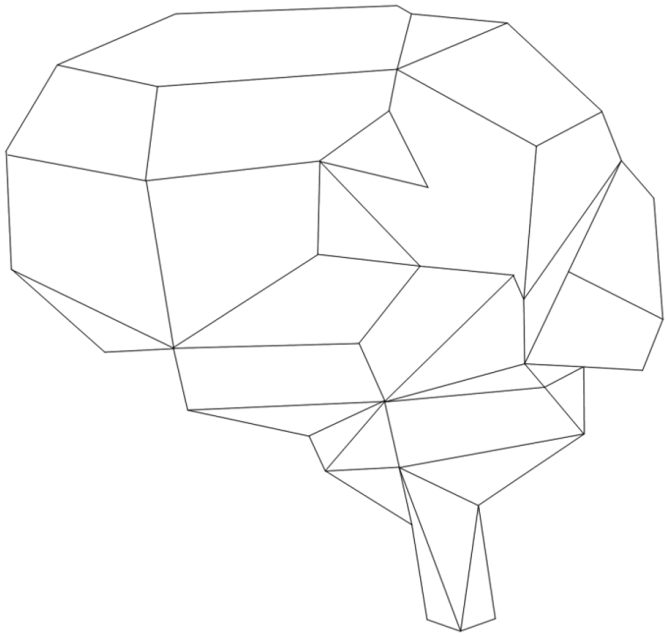
Stimulants and the developing brain

Schweren, Lizanne Johanna Stephanie

Chapter 10

GENERAL DISCUSSION



This thesis had two overall aims. Our first and foremost aim was to describe long-term effects, or the absence thereof, of stimulant treatment on the developing brain in children, adolescents and young adults with ADHD. Our second aim was to advance our knowledge about mechanisms underlying long-term effects of stimulant treatment in the brain. To these ends, we investigated associations between stimulant treatment history and various neural outcomes in a large cross-sectional ADHD sample (NeuroIMAGE, see page 17 Box 1), with special focus on the frontal-striatal system and on non-volumetric brain parameters. Furthermore, we evaluated differences in patient characteristics and exposure patterns that may influence long-term effects, with at its extreme the investigation of an adolescent recreational stimulant user group without ADHD (Youth At Risk, see page 17 Box 2).

In this final chapter, I first provide a summary of the findings in each of the chapters. Next, I describe how these findings relate to each other and to the existing literature, and how they provide answers to our two main research questions. After that, I discuss methodological strengths and limitations of our approach, potential clinical implications of our findings, and suggest directions for future studies.

SUMMARY PER CHAPTER

At the start of this thesis, in *chapter 2*, we reviewed the MRI literature regarding short- and long-term effects of stimulant treatment on brain structure and function. Evidence from structural MRI studies published up to 2011 suggested that those with ADHD who received long-term stimulant treatment had structural white matter, anterior cingulate cortex, thalamus, and cerebellum indices in that were less different or not different at all from those of healthy controls compared to children with ADHD that were untreated. Moreover, preliminary evidence suggests that methylphenidate treatment may normalize the trajectory of cortical development in ADHD. Similarly, functional MRI studies had shown that case-control differences in brain activation patterns during cognitive control, attention, and rest were attenuated after a single dose of methylphenidate, but evidence of long-term effects on these activation patterns was missing. The effects of methylphenidate on the brain appeared highly specific and dependent on numerous factors, including biological factors (e.g., age), and the environment (e.g., task difficulty).

In *chapter 3*, we investigated long-term effects of stimulant treatment on cortical thickness. ADHD had previously been associated with widespread changes in cortical thickness, and these changes had been suggested to be reduced or even disappear at older age, but also after stimulant treatment. In our sample, we found that medial temporal cortical thickness in both hemispheres was reduced in adolescents/young adults with ADHD compared to their typically developing peers.

These differences were associated with symptoms of hyperactivity and prosocial behavior. However, they were independent of stimulant treatment history. In fact, stimulant treatment was not associated with cortical thickness in any brain region. Furthermore, thinner medial temporal cortex was present throughout adolescence and young adulthood, and age-related changes in cortical thickness were the same for individuals with and without ADHD across the cortical mantle. Thus, we found no evidence of cortical normalization either with stimulant treatment or at older age.

