (Elsabbagh et al. 2009; 2012). These “brain-training” techniques could be modified in different ways to tackle problems unique to the disorders, such as reinforcing increased attention to the eye region of the face in ASD. Early intervention studies will also allow tests of causality between the various deficits reported in this thesis and help to develop individual-specific treatment strategies for complex comorbid cases. Drawing upon the shared risk factors could reduce the prevalence. It is likely that a combination of these approaches will prove useful in the treatment of the disorders. Ultimately, the study of psychiatric disorders alongside each other will help to identify novel biological targets and subsequent development of new and more effective treatments.

9.7 Concluding remarks

The contributions from this thesis emerge from an interdisciplinary approach combining quantitative genetic and cognitive-electrophysiological research in population and clinical samples. The identification of cognitive-electrophysiological parameters that are specific to ASD and ADHD, heritable and share genetic overlap with the disorders, provides the first step towards improved measures for use in genetic research and clinical assessment. In addition,
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despite these dissociations between them, both disorders are associated with multiple widespread impairments across arousal, executive function and social cognition processes. Consequently, the concept of multiple deficits arising from multiple pathophysiological pathways, and how their interaction with each other may differ between psychiatric disorders, requires further investigation using multi-level approaches. Future research incorporating genetically sensitive designs and molecular genetic approaches across all three conditions with larger sample sizes will move the field forward, providing further understanding of the similarities and differences underlying the high rates of co-occurrence between ASD and ADHD. Importantly, elucidating the basis of comorbid ASD+ADHD as an additive condition with the unique deficits of both disorders is likely to yield significant scientific and clinical benefit, in terms of classification and treatment possibilities.
References


APA (1968). *Diagnostic and Statistical Manual of Mental Disorder (2nd ed.)*. Washington, DC.


References


Christakou, A., Murphy, C. M., Chantiluke, K., Cubillo, A. I., Smith, A. B., Giampietro, V., et al. (in press). Disorder-specific functional abnormalities during sustained attention in youth with Attention Deficit Hyperactivity Disorder (ADHD) and with Autism. *Molecular Psychiatry*.


References


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References


References


Gizer, I. & Waldman, I. (in press). Double Dissociation Between Lab Measures of Inattention and Impulsivity and the Dopamine Transporter Gene (DAT1) and Dopamine D4 Receptor Gene (DRD4). *Journal of Abnormal Psychology*.


References


References


References


References


References


279


References


References


References


Shi, T., Li, X., Song, J., Zhao, N., Sun, C., Xia, W., et al. (in press). EEG characteristics and visual cognitive function of children with attention deficit hyperactivity disorder (ADHD). *Brain and Development*.


References


References


References


