Neural and genetic underpinnings of response inhibition in adolescents with attention-deficit/hyperactivity disorder
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Chapter 1

GENERAL INTRODUCTION
Attention-Deficit/Hyperactivity Disorder (ADHD) is a relatively common (the prevalence is about 5% worldwide (1)) and etiologically complex neurodevelopmental disorder. ADHD is characterized by the persistent presence of inattentive and/or hyperactive impulsive symptoms, coupled with impaired functioning in daily life (2). ADHD is thought to be related to cognitive deficits; specifically, executive functioning deficits have been found in subjects with ADHD as compared to healthy controls (3). Since relatively little is known about the biological mechanisms underlying ADHD, these executive functioning deficits are considered as an important target to further investigate the neurological and genetic underpinnings of ADHD.

In this thesis we took a specific cognitive process, i.e., response inhibition as a starting point to investigate the neurobiological and genetic alterations underlying ADHD. The heart of this thesis consists of four research articles, each using a specific method or specific outcome measure to further our insight into the nature of response inhibition and its relationship with ADHD. In this introduction I will first give a short overview of the key concepts that are central to these research articles, and in the general discussion I will reiterate and summarize my findings, and interpret the main conclusions we can draw from this research.

**Response inhibition**

Imagine standing at a street corner, ready to cross the street. Just when you are about to step off the sidewalk, a speeding car appears out of nowhere. At the last moment you are just able to stop yourself from making a fatal step off the sidewalk. This is an example of response inhibition in practice; the conflict you perceive in such a moment is caused by two neural processes vying for control, one trying to initiate a movement and the other trying to cancel that same movement.

Within the complex and quickly changing environment of modern society, we constantly need to be aware of our surroundings, and it is essential that we are able to quickly adapt our behavior in reaction to new incoming information. The ability to integrate external or internal novel information quickly enough to inhibit an already initiated response is one of the key features of cognitive control. The process of cancelling, interrupting or deviating an ongoing reaction is called *response inhibition* and is one of the main processes that are often found to be impaired in ADHD (4).

In particular, the lack of proper response inhibition is thought to be a core deficit underlying the impulsivity observed in patients with ADHD (5), as impulsive thoughts and actions are not evaluated quickly enough to still be withheld if they seem inappropriate. However, it
is not exactly clear at the moment what goes wrong in the neural processes underlying response inhibition in probands with ADHD.

Since ADHD is both an etiologically complex and heterogeneous disorder, it is not feasible to study the complete biological nature of the entire phenotype at once. Instead, we can look at the different cognitive processes that are affected in individuals with ADHD, and study their biological nature, in order to gain piecemeal insight into the etiology of the disease. In case of the response inhibition process, a large body of research is already available on the neurobiological and genetic basis in healthy subjects. We can use this knowledge of response inhibition in healthy subjects to observe which parts of the cognitive process are altered in ADHD.

**Stop-signal task**

One of the most commonly used methods to measure response inhibition performance is the stop-signal task (SST). First introduced by Logan and colleagues (6), this task continually presents a participant with stimuli to which they are required to respond with an action, the so called go-signals; however, in a subsample of the trials, the go-signal is followed after a short interval by another signal, the stop signal, indicating that the participant has to withhold his response. The time between the go and stop signal is called the stop-signal delay, or SSD. By varying the length of this delay, it is possible to determine the time a participant needs to successfully process the stop-signal and withhold his response in 50 percent of the trials. By subtracting this eventual delay from the mean reaction time (or MRT), we obtain the stop-signal reaction time (SSRT), or the time a subject needs to properly process the stop signal in half of the cases. The SSRT is the main measure of response inhibition performance, with a longer SSRT indicating poorer response inhibition.