In the next chapter, *chapter 4*, we used diffusion tensor imaging (DTI) to study white matter structural connectivity. Previous DTI studies had revealed subtle abnormalities in white matter connectivity in individuals with ADHD, and stimulant treatment may in the long term reduce such abnormalities. We investigated associations between treatment history and white matter connectivity within five dopaminergic circuits (orbitofrontal-striatal, orbitofrontal-amygdalar, amygdalar-striatal, dorsolateral-prefrontal-striatal and medial-prefrontal-striatal). Individuals with ADHD presented with reduced fractional anisotropy in the orbitofrontal-striatal pathway, indicative of impaired structural connectivity. In the same pathway, higher lifetime stimulant dose was associated with lower diffusivity, which may be indicative of improved structural connectivity at higher treatment levels.

Next, in *chapter 5*, we evaluated associations between stimulant treatment history and brain activation patterns during performance of a monetary incentive delay task, known to activate the striatum by eliciting reward-related dopaminergic neurotransmission. One previous study had reported indications of normalized striatal activation patterns in adult ADHD patients with a history of stimulant treatment. Here we found that, even though participants were in their non-medicated state during scanning, stimulant treatment history predicted brain activation patterns during reward processing. Individuals who had initiated treatment early and had received a relatively high dose showed more activation in the supplementary motor area and dorsal anterior cingulate cortex, compared to individuals with later onset and lower dose treatment. These changes may indicate compensatory recruitment of brain regions for higher-order integration of valence information in the intensely treated group. Contrary to our hypothesis, treatment history was not associated with (more normative) striatal activation patterns.

In *chapter 6*, we investigated long-term effects of treatment on striatum, hippocampus, and frontal cortex volumes, and how these may be moderated by age and/or by two common variants of dopaminergic genes (*DAT1*, encoding the presynaptic dopamine transporter; and *DRD4*, encoding the postsynaptic dopamine D4 receptor). Previous animal studies had shown exacerbated effects of methylphenidate (therapeutic dose) on brain structure when treatment was administered at younger age. Here, we found that striatum volume was not associated

with stimulant treatment history. In the frontal cortex and left hippocampus, however, *DRD4* genotype and age predicted the effects of stimulant treatment. At younger age and lower treatment-levels, but not at younger age and higher treatment levels, carriers of the *DRD4* 7R-allele showed decreased frontal cortex volumes compared to controls. At older age, individuals with ADHD showed lower frontal volumes irrespective of genotype or treatment history. Left hippocampal volume was similar to controls at average treatment levels, and increased with treatment only in carriers of the *DRD4* 7R-allele and at younger age. These findings may suggest that carriers of the *DRD4* 7R-allele may at younger age be sensitive to cortical remodeling after stimulant treatment.

Chapter 7 addressed combined stimulant and antipsychotic treatment, an off-label yet rather common augmentation strategy for the treatment of behavioral problems in ADHD. In animals, administration of antipsychotics had been shown to counteract the acute effects of stimulants in the striatum. Here, we compared volumes of brain regions involved in dopaminergic circuits between patients who had received combined treatment, patients who had received stimulants but not antipsychotics, and healthy controls. Patients in the combined treatment group, but not those in the stimulant-only group, showed a reduction in total cortical volume and frontal cortex volume compared to healthy controls. Further, the combined treatment group, but not the stimulant-only group, showed volume reduction in bilateral subthalamic nuclei and left thalamus. Structural abnormalities in the combined treatment group may have existed prior to treatment, perhaps in relation to behavioral problems or ADHD severity. However, our findings could also indicate that combined stimulant and antipsychotic treatment may result in volume reductions in the developing brain.

In *chapter 8* we took a sidestep and investigated the effects of non-medical, recreational use of psychostimulants such as amphetamines or cocaine. Recreational stimulant exposure patterns are very different from typical ADHD treatment patterns. The neural consequences of recreational stimulant use had previously been studied in healthy adults, but not in adolescents. In the prospective, repeatedly scanned Youth At Risk sample, we evaluated the effect of incidental, high-dose stimulant exposure on the development of hippocampus structure and memory performance. We found that learning/memory test performance changed differently over time for stimulant-exposed individuals compared to non-exposed individuals. Whereas nonusers (fully abstinent) and non-stimulant users (e.g., marijuana, but not stimulants) showed an upward trend in test performance over time which may reflect subtle learning effects, stimulant-users showed a downward trend. No changes in hippocampal volume, microstructure, or surface morphology were found in the stimulant-exposed group compared to both non-exposed groups.

