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Introduction
Chapter 1

This thesis focuses on neuropsychological dysfunctions that may mediate the relationship between genetic predisposition and disease symptoms in Attention-Deficit/Hyperactivity Disorder (ADHD). In the following sections, a more elaborate description is given of the clinical manifestation of ADHD and the genetic and environmental risk factors of ADHD. In addition, the neurobiological and neuropsychological deviations associated with ADHD are described. Thereafter, a theoretical framework is provided, combining the aspects above, that has guided the study design of the present thesis. Specific study aims will then be discussed as well as the relation of each chapter to these study aims.

Clinical Manifestation of ADHD

ADHD is an impairing neurodevelopmental disorder that affects around 4-5% of school-aged children (Buitelaar, 2002). It entails a core set of symptoms, namely symptoms of inattention, hyperactivity, and impulsivity. Inattention refers to forgetfulness, daydreaming, distractibility and difficulty sustaining attention. Hyperactivity encompasses restlessness, excessive talking, and fidgeting. Impulsivity is expressed as blunting out answers before the question has been completed, difficulty awaiting turn, and frequently interrupting others (American Psychiatric Association, 1994). Although most patients portray symptoms in all three domains, about 20% of patients do not (Buitelaar, 2002), and on this basis a distinction is made in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) between a predominantly inattentive subtype, predominantly hyperactive/impulsive subtype, and a combined subtype (American Psychiatric Association, 1994). Symptoms of inattention, hyperactivity, and impulsivity are already present before age seven, are persistent, and more severe than typically observed in individuals of the same age. As a consequence of these symptoms, patients are impaired in their social, academic, and/or occupational functioning.

The clinical manifestation of ADHD may be influenced by a variety of factors, including gender, age, comorbid disorders, and intelligence. ADHD affects more boys than girls (Barkley, 1990), with estimated ratios varying between 3:1 and 9:1 (Arnold, 1996; Gaub & Carlson, 1997). This appears largely due to genuine gender differences: In general, boys are more susceptible than girls for developing externalizing disorders (disorders characterised by problem behavior that is manifested outwards and, therefore, noticeable and problematic for the environment) (Kramer et al., 2007). It appears that girls may require a higher number of risk factors than boys before developing ADHD (Rhee et al., 1999). However, referral bias also plays a role: Girls are
less likely to portray symptoms of hyperactivity/impulsivity and to suffer from additional
externalizing psychopathology. This may lead to girls being less disturbing at home and in
school and, in turn, may lead to an underestimation of ADHD in girls.

The symptoms of ADHD seem to decline somewhat with age (Faraone et al., 2000c,
2006; Willoughby, 2003), although structural brain abnormalities may persist in some patients
(Castellanos et al., 2002) but not in all (Shaw et al., 2007). Nevertheless, about two third of the
individuals diagnosed with ADHD as a child no longer meet criteria for ADHD as an adult
(Spencer et al., 1998) and the prevalence estimations of ADHD in adults (0.5-2%) is lower than
the prevalence estimation in childhood (4-5%) (Buitelaar, 2002). It has been suggested that
acquired brain mechanisms during development may be beneficial for overcoming ADHD
symptoms to a certain extent (Halperin & Schulz, 2006) and that ADHD may be seen as a
delayed, yet normal development (Satterfield & Braley, 1977).