![Figure 1: Schematic overview of the stop-signal task. Stop-signal reaction time (SSRT) is derived by subtracting the stop-signal delay (SSD) from the reaction time at which a participant is able to correctly inhibit a response in 50% of the trials.](image-url)
Other outcome measures from the stop-task that have been examined in the current thesis include the number of errors on go-trials (errors), and reaction time variability (RTV). The number of errors on go-trials consist of omission errors, where a subject fails to make a response, and commission errors, where the subjects makes a response to the wrong side of the screen. Although errors are indicative of poor task performance, they are not thought to reflect response inhibition problems per se, but a more general attentional lapse causing a temporarily failure to comply with the task demands (7). Similarly, reaction time variability indicates how the reaction time differs between trials. High variability is therefore also indicative of large sways in task performance related to poor attentional control (8). Since both errors and reaction time variability have an indirect influence on the estimation of the SSRT (9), it is important to adjust for the attention related measures when investigating response inhibition between diagnostic groups.

A large number of studies have investigated the performance of subjects with ADHD on versions of the stop-task, many of them reporting impaired response inhibition performance, specifically in children with ADHD (4). However, a recent meta-analysis has shown that although these effects are generally stable across studies, they are not necessarily present in all subjects with ADHD (10). Specifically, about half of the subjects with ADHD have response inhibition scores on par with healthy subjects.

In order to take into account this variation in response inhibition performance, and to dissociate the influence of the different cognitive processes that may influence response inhibition performance, we have examined the neural correlates underlying response inhibition performance within this thesis, and compared these neural measures between probands with ADHD, their unaffected siblings, and healthy controls.

**Neurobiology of response inhibition**

The neurobiological substrate of response inhibition has been thoroughly investigated in healthy subjects. The neural correlates of response inhibition have been studied using a variety of measures, the most common of which being functional magnetic resonance imaging (fMRI). fMRI is a neuroimaging method which enables the detection of neural activation by measuring the associated changes in blood flow and oxygenation in a region. The neural activation patterns as measured with fMRI indicate that two major neural networks are implicated in the realization of the response inhibition process. The first network, the frontal-striatal network, consists of the inferior frontal gyrus, pre-supplementary motor area, and basal ganglia. The inferior frontal gyrus is generally thought to be involved in cue/salience detection (11), and within the response inhibition process is considered the central node to control and initiate the stopping process (12). The pre-supplementary motor area
and basal ganglia are mainly involved in the preparation of going and stopping actions, and the execution of the stop-process (13; 14).

Additional evidence for the involvement of the frontal-striatal network comes from a body of research using electrophysiological measures of neural activation or Transcranial Magnetic Stimulation (TMS). Unlike fMRI, TMS can be used to directly assess the causal relation of a specific cortical area within a cognitive process. TMS uses a magnetic pulse to temporarily inhibit or excite certain nodes to study their effect within a network. TMS studies on the response inhibition network have demonstrated the causal role of both the inferior frontal gyrus in the initiation of the inhibition process (11) and for the pre-supplementary motor area in the execution of the inhibition signal (15). Research using electroencephalography (EEG) further demonstrated the temporal dynamics of the neural activation, providing additional proof for a causal pathway from inferior frontal to supplementary motor areas and basal ganglia during response inhibition (16–18).

The second network typically associated with response inhibition is the frontal-parietal network, consisting of the temporal/parietal junction, opercular cortex, and superior frontal gyrus (19). This network has been shown to be involved in a variety of cognitive tasks, and is associated with both attentional processing and top-down (re-)direction (19). The dorsolateral part of the prefrontal gyrus also has been implicated in the process of task-set maintenance and top-down attentional redirection (12; 19; 20). The activation of the frontal-parietal network during response inhibition suggests that attentional processes may play a role in response inhibition performance (21–23). Specifically, failure of proper attentional reallocation to a stop-stimulus during response inhibition may lead the omission of a stop signal, or to the failure of initiating an inhibition. Thereby, lapses in early attentional processes play a role in response inhibition, and may indirectly cause higher stop-signal reaction times (9). The necessity of proper attentional control during response inhibition is further supported by TMS studies, which validate the causal role of the temporal/parietal nodes in attentional processes (24).

