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Neural mechanisms of reward processing in attention-deficit/ hyperactivity disorder

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Neural mechanisms of reward processing in attention-deficit/ hyperactivity disorder

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Chapter 1

General introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common, neurodevelopmental disorder that occurs early in life and is characterized by enduring problems in the ability to focus attention and to regulate impulsivity and motor activity (American Psychiatric Association 2013). Because ADHD impairs the individuals' personal, social and professional functioning, it is a significant problem for the quality of life of affected persons as well as for society, amongst other things because it has to pay the costs for treatment as it has to pay the costs for treatment. Experimental cognitive research in this field has robustly demonstrated that participants with ADHD have deficient executive functions (Willcutt et al. 2005). Only recently, deficits were also shown for reward-related processes providing other plausible accounts for the emergence of ADHD core deficits as impulsivity or distractibility (Luman et al. 2010). Studies on the neural processes underlying these reward-related deficits were mainly done in adult participants with ADHD and consistently reported deficient signaling in reward processing brain circuits. For young participants with ADHD, however, only a few studies with small sample size exist and empirical findings are inconsistent. Therefore, the overall aim of this thesis is to examine the behavioral and neural processes involved in the processing of reward in a large population of young participants with ADHD, providing additional clues about the neurobiological basis of ADHD.

Background: definition, prevalence, and clinical presentation of ADHD

With an estimated prevalence of 5% worldwide, ADHD is one of the most common psychiatric disorders (Polanczyk et al. 2007). According to the fourth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-4 (American Psychiatric Association 2000)), the reference manual for diagnostic classification for mental health professionals and researchers at the time the study was designed and conducted, it is characterized by an enduring (i.e. more than 6 months) pattern of symptoms of inattention, hyperactivity and impulsivity. Examples of such symptoms are for instance increased distractibility, difficulty to wait one's turn or to remain seated when one is expected to do so. Moreover, in order to be diagnosed with ADHD, symptoms need to be present in at least two domains of personal life (e.g. familial life, school performance and social relations) and lead to a reduction in quality of functioning in these domains. To meet classification criteria, ADHD symptoms have to be present early in life (i.e. by the age of 7). Depending on the kind of symptoms, DSM-4 distinguishes between, three different subtypes: the predominantly inattentive type (ADHD-I), predominantly hyperactive/impulsive type (ADHD-H), and the combined subtype (ADHD-C).

During the writing process of this thesis, the DSM has been replaced by a more recent version (DSM-5) and with it some criteria have been updated. This thesis therefore follows the currently valid DSM-5. Main differences between DSM-4 and -5 criteria are that the age of onset criterion has been extended from 7 to 12 years, and the replacement of 'subtypes' by 'presentations.' The latter change was introduced because clinical subtypes

have not proven to be valid categories that were stable across time. Symptoms may persist into adulthood. However, reported persistence of the disorder varies, depending on the definition of persistence, but it is with 20 - 40% overall high (Faraone et al. 2006). Box 1 displays a summary of the DSM-5 classification of ADHD.

BOX 1. DSM-5 diagnostic criteria for attention-deficit/hyperactivity disorder

A.
(1) Six or more symptoms of **inattention** for children up to age 16, or five or more for adolescents 17 and older and adults; symptoms of inattention have been present for at least 6 months, and they are inappropriate for developmental level:

Inattention
a Often fails to give close attention to details or makes careless mistakes in schoolwork, at work, or with other activities.
b Often has trouble holding attention on tasks or play activities.
c Often does not seem to listen when spoken to directly.
d Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (e.g., loses focus, side-tracked).
e Often has trouble organizing tasks and activities.
f Often avoids, dislikes, or is reluctant to do tasks that require mental effort over a long period of time (such as schoolwork or homework).
g Often loses things necessary for tasks and activities (e.g. school materials, pencils, books, tools, wallets, keys, paperwork, eyeglasses, mobile telephones).
h Is often easily distracted
i Is often forgetful in daily activities.

(2) Six or more symptoms of hyperactivity-impulsivity for children up to age 16, or five or more for adolescents 17 and older and adults; symptoms of hyperactivity-impulsivity have been present for at least 6 months to an extent that is disruptive and inappropriate for the person's developmental level:

Hyperactivity
a Often fidgets with or taps hands or feet, or squirms in seat.
b Often leaves seat in situations when remaining seated is expected.
c Often runs about or climbs in situations where it is not appropriate (adolescents or adults may be limited to feeling restless).
d Often unable to play or take part in leisure activities quietly.
e Is often "on the go" acting as if "driven by a motor".
f Often talks excessively.

Impulsivity
g Often blurts out an answer before a question has been completed.
h Often has trouble waiting his/her turn.
i Often interrupts or intrudes on others (e.g., butts into conversations or games).

In addition, the following conditions must be met:
B Several inattentive or hyperactive-impulsive symptoms were present before age 12 years.
C Several symptoms are present in two or more setting, (e.g., at home, school or work; with friends or relatives; in other activities).
There is clear evidence that the symptoms interfere with, or reduce the quality of, social, school, or work functioning.
D The symptoms do not happen only during the course of schizophrenia or another psychotic disorder. The symptoms are not better explained by another mental disorder (e.g. Mood Disorder, Anxiety Disorder, Dissociative Disorder, or a Personality Disorder).

Based on the types of symptoms, three kinds (presentations) of ADHD can occur:

– Combined Presentation: if enough symptoms of both criteria for inattention and hyperactivity-impulsivity were present for the past 6 months.
– Predominantly Inattentive Presentation: if enough symptoms of inattention, but not hyperactivity-impulsivity, were present for the past six months.
– Predominantly Hyperactive-Impulsive Presentation: if enough symptoms of hyperactivity-impulsivity but not inattention were present for the past six months.

As the criteria in Box 1 illustrate, the diagnosis of ADHD is completely based on behavioral observations and reports, which may result in subjective interpretations and misestimations. An observer has to estimate the age-dependent appropriateness of the behavior although there is no established golden standard for this. Unless an observer is extensively trained in such estimations, which would require training and produce additional costs, behavior is commonly evaluated on the basis of one's experience. This may create various forms of bias as for instance a teacher, who mainly sees typically developing children, may classify a vibrant child as clinically impulsive, whereas a child psychiatrist, who is continuously confronted with severe cases of ADHD, may classify the same behavior as within normal range. On top of that, the observer is dependent on how an individual presents him/herself at the moment of assessment. It might be that situational (e.g. a busy day) or intentional factors (e.g. individual wants to get treatment) influence the behavior of the observed person.

Even if the diagnostic procedure would provide a comprehensive and unbiased description of the symptoms, problems may also arise from the unified diagnostic category. The goal of the diagnostic procedure is to classify observed behavior as being either healthy or pathologic. By reducing observed behavior to a dichotomous classification, information is lost about the specific pattern of symptoms that lead to the individual diagnosis. This is problematic as different patterns of symptoms are mapped to the same diagnosis. Different symptoms, however, might be caused by different functional deficits and consequently, the clinical category ADHD is very unspecific and heterogeneous at the clinical and etiologic level.

An improved approach to classify participants would be to start conceptualizing the disorder in terms of affected functional systems that relate to the observed behavior. By doing so we could develop objective measures, that can be used for diagnostic purposes and that ideally also inform about its causation, which could improve therapeutic interventions.

Comorbidity of ADHD

On top of the previously described behavioral symptoms, ADHD is characterized by the high rate of comorbidity. This refers to the co-occurrence of two or more disorders at the same time in the same person, leading in most cases to more severe functional impairments and a worse outcome (Gillberg et al. 2004). Several studies have estimated that 60-100% of the ADHD population presents one or more comorbid disorders. The most common among these are; developmental coordination disorder (DCD; present in 50% of the population), depression (16-26%) and anxiety disorders (~12%), oppositional-defiant disorder (ODD; 30-60% (Biederman et al. 2007)), conduct disorder (CD; 30% (Jensen et al. 1997)) and autism spectrum disorder (ASD; 65-80% (Gillberg et al. 2004; Rommelse et al. 2011)). Also bipolar disorder, tic disorders, obsessive-compulsive disorders (OCD), substance use

disorders (SUD), personality disorders and language disorders (50% delayed onset of language) have been found at a higher rate in ADHD (see (Gillberg et al. 2004) for an overview).

Among all these comorbidities, ASD and ADHD have a special relation. At the clinical level, key symptoms defining both disorders create the impression that both disorders differ largely: ASD is characterized by severe problems in social interaction and verbal and non-verbal communication and rigid behaviors, and ADHD by problems in inattention and hyperactivity/impulsivity. However, other clinical indicators suggest a very close relationship between the two disorders: they are co-occurring very often (Rommelse et al. 2010) and are both considered to be neurodevelopmental disorders with onset early in life. Furthermore, it is a common observation that the clinical diagnosis alternates during development between both disorders (Fein et al. 2005). Moreover, findings from family and twin studies indicate shared genetic underpinning of both disorders (for review see (Rommelse et al. 2010)). By investigating ADHD and comorbid ASD at the same time, one might unravel whether the different disorders have a causal relationship, share cognitive and neural mechanisms or are independent of each other (Banaschewski et al. 2007; Rommelse et al. 2011).

Heritability of ADHD

Evidence from twin, adoption and family-genetic studies estimate the heritability of ADHD to be as high as 80% (Faraone and Mick 2010). This has led to many molecular genetic studies aiming at identifying specific genes that can be linked to ADHD. Based on the assumption that ADHD with its high prevalence is caused by common genetic variants most of these studies were gene association studies. While hypothesis-free genome wide approaches did not have enough power to associate genetic variants with ADHD (Franke et al. 2009), hypothesis-driven candidate gene approaches reported effects for common gene variants such as DAT1 and DRD4 (Gizer et al. 2009). Effects of these candidate genes on ADHD were small, which is in line with the hypothesis that ADHD is a multifactorial, polygenetic disorder (Franke et al. 2009).

Besides this, hypothesis-free approaches have recently also assessed rare gene variants. Such gene variants include chromosomal deletions or duplications known as copy number variants (CNV) or de novo mutations. CNVs that encompass relatively large genomic segments (>1 kb) were strongly associated with ADHD (Williams et al. 2010), interestingly in regions that overlap with those reported in participants with ASD (Thapar et al. 2012).

Despite these efforts, molecular genetic association studies have been proven complicated by the fact that genes interact with each other and with environmental factors. Moreover, it is known that environmental factors can change genetic expression in a dynamical way (i.e. epigenetics (Mill and Petronis 2008)). Therefore, alternatives to these association studies have been proposed. One alternative approach is the identification of endophenotypes. Endophenotypes (or intermediate phenotype) are heritable, quantifiable

traits that share genetic variance with the disorder and are more closely related to the neurobiological and genetic underpinning of the disorder than its symptoms (Gottesman and Gould 2003). Based on their hypothesized genetic predisposition, endophenotypes are expected to be present in non-affected family members (albeit to a lesser extent) who share on average 50% of the genes with affected family members. Cognitive measures and neural metrices that functionally relate to these cognitive measures are prominent candidates for endophenotypes of ADHD.

Cognitive deficits in ADHD

Besides the classification of ADHD based on the description of behavioral symptoms, numerous studies have characterized specific impaired cognitive functions in participants with ADHD. Such cognitive functions can be assessed by specific, and sometimes, experimental tasks. Such assessments have not only the advantage that they are quantitative and objective, but in addition, due to the fact that they break down complex behaviors to specific cognitive processes, they might help to better understand the emergence of those behaviors.

Given that the clinical presentation of ADHD include core symptoms as distractibility and impulsivity, research on cognitive functions associated with the disorder has focused on attention and inhibition processes (see e.g. (Barkley 1997; Willcutt et al. 2005)). These cognitive functions, hypothesized to quantitatively assess clinical symptoms of ADHD, included mental operations such as maintaining information in working memory, maintaining attention during prolonged periods (i.e. vigilance), planning and organization, switching between different cognitive tasks (i.e. set-shifting) and inhibition of a prepared motor responses (i.e. response inhibition). Together, they are also known as aspects of executive functions (EF).

Scientific efforts have led to the identification of deficits on all of these EFs (e.g. (Sergeant 2004; Willcutt et al. 2005)) in ADHD patients. The most consistent evidence hints at deficits in response inhibition as most participants with ADHD perform more poorly than healthy controls on stop-signal reaction tasks (SSRT). During this task participants are asked to respond as quickly as possible with a button press to a stimulus (i.e. response signal). Incidentally, an additional stimulus can occur with some latency (i.e. the stop signal) indicating the need to interrupt the response. While participants with ADHD are able to interrupt the planned motor response if the stop signal immediately follows the response signal, they are unable to inhibit their response for longer delays between response and stop signal. Apart from this response inhibition deficit, participants with ADHD have been found to consistently exhibit vigilance deficits as indicated by increased errors during a continuous performance task. There is also some evidence for planning problems, as indicated by an increased number of turns participants with ADHD require to solve the tower of Hanoi problem, and impaired visual and verbal memory.

The problem of EF tasks, however, is that they only explain a small amount of variance between ADHD participants and healthy participants (Willcutt et al. 2005), which becomes obvious when comparing effect sizes of between-group EF measures (d ~ .4–.6; (Cohen 1988)) with effect sizes of between-group symptom counts (d ~ 2.5–4.0). This fact inspired researchers to reconsider the role of EF deficits as the primary cognitive deficits of ADHD. The current view is that ADHD is characterized by deficits in different cognitive domains. Besides executive functions, deficits are observed for reward processing (Luman et al. 2010), temporal processing and timing (Toplak et al. 2005), speech and language (Tomblin and Mueller 2012), memory span, processing speed, response time variability (Kuntsi and Klein 2012), arousal/activation (Fair et al. 2012) and motor control tasks (Fliers et al. 2009). On top of that, different cognitive domains have been shown to interact with each other. For example, EF deficits in participants with ADHD have been found to depend on how performance was rewarded, suggesting that deficits in executive functions might be caused by motivational processes (Aarts et al. 2015).

From all these deficits in cognitive functioning, described in ADHD patients, behavioral and neural aspects of reward processing are of particular interest. Reward processing plays a central role in everyday behavior and has been linked to a relatively well-outlined neural circuitry. However, although the concept of reward processing and its neural correlates have been widely studied in healthy individuals, the implication of reward processing in ADHD patients is relatively unclear. Therefore, this thesis contributes to elaborating our knowledge of the reward-related neurobiological mechanisms underlying ADHD.

Unraveling reward processing

Reward processing is a central aspect of human behavior and refers to all processes that regulate behavior on the basis of reward. According to current views reward has three core principles: learning, motivation and affect (Berridge and Robinson 2003). Reward can be primary as for instance receiving food, but also secondary such as receiving monetary incentives.

First, the learning aspect of reward is necessary to establish relationships between actions or states and their consequences (i.e. Pavlovian learning) and the contingencies of these consequences (i.e. instrumental learning). Second, reward motivates the individual to act in favor of the reward. Third, reward consumption often elicits a hedonic or affective response in the rewarded individual. Accordingly, our behavior is highly dependent on the ability to establish proper predictions of when and where rewards are to be expected, the utilization of these predictions during decision-making and the amount of pleasure experienced when receiving a reward (Robbins and Everitt 1996). In this dissertation the focus will be on these last two aspects of reward processing (Chapter 3, 4, and 6).

In ADHD, poor behavioral performance on reward-related tasks is a typical finding, which has frequently been demonstrated. For example with increasing time children with ADHD show quicker devaluation of monetary rewards (i.e. temporal discounting; (Scheres et al. 2008)) and prefer immediate rewards above delayed reward even if the delayed reward is associated with a higher reward (delay aversion (Sonuga-Barke et al. 2008); for review see (Johansen et al. 2009)). Children with ADHD also seem to make suboptimal choices leading to decreased gains (Toplak et al. 2005) and higher losses (Drechsler et al. 2008). Moreover, they exhibit impaired reinforcement learning as indicated by displaying overly perseverative responses (making more errors) when these responses are not reinforced anymore (extinction) or are even punished (reversal learning) (Itami and Uno 2002). Finally, participants with ADHD do show motivation-related improvements on behavioral performance, which is not or less present in unimpaired children ((Uebel et al. 2010); for review see (Luman et al. 2005)). Interestingly, this increased sensitivity to reward in participants with ADHD is also to a lesser extend present in unaffected siblings, suggesting that reward processing is also an endophenotype (Uebel et al. 2010). Nevertheless, it has to be noted that some studies fail to replicate behavioral changes of reward processing in participants with ADHD. For example, no group differences have been reported in studies measuring decision-making or delay aversion ((Geurts et al. 2006; Solanto et al. 2007; Sjöwall et al. 2012); for review see (Luman et al. 2010)). One explanation for such inconsistencies is that studies use different study designs to assess reward processing. Experimental tasks and their specific parameters may affect the sensitivity with which they detect dysfunctional cognitive functions. Another explanation relates to the used analytical methodology. As Coghill and colleagues demonstrated ADHD is on a groups-level associated with a large variety of cognitive deficits including EFs as working memory and inhibition, reward processing and timing and variability (Coghill et al. 2014). Individuals, however, differ in what kind of deficits they display. Most participants with ADHD showed deficits in one or two cognitive domains, some have no deficits at all and very few have deficits in all domains. This observation suggests that ADHD is heterogeneous at the neurocognitive level.

The neuroscience of reward processing

Animal studies and studies in humans have linked reward processing to dopaminergic brain circuits located in frontal and subcortical regions of the brain (for review, see (Cools 2008) or (Haber and Knutson 2010)). These circuits mainly consist of mesolimbic projections connecting the ventral tegmental area (VTA) with the ventral striatum (VS; its main structure is the nucleus accumbens (NAcc)) and mesocortical projections connecting the VTA with the prefrontal cortex (PFC). Mesolimbic signals are also transmitted to the PFC via two pathways: 1) the direct (striato-nigral) pathway, which is a connection of the striatum with the internal globus pallidus and thalamus, and 2) the indirect (striato-pallidal) pathway that has an extra loop between globus pallidus externalis and subthalamic nucleus.

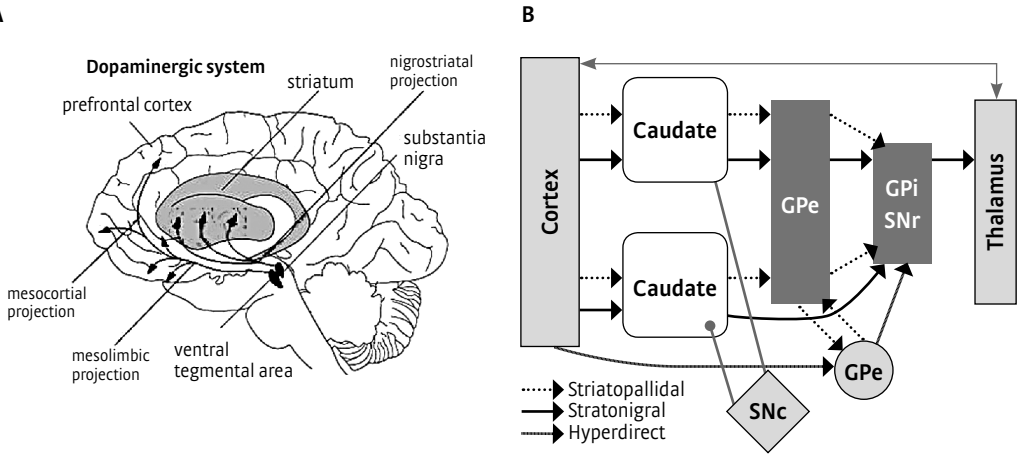

FIGURE 1. A) Main dopaminergic projections (in red) of the mesolimbic, mesocortical and nigrostriatal pathways (adapted from Cools, 2008) B) Schematic of the corticostriatal pathways. GPe: Globus pallidus external; GPi: Globus pallidus internal; SNr: Substantia nigra pars reticular; STN: Subthalamic nucleus; SNc: Substantia nigra pars compacta (adapted from Frank et al., 2011)

The two most crucial structures within these pathways for investigating the motivational and affective reward processes are the VS and orbitofrontal cortex (OFC; i.e. the most important reward-processing structure within the PFC).

Several lines of evidence have revealed the specific function of these structures in the context of each reward process. For example, non-invasive studies in humans using functional magnetic resonance imaging (fMRI; see Box 2) have found the VS and OFC to respond when participants received different rewards such as money, food and juice, indicating the role of these regions in affective reward processing (for review see (Sescousse et al. 2013) (McClure et al. 2004)).

BOX 2 Neuroimaging techniques

1) Functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) is a neuroimaging technique that allows mapping cognitive functions to brain structures at a whole brain level. It is a non-invasive technique that makes use of the fact that oxygenated blood has different magnetic characteristics than deoxygenated blood. When a brain region becomes active it will consume more oxygen than non-active regions. In order to restore the baseline level of oxygen, oxygenated blood will flow to the activated region. This change in oxygen levels is the blood-oxygen level dependent (BOLD) signal measured using fMRI.

Task-based fMRI
Task-based fMRI combines fMRI with the presentation of a specific task, for example a cognitive task. By synchronizing both measures, the effect of experimental manipulations on cognition can be correlated with neural signals.
The BOLD-signal is measured for the whole brain with a resolution in the cubic millimeter range, making it a good means to investigate neural activity in the spatial domain. However, there are downsides to the technique. The BOLD-signal, which is measured with fMRI, reflects a hemodynamic response to the neural activation, which is

elicited by a task manipulation. This change in neural blood flow has a delay of several seconds and peaks after 4-6 seconds, which limits the temporal resolution of the technique (Cohen and Bookheimer 1994); see Figure 2).

The most common analytical approach is to estimate the group-wise average BOLD response for a certain experimental condition and contrast it with another experimental group or condition. This can be done for an a-priori defined region (hypothesis-driven region of interest (ROI) analysis) or at the whole brain level (voxel-wise).

Resting state FMRI
Resting state fMRI (rs-fMRI) differs from task-based fMRI by being a measure of the brain at rest. Because no task-demands elicit neural changes at dedicated locations, targeted measure of this technique is the temporal synchronicity of the fluctuating neural activation. The basic assumption behind this technique is that regions with highly synchronized time courses communicate with each other, forming functional neural networks. Accordingly, opposed to task-based fMRI studies that focus on localizing cognitive function in local neural structures, this technique aims at investigating the network structure underlying cognitive functions (Poldrack 2011).

Commonly, rs-fMRI studies apply a seed-based analytical approach. This is a model-based approach meaning that a-priori an initial (seed) region is selected, for which the correlation with the time course of another (target) region of the brain is calculated. A target region may be another region with a-priori expected relationship (ROI) but can also be every voxels of the brain. Model-free approaches also exist such as independent component analysis (ICA). ICA is a blind-signal separation method. Based on the assumption that FMRI data is a composition of different mixed signals, it decomposes data based on their temporal synchronicity into independent components. Time courses of these components can subsequently be used to investigate whole-brain functional connectivity or communication between networks.
Other analytical approaches include e.g. the analysis of fluctuation amplitudes in the low-frequency band (0.01-1 Hz), assessment of homogeneity within local regions or clusters, analysis of functional connectivity connection density (FCDC; number of connections of a single voxel with other regions of the brain) and graph-theory based approaches (Oldehinkel et al. 2013).

2) Structural MRI and diffusion tensor imaging (DTI)

Structural MRI
Structural MRI is a magnetic resonance imaging (MRI) technique that provides information about the brains' anatomy. Based on their specific magnetic characteristics this technique is able to distinguish between different tissues in the brain, including grey matter (mainly cell bodies), white matter (long-range nerve fibers) and cerebral-spinal fluid (CSF).
On top of volumetric measures, common analytical approaches involve morphometric techniques that assess local changes of grey matter density (e.g. voxel-based morphometry; (Ashburner and Friston 2000)) and the surface area of the cerebral cortex (Fischl and Dale 2000).

Diffusion tensor imaging
Diffusion tensor imaging (DTI; (Pierpaoli et al. 1996)) is a technique that measures the diffusion characteristics of water molecules in brain tissue. Because the movement direction of water molecules is restricted for example by myelinated cell membranes, this technique is useful for imaging the fibrous organization of white-matter tracts. Several measures can be derived including mean diffusivity (MD) but also the directionality of white matter fibers (i.e. fractional anisotropy (FA)). In addition, large fiber bundles can be reconstructed to investigate white-matter connectivity of the brain (i.e. tractography) (Jones and Pierpaoli 2005).

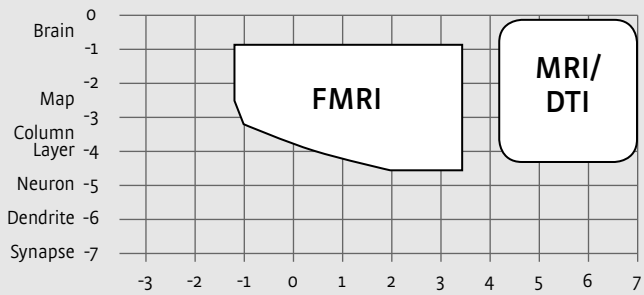

FIGURE 2 Overview of different neuroscience techniques with their associated temporal and spatial resolution as reviewed in this thesis in the context of ADHD (Courtesy of Christian Beckmann).

For motivational aspects of reward, animal studies have shown that dopaminergic neurons in the VS play a vital role (Bromberg-Martin et al. 2010). These neurons demonstrate constant (tonic) firing, which can be modulated by phasic increases and decreases. Firing of these neurons increases with unexpected primary rewards such as food and water, suggesting that firing reflects a reinforcement learning signal (Schultz et al. 1997). Moreover, signaling of these neurons is crucial for incentive motivation or 'wanting'. Incentive motivation refers to the attribution of motivation to an initially neutral stimulus, which promotes reward seeking (Berridge and Robinson 1998). As brain stimulation studies or pharmacological studies show, the more these neurons are stimulated the more "approach behavior" an animal will demonstrate (Wise 2004).

Empirical evidence has demonstrated that BOLD signal changes in the VS share many characteristics with dopamine neurons in the mesolimbic brain circuit. For example, increases of the BOLD response have selectively been found in the VS when participants observed reward-predicting cues (Knutson et al. 2001) or received unexpected rewards (Delgado et al. 2000). Moreover, the VS has been found to code for reward magnitude and probability, two basic parameters to estimate the value of an expected reward (Knutson et al. 2005; Yacubian et al. 2006). Together, these studies indicate that BOLD signal changes in the VS reflect changed firing of dopaminergic neurons in the same structure.

One widely used paradigm for studying reward processing is the monetary incentive delay (MID) task (see Figure 3). In this task participants have to respond with a motor response to the presentation of a target stimulus within a short time interval. The target is preceded by a cue that signals the possibility to gain a reward after successful (i.e. fast enough) response to the target. At the end of each trial, the outcome of the response and the gain for the whole task is presented.

This task allows to reliably elicit neural responses in reward-processing neural structures to the prediction of a rewarded stimulus (i.e. reward anticipation) and the affective response to its receipt, two key aspects of reward anticipation (Fairchild 2011).

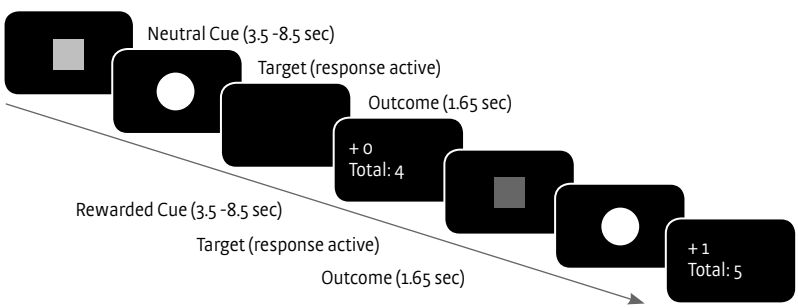

FIGURE 3 Illustration of the monetary incentive delay (MID) task as used in this dissertation (Chapter 3,4, and 6). Color of the cue indicate the possibility to gain a reward (■ = reward; ■ = neutral) after successful responses (in-time button press). Feedback indicates accuracy (0/+1) and total number of hits (20 cents per hit).

Neural correlates of reward processing in ADHD

Neuroimaging studies of reward processing in ADHD can be divided into three different types: structural imaging studies, functional imaging studies and imaging of the brain at rest (see Box 2).

Structural MRI studies in children with ADHD have reported reductions of grey matter volume in core brain regions associated with reward processing including the prefrontal cortex (Valera et al. 2007; Frodl and Skokauskas 2011) and the ventral striatum (Carmona et al. 2009) suggesting that functional impairments in ADHD may also be reflected by structural changes in reward-critical structures. However, to our knowledge, no DTI study has investigated the related question whether white matter tracts that connect the key neural structures of the reward-processing network are affected by ADHD.

FMRI studies provide evidence for functional changes in regions that are critical for reward processing. Most of these have been conducted in adults with ADHD using the MID task. These studies quite consistently reported attenuated brain responses in the ventral striatum during reward anticipation (see (Plichta and Scheres 2014) for review). One other study using the same paradigm reported additional increased responses of the OFC during reward receipt (Ströhle et al. 2008). Some studies investigated reward processing using different experimental tasks as for instance an intertemporal choice task (Plichta et al. 2009) or a classical conditioning paradigm (Furukawa et al. 2014), and replicated lower responses of the VS in ADHD. Still another study administered a card guessing task and found that the OFC in participants with ADHD was insensitive to different outcome values (Wilbertz et al. 2012). In the same OFC region decreased responses were reported in participants with ADHD using a rewarded continuous performance task (Cubillo et al. 2012).

Two studies applied the MID paradigm to study reward anticipation in adolescents with ADHD (Scheres et al. 2007; Kappel et al. 2014). One study replicated the results observed in adults and reported lower ventral striatal responses during reward anticipation in ADHD relative to controls (Scheres et al. 2007), the other reported no differences in this region (Kappel et al. 2014). Another study used a slightly different task to study reward anticipation and receipt (Paloyelis et al. 2012). In that task a cue indicated whether participants were rewarded or punished after a correct response consisting of a correct semantic classification of a picture depicting an either living or non-living stimulus. This study reported during reward anticipation the same ventral striatal response in participants with ADHD and healthy participants, during reward receipt increased responses of the VS in participants with ADHD were observed. Increased responses have also been reported in the VS for inescapable delays (Lemiere et al. 2012), a study with a small sample size (N=20), and in the OFC for reward during a rewarded continuous performance task (Rubia et al. 2009).

There are also some studies with larger sample size investigating functional integration using rs-FMRI in adolescents with ADHD. These studies found in young participants with ADHD relative to healthy controls weaker connectivity between VS and OFC (N=44,(Posner et al. 2013)). Opposed to this, other studies have reported increased synchronicity (N=100;(Costa Dias et al. 2013)) and connection density in children (N=551;(Tomasi and Volkow 2012)) between the VS and PFC.

Summary

To sum up, various imaging approaches indicate that ADHD is associated with changes in the neural system underlying reward processing. Structural studies showed volume reduction in the VS and PFC. As the exact functional implication of these volumetric differences is unclear, functional investigations of reward processing have been initiated. These efforts revealed consistent evidence for functional changes in adult participants with ADHD, mainly consisting of decreased brain responses to anticipated reward in the VS and aberrant signaling during receipt in the OFC. Signaling in the VS during reward anticipation has been argued to reflect either the predicted value of an expected reward (Schultz 2010) or incentive salience (Berridge and Robinson 2003), in both cases this suggests that participants with ADHD are impaired in correctly processing the motivational aspect of reward. Furthermore, responses of the OFC have been associated with signaling of reward values of stimuli in our environment (O'Doherty 2004; Sescousse et al. 2013), suggesting that adult participants with ADHD overreact to rewarded stimuli, which may result in imbalanced decision-making.

Evidence for neural changes underlying reward processing is less convincing for young participants with ADHD than for adults. Firstly, fewer studies have been conducted in young participants and reported findings are highly inconsistent with regard to where changes occur in associated neural structures and which direction these effects have (increases versus decreases). Secondly, those studies in young participants with ADHD that have been conducted tested smaller samples (the largest study in adults tested 136 participants (Hoogman et al. 2011) versus 68 in adolescents (Paloyelis et al. 2012)). Thirdly, most studies in young participants with ADHD only investigated reward anticipation. Consequently, more studies in young participants with ADHD are needed, investigating key aspects of reward processing, both as reward anticipation and also reward receipt. In addition, reported changes of functional connectivity in young participants with ADHD indicate that brain reward processing from a network perspective may provide additional cues about the neural changes underlying this disorder. Finally, as no study so far has investigated endophenotypic characteristics of neural measures underlying reward processing, familial studies are crucial to estimate the contribution of heriditary factors to these neural measures.

Outline, Aims and Hypotheses of this thesis

This thesis presents the NeuroIMAGE study, a prospective phenotypic, cognitive, genetic and MRI study in young participants with ADHD. Central elements of the thesis are four published research articles that shed light on different aspects of reward processes in ADHD. The first research article answers the question whether we can segregate neural processes during anticipation and receipt of reward that are altered in participants with ADHD and whether such measures are valid endophenotypes (Chapter 3). The second research article describes these reward-related neural processes from a network perspective and investigates, whether participants with ADHD show deficient functional integration of neural networks implicated in reward processing (Chapter 4). The third article shows whether the generic functional architecture of reward-related, attentional and motor control networks contribute to the functional neural changes observed in ADHD (Chapter 5). The final article addresses the question whether there is evidence for reward-related neural processes that are specific to either symptoms of ADHD, or symptoms of autism, or a combination of both disorders (Chapter 6).

The overall aim of the thesis is to expand our knowledge of the behavioral and neural correlates underlying reward processing in adolescents and young adults with ADHD and address inconsistencies reported in the literature.

My specific aims were:

1 To introduce the study sample, the NeuroIMAGE cohort, which was used to investigate neural and cognitive deficits in ADHD families (**Chapter 2**). Specifically, quality of the FMRI data was assessed in terms of in-scanner movement and strength of the BOLD-signal across sites. I hypothesized that our quality assurance policy would result in high data quality.

2 To investigate segregated neural processes implicated in reward processing in young participants with ADHD (**Chapter 3**). Specifically, I aimed to reconcile inconsistencies in the literature by investigating reward processing in a large sample of participants with ADHD and healthy participants. I hypothesized to find altered reward-related responses in frontostriatal brain circuits.

3 To investigate whether reward processing is an endophenotype of ADHD (**Chapter 3**). I expected to find alterations of neural firing in frontostriatal regions that were intermediate between those of participants with ADHD and healthy participants.

4 To explore the functional integration of reward-related brain networks including characteristics such as functional connectivity and network communication in healthy participants and in the context of ADHD by applying a network discovery approach on task-based fMRI data (**Chapter 4**).

5 To describe the functional architecture underlying reward processing and other aspects of behavioral control, and to understand the association between these neural networks and ADHD (**Chapter 5**). I expected to find altered resting state functional connectivity to be associated with ADHD, particularly for the ventral frontostriatal network.

6 To investigate the influence of comorbid traits of ASD on neural correlates of reward processing (**Chapter 6**). I hypothesized that the neural response to reward would be altered specifically for each disorder, with alterations in the frontostriatal circuit and insular cortex to be related to ASD and decreased responses in the VS to be related to ADHD.

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Chapter 2

The NeuroIMAGE study: a prospective phenotypic, cognitive, genetic and MRI study in children with attention-deficit/ hyperactivity disorder. Design and descriptives.

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Abstract

Background Attention-deficit/Hyperactivity Disorder (ADHD) is a persistent neuropsychiatric disorder which is associated with impairments on a variety of cognitive measures and abnormalities in structural and functional brain measures. Genetic factors are thought to play an important role in the etiology of ADHD.