Finally, in *chapter 9*, we addressed long-term effects of stimulant treatment in terms of clinical or behavioral outcomes longitudinally. In previous studies, adequately investigating such effects spanning multiple years had proven challenging, and findings had suggested clinical improvement but also worsening of symptoms after long-term treatment. Here, we compared the development of ADHD symptoms, social-emotional functioning, and test performance in three cognitive domains (all while participants were in their non-medicated state), between stimulant-treated and not stimulant-treated individuals with ADHD that were closely matched on baseline characteristics. All but two outcome measures (emotional problems and prosocial behavior) improved over the follow-up period of approximately six years. However, for all outcomes, improvement was the same for participants who had received treatment and those who had not, suggesting no long-term treatment effects.

WHAT ARE THE LONG-TERM EFFECTS OF STIMULANT TREATMENT ON THE DEVELOPING BRAIN?

Neurotoxicity and harmful effects

Concerns that stimulant treatment may adversely affect brain development are frequently voiced, especially in popular media that seem eager to emphasize similarities between ADHD medication and for instance hard drugs such as cocaine. Evidence for stimulant-induced damage to dopaminergic nerve terminals stems almost exclusively from animal studies, in which stimulants are typically administered in binge-like patterns at pharmacologically high dosages (Berman et al., 2009). However, two positron emission tomography (PET) radiotracer studies, allowing in vivo assessment of dopamine metabolism in humans, have also suggested long-term adverse effects of stimulant treatment in patients with ADHD. (Wang et al., 2013; Ludolph et al., 2008). MRI outcomes may indirectly reflect (correlates of) dopaminergic neurotransmission, but are far more distal. To date, MRI studies have reported no evidence of lasting detrimental treatment effects on basal ganglia volumes (e.g., Shaw et al., 2014; Semrud-Clikeman et al., 2006), cortical thickness (e.g., Hoekzema et al., 2012; Shaw et al., 2013), or brain activation patterns (e.g., Pliszka et al., 2006; Schlochtermeier et al., 2011; Stoy et al., 2011). If anything, MRI findings to date more likely reflect neutral or even beneficial treatment effects, which will be discussed in the next section.

In line with the existing MRI literature, our findings do not provide support for stimulant treatment negatively affecting brain development in children, adolescents, and young adults with ADHD. We found no indications that the development of cortical thickness may be impacted by stimulant treatment (*chapter 3*). Furthermore, stimulant treatment was not associated with alterations in striatal

volumes, nor with abnormal striatal activation patterns during reward (*chapter 5 and 6*). We also found no indication of detrimental changes in frontal-striatal white matter structural connectivity after stimulant treatment (*chapter 4*). Finally, there was no indication that stimulant treatment resulted in an exacerbation of ADHD symptoms when medication was (temporarily) discontinued, as had previously been suggested (Wang et al., 2013), nor did long-term stimulant treatment result in a decline in social-emotional or neuropsychological functioning (*chapter 9*).

Only in one instance did we find less normative brain structure in ADHD at higher treatment levels. In a specific subgroup of patients, namely those of young age and carrying the *DRD4* 7R-allele, hippocampus volume increased with more stimulant exposure, deviating away from control levels (*chapter 6*). Larger volume of the hippocampus, a medial temporal lobe structure critically implicated in memory and learning, is not typically associated with negative outcomes. For example, memory impairments in severely addicted stimulant abusers are associated with hippocampal volume reduction/atrophy rather than with increased volumes (Berman et al., 2008). The dose-response curve, with on its horizontal axis 'stimulant dose' and on its vertical axis 'hippocampus volume', could in theory follow an inverted U-shape, such that a lower/clinical dose results in enlarged hippocampi while a higher/abusive dose results in hippocampal volume loss. A similar inverted U-shape curve has previously been proposed to describe acute stimulant effects on cognition and brain activation patterns (Swanson et al., 2011). Acute stimulant-induced improvement of declarative memory has been reported in patients with ADHD (Verster et al., 2010), as have hippocampal volume reductions in adults with a history of childhood stimulant treatment compared to those without (Onnink et al., 2014; Frodl et al., 2010). ADHD is not typically associated with declarative memory problems, and perhaps for that reason declarative memory performance was not among the cognitive domains assessed in our sample. Previous findings, in conjunction with our findings of subtle decline in memory performance after recreational (typically high-dose) stimulant exposure (*chapter 8*) emphasize the need to further address potential lasting effects of stimulant treatment on the hippocampus and memory performance. At this point, we cannot exclude the possibility that enlarged hippocampi in young *DRD4* 7R-carriers could reflect damage to dopaminergic nerve terminals even at clinical dose.

In short, we detected no harmful long-term effects of stimulant treatment on frontal-striatal grey or white matter structure, cortical thickness, reward-related brain activation patterns, nor on any clinical, behavioral, or cognitive outcomes. In a subgroup of patients, high-dose stimulant treatment was associated with enlarged hippocampus volume, but it is unclear whether such changes should be interpreted as beneficial or harmful, and whether they have functional/clinical implications.