**Neurobiological alterations in response inhibition in ADHD**

Given the response inhibition deficits demonstrated by subjects with ADHD, a series of recent studies have investigated the potential differences in the neural correlates of response inhibition between probands and controls (e.g. (25–27)). These studies indicated that probands with ADHD generally show lower activation in the main nodes of the frontal-striatal network underlying response inhibition. Additionally, the functional connectivity between the main nodes of the frontal-striatal network has also been found to be lower in probands with ADHD. These alterations in both response inhibition performance and the
neural correlates of response inhibition have also been reported in the unaffected siblings of probands with ADHD. Since the unaffected siblings of children with ADHD share on average 50% of the genetic variance with their affected siblings, these patterns suggest that response inhibition deficits may be familial in nature (26; 28).

The involvement of the frontal-striatal circuits in response inhibition deficits in ADHD may provide a clue for the biological mechanisms underlying poor response inhibition performance in ADHD. This is particularly relevant given our knowledge of the neurochemical functioning of the frontal-striatal circuits in healthy subjects; specifically, previous studies have shown that proper functioning of the frontal-striatal network is largely dependent on appropriately balanced levels of the main monoamine neurotransmitters dopamine, noradrenaline (29; 30), and serotonin (31–33).

The use of positron emission tomography (PET) to investigate dopamine availability during response inhibition has indeed demonstrated that lower dopamine availability is associated with poorer response inhibition performance in ADHD (34; 35), providing direct evidence for the role of dopamine in determining response inhibition performance.

Similarly, pharmacological intervention studies have found evidence that methylphenidate, the main form of stimulant medication for ADHD and a dopamine and noradrenaline reuptake inhibitor, and atomoxetine, a non-stimulant noradrenaline reuptake inhibitor, improve both response inhibition performance (36; 37) as well as increase the neural activation in the frontal striatal network (38; 39) in both healthy controls as well as probands with ADHD (40). These results again support the causal relations between dopamine/noradrenaline availability, frontal-striatal activation and response inhibition. Less evidence has been accrued on the role of serotonin in this framework. Several acute tryptophan depletion (ATP) studies have indicated effects on impulsivity in general (41), however, without clear effects on response inhibition performance in particular (42); even though another study has shown neural activation in the inferior frontal areas to be decreased after tryptophan depletion (43).

These insights into the neurochemical modulation of the response inhibition network in ADHD, as well as the patterns of response inhibition performance observed in family members of probands with ADHD, provide us with potential genetic variants to investigate the biological underpinnings of response inhibition deficits in ADHD.
Genetic influences on response inhibition and ADHD

Evidence from both family, twin and adoption studies (44) have indicated a high heritability for ADHD. An estimated 76% of the phenotypic variance in ADHD is determined by heritable factors (45; 46). However, the search for genes underlying this heritability has so far rendered relatively few (replicable) genetic variants that are significantly associated with the ADHD phenotype. Furthermore, only a small part of the phenotypic variance underlying ADHD can be explained by currently known genetic variants (47; 48).

The main reasons that contribute to the difficulty of gene finding in ADHD are the etiological complexity and heterogeneity of the ADHD phenotype. In most cases ADHD is a multifactorial (polygenic) disorder, meaning that a large number of common genetic variants that each contributes only a very small part of the eventual phenotype. Only in a small minority of cases major rare variants such as CNV’s or other large scale mutations are thought to explain a significant part of the ADHD phenotype (49). The additional interactions between different genetic variants, as well as the interaction between environmental and genetic factors over the course of normative development make the straightforward interpretation of causal pathways between genetic, neurobiological, and behavioral markers of ADHD extraordinarily difficult.

Nevertheless, in recent years several types of genetic studies have contributed to our understanding of the genetic background of ADHD. First, a large set of risk gene studies have been performed to investigate a-priori defined candidate genetic targets. These studies target single-nucleotide polymorphisms (SNPs), variable number tandem repeat (VNTRs) polymorphisms or copy-number variations (CNVs) which influence gene expression in genetic systems which are involved in the regulation of cognition and behavior. Taken together, risk gene studies have indicated several consistent risk genes involved in ADHD (47), most of which are associated with the main monoamine systems, specifically the dopamine and serotonin neurotransmitter systems.

