ADHD is an impairing neurodevelopmental disorder characterized by inattention, hyperactivity, and impulsivity and is associated with various neurological, neurochemical, neurophysiological, and neuropsychological deviations. The disorder is largely determined by heritable factors (Faraone et al., 2005). However, the identification of risk genes for ADHD by linking chromosomal regions and/or susceptibility genes (the genotype level) to the behavioral symptoms (the phenotype level) has proven to be troublesome, because of the heterogeneity at both levels (Buitelaar, 2005; Gottesman & Gould, 2003). By focusing on neuropsychological dysfunctioning (endophenotypes), which may confer an increased (genetic) risk for ADHD, it may be possible to identify susceptibility genes for ADHD. That is, endophenotypes are considered to be less genetically complex than phenotypes, since they are etiologically ‘closer’ to the disease genes than phenotypes (Almasy & Blangero, 2001; Castellanos & Tannock, 2002; Waldman, 2005). Endophenotypes are statistically more powerful than phenotypes because they are quantitative traits and not diffuse syndromes as in DSM-IV. Endophenotypes may be more useful in exploring different pathways leading to the disorder than phenotypes (Waldman, 2005), because they may form more homogeneous subgroups of patients sharing an underlying deficit.

The overall aim of this dissertation was to examine the viability of neuropsychological measures as endophenotypes for ADHD by studying neuropsychological functions in a large, extensively phenotyped group of participants and adolescents with ADHD and their non-affected siblings from whom DNA was obtained. In addition, control participants were recruited, which resulted in a sample of 816 participants. The focus of the neuropsychological test battery was on two key domains of functioning in ADHD. Firstly on executive functions: inhibition, visuo-spatial and verbal working memory, and set shifting. Secondly on motor functions: speed, variability, control and timing of motor output. It was considered that this battery offered a strong chance of finding endophenotypic dysfunctions.

This dissertation is divided into three parts, in order to cover the primary three research questions. Chapters in Part 1 are all related to the research question of which neuropsychological functions formed candidate endophenotypes. It was studied whether a broad range of neuropsychological functions fulfilled several key characteristics of an endophenotype, such as whether participants with ADHD were on average impaired compared to control participants, whether comparable deficits were also present in the non-affected siblings, and whether siblings resembled each other on neuropsychological functioning. The chapters in Part 2 of this dissertation aimed at examining the research question of how the endophenotype and phenotype are related and what factors may influence
this relationship. It was tested whether group differences were equal in magnitude at the endophenotypic and phenotypic levels, and whether the relationship between endophenotype and phenotype was mediated and/or moderated by factors such as IQ, age, gender, and rater bias. It was also studied whether ADHD endophenotypes were related to comorbid disorders, and whether neuropsychological endophenotypes were more strongly linked to heritable factors than the ADHD phenotype itself. The chapters in Part 3 focused on the research question of whether neuropsychological functions were related to specific genes and chromosomal regions. The relationship between selected candidate genes (DAT1, DRD4, MAOA) and neuropsychological functions was explored, as well as the relation between chromosomal locations and neuropsychological functions.

Summary

Part 1
In Chapter 2, the most intensively studied aspects of executive function (EF) in ADHD (inhibition, visuo-spatial and verbal working memory) showed endophenotypic characteristics: EF deficits were not only present in participants with ADHD, but were observed also in their at-risk siblings. Similar results were found for Verbal IQ. Performance IQ appeared (nearly) normal in affected and non-affected siblings. Interestingly, most results indicated an independent segregation of EF and IQ deficits: Correlations and sibling cross-correlations were not significant between EF and IQ. Group effects in EF could not be explained by group differences on IQ and vice versa. Siblings resembled each other in their EF-IQ discrepancy instead of having generalized impairments across both domains: Siblings of probands with EF (but not IQ) problems displayed the same selective EF (but not IQ) deficit. In contrast, the three EF functions appeared to segregate together, as did Verbal and Performance IQ. Thus, it was concluded that EF and IQ impairments segregate relatively independently of each other.

Results reported in Chapter 3 revealed that time reproduction, a function strongly related to both inhibition and working memory, was impaired in affected participants and their non-affected siblings and correlated between siblings. As expected, longer test durations discriminated both groups even more from controls, suggesting that increasing demands on the ability to reproduce time made the performance deficit more prominent. Unexpected was the moderating influence of age on group difference for time reproduction: Participants with ADHD could be clearly dissociated from control participants up to the age of 9. After that age,
group differences were somewhat attenuated, although still present. Differences between non-affected siblings and controls were constant across the age range studied. These findings suggested that time reproduction may serve as a candidate endophenotype for ADHD, predominantly in younger children with (a genetic risk for) ADHD.