Normalization and beneficial effects

Contrary to the publicly endorsed concerns about harmful effects, the scientific community has in recent years reported mostly in favor of beneficial effects of long-term treatment on the brain. More specifically, it has been suggested that stimulant treatment may reduce or normalize brain changes typically associated with ADHD. Previous MRI studies have indicated normalizing treatment effects on structural changes in for instance the basal ganglia (Frodl & Skokauskas, 2012; Nakao et al., 2011), lateral prefrontal cortex (Shaw et al., 2009), and anterior cingulate cortex (Semrud-Clikeman et al., 2006), although there have also been negative findings (e.g., Onnink et al., 2014; Semrud-Clikeman et al., 2014; Shaw et al., 2014). Sustainable neural modifications may result from repeated modulation of monoaminergic neurotransmission, or from repeated manifestation of age-appropriate behaviors (i.e., paying attention in class, making non-impulsive decisions) increasing the strength of neural networks underlying these behaviors (Kasparek et al., 2015).

One chapter in this thesis provides partial support for normalizing effects of stimulant treatment. In chapter 5, we found that frontal cortex volume was reduced in individuals with ADHD compared to their typically developing peers, and that in young patients carrying the *DRD4* 7R-allele, higher levels of treatment were associated with increased frontal cortex volume. This may suggest treatment-induced normalization of frontal cortex volumes in a specific subgroup of patients. Much more frequently, however, we concluded that normalization with stimulant treatment did not occur. We found decreased medial temporal cortical thickness (*chapter 3*), and lower frontal-striatal fractional anisotropy (*chapter 4*), in both stimulant-treated and stimulant-naive participants. Striatal activation patterns during reward processing, that had previously been found to be altered in our ADHD sample, were not associated with treatment history either (*chapter 5*). Finally, although clinical and cognitive outcomes improved over time towards more normative levels, these improvements occurred in treated and non-treated individuals alike (*chapter 9*).

Rather than neural normalization, however, beneficial treatment effects may occur through compensatory strategies or processes. We found two indications of long-term beneficial brain changes in stimulant-treated patients with ADHD as compared to their typically developing peers, that were not also present at lower treatment levels or in stimulant-naive patients. First, during reward outcome, individuals with a history of early-onset and high-dose stimulant treatment, but not those with late-onset and low-dose treatment, showed higher activation in brain regions of cognitive control (*chapter 5*). Increased cognitive control is likely beneficial to individuals with ADHD who tend to respond impulsively to reward, and may

compensate for deficient striatal activation patterns that were not normalized by stimulant treatment. Second, patients with ADHD presented with compromised structural connectivity in the orbitofrontal-striatal pathway. In that same pathway, a negative association between cumulative stimulant dose and mean diffusivity indicated improved white matter structural connectivity at higher treatment levels, albeit through a different mechanism (mean diffusivity rather than fractional anisotropy; *chapter 4*). Potential mechanisms underlying such lasting treatment effects are further discussed below (see *What mechanisms underlie effects of long-term stimulant treatment in the brain?)*

In short, we found little evidence that stimulant treatment may reduce or normalize brain changes typically associated with ADHD, except in the frontal cortex where treatment may normalize volume reductions in a specific subgroup of patients. However, beneficial treatment effects may have occurred through compensatory mechanisms without affecting/normalizing the initial deficit.

The role of stimulant exposure patterns

The distinction between previously treated and stimulant-naive patients with ADHD is practical and often used, yet it may be an overly simplistic representation of complex stimulant treatment trajectories. Throughout this thesis, we went beyond this distinction and investigated whether different exposure patterns (e.g., younger or older age of treatment initiation, higher or lower daily dose, shorter or longer treatment duration, etc.) were associated with different long-term outcomes, as had been suggested in animal studies (e.g., van der Marel et al., 2014 and 2015). The effects of incidental, high-dose recreational stimulant exposure could be informative in this regard as well.

For the studies in this thesis, detailed pharmacy transcripts provided detailed information regarding stimulant exposure patterns, such as age of treatment, dose, continuity, etc. Disentangling how these various exposure parameters contributed to stimulant-induced brain changes in their unique and shared ways was challenging (see *methodological considerations*). All in all, when effects of treatment were found, they could typically not be attributed to a single treatment parameter. In *chapter 6*, where we reported a combined effect of age, genotype, and treatment on frontal cortex volumes, the treatment component in the model could be represented by either cumulative dose or treatment duration. For a similar combined effect in the hippocampus, the treatment component could be represented by cumulative dose, treatment duration, or age of treatment onset. It thus seems that the effects of these treatment indicators are shared rather than unique. Daily dose and recency of treatment appear to be of less importance.