Additional methods used to investigate genetic contributions to the ADHD phenotype include hypothesis-free methods, like genome-wide association studies (GWAS). GWAS are based on the analysis of a very large number of SNPs from the entire genome in large numbers of subjects to find significant associations between genetic variants and a disease phenotype. However, until this point GWAS studies have failed to find significant single gene associations with ADHD (50). This is in part due to the small effect sizes expected from these common polymorphisms which require huge sample sizes for their detection. The (future) pooling of samples from different consortia may aid in discovering novel risk genes for ADHD. Additionally, whole-genome or exome sequencing are novel methods which may aid in the discovery of rare genetic variants linked to increased ADHD risk in the near future.
These hypothesis-free methods are based on the entire genome (or exome) sequence of subjects, and will allow the comparison of the genome of subjects with ADHD with their direct family members without ADHD to discover (de-novo) genetic variations strongly linked with ADHD (51)s.

Alternative methods to increase the power of genetic studies in ADHD include the use of more advanced statistical methods. One example hereof is the functional gene group analysis, which combines variance from multiple genetic variants based on cellular function (52) to best explain variance in the phenotype. Similarly, bioinformatics pathway analyses have been used to identify underlying functional pathways from the available GWAS data on ADHD, leading to the identification of the neurite outgrowth pathway which encompasses many of the genes previously linked with ADHD (53). Further genetic pathway analysis used by Bralten et al. (54) has been employed to demonstrate the influence of cumulative variance within the dopaminergic, serotonergic, or neurite outgrowth pathway on the ADHD phenotype. Genetic variance within a pathway is not entirely independent, and since both common and rare genetic variants seem to converge within the same pathways (49). Therefore, these methods rely on combining the hypothesis driven selection of variants from an entire genetic pathway, and investigating the cumulative explanatory power of these many variants.

Lastly, there is the opportunity to use biomarkers as intermediate targets between the genetic and phenotypic levels. Specifically, combining hypothesis driven candidate gene selection with this so called endophenotype model will be the central method employed in the chapters three and four of the current thesis that focus on the genetic influences on response inhibition and ADHD.

Endophenotypes

Similar as the ADHD phenotype, response inhibition performance is highly heritable (estimated heritability of SSRT between 30% and 50%; (55; 56)), and the presence of response inhibition deficits is associated with ADHD diagnoses within families (57). Response inhibition has therefore been considered as one of the main endophenotypes for ADHD (55; 58–60).

Endophenotypes are heritable biomarkers that are more closely related to the genetic underpinnings of a disorder than the disorder itself (61) and may facilitate the search for causal genetic variants of a disorder (62), by acting as an intermediate level of description on the causal pathway between genetic variants and the disease phenotype (see also (63) for a critical review of the endophenotype concept).
Given the heterogeneous and complex nature of the ADHD phenotype, the use of response inhibition as an endophenotype may allow us to scaffold the causal pathways from genetic variants via neurobiological alterations to behavioral deficits in ADHD. The current thesis tried to unravel a part of the causal nature of ADHD by following the pathway from disease phenotype to the neural correlates of response inhibition, behavioral response inhibition performance, and finally the genetic underpinning of these neural and behavioral measures.

Importantly, the pattern of familial transmission assumed by endophenotype models is that unaffected siblings of probands with ADHD, which share on average 50% of the genetic risk factors present in the probands, also differ on the endophenotypic measures as compared to healthy controls. Apart from the primary requirement of differentiating between patients and controls, a less strict requirement of the endophenotype model is that siblings show on average intermediate levels of impairment on the endophenotypic measure, due to the dose effect of genetic risk factors, even in the absence of ADHD symptoms. The sample used in the current project therefore consisted of probands with ADHD, as well their unaffected siblings. This greatly increases the possibilities to investigate the familial nature of a biomarker, and has been a central point in the current thesis.