ADHD is frequently associated with a range of other psychiatric and neurological
disorders. It is estimated that around 60-100% of children with ADHD also exhibit one or more
comorbid disorders (Gillberg et al., 2004) that often continue into adulthood (Biederman, 2004;
Kessler et al., 2006). Around 42-90% of children meet criteria for Oppositional Deviant Disorder
(ODD) and/or Conduct Disorder (CD) (Bauermeister et al., 2007; Cunningham & Boyle, 2002;
Gillberg et al., 2004; Jensen et al., 1997), disorders characterized by externalizing behavioral
problems, such as aggressive behavior, difficulty with authority (ODD) or lying, stealing, and
vandalism (CD). Furthermore, between 13-51% of ADHD patients suffer from internalizing
disorders, including anxiety and depression (Bauermeister et al., 2007; Gillberg et al., 2004;
Jensen et al., 1997). Currently, the DSM-IV rules out a diagnosis of autism in ADHD.
Nevertheless, a large percentage (65-80%) of children with ADHD portrays symptoms in the
autistic spectrum (Clark et al., 1999; Gillberg et al., 2004). Disorders outside the psychiatric
spectrum are frequently observed in patients with ADHD, such as dyslexia (25-40%), motor
coordination problems (50%), dyscalculia (10-60%), sleep disorders (25-50%), and enuresis
and/or encopresis (30%) (Bhatia et al., 1991; Gillberg et al., 2004; Owens, 2005; Willcutt et al.,
2005b). ADHD patients with comorbid problems compared to ADHD patients without comorbid
problems appear to have a more severe form of ADHD, are often more impaired in their daily
functioning, and have a poorer long term prognosis (Bauermeister et al., 2007; Biederman et al.,
1996; Conor et al., 2003; Gillberg et al., 2004).

A frequently reported finding is that children with ADHD have on average a lower IQ
than controls (Antshel et al., 2006; Frazier et al., 2004; Kuntsi et al., 2004). ADHD patients with
a lower intelligence seem to have a higher severity of ADHD symptoms, tend to be more
impaired in their daily functioning, and have a worse prognosis than ADHD patients with a normal to above normal intelligence (Aman, 1996).

The clinical manifestation of ADHD may be influenced by the gender, age, comorbid disorders, and intelligence of the patient. The observable (number and severity of) ADHD symptoms is called the ‘phenotype’ of a patient. Phenotype comes from the Greek phainein, meaning ‘to show’ (Almasy & Blangero, 2001) and may be defined as the observable, outward, physical manifestation. The phenotype of an individual determines if he/she eventually will or will not be diagnosed with ADHD according to the criteria such as those detailed in the DSM-IV.

Genetic and Environmental Risk Factors of ADHD

ADHD clusters in families (Faraone et al., 1995a), with siblings of an affected individual having a three to fivefold increased risk for developing the disorder (Biederman et al., 1992; Faraone et al., 1993a). The role of genetic factors appears more important than that of environmental factors in increasing the risk for ADHD (Derks et al., 2008; Faraone et al., 2005; Polderman et al., 2007a). Numerous twin and adoption studies have been conducted in order to disentangle the roles of genetic factors as well as shared environment and unique environment in determining ADHD. Estimations of the contributions of these factors vary, but around 76% of the variability between individuals in ADHD seems to be attributable to heritable factors (Faraone et al., 2005). These estimations are fairly constant across different ethnic cultures studied and indicate ADHD is largely related to a genetic predisposition for the disorder (Faraone et al., 2005). In the initial genetic models of ADHD, a single dominant gene was believed to be important (Faraone, 2000c; Smalley 1997). Later genetic models entailed multiple genes acting in an additive manner, each with a small individual effect (Brookes et al., 2006; Faraone et al., 2001). These susceptibility genes may result only in the full disorder if a minimum number or certain combination of susceptibility genes is present. Linkage studies using affected sibling-pairs (both siblings have ADHD) have been conducted to identify chromosomal regions that are more frequently shared by affected siblings than the average frequency of sharing loci by siblings, hence possibly conferring ADHD risk genes. Such studies have identified several candidate chromosomal regions that may be related to ADHD, although little consistency exists between studies and no genes have been identified in these regions that may account for the linkage signals (Elia & Devoto, 2007). Candidate gene studies have been conducted, aimed to test for an association
between biologically plausible candidate risk genes and ADHD. These studies have mainly focused upon genes involved in the regulation of catecholamine and other neurotransmitter pathways, in which genes involved in the regulation of dopamine have received the most attention, since the dopaminergic systems seem to play an important role in the etiology of ADHD (Jucäite et al., 2005; Spencer et al., 2007). For example, association between ADHD and the dopamine transporter gene (DAT1 / SLC6A3), two dopamine receptor genes (DRD4 and DRD5), and a mitochondrial enzyme that regulates dopaminergic signals in the pre-synaptic region (MAOA) has been reported (Brookes et al., 2006; Faraone & Khan, 2006; Kuntsi et al., 2006a). In addition, associations have been reported between the serotonin transporter gene (5-HTT / SERT / SLC6A4), serotonin receptor gene (HTR1B), and other genes that regulate neurotransmission (e.g. COMT, SNAP-25). For an overview we refer to Brookes et al. (2006) and Faraone and Khan (2006).