Another indication that the different exposure parameters may have shared rather than unique effects was provided in *chapter 5*, where we performed a multivariate data-driven classification of stimulant-treated participants based on their lifetime exposure trajectories. In the optimal solution, the vast majority of stimulant-treated participants was grouped in one of only two classes. Over 90% of stimulant-treated participants could best be classified as either a) early treatment onset, long treatment duration, and high maximum and total dose, or b) late treatment onset, short treatment duration, and low maximum and total dose. Other exposure patterns (e.g., late onset and high dose) were highly uncommon. Thus, although we were able to compute various detailed exposure parameters from the pharmacy transcripts, within patients with ADHD these parameters were very highly correlated which hampered meaningful distinction between their potentially unique effects in the brain. Furthermore, although the distinction between stimulant-treated and stimulant-naive patients may indeed be overly simplistic, virtually all stimulant-users could be classified as either moderate-users or intense-users, indicating that differentiation between stimulant-users is not necessarily very complex either. It is important that the prevalence and relevance of different exposure patterns are replicated in independent samples, preferably in samples based in different health care realities.

Finally, there was the group of adolescent recreational stimulant users, who typically consumed stimulants in a binge-like pattern (incidental, high-dose exposure to cocaine or MDMA). Note that we did not directly compare stimulant exposure effects in recreational users to those in patients with ADHD. However, the effect of stimulant exposure on hippocampus volume was evaluated in both groups, and in both groups we found no exposure effect. High-dose exposure did negatively affect declarative memory performance in the recreational users group. Unfortunately, declarative memory performance was not tested in the ADHD sample with low-dose exposure, hence we are unable to conclude whether subtle memory changes occur specifically after high-dose exposure patterns.

Conclusion

Taken together, we may reasonably conclude that long-term effects of stimulant treatment on the developing brain in ADHD are subtle. Findings of long-term treatment effects were outnumbered by findings of no effects of treatment. We found no indications of harmful long-term effects of stimulants on brain structure or function, although our findings of hippocampal changes do ask for further investigation. At the same time, we also found little evidence that brain changes typically associated with ADHD are reduced or even disappeared after stimulant

treatment, except in the frontal cortex where treatment may normalize volume reductions in a younger patients carrying the *DRD4* 7R-allele. In two instances, however, we found that beneficial effects of long-term treatment may have occurred through compensatory mechanisms. Importantly, even if stimulant treatment indeed caused either beneficial or harmful brain changes, such changes were not accompanied by better or worse outcomes in terms of ADHD symptoms, social-emotional functioning, or cognitive performance. In terms of exposure patterns, it appears that treatment duration, cumulative dose, and age of treatment onset have shared rather than unique contributions to long-term brain changes.

WHAT MECHANISMS UNDERLIE EFFECTS OF LONG-TERM STIMULANT TREATMENT IN THE BRAIN?

The second aim of this thesis was to advance our understanding of how long-term effects of stimulant treatment in the developing brain come about. Long-term effects may be caused by the same mechanism that also underlies acute stimulant effects in the brain (i.e., blockade of dopaminergic autoreceptors in the striatum), but we propose that long-term effects of treatment are likely the result of a different mechanism, on two grounds. First, previous PET studies have repeatedly shown that acute stimulant effects are localized within the striatum (e.g., Cherkasova et al., 2014), whereas here we found no evidence of treatment-induced changes in striatal volume (*chapter 6*) or activation during reward (*chapter 5*). Rather, effects of long-term treatment were localized in the frontal cortex, hippocampus, and supplementary motor area/dorsal anterior cingulate cortex. Second, if acute and long-term effects would reflect the same mechanism, then a genetic variant predisposing acute stimulant effects (*DAT1*; Aarts et al., 2014, Kasparbauer et al., 2015) would be expected to predict the occurrence of long-term changes as well. However, in our sample, *DAT1* genotype was not predictive of long-term changes in striatum volume (*chapter 6*), nor in frontal cortex or hippocampus volume (additional analyses, not shown).