Methods The NeuroIMAGE study is a follow-up of the Dutch part of the International Multicenter ADHD Genetics (IMAGE) project. It is a multi-site prospective cohort study designed to investigate the course of ADHD, its genetic and environmental determinants, its cognitive and neurobiological underpinnings, and its consequences in adolescence and adulthood. From the original 365 ADHD families and 148 control (CON) IMAGE families, consisting of 506 participants with an ADHD diagnosis, 350 unaffected siblings, and 283 healthy controls, 79% participated in the NeuroIMAGE follow-up study. Combined with newly recruited participants the NeuroIMAGE study comprehends an assessment of 1069 children (751 from ADHD families; 318 from CON families) and 848 parents (582 from ADHD families; 266 from CON families). For most families, data for more than one child (82%) and both parents (82%) were available. Collected data include a diagnostic interview, behavioral questionnaires, cognitive measures, structural and functional neuroimaging, and genome-wide genetic information.

Conclusions The NeuroIMAGE dataset allows examining the course of ADHD over adolescence into young adulthood, identifying phenotypic, cognitive, and neural mechanisms associated with the persistence versus remission of ADHD, and studying their genetic and environmental underpinnings. The inclusion of siblings of ADHD probands and controls allows modeling of shared familial influences on the ADHD phenotype.

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder affecting about 5% of children and 3-4% of adults (Fayyad et al. 2007; Polanczyk et al. 2007). Its main characteristics are a pervasive pattern of inattention and/or hyperactive-impulsive behaviors that occur early in life and lead to impaired social functioning and educational and occupational achievements (American Psychiatric Association 2000). ADHD persists into adulthood in 15-60% of cases, depending on the definition of remission (Biederman 2000). Adoption and twin studies have indicated substantial involvement of genetics in the causation of ADHD, with additive heritability estimated around .70-.80 (Faraone et al. 2005). ADHD is a complex disorder, in which different combinations of genetic and environmental factors contribute to the overall risk of developing the disorder (Franke et al. 2009). The genetic model underlying most cases of ADHD is likely one in which multiple genetic factors of small to moderate effect size contribute to disease etiology. In a small number of cases rare genetic variants with moderate to strong effect size have been identified (Lionel et al. 2011; Williams 2012).

Much research has focused on cognitive and neural mechanisms underlying ADHD. One of the most consistent findings in cognitive ADHD research refers to deficient top-down executive functions such as response inhibition deficits (Willcutt et al. 2008). Other cognitive domains involved include reward processing (Luman et al. 2005) and temporal processing/response variability (Toplak et al. 2006). ADHD is also associated with various changes in brain structure and function. Structural changes of grey matter consist of a reduction of total brain volume in ADHD, with greatest reductions in frontal regions, the basal ganglia (caudate nucleus), cerebellum, and corpus callosum (Valera et al. 2007; Nakao et al. 2011; Frodl and Skokauskas 2011). Changes of white matter, as measured with diffusion tensor imaging (DTI), have most consistently reported alterations in the corona radiata, corpus callosum, internal capsule, and cerebellum (van Ewijk et al. 2012). Most functional imaging studies on ADHD have reported changes in fronto-striatal brain circuits, but changes in sensorimotor circuits and the default network have also been documented (Cortese et al. 2012). Converging evidence suggests that some of these abnormalities normalize due to stimulant medication use (Spencer et al. 2013).

Although various genetic, cognitive, and neural factors have been associated with ADHD, most evidence about these factors and their interplay is inconsistent (Scheres et al. 2001; Scheres et al. 2007; Gizer et al. 2009; Paloyelis et al. 2012). This may be explained by 1) the substantial clinical and etiological heterogeneity of ADHD; and 2) methodological differences in study design (i.e. instructions, task parameters) and analysis methodologies (e.g., outcome measures in brain imaging data analysis). Large sample sizes could resolve inconsistent findings and segment ADHD into more homogenous subgroups, which may allow dissection of the cognitive, neural, and genetic mechanisms involved in subtypes of ADHD.

Accordingly, several large-scale ADHD projects at the national and international level have been initiated, e.g. the International Multicentre persistent ADHD Genetics CollaboraTion (IMpACT; http://impactadhdgenomics.com/nl/) and ADHD-200 (http://fcon_1000.projects.nitrc.org/indi/adhd200/index.html). One of the first international programs on ADHD is the International Multicenter ADHD Genetics (IMAGE) project that has been designed to identify molecular-genetic factors involved in ADHD (Brookes et al. 2006; Rommelse et al. 2008a). The IMAGE project collected DNA and detailed information on the phenotype of ADHD and relevant comorbidities as well as site-specific cognitive performance of 5758 subjects from 1401 ADHD families in 8 countries in Europe and Israel between 2003 and 2007. This effort has led to candidate-gene studies (Brookes et al. 2006; Asherson et al. 2007), linkage (Asherson et al. 2007; Rommelse et al. 2008c; Zhou et al. 2008a) and genome-wide association analyses (Lasky-Su et al. 2008a; Lasky-Su et al. 2008b; Neale et al. 2008), meta-analyses (Zhou et al. 2008b; Neale et al. 2010) and several cognitive studies (Andreou et al. 2007; Rommelse et al. 2007b; Rommelse et al. 2008b). Beyond these efforts the Dutch site of the IMAGE project also collected cognitive measures on unaffected siblings of ADHD probands and of control children that allowed analyzing whether dysfunctions in such measures are familial and would qualify as an endophenotype of ADHD. The concept of endophenotype refers to quantitative and objective measures of (psychiatric) disorders that represent heritable vulnerability traits and are intermediate on the pathway from genotype to phenotype (Gottesman and Gould 2003; Rommelse et al. 2011). Moreover, because of their assumed heritability, a valid endophenotype should be found at a higher rate in unaffected family members than in the general population (Gottesman and Gould 2003).

Probands, siblings and healthy control subjects of the Dutch, German and Belgian sites of IMAGE were re-invited to participate in an intermediate follow-up study, focusing on substance-related disorders (Groenman et al. 2013a) as well as medication treatment (Groenman et al. 2013b). Approximately 6 years after original study entry at the Dutch site, an additional follow-up has been initiated, the NeuroIMAGE project (http://www.neuroimage.nl/), which is described in the current paper. NeuroIMAGE comprised re-evaluation of the ADHD phenotype and relevant comorbidities, repeated cognitive assessment and acquisition of functional and structural magnetic resonance imaging (MRI) of the brain, as well as phenotypic and cognitive assessments of the parents of affected and healthy participants. Together with all previous measures, the NeuroIMAGE study incorporates longitudinal cognitive and phenotypic data, information about genotype, neural structure and function, medication history as well as phenotypic family data for probands with childhood ADHD and normal developing children. As a result, the study is an invaluable resource for the examination of the course and consequences of ADHD from childhood over adolescence into adulthood, for the identification of cognitive and neural mechanisms associated with persistence versus remission of ADHD, and for the study of genetic and environmental factors involved. Assessing the phenotype and cognitive performance of parents can enrich our understanding of the risk of ADHD transmission through genes and the familial environment.

Methods

The Cohort

Original IMAGE Cohort (2003-2006)

Participants for NeuroIMAGE were selected from the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study, conducted between 2003 and 2006 (as described previously (Rommelse et al. 2008a; Nijmeijer et al. 2008; Müller et al. 2011a; Müller et al. 2011b)). In the Dutch part of IMAGE 365 families with at least one child with combined subtype ADHD and at least one biological sibling (regardless of ADHD diagnosis) were recruited, in addition to 148 control families with at least one child, with no formal or suspected ADHD diagnosis in any of the first-degree family members.

Intermediate Follow-up (2008-2009)

Here, the intermediate follow-up will be described in short (for a full description of the intermediate recruitment procedures see (Groenman et al. 2013a)). During the intermediate follow-up (2008-2009) probands, siblings and healthy control subjects and parents of the Dutch, German and Belgium sites of IMAGE were re-invited to participate on average 4.4 years (SD=.71) after original study entry. The complete cohort of the intermediate follow-up comprised 415 ADHD families (1001 children and 727 parents) and 141 control families (119 children and 253 parents). This resulted in a retention rate of 86.9% of original families during the intermediate follow-up.

NeuroIMAGE (2009-2012)

For NeuroIMAGE, all family members, including those who did not participate in IMAGE, were invited for follow-up measurement and (re)assessed between 2009 and 2012. The follow-up was conducted at two test sites: the VU University Amsterdam/VU University Medical Centre in Amsterdam and the Radboud University Medical Centre in Nijmegen. The time between the IMAGE and NeuroIMAGE measurements ranged between 3.5 and 8.9 with a significant longer interval between measurements for ADHD families (overall: 5.9 years (SD=.74); ADHD: 6.1 years (SD=.6); Controls: 5.4 years (SD=.7); $F(1, 401) = 106$; $p<.001$)). Additionally children with ADHD (foremost girls) and healthy control boys were newly recruited to balance the distribution of gender and age between the ADHD and healthy control groups in NeuroIMAGE. Inclusion criteria were largely consistent with criteria during IMAGE: participants had to be between 5-30 years, of European Caucasian descent, have an IQ $\geq$ 70, and no diagnosis of autism, epilepsy, general learning difficulties, brain disorders, and known genetic disorders (such as Fragile X syndrome or Down syndrome). Different from the original inclusion criteria used in IMAGE, we allowed inclusion of children with any subtype ADHD in the current study. This was done to closely match the original cohort that included participants with partly remission of ADHD symptoms. The newly recruited patients had significantly more inattentive and hyperactive symptoms than the original cohort (overall: 4.8 inattentive symptoms (SD=3.1); newly recruited: 6.3 inattentive symptoms (SD=2.7); original cohort: 4.7 inattentive symptoms

(SD=3.4); F(1, 555) = 5.5 ; p < 0.02); overall: 3.9 hyperactive symptoms (SD=3.2); newly recruited: 5.7 hyperactive symptoms (SD=2.8); original cohort: 3.8 hyperactive symptoms (SD=3.2); F(1, 555) = 10.3 ; p < 0.001). Figure 1 provides an overview of the NeuroIMAGE sample composition.

Families

Image I	ADHD	Control	Total
Included families	365	148	513
Retained families	288	119	407
%	79	80	79

NeuroIMAGE	ADHD	Control	Total
	331	153	484

Newly recruited	ADHD	Control	Total
	43	34	77

Individuals

Children		ADHD[a]	Control[a]	Total
Children from Image I	N	700	270	970
Newly recruited children	N	51	48	99
	Total	751	318	1069
	% male	58	51	56
	Age[b]	17.1 (3.7)	16.7 (3.9)	17.0 (3.7)
	IQ[b]	98 (16)	105 (14)	100 (16)
	SES[bc]	11.4 (2.3)	12.8 (2.7)	11.8 (2.5)

[a] ADHD / Control families
[b] Mean and standard deviation
[c] Average of parents' corrected years of education

Parents		ADHD[a]	Control[a]	Total
Parents from Image I	N	504	208	712
Newly recruited parents	N	78	58	136
	Total	582	266	848
	% male	46	44	46
	Age[b]	47.2 (5.5)	48.7 (4.6)	47.7 (5.3)
	IQ[b]	103 (16)	112 (16)	105 (16)
	SES[bc]	11.5 (2.9)	13.0 (3.3)	12.0 (3.1)

[a] ADHD / Control families
[b] Mean and standard deviation
[c] Average corrected years of education

FIGURE 1 Overview of the NeuroIMAGE sample composition. In this figure, an overview of the participating families is displayed (left) as well as well as demographic characteristics of the individual participants (right) segregated by family type (ADHD vs CON) and type of family member [child (above) vs. parent (below)].

Including the newly recruited families, the complete NeuroIMAGE cohort comprised testing of more than 1000 children and approximately 850 tested parents. Retention rate from the original IMAGE study was high (79%) with significantly higher rates for participants from control families compared with participants from ADHD families (84% vs 77%, Chi^2(df =1, N= 970) = 6.6 , p < 0.05). The most important reasons for drop-out were being too busy, family problems, and time consumption of the study (for a full measurement the whole family needed to spend an entire day at the test site). Dropped-out participants only differed from followed-up participants on estimated IQ (M=99, SD=11 vs 103, F(1,942) = 7.1, p < 0.01). No differences were present in terms of age, or number of inattentive or hyperactive symptoms (for a more extensive comparison of drop-out and follow-up participants see (van Lieshout et al. 2013). For 82% of the families we were able to collect data for two or more siblings (87% ADHD, 72% controls) and for both parents (85% ADHD, 76% controls).

Measurements

Measures During Intermediate Follow-up

Measures at the intermediate follow-up included questionnaires and a structural interview. Questionnaires were used to assess: (a) ADHD symptom severity of both parents and offspring (Conners' Adult ADHD Rating Scale (CAARS R-L (Conners et al. 1999)); Conners' Parent Rating Scale (CPRS R:L (Conners et al. 1998a)); Conners Wells' Adolescent Self-Report Scale: Short Form (CASS:S (Conners et al. 1997))), (b) Substance use disorders (SUDs) assessed by self-reported alcohol dependence (Alcohol Use Disorders Identification Test (AUDIT (Saunders et al. 1993))), drug abuse (Drug Abuse Screening Test – 20 (DAST-20 (Gavin et al. 1989))), and nicotine dependence (Fagerström Test for Nicotine Dependence (FTND (Heatherton et al. 1991))), (c) Alcohol, and tobacco consumption over the past month (Timeline Follow Back Interview (TLFBI (Sobell et al. 1996))), (d) Lifetime alcohol related problem behavior (The Michigan Alcohol Screening Test (MAST (Selzer et al. 1975))), (e) Gambling problems (The South Oaks Gambling Screen (SOGS (Lesieur and Blume 1987))) and (f) Driving behavior (Driving Behavior Questionnaire (DBQ (Parker et al. 1995))). Furthermore, parents of participants were interviewed about their children using the SUD module of the Diagnostic Interview Schedule for Children (DISC-IV (Shaffer et al. 2000)). A final set of measures was taken to determine patterns of use of prescribed drugs: (1) Medication use (ADHD Medication Use Questionnaire (AMUQ (Johnston et al. 1998))), and (2) The misuse and diversion of ADHD medication (MGH ADHD Medication Misuse and Diversion Assessment (MAMMDA (Wilens et al. 2006))).

Measures During NeuroIMAGE

Assessments for NeuroIMAGE included behavioral questionnaires, a semi-structured clinical interview (Dutch translation of the Schedule for Affective disorders Schizophrenia – present and Lifetime version (K-SADS (Kaufman et al. 1997))), several cognitive measures, acquisition of saliva and somatic measures obtained in all family members. In addition, all children older than 7 years, without contraindication for an MRI measurement (e.g. implanted metal, medical devices, or pregnancy) and willingness to participate underwent an MRI scanning session. Figure 2 outlines all measurements.

Questionnaires

Questionnaires assessed several domains of functioning, including: (a) ADHD symptoms and comorbidities including anxiety, depression and oppositional behavior (Conners et al. 1998b; Kessler et al. 2002; van Widenfelt et al. 2003; Rommelse et al. 2007b; Rommelse et al. 2008b; Donker et al. 2010), (b) autism spectrum symptoms (Tremblay et al. 1991), (c) medication history, (d) severe life events and severe chronic adversity (Oldehinkel et al. 2008; Bosch et al. 2012), (e) peer relationships (Walden et al. 2004), (f) antisocial and criminal behavior (Loeber et al. 1993; Kimonis et al. 2008; Geluk et al. 2012), (g) body development (Brooks-Gunn et al. 1987), (h) motor coordination (Wilson et al. 2000), (i) academic achievement, (j) parenting and parental supervision (Brown 1966; Kerr and Stattin 2000), and (k) personality traits (Goldberg 1992; Klimstra et al. 2009). For participants using medication, ADHD ratings were collected about the participants' func-

tioning off medication. For children younger than 12 years their parents or researchers assisted the completion of the self-report questionnaires for the child.

Regarding medication history parents provided detailed information about lifetime use of psychoactive medication for themselves and their children. Additionally we asked them for written consent to obtain information from the pharmacy records about all delivered psychoactive medications over the last 6 years.

CHILDREN

Questionnaires

ADHD symptomatology and comorbidities
- Conners' Adult ADHD Rating Scale (CAARS R-L)
- Conners' Parent Rating Scale (CPRS R-L)]
- Conners' Teacher Rating Scale (CTRL R-L)
- Strength and Difficulties Questionnaire (SDQ)

Autism spectrum disorder
- Children's Social Behaviour Questionnaire (CSBQ)

Medication history / pharmacy records

Severe life events and severe chronic adversity
- Long Term Difficulties Questionnaire
- Life Events Questionnaire

Peer relationships
- Friends Inventory

Antisocial and criminal behaviour
- Self-Report of Antisocial Behavior Scale
- Callous Unemotional Traits (CU-Traits)

Body development
- Pubertal Development Scale (PDS)

Motor coordination
- Developmental Coordination Disorder Questionnaire (DCD-Q)

Personality
- Goldberg's Big Five Questionnaire

Academic achievement

Parenting and parental supervision
- Parental Expressed Emotions
- Parental Supervision Questionnaire

Diagnostic interview

- Kiddie - Schedule for Affective Disorder and Schizophrenia Present and Lifetime Version (K-SADS-PL)

Somatic and other measures

- Blood Pressure
- Heart Beat
- Head Circumference
- Length
- Saliva
- Waist Circumference
- Weight

Cognitive assessment

Intellectual functioning
- Block design (WISC / WAIS)
- Vocabulary ((WISC / WAIS)

Executive functions
- Digit Span (WISC / WAIS)

Information processing speed
- Baseline (Motor) Speed

Emotional processing
- Identification of Facial Emotions

Reward processing
- Reversal Learning
- Temporal Discounting

Temporal processing
- Timetest Reproduction
- Motor Timing

Reading fluency
- One Minute Reading Task

Visuomotor integration
- Prosody
- Pursuit
- Tracking

Magnetic Resonance Imaging

Executive functions
Visuospatial Working Memory (WM)
Response Inhibition (Stop)

Reward processing
- Monetary Incentive Delay Task (MID)

- Anatomical MRI
- Diffusion Tensor Imaging (DTI)
- Resting State MRI (R-FMRI)

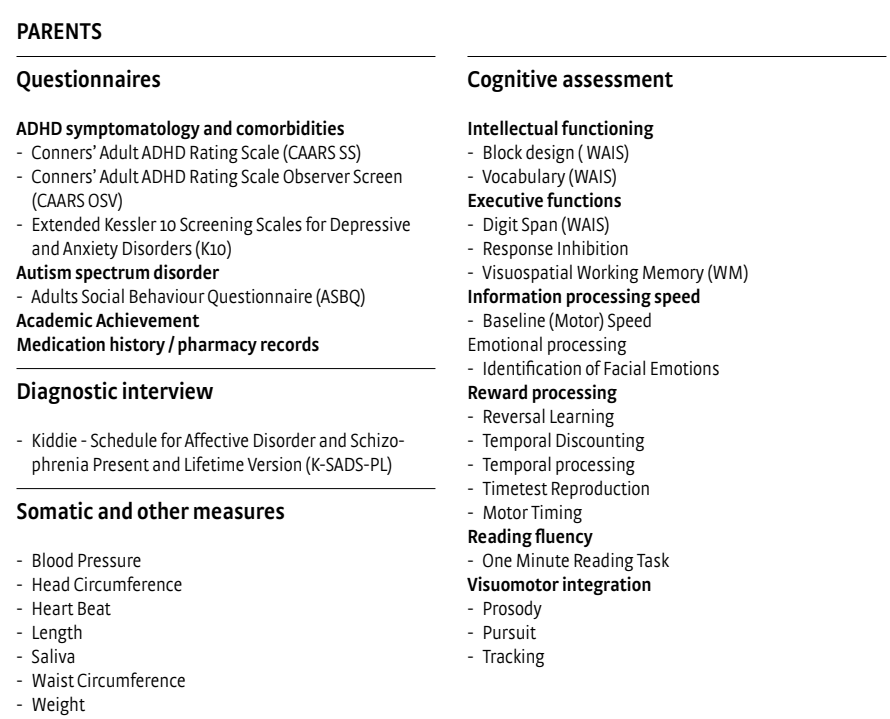


FIGURE 2 Assessment protocol NeuroIMAGE. This figure indicates NeuroIMAGE's full assessment protocol for children (left) and parents (above)

Diagnosis

Diagnostic Interview

All participants of NeuroIMAGE, were interviewed using the K-SADS. The K-SADS is a semi-structured diagnostic interview and designed to assess current and past episodes of psychopathology in children, adolescents, and adults according to DSM-IV criteria. It provides operational definitions of individual symptoms as well as diagnosis-relevant questions such as symptom onset and impairment. It is separated into screen items, reflecting core symptoms of a disorder, and supplementary modules, consisting of a full assessment of that disorder. Lastly, by interviewing both the parents, and the child, the K-SADS diagnosis is based on different informants. For this study, we included assessments for affective disorders, anxiety disorders, behavioral disorders and tics disorders. The presence of psychiatric disorders within these domains except ADHD (i.e. oppositional defiant disorder (ODD), conduct disorder (CD), chronic or transient motor or vocal tic disorder, Tourette's disorder, major depression (MD), dysthymic disorder, generalized anxiety disorder, social phobia, separation anxiety and panic disorder) was evaluated in all participants using a procedure similar to the ADHD interview. Participants with elevated scores on one or more screen items were administered a full supplement. Final diagnosis was based on DSM-IV criteria for that specific disorder.

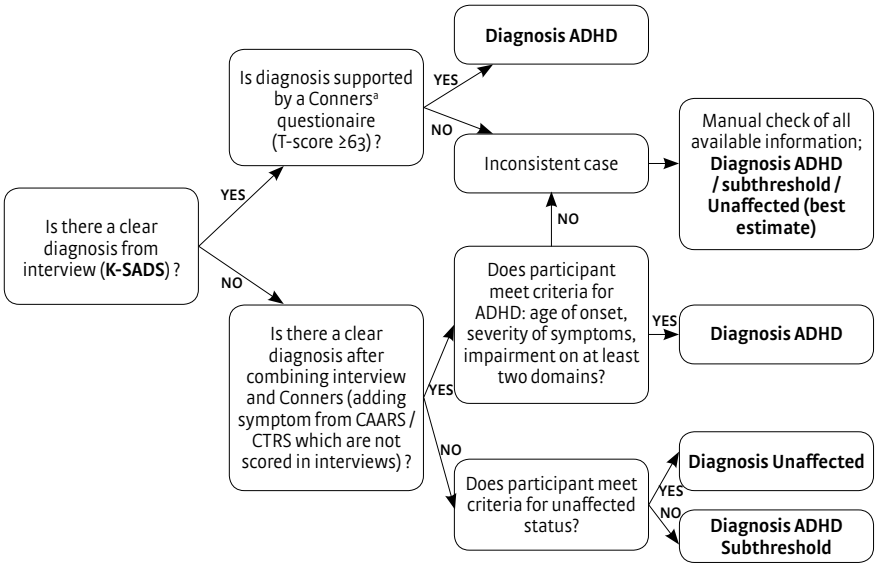
Algorithm

To determine ADHD diagnosis at the time of participation in NeuroIMAGE, we used a diagnostic algorithm, which combined the diagnostic interview (K-SADS) with the Conners rating scales. The interview served as the fundament for diagnosis Participants were diagnosed with ADHD provided they a) had ≥ 6 hyperactive/impulsive and/or inattentive symptoms, b) met the DSM-IV criteria for pervasiveness and impairment (measures derived from the K-SADS), and c) showed an age of onset before 12 (following the proposed changes for the DSM-5; see (Polanczyk et al. 2010)). To account for a possible underestimation of ADHD symptomatology in a familial setting we complemented information from the interview with symptom counts from the Conners' ADHD questionnaires (CTRS-R:L for participants < 18 years or CAARS-S:L for participants ≥ 18). To prevent an artificial inflation of ADHD diagnosis, this was only done when at least 2 symptoms were reported on the questionnaire. When a participant met these criteria, it was checked whether they received a T-score ≥ 63 on at least one of the DSM-IV ADHD scales on either one of the Conners ADHD questionnaires (DSM Inattentive behaviour (scale L of the CTRS-R:L; scale E of the CAARS-S:L), DSM Hyperactive/Impulsive behaviour (scale M of the CTRS-R:L; scale F of the CAARS-S:L), and DSM Total (scale N of the CTRS-R:L; scale G of the CAARS-S:L)) filled out about a period without medication. Cases with inconsistent information (N=73 (7%)) from these two sources of information were evaluated by a team of experts (psychiatrist JB and 8 psychologists) to derive a consensus (best-estimate) diagnosis.

To be considered unaffected, participants were required to exhibit a T < 63 on each of the subscales of each of the Conners questionnaires and to have ≤ 3 symptoms derived from the combined symptom counts of the K-SADS and CTRS-R:L/CAARS-S:L. All participants who did not meet our requirements for either ADHD or unaffected status were classified as subthreshold ADHD and need to be excluded from case control comparisons.

Criteria were slightly adapted for adults (≥ 18 years) such that a symptom count of 5 symptoms was sufficient for a diagnosis (Polanczyk et al. 2010). Adults were considered unaffected when they exhibited ≤ 2 ADHD symptoms on the symptom counts. Figure 3 illustrates the steps leading to diagnostic classification. The distribution of diagnostic groups is provided in Table 1.

We were able to determine the diagnostic status for 1023 (96%) children who completed the full diagnostic procedure. The remaining cases were not willing to participate in an interview.



[a] Children: CPRS-RL or CAARS –SL (age ≥ 18) / CTRS –RL (age < 18); Parents: CAARS-OSV

FIGURE 3 Flow chart of the diagnostic algorithm for children and parents

TABLE 1 Demographic information and number of measures in assessed children segregated by diagnosis

Diagnostic groups	ADHD	Unaffected Sibs	Controls	Affected Controls[d]	Subthreshold[f]	Not diagnosed
N (% of sample)	412 (39)	227 (21)	262 (24)	41 (4)	81 (8)	46 (4)
% male	68	42	49	66	52	52
Age[a]	16.6 (3.4)	17.4 (4.1)	16.6 (3.7)	17.6 (5.3)	18.4 (3.7)	16.9 (2.9)
IQ[ab]	96 (16)	101 (15)	106 (14)	102 (12)	102 (14)	NA
SES[ac]	11.4 (2.3)	11.5 (2.4)	12.9 (2.7)	11.8 (2.7)	11.4 (2.2)	11.6 (2.5)
Inattentive symptoms[ae]	7.3 (1.7)	0.6 (1.4)	0.5 (1.4)	4.6 (2.6)	3.7 (1.4)	NA
Hyperactive symptoms	6.0 (2.4)	0.5 (1.0)	0.4 (1.0)	3.1 (2.4)	2.9 (1.6)	NA
Questionnaires[g]	412 (100)	227 (100)	262 (100)	41 (100)	81 (100)	46 (100)
Neuropsychological data[g]	380 (92)	207 (91)	239 (91)	34 (83)	72 (89)	0 (0)
MRI data[g]	328 (80)	171 (75)	211 (81)	31 (76)	59 (73)	0 (0)
Somatic measures[g]	369 (90)	206 (91)	233 (89)	34 (83)	71 (88)	0 (0)

[a] Mean and standard deviation
[b] Estimated IQ
[c] Corrected years of education
[d] Affected controls are follow-up children that have developed symptoms between IMAGE 1 and NeuroIMAGE
[e] Based on combined symptom count
[f] Probands with less symptoms than needed and healthy controls with more symptoms than allowed
[g] Numbers in each cell represent absolute and relative (within parentheses) amount of available data per diagnostic group

For participating parents, diagnostic procedures were similar to those applied to children ≥ 18 years old. Parents were administered the K-SADS and, if possible, their partners completed the CAARS-O:SV. A retrospective childhood diagnosis was established in addition

to a current diagnosis using the same diagnostic algorithm used for young adults except that for childhood diagnosis a minimum of 6 symptoms was required. Moreover, the DSM Inattentive, DSM Hyperactive-Impulsive, and DSM Total subscales of the CAARS-O:SV were used to validate the current diagnosis ADHD. Based on the combination of childhood and current diagnosis, different types of ADHD could be differentiated for participating parents. Parents with a childhood diagnosis of ADHD could either be persistent (current diagnosis ADHD), residual (current diagnosis ADHD subthreshold), or remittent (current diagnosis unaffected). For parents without a childhood diagnosis of ADHD, the diagnosis was either ADHD late onset (current diagnosis ADHD) or unaffected. Because there were no participants with a childhoold diagnosis subthreshold ADHD, this resulted in seven diagnostic categories which are shown in Table 2.

TABLE 2 Demographic information and number of measures in assessed parents segregated by diagnosis

Diagnostic groups	Persistent	Residual	Remittent	Late onset	Unaffected	Controls	Affected controls[e]	Subthreshold[f]	Not diagnosed
N (% of sample)	101 (12)	40 (5)	9 (1)	22 (3)	319 (38)	224 (26)	19 (2)	56 (7)	58 (7)
% male	56	55	78	32	41	44	37	46	55
Age[a]	47 (5)	47 (5)	45 (4)	47 (5)	47 (5)	49 (5)	49 (5)	47 (6)	49 (6)
IQ[ab]	104 (16)	99 (13)	114 (25)	106 (18)	103 (15)	112 (16)	113 (16)	104 (16)	NA
SES[ac]	11.78 (2.97)	11.2 (2.16)	11.79 (3.8)	12 (2.69)	11.55 (3.08)	13.06 (3.33)	11.17 (2.02)	11.33 (2.57)	NA
Inattentive symptoms[a]	5.4 (3.2)	1.6 (2.3)	0.6 (0.9)	3.5 (2.8)	0.4 (1.2)	0.1 (0.4)	1.3 (1.6)	1.7 (1.6)	NA
Hyperactive symptoms[a]	5.2 (3.2)	2 (2.2)	0.6 (0.9)	3.7 (2.8)	0.4 (1.1)	0.2 (0.6)	2.3 (2.1)	2.3 (1.8)	NA
Questionnaires	101 (100)	40 (100)	9 (100)	22 (100)	318 (100)	224 (100)	19 (100)	56 (100)	58 (100)
Neuropsychological data	78 (77)	35 (88)	4 (44)	20 (91)	245 (77)	138 (62)	15 (79)	46 (82)	0 (0)
Somatic measures	77 (76)	34 (85)	4 (44)	17 (77)	241 (76)	136 (61)	15 (79)	46 (82)	1 (2)

[a] Mean and standard deviation
[b] Estimated IQ
[c] Corrected years of education
[d] Based on combined symptom count
[e] Affected controls are follow-up parents that have developed (subthreshold) ADHD between IMAGE 1 and NeuroIMAGE
[f] Parents with less symptoms than needed for a diagnosis and healthy controls with more symptoms than allowed
[g] Numbers in each cell represent absolute and relative (within parentheses) amount of available data per diagnostic group

Cognitive Assessment

All participants (children and parents) completed a comprehensive protocol of cognitive tasks measuring (a) intellectual functioning (Wechsler 2000; Wechsler 2002), (b) executive functions (Logan et al. 1984; Wechsler 2000; Klingberg et al. 2002; Wechsler 2002; McNab et al. 2008), (c) information processing speed (De Sonneville 1999), (d) emotional processing (De Sonneville 1999), (e) temporal processing (Rommelse et al. 2007c; Rommelse et al. 2007a),(f) reading fluency (Brus and Voeten 1973), (g) visuomotor integration (De Sonneville 1999), and (h) reward processing (Knutson et al. 2001; Itami and Uno 2002; Scheres et al. 2006). Except the subtests of the WISC/WAIS and the reading test, all tasks were computerized.

MRI Measures

Participating children completed a session in a magnetic resonance imaging (MRI) scanner. At the two test sites comparable 1.5T MRI scanners were employed (Siemens SONATA and Siemens AVANTO; Siemens, Erlangen, Germany), using identical head coils (8-channel Phase Array Head Coil). A scanning session included two anatomical T1 scans, a diffusion tensor imaging scan (DTI), a resting state functional MRI (R-FMRI) scan, and three functional imaging tasks including a visual working memory task (Klingberg et al. 2002; McNab et al. 2008), a stop signal reaction task (Logan et al. 1984) and a monetary incentive delay (MID) task (Knutson et al. 2001). MRI scanning sequences were closely matched across the two scanning sites (Table 3).

TABLE 3 Scan sequences

Sequence	TR/TE/T1	Field of view	Matrix	Voxel size (mm)	Gap (%)	GRAPPA	b-value	Directions / bo's
	mm	mm	RL/AP/slices			factor		
T1	2730/2.95/1000	256	176/256/256	1.0 x 1.0 x 1.0	50	2	NA	NA
R-FMRI	1960/40/-	224	64/64/37[a]-38[b]	3.5 x 3.5 x 3.0	17	none	NA	NA
Functional Tasks	2340/40/-	224	64/64/37[a]-38[b]	3.5 x 3.5 x 3.0	17	none	NA	NA
DTI	8500/97/-	256	128/128/60	2.0 x 2.0 x 2.2	0	2	1000	60/5

[a] Nijmegen
[b] Amsterdam

Because of limited time for scanning we were unable to collect all MRI measurements for each participant. Therefore, we differentiated between four acquisition protocols. All protocols included two anatomical T1 scans. Additionally, three of the four protocols included two of the three functional imaging tasks, the DTI, and the R-FMRI measurements (thus dropping one task). The fourth protocol contained all three functional imaging tasks (thus dropping the DTI and R-FMRI measurements). Following this procedure we were able to measure brain anatomy for 800 participants (100%), reward processing for 564 (70%) participants, response inhibition for 533 (67%) participants, working memory for 648 (81%) participants, R-FMRI for 536 (67%) participants, and DTI for 591 (74%) participants. We balanced the order of tasks across protocols and the order of the used protocols was pseudo-randomised across families to achieve an equal distribution of protocols across site and family type.

Genetic Determinants

Genetic material and data available for the NeuroIMAGE sample

Participants whose genotypic information was not collected during IMAGE, provided saliva for DNA analysis. We were able to obtain genetic data for almost every participant in the NeuroIMAGE study, except for 5 participants who did not did not provide a saliva sample.

DNA Isolation

An extensive description of DNA extraction and genotyping in IMAGE is provided elsewhere (Brookes et al. 2006). Briefly, for the IMAGE sample DNA was extracted from blood

samples or immortalized cell lines at Rutgers University Cell and DNA Repository, New Jersey, USA as well the Human Genetics department of the Radboud University Medical Centre in Nijmegen. Additional NeuroIMAGE samples were collected in the form of a saliva sample. DNA was isolated from saliva using Oragene containers (DNA Genotek, Ottawa, Ontario, Canada) according to the protocol supplied by the manufacturer at the Radboud's department of Human Genetics.

Genetic Linkage Data

As described by Asherson et al. (Asherson et al. 2008), a total of 5545 autosomal single-nucleotide polymorphisms (SNPs) from the Illumina Linkage IVb SNP panel were successfully assayed, with a call rate of 99.6% and a reproduction rate of 99.994%. After data cleaning, 5407 autosomal SNPs with an average resolution of 1.66 SNPs per centimorgan were available for linkage analyses. In total linkage data was available for 322 subjects with ADHD (144 combined type, 147 with predominantly inattentive type, 31 with predominantly hyperactive-impulsive type), 189 unaffected individuals, 64 subjects subthreshold for ADHD and 28 with unknown status.

Genome-wide Genotyping Data

Genome-wide genotyping of the IMAGE probands (n=231) and their parents (n=445) was performed as part of the GAIN study using the Perlegen genotyping platform of 600,000 tagging single nucleotide polymorphisms (SNPs) (for details on genotyping, data cleaning, and quality control procedures see (Neale et al. 2010)). For NeuroIMAGE, genotyping was performed for affected, unaffected and control children who had not been genome-wide genotyped before (n=492); this was done using the HumanCytoSNP-12 genotyping chip with 200,000 tagging SNPs. Quality control steps were performed for the genotype data. SNPs were excluded if the call rate per SNP was less than 95%, the minor allele frequency was less than 1%, or the SNPs failed the Hardy-Weinberg equilibrium test at a threshold of $p \leq 10–6$ (genome-wide). Participants were excluded if the call rate per individual was lower than 95%. To increase genomic coverage and to harmonize genotyping, imputation was performed in the different datasets using the 1000 Genomes Reference data. In total we have genome-wide data available for 331 subjects with ADHD (150 combined type, 143 with predominantly inattentive type, 38 with predominantly hyperactive-impulsive type), 301 unaffected individuals (unaffected siblings and healthy controls), 78 subjects with subthreshold ADHD and 13 not diagnosed.