**Description of our sample**

The research sample described in this thesis is part of the NeuroIMAGE project; the Dutch follow up of the International Multicenter ADHD Genetics (IMAGe) cohort study. For the NeuroIMAGE project, 331 families with at least one proband with ADHD and one sibling, as well as 153 control families, were tested as part of this follow up. Families were invited at one of two test sites at the VU Amsterdam or University Medical Centre Nijmegen, where they performed an extensive battery of neuropsychological tests, as well as a range of questionnaires, a diagnostic interview, genetic testing and, in case of the children, structural and functional MRI scans. The sample for the current thesis was based on those participants who performed the stop-task within the fMRI scanner. Detailed recruitment, diagnostic and testing procedures for the entire sample have been described in the main methods and design paper for the NeuroIMAGE project (64) and can also be found at www.neuroimage.nl.

**Aims of this thesis**

The aims of this thesis were twofold. First, we aimed to expand our understanding of the neural correlates of response inhibition in ADHD. Therefore, we investigated both neural activation and functional connectivity during response inhibition within the NeuroIMAGE
sample. This allowed us to determine potential neural alterations within adolescents and young adults with ADHD, while the inclusion of the unaffected siblings of probands with ADHD in this design ensured that we could investigate the familial nature of these neural alterations. Additionally, we were able to investigate the added value of neural activation and connectivity measures as an endophenotype for ADHD relative to the behavioral performance on the task.

The second aim of this thesis was to further investigate the genetic basis of the neurobiological correlates of response inhibition. Specifically, we assessed the influence of several risk genes from both the dopamine and serotonin pathways on fMRI activation during the stop-task. This allowed us to examine the influence of these risk genes on the neural activation measures during response inhibition. Further, it enabled us to determine whether the neural alterations within probands with ADHD and their unaffected siblings were linked to variance within these genes.
Chapter Outline

Chapter 2: In the first chapter of this thesis we explored the differences in stop-task performance within a large sample of adolescents with ADHD, their unaffected siblings and healthy controls. Additionally, we used fMRI to measure the neural activation differences during response inhibition, and the alterations in neural activation in subjects with ADHD. By incorporating the unaffected siblings we could subsequently also investigate the familial nature of both behavioral and neural measures and response inhibition. Furthermore, we investigated if the differential neural activation between diagnostic groups is predictive of either task performance or ADHD severity.

Chapter 3: In the second chapter of this thesis we returned to the differences in neural activation between probands with ADHD, unaffected siblings and controls. We used the nodes which showed maximally dissociate activation as seed regions for a psycho-physiological interaction analysis, thereby uncovering the patterns of neural connectivity underlying response inhibition. Here too we could investigate the alterations in neural connectivity underlying response inhibition in ADHD, as well as the familial nature of these alterations and the availability of potential compensatory methods in unaffected siblings. We also investigated if neural connectivity patterns are predictive of task-performance and ADHD severity.

Chapter 4: In the third chapter of this thesis, we investigated the association between several catecholamine regulation gene variants, response inhibition and ADHD. Specifically, we employed a whole-brain, hypothesis free analysis method to investigate the effect of DAT1 and COMT variants on neural activation, additionally, we investigated if these variants were associated with ADHD diagnosis, stop-task performance, and the neural activation differences we described in chapter one.

Chapter 5: In the fourth chapter of this thesis, we investigated the association between several serotonin genes, response inhibition and ADHD. Here, we investigated the role of the 5HTT and HTR1B variants on whole-brain neural activation during response inhibition. Additionally, we investigated the role of these variants in predicting ADHD diagnosis, response inhibition performance and the neural activation differences as described in chapters one.

Chapter 6: In the discussion chapter, we reiterated the main findings of this thesis, and interpreted them within the relevant theoretical frameworks. We interpret the contribution of this thesis within the bigger framework of ADHD research in neuroscience, and look at some future research and applications.
References