If different from the mechanism underlying acute stimulant effects, what could the process causing long-term brain changes entail? Our findings of cortical remodeling after stimulant treatment in younger patients carrying the *DRD4* 7R-allele may be especially informative in this regard. The *DRD4* gene encodes postsynaptic dopamine D4 receptors, that play an important role in maintaining tonic dopamine levels and are crucial for the occurrence of neural plasticity (Asghari et al., 1995; Goto et al., 2010; Padmanabhan & Luna, 2013). Thus, the frontal cortex of young *DRD4* 7R-carriers may exhibit postsynaptic characteristics allowing for long-term neural plasticity, i.e., structural adaptation of the brain, in the event of exposure to stimulants. Interestingly, *DRD4* 7R-carriership has previously been associated with

better clinical outcome in older adolescents with ADHD of whom at least 78% had received stimulant treatment at some point in life (Shaw et al., 2007b). Moreover, *DRD4* genotype and social environment together, but not *DRD4* genotype alone, predicted prefrontal cortex activation during response inhibition (Richards et al., 2016). This may suggest a more general mechanism of *DRD4* 7R-carriers being more sensitive to lasting brain changes when circumstances (such as stimulant treatment and/or a positive environment) are optimal. I do wish to emphasize that our findings of age- and genotype specific treatment effects await replication in an independent sample. Moreover, and more generally, our thoughts about underlying mechanisms remain highly speculative at this point, and require further investigation using other modalities such as PET or SPECT.

METHODOLOGICAL CONSIDERATIONS

Treatment effects in an observational study design

Any investigation of treatment or exposure effects spanning multiple years, including the current investigation, is inevitably naturalistic by design. An observational study design comes with limitations as well as opportunities. Associations between treatment and outcomes that are identified through observation may represent true effects of treatment, but could also reflect bias and/or confounding (Jepsen et al., 2004). We aimed to reduce information bias, occurring in observational studies when treatment itself is associated with the accuracy of reported outcomes (e.g., parent-rated ADHD symptom scores while off-medication may be less accurate after the child has consistently been using medication for some time), by the use of multiple informants. Furthermore, we attempted to statistically minimize the influence of confounding factors throughout this thesis, e.g., through matching and covariate adjustment. Nevertheless, bias and confounding cannot be ruled out. Especially confounding through unmeasured characteristics and confounding-by-indication / susceptibility bias (i.e., when treatment is preferentially prescribed to patients with worse prognosis, due to e.g., pretreatment severity) are difficult to address. Propensity score adjustment, i.e., statistical adjustment for the estimated propensity or likelihood that a given patient will receive treatment, could be valuable in this regard. Unfortunately, propensity estimates could not be calculated based on our data.

Another drawback of observational study designs is their tendency to result in imbalanced exposure groups. Only a handful of participants within our ADHD sample were naïve to stimulant treatment, which is in accordance with high prescription rates in the Netherlands. In contrast, in the Youth At Risk sample,

recreational stimulant users were scarce which is in accordance with the relatively low incidence of recreational stimulant use amongst adolescents. Therefore, in both samples, attempts towards matching were often unsuccessful, or resulted in the exclusion of a substantial number of participants.

Despite these limitations, observational cohort studies also have advantages. Results from observational studies are generally more consistent compared to those from randomized controlled trials, and concerns about susceptibility bias resulting in an overestimation of treatment effects have been invalidated in recent years (Concato et al., 2000; Concato, 2013). Moreover, large-scale observational studies are especially suitable for the study of heterogeneous clinical populations such as patients with ADHD. Not only do such samples enhance generalizability of findings, but they also provide researchers with much-needed statistical power to study within-group variability. For example, the investigation of differential treatment effects at different ages, or at a wide range of doses, could not have been achieved in a randomized controlled trial.

Effects of treatment in a cross-sectional study design

Both the IMAGE-NeuroIMAGE and the Youth At Risk cohort are longitudinal by design. Unfortunately, however, several aspects of the NeuroIMAGE study design prevented optimal longitudinal investigation of stimulant treatment effects on the brain. First, the baseline assessment (IMAGE, approximately six years prior to NeuroIMAGE) did not include an MRI session. Therefore, at the level of brain outcomes, the available data were cross-sectional. Cross-sectional data are far from optimal for the investigation of developmental disorders, and perhaps even more problematic for the investigation of effects of treatment, as there is a substantial risk for confounding (Kraemer et al., 2000). Ideally, brain data is collected prior to initiation of treatment, to allow analysis of (and adjustment for) brain indices that at baseline distinguish between patients who will later receive stimulant treatment and those who will remain untreated. The cross-sectional nature of our study should be kept in mind when interpreting associations with treatment and age. More conclusive interpretation of such effects can only be derived from longitudinal studies. Second, a substantial proportion of participants with ADHD had already received stimulant treatment prior to enrollment in the IMAGE study. Thus, for these participants, clinical baseline measurements did not represent their pre-treatment state, and their pre-treatment status was not assessed. As a result, we were unable to statistically adjust for pre-treatment characteristics such as symptom severity, which would have reduced the risk of susceptibility bias. Note that these two limitations do not apply to the analyses presented in chapter 8, as in the Youth At Risk sample all participants

underwent MRI scanning at baseline when they were drug-naive. Ideally, longitudinal studies with repeated scanning should be prospective, i.e., starting prior to treatment onset.