Somatic and other measures

To obtain an estimate of possible unhealthy eating habits, abnormal growth or other physiological abnormalities, we measured body length and weight, head and waist circumference, blood pressure, and heart rate at rest.

Procedures

Ethical Approval

This study was approved by the regional ethics committee (Centrale Commissie Mensgebonden Onderzoek: CMO Regio Arnhem – Nijmegen; 2008/163; ABR: NL23894.091.08) and the medical ethical committee of the VU University Medical Center. We obtained written informed consent for every participant. For children 12 to 18 years old, both parents and children gave consent, for children younger than 12 parents gave consent for their children. In case a participant retracted consent, all data of that participant were removed from the database and withheld from further analysis. Participating families were regularly informed with a newsletter about study progress and resulting publications.

Assessment

After an initial contact by telephone or through public schools, interested families received an information package including general project information, informed consent forms and questionnaires. Minimal requirement for participation was that a participant was willing to fill out questionnaires. In case of participation, a clinical interview for each family member was done by telephone. During this screening we asked participants to withhold use of psychoactive drugs or drugs with potential effects on test performance for either 48 hours before the test day or according to the washout period of the drug.

If feasible we organized a single test day for each family covering all assessments; otherwise testing spanned several days. For families that were not willing to come to the test sites, we offered a test day without MRI at the family's home, which occurred only in very few cases. In Table 2 and 3 available data per diagnostic group are indicated. During this day parents and children older than 12 years were interviewed using the K-SADS; children below the age of 12 were not interviewed. Participants with elevated scores on screen items of the interview (score: 3) were administered the full supplementary module of that disorder. Cognitive tests were administered in a fixed order and due to its length divided in two parts. Across families the administration of both parts was counter-balanced. All children participating in an MRI session were prepared for scanning using a mock scanner. Each testing day ended with a short debriefing. The monetary reward of €50 was granted to every participating child, and travel cost were reimbursed to parents. Children who completed an MRI session were also offered a copy of the anatomical MRI scan. Moreover, all participants received the monetary reward gained during cognitive assessment and, on demand, a short report of their performance on the IQ test and questionnaire/interview scores. An example of a test day can be found in the supplementary material.

Staff Training and Supervision

Test staff consisted of PhD students and research assistants. The whole staff carried out cognitive testing, diagnostic interview and MRI scanning was restricted to PhD students who had received training at forehand. The MRI scanning training consisted of practicing to operate the scanner, learning security procedures and monitoring quality of the data (e.g. spike identification). For the diagnostic interview, a PhD student had to attend psychiatric diagnostic intake sessions of ADHD children at local child Psychiatry departments (Karakter, Nijmegen; Accare, Groningen) or interview sessions led by a trained interviewer. Moreover, in practice interviews, PhD students conducted diagnostic interviews under supervision of a clinician or trained professional. For quality control, monthly meetings were held to discuss controversial cases and to maintain agreement about ADHD symptoms. In addition, every interviewer was filmed during an interview and evaluated by other interviewers. By comparing symptom-wise the evaluations of the filmed interviewer with ratings of the other interviewers, we were able to determine the inter-rater reliability (IRR). For ADHD, on average seven raters contributed to each symptom evaluation, for ODD and CD, at least five raters contributed to each evaluation. For ADHD, ODD; and CD, IRR across all raters and interviews was excellent (ADHD: 0.94; ODD: 0.89; CD: 0.95).

To standardize cognitive testing and neuroimaging as much as possible written standard operating procedures (SOPs) for administration of cognitive tests and MRI assessments were developed. All researchers received training to administer the test battery using the SOPs before they were allowed to test during a test day. The first sessions of research assistance were conducted under supervision of an experienced PhD student.

Data Management and Quality Control

We encoded every participant with an anonymous identifier number to separate personal from scientific data. Data collection was documented with a case report enlisting all available data for that person and notes regarding factors that might have influenced the data acquisition. Moreover, all digital data were securely uploaded to a central storage server which was backed-up to tape daily and archived in at least two different locations. To check data integrity, we compared the presence of the uploaded data with what was expected from the digitized case reports. In addition, we obtained demographic information from multiple sources (e.g. information about gender and age from self-reports and data entry by the researcher during scan session; for gender also from genotypic analysis) to assure that data from different modalities (MRI, genotype, behavioral data, self-reports) were associated with the correct corresponding participant.

The research team digitized all questionnaires. After entering these data, quality checks for a random sample of questionnaires were conducted. When the error rate of a questionnaire was below 1%, the data were accepted as valid. In case of higher error rates,

all data for that specific questionnaire were checked with the original paper version and corrections were made where needed.

For MRI data, we also implemented several data checks to assess the quality of the collected scans. For every MRI sequences we calculated the signal-to-noise ratio and the amount of spurious spikes in the signal. For the T1 anatomical scans two independent raters evaluated quality of both scans on a 4-point scale (1 = good; 2 = useable; 3 = poor; 4 = very poor). Consistency between both raters was sufficient to good (ICC: 0.59) and the evaluated quality of the scans was good: From 1559 scans only 105 (6.7%) were rated other than good or usable by one of the raters, leaving 767 (96%) participants with at least one useful structural scan.

MRI Movement Artifacts

Head movement during MRI scans can greatly impact the quality of the data collected (Van Dijk et al. 2012; Power et al. 2012; Satterthwaite et al. 2012). Therefore we undertook several steps to minimize movement during scanning, and to assess data quality afterwards. Before the MRI session all participants were trained in a mock scanner to keep their head still while images are being acquired. During the structural scans, participants were offered to watch a short movie or to listen to their favorite music, thereby distracting them from scanning, while helping them to stay still. During functional MRI scans we monitored participants' movements by performing real-time calculations of the head rotation and translation parameters. When participants moved excessively, we gave feedback and encouraged participants to stay still for the next scan. Finally, given the importance of the anatomical scan for processing the other scan types (i.e., to allow correct normalization to a common space), we administered the T1 anatomical scan twice during the MRI session.

We also made a quantitative between-group comparison of head movement during functional MRI scans. To this end we calculated the 3 head rotation (degrees) and 3 translation parameters (millimeters) using SPM8 software (Wellcome Trust Centre for Neuroimaging, UCL). Rotation parameters were converted to distances (in millimeters). By taking the summed absolute image-to-image displacement per parameter and adding these up, we constructed a summary score of the total movement over the time series per participant. As displayed in Figure 4, peaks of these distributions are slightly shifted between ADHD cases and controls, suggesting that the ADHD cases moved a bit more during scanning. However, for all sequences we observed an almost complete overlap of distributions indicating that within-group variance was much larger than between-group variance. This is also illustrated by the computed Cohen's effect sizes that, varying between 0.10 and 0.51, appear to be small to moderate. We concluded from these observations that movement is not very likely to confound our case-control comparisons and we therefore decided to deal with movement in a standard fashion (i.e. statistical correction using realignment parameters in 1st/2nd level analysis, exclusion of extreme movers / outliers, post-hoc analysis whether movement does confound a specific analysis).

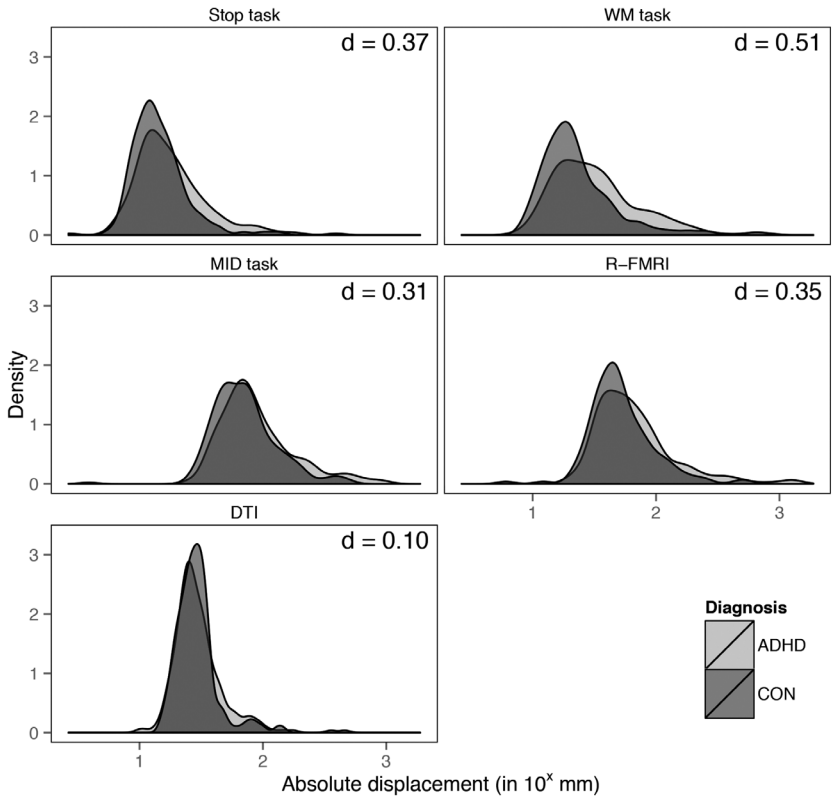


FIGURE 4 Distribution of the summarized movement parameters per MRI scan with time series [response inhibition (Stop), reward processing (MID) and visuospatial working memory (WM) task, resting state fMRI (R-FMRI), diffusion tensor imaging (DTI)] stratified by diagnostic group (ADHD vs. CON). The numbers in the upper part of each facet indicate effect sizes (cohen's d) of between-group differences of the mean

Site Effects

Data acquisition was carried out at the VU University Amsterdam and VU University Medical Centre, or at the Radboud University Medical Centre and Donders Centre for Cognitive Neuroimaging in Nijmegen. This has implications for data analysis, as multi-site data acquisition induces non-specific variability in the data (e.g. differences in test rooms and scanner properties, slight variations in instructions). Several steps were taken to minimize site-effects, such as using SOPs, equal (or similar) equipment, standard scan protocols at both sites, training experimenters on cognitive testing and conducting interviews in a standard manner. Table 4 displays an overview of the demographic information of the sample with cognitive data including site information. It is apparent that the number of participants per site, percentage of males, IQ and socioeconomic status are not matched across sites, and analyses will need to be adjusted for potential sites effects.

TABLE 4 Sample distribution per measuring site

N tested	Total 934						
Tested per site	Nijmegen 472 (51%)		Amsterdam 462 (49%)				
	ADHD	Control	ADHD	Control	Main effect site	Main effect family	Interaction site x family
N (%)	379 (80)	93 (20)	280 (61)	180 (39)		p < 0.001	p < 0.001
% male	53	42	64	57		p < 0.002	p < 0.001
Age[a]	16.8 (3.7)	16.2 (3.5)	17.3 (3.7)	16.8 (4.1)			
IQ[a]	99 (16)	109 (15)	97 (16)	104 (13)	p < 0.03	p < 0.001	
SESa	11.3 (2.2)	13.8 (2.9)	11.8 (2.4)	12.4 (2.4)	p < 0.003	p < 0.001	p < 0.001
Inattentive symptoms[ab]	4.8 (3.3)	0.6 (1.4)	4.7 (3.6)	1.3 (2.4)		p < 0.001	
Hyperactive symptoms[ab]	4.2 (3.2)	0.5 (1.3)	3.6 (3.1)	0.8 (1.6)	p < 0.01	p < 0.001	p < 0.04

[a] Mean and standard deviation
[b] Based on combined symptom count

Although we aimed to match imaging protocols between sites, we were unable to completely match the scanner types (Siemens Avanto versus Siemens Sonata). Such difference in hardware can be expected to yield between-site differences imaging quality or parameters. To estimate these differences and assess them in light of between-subject variability, we compared image quality measures and, most importantly, dependent measures of the experimental designs for each scanning modality (T1 anatomical, all functional tasks, R-FMRI, DTI) between gender and age matched control participants from both sites. Representing image quality, for the T1 anatomical and the DTI scans we calculated the signal-to-noise ratio (SNR), defined as mean imaging signal within the brain divided by the noise level, i.e. the mean standard deviation of the signal in the air divided by 0.655 (Henkelman 1985). For the functional MRI scans we calculated temporal SNR by the brain averaged ratio of the mean and standard deviation of the signal over time. Temporal SNR was calculated on the raw data after applying realignment to correct for gross head movements. To evaluate potential site effects within our experimental design, we selected one measure of interest for each imaging modality. For the anatomical scan we selected relative grey matter volume (grey matter divided by the total brain volume as estimated by SPM). For DTI we calculated the mean FA value within the posterior corpus callosum (extracted using the FreeSurfer software package). For each functional task we assessed task-related activity in a region of interest (ROI). We assessed the ventral striatum during rewarded anticipation (vs nonrewarded anticipation), right inferior frontal gyrus for successful response inhibition (vs noninhibited responses) and inferior frontal gyrus for working memory demanding periods (vs baseline). For each region we extracted mean activity from the normalized contrast maps of the first-level parameter estimates for the specified contrast. For the R-FMRI scan we quantified the identifiability of the default network by calculating the ratio between the connectivity strength within a default-mode mask and connectivity strength outside this mask. Connectivity measures were obtained by dual regression using 10 well-defined networks (Filippini et al. 2009). The distribution of each measure per imaging site is plotted in Figure 5. As expected, differences between sites could be observed in the distribution of all measurements. However,

all measures exhibited large overlap (Cohen's d was in the range between (+/-) 0.12 and 0.76, with a mean around 0.50) between sites, suggesting that between subject variability within site outweighed any systematic between-site differences. Importantly, compared to the effect on raw image quality, site had a considerably smaller effect on most derived measures indicating that in our study site effects are likely to play a less important role when answering experimental questions.

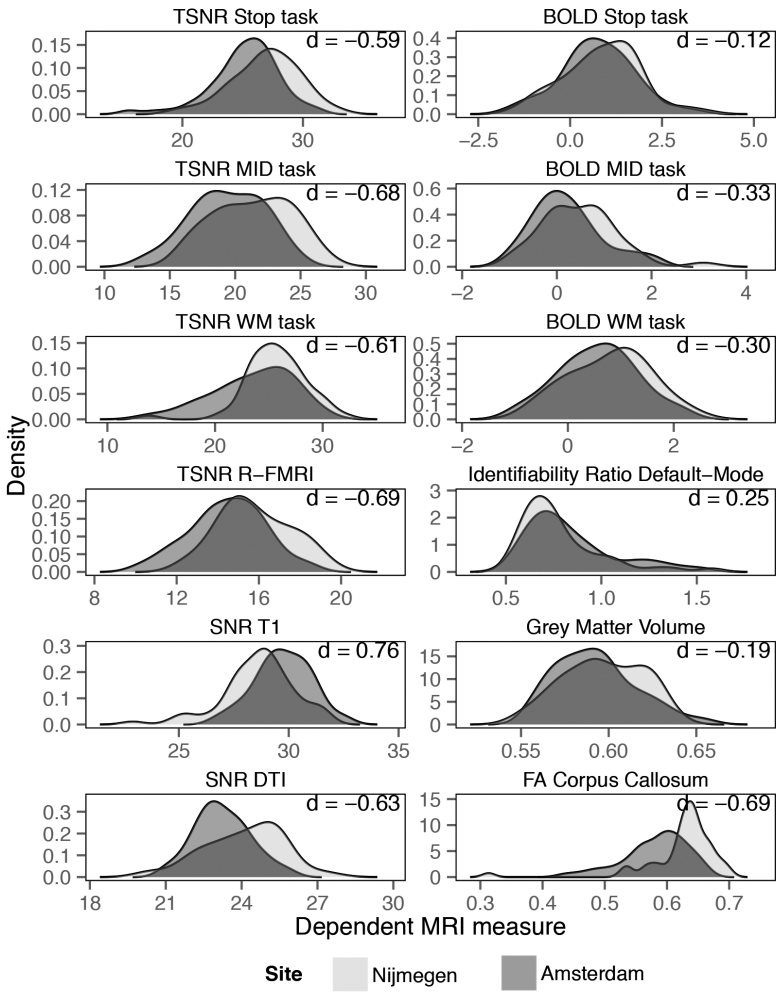


FIGURE 5 Distribution of dependent MRI measures stratified by scan site. This figure shows the density plots of all MRI measures for a global (left) and specific (right) dependent measure. Measures comprise signal-to-noise ratios (SNR) of the anatomical T1 and DTI scan, temporal signal-to-noise ratios (TSNR), blood oxygen-level dependent (BOLD) responses for all functional tasks [response inhibition (Stop), reward processing (MID) and working memory (WM)], identifiability measure (log10 transformed) of a R-FMRI default network, relative grey matter volume, and the mean fractional anisotropy (FA) value of the corpus callosum. Effect sizes (cohen's d) of between-group differences are indicated in the upper part of each facet.

Age- and Gender-specific Templates for MRI Analyses

Each participant's brain is different in size and structure. Accordingly, between-subject comparisons necessitate transformation of each participant's MRI data to a common analysis space. This allows making inferences about group differences in specific brain structures or functions, based on the assumption that the transformation has aligned similar brain structures across participants. A typically used transformation is the alignment of a participant's brain to a template from the Montreal Neurological Institute (MNI152). This template represents the average healthy adult brain and is used in most MRI studies. This approach has the advantage that brain regions described in one study can be compared to brain regions described in another. However, given the wide age-range of the NeuroIMAGE sample (8-30 years) and the developmental phase in which our participants fall, a possible transformation bias may exist, in that brains of older participants will need less transformation to match the MNI152 template compared to brains from younger participants. Similarly it is possible that structural brain differences between ADHD and controls translate into functional differences detected with fMRI. This could be due to differences in transformation to the MNI152 space caused by the underlying structural differences between ADHD and controls. To counteract such biases, we developed a transformation procedure that ultimately transforms participants' brains to a 'neutral midspace'. Such a midspace is determined by the participants included in a specific analysis. For instance, when conducting a case-control comparison for a certain task all participants with ADHD and all healthy participants who performed that task are used to calculate a neutral midspace. In short, the midspace was obtained through as stepwise normalization of each participant's brain to 1) MNI152 space, 2) a study template based on the average of all participant's brains in MNI152 space, 3) a specific subtemplate (e.g., based on all participants with ADHD, or all males). Transformation of a participant's brain to the desired midspace was then accomplished by concatenating the transformation of the participant's brain to the study template with a weighted transformation of the study template to a combination of subtemplates. As an example, in case of 60 controls and 40 patients with ADHD, bringing a participant to a diagnosis neutral midspace would entail transformation of that participant's brain using a concatenation of the participant to study template transformation with (0.6 x transformation of study template to control template) + (0.4 x transformation of study template to ADHD template). Because the midspace accounts for demographic characteristics of the analyzed population, transformation of imaging data to that space minimizes possible confounders as gender, age and diagnosis. Importantly, the midspace and templates are aligned with the MNI152 template space. Thus coordinates of results obtained with this procedure are comparable to coordinates obtained using the traditional transformation to MNI152 space.

Conclusion: Anticipated Outcome and Opportunities of NeuroIMAGE

The NeuroIMAGE database offers the opportunity to study several key aspects of ADHD in a large family-based sample: 1) the course of ADHD, 2) its neurocognitive and genetic underpinning, and 3) its heterogeneity. By assessing cognitive systems during the critical period from childhood to adulthood we can investigate which cognitive and associated neural systems are stable and which undergo developmental changes. Finding a profile of cognitive, neural and/or genetic markers linked to remitting or persistent ADHD would significantly deepen our understanding of the biological mechanisms involved in the course of ADHD. Very importantly, on a clinical level, it would provide a means of identifying children with ADHD who are at risk for a persistent course into adulthood and poor clinical outcome. In turn, this should provide a basis for the development of more powerful treatment approaches for this group of patients and monitoring treatments more effectively.

ADHD is likely to be a combination of multiple etiologically distinct subtypes with overlapping symptom presentations. By bringing together diverse measures we may be able to identify specific subtypes on the basis of cognitive and/or brain measures, where behavioral measures alone might have been unsuccessful. Furthermore, NeuroIMAGE encompasses a comprehensive assessment of other psychopathological domains (e.g., ODD/CD, ASD), which allows exploring the specificity of genetic, cognitive and neural correlates of ADHD. Detailed medication use data enables the study of brain correlates associated of long-term medication use. Using empirical modelling techniques like latent class analysis (LCA) and recent extensions of these techniques one may identify groups of participants who have very similar patterning of scores on cognitive and brain measures reflecting biologically relevant, distinct etiological pathways towards disease (Bureau et al. 2011; Fair et al. 2012).

While being too small for gene-finding studies, the NeuroIMAGE database forms an excellent resource for mapping biological pathways from gene to disease. For gene-finding studies, for which even larger samples are needed, NeuroIMAGE contributed its data to meta- and mega-analyses in international initiatives like those of the Psychiatric Genomics Consortium (PGC ; https://pgc.unc.edu/) and the ENIGMA consortium (Enhancing NeuroImaging Genetics through Meta-Analysis, see enigma.loni.ucla.edu). In addition to that, the NeuroIMAGE database forms an international scientific resource which may be accessed by other researchers in the field (for requests regarding access to the NeuroIMAGE data see www.neuroimage.nl).

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Chapter 3

Increased Neural Responses to Reward in Adolescents and Young Adults With Attention-Deficit/Hyperactivity Disorder and Their Unaffected Siblings

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Commentary on the adapted task

Measuring the Neural Basis of Reward Anticipation and Reward Receipt in Attention-Deficit/Hyperactivity Disorder: The Importance of Task Design

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Abstract

Objective Attention-deficit/hyperactivity disorder (ADHD) is a heritable neuropsychiatric disorder, associated with abnormal reward processing. Limited and inconsistent data exist about the neural mechanisms underlying this abnormality. Furthermore, it is unknown whether reward processing is abnormal in unaffected siblings of participants with ADHD.

Method We used event-related functional magnetic resonance imaging (fMRI) to investigate brain responses during reward anticipation and receipt with an adapted monetary incentive delay task in a large sample of adolescents and young adults with ADHD (n=150), their unaffected siblings (n=92), and control participants (n=108), all of the same age.

Results Participants with ADHD showed, relative to control participants, increased responses in the anterior cingulate, anterior frontal cortex and cerebellum during reward anticipation, and in the orbitofrontal and occipital cortex during reward receipt. Responses of unaffected siblings were increased in these regions as well, except for the cerebellum during anticipation and the orbitofrontal cortex during receipt.

Conclusion ADHD in adolescents and young adults is associated with enhanced neural responses in frontostriatal circuitry to anticipation and receipt of reward. The findings support models emphasizing aberrant reward processing in ADHD, and suggest that processing of reward is subject to familial influences. Future studies using standard MID task parameters have to replicate our findings.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a neuropsychiatric disorder affecting about 5% of children worldwide (Polanczyk et al. 2007) and is characterized by a pattern of impairing and persistent inattention and/or hyperactivity and impulsivity (American Psychiatric Association 2013). Research on cognitive aspects of ADHD has focused longtime on executive functions such as working memory and response inhibition (Willcutt et al. 2005). However, more recent cognitive models of ADHD have implicated deficits in reward processing (Luman et al. 2010). Children with ADHD appear to be more sensitive to the positive effects of reward on performance (Luman et al. 2005; Uebel et al. 2010), make more risky decisions to obtain rewards (Groen et al. 2013), have stronger preference for immediate compared to delayed rewards (Marco et al. 2009; Bitsakou et al. 2009), and show steeper temporal discounting compared to control participants (Scheres et al. 2008; Demurie et al. 2011). However, reports on behavioral measures of reward processing are inconsistent, and findings often remain un-replicated (e.g. (Solanto et al. 2007; Sjöwall et al. 2012; Groen et al. 2013)). Little is known about the neural underpinnings of reward processing in particular in adolescents with ADHD. Our study aimed to investigate the neural mechanisms underlying reward processing in adolescents and young adults with ADHD, their unaffected siblings, and control participants.

Fronto-striatal brain networks, including the orbitofrontal cortex, medial prefrontal cortex, and the ventral striatum (VS) play a crucial role in reward processing (for review see (Haber and Knutson 2010)). Accordingly, studies investigating reward processing using a monetary incentive delay (MID) task have found alterations in VS signaling in both healthy populations and participants with ADHD (for review see (Plichta and Scheres 2014)). However, the manner in which VS signaling is altered is dependent on the studied population. Control participants with impulsive traits showed an increase of the striatal response to reward, whereas participants with ADHD mostly had decreased striatal responses to reward. VS responses during reward anticipation for adolescents with ADHD were observed to be lower than for control participants, but no differences were observed during reward receipt (Scheres et al. 2007). However, an increased response in the same VS area during reward receipt but not during reward anticipation has been reported as well (Paloyelis et al. 2012). This inconsistency may be related to the small to moderate sample sizes and differences in task and study design. We aimed at resolving this discrepancy by assessing reward anticipation and reward receipt using an adaptation of the MID task in a large population of adolescents and young adults with ADHD and control participants. The MID task has been repeatedly shown to elicit a neural response in the VS to both reward anticipation and receipt (for review see (Hermans et al. 2010; Hoogman et al. 2011; Sescousse et al. 2013; Plichta and Scheres 2014)).

ADHD has a strong genetic loading with an estimated heritability of about 80% (Faraone and Mick 2010). Siblings of participants with ADHD, who share on average 50% of their genetic information, have a two- to eight-fold elevated risk of ADHD relative to control

participants (Faraone and Doyle 2001). Despite the high heritability of ADHD, identification of genes that contribute to the etiology of the clinical phenotype has proven challenging. The identification of endophenotypes may be helpful in unraveling the genetic component of ADHD. Endophenotypes are objective measures that represent heritable vulnerability traits associated with the disorder in the population and are thought to be intermediates on the pathway from genotype to phenotype (Gottesman and Gould 2003). Importantly, because of their assumed heritability, it has been proposed that valid endophenotypes can be found at a higher rate in unaffected family members than in the general population (Gottesman and Gould 2003; Rommelse et al. 2011). So far, two studies have investigated the familiality of behavioral measures of reward processing in the context of ADHD. These studies have reported oversensitivity to reward and abnormal preference for immediate reward in unaffected siblings (Marco et al. 2009; Uebel et al. 2010). Moreover, genetic effects on reward processing in control participants have been described (Dreher et al. 2009). Therefore we investigated whether neural measures of reward processing in unaffected siblings are intermediate between those of participants with ADHD and control participants, thus supporting their role as an endophenotype of ADHD.

Methods

Participants

This study was approved by the local ethics committee of participating centers. Written informed consent was obtained from all participants or their legal guardians (for participants <12 years). We considered data from 571 participants of the NeuroIMAGE cohort, a large-scale cohort of families with one or more children with ADHD and control families recruited for the International Multicenter ADHD Genetics (IMAGE) study (Brookes et al. 2006; Rommelse et al. 2008). Detailed recruitment and testing procedures for NeuroIMAGE have been described elsewhere (von Rhein et al. 2014).

At the time of follow-up, clinical status was re-assessed by a trained professional administering the Schedule for Affective Disorders and Schizophrenia for School-Age Children (KSADS) (Kaufman et al. 1997) to parents and children and complemented by ADHD questionnaires (Conners' Parents and Teacher Rating Scales (Conners et al. 1998; Conners et al. 1999); see (von Rhein et al. 2014) for detailed diagnostic procedures). Diagnosis was based on DSM-5 criteria (American Psychiatric Association 2013). Both unaffected siblings and control participants were free of ADHD.

The descriptive characteristics of the sample are summarized in Table 1. After applying exclusion criteria (see Supplement 1, available online) we were able to analyze 350 individuals: 150 participants with ADHD (68 predominantly inattentive, 21 predominantly hyperactive-impulsive, and 61 combined type), 92 unaffected siblings, and 108 control participants. Age was not different between groups (Table 1), while gender was unequally

distributed with a higher percentage of men with ADHD compared to the other groups (Chi2(2)=23.3; p<0.01).

As expected in a clinical sample of participants with ADHD, the majority had a history of treatment with ADHD medication (n=114 of 150). ADHD medication consisted of treatment with methylphenidate with immediate release (MPH-IR; n=103), methylphenidate with extended release (MPH-ER; n=84), atomoxetine (n=14), and/or dextroamphetamine (n=8). All participants had discontinued use of medication prior to testing for 48 hours.

TABLE 1 Demographic and behavioral data of the NeuroIMAGE sample. Numbers represent count (N), percentage (%), mean (M) and standard deviation (SD). Group comparison refers to post-hoc group-wise comparisons of participants with ADHD (A), unaffected siblings (S) and control participants (C)
ODD = oppositional defiant disorder; CD = conduct disorder.

	Participants with ADHD		Unaffecd Siblings		Control Participants		
Demographics	**N**	**%**	**N**	**%**	**N**	**%**	**Group comparison**
Sample	150	43.2	92	26.5	108	30.3	
Comorbid	34/8	23/5	0	0	0	0	
Male	105	70	42	45.7	44	41.9	
		M	**SD**	**M**	**SD**	**M**	**SD**
Age	17.7	3.0	18.5	3.8	17.2	3.0	A=S=C
IQ[a]	97.9	15.3	99.8	15.6	107.7	13.9	(A=S)<C
Inattentive symptoms[b]	7.2	1.8	0.6	1.4	0.5	1.3	A>(S=C)
Hyperactive/impulsive symptoms[b]	6.0	2.4	0.6	1.0	0.3	0.8	A>(S=C)
Behavior							
Reaction times reward (in ms)	293	36	296	34	296	32	
Reaction times neutral (in ms)	325	47	324	39	320	38	
Difference Reaction times							F(2,328)=2.7; p<0.07 A=S=C
Coefficient of variation reward	0.188	0.104	0.180	0.053	0.173	0.061	
Coefficient of variation neutral	0.228	0.103	0.214	0.079	0.192	0.053	
Difference Coefficient of variation	0.04	0.09	0.03	0.08	0.02	0.09	F(2,275)=3.1; p<0.05 A>C;A=S;S=C

[a] Estimated on basis of vocabulary and block-design sub-tests
[b] Symptoms based on combination of the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS) and the Conners' Parent Rating Scale (CPRS)

Reward Anticipation Paradigm

We used a modified version of the MID task (Knutson et al. 2001; Hoogman et al. 2011) participants were asked to respond as quickly as possible to a target by pressing a button. Prior to this target, a cue indicated the possibility to gain a reward after a button press within a given time window. Every trial ended with a feedback screen informing about the outcome of the current trial. Depending on the participants' performance the response window for a correct response was adapted in the next trial resulting in an expected hit rate of 33%. The experiment lasted 12 minutes and a total of 5 Euros could be gained. At the end of the experiment, the awarded money was paid to the participant (see supplementary material and Figure S1 for a detailed description of the task).

Compared with the original task, our version differed on two main aspects: hit rate (33% vs 66%) and reward magnitude (20 cents vs 5 Dollar). The rationale behind these adaptations was to increase the demands of the task with stronger task engagement as result. Secondly, our adaptations aimed at meeting the practical constraints of our study. Considering that we limited ourselves to rewarded and neutral conditions, rewarding participants according to the original task parameters would have led to disproportional monetary rewards (~80 euro's), which was a concern for us and our ethical review board.

Behavioral measures

Behavioral outcome measures were reaction time and coefficient of variation (CV) in the rewarded and neutral conditions. Based on trials with correct responses (i.e. no premature responses (RT<100 ms), too many (>1) or too early (i.e. before target onset) button presses or no response at all) we calculated mean reaction times (RT). The coefficient of variation (CV) was defined as the standard deviation divided by the mean. Values were log10-transformed to improve normal distribution of the data.

Image analysis

After image acquisition, preprocessing, and initial nuisance regression (see Supplement 1, available online), statistical parametric maps were estimated for each participant with a general linear model (GLM; FSL FEAT). First-level regressors included six regressors of interest (onset times of rewarded and neutral cues, hits, and misses, each with a duration of 0 seconds) and 6 regressors of no interest. The latter regressors comprised a) onsets of rewarded and neutral targets, b) cue, target, and outcome onsets of error events, and c) a motion regressor. Error events comprised events of trials with incorrect responses. The motion regressor was inserted to control for possible movement artifacts (Keulers et al. 2012). Head movements from one image to the next exceeding 0.5 mm in either the x, y or z direction were considered movement artifacts. Onset of this error event was set to 8 seconds before the movement and all events within this 8 seconds interval were discarded. To ensure we had a sufficient amount of events to model our regressors of interest we

only included participants with at least 5 events per event type (see Table S1, available online). All regressors and their temporal derivatives were convolved with a canonical hemodynamic response function (HRF). Finally, the estimated beta maps for each participant were normalized to a common space (MNI152) for group comparisons.

Group comparison was divided into two steps: identification of regions that show sensitivity to ADHD-control differences and testing of endophenotypic characteristics within these regions including participants with ADHD, unaffected siblings and control participants. The first group comparison included participants with ADHD and control participants only. We chose this approach opposed to a more conservative approach of assessing diagnostic effects within a general linear model including all three groups in order to be maximally sensitive to diagnosis-dependent effects. An ADHD-control comparison derived from a model including the unaffected siblings would have been less sensitive as the included siblings would increase the error variance. Alternatively, investigating only the three-group contrast would reveal only regions showing endophenotypic characteristics (for results following this latter strategy see Supplement 1 and supplemental Table S2, available online).

Region identification

To identify brain regions that showed deviant BOLD responses during reward anticipation and receipt, normalized contrast maps of the first-level parameter estimates were taken to second-level random effect analyses (FSL FLAME). For anticipation, we contrasted response maps for rewarded cues with response maps for neutral cues. For monetary reward receipt, we assessed the interaction of accuracy (hits versus misses) and reward (rewarded versus neutral trials (rewarded hits: 1; rewarded misses: -1; neutral hits: -1; neutral misses: 1). This contrast was thought to have highest sensitivity to responses of the VS, signaling the need to adapt behavior in order to maximize reward gain and minimize punishment, commonly referred to as the reward prediction error (Sescousse et al. 2013). Group (ADHD versus control) was entered as a between-subject factor in both anticipation and receipt analyses. Scan location, age, gender, comorbidity with ODD/CD, and summary movement parameters (sum of all realignment parameters, and the number of movement-related error events) were added as regressors to account for effects of no interest. After initial thresholding at the voxel level ($Z > 2.3$) statistical inference was done at a cluster level using Gaussian Random Field (GRF) theory-based significance testing (FSL (Smith et al. 2004); $p < 0.025$ to correct for testing during anticipation and receipt) within a whole-brain search-space (for results of analyses restricted to an ROI search-space see Supplement 1 and supplemental Table S3, available online).

Assessment of unaffected siblings

To subsequently examine the endophenotypic characteristics of regions identified by the procedure above, we tested the influence of familiality in each identified region using an ANOVA with group (ADHD, unaffected siblings and control) as between-subject factor. We

added scan location, age, gender, comorbidity with ODD/CD and movement summary scores as covariates. Of note, in familial study designs that include more than one participant per family the assumed independence of data is violated, potentially underestimating inter-individual variance in standard GLMs. The current study allowed inclusion of more than one participant with ADHD from one family. We therefore corrected for non-independence of data by adding family as random effect. This was done in R (R 2.15.3 using the lme4 package (lme4 1.0.4 (Bates et al. 2013)). All p-values were Bonferroni corrected ($p<0.0083$) for the number of clusters.

In addition to the regions identified by the ADHD-control comparison we specifically examined the role of the VS using region-of-interest (ROI) analyses (Sescousse et al. 2013). To avoid non-independent voxel selection, we defined our ROI based on anatomical information. Each participant's anatomical MRI scan was segmented using an automatic subcortical segmentation tool (FIRST v1.2; (Patenaude et al. 2011)). From these segmented structures, we selected the bilateral VS labelled as nucleus accumbens (NAcc), aligned them with the functional images, and extracted the mean of the parameter estimates from the contrast images for reward anticipation and receipt. We tested for endophenotypic characteristics of each of these 2 measures using an analysis of variance with the same design as described above.