Despite the fact that genetic factors are more important in relation to ADHD, certain environmental risk factors have also been identified as being related to ADHD. Epilepsy, brain infections and complications during pregnancy and delivery are more likely to have occurred in children that develop ADHD (Cantwell, 1981; Holdsworth & Whitmore, 1974; Werner & Straus, 1941). Exposure to alcohol, drugs, and tobacco as a foetus increases the risk for ADHD in a child as well as being born prematurely and having a low birth weight (Max et al., 2002; Milberger et al., 1997). Also lead contamination and food additives/diet constitute an increased risk (Banerjee et al., 2007). Furthermore, low socio-economic status, family conflict, maternal psychopathology, poor parental coping, and high levels of expressed emotion may all relate to ADHD, even after adjustment for the effects of parental ADHD (Biederman et al., 2002).

Thus, ADHD seems largely determined by genetic factors. The genetic make-up of an individual may be labelled the ‘genotype’. This genotype may confer several potential genes that increase the risk for an individual to develop ADHD. Since ADHD is a relatively common disorder, it appears that these susceptibility genes are also common in the general population (Faraone et al., 2005). Susceptibility genes and environmental risk factors (inter)act and influence the ADHD phenotype (Faraone et al., 2005).
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Neurobiological Deviations in ADHD

It is hypothesized that ADHD risk genes influence the ADHD phenotype through neurobiological and neuropsychological deviations frequently observed in individuals with ADHD.

Structural Neurological Deviations

Initially, brain damage was thought to be the primary cause of ADHD, since epilepsy, brain infections and complications during pregnancy and delivery were more common among ADHD patients than normal (Cantwell, 1981; Holdsworth & Whitmore, 1974; Werner & Straus, 1941). However, the majority of ADHD patients do not suffer from any of these predisposing features, making it unlikely that brain damage constitutes the main cause of ADHD (Rutter, 1977). In later years, the similarities in symptoms between ADHD patients and patients with frontal lobe damage have led to the hypothesis that pathology in the frontal lobe accounted for symptoms of ADHD (Benton, 1991; Satterfield & Dawson, 1971). Anatomical imaging studies have indeed revealed that ADHD is associated with smaller volumes of the (pre)frontal lobes (Castellanos et al., 1996; Filipek et al., 1997). The connections between the frontal lobes and various subcortical areas appear altered in ADHD (Casey et al., 1997; Vaidya et al., 1998). However, structural abnormalities in ADHD are widespread and not confined to the frontal lobes alone (Durston, 2003), since many studies have documented structural abnormalities outside the frontal lobes, such as reductions in caudate volumes (Castellanos et al., 1994; Filipek et al., 1997; Semrud-Clikeman et al., 2000), corpus callosum volumes (Baumgardner et al., 1996; Giedd et al., 1994), cerebellar volumes (Castellanos et al., 1996, 2001), and retrocallosal regions that include parietal, temporal and occipital areas (Filipek et al., 1997).

Functional Neurological Deviations

Functional imaging studies have shown a decreased/more diffuse global activation of the (pre)frontal lobes in ADHD patients (Rubia et al., 1999a; Schweitzer et al., 2000). However, abnormal activation in multiple cerebral areas other than the frontal lobes during executive functions has also been reported (Bush et al., 2005). Even the baseline brain activity (without taxing executive functions) appears altered. For example, the resting-state activity appears higher in sensory and sensory-related cortices in adolescents with ADHD (Tian et al., 2007). Differential patterns of baseline brain activity appear widespread and can be found in the frontal cortex, sensorimotor cortex, cerebellum, and anterior cingulate cortex. This suggests that the
spontaneous neuronal activity is different in ADHD patients compared to controls, which may be related to the pathophysiology of ADHD.