Chapter 4 illustrated that deficits in inhibition and visuo-spatial working memory may also be revealed using eye tracking paradigms, reflected by an increase in anticipatory saccades and impaired accuracy in memory-guided saccades, respectively. Intriguing was the finding of a tendency to under/overshoot the memorized location: Normal controls tended to undershoot the memorized location (which has been reported previously in healthy participants). Participants with ADHD and their non-affected siblings showed this tendency to a lesser degree, which resulted in an overshoot of the memorized target in affected participants. This tendency to overshoot saccades relative to controls was not related to the measures of inhibition and visuo-spatial working memory, suggesting that neuropsychological endophenotypes may also be found outside the spectrum of the frequently studied EF domain. It was concluded that memory-guided saccade deficits may relate to a familial predisposition for ADHD.

Endophenotypes outside the EF domain were found in the following chapters. In Chapter 5, participants with ADHD and their non-affected siblings committed more errors than controls on a baseline measure of responding (i.e. simple reaction time task). Moreover, compared to their baseline speed and accuracy of responding, participants with ADHD and their non-affected siblings were not disproportionally slower or more inaccurate, when demands for motor inhibition or cognitive flexibility were added to the task. This suggested that poorer performance on EF tasks in participants with ADHD and their non-affected siblings may result from deficiencies in lower order cognitive processes (such as motor functioning) and not (only) from higher order cognitive processes/executive functions.

Additional evidence for endophenotypes outside the EF domain, but within the motor domain, was found in the following two chapters. In Chapter 6 the accuracy and variability of motor timing were studied. Two basic motor tasks were administered alongside the motor timing task to account for generalized deficiencies in speed and variability of motor output. Variability of motor timing convincingly met all required characteristics of an endophenotype, though accuracy (tendency to under-produce the time interval) appeared predominantly present in affected participants and not in non-affected siblings. Interestingly, slow and variable motor output without a timing component was only present in affected participants and not in non-affected siblings, suggesting that slow and variable basic motor output is not convincingly
associated with a familial vulnerability for the disorder. In addition, self-generated motor output was normal in affected participants (and non-affected siblings). This suggests that the other two tasks may have required some cognitive processing (e.g. registering a stimulus and responding to it), whereas self-generated motor output only required executing a motor action. The findings in this chapter suggested that abnormalities in motor timing were predominantly related to deficient motor timing processes and not to a generalized deficient motor functioning.

Chapter 7 attempted to replicate previous findings of abnormal higher-order controlled motor deficits in participants with ADHD and their non-affected siblings (Slaats-Willemse et al., 2005b). Like Slaats-Willemse and colleagues, we found that participants with ADHD were less precise and stable in their motor control. However, findings differed with respect to the non-affected siblings: Slaats-Willemse et al. reported non-affected siblings to be selectively impaired in motor control requiring continuous adaptation, whereas we found non-affected siblings to be selectively impaired in motor control requiring following a known pathway. In addition, the findings here suggested that group differences were moderated by an effect of hand not reported by Slaats-Willemse et al.: No group differences emerged, when the right hand was used. Group differences did emerge when the left hand was used. This finding was possibly related to the known right hemispheric brain pathology in participants with ADHD and/or to differential effects of daily practice with both hands. Imprecision and instability of motor control of the left hand appeared to be useful as an endophenotype.

The final chapter of Part 1, Chapter 8, tested whether possible alterations in somatosensory functioning (i.e. processing of tactile and kinesthetic stimuli) in ADHD, could be found in non-affected siblings, hence supporting the viability of non-EF endophenotypes. Further, the subjective experience of pain was assessed. Results suggested that tactile perception, but not kinesthesia, was deviant in participants with ADHD and their non-affected siblings. Only non-affected siblings, but not affected participants, reported a lower level of emotionality and intensity of previously experienced pain compared to controls. The ‘objective’ tests of somatosensory functioning did not relate to the subjective sensation of pain. These findings suggested that alterations in tactile perception may relate to a familial susceptibility for ADHD. Furthermore, clinicians should be aware of possible under-reportage of experienced pain in siblings of children with ADHD.
In Chapter 9 several issues were investigated. First, the predictive validity for the ADHD diagnosis was assessed of the endophenotype of an aggregated component score combining ten neuropsychological task measures. The endophenotypic construct classified children with moderate accuracy (about 50% classified correctly in each of the three groups), suggesting substantial overlap between endophenotypic functioning in the groups of affected children, non-affected siblings, and controls. A second issue studied was whether group differences at the endophenotypic and phenotypic level were comparable in their magnitude. This was the case, when non-affected siblings were compared to controls. It was found that their subtle neuropsychological deviations were proportionally related to subtle behavioral deviations. However, group differences differed in magnitude, when affected participants were compared to controls: Affected participants displayed a more severe phenotype than endophenotype. This suggests that other factors aggravate the ADHD symptoms in participants. A third topic was whether the relation between endophenotype and phenotype was mediated and/or moderated by gender, age, IQ, and rater bias. A potentially moderating effect (age) was found as well as several mediating effects (gender, age, IQ). However, none of the effects studied could account for the finding that affected participants had a more severe phenotype than endophenotype. These findings suggest that neuropsychological endophenotypes are moderately predictive of ADHD diagnosis, but other factors may aggravate ADHD symptoms in affected participants. In addition, the relationship between endophenotype and phenotype is similar for boys and girls, both when ADHD ratings are made by parents or teachers, and across the IQ range. This relation is probably similar in the age range of 5 to 19 years.