Finally, we estimated the influence of group on RT and CV difference scores (neutral RT/CV minus rewarded RT/CV)

Age, Family gradient and Sensitivity analyses

Because of the wide age range of the studied sample and the divergent findings in literature including older participants we investigated age-related effects in our sample. Therefore, we conducted two analyses. First, we divided our sample in two age groups (<18, 18+). We used this division as an additional between-subject factor, resulting in an age by diagnosis design with comorbidity with ODD/CD, scan site, gender and motion as nuisance regressors. All reported clusters inclusive the ROI were treated as dependent variables. Second, we added the interaction of age as a continuous measure with diagnosis to the statistical model.

We also assessed familiality of our neural measures by calculating family gradients. Results of these analyses as well as additional post-hoc assessment of potential confounding factors such as medication use and testing at multiple sites and main effect of task separately in all three diagnostic groups are reported in the supplements (see Supplement 1 and supplemental Table S6, S8-S11 and Figure S3, available online).

Results

Behavioral results

Task performance is summarized in Table 1 and plotted in Figure 1. There was a main effect of cue type with faster reaction times for rewarded trials compared to neutral trials (295 ms versus 323 ms; $t(335)=-19.4$; $p<0.001$). We did not observe a significant group difference ($F(2,328)=2.7$; $p<0.07$). Regarding CV we found a main effect of cue, with more variability during neutral trials (0.186 versus 0.235; $t(349)=5.9$; $p<0.001$). We also observed a significant group effect ($F(2,275)=3.1$; $p<0.04$). Pair-wise comparison revealed that reward-related reductions in variability were larger in participants with ADHD compared with control participants ($t(236)=-2.4$; $p<0.02$). Unaffected siblings had CV scores that were similar to those of control participants and their affected siblings.

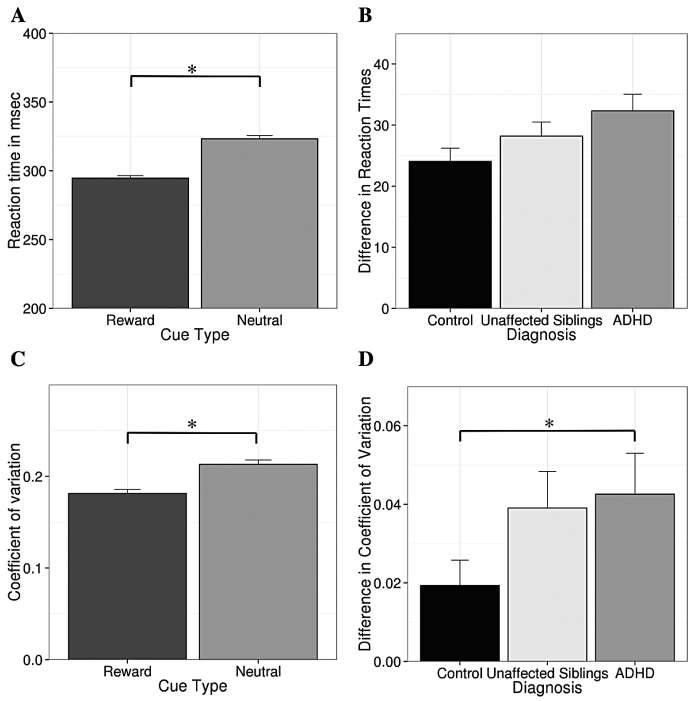

FIGURE 1 Behavioral effects of reward on reaction times (RT) and coefficient of variation (CV) across all participants (A + C) and for each diagnostic group (B + D). *$p<0.05$

General task effects

After family-wise error correction for multiple comparisons within the whole brain, we established a significant effect of reward anticipation for the contrast rewarded versus neutral cue. Regions that showed response to the task manipulation included the basal ganglia inclusive the VS, anterior cingulate cortex (ACC), insular cortex, visual cortex and

cerebellum (Figure 2). For reward receipt, we detected significant BOLD signal increases for rewarded relative to neutral hits (versus misses) in the reward system, including the VS and frontal regions, motor cortex and visual cortex.

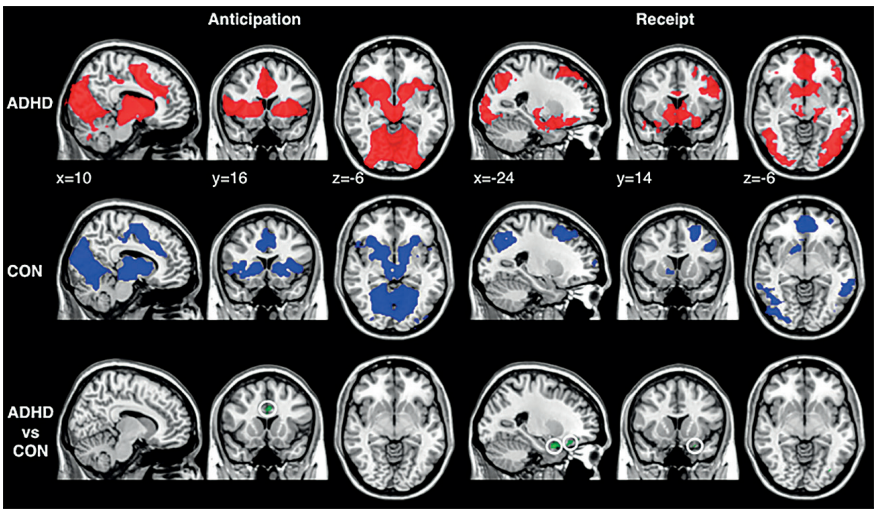

FIGURE 2 Brain responses to rewarded versus neutral cues (Anticipation; left) and rewarded vs neutral accuracy (hits versus misses) (right) in participants with ADHD (red) and control participants (blue), and the contrast ADHD versus control participants (green). All maps show GRF-theory based clusters significance at $p<0.025$ within a whole-brain search-space.

Main effects of ADHD diagnosis

During anticipation we observed a significant main effect of ADHD (ADHD vs. control comparison) in three clusters including the ACC, frontal pole, and cerebellum (Figure 2; for results of three-groups group analysis see Supplement 1, available online). During receipt, the same comparison revealed a significant main effect of ADHD in the occipital cortex and two regions in the OFC, one of them extending into amygdala. All significant clusters are summarized in Table 2 (post-hoc tests of the interaction that is implicitly modeled in this comparison can be found Tables S4-S5 and are illustrated in supplemental Figure S2, available online).

TABLE 2 Overview of brain regions used to test for group differences. Regions comprised significant clusters (cluster p<0.025) from the ADHD-control comparison and one region-of-interest (ROI). Indicated p-values are corrected for non-independence.

	Region	Size[a]	Side[b]	Z	X	Y	Z	Omnibus-test	Pairwise-comparison[c]
Anticipation	Frontal pole	86	R	1.63	30	36	36	F(2,307)=8.1; p<0.001*	(A=S)>C
	Anterior Cingulate Cortex	89	L/R	2.24	-4	16	30	F(2,331)=10.0; p<0.001*	(A=S)>C
	Cerebellum	91	L	3.91	-26	-58	-28	F(2,306)=8.5; p<0.001*	A>(S=C)
ROI	Nucleus Accumbens		L/R					F(2,333)=1.0; p<0.1	A=S=C
Receipt	Occipital cortex	90	L	2.14	-44	-64	2	F(2,287)=9.3; p<0.001*	(A=S)>C
	Orbitofrontal cortex	100	L	2.87	-30	26	-18	F(2,335)=9.7; p<0.001*	(A=S)>C
	Orbitofrontal cortex	127	L	4.64	-22	4	-24	F(2,288)=20.0; p<0.001*	A>(S=C)
ROI	Nucleus Accumbens		L/R					F(2,332)=3.1; p<0.04*	A>C;A=S;S=C

[a] in Voxel
[b] Left(L) / Right(R)
[c] A=ADHD; S=Unaffected Siblings; C=Control Participants

Familiality analyses

Results of statistical group analysis (ADHD, unaffected siblings, control) in the significant clusters are presented in Table 2. Two different response patterns were observable. For the majority of tested regions both participants with ADHD and unaffected siblings had increased responses relative to control participants (anticipation: ACC, frontal pole; receipt: occipital cortex, OFC). In two other regions (anticipation: cerebellum; receipt: OFC) participants with ADHD had increased brain responses relative to their unaffected siblings and control participants; the responses of unaffected siblings and control participants did not differ from each other. Group-specific mean responses are displayed in Figure 3.

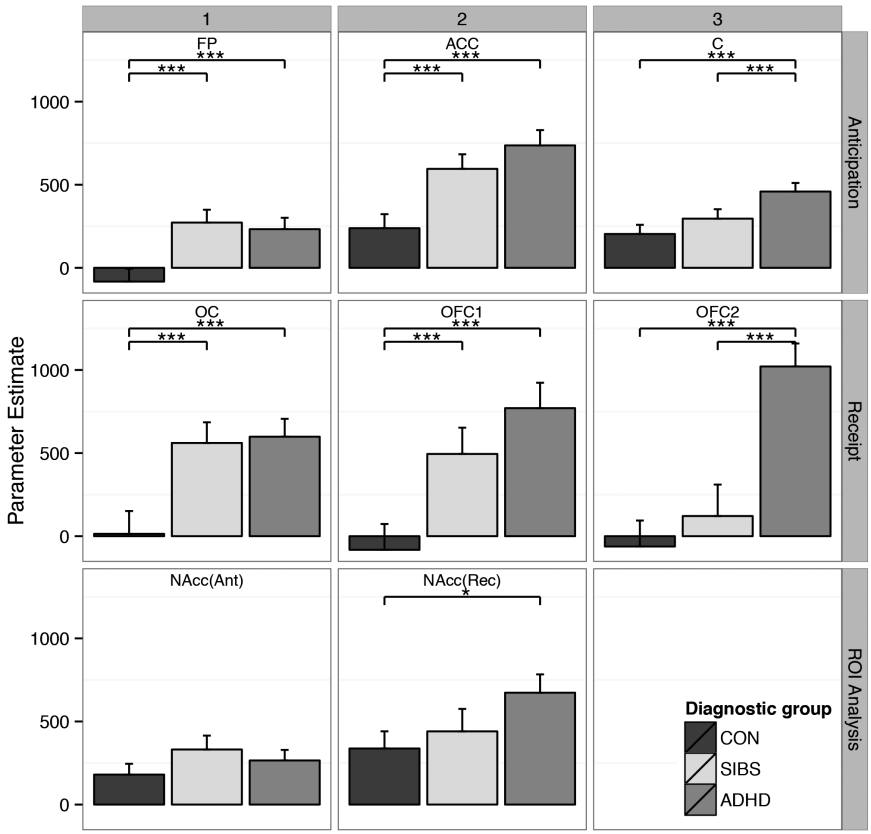

FIGURE 3 Group means for significant clusters from the ADHD-control comparisons and regions-of-interest (NAcc) analyses. Regions are frontal pole (FP), cerebellum (C), anterior cingulate cortex (ACC), occipital cortex (OC), orbitofrontal cortex (OFC), OFC extending into amygdala (OFC2). Error bars indicate standard error of the mean. *p<0.05 ***p <0.001

Results of the ROI analysis are also indicated in Table 2 and Table S10 and S11, available online. For reward anticipation and receipt, analysis of the VS revealed a main effect of task with increased BOLD response for rewarded trials compared with neutral trials (anticipation: $t(257)=5.0$; $p<0.001$; receipt: $t(257)=5.8$; $p<0.001$). For receipt, we additionally observed an ADHD effect ($F(2,334)=3.2$; $p<0.04$), with an increased BOLD response in ADHD compared to control participants ($t(334)=2.44$; $p<0.04$). Unaffected siblings did not differ from their affected siblings and the control participants. During anticipation BOLD responses were equal between all groups ($F(2,336)=1.1$, $p<0.4$).

Age analyses

None of the brain regions (i.e. clusters from the ADHD-control comparison and NAcc during anticipation and receipt) showed a significant effect of age or interaction between age and diagnosis (see Supplement 1 and supplemental Table S7, available online).

Discussion

The aim of this study was to investigate the neural mechanisms of reward processing in ADHD and its potential as endophenotype of ADHD. Our results revealed that ADHD is characterized by increased reward-related neural responses during anticipation and receipt as well as reduced variability of behavioral responses for rewarded cues. These findings extend previous observations of increased impact of reward on behavior in adolescent ADHD (Luman et al. 2005). Further, unaffected siblings of participants with ADHD had also increased neural responses during anticipation and during receipt, suggesting that familial factors play a role in this increased sensitivity of the reward system.

Participants with ADHD specifically displayed an increased response to reward in the frontal pole and orbitofrontal cortex. During anticipation this increased response extended to the ACC, which together with the basal ganglia forms the frontostriatal reward network. Here, the orbitofrontal cortex has been put forward as the central structure for representing the value of an expected outcome (O'Doherty et al. 2001), whereas the ACC has been associated with performance monitoring (Kennerley et al. 2006). Consequently, it might be that participants with ADHD overestimate the expected value of reward outcome. Additionally, they might recruit more resources to monitor actions or prepare a response.

Participants with ADHD also exhibited an increased response to reward receipt in the VS. VS responsiveness has been considered to code either for the hedonic value representation of reward (i.e. the amount of subjective pleasure an individual experiences) and to represent reward prediction error coding (i.e. the difference between expected and actual reward (Sescousse et al. 2013)). This would suggest that participants with ADHD might be hypersensitive to reward because they experience receiving a reward relative to no reward as more pleasurable than control participants. Alternatively, they might be unable to correctly establish an association between a reward-predicting cue and the receipt leading to an increased prediction error response during reward receipt relative to non-reward. A third explanation would be that participants with ADHD experience the unability to gain reward as overly aversive which may result in a stronger signal of the brain to avoid such a situation in future (Lemiere et al. 2012).

A novel aspect of the current study was the assessment of familiality of the neural mechanisms of reward processing in ADHD. For most of these brain regions the unaffected siblings exhibited increases of brain responses that made them comparable to affected participants. For two regions (cerebellum, OFC), we found that their brain responses were more similar to the control participants. Together our results suggest that unaffected siblings show part of the disorder-specific changes in neural functioning, however, these changes were present to a lesser extent. The observed changes in the neural substrate underlying reward processing in symptom-free family members of participants with ADHD suggest that familial factors (i.e. genes and/or shared environment) contribute to alterations in the reward system. Interestingly, genes such as DAT1, which can confer risk

to ADHD, have been found to affect reward processing in control participants (Dreher et al. 2009) and participants with ADHD (Paloyelis et al. 2012). As a result, altered reward processing is a potential new endophenotype of ADHD. Of note, we used strict diagnostic criteria to guarantee that unaffected siblings neither exhibited subthreshold ADHD nor differed from control participants on ADHD symptom measures.

Our data replicate behavioral reports of altered reward sensitivity in adolescents with ADHD (Luman et al. 2005). However, they are in contrast with reports of decreased responsiveness of the VS in anticipation of reward in adults with ADHD (Plichta and Scheres 2014) as well as with one study with adolescents with ADHD (Scheres et al. 2007). Age may have a critical influence on the functioning of the reward system. Evidence supporting this account comes from studies showing developmental changes in neural firing of the reward system. These studies have mainly shown hypersensitivity of the reward system in adolescents (Galvan 2010). Adolescence is thought to be characterized by behavioral changes such as increased risk-taking and behavioral impulsivity (Arnett 1999; Casey and Jones 2010) putatively caused by an imbalance of basal ganglia and prefrontal cortex maturation. Moreover, impulsivity is in the healthy population associated with increases in sensitivity to reward (Plichta and Scheres 2014). Given that healthy adolescents and participants with ADHD share increased behavioral impulsivity, we speculate that both adolescents and participants with ADHD might experience an age-related imbalance of neural development. At later ages, when regulatory functions of the prefrontal regions have matured in healthy populations (Casey et al. 2008), signaling in striatal regions may not only be normalized in participants with ADHD but may even be suppressed, resulting in a hypo-responsive reward system. However, we did not find support for this interpretation in our study since effects of age or an interaction of age by diagnosis were non-significant. This may be because our participants are mainly late adolescents and young adults. Accordingly, it might be that striatal hypo-responsiveness occurs at a later age. Another possibility is that adult ADHD is different from adolescent ADHD in terms of neural underpinnings. Longitudinal studies are needed to differentiate between these possible explanations.

The findings reported here should be interpreted in the context of strengths and limitations of our study. We examined reward processing in a large sample of carefully phenotyped participants using a family-design. A modified version of the well-established monetary incentive delay paradigm was employed, which induced clear behavioral and neural activation effects as both whole-brain and region of interest analyses indicated. Another strength of the study was that all participants were scanned while off medication. Furthermore, we were able to rule out the effects of common confounds such as medication use, gender, and comorbidity with ODD/CD.

Our task was modified compared to the traditional versions of the MID (Knutson et al. 2001). Specifically, we included only one level of reward with relatively low reward magnitude and lowered hit probability. Evidence from imaging studies on reward processing in healthy participants suggests that reward processing in the striatum is dependent on

both of these parameters (Yacubian et al. 2006). Considering that previous studies with children and adults with ADHD have reported differences in striatal responses for a high reward condition only, it may be that inconsistencies between these studies and ours might be due to differences in reward magnitude. Indeed, signals in the striatum are most robust when high rewards are at stake, while responses to lower rewards are present, yet less reproducible (Wu et al. 2014). Although we cannot exclude this possibility, we argue that this is unlikely given observations that dopaminergic midbrain neurons code for the relative rather than absolute value of a reward. Indeed, reward-related responses adapt to the context in which a reward is presented (Tobler et al. 2005), in the sense that they depend on a combination of the overall expected value and their variance rather than the absolute magnitude of reward value. As is the case for dopamine neurons (Tobler et al. 2005), reward-related BOLD signals should maintain their sensitivity over a large range of reward values. This argument is supported by the clear and strong reward-related responses we observed in regions that are typically associated with reward processing (see Figure 2) (Knutson et al. 2001).

Another aspect of the changed task parameters relates to the underlying cognitive process. Specifically, it might be that due to the infrequent hits our task was generally experienced as too difficult leading to frustration and surprise rather than anticipating and receiving reward. Indeed, striatal structures are known to show biphasic responses, making them capable to respond to positive (reward) as well as negative (punishment) stimulation (Liu et al. 2011). Moreover, such an account would be in line with experimental findings demonstrating increased responses in reward processing brain regions to delayed rewards (Lemiere et al. 2012). Yet, both reward magnitude and reward probability are coded relatively rather than absolute (Tobler et al. 2005). This implies that the perception of a cue as rewarding or punishing depends on the context. Accordingly, our hit rate of 33% would have been experienced as frustrating only when participants were able to compare this with experimental paradigms or conditions with a higher hit rate. Secondly, we observed faster and more stable responses on rewarded trials for all participants indicating that participants were aware of the reward component of the task rather than being surprised by the infrequent hits. Nevertheless, future studies need to confirm our findings. Moreover, given that another study reporting no VS differences in the anticipatory phase (Paloyelis et al. 2009) applied a paradigm that also differed on reward probability suggests that this parameter is a crucial task parameter and deserves systematic investigation in future.

Finally, our task was originally designed to investigate reward anticipation and was not optimized for assessing reward receipt processing. The amount of trials used to estimate responses during reward receipt was relatively small and, with twice as many misses as hits, unequally distributed (for details see Table S1, available online). This may have resulted in a suboptimal estimation of receipt-related effects. Nevertheless, we are confident that the receipt-related effects reported here are robust and reliable based on the size of the sample we used as well as the clear main task effect of receipt (irrespective

of group) that is in line with previous reports (Liu et al. 2011). A final note of caution concerns the functional specificity of the observed effects to reward processing. Specifically, we cannot exclude the possibility that the reported hyper-activation during reward anticipation and receipt reflects non-reward specific effects of ADHD on the processing of salient events (Zink et al. 2003).

To summarize, adolescents and young adults with ADHD, compared to control participants, had increased responses of the reward system (even when relatively low rewards were at stake). Unaffected siblings of the participants with ADHD showed the same altered response to reward anticipation (frontal pole; ACC) and receipt (occipital lobe; OFC) as their affected siblings whereas no changes could be observed in the cerebellum (anticipation) and the OFC (receipt). Our findings highlight that familial factors contribute to the pathogenesis of ADHD by affecting the reward system, and suggest that altered reward processing is a promising endophenotype of ADHD.

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Measuring the Neural Basis of Reward Anticipation and Reward Receipt in Attention-Deficit/Hyperactivity Disorder: The Importance of Task Design

Michael Plichta, Anouk Scheres

In the May 2015 issue of the Journal, von Rhein et al. (von Rhein et al. 2015) investigated ventral-striatal (VS) response during monetary reward anticipation and receipt using an impressively large sample (N = 350) of adolescents and young adults with attention-deficit/hyperactivity disorder (ADHD), their unaffected siblings, and typically functioning controls. The goal was to resolve putative inconsistent findings in previous studies by using a statistically well-powered, multicenter, large-scale functional magnetic resonance imaging study. The authors reported group differences during reward receipt but not during reward anticipation.

Our concern is that the study did not clearly resolve previous inconsistencies because of modifications to the experimental task used. The authors used the Monetary Incentive Delay task (Knutson et al. 2005), which is one of the most frequently applied functional magnetic resonance imaging tasks, and one with high test-retest reliability (Wu et al. 2014). However, the authors changed 2 parameters that are essential to the psychological process and brain activation measured with this task.

First, the gain amount per trial was decreased from $5 (original task) to €0.20. This is disadvantageous, because low monetary gains have been associated with low test- retest reliability of the VS response during gain anticipation (Wu et al. 2014). Obviously, this makes the task less efficient regarding VS activation, and the reported main contrast effect sizes are small compared with other studies using this task (e.g., Wu et al. (Wu et al. 2014)). Although the authors argued that lowering gains was necessary, other solutions exist, including using loss trials or occasional boost trials.

The second parameter is the probability of winning on a successfully performed trial (hit rate). In the standard task, an adaptive algorithm adjusts the hit rate to remain at approximately 66%. This is a well-thought-out value because sustained dopamine activation between cue and reward is highest at 50% and decreases as the probability of the outcome approaches 0% or 100% (Fiorillo et al. 2003). As a consequence, a 66% hit rate leads to high dopamine activation and further establishes the positive association between cue and reward. Von Rhein et al. lowered the hit rate to 33%. What are the potential consequences? First, participants may become frustrated over time because they do not actually win on two thirds of the win-trials and might experience the task as out of their control.

Because subjective ratings were not reported, it is hard to rule out these possibilities. From the participant's perspective, the gain cue might not signal an upcoming reward but rather a non-gain. This notion is supported by the observation that people can ap-

proximately estimate the hit rates of various cue types (Knutson et al. 2005). Also, research suggests that behavioral reward anticipation and VS activity increase with increasing probabilities (Abler et al. 2006). Therefore, a hit rate of 33% would appear suboptimal when aiming to measure reward anticipation and associated VS activity. This lower probability of success, in combination with low monetary gains, resulted in low expected values, which also has been associated with VS activation (Knutson et al. 2005). Because these modifications have not been validated previously, we believe it is difficult to draw accurate interpretations from the findings presented here. To generalize, one would need to refer to studies that systematically compare between manipulated hit rates, and few of these exist.

Despite these concerns, the finding of a relative VS over- activation in patients with ADHD during reward receipt is of special interest and importance because the current task is potentially more optimal for eliciting neural responses to reward receipt than during anticipation. The reason for this is that low hit-rates might trigger stronger prediction error signals than standard versions of the Monetary Incentive Delay task would (Abler et al. 2006). These findings might suggest an important role of learning and associated neural responses in individuals with ADHD.

Overall, although we believe that large-scale studies are needed, we recommend that investigators build incrementally from existing validated paradigms for comparability and carefully consider tradeoffs between efficiency and scientific interpretability.

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Reply by the authors

Daniel von Rhein, Roshan Cools, Jan Buitelaar

Drs. Plichta and Scheres have provided a thoughtful comment on our article about increased neural responses to reward in adolescents and young adults with attention-deficit/hyperactivity disorder (ADHD) and their unaffected siblings (von Rhein et al. 2015). In their commentary, the authors express concerns about the interpretability of our findings in light of the specific version of the Monetary Incentive Delay (MID) task that we administered. Specifically, in our version of the MID task, we opted for a smaller gain amount per trial and a lower hit rate (i.e., probability for success) compared with the original version as described by Knutson et al (Knutson et al. 2001).

We had a clear rationale for these modifications. First, our adaptation of the hit rate was aimed at increasing the demands of the task because the original hit rate of 66% appeared to be rather easy in pilot experiments. A lower hit rate resulted in stronger task engagement. Second, our adaptations were motivated by the practical constraints of our study. From all previous studies investigating reward processing in ADHD, no study has reported diagnosis- specific changes in neural functioning in critical reward- processing structures, including the ventral striatum (VS) and orbitofrontal cortex (OFC) for the punishment condition (Scheres et al. 2007; Stoy et al. 2011; Paloyelis et al. 2012). In consequence, we chose to focus on the motivational aspect of reward processing and omitted a punishment condition. Therefore, without a punishment condition, hits on rewarded trials add up to a large sum that is not decreased by losses. Provided that the task parameter of our study would have been the same as in the original article (V5 per hit, hit rate 66% of all [25] responses), we would have had to pay each participant of the task approximately V80. This disproportional monetary reward was a concern for us and our ethics review board. By choosing a hit rate of 33% and 20 cents per hit, we could solve the problem of disproportional gain (about 1.60 euro's paid per participant).

We believe it is highly unlikely that the lack of an ADHD effect on reward-anticipation–related blood oxygenation level-dependent (BOLD) signal in the VS reflects these changes to the paradigm. First, there were strong main effects of task, with the VS responding strongly during reward anticipation (Figure 1). Second, we obtained significant effects of ADHD on reward-anticipation–related BOLD signal in other parts of the brain. Third, our version of the paradigm has been used successfully in previous studies that showed BOLD signal changes in the VS (Hermans et al. 2010; Hoogman et al. 2011). Fourth, the suggestion that the lower reward magnitude would have rendered the task insensitive to subtle effects of ADHD is counteracted by the much larger sample used in the current study compared with previous studies. In any case, there is strong evidence that dopamine neuron activity is coded adaptively, in a context-dependent manner, as a function of the average expected value. The degree to which medium-size rewards elicit more activity than small rewards is equal to the degree to which large rewards elicit more activity than

medium-size rewards (Tobler et al. 2005). Accordingly, there is no reason to hypothesize that lower overall reward magnitude would elicit lower motivation or lower VS signal. Fifth, there is evidence that dopamine neurons exhibit the strongest sustained activity if the reward is most uncertain (Fiorillo et al. 2003). Thus, by analogy, there is no reason a priori to hypothesize that the lower reward probability would elicit lower motivation or lower VS BOLD signal.

Plichta and Scheres dispute that we were able to demonstrate that the modified MID task led to clear and significant predicted task effects at the behavioral and neural levels as described in the article (von Rhein et al. 2015). We observed faster reaction times in rewarded compared with neutral trials (von Rhein et al. 2015) (Figure 1 of the original article). Reward anticipation for the contrast rewarded versus neutral cue was associated with increased responses in the basal ganglia, including the VS, anterior cingulate cortex, insular cortex, visual cortex, and cerebellum (von Rhein et al. 2015) (Figure 2 of the original article). For reward receipt, we detected significant BOLD signal in- creases for rewarded compared with neutral hits (versus misses) in the reward system, including the VS and frontal regions, motor cortex, and visual cortex (von Rhein et al. 2015) (Figure 2 of the original article).

Accordingly, when we compare the results of our study with those of the original study (Knutson et al. 2001), the response map of our contrast reward cue versus neutral cue resembles the response map for reward anticipation of the original task. We illustrate this in Figure 1, where we show results of the original MID task (right) and our main effect of reward anticipation for the healthy controls (left; blue) and participants with ADHD (left; red) aligned with the coordinates of the peak of the effect of reward anticipation (as reported in the original article (Knutson et al. 2001)).

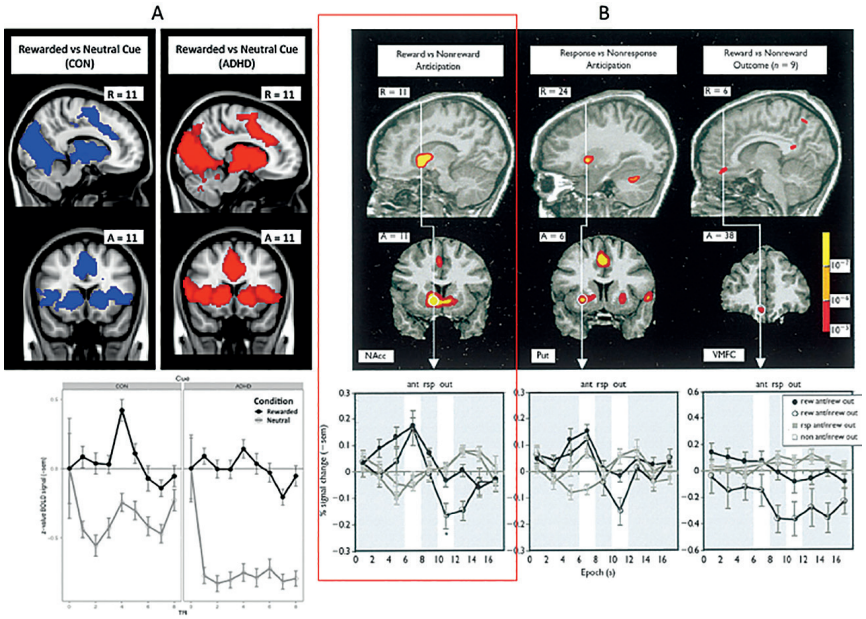

FIGURE 1 Spatial maps for the contrast rewarded versus neutral cue for participants with attention-deficit/hyperactivity disorder (ADHD) and healthy participants of the study by (A) von Rhein et al. and as reported by (B) Knutson et al. Both figures also show average time courses of the blood oxygenation level-dependent (BOLD) response in the ventral striatum (VS). Note: Red box on the previously published image highlights the information pertinent to discussion. Black and gray lines indicate VS responses after the presentation of rewarded and neutral cues. Ant = anticipation phase; CON = control; NAcc = nucleus accumbens; non ant = non-rewarded anticipation; nrew out = non-rewarded outcome; out = receipt phase; rew ant = reward anticipation; rew out = rewarded outcome; rsp = response phase; rsp ant = no response requirements; SEM = standard error of the mean; TR = repetition time. Figure 1B is reprinted with permission from Knutson B, Fong GW, Adams CM, Varner JL, Hommer D. Dissociation of reward anticipation and outcome with event-related fMRI. NeuroReport 2001;12:3683-3687.

Figure 1 shows that the location of task effect is exactly the same for the 2 studies. In addition, we calculated the mean time course of the VS for rewarded (indicated in black) and neutral (gray) trials with the onset of the cue at 0 on the x-axis and the BOLD response at that time point as baseline. As shown, the BOLD response increases from onset of the rewarded cue until it peaks after approximately 4 repetition times (9.2 seconds). For neutral cues, the BOLD response decreases. These findings replicate previous studies that successfully implemented the same adaptations leading to interpretable and valid findings (Hermans et al. 2010; Hoogman et al. 2011) and support our claim that the task manipulation worked.

It should be noted that we studied reward processing in a sample of participants with ADHD and controls that was substantially larger than samples of prior studies. In this way, our study was much more protected from false-positive findings than previous, smaller-scale studies. Adequate sample size is important for at least 2 reasons. First, giv-

en the significant heterogeneity of ADHD at the etiologic, clinical, and neural levels, findings from small samples might be difficult to generalize to the entire ADHD population. Second, underpowered studies lead to overestimation of effect sizes (Button et al. 2013).

Plichta and Scheres appropriately state that careful interpretation and assessment is warranted when adjusting task parameters. However, in this comment and the supplementary material of our article, we show that our modified version of the MID task elicits similar task-related activity as the original version presented by Knutson et al (Knutson et al. 2001). In this context, we believe our findings provide an important contribution to the current literature on the neural dysfunctions of ADHD.

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Chapter 4

Network-level assessment of reward-related activation in participants with ADHD and healthy participants

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Abstract

Introduction Reward processing is a key aspect of behavioral control processes, putatively instantiated by mesolimbic and mesocortical brain circuits. Deficient signaling within these circuits has been associated with psychopathology including ADHD. We applied a network discovery approach to assess specific functional networks associated with reward processing and their relation with ADHD.

Methods To describe task-related processes in terms of integrated functional networks we applied independent component analysis (ICA) to task response maps of 60 healthy participants who performed a monetary incentive delay (MID) task. Resulting components were interpreted on the basis of their similarity with group-level task responses as well as their similarity with brain networks derived from resting state FMRI analyses. ADHD-related effects on network characteristics including functional connectivity and communication between networks were examined in an independent sample comprising participants with ADHD and healthy controls.

Results We identified 23 components to be associated with 4 large-scale functional networks: the default-mode, visual, executive control, and salience networks. The salience network showed a specific association with reward as well as the highest degree of within-network integration. ADHD compared to healthy controls was associated with decreased functional connectivity between salience and executive control network and peripheral brain regions.

Conclusions Reward processing as measured with the MID task involves one reward-specific and three general functional networks. Participants with ADHD exhibited limited alterations in connectivity of the salience and executive control networks with associated brain regions during performance of the task.

Introduction

Reward processing is essential to human behavior. It motivates by eliciting approach behavior and facilitates learning as we try to maximize rewards, thereby directly impacting behavioural control. Reward further impacts affective experiences associated with behavior, for example by inducing pleasure when receiving a reward. It is clear that these processes affect how and what kind of decisions we make, what kind of preferences we have, and, as it is directly related to cognitive functions, how many cognitive resources we allocate to perform in a given situation.

Given its central role in behavioral control, abnormal reward processing has been associated with various forms of psychopathology, including attention-deficit/hyperactivity disorder (ADHD). Reward processing in ADHD, compared to healthy controls, is characterized by a preference for small but immediate rewards above larger but delayed rewards, and increased reward-induced effects on cognitive task performance (Sonuga-Barke 2002; Luman et al. 2005; Drechsler et al. 2008).

Behavioural alterations of reward processing in ADHD are accompanied by aberrant signaling in those structures of the brain that are thought to govern reward processing. These structures include mesolimbic and mesocortical brain circuits consisting of midbrain, ventral striatum, anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) (Frank and Fossella 2011). In participants with ADHD brain activity in those regions is altered compared to control participants during reward anticipation and reward receipt (Plichta and Scheres 2014; von Rhein et al. 2015). Other studies investigating reinforcement learning (Hauser et al. 2014) or other reward manipulations such as temporal discounting (Chantiluke et al. 2014) or rewarded continuous performance (Rubia et al. 2009) report converging findings of abnormal activations in these same structures in ADHD. Together these findings provide support for the role of dysfunctional mesolimbic and mesocortical brain networks in ADHD.

The functional units of the brain are brain networks, i.e. networks of specialized, neural structures that communicate with each other (Mesulam 1998; Poldrack 2011). However, investigation of network characteristics using conventional task-based analytical approaches is limited. Such approaches commonly use univariate models that rely on calculating averaged responses of the brain to manipulations, either for a region of interest (ROI) or at the whole-brain level (voxel-wise). Although such approaches are powerful in localizing cognitive functions based on the blood oxygen-level dependent (BOLD) response amplitude, they are blind to relational aspects between neural structures. These aspects are, however, crucial to fully describe the functional properties of a neural circuit underlying a cognitive function. Accordingly, a model that takes the brain's highly connected neural structure into account will provide a biologically more valid description of neural processes compared to a model that assumes functional independence (Sporns et al. 2004).