**Neurochemical Deviations**
The neurotransmission of dopamine, serotonin, and noradrenalin appears abnormal in ADHD (Biederman & Spencer, 1999; Gainetdinov et al., 1999; Spivak et al., 1999). Mainly dysfunctional dopaminergic neurotransmission has been frequently implicated in ADHD. For example, pharmacologic agents that influence the dopamine system have been effective in the treatment of ADHD (Faraone et al., 2006). In addition, studies using Positron Emission Tomography (PET) have shown a deregulation of dopaminergic neurotransmission in ADHD patients (Jucaite et al., 2005; Spencer et al., 2007) and animal studies have illustrated that various changes in dopaminergic neurotransmission, such as blockage of the dopaminergic receptors and reduced dopamine synthesis, lead to ADHD-like behavior (i.e. hyperactivity, disinhibition) in mice (Russell, 2007; Van der Kooij & Glennon, 2007).

**Neurophysiological Deviations**
Using electroencephalography (EEG) and event related potentials (ERP), studies have investigated neurophysiological abnormalities in ADHD. Abnormalities in the resting state EEG have been reported, like increased theta (and sometimes alpha) activity and decreased beta activity, suggesting an increase of slow wave activity in ADHD (Lazzaro et al., 1999). An altered theta/beta ratio appears to be a commonly observed trait in ADHD relative to normal controls (Snyder & Hall, 2006). Reduced ERP amplitude has been documented in patients with ADHD during various cognitive tasks. A frequently reported finding is a reduced P3 amplitude, reflecting reduced activity of the anterior attention system, during the Go/NoGo task (Rodriguez & Baylis, 2007), during the Continuous Performance task (Overtoom et al., 1998), and during the Stop task (Liotti et al., 2005).

**Neuropsychological Dysfunctions**
Neuropsychological dysfunctions are defined as dysfunctions in specific psychological processes and/or overt behaviors that are related to structural and functional brain deviations. In the ADHD neuropsychological literature, the focus has been predominantly on so called ‘executive functioning’ (EF) (Boonstra et al., 2005b; Doyle, 2006; Pennington & Ozonoff, 1996; Seidman et al., 2004; Sergeant et al., 2002). EF has been defined as “those capacities that enable a person to engage successfully in independent, purposive, self-serving behavior” and is highly related to
prefrontal cortex functioning (Lezak, 1995). EF impairments have been reported in many studies with ADHD patients, with problems in inhibition and working memory being the most frequently replicated (Boonstra et al., 2005b; Doyle, 2006; Pennington & Ozonoff, 1996; Seidman et al., 2004; Sergeant et al., 2002). Deficits in the ability to reproduce time intervals (>1 second) found in patients with ADHD have been hypothesized as also relating to/resulting out of deficits in executive functioning, since time reproduction loads heavily on working memory and inhibition processes (Barkley et al., 2001b). It has been proposed that disinhibition or executive dysfunction form the core deficit in ADHD (Barkley, 1997; Pennington & Ozonoff, 1996) and that executive problems are related to abnormalities in the frontal lobe and frontal-subcortical structures found in patients with ADHD (Castellanos & Tannock, 2002; Durston, 2003).

However, disinhibition or executive dysfunction as core deficit of ADHD cannot account for the numerous findings of non-executive deficits found in patients with ADHD. A general slowness and variableness of information processing is already apparent at young age even in borderline ADHD cases (Kalff et al., 2005). Furthermore, deficits have been reported in encoding, perception, language, visuomotor integration, motor functioning, learning, memory, temporal processing, word-reading, color-naming, and pattern- and spatial recognition (August & Garfinkel, 1989; Banaschewski et al., 2005; Blondis, 1999; Boonstra et al., 2005b; Carte et al., 1996; Dowson et al., 2004; Tannock et al., 2006; Van Mourik et al., 2005). Mainly the findings of problems in the spatial and temporal organisation of motor output are multitudinous and are believed to be related to abnormalities in structure and/or function of the cerebellum and basal ganglia found in ADHD (Barquin et al., 1998; Castellanos et al., 1996). For example, previous research has shown that many children with ADHD experience problems in gross (Carte et al., 1996; Piek et al., 1999) and fine motor skills (Korkman & Pesonen, 1994; Marcotte & Stern, 1997; Pitcher et al., 2003; Whitmont & Clark, 1996). Even young children at risk for ADHD perform less accurate and more variable at motor tasks compared to normally developing peers (Kalff et al., 2003). Deficits in the timing of motor output have also frequently been reported (see for review Toplak et al., 2006). All in all, studies on neuropsychological functioning of ADHD patients have revealed both executive as well as motor dysfunctions, and it thus seems important not to focus exclusively on EF deficits being the core deficit in ADHD (Barkley, 1997), but also to take into account possible motor deficits.