Chapter 10 focused on the relationship between the ADHD-endophenotype of an aggregated component score combining ten neuropsychological task measures and comorbid disorders. ADHD was strongly related to oppositional defiant behaviors, followed by autistic traits, and succeeded by motor coordination problems, anxiety, and reading problems. Importantly, the neuropsychological composite ADHD-endophenotype was related to the comorbid problems and cross-related to comorbid problems in siblings, even when correcting for the presence of ADHD. This may point to a shared etiology of ADHD and comorbid problems. Further support for this interpretation is given by the fact that ADHD in combination with a comorbid problem did not appear to be a distinct subtype of ADHD, but rather ‘more of the same’ of both disorders.

A final issue examined in this section was whether neuropsychological functions as endophenotypes were more useful than phenotypic measures of ADHD with respect to the familiality of the disorder. This was tested in Chapter 11. Results revealed that six of the ten
neuropsychological measures correlated more strongly between siblings than an ADHD composite, suggesting that these measures may have a larger heritability than ADHD symptomatology itself. Furthermore, significant sibling cross-correlations also suggested that six of the ten neuropsychological measures were related to similar familial (and heritable) factors as ADHD, thus indicating these measures to be useful for genetic research in ADHD. An aggregated neuropsychological composite appeared to be the most powerful, since it correlated more strongly between siblings than individual task measures. These findings suggest that genetic research in ADHD may be facilitated by including neuropsychological functions.

Part 3

Chapter 12 attempted to shed light on the mechanisms of DAT1 (the gene coding for dopamine transporter 1) effects on ADHD by reviewing previous studies linking this gene to neurophysiological and neuropsychological functions and by studying the relation of DAT1 with neuropsychological functions collected in this dissertation. The review indicated that the majority of studies did not find a relation between DAT1 and neurophysiological and neuropsychological measures. In the Dutch IMAGE sample, several of the polymorphisms of DAT1 were associated with ADHD, and ADHD was associated with impaired neuropsychological functioning. However, none of the DAT1 polymorphisms was convincingly associated with neuropsychological dysfunctioning. This suggested that the effect of DAT1 on ADHD was not mediated by neuropsychological functions as assessed here. It may, however, be hypothesized that DAT1 has an effect on neuropsychological processes not examined in our study or previous studies, such as delay aversion, since DAT1 is mainly expressed in the striatum and not in the prefrontal cortex. Hence, DAT1 may influence striatum related functions (such as delay aversion and motor functions) more heavily than prefrontal related functions (such as executive functions). Some support for this hypothesis was found in the more detailed analysis of individual neuropsychological tests: If there was any association between DAT1 and neuropsychological functioning in our sample, it was within the domain of motor functioning and not within the executive domain (however, most of these associations did not survive correction for multiple testing). Interestingly, associations between DAT1 with ADHD were only found in adolescents, which may suggest that DAT1 mainly exerts its effect in adolescence, and/or that having a more persistent form of ADHD may mark a more severe or homogeneous genetic form of the disorder.
Chapter 13 illustrated that DRD4 (the gene coding for dopamine receptor D4) was associated with overall neuropsychological functioning, but this association was reversed in adolescents and children; specific for non-affected siblings and not affected participants; and robustly carried by cognitive functioning and not by motor functioning. In children, the 7-repeat allele of the exon polymorphism of DRD4 was related to better cognitive functioning, whereas the reverse was true in the adolescents. These results suggest that the effect of the DRD4 7-repeat allele on neuropsychological functioning is dependent on age and ADHD status and robustly carried by cognitive and not motor functioning. The latter finding may be explained by the fact that DRD4 is mainly expressed in the prefrontal lobes (strongly related to executive/cognitive functions) and not, or to a lesser extent, in other parts of the brain underlying motor functioning.