Here we provide a network discovery approach to define brain areas implicated in reward processing and to assess whether reward-related network characteristics differ between ADHD and controls. Specifically, we performed an independent component analysis (ICA) on participant-level reward-related activation maps to define those regions that co-activate across participants. ICA is a data-driven approach that separates neural data into a set of spatially independent components (ICs). By identifying components that reflect neuroanatomical systems we will be able to describe task responses in terms of associated larger functional networks. Because these components are determined based on the consistency of brain response patterns, this method is also sensitive to responses with low amplitude if they are consistent across participants. Finally, component selection allows focusing further investigations on specific components of interest, thus avoiding interpretation of noise components or e.g., components that are invariant to task demands.

Investigation of network characteristics in the context of ADHD is of particular interest as studies applying network analyses using resting state-fMRI report ADHD effects on a network level (for review see (Oldehinkel et al. 2013)). For example, ADHD has been associated with decreased connectivity between ventral striatum and orbitofrontal cortex (OFC) (Posner et al. 2013). Moreover, several studies report aberrant connectivity in the default-mode network (DMN) in ADHD (for review see (Konrad and Eickhoff 2010; Posner et al. 2014). By applying network analysis to reward-related task responses we will be able to specifically assess the association between ADHD and network characteristics of reward-related functional networks.

Methods

To identify reward-related functional networks based on spatially coherent response patterns we performed independent component analysis on task-based response maps derived from 60 control participants. We refer to these participants as the '**discovery sample**'. In addition, we used an independent '**test sample**' including 48 controls and 150 participants with ADHD to investigate ADHD-control differences in the spatial and temporal characteristics of the derived functional networks. An overview of the analytical steps is illustrated in the supplementary material (Figure S1) and each step is described in detail below.

Participants

Both the participants for the discovery sample as for the test sample were selected from the NeuroIMAGE study (www.neuroimage.nl). Detailed description of the recruitment and selection procedure for the entire cohort can be found in the supplements and in (von Rhein et al. 2014). Here, we included all participants from ADHD and control families who underwent a MRI scan session that included administration of a monetary incentive delay (MID) task (N=370). Participants with ADHD were required to have a current ADHD diagnosis according to the DSM-5 definition, healthy controls no more than 2 ADHD-re-

lated symptoms (see von Rhein et al (von Rhein et al. 2014) for detailed diagnostic procedure). As the study was conducted in families, participants included siblings (n=45). Exclusion criteria were use of medication (other than ADHD medication) or drugs (n=7), acute psychiatric conditions such as psychosis (n=3) and qualitatively insufficient or incomplete data (n=102; see Supplements for details). Applying these selection criteria resulted in the inclusion of 258 participants in the current analyses (150 ADHD, 108 healthy controls). These participants were the same as described in a univariate task-based case-control comparison presented elsewhere (von Rhein et al. 2015).

As indicated above the 258 participants were divided into a discovery and test sample. The discovery sample was entirely composed out of control participants to ensure an unaffected definition of reward-related networks. Sex was unequally distributed between controls and ADHD participants with more girls in the control group and more boys in the ADHD group. To ensure that at least 25% of participants in each sample were male we randomly assigned 25% (n=15) of the control boys to the discovery sample. All remaining control boys (n=33) were assigned to the control test sample. Subsequently, we added female control participants to the control test sample until the ADHD and the healthy control test sample had a comparable percentage of male participants (~70%). This was achieved by adding 15 randomly selected females to the control test sample. All other control females were added to the discovery sample. Demographics for all samples are displayed in Table 1.

TABLE 1 Demographics of the three study samples

Discovery sample	Test samples		
Controls	**Controls**	**ADHD**	
60	48	150	N
0	0	34/8	Comorbid (ODD/CD)
15 (25)	33 (69)	105 (70)	Number of males (%)
Mean (SD)	**Mean (SD)**	**Mean (SD)**	
17.3 (2.8)	16.9 (3.2)	17.7 (3)	Age
108 (15)	107 (13)	98 (15)	IQ
0.3 (1.1)	0.8 (1.5)	7.2 (1.8)	Inattentive symptoms
0.1 (0.4)	0.6 (1)	6 (2.4)	Hyperactive symptoms

Paradigm

Participants were performing a monetary incentive delay (MID) task, in which they need to respond to the occurrence of a target stimulus by pressing a button. The core manipulation of this task relates to the target-preceding cue. The color of this cue informs the participant whether a button press is potentially rewarded or not. Difficulty of the task is adapted to the performance of each participant by adjusting the time window in which

participants are allowed to respond (20 milliseconds shorter after hits and 10 milliseconds longer after misses), separately for rewarded and neutral cues. This resulted in an expected hit rate of 33% for each trial type. Common measures of the MID task are reaction times and reaction time variability as well as neural responses in the reward processing brain structures including orbitofrontal cortex (OFC) and ventral striatum (VS) during reward anticipation and receipt (Knutson et al. 2001; Fairchild 2011). A detailed description of the task is available in the supplements.

Brain network analysis of task-related responses

Network identification and decomposition

To recover networks implicated in reward processing we focused on task-based activation maps of the participants in the discovery sample. First, we identified all brain regions dedicated to reward processing by performing a typical task-based fMRI analysis (first-level) on preprocessed fMRI data with onset times of rewarded and neutral cues (regressor 1 and 2), hits (regressor 3 and 4), and misses (regressor 5 and 6) as regressors of interest (see von Rhein et al (von Rhein et al. 2014) and supplements for details of data acquisition / preprocessing and first-level statistics). This analysis resulted in participant-level spatial maps (zstat) for these six regressors. To investigate the relationship between these simple activation maps and higher-order contrasts hypothesized to capture key reward processes we included the within-subject contrasts reward cue versus neutral cue (reward anticipation; spatial map 7) and rewarded vs neutral accuracy (hit versus miss; reward receipt, spatial map 8). All eight maps were transformed to a common space (MNI152) for group analysis (see supplementary material for details).

To decompose the reward network into independent sources, we concatenated unthresholded participant-level zstat maps to one time series. On these data, we applied independent component analysis (ICA) as implemented in FSL MELODIC (Jenkinson et al. 2012). To maximize component reliability we ran 50 ICA decompositions, each including data from 40 randomly selected participants from the discovery sample. We requested extraction of 15 independent components (ICs) for each ICA decomposition. We chose this number to allow sufficient differentiation between noise and non-noise components, while avoiding unreasonably scattered functional networks. The spatial maps of all ICs gained from these multiple ICA decompositions were thresholded by means of mixture modeling at $p<0.5$ (Woolrich et al. 2005) and entered into a meta-ICA decomposition with a dimensionality of 30 components. The 30 resulting components were again thresholded using mixture modeling at $p<0.5$ (Woolrich et al. 2005) to reveal the final spatial maps. Based on these spatial maps we determined by visual inspection whether components were considered as noise components.

Network Interpretation

To facilitate interpretation of the obtained meta-ICA components in terms of their relation with task aspects, we investigated spatial similarity between each component and

the group-level task response maps. The group-level task response maps were derived from group-level statistical analysis on the participant-level zstat maps with age, gender and scan site as covariates. Group-level statistical maps were thresholded using Gaussian Random field (GRF) theory based cluster statistics ($p < 0.05$) after initial thresholding ($Z > 2.3$). We obtained group-level maps for the following eight contrasts: rewarded cue, neutral cue, rewarded hit, neutral hit, rewarded miss, neutral miss, reward cue versus neutral cue and rewarded (hit versus miss) versus neutral (hit versus miss) receipt.

We conducted a spatial regression for each of the obtained group response maps against all 30 ICs from the meta-ICA. This provided a unique loading (beta) for each IC on the task response maps, effectively indexing the spatial similarity of each component with each of the eight task response maps. Combining the eight beta weights into one vector per IC yielded for each IC a profile that resembled similarity between the IC and the response maps. Noise components were excluded from further analyses (see supplementary Figure S4 for reference).

Since many obtained profiles showed similar patterns, we reduced the number of profiles using a k-means clustering algorithm. This algorithm allocates individual data points to clusters by means of maximizing between-cluster differences and minimizing within-cluster differences. The number of clusters (k) was determined iteratively (n=1000) and by comparing the explained variance of all possible k's for our data to explained variance obtained for randomly generated data (see supplements for details). We performed clustering with k ranging from two to the amount of non-noise components (i.e., 2-23). This step would yield a limited number of distinct profiles represented by cluster-averaged beta weights.

To identify the larger functional networks represented by the distinct profiles resulting from the clustering procedure we calculated averaged spatial maps of the corresponding network components and mapped these visually on functional networks gained from resting-state functional connectivity analyses (Seeley et al. 2007; Smith et al. 2009).

Network characteristics in healthy controls and participants with ADHD

The second aim of our study was to assess the effects of ADHD on reward-related brain networks. To this end we used the networks identified using the discovery sample to investigate network-related metrics in an independent sample (the test sample) of healthy control participants and adolescents with ADHD.

Network integrity

To investigate integrity of the identified network components we applied a dual regression analysis using all ICs from the meta-ICA (Filippini et al. 2009). This analysis consists of two stages. In the first stage we used every participant's full task-related time series to derive the time series for each of the ICs, by entering all ICs as spatial regressors in a mul-

tivariate GLM. In a second step, the obtained time courses were used as temporal regressors in a multivariate GLM to calculate spatial maps of each component for each individual. For further group comparisons these spatial maps were transformed into MNI152 space using a custom study template (see supplementary material).

To investigate diagnostic effects we applied a group-level GLM to the subject-specific spatial maps with diagnosis, age, gender, scan site, comorbidity with ODD/CD, and head motion summary scores as covariates. Significance for the effect of group was assessed using permutation testing (FSL randomize) with 10000 permutations. Clusters were considered significant if they comprised at least 20 voxels and an FDR-corrected p value < 0.05 (Smith et al. 2004).

Communication between network components

Finally, we investigated the synchronicity of brain responses within and between different networks. For this analysis we used the FSLnets toolbox (fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets). This matlab-based toolbox uses the time course of each IC (result of the first step of dual regression described above) to calculate normalized regression coefficients for each IC-IC pairing (ridge regression).

To test regression coefficients at the group-level we modeled group as between-subject factor and age, gender, scan site, comorbidity with ODD/CD and head motion summary scores as covariates. Statistical inference for the group contrast (ADHD vs CON) was done using permutation testing with 10000 permutations. Coefficients of IC-IC pairings were considered significant if they exhibited a p-value < 0.05 (FDR-corrected).

Results

Results brain network analysis

Network identification and decomposition

The meta-ICA applied to the ICA components resulting from iterative decomposition on the participant-level task-related maps yielded 30 components. Of those 7 components were considered noise components based on visual inspection (see Figure S4 in the supplemental material), leaving 23 components for further investigation. Figure 1 illustrates the spatial maps for each non-noise component.

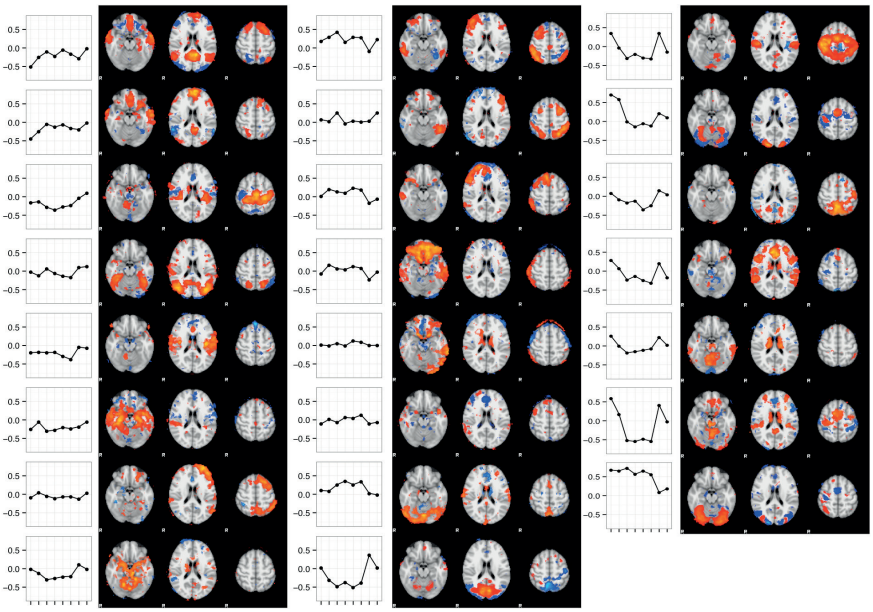

FIGURE 1 Profiles and spatial maps of all non-noise ICs gained from meta-ICA. Profiles indicate relation between task response maps and ICs expressed as beta estimates (y-axis) of the multiple linear regression with task response map (x-axis) as dependent measure and ICs from the meta ICA as independent measure. Task response maps are (from left to right): rewarded cue, neutral cue, rewarded hits, rewarded misses, neutral hits, neutral misses, reward anticipation and reward receipt.

Network interpretation

To interpret the components resulting from the meta-ICA we compared the component spatial maps to group-level task activation maps. Group-level activation maps are shown in Supplementary Figure S5. Using spatial multiple regression we determined the correspondence between the component spatial map and each of the task contrast maps. The resulting beta loadings for each component on each of the task contrasts are depicted in Figure 1.

Applying k-means clustering to all 23 series of beta-loadings displayed in Figure 1 yielded an optimal number of four specific clusters (see supplement for detailed clustering results). These clusters are summarized in Figure 2 showing the average cluster beta-series as well as aggregated brain maps. This figure indicates that the first three clusters, containing eight (cluster 1), seven (cluster 2) and one (cluster 3) component respectively, showed a general loading on almost all task aspects. Components included in the first cluster were located in posterior cingulate gyrus (PCC), bilateral lateral parietal cortex, and ventromedial prefrontal cortex. Compared with functional networks gained from resting-state functional connectivity (RSFC) studies, these structures primarily resemble the default-mode network (DMN) (Buckner et al. 2008; Smith et al. 2009). Yet, few other regions were also associated with this cluster including cortical structures such as bilateral temporal cortex, motor cortex, and subcortical regions including putamen, hippocampus, and amygdala.

The second cluster was associated with dorso- and ventrolateral PFC and lateral parietal cortex. These regions fit best with the executive control network (ECN) (Seeley et al. 2007). Other regions showing significant association with this cluster were ventromedial PFC, inferior temporal gyrus, and cerebellum.

The third cluster was formed by one single component. It strong loaded on all task-event response maps and was strongly associated with the lateral visual cortex. Further including bilateral thalamus, this cluster fits best with a lateral visual network (Seeley et al. 2007). Other regions associated with this cluster were bilateral putamen, inferior and superior frontal gyrus, and ventromedial PFC.

Finally, a fourth cluster was formed by seven components and demonstrated reward-specific loadings. This cluster had strong loadings on response maps for rewarded cues and the reward anticipation contrast and moderate loading on all receipt events. Associated regions comprised anterior cingulate cortex (ACC), supplementary motor cortex, bilateral fronto-insular cortex, nucleus accumbens, putamen, brain stem, and thalamus. Compared with functional networks at rest, this cluster showed high similarity with the salience network (Seeley et al. 2007). Additional involved regions were motor cortex, visual cortex, dlPFC, and cerebellum.

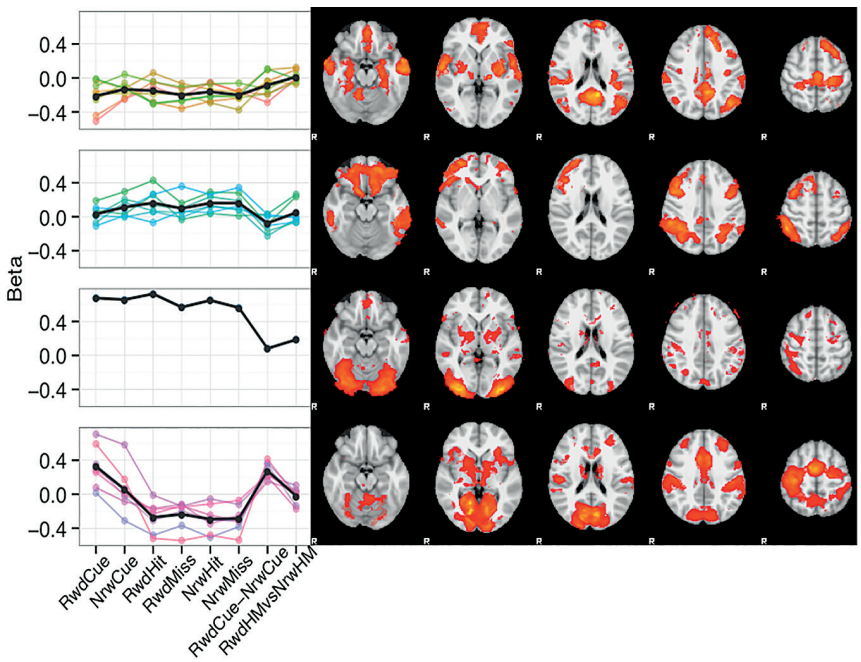

FIGURE 2 K-means clustered profiles and spatial maps of non-noise components. Black lines in profile plots indicate mean for each cluster. Spatial maps of independent components are averaged across cluster and thresholded (z>2.3). Major networks that correspond with the different clusters are: 1) Default-Mode, 2) Executive control 3) Lateral Visual and 4) Salience network.

ADHD-related effects on network characteristics

Network integrity

Group maps of the functional connectivity analysis in the test sample are displayed in supplementary Figure S5. These maps replicate network components identified with the meta-ICA in the discovery sample (Figure 2). Three of these networks exhibited extensions into adjacent regions. For the executive control network adjacent regions included bilateral caudate nucleus and medial visual cortex. The visual network included precentral gyrus, dorsomedial PFC, and cerebellum; the salience network further included posterior cingulate gyrus, and caudate nucleus.

Statistical testing for group differences revealed that connectivity of four network components was sensitive to effects of diagnosis (i.e., ADHD vs. controls). As indicated in Table 2, two of these components were associated with the salience clustered network and two with the executive control network. We observed significantly stronger connectivity for control participants relative to participants with ADHD between the executive control network and inferior frontal gyrus (IFG) and cerebellum, and between the salience network and inferior temporal gyrus. In addition, in participants with ADHD the salience network had significantly stronger connections with the cerebellum. Of note, all affected regions exhibiting altered connectivity were rather small (voxel sizes <= 31) with exception the IFG (88 voxels). An overview of all significant clusters is given in Table 2 and in supplementary Figure S7.

TABLE 2 Significant clusters from whole-brain connectivity analysis

Comparison	IC	Size	Z-score	X	Y	Z	Region	Network
CON vs ADHD	0	27	3.82	62	-48	-12	Inferior Temporal Gyrus	Salience
	25	88	3.61	56	16	4	Inferior Frontal Gyrus	Executive control
	28	31	4.13	-18	-62	-52	Cerebellum	Executive control
ADHD vs CON	5	20	4.41	6	-50	-16	Cerebellum	Salience

Network communication

A matrix with the regression coefficients of all IC-IC pairings is displayed in Figure 3. The regression coefficients illustrate connectivity between the ICs included in each cluster, illustrating both within as well as between cluster communication. The salience network exhibited strong within-cluster integration between its composing ICs, as the majority of within-cluster connections was positive (76%). Connections between ICs in the salience cluster and ICs from other clusters were more variable with 36% positive connections between the salience ICs and DMN ICs, 35% positive connections with ECN ICs, and 43% positive connections with lateral visual ICs. This result indicates that ICs within the salience network exhibited specific time-courses that were different from the time-course of ICs composing the three other networks.

The within- and between-cluster integration was less clear for the ICs composing the DMN, and ECN. Only 50% (DMN), and 62% (ECN) of within-cluster connections and 43% of the between-cluster connections were positive. Finally, statistical comparison of the IC-IC matrices obtained for both groups did not reveal significant diagnosis-related differences.

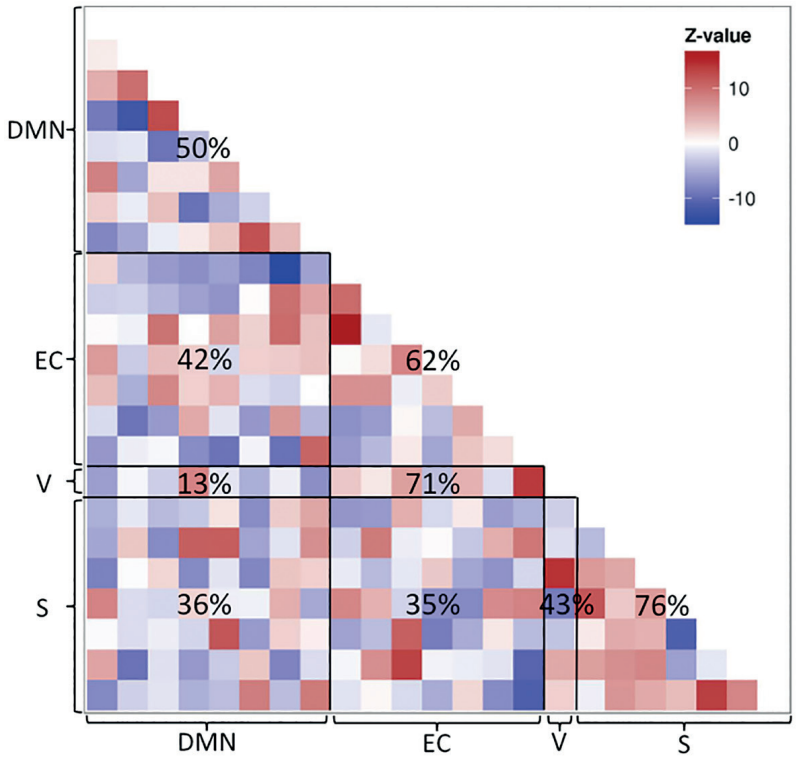

FIGURE 3 Matrix of the regression coefficients of all IC-IC pairings. Letters at x- and y-axis indicate cluster to which IC belongs (DMN: default-mode network, EC: executive control network; V: lateral visual network; S: Salience network). Digits in matrix indicate percentage of positive connections within or between clusters.

Discussion

In this study we set out to identify functional networks on the basis of reward-related task responses to describe reward processing in terms of a limited set of larger brain networks. We recovered four major brain networks. Three of them resembled general, reward-independent task-responses, while one was specifically reward processing oriented. Comparison of these networks with existing resting-state functional connectivity studies revealed that they reflect the executive control, default-mode, lateral visual network, and salience networks. We subsequently used this network-based characterization of reward processing to investigate the potential impact of ADHD resulting network characteristics.

The network that was selectively associated with rewarded task responses was the salience network. The salience network showed strong similarity with brain responses to rewarded cues and the reward anticipation contrast and slightly negative similarity with responses to receipt. The main functions of the salience network are to integrate information of different modalities such as sensory information and bodily states in order to establish goal-directed behavior, and to process emotion- and reward-related information (Seeley et al. 2007). The association of this network with rewarded cues might therefore reflect brain processes signaling the salience of the cue, but maybe also the need to perform well, elaborating optimal response strategies, or increasing the readiness to respond.

The other three brain networks showed general similarity with all task responses, independently of whether they involved reward manipulations or not. These networks were the default-mode network (DMN), the executive control network, and a lateral visual network. The executive control network exhibited a positive association with all task responses. For cue events, involvement of the executive control network might be related to attentional control processes necessary for an adequate behavioral response as for instance inhibition of a button press before the target occurs. Further, activation of this network during receipt events suggests that operability of this system is maintained during the whole trial.

The positive association between the executive control network and all task responses was paralleled by a negative association between the DMN and all task responses. The DMN is thought to be implicated in the internal organization of the brain and undirected thoughts and has been found to be dynamically linked with the executive control network (Fox et al. 2005; Kelly et al. 2008) suggesting that the brain needs to deactivate the DMN to allocate attentional resources.

The third general network, which showed the strongest positive association for all task aspects, was the lateral visual network. The fact that this network occurred in our analyses might best be explained by the modality of our task, which necessitates processing color information, detecting changes (occurrence of target), and reading feedback information during reward receipt.

Further investigating the connectivity between components constituting the four networks provided additional information about within- and between-network characteristics. The salience network exhibited the most consistent within-network integration, observed as positive connectivity between all components constituting this network. When investigating between-network communication, we observed one very consistent negative relation, namely between DMN and the visual network, where only 13% of connections between components were positive. In contrast, the relationships between the executive control and the visual network were mainly positive. This result suggests that whereas the visual network integrates with the ECN, both are rather segregated from the DMN. Similarly, the relation between the salience and executive control networks

was also negatively oriented, suggesting an absence of information integration. Further research could include adequate modeling of these relationships within for instance a Dynamic Causal Modeling context (Friston et al. 2003). The individual components as recovered through our approach would provide excellent regions of interest to define causal models.

Analyses were done on a local data set investigating reward anticipation and receipt. However, the approach is easily extendable to other datasets. One interesting next step would be to acquire imaging data from different reward-related functional tasks including e.g. probabilistic reversal learning and decision-making paradigms, and recover common reward-related networks from those. Data sharing initiatives exist that might provide repositories for such efforts (e.g. Neurovault.org; OpenfMRI.org). Comparable attempts have proven to be fruitful. For instance, Smith and colleagues identified with the same data-driven, hypothesis-free approach network components from task response maps of a large database (>7000 maps) and compared these with functional networks obtained from a resting-state network analysis (Smith et al. 2009)). They were able to match the majority of task-based networks with resting-state networks, thereby demonstrating a close link between functional networks underlying diverse cognitive functions and the functional architecture of the brain. Our approach is similar in the sense that we also used task-based activation maps as our starting point. However, our input maps were unthresholded z-statistic maps related to a specific cognitive paradigm, compared to Gaussian spheres modeled at peak locations recovered from a database of studies as in Smith et al. As such, we believe that our approach allowed a more comprehensive and specific assessment of reward-related networks.

We used this specific network-based approach to investigate the effects of ADHD of network integrity. In an independent test sample we revealed that ADHD participants relative to healthy controls exhibited decreased connectivity of the executive control network with the interior frontal gyrus (IFG) and cerebellum, as well as changed connectivity of the salience network with decreases in the inferior temporal gyrus and increases in the cerebellum. In contrast, we observed no group-related differences in the between- and within-network communication characteristics.

The limited spatial extent of our results is in contrast to earlier resting-state fMRI studies evidencing large neural changes in core structures of reward processing brain networks in ADHD (Costa Dias et al. 2013; Posner et al. 2013). However, they are in line with the ADHD-related effects observed in a typical task-based analysis on the same data (von Rhein et al. 2015). Using the same sample as used in these analyses, this study reported moderately increased reward-related activity in VS and OFC, core reward processing structures. It should be noted however, that reward processing is a heterogeneous concept including different processes (see (Berridge and Kringelbach 2008)), which are associated with signaling in different neural structures. Accordingly, we might have pooled distinct functional networks as one (i.e. salience) network. The use of such a unified network, however, might not be specific enough to capture subtle differences between ADHD participants and healthy

controls in particular aspects of reward processing. Taken together the results from both our studies suggest that ADHD might be related to aberrant functionality of otherwise intact reward-related processing functional architecture.

An important point of discussion is the heterogeneity of the network components that we recovered from the meta-ICA analysis. To simplify our reported network decomposition, we summarized the large amount of components by clustering them together. We interpreted these clusters of components as unified networks assuming shared/common functionality of components within each cluster. This, however, might not be the case as for instance the proportion of positive within-network correlations indicates. Some components within a network cluster were negatively correlated, which we interpret as a lack of integration of those components within the network. Alternatively, it might be that the clustered networks include structures that do not belong to the functional network, thereby reducing the homogeneity of the clustered networks.

A second discussion point relates to the inclusion of the higher-order contrasts (rewarded cue versus neutral cue and rewarded versus neutral accuracy (hit versus miss)) in the component identification procedure. Components constituting the three general networks did not show high loadings for these higher-order contrasts questioning their additional value. For the salience network, however, we see that the loading of the reward anticipation contrast mirrored the positive loading observed for rewarded cues. This shows that the inclusion of higher-order contrasts enable us to investigate which task aspect is captured by a contrast, thereby facilitating its interpretation. For instance in case of the contrast rewarded versus neutral cue, finding high spatial similarity with rewarded cues supports the interpretation that this contrasts captures reward processes.

To conclude, we discovered brain networks on the basis of reward-related task responses. Using a data-driven approach we were able to recover four major brain networks involved in reward processing: the salience and executive control network, the lateral visual network and the default mode network. This finding provides a comprehensive picture of involved brain networks and their specific task-related role. Only the salience network was selectively associated with rewarded task-aspects whereas the other networks were more generally associated with the task. Between component connectivity analysis revealed a high degree of network integrity in the salience network, which was less evident in other networks. Comparison of ADHD participants with healthy participants revealed changes of functional connectivity within the salience and executive control network.

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Chapter 5

Aberrant Local Striatal Functional Connectivity in Attention-Deficit/ Hyperactivity Disorder

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Abstract

Background Task-based and resting state fMRI studies report ADHD-related alterations in brain regions implicated in cortico-striatal networks. We assessed whether ADHD affects the brain's global cortico-striatal functional architecture, or whether effects are limited to local, intra-striatal functional connections.

Methods We included a cohort of adolescents with ADHD (N=181) and healthy controls (N=140) and assessed functional connectivity of nucleus accumbens, caudate nucleus, anterior putamen, and posterior putamen. To assess global cortico-striatal functional architecture we computed whole-brain functional connectivity by including all regions of interest in one multivariate analysis. We assessed local striatal functional connectivity using partial correlations between the timeseries of the striatal regions.

Results Diagnostic status did not influence global cortico-striatal functional architecture. However, compared to controls, participants with ADHD exhibited significantly increased local functional connectivity between anterior and posterior putamen ($p=0.0003$; ADHD: $z=0.30$, controls: $z=0.24$). Results were not affected by medication use or comorbid oppositional defiant disorder and conduct disorder.

Conclusions Our results do not support hypotheses that ADHD is associated with alterations in cortico-striatal networks, but suggest changes in local striatal functional connectivity. We interpret our findings as aberrant development of local functional connectivity of the putamen, potentially leading to decreased functional segregation between anterior and posterior putamen in ADHD.

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) has been associated with deficits in executive functions such as response inhibition, working memory (Willcutt et al. 2005), reward processing (Sonuga-Barke 2005), and motor function (Stray et al. 2013). Key brain regions associated with these functions are located in the striatum, including three main nuclei: nucleus accumbens (NAcc), caudate nucleus, and putamen. Each striatal structure receives projections from distinct cerebral regions (Alexander et al. 1986; Di Martino et al. 2008; Helmich et al. 2010). NAcc forms a network with anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC), associated with reward processing and motivational control (Haber and Knutson 2010). Caudate nucleus regulates cognitive control processes via connections with dorsolateral prefrontal cortex (DLPFC (Levy et al. 1997)). Finally, putamen regulates motor function through connections with motor cortices (Alexander et al. 1986). In addition, it is hypothesized that putamen can be subdivided into a functionally distinct anterior and posterior region (Tricomi et al. 2009; Aramaki et al. 2011). Anterior putamen has been associated with higher-order cognitive aspects of motor control including learning and initiating new movements (Aramaki et al. 2011), through connections with pre-supplementary motor area and ACC (Helmich et al. 2010). Posterior putamen has been related to the execution of well-learnt, skilled movements (Tricomi et al. 2009), via connections to primary and secondary motor areas (Helmich et al. 2010).

As these cortico-striatal networks are implicated in behavior that is often impaired in patients with ADHD, they have been suggested as potential neural underpinnings of ADHD-related deficits (Cubillo et al. 2012). Task-based fMRI studies support the involvement of cortico-striatal networks in ADHD. Patients with ADHD showed aberrant brain responses in DLPFC, ACC, caudate nucleus, and supplementary motor area during response inhibition and attention, and in NAcc and OFC during reward processing (Cubillo et al. 2012; Cortese et al. 2012). Several resting state fMRI (rs-fMRI) studies have demonstrated aberrant functional connectivity of ACC, frontal cortex, caudate, putamen, NAcc, and motor regions in ADHD (for review see (Oldehinkel et al. 2013)). Furthermore, atypical functional connectivity of putamen, OFC, and NAcc, has been associated with severity of symptoms of hyperactivity/impulsivity and inattention (Cao et al. 2009; Tomasi and Volkow 2012; Costa Dias et al. 2013).

Results from these fMRI studies suggest dysfunction of cortico-striatal networks in ADHD. However, the observation that one or more regions within a cortico-striatal network show aberrant brain responses does not necessarily imply dysfunction of the entire network. Instead, the observed dysfunctions might be primarily related to impairments in within-striatum cross-talk, based on the assumption that striatal regions modulate each other via striato-nigro-striatal connections (Haber et al. 2000; Aarts et al. 2011). Studies of brain anatomy provide evidence for local striatal abnormalities in ADHD as reduced volume has been reported for caudate nucleus, NAcc, and putamen (Cubillo et al. 2012). Only few studies report on local, intra-striatal functional connectivity and its relation to ADHD.

Using regional homogeneity and degree centrality, aberrant local functional connectivity in caudate nucleus (Cao et al. 2009; Costa Dias et al. 2013) and putamen was demonstrated using rs-fMRI in ADHD (Costa Dias et al. 2013). One of these studies also reported atypical local functional connectivity between putamen and NAcc (Cao et al. 2009). Based on these findings, we hypothesize that aberrant local connectivity between striatal structures contributes to ADHD symptomatology. As different striatal regions can interact with each other via their midbrain connections such local changes of connectivity might also account for changes in associated cortico-striatal networks (Haber et al. 2000).

In light of this hypothesis we investigate whether ADHD is primarily associated with changes in global cortico-striatal functional architecture or is also evident in changes to local functional connectivity between substructures within striatum. To this end we examine resting state functional connectivity of NAcc, caudate nucleus, anterior putamen, and posterior putamen in a large sample of participants with ADHD and healthy controls using comprehensive multivariate and partial correlation analyses.

Methods

Participants

All participants were part of the NeuroIMAGE cohort (von Rhein et al. 2014), the Dutch follow-up study of the large-scale International Multicenter ADHD Genetics (IMAGE) study (Müller et al. 2011). The NeuroIMAGE cohort consists of families with children diagnosed with ADHD and control families. Here, we included participants from ADHD families with a DSM-5 based ADHD diagnosis and participants from control families who completed both a structural MRI scan and a rs-fMRI scan (N=356). Diagnoses of ADHD and comorbid disorders, including oppositional defiant disorder (ODD), conduct disorder (CD), anxiety disorders, and depression were assessed by a trained professional using the Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS (Kaufman et al. 1997), complemented with Conners' ADHD questionnaires (Conners et al. 1998; Conners et al. 1999). The full diagnostic algorithm and inclusion criteria are described in the Supplementary information, further details about the NeuroIMAGE study and its diagnostic and general testing procedures are described elsewhere (von Rhein et al. 2014). Our study was approved by the local ethical committees of the participating centers; written informed consent was obtained from all participants (for participants >12 years) and their legal guardians (for participants <18 years).

We excluded participants for head-motion (N=21) as determined by frame-wise displacement ((Power et al. 2012); cut-off=0.73 RMS-FD, corresponding to the 5% highest movers in the total sample), and participants with insufficient brain coverage during the rs-fMRI scan (N=14). Our final analyses included 181 participants with ADHD and 140 healthy controls. It should be noted that both the ADHD and control group included participants

of whom a sibling was present in the same group (ADHD: n=35; controls: n=53). Group characteristics are specified in Table 1 and Supplementary Figure S1. Groups were not balanced with respect to IQ, gender, scan location, and comorbid disorders. Within the ADHD group, 133 participants had used medication prescribed for ADHD during at least six months in their lives. All participants were asked to withhold medication use for 48 hours before the day of assessment.