The documented neurological and neuropsychological deviations in ADHD patients may be termed 'endophenotypes'. The Greek word 'endo' means 'within' or 'inside' (Almasy &
Blangero, 2001; Gottesman & Gould, 2003). Endophenotypes may be seen as phenotypes that are not (directly) observable but form an underlying liability to develop the ADHD phenotype (Castellanos & Tannock, 2002). However, since techniques to study neurobiological and neuropsychological aspects have advanced in the recent decade and the invisible is no longer invisible, the terms ‘intermediate’ or ‘latent’ phenotypes may be more accurate.

**Etiological Model of ADHD**

The interplay between genotype, endophenotype, and phenotype can be portrayed in a model. This model is not uniquely applicable to ADHD but is more widely applied to psychiatric and non-psychiatric disorders (Almasy & Blangero, 2001). A comprehensive summary of this model, applied to schizophrenia, has been published by Cornblatt and Malhotra (2001). Here, this model has been altered slightly and adapted for ADHD (see Figure 1.1).

Grossly simplifying the real nature of events, the model portrays the hierarchical chain of events leading up to a (psychiatric) disorder. At the top of the figure, both genetic factors and environmental factors play a role in determining the brain morphology of a child. These factors can both be positively and negatively influencing the (mental) health of a child. In the case of ADHD, multiple genes may add up (and interact, i.e. the effect of a gene depends on the effect of another gene) and produce an increased susceptibility of a child to develop ADHD. In addition, environmental factors may further increase (or decrease) the risk for developing ADHD. For example, pre- and perinatal complications occurring in a child already genetically at risk, may contribute to the underlying neurobiological abnormalities and, with that, may increase, for example, neuropsychological dysfunctions in inhibition, working memory, motor coordination, etcetera. These neuropsychological dysfunctions may lead, in turn, to observable problems in attention and hyperactivity/impulsivity to the extent that the problems get so severe and impairing, a diagnosis of ADHD is warranted. Thus, in this model, neurobiological and neuropsychological impairments form a link between susceptibility genes and ADHD.

However, even though this model gives a clear and interpretable illustration of the chain of events leading up to a disorder, in reality, it is not so simple. First of all, genetic and environmental risk factors do not simply add up, but may also correlate and interact (Jaffee & Price, 2007). For example, parents passing on their ‘ADHD-genes’ may also provide adverse environmental conditions, such as smoking or drinking during pregnancy (Jaffee & Price, 2007).
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Second, the influence of genes and environment is not constant during the development (Brown, 2002). Genes may ‘switch on and off’ during the lifespan and the effect of genes can be influenced by certain environmentally induced effects like stress (Brown, 2002). Passing the threshold and being diagnosed with ADHD is, therefore, not definite for life, since the ADHD symptoms may decrease with age and positive environmental factors such as treatment, may lead to a remission of the disorder.

Third, the arrows in the figure suggest a one-way chain of events, whereas arrows between all the different levels in the forward and backward directions would be more accurate. For example, having the disorder may create an adverse environment because the behaviour of the child might provoke negative reactions from teachers, parents, and other children. The child is being expelled more often, which reduces his/her chances on a good education. This adverse environment may, in turn, aggravate the ADHD symptoms.