Fortunately, there is an advantage to the current cross-sectional study as well. The NeuroIMAGE cohort stands out through the inclusion of older adolescents and young adults, which in a longitudinal design starting in childhood would be very time-consuming and prone to dropout. The late adolescent/early adult phase is often characterized by marked clinical changes (e.g., reduction of hyperactivity symptoms) as well as normative developmental changes (e.g., improved executive functioning), yet has received very little attention in previous studies of ADHD. Specifically in terms of effects of treatment, the inclusion of older adolescents and young adults was an asset of our study as it allowed the investigation of stimulant effects occurring only after multiple years of treatment, and/or occurring long after treatment had been discontinued.

The shared and unique effects of different treatment characteristics

Naturally, characteristics of stimulant treatment history are highly correlated amongst each other. For instance, individuals who have started treatment at early age are likely to have longer treatment duration and higher lifetime cumulative dose during adolescence, when these parameters were calculated. Associations between treatment and age add to the complexity, as for example treatment duration but also daily dose tend to increase with age. Finally, treatment characteristics may also interact with each other and with age to affect brain development. For example, as was shown in animal studies (van der Marel et al., 2014 and 2015) and in chapter 6, a certain stimulant dose may cause lasting brain changes when administered at young age but not when administered at older age. Disentangling the influences of individual treatment parameters on brain development is very challenging, if not impossible.

Nevertheless, throughout this thesis, we made several attempts to achieve such differentiation. In most of the empirical chapters, we initially evaluated associations between one encompassing stimulant treatment parameter on the one hand (i.e., age-adjusted cumulative stimulant dose, or CSI_{ADJ}), and brain changes on the other. CSI_{ADJ} represented each of the other treatment indicators at least to some extent, while being independent of age at time of scan. We anticipated that CSI_{ADJ} was informative in itself, but would also serve as a proximate measure of other treatment parameters, which we would attempt to tease apart in second instance. As a benefit to this approach, the alpha level of statistical testing does not require adjustment for multiple comparisons, which is necessary when all treatment indicators would be tested one by one. However, as a disadvantage, those treatment parameters that are

less correlated with CSI_{ADJ} are less likely to have been detected. This shortcoming should be kept in mind when considering the unique and shared effects of stimulant treatment parameters (see *The role of stimulant exposure patterns*). We concluded that, when treatment effects were found, they could typically not be attributed to a single treatment parameter but rather represented an overall effect of cumulative dose, treatment duration, and onset age. Dose and recency of treatment appeared to be of less importance. Note that the lack of findings regarding dose and recency may also be reflective of lower correlations between these treatment parameters and CSI_{ADJ}. Detection of brain changes associated with treatment recency and daily dose may require univariate modeling of these parameters.

CLINICAL IMPLICATIONS

I wish to discuss our findings in terms of potential implications for clinical practice. After all, concerns about long-term consequences of stimulant treatment have dominated the public debate about such medications. Clinicians, parents, and patients, including those who dedicated their time to participate in our study, are longing for information that may aid their decision-making. At the start of this section, however, I do want to emphasize once more that we were able to study *correlates* of long-term stimulant treatment, that may or may not reflect long-term *consequences*. Moreover, be advised that the brain is just one organ. For clinical decision-making, long-term risks and benefits for other organs, bodily functions, and other aspects of well-being should be considered as well.

Concerns that stimulant treatment may adversely affect brain development are frequently voiced. Quite contrarily, it has also been suggested that stimulant treatment may positively affect brain development. All in all, our findings provide little to no support for adverse long-term treatment effects, while they are partially supportive of beneficial effects. At the neural level, potential subtle long-term benefits may include 1) increased activation in cognitive control areas during reward processing; 2) improved orbitofrontal-striatal structural connectivity; and 3) for a subgroup of patients, normalization of frontal cortex volume. Especially for clinicians, however, it is important to realize that even if certain aspects of brain structure or function improve after treatment, these changes may or may not ultimately benefit the child's functioning. In *chapter 9*, we report a striking absence of lasting effects of treatment on ADHD symptoms, social-emotional functioning, and cognitive functioning across various domains. Clinicians may be advised to communicate to parents and patients that stimulant treatment is likely to cause symptomatic relief, but is not expected to cause lasting improvement of ADHD pathology.