TABLE 1 Participant characteristics

	ADHD (N=181)		Controls (N=140)		Test statistic	p-value
	Mean	SD	Mean	SD		
Age (years)	17.73	3.10	17.07	3.35	t(319)= 1.814	0.07
Estimated IQ[a]	96.13	15.43	106.20	13.86	t(315)= -6.019	**
Inattentive symptoms[b]	7.36	1.52	0.44	1.31	t(319)= 42.91	**
Hyperactive/Impulsive symptoms[b]	5.79	2.42	0.37	0.88	t(319)= 25.28	**
Medication use (years)	5.44	4.55	-	-	-	-

	N	%	N	%		
Number of males	133	73.48	64	45.71	X2= 25.67	**
Scan site Nijmegen	98	54.14	50	35.46	X2= 10.79	**
ODD diagnosis[c]	49	27.07	1	0.71	X2= 41.70	**
CD diagnosis[d]	7	3.87	-	-	-	-
Lifetime medication use[e]	133	74.48	-	-	-	-

[a] Estimated IQ based on Wechsler Intelligence Scale for Children or Wechsler Adult Intelligence Scale–III Vocabulary and block design.
[b] Symptom count based on K-SADS interview (Kaufman et al., 1997) and Conners' questionnaires (Conners et al., 1998, 1999); Maximum of 9 symptoms per dimension (≥6 is clinical threshold).
[c] Oppositional Defiant Disorder.
[d] Conduct Disorder. e Participants that used medication prescribed for ADHD during at least six months in their lives.** p≤0.001.

MRI acquisition

MRI data were acquired at two scanning sites on 1.5 Tesla Siemens scanners (Siemens AVANTO at the Donders Institute for Brain, Cognition and Behavior in Nijmegen and Siemens SONATA at the Free University Medical Centre in Amsterdam). Identical Siemens 8-channel head coils and matched scanning protocols were used at both locations. Structural images were obtained using an MPRAGE sequence (TR=2730ms, TE=2.95ms, T1=1000ms, voxel size=1x1x1mm, flip angle=7, matrix size=256x256, FOV=256mm, 176 slices). A gradient echo-planar imaging (GE-EPI) sequence was used for the acquisition of rs-fMRI data (TR=1960ms, TE=40ms, flip angle=80, matrix size=64x64, in-plane resolution=3.5mm, FOV=224mm, 35 axial slices, slice thickness/gap=3.0mm/0.5mm, 265 volumes). During the rs-fMRI scan participants were instructed to keep their eyes open while not thinking of anything in particular.

Preprocessing

The rs-fMRI data were preprocessed using tools from the FMRIB Software Library (FSL version 5.0; http://www.fmrib.ox.ac.uk/fsl) and included removal of the first five volumes to allow for signal equilibration, head movement correction via realignment to the middle volume (MCFLIRT; (Jenkinson et al. 2002)), grand mean scaling, spatial smoothing using a 6mm FWHM Gaussian kernel, and high-pass filtering (0.01 Hz). Functional data were denoised for motion-related artifacts using automatic noise selection as implemented in ICA-AROMA (Pruim et al. 2015b). Nuisance regression was conducted to remove signal from white matter and cerebrospinal fluid. The rs-fMRI images were co-registered to the participant-level high resolution anatomical images using boundary-based registration (Greve and Fischl 2009) implemented in FSL FLIRT. For each participant we calculated the non-linear transform from the high-resolution anatomical image to a custom study template using FSL FNIRT (Jenkinson et al. 2002). The custom group template (voxel size 2x2x2mm) was generated by averaging across T1-scans (after non-linear normalization to MNI152 standard space) of all participants in the NeuroIMAGE study (N=787).

Seed selection

We obtained participant-specific masks for NAcc, caudate nucleus, and putamen using automated subcortical segmentation of the individual structural scans as implemented in FSL FIRST (Patenaude et al. 2011). The masks for NAcc and caudate nucleus were directly transformed to the participants' native functional space and binarized. The putamen mask was first transformed to MNI152 standard space to allow automatic separation into an anterior and posterior division similar to the method implemented by Helmich and colleagues (Helmich et al. 2010). The anterior commissure was used as a border to separate both regions. A gap of two voxels was inserted between the anterior and posterior division of putamen by excluding voxels located directly anterior and posterior of the anterior commissure. This procedure avoided partial voluming effects arising from potential overlap of both seed regions near the commissure. Next the anterior and posterior putamen masks were transformed back to participant native functional space and binarized.

Global striatal functional connectivity analyses

We used the structurally defined masks to extract timeseries from the rs-fMRI data for each striatal seed. To this end we extracted the timeseries for all voxels within each mask and applied singular value decomposition. We obtained the first eigenvariate from this decomposition and used the associated timeseries for further analyses.

Based on these timeseries, we obtained participant-level whole-brain voxel-wise functional connectivity estimates for all seed-regions by means of multiple regression. By applying a multiple regression approach (instead of a univariate analysis for each striatal seed separately), variance that is shared between striatal seed regions is not attributed

to any of the striatal regions. The multiple regression approach thus resulted in unique whole-brain voxel-wise functional connectivity maps for each striatal seed unconfounded by contributions of the other seeds. In addition to whole-brain connectivity maps for each seed, we computed connectivity difference maps for anterior versus posterior putamen to test the hypothesis of a functional distinction between these two regions. Resulting connectivity maps were transformed to the study template for group analysis.

We compared participants with ADHD to healthy controls in a group level analysis for each of the obtained regression maps using permutation testing (1000 permutations) as implemented in FSL Randomise. Covariates were included for age, gender, IQ, scan-site, and comorbid diagnosis (ODD and/or CD). We applied threshold-free cluster enhancement as implemented in FSL (Smith and Nichols 2009) and statistical significance was determined by means of a family-wise error (FWE) threshold of $p<0.05$.

Local striatal functional connectivity analyses

Local functional connectivity between the striatal seeds was assessed by calculating full (Pearson) correlations and partial correlations between the eigenvariate timeseries for every combination of seeds (six pairs). By using partial correlations, variance that is shared between striatal regions is not attributed to any of the striatal regions. Partial correlations thus reflect unique local functional connectivity between each pair of striatal regions. Computing partial correlations between the different striatal seeds can hence be interpreted as the local functional connectivity equivalent of using a multiple regression analysis to compute whole-brain functional connectivity of the striatal seed regions.

Both full and partial correlations were transformed into normally distributed values using Fisher's r-to-Z-transformation. Significant differences in correlation strength between the ADHD and control group were tested using permutation testing with 5000 permutations for each seed-pair. P-values were obtained by calculating the proportion of permuted samples that yielded a difference between the ADHD and control group higher than the observed difference. Correction for multiple comparisons was implemented using Bonferroni correction. Differences were considered statistically significant if $p<0.008$ (=0.05/6 seeds pairs).

Sensitivity analyses

For regions that showed significant ADHD versus control differences in the global or local striatal analyses we examined in ADHD patients whether results were related to ADHD symptom severity. We calculated partial correlations (i.e., corrected for effects of age, site, gender, IQ, and ODD/CD comorbidity) between functional connectivity and symptom count. ADHD symptom count was assessed by the K-SADS diagnostic interview complemented with Conners' ADHD rating scales. The DSM-Inattentive behavior scale (0-9 symptoms), the DSM-Hyperactivity/Impulsive behavior scale (0-9 symptoms) and DSM-Total symptom scale (0-18 symptoms) were used (see Supplementary information

for further details). Finally, we ensured that group differences in connectivity were not influenced by ADHD subtype, medication history, IQ, gender, scan site, and comorbid ODD/CD (see Supplementary information).

Results

Global functional connectivity of four striatal regions

Group connectivity maps of NAcc, caudate, anterior putamen, and posterior putamen in both the ADHD and control group replicated the major cortico-striatal networks (Alexander et al. 1986; Di Martino et al. 2008; Helmich et al. 2010). Figure 1 displays regions exhibiting functional connectivity with the four striatal seed regions in both groups. For a description of connectivity patterns see the Supplementary information.

We did not observe significant differences between the ADHD and control group in the whole-brain functional connectivity maps. To replicate previous studies we also investigated cortico-striatal connectivity with one seed at a time (as opposed to our multivariate model). Similar to the multivariate analyses, these univariate analyses did not yield differences between our ADHD and control group (see Supplementary Figure S2).

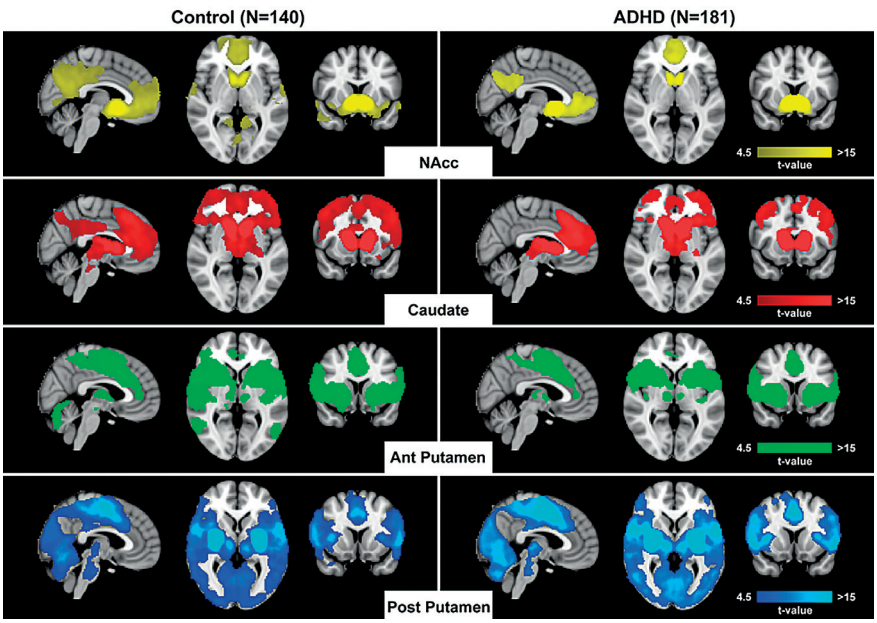

FIGURE 1 Global Striatal Connectivity

Whole-brain functional connectivity maps for nucleus accumbens, caudate nucleus, anterior putamen, and posterior putamen in the control (left) and ADHD group (right). Significant activation is shown (FWE-corrected, $p<0.05$). We observed no difference between the ADHD and control group.

Local striatal functional connectivity

Local connectivity assessed using full correlations (i.e., uncorrected for global striatal effects) revealed significant group differences for four of the six striatal seed-pair combinations (see Figure 2). Significantly increased intra-striatal correlations were observed in participants with ADHD compared to healthy controls for the seed pairs 1) NAcc – anterior putamen (p=0.004; ADHD: z=0.25, controls: z=0.20), 2) caudate - anterior putamen (p=0.004; ADHD: z=0.41, controls: z=0.34), 3) caudate - posterior putamen (p=0.008; ADHD: z=0.26, controls: z=0.20), and 4) anterior putamen - posterior putamen (p=0.00006; ADHD: z=0.39, controls: z=0.31).

When controlling for global striatal effects using partial correlations, we observed that local functional connectivity between anterior putamen and posterior putamen was significantly increased in the ADHD group compared to the control group (see Figure 2; p=0.0003; ADHD: z=0.30, controls: z=0.24). Post-hoc analyses revealed that this finding was independent of ADHD subtype and not influenced by medication use, imaging site, gender, IQ, or ODD/CD comorbidity (Supplementary Figures S3 and S4).

Finally, we confirmed that the obtained ADHD-related result was restricted to local connectivity by directly comparing the whole-brain connectivity maps obtained for anterior and posterior putamen. We observed no significant differences between ADHD and controls in this analysis (see Supplementary Figure S6).

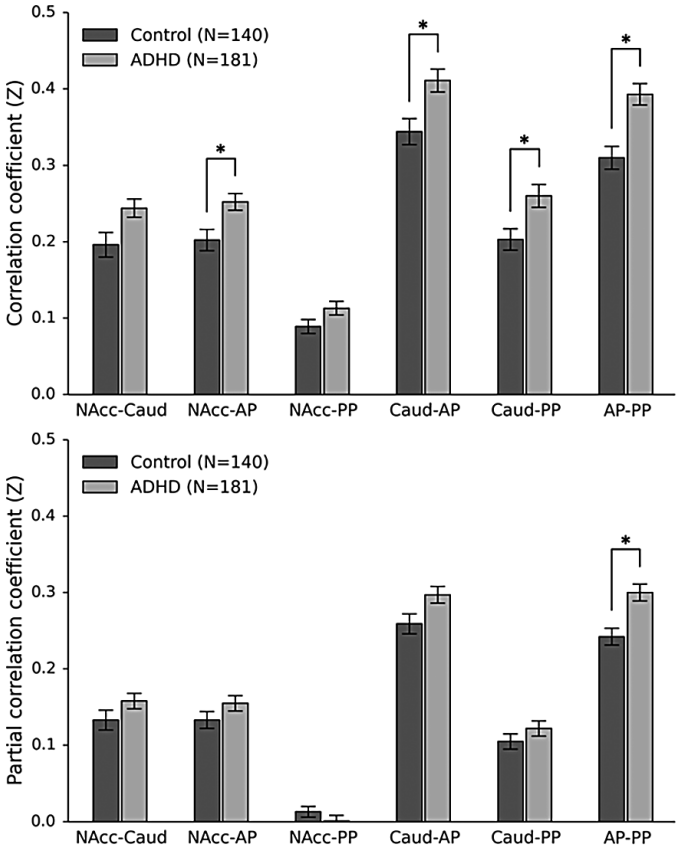

FIGURE 2 Local Striatal Connectivity

Mean Fisher-z transformed correlation coefficients indexing local, between seed functional connectivity. Full correlations are shown in the top graph. Significantly increased correlations were observed in the ADHD compared to control group for NAcc - anterior putamen (p=0.004; ADHD: z=0.25, controls: z=0.20), caudate – anterior putamen (p=0.004; ADHD: z=0.41, controls: z=0.34), caudate - posterior putamen (p=0.008; ADHD: z=0.26, controls: z=0.20) and anterior putamen – posterior putamen (p=0.00006; ADHD: z=0.39, controls: z=0.31) connectivity. Partial correlations are shown in the bottom graph. A significantly increased partial correlation between anterior putamen and posterior putamen connectivity was found in the ADHD group (p=0.0003; ADHD: z=0.300, controls: z=0.242). Error bars indicate standard error of the mean. Abbreviations: NAcc = nucleus accumbens, Caud = caudate nucleus, AP = anterior putamen, PP = posterior putamen. Statistical differences were assessed using permutation testing and a Bonferroni-corrected alpha level of $p<0.008$ (=0.05/6 seeds pairs).

Relationship with symptom severity

We did not observe significant relationships between anterior-posterior putamen connectivity and inattentive symptoms (r=-0.078, p=0.306), hyperactive/impulsive symptoms (r=-0.028, p=0.714), or total symptom count (r=-0.065, p=0.398).

Discussion

We investigated local and global cortico-striatal connectivity in a large sample of youth with ADHD and healthy controls. Contrasting previous work, we did not replicate ADHD-related alterations in the major cortico-striatal networks. Conversely, ADHD was associated with aberrant local functional connectivity between the anterior and posterior division of putamen.

Consistent with existing theories, we identified the four major cortico-striatal networks in both participants with ADHD and healthy controls (Alexander et al. 1986; Di Martino et al. 2008; Helmich et al. 2010). However, the whole-brain functional networks of NAcc, caudate, anterior putamen, and posterior putamen did not yield differences between the ADHD and control group. As such, our results do not replicate previous task-based fMRI (see (Cubillo et al. 2012; Cortese et al. 2012)) and rs-fMRI studies (Cao et al. 2009; Mennes et al. 2011; Tomasi and Volkow 2012; Costa Dias et al. 2013) that reported ADHD-related global dysfunction and atypical functional connectivity in cortico-striatal networks. For example, task-based studies have reported increased activation in NAcc and OFC during reward processing (von Rhein et al. 2015), and decreased activation in putamen, caudate, ACC, and DLPFC during response inhibition and attention tasks in ADHD (Cubillo et al. 2012). Furthermore, reduced functional connectivity of putamen with frontal cortex, temporal cortex, and precuneus (Cao et al. 2009) as well as increased functional connectivity between caudate and ACC has previously been demonstrated (Mennes et al. 2011). In addition, decreased functional connectivity between NAcc and frontal cortex was found to correlate with increased impulsivity scores (Costa Dias et al. 2013).

One explanation for differences with results from task-related studies might be that rs-fMRI, as used in the current study, measures the brain when cognitive load is low. When cognitive load increases, as typically induced in task-based fMRI measurements, deficits might become evident in aberrant recruitment of brain regions. This hypothesis corresponds with effort-related deficits in ADHD as proposed by the cognitive-energetic model (Sergeant 2000). Further, differences between previous rs-fMRI studies and our study might be related to differences in methodology. Previous studies reporting atypical global connectivity of striatal regions applied univariate analysis (Cao et al. 2009; Mennes et al. 2011; Tomasi and Volkow 2012; Costa Dias et al. 2013). Yet, when implementing this type of analyses we also failed to reveal significant group differences (see Supplementary Figure S2). However, we can for instance not exclude variability in earlier findings related to insufficient control for head motion artifacts (Van Dijk et al. 2012; Pruim et al. 2015b), which was rigorously implemented in the current study (Pruim et al. 2015a).

The absence of ADHD versus control differences in whole-brain connectivity could also be related to heterogeneity within our sample. Heterogeneity in terms of phenotypic (Sonuga-Barke 2002) as well as cognitive characteristics (Fair et al. 2012) is a common observation in ADHD as well as healthy populations. This problem is partially mitigated by re-

cruiting participants with similar demographic characteristics. Indeed, previous studies have specifically selected participants without stimulant treatment (Cao et al. 2009), or only participants with combined (Costa Dias et al. 2013) or non-hyperactive subtype (Mennes et al. 2011). Moreover, these studies focused on participants within a small age range. In contrast, our population study included the broad clinical phenotype with all subtypes, with and without stimulant treatment, and participants within a broad developmental age range. This approach may however, wash out effects previously reported in smaller, more homogeneous samples. Yet, within our local findings we did not observe differences between the different ADHD subtypes (see Supplementary Figure S4).

In contrast to the absence of ADHD-related effects on the major cortico-striatal networks, we did observe associations between ADHD diagnosis and functional connectivity locally within the striatum. Local connectivity between several striatal regions was increased in participants with ADHD compared to controls (full correlation results). Subsequent partial correlation analysis suggested that these effects were attributable to a specific increase in functional connectivity between anterior and posterior putamen in participants with ADHD. We interpret this finding as decreased functional segregation of anterior and posterior putamen in ADHD.

Taking into account the cognitive functions attributed to anterior and posterior putamen, our results lead to new, testable hypotheses. Anterior putamen has been associated with higher-order cognitive aspects of motor control such as learning and initiating new movements (Aramaki et al. 2011). Posterior putamen on the other hand, has been implicated in the execution of well-learned, skilled movements (Tricomi et al. 2009). In this context, it is possible that decreased functional segregation of the neural correlates for 'learning and initiating new movements' and 'execution of skilled movements' might be related to the various motor skill deficits observed in ADHD, such as delays in gross motor milestones (sitting, crawling, walking), clumsiness, and poor fine motor control (Vasserman et al. 2014). Accordingly, our results warrant research into the hypothesis that the difference between 'learning and initiating new movements' and 'execution of skilled movements' is less distinctive in participants with ADHD compared to healthy controls. As a preliminary examination we assessed general motor function using the Developmental Coordination Disorder Questionnaire (DCD-Q (Wilson et al. 2000), see Supplementary information). Although motor skills were significantly impaired in the ADHD compared to the control group ($p < 0.002$), motor skills were not related to anterior-posterior putamen connectivity ($-0.038 < r > 0.037$; $p > 0.52$ for all scales). In light of our hypothesis this result is not unexpected, as the DCD-Q might not be the best instrument to distinguish 'learning and initiating new movements' from 'execution of skilled movements'.

The observed increased local functional connectivity between anterior and posterior putamen in the ADHD group can also be interpreted in a developmental context. Typical development or maturation of functional brain networks has been characterized by both a decrease in short-range, local connectivity strength (segregation) and a simultaneous

increase in the strength of long-range, global functional connectivity (integration) (Kelly et al. 2009; Fair et al. 2009). According to the delayed maturation hypothesis for ADHD, local connectivity would be increased and global connectivity decreased in youth with ADHD, while connectivity would normalize at a later age. Although not significant, supplementary analyses exploring the effects of age hinted that local anterior-posterior putamen connectivity decreased with age in the control group but not in the ADHD group (see Supplementary Figure S5). These findings suggest aberrant development of local connectivity in the ADHD group, potentially resulting in local 'over-connectivity' in ADHD.

When comparing our whole-brain functional connectivity results with previous rs-fMRI studies we note that our methodology improved several key aspects. First, we did not investigate functional connectivity of a single region, but included four striatal regions in one analysis. We thereby increased the specificity of our findings: variance that was shared between striatal seeds was not assigned to any of the striatal seeds. As a result, we obtained unique whole-brain functional connectivity maps for each region that were not confounded by possible global alterations in connectivity. This approach echoed in the partial correlation analyses. Second, we did not define seed regions based on an anatomical atlas or standard coordinates. Instead we used subject-specific regions of interest based on an anatomical segmentation of each individual brain. Accounting for inter-individual differences in striatal anatomy, we increased the specificity of our analyses. Third, we used an advanced data-driven method for secondary motion denoising resulting in functional connectivity maps that are minimally confounded by motion (Pruim et al. 2015b).

When interpreting our results, limitations have to be considered. Within the ADHD group differences existed regarding dose and type of medication. Stimulant medications are effective in suppressing ADHD symptoms (Swanson et al. 2011) and have been demonstrated to have acute effects on brain function (Rubia et al. 2014). All participants in our study were, however, free of medication starting 48 hours before the rs-fMRI scan, which should have eliminated acute effects of medication on brain function. Furthermore, it should be noted that the control group and ADHD group differed significantly in gender, scan site, IQ, and ODD/CD comorbidity. However, sensitivity analyses revealed no influence of medication use, gender, scan site, IQ, and ODD/CD comorbidity on our findings.

Conclusion

We observed increased local functional connectivity between the anterior and posterior region of putamen in participants with ADHD relative to controls. We interpret this finding as a decreased functional segregation of both putamen regions in ADHD, which might be related to motor deficits in ADHD.

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Chapter 6

Distinct effects of ASD and ADHD symptoms on reward anticipation in participants with ADHD, their unaffected siblings, and healthy controls: a cross-sectional study

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Abstract

Background Autism spectrum disorder (ASD) traits are continuously distributed throughout the population, and ASD symptoms are also frequently observed in patients with attention-deficit/hyperactivity disorder (ADHD). Both ASD and ADHD have been linked to alterations in reward-related neural processing. However, whether both symptom domains interact and/or have distinct effects on reward processing in healthy and ADHD populations is currently unknown.

Methods We examined how variance in ASD and ADHD symptoms in individuals with ADHD and healthy participants was related to the behavioral and neural response to reward during a monetary incentive delay (MID) task. Participants (mean age: 17.7 years, range: 10-28 years) from the NeuroIMAGE study with a confirmed diagnosis of ADHD (n=136), their unaffected siblings (n=83), as well as healthy controls (n=105) performed an MID task in a magnetic resonance imaging (MRI) scanner. ASD and ADHD symptom scores were used as predictors of the neural response to reward anticipation and reward receipt. Behavioral responses were modeled using linear mixed models; neural responses were analyzed using FMRIB's Software Library (FSL) proprietary mixed effects analysis (FLAMEO).

Results ASD and ADHD symptoms were associated with alterations in BOLD activity during reward anticipation, but not reward receipt. Specifically, ASD scores were related to increased insular activity during reward anticipation across the sample. No interaction was found between this effect and the presence of ADHD, suggesting that ASD symptoms had no differential effect in ADHD and healthy populations. ADHD symptom scores were associated with reduced dorsolateral prefrontal activity during reward anticipation. No interactions were found between the effects of ASD and ADHD symptoms on reward processing.

Conclusions Variance in ASD and ADHD symptoms separately influences neural processing during reward anticipation in both individuals with (an increased risk of) ADHD and healthy participants. Our findings therefore suggest that both symptom domains affect reward processing through distinct mechanisms, underscoring the importance of multidimensional and multimodal assessment in psychiatry.

Background

Autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder characterized by social, communicative, and behavioral deficits (American Psychiatric Association 2013). ASD traits are continuously distributed in the general population, with symptomatology below the clinical threshold for diagnosis relatively common (Constantino and Todd 2003; Constantino and Todd 2005; Ronald et al. 2006). Research into the broader ASD-related phenotype is especially relevant since ASD symptoms are elevated in various clinical populations, particularly in patients with attention-deficit/hyperactivity disorder (ADHD).

ADHD, a neurodevelopmental disorder characterized by inattentiveness and/or hyperactivity and impulsivity, has been associated with high ASD comorbidity and elevated levels of ASD symptoms compared to the general population (Reiersen et al. 2007; Mulligan et al. 2009; Taurines et al. 2012). The high levels of comorbidity of ADHD and ASD could be due to a shared aetiology, and studies have indeed shown psychopathological, neuropsychological, neuroimaging and genetic overlap between the disorders (Banaschewski et al. 2011; Rommelse et al. 2011; van der Meer et al. 2012). How the two symptom domains interact in their effects on cognition, however, remains largely unknown.

One area where ASD and ADHD effects could interact is during the processing of reward. Both disorders have been linked to abnormalities in the frontal-striatal neural circuits associated with reward processing; whether this is the result of similar pathophysiological mechanisms is unclear (Taurines et al. 2012; Kohls et al. 2014). Summarizing the literature, it appears that ASD is related to abnormalities in the processing of certain types of reward rather than associated with a general reward processing deficit (Dichter 2012; Kohls et al. 2014). The processing of social and monetary reward has generally been associated with diminished activity in fronto-striatal areas in ASD versus control participants (Scott-Van Zeeland et al. 2010; Delmonte et al. 2012; Dichter et al. 2012a; Kohls et al. 2013; Richey et al. 2014); in contrast, some studies have also reported ASD-related hyperactivity during monetary reward processing in brain regions outside the traditional reward circuit (Dichter et al. 2012a; Dichter et al. 2012c). Increased responses to other types of reward (food cues, faces and images of personal relevance) have also been observed in participants with ASD in the insula (Cascio et al. 2012; Dichter et al. 2012c), amygdala (Dichter et al. 2012c) and (pre)frontal cortex (Dichter et al. 2012c; Dichter et al. 2012b). Reduced motivation to obtain social and monetary rewards (Chevallier et al. 2012) combined with increased motivation to pursue personally relevant stimuli could explain these bidirectional findings in ASD (Cascio et al. 2012; Dichter et al. 2012b).

In ADHD, both hypoactivity and hyperactivity in reward circuits in response to reward has been reported. The current consensus is that ADHD is characterized by decreased striatal activation during reward anticipation (Plichta and Scheres 2014), but increased prefrontal and striatal responses during reward receipt, compared to typically developing controls (Ströhle et al. 2008; Gatzke-Kopp et al. 2009; Rubia et al. 2009; Paloyelis et al. 2012).

Only a few neuroimaging studies on reward processing to date have included participants with ASD and ADHD (Chantiluke et al. 2014; Kohls et al. 2014; Chantiluke et al. 2015). In a study by Kohls and coworkers participants with ADHD displayed increased striatal and prefrontal activation during receipt of monetary reward (compared to control and ASD groups), whereas the presence of ASD was associated with striatal hypoactivity for both monetary and social reward conditions, in line with previous research in ADHD and ASD samples (Kohls et al. 2014). Chantiluke and colleagues compared the association between behavioural and neural responses related to temporal discounting in ADHD, ASD, comorbid ADHD / ASD and healthy controls. Besides shared abnormalities in all patient groups, they also found ASD-specific differences in the insula, and cerebellar deviations partially shared between ADHD and comorbid participants (Chantiluke et al. 2014). A pharmacological study from the same lab showed that fluoxetine (a selective serotonin reuptake inhibitor) had different effects on the neural signatures of reward reversal in ASD and ADHD (Chantiluke et al. 2015). ASD-related hypoactivation of medial prefrontal cortex (mpFC) under placebo was normalized under fluoxetine conditions, whereas participants with ADHD displayed mPFC activation similar to controls under placebo but hypoactivation after taking fluoxetine. In addition, both ASD and ADHD showed hypoactivation of the precuneus during reward reversal under placebo conditions compared to controls, suggesting that reward reversal is associated with both common and dissociative neural abnormalities in ADHD and ASD (Chantiluke et al. 2015).

The previous studies therefore suggest that ASD and ADHD can have both shared and distinct effects on reward processing. In addition, some evidence suggests that the cognitive dysfunctions of comorbid ASD and ADHD are not simply a combination of those of ADHD and ASD, but can be qualitatively different and/or more severe (Nydén et al. 2010; van der Meer et al. 2012; Chantiluke et al. 2014). However, much is still unknown about the specific and combined effects of ASD and ADHD symptoms on reward processes within the same study population.

Hence, in the current study we investigated these effects in a large well-described sample that included individuals with ADHD, their unaffected siblings and healthy control participants. Unaffected siblings were included as they are known to present with increased ASD symptom levels compared to healthy controls (O'Dwyer et al. 2014). Reward processing was measured using functional magnetic resonance imaging (fMRI) during a monetary incentive delay (MID) task, a commonly used reward task that reliably elicits activity in reward circuits (Knutson and Cooper 2005). By measuring both ADHD and ASD dimensionally, we could systematically investigate the separate and cumulative impact of both factors. With this approach we aimed to gain insight into both the effects of comorbid ASD and ADHD symptoms in individuals with ADHD, and improve our understanding of the impact of ASD and ADHD traits in unaffected populations.

We expected that higher levels of ASD symptoms would be associated with activity changes in fronto-striatal regions and the insula during reward anticipation and receipt based on previous studies (Scott-Van Zeeland et al. 2010; Cascio et al. 2012; Delmonte et al. 2012; Dich-

ter et al. 2012b; Dichter et al. 2012c; Dichter 2012; Kohls et al. 2013; Richey et al. 2014; Chantiluke et al. 2014; Chantiluke et al. 2015). In contrast, ADHD symptoms were expected to relate to decreased striatal activation during reward anticipation, and increased fronto-striatal activation during reward receipt (Plichta and Scheres 2014; Kohls et al. 2014).

Based on our analyses, we can report two main findings in this article. First, we observed that participants with more ASD symptoms showed increased activity in the insula during reward anticipation. Second, we found that higher ADHD symptom levels were associated with decreased activity in the dorsolateral prefrontal cortex during reward anticipation. We found no effects during reward receipt or any interaction between the ADHD and ASD effects.

Methods

Setup

The current investigation was conducted as part of the Dutch multisite NeuroIMAGE project ((von Rhein et al. 2014), http://www.neuroimage.nl/). The NeuroIMAGE study was approved by the local ethics committee (CMO Regio Arnhem – Nijmegen; 2008/163; ABR: NL23894.091.08). For details on recruitment of participants and a description of all study procedures in NeuroIMAGE, see (von Rhein et al. 2014). Critically, this cross-sectional study uses a subsample of the dataset used in von Rhein and colleagues (von Rhein et al. 2015), in which reward processing was compared between healthy participants and participants with a clinical diagnosis of ADHD (Additional File 1).

Monetary Incentive Delay task

Participants performed a monetary incentive delay (MID) task while undergoing MRI (Knutson et al. 2001). Participants were instructed to respond as quickly as possible to a target (a white circle) by pressing a button. Responses were correct when given within 270–500 ms after target onset; specifically, the response window was adapted to approximate a 33% hit rate. Although MID tasks typically use higher reward probabilities, the current task design has been used successfully in the past and has been shown to reliably engage the fronto-striatal reward circuit (Hermans et al. 2010; Ossewaarde et al. 2011). Each correct response ("hit") shortened the window by 20 ms while each incorrect response ("miss") increased it by 10 ms. Response windows were adapted for reward and non-reward conditions separately to equalize the amount of hits on both trial types. Although this method minimized differences in hit rates between conditions, it did so at the cost of losing hit rate as a useful index of behavioral performance. Behavioral outcome was therefore assessed using the reaction times in the reward and non-reward condition. Targets were preceded by a cue (a filled square, duration: 3.5–8.5 seconds) with variable colour coding (green for reward, red for no reward) that deterministically

predicted whether a reward could or could not be obtained on the current trial. Reward consisted of 0.20 € per correct response in the reward condition. The outcome of each trial was displayed for 1650 ms after response. Trials were concluded by a fixed inter-trial interval (the presentation of a blank screen) of 500 ms. Timing between events was not jittered. In total, the task consisted of 25 reward and 25 non-reward trials, supplemented by 25 null events. Null events were trials with only a fixation cross and required no response. Participants were given standardized instructions and performed a block of practice trials before starting the task. Trial order was pseudo-randomized and the total duration of the experiment was 12 minutes. The experiment concluded by showing the total amount of money awarded on the screen; this reward was subsequently transferred to the participant's bank account.

Participant selection

MRI data for the MID task was available for 564 participants from NeuroIMAGE, which represented all NeuroIMAGE participants who had no MRI contraindications and were willing to undergo MR scanning (von Rhein et al. 2014). Here, participants were included in the analyses if they either 1) had a diagnosis of ADHD, 2) were unaffected siblings of participants diagnosed with ADHD or 3) were unrelated and unaffected control participants. Healthy participants with siblings with ADHD were not included in the control group. Participants suffering from acute psychiatric conditions other than ADHD were excluded. Furthermore, participants were excluded if technical problems occurred during MRI or any scientifically or clinically relevant incidental findings were observed. Additionally, participants who displayed excessive movement (3 movements of 4 mm or more) during MRI were excluded to safeguard data quality. Only 1 participant was included with more than 0 but less than 3 large movements; this participant showed 1 such shift during MRI acquisition. Participants excluded for excessive motion were generally younger, had a higher chance of being diagnosed with ADHD, and generally displayed higher Conners Hyperactivity scores than included participants (Additional File 2). Although removal of these participants from our analyses could result in a slight bias, it is a practical reality that such a subpopulation is not optimally suited for MRI studies, and acquiring data of a quality sufficient for analysis was considered more important. Behaviourally, any participant with <5 correct responses in the reward or non-reward condition was excluded to improve our statistical power to detect differences. Participants excluded for this reason tended to be younger; participants with ADHD were overrepresented in the excluded sample but excluded participants with ADHD did not differ in ADHD or ASD symptom scores (or other demographics besides age) compared to included participants with ADHD (Additional File 2). The small bias in the sample used for our analyses that resulted from this exclusion procedure was again preferred over including participants for whom the reward-related neural processes could not be estimated satisfactorily. Finally, only participants were included for whom complete CSBQ questionnaire data were available. A complete exclusion flowchart can be found in Additional File 1.

After this exclusion process, 136 participants with ADHD ('ADHD'), 83 siblings ('SIBS') and 105 healthy controls ('CON', total N=324) were available for analysis (for demographics, see Table 1). Participants with ADHD were confirmed to have a clinical diagnosis of ADHD (Additional File 3). A subset of the ADHD group also had comorbid diagnoses of Oppositional Defiant Disorder or Conduct Disorder (ODD/CD) (n=39). Participants had no other diagnosis of any neurological disorder or learning disability, were 10-28 years of age, had an IQ≥70, had no MRI contra-indications, were confirmed to be off-medication at the time of testing for at least 48 hours and were of European-Caucasian descent. Written informed consent was obtained from all participants.