Fourth, the figure may falsely give the impression that the chain of events leading up to ADHD is independent of the chain of events leading up to other disorders. This is certainly not the case (Cornblatt & Malhotra, 2001). As has been described above, ADHD is frequently accompanied by other (psychiatric) disorders of which the causal chain of events is intertwined with that of ADHD. At a genetic level, ADHD may overlap with several of these comorbid conditions, such as other externalizing disorders, dyslexia, motor coordination problems, and autism spectrum disorders (Banaschewski et al., 2005; Coolidge et al., 2000; Dick et al., 2005; Nadder et al., 2002; Stevenson et al., 1993). Certain genes may give rise to an enhanced risk for several disorders. The same may be said for environmental risk factors. It is, therefore, not surprising that at a neurobiological and neuropsychological level, dysfunctions found in ADHD are also found in several of the comorbid disorders (Banaschewski et al., 2005; Coolidge et al., 2000; Geurts et al., 2004). Moreover, having a comorbid disorder alongside ADHD may hypothetically even aggravate the ADHD symptoms. A child with ADHD and reading difficulties may be even more easily distracted than a child with only ADHD, because reading is not a fully automatic skill and requires more effort than normal (Pennington et al., 1993).

Fifth, the diagnosis of ADHD, as well as other (psychiatric) diagnoses, relies on somewhat arbitrary criteria. The DSM views psychiatric disorders as either present or absent. Criteria have to be met to be diagnosed as having ADHD. This leads to the ambiguous situation in which an individual may meet five rather than the required six symptoms of inattention or hyperactivity/impulsivity and is labelled as unaffected, even though this individual clearly shows vulnerability for ADHD (Lahey et al., 2007). Psychiatric disorders may, therefore, be best viewed as the extreme of continuous traits present in the general population (Reich et al., 1975).
Figure 1.1 Etiological model of ADHD inspired by Corinhal and Malhotra (2001).
Sixth, aside from the general complexities in using the etiological model of psychiatric disorders, there may be certain difficulties specific to ADHD. Perhaps more than other psychiatric disorders, the diagnosis of ADHD may be ambiguous. For instance, parental and teacher ratings on inattentive and hyperactive-impulsive behaviour correlate only moderately (Mitsis et al., 2000). This may be (partly) due to the difference in settings in which the ratings are being made, which limits the comparisons of ratings between parents and teachers (i.e., attending in a classroom full of other children may not be comparable to attending to a conversation with one of the parents). However, the sometimes discrepant observations may also be related to observer specific characteristics (Mitsis et al., 2000). To some degree, ADHD is in the eye of the beholder: What one adult would view as abnormally active or inattentive may be viewed as normal by another adult.

To conclude, the etiological model of ADHD provides a comprehensive theoretical framework for studying the genotypic, environmental and endophenotypic factors that underlie ADHD. However, care should be taken when using this model, since complex relations exist between the factors.

A Focus on Endophenotypes
In order to shed light on the etiology of ADHD, a focus on endophenotypes may be necessary. The identification of susceptibility genes for ADHD by linking the genotype to the phenotype has proved to be troublesome, partly because of the heterogeneity at both levels (Buitelaar, 2005; Gottesman & Gould, 2003). Focusing on endophenotypes, instead of phenotypes, may contribute to the identification of susceptibility genes in ADHD in several ways. First, endophenotypes are proposed to be more heritable than phenotypes because they are etiologically ‘closer’ to the disease genes than phenotypes (Almasy & Blangero, 2001; Castellanos & Tannock, 2002; Waldman, 2005). Therefore, using endophenotypic measures instead of (or in addition to) phenotypic measures may result in stronger linkage and association signals. Second, because endophenotypes are quantitative traits and not dichotomous entities like DSM diagnostic categories, variance is maximized and statistical power is gained. Third, it has been hypothesized that endophenotypes may be useful in exploring different pathways leading up to the disorder. Creating more homogeneous subgroups of patients based on a shared endophenotypic dysfunction, it has been hypothesized that these patients are also more alike with respect to genetic underpinnings, hence improving genetic signals (Almasy & Blangero, 2001).
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In the last two decades, substantial attention has been given to studying endophenotypes of psychiatric disorders. This has led to the discussion of what exactly constitutes an endophenotype and what criteria must be met for a trait to be useful as candidate endophenotype (Gottesman & Gould, 2003; Waldman 2005). Since an endophenotype forms a link between susceptibility genes and the disorder, it follows that: (1) the trait is heritable (and familial), in which partly the same genes influence the endophenotype and phenotype; (2) the trait is associated with the disorder (i.e. present in affected individuals); (3) the trait is observable in non-affected first-degree relatives of an affected individual, because first-degree relatives are likely to carry some of the susceptibility genes of the disorder. This last criterion also provides the opportunity to shed some light on “the chicken or the egg” problem: Are neuropsychological dysfunctions causally related to ADHD, or is the presence of ADHD causing a poor task performance? Non-affected siblings portray normal levels of inattention and hyperactivity / impulsivity. In case non-affected siblings show (some of) the neuropsychological deficits also observed in their affected siblings, then it is less likely that the neuropsychological deficits are caused by the presence of ADHD itself.