Clinicians may be interested to learn which patients benefit most from treatment, and which patients are at highest risk for harmful effects. In our studies, effects of treatment (if any) were the same for boys and girls. We found one indication that young patients possessing the *DRD4* 7R-risk allele may be more sensitive to lasting treatment effects in the brain. However, even within this specific group, there are substantial differences between patients. At this moment, the prediction of lasting effects of treatment for an individual patient based on their age and genotype is far from likely. Clinicians may also be interested to learn what characterizes optimal stimulant treatment. Should treatment be initiated at early age? What is the optimal dose, and when should treatment be discontinued? Overall, when effects of treatment were found, they appeared to be more pronounced when lifetime total dose was higher, treatment duration was longer, and start age was earlier; however, not one of these characteristics could be identified as being the most important.

Finally, I wish to draw the clinician's attention to two preliminary findings in this thesis that, especially if replicated, could have clinical implications. First, our findings suggested that stimulant exposure could, in certain patients and/or at high dose, be associated with changes in hippocampus volume and declarative memory performance. Memory performance is not usually addressed during clinical assessments for ADHD. While awaiting further studies, clinicians should be vigilant of subtle changes in memory performance in stimulant-treated patients, especially those who received a high dose at young age. Second, I wish to address prescription practices regarding combined stimulant and antipsychotic treatment. Combined stimulant and low-dose antipsychotics treatment has been recommended, albeit off-label, for short-term treatment of severe behavioral problems (Kutcher et al., 2004). In our sample, the suggested treatment duration of several weeks was exceeded *en mass*. Our findings indicating brain changes in this specific combined treatment group, suggest that antipsychotic treatment, especially for prolonged time, is at this point not commendable.

FUTURE DIRECTIONS

In this final section, I suggest three major recommendations for future investigations into long-term stimulant treatment effects in ADHD. First, evidently, there is a need for prospective longitudinal neuroimaging data, that includes a baseline assessment prior to treatment onset. In the two largest longitudinal ADHD cohorts to date (NIMH, and NeuroIMAGE) only a minority of participants were naive to stimulants at their initial MRI scan. Brain scans of stimulant-naive patients will provide essential information about non-treated ADHD that is now lacking. As the majority of these initially stimulant-naive patients will at some point commence

treatment, targeted inclusion of additional stimulant-naive participants during the follow-up phase may be needed. I further suggest that prospective longitudinal studies, where possible, employ alternative neuroimaging techniques such as single photon emission computed tomography (SPECT) and pharmacological MRI (phMRI). (Semi-) quantitative methods have previously suggested deleterious long-term effects of treatment on dopamine metabolism (Ludolph et al., 2008; Wang et al., 2013), which may not be measurable using conventional MRI. Commendable efforts have been made by another Netherlands-based research group to employ such alternative neuroimaging methods (e.g., Bottelier et al., 2014; van der Marel et al., 2014 and 2015).

The second recommendation inclines additional effort to investigate functional rather than structural long-term correlates of stimulant treatment. Functional neuroimaging studies on this topic are still sparse, especially compared to the substantial number of structural MRI studies. In this thesis, we found that stimulant treatment history was associated with altered activation patterns in cognitive control areas during reward processing, in the absence of structural brain changes in this area. Changes in neural activation patterns, that last when stimulant treatment is temporarily ceased, may provide an intermediate level between long-term structural brain changes and acute behavioral or clinical treatment effects. Evaluating long-term treatment effects on brain activation patterns would thus provide novel information regarding the long-term risks and benefits of stimulant treatment.

Finally, third, I recommend that future studies specifically address long-term stimulant treatment effects on learning and memory performance, and on hippocampal structure and function. Perhaps as a result of their questionable link with ADHD symptoms, declarative memory and the hippocampus are not typically targeted in the study of ADHD. As an illustration, the Pubmed query 'ADHD declarative memory' yielded only 16 hits; a far cry from the 799 hits for 'ADHD response inhibition'. Subtle changes in declarative memory performance after stimulant treatment, either detrimental or beneficial, could thus easily have gone undetected. By contrast, the hippocampus and learning/memory performance have traditionally been an important focus of (animal) research into stimulant-induced neurotoxicity and addiction. Future studies would benefit from transdisciplinary efforts, in which expertise from the fields of neurotoxicity/addiction and child and adolescent psychiatry are combined.