Children's Social Behavior Questionnaire

ASD symptoms were measured with the Children's Social Behavior Questionnaire (CSBQ, (Hartman et al. 2007)). The CSBQ was developed to measure the whole spectrum of ASD, including milder, subclinical symptoms, and includes items that directly refer to the DSM-5 criteria for ASD as well as items that measure additional symptoms associated with ASD (Hartman et al. 2006). It consists of 49 items divided into 6 subscales. The six subscales are (1) "Not Tuned" (deficits in tuning emotions and behaviour to the current situation), (2) "Lack of Social Interest" (reduced social interest, motivation and reciprocity), (3) "Orientation Problems", (problems with orientation in space and time), (4) "Not Understanding", (problems with understanding social context), (5) "Stereotypic Behaviour", (repetitive motor and sensory behaviour and stereotypy), and (6) "Resistance to Change" (fear and resistance to change). CSBQ items from subscale 2, 4, 5 and 6 refer directly to the clinical criteria for ASD from DSM-5; subscale 1 and 3 instead index other impairments typically associated with ASD but not specific to this disorder (e.g., executive functioning deficits and social-disruptive behaviour) (Volkmar 2012). Items are scored by parents or legal guardians on a three-point scale ranging from "does not apply" via "occasionally applies" to "clearly or often applies". Subscale scores are calculated by summing up the scores of all contributing items. In this study, a composite score was used of the four CSBQ subscales that target deficits specific to ASD (CSBQASD, the sum of scores on scale 2, 4, 5 and 6) to isolate the contribution of ASD symptoms from those of other disorders.

Conners Parent Rating Scale

As an analog to the dimensional CSBQASD score, we used the Conners Parents Rating Scale Revised-Long Version (CPRSR-L) (Conners et al. 1998; Conners et al. 1999) as a dimensional index of ADHD severity. Specifically, we used the combined raw score of the ratings on the DSM inattentive and DSM hyperactive/impulsive subscales as our measure of ADHD symptoms. In addition, we investigated the individual impact of the subscales by including their raw scores as separate regressors in supplementary analyses (Additional File 4).

Medication status

Although all participants with ADHD were off medication for at least 48 hours before our measurements were taken, their history of medication use was not equal. Permission was sought from each participant to obtain pharmacy records describing their lifetime stimulant use. No distinction was made between different stimulant drugs. Permission could not be obtained from 39 participants (17 ADHD; 7 SIBS; 15 CON). For all other participants medication records were acquired. Records confirmed that no control participants had a history of stimulant use. A few unaffected siblings had a history of stimulant use: 5 siblings had used stimulants up to 2 years, whereas 3 had used stimulants for more than 2 years. It is important to note that these siblings were not on medication around the time of testing. Amongst the 119 participants with ADHD, 102 had a history of stimulant use (with 22 having used/using stimulants for up to 2 years, and 80 having used/using stimulants for more than 2 years). 14 participants with ADHD were stimulant drug-naive.

Behavioral analysis of the MID

Reaction times (RT) for both reward and non-reward conditions were transformed by a log10 transformation to conform to the equality of variance assumption. Trials with responses faster than 100 ms were excluded. RT for Reward and Non-reward hits were first compared across all participants in a paired t-test using SPSS (version 21, IBM Corporation, Armonk, New York, USA). Subsequently, linear mixed models were run in SPSS to model the effects of various factors on the RT for Reward Hits, Non-reward Hits and the Reward Hit - Non-reward Hit RT difference. Models included the relevant RT as its dependent variable and used dimensional scores of ASD and ADHD symptoms and their interaction, Age, Sex, IQ, and Scan Site as fixed effects with Family ID (to control for familial effects) modeled as a random effect. The ASD x ADHD interaction term was calculated as the element-wise multiplication of the centered ASD and ADHD variables. Medication use (cumulative stimulant medication duration) was added as an additional random effect in a separate model that included data from all participants for whom medication data was available. This model served as a sensitivity analysis to investigate the influence of medication usage. .

MRI acquisition

Functional magnetic resonance imaging (fMRI) data was recorded at two separate scan sites using nearly identical acquisition parameters. At the VU University in Amsterdam, data was acquired on a 1.5 T Siemens Sonata scanner; at the Donders Institute for Brain, Cognition and Behaviour in Nijmegen, data was acquired on a 1.5 T Siemens Avanto scanner (both: Siemens Medical, Erlangen, Germany). Whole-brain T2*-weighted images were acquired using an echo-planar imaging (EPI) sequence (37 slices in Nijmegen/ 38 slices in Amsterdam, repetition time = 2340 ms, echo time = 40 ms, field of view = 224x224 mm, voxel size=3.5x3.5x3.0 mm, matrix = 64x64, slice thickness = 3 mm, 17% gap). Whole-brain

T1-weighted anatomical images were acquired at both sites using a magnetization-prepared, rapid-acquisition gradient echo (MPRAGE) sequence (176 slices, repetition time = 2730 ms, echo time = 2.95 ms, inversion time = 1000 ms, voxel size = 1.0x1.0x1.0 mm, field of view = 256 mm). To control for site effects in the neuroimaging data, analyses included scan site as a covariate of no interest.

MRI preprocessing

Functional and structural imaging data were preprocessed and analyzed using the FMRIB Software Library (FSL, version 5 (Jenkinson et al. 2012)). The first 5 functional volumes of each participant were discarded to allow for T1 equilibrium. All other volumes were realigned to the first remaining volume to correct for head motion. The resulting extended realignment parameters plus the extracted time courses of regions containing white matter and cerebral spinal fluid were then used for nuisance regression. Subsequently, images were spatially smoothed using a Gaussian kernel with a Full Width at Half Maximum (FWHM) of 6 mm and high-pass filtered at 0.001 Hz.

Functional images were spatially co-registered to their associated structural image using FSL FLIRT and normalised to MNI152 standard space after 1st level statistics had been performed. Considering the wide age range of our sample, we opted to register all participants' brains to a custom study template that was generated by averaging all T1-scans of participants in the NeuroIMAGE study (n = 787), with a resolution of 2 x 2 x 2 mm after transforming it non-linearly to MNI152 space with FSL FNIRT. For each participant, a non-linear warp-field for normalization from T1 to the custom template was calculated and subsequently applied. This procedure minimized the bias towards adult brains and provided a better brain registration for younger participants.

MRI 1st level analysis

For every subject, statistical parametric maps were estimated using a general linear model that included all relevant features of the MID trials (FSL FEAT). Six regressors of interest were included, containing the onsets for reward cues, non-reward cues, reward hits, reward misses, non-reward hits and non-reward misses, with all events modeled with a duration of zero. In addition, six regressors of no interest were included. These regressors modeled 1) movement artifacts; 2,3) the onsets of target presentation for reward and non-reward trials; and 4,5,6) the onsets for the cue, target and outcome of error trials. Movement artifacts were head movements from one image to the next that exceeded a threshold of 0.5 mm in any direction. Event onset of these artifacts was set to 8 seconds before the movement and all events of interest within this 8 seconds interval were discarded (Keulers et al. 2012). Error trials were trials with premature responses (RT<100 ms), too many responses (> 1 button press) or no response. All these regressors were modeled including their temporal derivatives and subsequently convolved with a canonical hemodynamic response function (HRF).

The 1st level models of the MID task provided two contrasts of interest. The neural effect of reward anticipation was obtained by contrasting BOLD activity evoked by reward and non-reward cues (reward cue > non-reward cue). Reward outcome–related activity was quantified by contrasting the effect of correct responses during reward trials with that observed during non-reward trials ([Reward hit – reward miss] > [non-reward hit – non-reward miss]). Estimated beta maps for both contrasts were normalized to MNI152 standard space for each participant for subsequent group comparisons.

MRI 2nd level analysis

Group-level analyses modeled neural activation across the full sample of participants (thus including participants with ADHD, their siblings and control participants in the same model). 2nd level activation maps were calculated with FSL FLAME using the normalized beta maps from the 1st level analyses. Neural responses during reward anticipation and reward outcome were modeled separately at the 2nd level, and included the 1st level variance estimates to account for between-subject differences in the quality of parameter estimation. The 2nd level model included the participant-specific ASD symptoms, ADHD Symptoms, the ASD x ADHD interaction (the element-wise multiplication of the previous two variables), Age, Sex, IQ, Scan Site, and ODD/CD comorbidity as explanatory variables (EVs). The factor Group (i.e. ADHD, siblings or control) was only present as EV in post-hoc sensitivity analyses. Additional models including separate regressors for ADHD hyperactive/impulsive and inattentive symptoms were run as supplementary analyses (Additional File 4). All EVs were demeaned (using the overall sample mean) before inclusion. The 2nd level models were calculated using the FSL flameo command and included automatic detection and de-weighting of outliers (Woolrich et al. 2009).

Statistical thresholding

All results reported were based on an initial uncorrected voxel-level threshold of $Z > 2.3$, corrected for the whole brain at the cluster level using $p < 0.025$ (FWE, corrected for testing both reward anticipation and reward receipt).

Post-hoc analyses of 2nd level MRI results

The mean time-series of each cluster that survived cluster-level correction were extracted for each participant using FSL for post-hoc analyses in SPSS. In these analyses, results were corrected for familiality (i.e. the non-independence of data from participants belonging to the same family due to shared genetic and environmental influences). Time-series for each cluster were entered as the dependent variable in a linear mixed model that included ASD Symptoms, ADHD Symptoms, the ASD x ADHD interaction, Age, Site and Sex as fixed effects and Family ID as a random effect. Moreover, this analysis was repeated for the subset of participants for whom medication data was available, additionally including their total stimulant use duration as a random effect to control for the effect of

medication use. This extra model served as a sensitivity analysis to investigate whether our findings were influenced by medication usage. Finally, the presence of interactions between any CSBQASD or CPRS-L effect and experimental group (ADHD, SIBS or CON) was checked by running separate models that included Group and a CSBQASD by Group interaction in addition to all previous fixed and random effects. These models served to test whether the observed effects of ASD and ADHD symptoms and the parameter estimates in the regions under investigation differed in the three experimental groups. Alpha was set at p=0.05 for all post-hoc analyses.

TABLE 1 Participants in the current study

	ADHD	Siblings	Control	
	N=136	N=83	N=105	
	Mean±SD	Mean±SD	Mean±SD	Comparison*
Age (years)	17.71±3.04	18.36±3.78	17.18±3.01	ADHD=SIBS+CON SIBS>CON
IQ	98.40±14.80	99.11±14.00	107.77±13.91	CON>(ADHD=SIBS)
Conners Score (Combined Scales)	69.76±12.92	47.73±6.69	45.38±4.52	ADHD>SIBS>CON
CSBQ ASD	10.56±9.44	9.14±10.10	3.43±4.54	(ADHD=SIBS)>CON
CSBQ Lack of Social Interest	3.89±4.35	3.27±4.14	1.12±2.12	(ADHD=SIBS)>CON
CSBQ Problems with Social Understanding	3.93±3.80	3.36±3.75	1.38±2.12	(ADHD=SIBS)>CON
CSBQ Stereotypical Behavior	1.43±2.32	1.42±2.23	0.45±1.06	(ADHD=SIBS)>CON
CSBQ Resistance to Change	1.32±1.65	1.10±1.67	0.48±1.01	(ADHD=SIBS)>CON
Adult	49.3%	44.6%	39.0%	Equal**
Site	40%	40%	59%	Unequal**
Sex	69% M	45 % M	45 % M	Unequal**

* Comparisons were made using Independent Sample T-tests at p<0.05.

**Equality of the distributions across participant groups was tested using Pearson's Chi Square Tests at p<0.05. SD=Standard deviation; ADHD=participants with ADHD; SIBS = unaffected siblings, CON= unrelated control participants. Adult= % of participants aged 18 years or older. Site = % of participants scanned in Amsterdam (the remainder was scanned in Nijmegen). M=Male.

Results

Demographics

Table 1 lists the demographics of the experimental sample. Participants with ADHD were of similar age compared to their siblings and healthy controls (ADHD vs SIBS t_{217}= 1.33, p=0.186; ADHD vs CON t_{239}=1.35, p=0.179); unaffected siblings were older than controls (SIBS vs CON, t_{186}=2.32, p=0.022). IQ was similar in participants with ADHD and their siblings (ADHD vs SIBS, t_{217}=0.36, p=0.725), but was higher in controls than the two other groups (CON vs ADHD, t_{239}=5.04, p<0.001; CON vs SIBS, t_{186}=4.22, p<0.001). Scores on the

CPRSR-L were highest for ADHD, as expected (ADHD vs SIBS, t_{217}=16.58, p<0.001; ADHD vs CON, t_{239}=20.47, p<0.001), but were also elevated in siblings compared to controls (SIBS vs CON, t_{186}=2.75, p=0.007). Finally, CSBQASD scores were higher in the ADHD group and their siblings compared to controls (ADHD vs CON, t_{239}=7.73, p<0.001; SIBS vs CON, t_{186}= 4.79, p<0.001), but not significantly different between the former groups (ADHD vs SIBS, t_{217}=1.03, p=0.305). In summary, participants with ADHD showed on average the highest severity of both ADHD and ASD, their unaffected siblings were similar to the ADHD group in ASD but not ADHD severity, and the healthy controls scored lowest on both ADHD and ASD dimensions.

Behavioral analysis of the MID task

Correct responses were faster for Reward versus Non-Reward hits (Reward Hit RT±Standard Error of the Mean (SEM) = 287.41±3.1 ms; Non-Reward Hit RT±SEM = 317.18±3.8 ms; Paired T-test on the log transformed data: t_{323} = -12.73, p<0.001). Subsequent mixed model analyses (controlling for age, IQ, sex, scan site, familial effects and ADHDxASD interactions) showed no significant association between the CSBQASD or CPRSR-L scores and any RT measure (Reward Hits RT, Non-Reward Hits RT, or the RT difference between correct Reward and Non-Reward trials, all p>0.05). In summary, although we found that reward trials showed the expected speeding of responses for all participants, we found no evidence that ASD or ADHD symptoms modulated the behavioral response of our participants in the MID task.

fMRI analysis of reward anticipation

All fMRI analyses were controlled for effects of age, sex, IQ, scan site, and ODD/CD comorbidity. Reward anticipation (Reward Cue > Non-Reward Cue) was associated with significant activation in a network of brain areas including the ventral striatum, amygdala, insula, cingulate cortex, and visual areas (Figure 1A, Additional File 5). Non-reward anticipation (Non-Reward Cue > Reward Cue) was related to stronger activity in the posterior cingulate and bilateral inferior parietal cortex.

ASD scores were positively correlated with activity in bilateral insula and the left superior frontal gyrus during reward anticipation. However, only the association between left insula activity and ASD scores remained significant after correction for familial non-independence and medication use (Figure 1B, Additional File 5). This effect persisted when restricting our analysis to participants below 18 years of age, and was not significantly different in adults and children (Additional File 6). Moreover, we found no significant effect of Group (ADHD, siblings or controls) on left insula activity or on the effect of ASD symptoms in the left insula (Additional File 7). In contrast, ADHD symptoms were negatively correlated with activity in posterior parietal and left dorsolateral prefrontal cortex (dlPFC) during reward anticipation. This negative correlation remained significant in the dlPFC after correction for familial non-independence and medication use (Figure 1C, Ad-

ditional File 5). This effect of ADHD symptoms was significant in participants below and above the age of 18 years when analyzed separately, and was not significantly different in these age groups (Additional File 6). No significant interactions between ASD and ADHD effects on reward anticipation were found. Again, we found no significant effect of Group on left dlPFC activity or on the effect of ADHD symptoms in the dlPFC (Additional File 7). Supplementary analyses looking at the distinct impact of ADHD hyperactice/impulsive and inattentive symptoms showed that the former was associated with reduced parahippocampal and lingual gyrus activity, and the latter with reduced caudate activity during reward anticipation (Additional File 4).

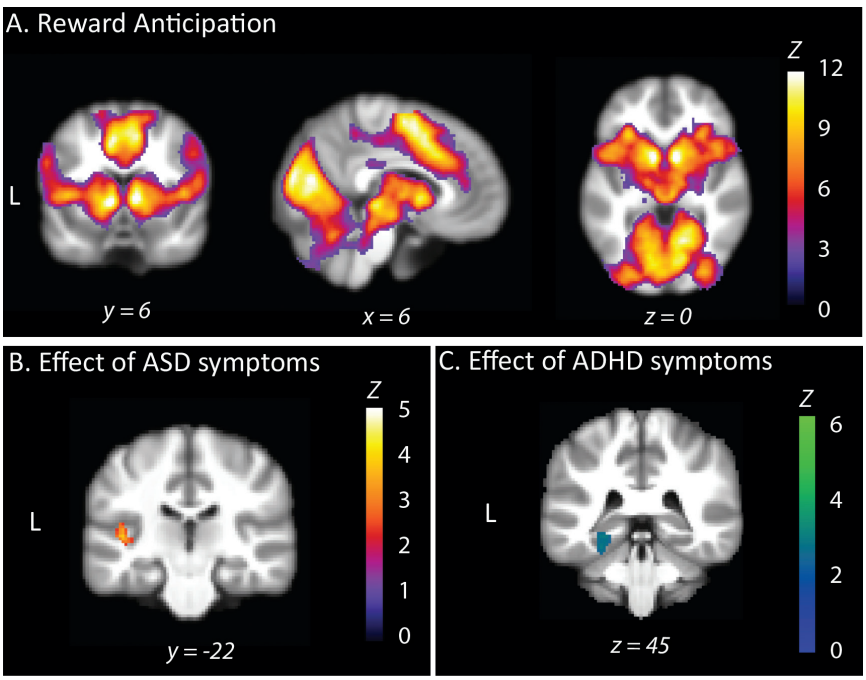

FIGURE 1 Neural responses associated with reward anticipation

A **Reward anticipation: activation stronger for Reward versus Non-Reward Cues.** Activation plotted represents the linear contrast between reward and non-reward cues from the time of cue onset. Reward anticipation was associated with stronger response in a network of brain areas including the striatum, medial (pre)frontal cortex, bilateral insula and parahippocampus, as well as posterior occipital and parietal regions.

B **ASD symptom scores were positively correlated with left insula activity during reward anticipation.**

C **ADHD symptom scores were negatively correlated with left parahippocampal cortex activation during reward anticipation.**

All activation shown was initially thresholded at the voxel level at $Z>2.3$, followed by whole-brain correction at the voxel level at $p<0.025$ (FWE). The clusters shown in panel B and C are significant after correction for familial non-independence and medication use. Results are plotted on representative slices of the NeuroIMAGE study template brain; coordinates are given in MNI space. See Additional File 3 for coordinates, p-values and cluster extent. Z= Z-value.

fMRI analysis of reward outcome

The neural response to reward outcome was investigated using the contrast between Rewarded and Non-Rewarded outcomes (Reward Hit-Miss > Non-Reward Hit-Miss). Rewarded hits were associated with significantly stronger activation than non-rewarded hits in the ventral striatum, anterior cingulate and orbito-frontal cortex, posterior cingulate and parietal cortex, as well as posterior visual areas (Figure 2). Non-rewarded outcomes were not linked to significant increases in activation compared to rewarded outcomes. No significant associations were found between ASD or ADHD scores and neural responses during reward receipt. Supplementary analyses related to distinct effects of ADHD hyperactive/impulsive and inattentive symptoms similarly did not result in significant findings.

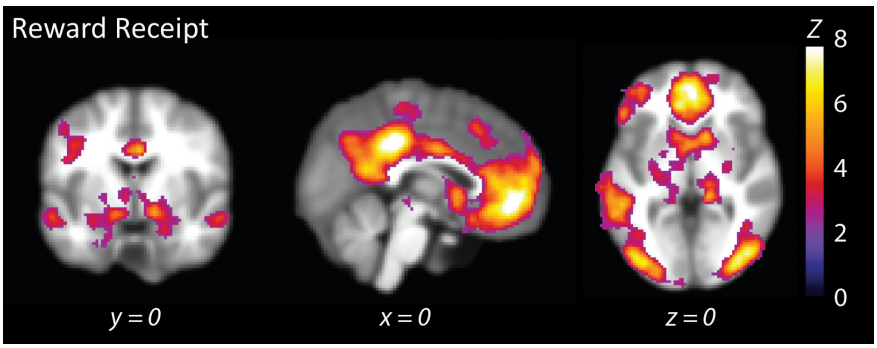

FIGURE 2 Reward outcome: activation stronger for Rewarded versus Non-rewarded Outcomes
Activation plotted represents the linear contrast (Reward Hit – Reward Miss) > (Non-reward Hit – Non-reward Miss). Reward outcome was associated with increased activity in the striatum, orbitofrontal and prefrontal cortex; bilateral posterior and inferior parietal cortex; posterior, mid and anterior cingulate gyrus, and bilateral amygdala and hippocampus. No significant increases in activation were observed for non-rewarded outcomes. All activation shown was initially thresholded at the voxel level at $Z>2.3$, followed by whole-brain correction at the voxel level at $p<0.025$ (FWE). Results are plotted on representative slices of the NeuroIMAGE study template brain; coordinates are given in MNI space. See Additional File 3 for coordinates, p-values and cluster extent. Z= Z-value.

Discussion

In this study we present evidence that variation in ASD and ADHD symptoms is related to specific changes in the neural signatures of reward processing in patients with ADHD, their unaffected siblings, and healthy controls. We found that ASD symptoms were positively related to left insula activity during reward anticipation across the three experimental groups. In contrast, ADHD symptoms were negatively related to activity in left dlPFC during reward anticipation. Both findings could not be explained by effects of age or sex. No effects of either ASD or ADHD were found during reward outcome.

Neural hyperactivity during the processing of reward in ASD is not a common finding, but has been demonstrated previously (Cascio et al. 2012; Dichter et al. 2012c; Dichter et al. 2012b). Our findings are in line with previous findings by Cascio and colleagues, who also found ASD-related hyperactivity in the insula during reward anticipation (Cascio et al. 2012). Our results also match well with the proposed role of the insula in interoceptive and motivational processes (Craig 2002; Critchley et al. 2004; Craig 2009) and its theorized relevance to decision-making and abnormal reward-seeking behaviour (Naqvi and Bechara 2010). Anatomical evidence suggests that ASD is characterized by structural abnormalities in the insula that could relate to heightened interoception, and/or a more internally oriented focus (Santos et al. 2011; Allman et al. 2011). Taken together, increased insula activity in individuals with higher levels of ASD symptoms might be related to altered motivational processes and/or greater interoception. As such, activity in the insula might be considered a possible marker of cognitive dysfunction in milder forms of ASD.

ADHD symptoms were associated with reduced left dlPFC activity during reward anticipation. ADHD has previously been linked to hypoactivity during anticipation of monetary reward, although primarily in striatal regions (Hoogman et al. 2011; Dichter et al. 2012a; Plichta and Scheres 2014; Kohls et al. 2014). Although the dlPFC is not considered a central part of the neural reward circuit, in our study it was involved in reward anticipation (i.e. more strongly activated during anticipation of reward versus no reward across the sample). This finding would suggest that the dlPFC is differentially responsive to rewarded versus non-rewarded contexts. Furthermore, the dlPFC has shown ADHD-related abnormalities in various cognitive contexts, due its proposed role in attentional and motivational processes, and our finding could thus reflect more general neurocognitive alterations associated with ADHD (Arnsten 2011; Arnsten and Rubia 2012). In addition, exploratory analyses of the reward anticipation phase using separate ADHD subscales indicated that hyperactive/impulsive symptoms were associated with reduced activity in the parahippocampal and lingual gyrus, whereas inattentive symptoms were linked to reduced caudate activity. These findings provide initial evidence that these subscales might modulate the neural response to reward anticipation differentially.

Against expectation, we found no evidence for striatal effects of ASD symptoms in our study. Although striatal deficits related to reward processing have been observed in multiple ASD studies (Dichter et al. 2012a), other studies did not find striatal abnormalities in monetary reward conditions in ASD and (Schmitz et al. 2008; Scott-Van Zeeland et al. 2010; Delmonte et al. 2012). We can only speculate about the reasons why striatal functioning was unaffected by ASD symptoms in our study. Our large sample size provided enough power to detect effects. Instead, differences in task parameters (e.g. dimensional measures versus categorical definitions of ASD; differences in reward probability and amount of reward) could provide an explanation. In addition, striatal abnormalities might be characteristic of clinical ASD only, and not clearly apparent in less affected populations. Note that we did find that the ventral striatum and other areas of the frontal-striatal reward circuit

were robustly activated during reward anticipation and receipt, in line with other studies, indicating that our task manipulation was successful (Knutson and Cooper 2005; Haber and Knutson 2010).

We did not find evidence for effects of ASD or ADHD symptoms during reward receipt, in the outcome phase of the MID task. Although it is difficult to speculate about the reasons for a null-finding, it could be in part due to the lower number of trials available for the outcome condition. However, we believe that our large sample offers substantial protection against this potential problem, and we do find strong reliable reward outcome-related neural responses. It could nevertheless be that dimensional effects of ADHD and ASD during the reward outcome phase are smaller than those during reward anticipation.

In addition to the expected positive effects of reward anticipation in fronto-striatal regions, we also observed significant activation differences in the reversed contrast (i.e. stronger activity for anticipation of no reward versus reward). These effects were localized in brain areas previously associated with the so-called Default Mode Network and could therefore reflect reduced task engagement (and increased mind-wandering) during non-reward versus reward anticipation (Raichle and Snyder 2007).

The results of this study should be seen in the context of some strengths and limitations. First, although a large part of the autistic spectrum was covered in our experiment due to the inclusion of three groups of participants with varying degrees of ASD symptoms, we did not measure the extreme end of the spectrum by including participants with a clinical diagnosis of ASD. This makes it difficult to translate our findings to more severely affected populations. However, our results remain relevant for individuals with ADHD and the general population, where milder forms of ASD are commonly observed. To extend our findings and provide converging evidence for the results of the current study, we are at the moment conducting research using participants with a clinical diagnosis of ASD who are also evaluated for ADHD symptomatology in the EU-AIMS project (www.eu-aims.eu).

We did not find behavioral effects of ASD or ADHD on the MID task in this study. We can therefore only speculate on the behavioral relevance of the current findings. Nevertheless, the absence of behavioral effects also erases a potential confound for the interpretation of the neuroimaging findings, and suggests that both ASD and ADHD symptoms are affecting the neural substrates of reward processing in a way that cannot simply be explained by differences in behaviour. This notwithstanding, the direct clinical relevance of our findings is not immediately apparent and will require future study.

An important strength of the current study was the use of dimensional measures of ADHD and ASD symptoms. These measures allowed for a more refined analytic approach relative to traditional categorical comparisons between populations. Similar approaches are becoming more and more common in psychiatry, as the relevance of dimensional aspects of many psychiatric disorders becomes increasingly apparent (Hudziak et al. 2007). Future

studies in ASD and ADHD populations could therefore benefit from including similar designs to further disentangle the contribution of ASD and ADHD symptoms to reward processes.

Unfortunately, this study could not investigate whether ASD (and ADHD) symptoms affect social (and other types of) reward differently from monetary reward. Further research is needed to investigate the specificity of our findings. Follow-up research could clarify whether social reward paradigms show similar effects. Given the ongoing discussion about the special relevance of social reward deficits in ASD and ADHD, such studies could provide valuable novel insights (Rommelse et al. 2011; Chevallier et al. 2012).

Since data was available from participants with ADHD (who scored high on ADHD and ASD), their unaffected siblings (who scored high on ASD but not ADHD), and healthy controls (who scored low on ADHD and ASD), our sample included a wide range of ASD and ADHD symptoms. This design, in combination with our large sample size, enabled us to separate effects of ADHD and ASD, and study whether both symptom dimensions interacted. We found no evidence for an interaction between ADHD and ASD symptom scores, nor did we find that the neural effects of ASD and ADHD symptoms differed in the three experimental groups. Our findings therefore suggest that ASD and ADHD symptoms affected all types of participants equally (at a given level of severity), and that ASD and ADHD did not have multiplicative effects in our sample. Practically, this would mean that ASD and ADHD affect reward processing in distinct ways, and via different (neural) mechanisms. Our findings thus do not directly support theories of a shared aetiology between ASD- and ADHD-related reward dysfunction, nor do they point towards a qualitative difference in reward abnormalities in individuals who score high on both ASD and ADHD symptom measures. However, our sample did not include individuals with clinical levels of ADHD and ASD, so we cannot rule out that such individuals would show specific abnormalities in line with previous studies (Nydén et al. 2010; van der Meer et al. 2012; Chantiluke et al. 2014).

Conclusions

With this study we provide evidence that variation in ASD- and ADHD-related symptomatology can modulate the neural response to reward anticipation in participants with ADHD, their unaffected siblings and healthy controls. Taken together, these results underscore the importance of multidimensional assessment for clinical and healthy populations in general and for the characterization of ADHD- and ASD-related effects on reward processing in particular.

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Chapter 7

Summary of main findings and discussion

The aim of this thesis was to expand our knowledge about the behavioral and neural correlates of reward processing in adolescents and young adults with ADHD and to address inconsistencies in the literature. To achieve this goal behavioral and functional imaging data was acquired and analyzed of the NeuroIMAGE cohort, a uniquely large sample including over 300 families with at least one child with ADHD, and over 150 control families.

Every chapter addressed reward processing from a different angle with the aim to illuminate specific aspects of the associated functional system.

In **Chapter 2** the NeuroIMAGE cohort, one of the largest samples of ADHD participants with neuroimaging data worldwide, was introduced. Part of this introduction was the description of the comprehensive quality assurance policy that addressed specific problems of multi-site studies. It was also tested whether group or site characteristics compromised data quality. First, our results demonstrated that participants with ADHD and healthy participants were very similar in how much movement they exhibited in the scanner. Second, neural measures such as the critical contrast reflecting reward anticipation were more variable between participants than between sites suggesting that non-specific site effects were less likely to blunt group differences for the acquired neural measures. Together, these results indicate that the NeuroIMAGE sample is an invaluable resource to investigate neural mechanisms of ADHD at a large scale. The use of such a large sample can assist resolving inconsistent findings related to cognitive and neural processes associated with ADHD as it provides a lot of statistical power for data analysis, which prevents the misestimation of effect sizes. Moreover, large samples enable researchers to detect small effects and segment ADHD into more homogenous subgroups.

In **Chapter 3** behavioral and neural responses to reward were investigated by applying a monetary incentive delay (MID) task. Core behavioral measures of this cognitive task included reaction time (RT) and reaction time variability (RTV). Neural measures were task-induced neural responses reflecting reward anticipation and receipt. We observed clear effects of reward on behavior as indicated by reward-dependent speeding and decreases in response variability. Reward also elicited brain responses in reward processing brain structures including the VS and OFC during reward anticipation and receipt. ADHD was associated with behavioral changes consisting of larger reward-related decreases in response variability as well as increased neural responses during reward anticipation and receipt. Increased neural responses were located in reward processing structures such as the VS and OFC during reward receipt, in the ACC, frontal pole and cerebellum during reward anticipation and in visual areas during reward receipt. Somewhat surprisingly, unlike previous studies with smaller sample sizes, our larger study revealed no change in activity in the VS during reward anticipation. These results indicate that ADHD affect neural processes that underlie reward processing, specifically during reward receipt.

Investigation of unaffected siblings of participants with ADHD revealed that this group exhibited the same increased neural responses in some of the brain areas that were af-

fected in participants with ADHD, including prefrontal cortex, occipital cortex and anterior cingulate cortex. Other areas in prefrontal cortex, VS and cerebellum were unchanged. These results indicate that neural mechanisms underlying reward processing are subject to familial factors.

In **Chapter 4** the task-induced brain responses reported in chapter 3 were reinvestigated from a brain network perspective. By applying a data-driven, hypothesis-free analytical approach the aim was to identify a set of functional networks based on the task-related brain responses. These networks were used to explore ADHD-related effects on network characteristics such as functional integration and communication between networks implicated in reward processing. We were able to recover four networks mainly associated with the task in healthy participants, namely the default-mode, the executive control, the lateral visual and the salience network. The first three networks were reward-independent and associated with all task aspects; only the salience network was selectively associated with rewarded cues. The salience network was the network showing the highest degree of within-network integration whereas it was highly segregated from the other networks. Participants with ADHD had very similar network characteristics compared to those reported for healthy participants. Diagnosis-related differences comprised small reductions of connectivity of the executive control and salience network with peripheral neural structures, suggesting a very similar configuration of reward-related functional networks in ADHD. Furthermore, finding a rather unaltered functional network configuration in ADHD whereas we observed task-induced local changes of the brain response in reward-critical brain regions as reported in the previous chapter suggests that, although some selective regions respond hypersensitive to reward, the communication of such reward information within and between reward-related functional networks is unaltered in ADHD.

In **Chapter 5** the intrinsic organization of neural networks, putatively controlling key aspects of human behavior such as motivational, attentional and motor control, were assessed. By investigating resting-state functional connectivity using a seed-based approach with different striatal regions as seeds in healthy controls and participants with ADHD, we were able to parcel the brain into different fronto-striatal networks. We observed no changes in connectivity within these networks in ADHD, indicating that the neural architecture of these functional networks is the same for participants with ADHD and healthy controls. In contrast, ADHD was associated with increased local intra-striatal connectivity in motor- and attention-related striatal nodes compared with healthy controls. This finding suggests that ADHD is associated with decreased functional segregation of neural circuits underlying attentional and motor functions, which might provide a neural basis for motor deficits frequently observed in ADHD.

Chapter 6 addressed the clinical heterogeneity of ADHD by investigating the unique association between neural mechanisms underlying reward processing and symptoms of ADHD, subclinical symptoms of ASD, and the combined effect of both symptom clusters.

This analysis was based on dimensional measures of ADHD and ASD symptom severity and showed that there were neural changes specific to each symptom domain. ASD symptomatology was uniquely related to aberrant signaling in the left insular cortex, whereas ADHD symptomatology was related to decreased signaling in the parahippocampal cortex. Compared with the results from the ADHD vs. control comparison presented in Chapter 3, this finding suggests that the parahippocampal cortex exhibits a linear relationship between ADHD symptoms and aberrant signaling in reward processing structures across the whole population, whereas reward-critical brain regions are sensitive to categorical ADHD-control differences.

There was no interaction effect of both symptom clusters on the neural measures. Together these results indicate that comorbidities of ADHD such as ASD symptomatology are associated with specific alterations of neural functioning, suggesting, to some degree, independent etiologic pathways of the two disorders.

Neurobiological mechanisms underlying reward processing in ADHD

The overall aim of this thesis was to test the hypothesis that ADHD implicates abnormalities in the neural mechanisms of reward processing. Therefore I applied conventional and network analyses to task-based fMRI data as well as seed-based functional connectivity analysis to rs-fMRI data. Of all studies presented in this thesis the task-based fMRI study provided most evidence for the hypothesized association between reward-processing brain networks and ADHD by revealing local increases of neural responses in participants with ADHD. Participants with ADHD exhibited increased reward-related activity in brain regions primarily associated with reward processing such as the VS and OFC, and other regions such as the cerebellum and the occipital lobe. The OFC has been associated with the representation of reward value, accordingly increased responses of the OFC suggest that reward magnitude is overrepresented in participants with ADHD during reward outcome (O'Doherty et al. 2001). Responses of the VS during reward receipt have been associated with affective responses to reward, suggesting that participants with ADHD are hypersensitive to reward information because they experience consumption of rewards as more pleasurable than healthy controls (Sescousse et al. 2013). Increased neural responses in other areas as occipital cortex and the cerebellum are more difficult to interpret, as these regions do not primarily belong to reward-processing brain networks. I speculate that neural changes in these regions are related to the primary function of these regions, namely visual perception and coordinated movement (although the cerebellum recently also has been implicated in non-motor function (Strick et al. 2009)). Accordingly, these effects might represent modulation by ADHD of the regulation of visual and motor processes during reward anticipation and receipt, which might be associated with altered task performance indirectly.