Thus far, knowledge of neuropsychological ADHD-endophenotypes is limited and inconclusive. Two studies have failed to find neuropsychological impairments in parents of children with ADHD (Asarnow et al., 2002; Murphy & Barkley, 1996) and two studies found no conclusive evidence of neuropsychological dysfunctioning in non-affected siblings (Nigg et al., 2004; Seidman et al., 2000). However, one study did find evidence for certain neuropsychological functions as endophenotypes (Bidwell et al., 2007) and other studies, specifically targeting inhibition or interference control, found evidence for these functions as endophenotypes (Crosbie & Schachar, 2001; Doyle et al., 2005; Schachar et al., 2005; Slaats-Willemse et al., 2003, 2005a). Less attention has been given to studying motor functions as ADHD-endophenotypes. In one study, it appeared that non-affected siblings had subtle motor problems, similar to their affected siblings (Slaats-Willemse et al., 2005b), suggesting endophenotypes for ADHD may also lie inside the motor domain. Clearly, much work needs to be done in this area of research.

Several studies have examined the relationship between ADHD candidate genes and neuropsychological measures. The majority of these studies are underpowered, so no firm conclusions can be drawn based on the findings. The largest study conducted thus far ($N = 540$) (Loo et al, submitted) investigated the relationship between several dopaminergic genes ($DAT1$, $DRD4$, $DRD5$) and measures of executive functioning and intelligence. The 7-repeat risk allele of $DRD4$ was related to poorer executive functioning and lower intelligence, but the $DRD5$ and
DAT1 genes were not conclusively associated with executive functioning. The second largest study (N = 122) (Barkley et al., 2006) reported opposite findings: DRD4 was not associated with ADHD or neuropsychological measures, whereas DAT1 was related to ADHD and poorer performance on some neuropsychological measures. For a comprehensive summary on the relation between DAT1, DRD4, and MAOA and neuropsychological measures, readers are referred to Chapters 12, 13, and 14, respectively. In general, associations between risk genes and neuropsychological measures are small, as would be expected given the polygenic model of ADHD. However, associations found in one study are often not replicated in other studies, or the reverse association is found (i.e. risk genotype is associated with better neuropsychological performance). Additional studies applying larger samples are warranted.

The Aim and Structure of This Thesis
The overall aim of this thesis is to examine the viability of neuropsychological measures as endophenotypes for ADHD, by studying neuropsychological functions in a large, well phenotyped group of children and adolescents with ADHD and their non-affected siblings for whom also DNA was obtained. Neuropsychological measurements are, compared to brain imaging techniques, easier to administer, less invasive for the participant(s), and cheaper. These benefits allowed us to examine neuropsychologically 816 children. The focus of the neuropsychological test battery is on executive functions (inhibition, visuo-spatial and verbal working memory, and set shifting) and motor functions (speed, variability, control and timing of motor output). The rationale for this battery is to examine the viability of the primary endophenotypic candidates of ADHD (executive functions), but also to take into account the possibility that motor dysfunctions frequently found in ADHD may form candidate endophenotypes. Furthermore, the wide age range of our subjects allows us to investigate whether the viability of neuropsychological endophenotypes is constant across age.