A third candidate gene that was studied in relation to neuropsychological functioning measures was MAOA (coding for a mitochondrial enzyme involved in the pre-synaptic degradation of monoamines [monoamine oxidase A]). Results are described in Chapter 14. It was hypothesized that sex differences for ADHD might be related to genes on the X-chromosome, like the MAOA gene. That is, unlike girls, boys do not have a spare X-chromosome, making the effect on an X-linked gene on cognition and behavior possibly stronger in boys compared to girls. Analyses were conducted using a haplotype based on three single nucleotide polymorphisms. Two haplotypes (GGC and ATT) captured 97% of the genetic variance in the investigated MAOA SNPs. The ATT haplotype was more common in non-affected siblings, conferring a protective effect for ADHD in both boys and girls. The ATT haplotype was associated with poorer motor control in boys, but with better visuo-spatial working memory in girls, suggesting the target and direction of the MAOA effect on neuropsychological functioning may be different in boys and girls. These differences were hypothesized as relating to many biological differences between males and females in serotonin neurotransmission. MAOA mainly influences the metabolization of serotonin; similar serotonin levels may produce opposite behavioral and cognitive effects. It is thus feasible that the effect of MAOA (through serotonin levels) on neuropsychological functions may not necessarily be comparable between boys and girls with ADHD. These findings suggest the genetic and neuropsychological mechanisms underlying ADHD may be different in boys and girls and underlines the importance of taking into account sex effects when studying ADHD.

Using a whole-genome QTL linkage analysis in Chapter 15, effort was made to identify chromosomal regions possibly conferring risk genes for ADHD. Two genome-wide significant linkage peaks were found, one for Motor Timing on chromosome 2q21.1 (LOD score: 3.944)
and one for Digit Span on 13q12.11 (LOD score: 3.959). Ten suggestive linkage peaks were found (LOD scores ≥ 2) on chromosomes 2p, 2q, 3p, 4q, 8q, 12p, 12q, 14q, 17q. The suggestive linkage signal for the component score that was found at 2q14.3 (LOD score: 2.878) overlapped with the region linking to Motor Timing. Several candidate genes were located in the region of the two significant linkage signals, which may prove useful in further association analyses. Several of the linkage peaks overlapped with previously reported linkage signals for reading disability and autism, possibly pinpointing to pleiotrophic loci that relate to neuropsychological deficits seen in both patients with ADHD and reading disability and/or autism. Some overlap was present in linkage signals with previous studies using cognitive measures in linkage analyses. In conclusion, our results suggest that neuropsychological candidate ADHD-endophenotypes may aid in the discovery of novel ADHD genes through linkage analysis.

**Key findings**

- A large array of neuropsychological functions (ranging from executive functions to motor functions and to the processing of tactile stimuli) form candidate ADHD endophenotypes, hence supporting the role of neuropsychological functions in heritability research of ADHD;
- Executive functions appear somewhat more viable as ADHD endophenotypes than motor functions;
- Nevertheless, executive and motor functions are interrelated and problems in motor functioning may sometimes explain poor performance on tasks aimed to measure executive functioning;
- On the majority of neuropsychological and phenotypical measures, non-affected siblings differ quantitatively, but not qualitatively, from their affected siblings, supporting the hypothesis that ADHD forms the extreme end of a continuum;
- Group differences at the endophenotypic and phenotypic levels are equal in magnitude for non-affected siblings and controls, but unequal for affected participants and controls: Affected children display a more severe phenotype than endophenotype, suggesting that other factors aggravate the ADHD symptoms in affected participants;
- The relationship between endophenotype and phenotype is similar for boys and girls, similar when ADHD ratings are made by parents and teachers, similar across the IQ range, and probably similar across the ages 5 to 19;
- ADHD endophenotypes also relate to comorbid disorders, possibly indicating a shared etiology of ADHD and comorbid problems;
- Some neuropsychological measures are more familial than measures of ADHD, making them useful tools in genetic research of ADHD;
- The effect of dopamine genes appears not comparable in children and adolescents, with reversed or stronger effects in adolescence than childhood ADHD;
- DAT1 has no effect on executive functions, but may have a small effect on motor functions, whereas the reverse is true for DRD4;
- The effect of the X-linked gene MAOA on neuropsychological functioning is not comparable in boys and girls, suggesting that the genetic and neuropsychological mechanisms underlying ADHD may be different in boys and girls;
- Using neuropsychological functions, two genome-wide significant linkage peaks and several suggestive linkage peaks are identified, possibly aiding in the discovery of novel ADHD genes.