Behaviorally, reward-related effects on brain responses were accompanied by stronger improvement of task performance as frequently observed in the literature (Luman et al. 2005; Drechsler et al. 2008). Participants with ADHD showed particularly poor performance on neutral trials (as indicated by more variable reaction times), and recovered from this deficit on rewarded trials, reaching the level of healthy participants. This finding suggests that in ADHD neural processes involved in reward processing are rather overly efficient than impaired, which results in enhanced behavioral performance. Observing increased neural responses in ADHD in reward-critical structures such as VS and OFC during reward receipt provides a neurobiological account for the hypothesized alteration of reward-related neural processes in ADHD. It also raises questions regarding the current view that ADHD is associated with decreased neural signaling in the VS during reward anticipation (Plichta and Scheres 2014). An aspect possibly explaining the inconsistency between previous study results and ours is that most of the previous studies investigating reward anticipation in ADHD were performed in adult populations. To my knowledge, only three studies investigated adolescent participants, one evidencing hypoactivation (Scheres et al. 2007) and the other two reporting no differences (Paloyelis et al. 2012; Kappel et al. 2014). Accordingly, there is limited evidence for deficits during reward anticipation in young participants with ADHD and age may be a critical factor determining whether participants with ADHD exhibit altered reward-related neural processes. Although my studies did not speak to this explanation, longitudinal studies have indicated diagnosis-specific developmental effects on the anatomy of brain structures implicated in reward processing (see (Casey et al. 2005)). Longitudinal studies are needed to investigate potential developmental effects on functional neural measures implicated in reward processing.

In addition, my finding highlights that ADHD-related alterations of neural signaling in reward processing brain structures are specifically present during reward receipt. Relatively few studies (6 out of 10) have investigated the neural correlates of these affective reward processes in ADHD, providing highly inconsistent evidence regarding location and direction of the reported effects (Scheres et al. 2007; Ströhle et al. 2008; Stoy et al. 2011; Wilbertz et al. 2012; Paloyelis et al. 2012; Edel et al. 2013). In adults, the majority (3 out of 4) of studies reported altered responses in the OFC (Ströhle et al. 2008; Wilbertz et al. 2012; Edel et al. 2013) with two studies reporting hypoactivation (Wilbertz et al. 2012; Edel et al. 2013) and one study reporting hyperactivation (Ströhle et al. 2008). In adolescents, one study reported neural changes in the striatum (Paloyelis et al. 2012), the other reported no difference (Scheres et al. 2007). Our study complements results of studies in adult participants with ADHD regarding the location where changes in neural firing were observed (i.e the OFC), suggesting that in both adolescent and adult participants with ADHD rewards are differently represented in the brain. However, more studies are needed to support our finding and unravel its direction.

My investigation of the intrinsic organization of reward-processing neural networks provided only indirect evidence for why the brain of participants with ADHD responds hypersensitive to reward. When we used resting-state fMRI to assess functional connectivity of the reward-critical VS with all other regions of the brain we did not see any ADHD-relat-

ed differences in functional integrity of this reward-related network. For example, communication between VS and OFC was the same for participants with ADHD and healthy participants. Also, the communication between the VS and other cores of the basal ganglia, which are associated with other aspects of behavioral control such as attention and motor control, was unchanged. Together, this indicates that the functional architecture of the reward-processing network is not altered in ADHD. Consequently, my results do not support that an altered functional architecture may account for the increased brain responses I observed in participants with ADHD relative with healthy participants during reward receipt in VS and OFC (von Rhein et al. 2015). Finding increased neural responses in reward processing regions during task performance whereas communication between the very same regions at rest is unaltered suggests that the affected regions are overly recruited only at the moment the associated cognitive system is in use (i.e. during reward processing).

To take into account that reward processing structures form a distributed network that depends on efficient communication between different network nodes we also investigated network characteristics of networks implicated in reward processing. In general, there was limited evidence for abnormalities of the functional integration of neural networks implicated in reward processing in ADHD. When investigating functional connectivity of brain networks derived from task responses during reward processing we observed subtle differences in network coherence of networks associated with reward processing. Networks exhibiting altered functional connectivity consisted of the salience network, which we found to be specifically associated with reward-aspects of the task, and the reward-independent executive control network (Seeley et al. 2007; Smith et al. 2009). Compared with healthy participants, we observed in participants with ADHD both networks to be less connected with cortical brain areas such as the inferior frontal and temporal gyrus suggesting decreased coherence of these networks. Only the cerebellum, a region that has already been associated with impairments in temporal prediction of environmental cues in ADHD (Durston et al. 2007), was both more (with the salience network) and less (with the executive control network) connected. Finding altered functional connectivity in ADHD has two important implications. First, altered connectivity in these regions are potential biomarkers of ADHD. Future research has to clarify the functional relevance of these regions in the context of reward processing. Second, finding only subtle changes in non-vital structures of the reward-processing network for a large test population suggests that the association between reward-related functional integration and ADHD is, at least at a group level, weak. In general, it appears that the global network configuration of reward processing networks including communication between networks was more or less the same for healthy controls and participants with ADHD.

Finding rather unaltered integrated, reward-related functional networks in ADHD is inconsistent with earlier reports of altered functional connectivity in related structures such as between VS and frontal cortex (Costa Dias et al. 2013) or between VS and putamen (Cao et al. 2009). One possible explanation for this inconsistency is the high phenotypical

heterogeneity in our sample compared to earlier studies that investigated either only participants with combined presentation of ADHD (Costa Dias et al. 2013) or only boys (Cao et al. 2009). In contrast, our study included boys and girls with ADHD, including all possible presentations of the disorder.

So far, we have discussed the involvement of the reward system in ADHD at a group level. However, other lines of research indicate that specific phenotypic characteristics contribute to deficits of this functional system. For example, Scheres and colleagues reported reward deficits such as steep temporal discounting specifically for participants diagnosed with ADHD combined type (ADHD-CT) and not for the inattentive type (ADHD-I) (Scheres et al. 2010). Moreover, inattentive participants with ADHD (ADHD-I) exhibit specific impairments on response inhibition when high rewards are at stake (Huang-Pollock et al. 2007). With regard to gender, no study so far has reported between-sex differences for ADHD participants on reward measures. However, effects of sex on delay aversion were demonstrated in healthy participants. Together, these findings suggest that reward-related deficits might be specific for a certain subgroup within the ADHD population and, accordingly, changed functional integration may only be visible in a specific subgroup.

In the context of my reported regional ADHD-related changes of neural firing (Chapter 3), one might also have expected to find alterations in reward processing structures at the network level. This, however, is not necessarily the case as both analyses focus on different aspects of neural functioning. Whereas analyses performed in Chapter 3 aimed at assessing functional specificity, thus the segregation of functionally distinct regions, my network analysis aimed at assessing functional integrity or how neural regions communicate with each other. Together, my findings suggest that, although reward-processing regions respond hypersensitive to reward, the communication of that reward information within and between networks is unaltered.

Our task-based study of ADHD participants with comorbid symptoms of ASD supported the interpretation that the characteristics of neural mechanism underlying reward processing are dependent on the specific phenotypic characteristics of the investigated sample. In that study we observed that local neural processes in the insular cortex were, corrected for the influence of ADHD, associated with subclinical autistics symptoms. In contrast, the parahippocampal cortex was specifically associated with ADHD symptoms. This finding suggests that ADHD and ASD symptoms are differentially linked to neural regions of reward processing. Finding neurobiological mechanisms that can be uniquely related to each disorder provides evidence for an independent, non-causal etiology of these comorbidities (Banaschewski et al. 2007). However, because we did not investigate whether there are neural mechanisms that are associated with both symptom clusters, we cannot refute the hypothesis that both disorders also share etiologic pathways.

Although I did not replicate, with this dimensional approach, results from my case-control comparison, both studies do not contradict each other; they answer different experi-

mental questions. The case-control comparison was conducted to identify brain regions that are sensitive to effects of ADHD, whereas the dimensional approach assessed neural regions exhibiting a linear relationship with ADHD symptoms. Both studies define ADHD differently: according to the categorical approach, ADHD is operationalized as an entity separate from the normal population; the dimensional approach operationalizes ADHD as an extreme form of characteristics that occur in the normal population. Accordingly, results from both studies need to be interpreted. Finding a (negative) linear relationship between ADHD symptoms and the parahippocampal cortex indicates that firing of this region is, like a trait, associated with ADHD across the whole population. Other regions just as the reward-critical structures VS and OFC suggest qualitative differences in neural firing of these regions for the ADHD population.

Endophenotypes and heritability of ADHD

An innovative question I investigated in this thesis was whether neural measures associated with reward processing are also endophenotypes of ADHD. This implies that these measures are not only related to the neurobiological mechanisms of ADHD, in addition they would be required to share genetic variance with ADHD and be more related to the genetic underpinning of the disorder than its symptoms. Neural measures implicated in reward processing are in this context of special interest as the same genetic variants have been associated with signaling in this functional system in healthy participants (Forbes et al. 2009; Hahn et al. 2011) as well as with the ADHD phenotype (Gizer et al. 2009).

Target for my investigation of endophenotypes were unaffected siblings of participants with ADHD. This group is particularly useful for that purpose because unaffected siblings share on average 50% of the genes with their affected siblings and are exposed to highly similar (i.e. shared) environmental factors, yet they have no ADHD diagnosis. We specifically investigated whether these siblings show neurobiological functioning intermediate between controls and participants with ADHD. Unaffected siblings exhibited abnormally increased neural responses in some of the neural structures, observed to be affected in participants with ADHD, thereby providing evidence for intermediate changes in reward-related neural brain regions. Interestingly, I observed these endophenotypic characteristics of reward processing only at a neural level; behavior was not affected in the unaffected siblings. This might be explained if we assume that behavioral measures of reward processing are, just as symptoms of ADHD, less strongly influenced by genes, whereas neural responses are closer to the biological level, at which genetic factors become more apparently visible.

Further exploration is needed to answer the question to what extent genetic, environmental or gene-environment interactions (GxE)) account for the observed neural changes. Excellent candidates for the investigation of genetic factors are genetic variants such as the DAT1, DRD2 and DRD4 polymorphism (Gizer et al. 2009; Hahn et al. 2011), which in case

of genetic effects, might provide a molecular-biological, etiological model of ADHD. However, two important considerations need to be noted. First, a pathway model, in which genetic variants explain neural measures, which in turn explain ADHD symptoms, is only testable if all three 'type'-measures (genotype, endophenotype and phenotype) are assessed at the same time, specifically testing for the mediating effects of the neural measures. Otherwise, the presumed endophenotypic measures can simply be co-occurences (i.e. epiphenomena) of a genetic variant without a causal relationship (for extensive discussion of different endophenotypic models see Kendler and Neale (Kendler and Neale 2010)). Second, previous studies elucidating the genetic underpinning of neural mechanism underlying reward processing are inconsistent. For example for DAT1, six studies investigated in healthy participants gene effects on reward–related neural responses in the striatum (Hoogman et al. 2013). Three of these six studies demonstrated gene-related effects on striatal responses. Considering that applied cognitive tasks and task parameters varied between studies, we first need to uncover which aspect of reward processing is associated with a specific genetic variant in healthy populations.

Clinical implications

Our studies of neural mechanisms underlying reward processing were mainly focused on fundamental aspects of ADHD. Nevertheless, our findings have implications for clinical practice.

As described earlier, I have identified a reward-related neural mechanism that is associated with ADHD: participants with ADHD overrepresent reward values and exhibit an overly affective response to reward which leads to a normalization of impaired behavioral performance observed during unrewarded actions. To answer the question of how this mechanism might explain symptomatic behavior, let's consider behavior in an educational setting, one of the domains, where participants with ADHD (especially of age of our tested sample) often show clinical symptoms such as inattention. For instance, participants with ADHD may suffer from distractibility when they try to do homework/study for an undesired study topic. From a motivational perspective, they may perceive studying as relatively unrewarding, in particular when compared with immediately rewarding activities such as gaming on mobile devices or social interactions with peers. Based on our observed neurobiological changes in ADHD it may become evident why particularly participants with ADHD have difficulty engaging or keep up with studying. They might assign an abnormally high value to the rewarding behavior and/or experience rewarding activities as relatively much more pleasurable than less rewarding activities.

For participants with ADHD with dysfunctional signaling in reward-related neural networks clinical interventions may be useful that specifically tackle the reported altered neurobiological mechanism. In our example, a therapist could instruct a patient with ADHD having problems to study to explicitly couple initially non-rewarding behavior (e.g. study-

ing for the undesired topic) with rewards. For example, he could ask the parents of participants with ADHD only to pay pocket money in return for study hours. A non-monetary incentive alternative would be to provide credits for study hours that can be exchanged for desired pleasurable activities. Such an approach would reflect token-economy, an intervention technique already applied in cognitive behavioral therapy, which might be an expansion or possibly an alternative for pharmacotherapy with methylphenidate.

A further implication can be derived from our observation that neural processes implicated in reward processing were specifically associated with either ASD or ADHD symptoms. First, finding a unique neurobiological basis for each of these two disorders indicates that comorbid participants exhibit additional neural changes to those of ADHD participants. This implies that comorbid participants might require other or additional clinical interventions than participants with pure ADHD. Such interventions should aim at addressing etiologic deficits that are specifically associated with the comorbidity. Second, although most participants of our study had no clinical diagnosis of ASD, we observed functional changes in brain regions to be associated with autistic symptoms. Regarding that disorder-specific functional changes of the brain are often accompanied by behavioral impairments, our finding highlights the necessity to standardly assess comorbid symptoms in ADHD populations, also at a subclinical level.

The final implications relate to the strength of the observed association between ADHD and our neural measures. At the group-level we have demonstrated that the diagnosis of ADHD is accompanied by neural deviations in the functional network associated with reward processing. By this we provide a handle to describe the disorder in terms of an affected neurobiological system instead of pure behavioral observations. However, our measure also showed a high degree of within-group variability. This implies that reward processing is less affected in ADHD than proposed by recent theories of ADHD that stress the role of reward-related deficits for the etiology of ADHD (e.g. (Sonuga-Barke 2002; Sagvolden et al. 2005)). Our empirical data provides evidence that reward-processing deficits may rather lead to behavioral enhancement in a subgroup of participants with ADHD than a general deficit, accordingly the proposed clinical interventions may only apply to a subgroup of participants that exhibit these specific reward-related neural deficits. Moreover, high variability implies that our measures are currently of limited value for diagnostic purposes.

Strengths and limitations of the presented studies

For all analyses presented in this thesis the NeuroIMAGE sample has been used. This sample is one of the largest clinic-based ADHD samples in the world, for which a large variety of data inclusive functional imaging, familial, and genetic data is available.

The use of this sample had advantages and disadvantages. The most important advantage is its large sample size, which helps to address an important problem in the field:

inconsistent findings. Most studies investigate neural mechanisms of ADHD rely, with approximately 15 to 50 participants per diagnostic group, on small samples. These studies have provided valuable insights in affected neural functional networks. However, studies with small samples can easily become a target of biases. For instance, outliers have much more impact on group averages when groups are small. Also, a selected group might by chance have certain characteristics that affect the acquired measurements. Most importantly, small studies suffer from a bias known as 'the winner's curse' (Ioannidis 2008). This bias refers to the phenomenon that initial scientific discoveries most probably overestimate effect sizes, which makes it hard for future studies to replicate reported findings (Button et al. 2013). In contrast, investigations in larger samples such as NeuroIMAGE are less affected by sampling bias (e.g. specific sample characteristics) and analytical degrees of freedom (e.g. outlier handling). Moreover, effect sizes are estimated more realistically, which increases the robustness of the reported results and the likelihood that these will be replicated in future studies. In summary, a large study sample as NeuroIMAGE provides an invaluable resource to robustly assess the association between neural mechanisms and ADHD. With its high statistical power, the sample allows detection of subtle effects with small effect size. On the basis of such identified neural and cognitive deficits, it might then be possible to address the problem of heterogeneity by forming more homogeneous subgroups of ADHD with shared underlying neurocognitive deficits.

The unique value of the NeuroIMAGE sample becomes clear when one considers the restricted financial and personal resources of most research labs, which simply cannot afford large-scale studies. In order to bridge the gap between required large-scale imaging studies and constraints of resources data sharing have been initiated (e.g. ADHD-200 (HD-200 Consortium 2012)). These initiatives pool functional data from several studies in order to gain large data sets with high statistical power. Downside of these efforts is that additional noise is induced e.g. by use of different scanners, scan sequences, experimental tasks, etc. Every non-standard setting adds variability to the data (i.e. larger standard deviation), which decreases the ability to detect between-group differences. There are ways to account statistically for this, however, most likely such attempts will be at the cost of sensitivity of the measures (e.g. more degrees of freedom).

Site-effects are also an issue for the NeuroIMAGE sample as data was acquired at different sites. Nevertheless, compared with data-sharing initiatives the NeuroIMAGE cohort has the advantage that the same metrics were collected on basis of the same protocols across different sites, thereby reducing potential confounding factors between sites and increasing data quality.

One last characteristic of the NeuroIMAGE sample that needs to be discussed is the study design. Most fundamental studies of ADHD investigate experimental groups that are balanced regarding their demographic characteristics such as IQ, age and gender. From an experimental point of view, this makes sense as balancing reduces potential statistical bias that can account for group differences. Especially, when a variable (e.g. IQ) covaries with

another variable of interest (e.g. ADHD symptoms), we cannot disentangle completely the effect of both because statistical correction methods are limited (Miller and Chapman 2001).

The NeuroIMAGE sample was unbalanced as IQ was higher for healthy participants compared with participants with ADHD. Also more boys than girls with ADHD were tested although gender was equally distributed in the healthy group. Accordingly, it is impossible to completely separate diagnostic effects from IQ and gender. The question is whether it would have been better to balance the groups because we particularly collected data of a sample that is close to a population sample. Because our sample resembled the distribution of the population our results are more likely to be externally valid (i.e. generalizable to the general population of ADHD participants) and this might outweigh the disadvantage of an unbalanced design. Particularly for reward processing, imbalanced IQ is unlikely to account for group differences, as reward-related tasks do not require high cognitive demands.

Recommendations for future research

In this thesis we have performed several group-level analyses on neural mechanisms implicated in reward processing. By using sensitive neural measures and an extraordinary large sample of participants, we have found evidence for a neurobiological mechanism underlying ADHD, as evidenced by hypersensitive responses of reward processing structures during reward anticipation and receipt.

However, considering that our concentrated efforts to study the neural mechanisms of reward processing revealed only subtle diagnostic effects with small effect size it seems necessary to discuss the value of investigating this neural system in ADHD. Despite the possibility that changed parameters of our task including reward magnitude and probability might have reduced the magnitude of the neural responses (for extensive discussion see Chapter 3), a simple explanation for the reported small effects is that the neural mechanisms implicated in reward processing are only partly disabled in participants with ADHD. Such an interpretation suggests that we might rather focus on different neural systems in ADHD. This, however, is not satisfactory as reward-related deficits are at a behavioral level evident in ADHD. A slightly different explanation is that neural signaling in this functional system happen at a faster time-scale and the BOLD response, with a temporal resolution in the order of seconds, might be too sluggish to capture diagnostic changes. Accordingly, future research might be improved by smarter incorporation of imaging methods that have a higher temporal resolution. Examples of such efforts exist, as for instance done by Plichta and colleagues (Plichta et al. 2013) who coupled measuring fMRI measures during a MID task with electroencephalography (EEG). They were able to relate brain responses in key areas associated with reward processing to an electrophysiological process and infer on basis of this relation the causal relationship between the involved neural structures. Accordingly, such measures might also provide additional cues for characterizing ADHD in terms of altered neurophysiological processes.

Another consideration for future research is that group-level fMRI analyses may not be the most appropriate approach to study neural mechanisms associated with ADHD. ADHD is a complex psychiatric spectrum disorder with a high degree of heterogeneity, which can occur at different levels: at a demographic level (age range, IQ), at a phenotypic level (kind of symptoms) and at a neural level (brain responses). Accordingly, it might be challenging to identify neural mechanisms that commonly apply to all or at least most of the members of the diagnostic group. Support for this interpretation comes for example from another study using structural MRI in the same sample. This study, investigating approximately 1000 participants with and without ADHD, reported significant changes of brain volume of as much as 3% (Greven et al. 2015). Finding a small, unspecific reduction of brain volume in a very large test population might indicate that brain volume is only weakly associated with ADHD. Alternatively, it might be that within–group heterogeneity of the ADHD and control group blurs ADHD-control differences at the group-level.

There are approaches to study subgroups of ADHD. With additional analysis as for instance latent class analysis we might be able to stratify ADHD with respect to which specific symptoms are associated with impairments on reward-related neural processes (Bureau et al. 2011). Alternatively, Fair et al demonstrated that both healthy and ADHD participants are quite heterogeneous groups and can better be characterized based on their neurocognitive profiles (Fair et al. 2012). Accordingly, the NeuroIMAGE data could be used to derive neural and cognitive measures of different functional systems and form groups based on neural or cognitive profiles. Apart from that, multivariate pattern analysis (MVPA) could be used to detect algorithm-based neural activity that help to classify participants as being either participants or healthy controls (Haynes and Rees 2006).

Conclusions:

- In participants with ADHD, reward processing regions in the striatum, including VS, and OFC are overly sensitive during reward anticipation and receipt. (Chapter 3)
- This hypersensitivity appears to be partly subjected to familial-genetic factors, which hints at a genetic underpinning of this alteration. (Chapter 3)
- In participants with ADHD, the executive control and salience network have altered connectivity with the cerebellum, frontal cortex and temporal cortex. (Chapter 4)
- The general, whole-brain functional architecture of regions within the striatum is unaltered in ADHD and cannot account for the reported task-related hypersensitivity to reward. (Chapter 5)
- Both ADHD and ASD have syndrome specific neural underpinnings of reward processing. (Chapter 6)

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Nederlandse samenvatting (Dutch summary)

ADHD is de afkorting voor de Engelse term Attention-Deficit-Hyperactivity Disorder en wordt in het Nederlands ook wel aandachtstekort-hyperactiviteitsstoornis genoemd. Het is een vaak voorkomende psychiatrische aandoening waarbij patienten in dusdanige mate moeite hebben met concentreren, overmatig beweeglijk en/of heel erg impulsief zijn, dat er sprake is van disfunctioneren in het dagelijks leven. Oorspronkelijk werd deze aandoening alleen bij kinderen vastgesteld, maar tegenwoordig wordt het ook vaak bij volwassenen gediagnosticeerd.

Erfelijke factoren lijken een belangrijke rol te spelen in de ontwikkeling van ADHD. Welke genen hierbij precies een rol spelen en hoe ze samenwerken met zogenaamde omgevingsfactoren, is tot op heden helaas maar in heel beperkte mate bekend.

Naast erfelijke en genetische factoren vermoedt men dat ook afwijkingen in het cognitief functioneren een verklaring bieden voor de uiteindelijke ontwikkeling van ADHD. Door meer inzicht te verkrijgen in de verschillen in cognitieve functies, bijvoorbeeld door het bestuderen van de hiermee geassocieerde neurale processen in de hersenen van patienten met ADHD, hopen we deze stoornis en haar beloop beter te begrijpen, met als ultieme doel een betere behandeling te kunnen bieden.

Doel van dit onderzoek

In dit proefschrift wordt het verwerken van beloningsinformatie bij patienten met ADHD onderzocht. Verwerking van beloningsinformatie verwijst naar de neurocognitieve processen van ons gedrag waarbij de waardering van ons handelen (kosten/baten) gerepresenteerd zijn in de hersenen en uiteindelijk leiden tot gedragsaanpassingen bij het individu. Uit de literatuur blijkt dat patiënten met ADHD beloning anders waarderen dan gezonde personen in vergelijkbare populaties. Ook lijkt het dat hersengebieden, bijvoorbeeld het ventrale striatum en de prefrontale cortex, die hierbij een belangrijke rol spelen verschillend functioneren.

In de in dit proefschrift gepresenteerde onderzoeken bekijk ik in een grootschalige populatie van jonge patiënten met ADHD specifiek deze neurocognitieve processen met behulp van zogenaamde fMRI scans. Op basis van verschillende hersenmaten (o.a. de hersenactiviteit die zichtbaar is tijdens de verwachting en het verkrijgen van beloning) probeer ik uitspraken te doen over mogelijke neurale mechanismen die geassocieerd zijn met ADHD.

Samenvatting per hoofdstuk

In **hoofdstuk 2** beschrijf ik de methoden en daarnaast de populatie die ik getest heb en waarop alle analyses van de volgende hoofdstukken zijn gebaseerd. De populatie be-

stond uit het NeuroIMAGE cohort en was een follow-up van een eerdere internationale studie over de erfelijkheid van ADHD. Het complete onderzoekscohort omvatte 331 families, waarvan op zijn minst 1 kind met de diagnose ADHD en een of meerdere broers en/of zussen, en 153 controle families. Alle families doorliepen een uitgebreide reeks aan onderzoeken waarbij phenotypische (diagnostische), neurocognitieve en genetische data verzameld werd. Naast de kansen en voordelen van dit grootschalig onderzoek belicht dit hoofdstuk ook de haken en ogen van een dergelijk onderzoek dat op meerdere onderzoekslokaties uitgevoerd wordt en biedt het handvaten voor de correcte analyse voor zulke data.

In **hoofdstuk 3** beschrijf ik de neurale processen tijdens het verwachten en het verkrijgen van beloning in de vorm van geld. Ik heb deze processen bij jonge patiënten met ADHD, onaangedane broers en zussen van de patiënten en bij gezonde controles bekeken. Het blijkt dat patiënten met ADHD in het algemeen verhoogde hersenactiviteit laten zien in de gebieden die belangrijk zijn voor de verwerking van beloninginformatie, onder andere het ventrale striatum, de prefrontale cortex en de anterior cingulate, ten aanzien van gezonde proefpersonen. Er bleek een effect tijdens beide processen te zijn, echter in verschillende hersengebieden. Onaangedane, dus symptoomvrije broers en zussen lieten in een aantal van deze gebieden dezelfde afwijkingen zien, in andere gebieden leken hun hersenen op die van de gezonde proefpersonen zonder ADHD in het gezin. Uit deze bevindingen kunnen we twee conclusies trekken: Allereerst lijken de hersenen van patienten met ADHD overgevoelig voor beloning. Ten tweede lijkt deze overgevoeligheid ook aanwezig te zijn in bij de onaangedane broers en zussen, hetgeen duidt op een mogelijke genetische causale factor.

Tot slot viel op dat ADHD patiënten voornamelijk slecht presteerden tijdens de onderzoeken op momenten dat er geen geld te winnen viel, hetgeen er mogelijk op wijst dat ADHD patiënten beloning nodig hebben om goed (normaal) te kunnen functioneren.

Hoofdstuk 4 is een methodologisch geavanceerde uitbreiding van de analyses van hoofdstuk 3. Hier heb ik geprobeerd op een hypothese-vrije manier beloningsverwerkende hersenstructuren in onafhankelijke componenten onder te verdelen. Doel was om erachter te komen welke grotere neurale netwerken betrokken zijn bij het verwerken van beloning, met welke andere gebieden in het brein deze netwerken communiceren en hoe deze netwerken onderling communiceren. Deze neurale netwerken heb ik vervolgens onderzocht op een verband met ADHD. De resultaten tonen het volgende: er zijn vier grotere neurale netwerken betrokken bij het verwerken van beloning: het gedrags-controle, het motivationele, het laterale visuele en het default-mode netwerk. In patiënten met ADHD lieten twee van deze netwerken veranderde connectiviteit zien met andere hersenengebieden. Dit duidt erop dat niet alleen de neurale netwerken die geassocieerd zijn met het verwerken van beloning in ADHD zelf aangetast zijn, maar ook de communicatie van deze gebieden met andere domeinen in het brein.

In **hoofdstuk 5** heb ik naar de functionele architectuur van de hersenen gekeken. Aan de hand van natuurlijke bloedfluctuaties in rust is het mogelijk om te bepalen welke gebieden tegelijk actief worden en netwerken vormen. Dit heb ik toegepast om te kijken hoe de subcorticale kernen in de basale ganglia met andere gebieden verbonden zijn, bijvoorbeeld het ventrale striatum met de frontale cortex. Daarnaast heb ik naar de communicatie van de kernen onderling gekeken. Beide maten heb ik vervolgens in ADHD patiënten en gezonde proefpersonen onderzocht. Hieruit blijkt dat voornamelijk de communicatie tussen de kernen sterker is in ADHD, de grotere functionele netwerken blijken hetzelfde verbonden in beide groepen.

In **hoofdstuk 6** heb ik geprobeerd het probleem van de fenotypische heterogeniteit (de uitgebreide comorbiditeit) binnen de ADHD populatie aan te gaan door ADHD symptomen te vergelijken met autistische symptomen. Specifiek heb ik naar het unieke verband tussen hersenenactiviteit tijdens het verwerken van beloningsinformatie gekeken. Dit heb ik op een dimensionele manier gedaan, dus alleen kijkend hoeveel symptomen iemand vertoont zonder onderscheid te maken tussen het wel of niet hebben van een diagnose van ADHD/ autisme. De resultaten laten zien dat beide soorten ziektegerelateerde symptomen geassocieerd zijn met typerende, niet overlappende afwijkingen in hersenenactiviteit.

Conclusies

Deze dissertatie bevat verschillende bevindingen over de verwerking van beloningsinformatie bij jonge patiënten met ADHD. Een van de kernconclusies is dat bij patiënten met ADHD hersenengebieden die betrokken zijn bij het verwerken van beloning hypersensitief reageren. Het feit dat we deze overgevoeligheid ook in niet aangetaste biologische verwanten van de patiënten zien, wijst erop dat deze neurale verandering genetisch bepaald is. Veranderingen van het beloningssysteem zijn ook op netwerkniveau te zien. De aandachtscontrole (executive control) en motivationele (salience) netwerken van patiënten met ADHD tonen veranderingen in de verbindingen met andere corticale en subcorticale gebieden. De functionele architectuur van het beloningssysteem is niet veranderd. In plaats daarvan zien we dat de communicatie tussen functionele netwerken lokaal wel veranderd is. Tenslotte blijkt dat ADHD en autisme specifieke neurale afwijkingen vertonen op populatieniveau (in de parahippocampale cortex bij ADHD en de insula cortex bij autisme).

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Mijn dank gaat uiteraard ook uit naar alle dataverzamelaars van het NeuroIMAGE team. Zonder onze gezamenlijke inspanningen was het nooit gelukt een zo groot aantal gezinnen te testen. Weekend of geen weekend, de MRI draaide door. In het bijzonder wil ik jou bedanken Andrieke, voor het samen met mij managen van de site Nijmegen. Maar natuurlijk ook de anderen: Jennifer, Winke, Marianne, Marloes, Hanneke, Annabeth en Siri.

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De onderzoekers van het UMC. Jullie waren een heel gezellige groep, een geweldige laagdrempelige omgeving voor het discussiëren van studies met een klinisch perspectief . De retreat in Kranenburg was echt geweldig (Marloes, Desiree, Boudewijn, Jana).

De TG bestaande uit Marek, Hong, Rene, Erik, Bram (leren programmeren was geweldig). Paul, in de drukste tijd van meten voelde ik me helemaal thuis bij jou in de kelder. En natuurlijk bedankt voor je support tijdens mijn zo hoognodige vakantie. Zonder jou was ik waarschijnlijk met een burn-out uitgevallen.

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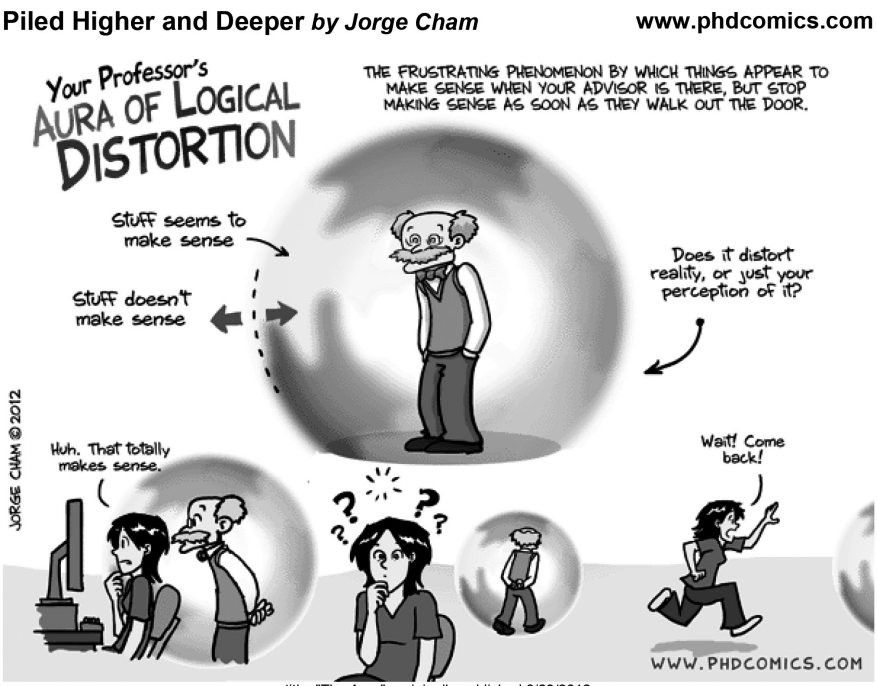
En dan zijn er nog onmisbare vrienden die mij gesteund hebben tijdens deze (str)tijd, van veraf en dichtbij: Christian en Christian, Roos en Hugo, de 'gezellige mensen' groep, Matthias, Tilman, Daniel. Dank voor al jullie begrip, luisterend oor en hulp waar nodig. Hopelijk komen er nu weer tijden aan met wat meer vrije tijd!

Daan, je bent terecht verbaasd als je je naam eerder verwacht hebt. Jij hoort bij bijna elke groep genoemd te worden: coauteur, dataverzamelaar, PhD binnen NeuroIMAGE en de groep van Roshan en het UMC, reviewer van mijn proefschrift en bovenal steunende vriend. Aan jou heb ik tijdens mijn promotie echt super veel gehad en ik ben heel blij je te hebben leren kennen!

Ik eindig mijn dankwoord met Maud, mijn geliefde vrouw en moeder van mijn twee kinderen. Er is een gezegde dat achter elke succesvolle man een sterke vrouw staat. Als deze zin op iemand van toepassing is dan op jou...



** De lach van mijn dag, Jan deze ging over ons...*

Curriculum vitae

Daniel von Rhein was born in Herten, Germany, on the 25th of May 1980. After finishing secondary school at the Heinrich-Heine-Gymnasium in Bottrop, he moved to Nijmegen to study psychology at the Radboud University Nijmegen. During this time, he developed a strong interest in neural processes that underlie human behavior. Accordingly, he followed the Cognitive Psychology Master's program and finished this track in 2008 with an internship at the Nijmegen Institute of Cognitive Information (NICI). Subject of his thesis was the neural mechanisms of error processing.

After gaining clinical experience with psychiatric populations in 2008/2009, he started his PhD project at the department of Psychiatry of the Radboud University Medical Center and Donders Center for Cognitive Neuroimaging. Together with other colleagues of the NeuroIMAGE project, he created one of the biggest neuroimaging databases in the field of ADHD that also contains familial, molecular genetic, and cognitive data. He is currently an employee of the Max-Planck institute in Nijmegen.

List of publications

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von Rhein, D.*, Oldehinkel, M.*, Beckmann, C., Oosterlaan, J., Heslenfeld, D., Hartman, C., Hoekstra, P., Franke, B., Cools, R., Buitelaar, J., Mennes, M. (submitted). Aberrant Local Striatal Functional Connectivity in Attention-Deficit/ Hyperactivity Disorder.

Sokolova, E., Groot, P., Claassen, T., **von Rhein, D.**, Buitelaar, J., Heskes, T. (submitted). Handling hybrid and missing data in constraint-based causal discovery to study the etiology of ADHD.

Richards, J., Arias Vásquez, A, **von Rhein, D**., Franke, B., Heslenfeld, D., Oosterlaan, J., Faraone, S., Buitelaar, J., Hartman, C. (submitted). Adolescent Behavioral and Neural Reward Sensitivity: A Test of the Differential Susceptibility Theory.

Schweren, L., Groenman, A., **von Rhein, D**., Weeda, W., Heslenfeld, D., Franke, B., Buitelaar, J., Oosterlaan, J., Hoekstra, P., Hartman, C. (submitted). Early and high-dose stimulant treatment of attention-deficit hyperactivity disorder is associated with altered neural reward responsiveness.

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