This thesis is divided into three parts, in order to cover the primary three research questions of this thesis:
1. Which neuropsychological measures form candidate endophenotypes by fulfilling several key characteristics of an endophenotype?
2. How do endophenotypic vulnerabilties translate into phenotypic problems?
3. Are neuropsychological measures related to specific genes and chromosomal regions?
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Part 1: Chapters 2-8
Thus far, limited knowledge has been gathered on candidate neuropsychological endophenotypes in ADHD. The aim of the first part of the thesis is, therefore, to examine whether executive and motor functions meet key criteria of an endophenotype: (1) siblings resemble each other on the measure; (2) children with ADHD are on average impaired compared to normal control children; (3) comparable deficits are also present in the non-affected siblings? All chapters in Part 1 deal with these criteria, but differ in their neuropsychological focus. Chapter 2 deals with the well-investigated executive functions in ADHD, namely inhibition, verbal and visuo-spatial working memory. Also the viability of intelligence as endophenotype was investigated. Chapter 3 studies the viability of time reproduction as endophenotype, a function heavily related to inhibition and working memory. Chapter 4 focuses on a subsample of ADHD-families to examine whether executive dysfunctions (inhibition and visuo-spatial working memory) are also measurable using eye-tracking devices. This also allows us to analyze the more basic metrics and dynamics of oculomotor control in ADHD-families. Chapter 5 aims at answering whether deficits in executive functions are also evident when a baseline measure of speed and accuracy of responding is taken into account. Chapters 6 and 7 present data on motor functions in ADHD and control families, namely the speed, variability, timing, and control of motor output. Chapter 8 investigates whether somatosensory functioning is abnormal in a subsample of children with ADHD and their non-affected siblings.

Part 2: Chapters 9-11
The relationship between endophenotype and phenotype is explored in Part 2 of this thesis. For example, a criterion of an endophenotype is that it is present in non-affected relatives in the absence of the disorder itself. But what causes a child to be phenotypically unaffected, yet endophenotypically affected? Previous research has established that certain factors, such as gender, age, and intelligence, may influence the clinical manifestation of ADHD. For instance, more boys than girls have ADHD, the severity of ADHD seems to lessen with age, and a higher intelligence is associated with a lower severity of ADHD. How these factors influence the relationship between endophenotype and phenotype has thus far not been investigated, but is in Chapter 9. In Chapter 10, it is examined whether the endophenotypes investigated in Part 1 also relate to comorbid disorders. This may offer insight into whether these comorbid problems share similar endophenotypic (and possibly genetic) roots with ADHD. There is reported to what degree ADHD is related to the different comorbid disorders and whether ADHD-endophenotypes relate to qualitatively different phenotypes if ADHD co-occurs with comorbid
problems, which may suggest that ADHD in combination with a comorbid disorder is a qualitatively, and not quantitatively, different phenotype. Lastly, in Chapter II, it is analyzed whether neuropsychological endophenotypes are more strongly linked to heritable factors than the ADHD phenotype itself, as would be expected based on the theoretical model of psychiatric disorders. It also reports on the degree of shared heritability of the ADHD phenotype and neuropsychological endophenotypes, and whether a composite measure of individual neuropsychological measures is viable as endophenotype. The latter is tested, because it is hypothesized that a composite score is a more robust measure as it entails less error variance than individual measures.

Part 3: Chapters 12-15
In Part 3, an examination is presented of the role of certain candidate genes and chromosomal regions in neuropsychological dysfunctioning associated with ADHD. Chapter 12 examines the relation between the gene coding for the dopamine transporter (DAT1) and neuropsychological dysfunctioning, and Chapter 13 examines the relation between the gene coding for a dopamine receptor (DRD4) and neuropsychological dysfunctioning. In Chapter 14, it is tested whether the X-linked gene involved in the degradation of the monoamines serotonin, norepinephrine and dopamine (MAOA) relates to neuropsychological functioning in ADHD and whether this relationship is moderated by sex. Last, in Chapter 15 a whole genome linkage analysis is performed in order to discover possible new linkage regions which may harbour susceptibility genes for neuropsychological deficits associated with ADHD.