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Detecting dopamine dysfunction with pharmacological MRI

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Chapter

11

Summary and general discussion

The development of novel neuroimaging methods over the past decades has allowed us to study dopamine function and dysfunction in more detail. This thesis aimed to further characterize the neurobiological origins underlying the pharmacological magnetic resonance imaging (phMRI) signal. Our second aim was to use this knowledge to investigate the age-dependency of methylphenidate (MPH) effects on the dopamine (DA) system in patients with attention-deficit/hyperactivity disorder (ADHD). This final chapter discusses the specific choices that were made in the studies presented, discusses the general results and provides future directions focusing on methodological advancements and possible perspectives for clinical research.

PART I - CHARACTERIZING THE DA PHMRI SIGNAL

phMRI in dopamine dysfunction

In **Chapter 2** we reviewed findings of three models of DA neurotoxicity. Lesion models are necessary to assess the construct validity of a technique; i.e. does phMRI indeed detect damage to a specific neurotransmitter system? Three commonly employed models were reviewed: the 6-hydroxy-dopamine (6-OHDA) model, the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model and the dexamphetamine/methamphetamine (dAMPH/METH model). We compared phMRI to positron emission tomography (PET) / single photon emission computed tomography (SPECT) and although the first two models show highly comparable results, limited phMRI studies were available for the comparison with PET/SPECT for the dAMPH/METH model. 6-OHDA and MPTP mediated neurotoxicity provided a good model for DA loss by mainly targeting the neurons in the substantia nigra (SN), similar to what occurs in Parkinson's disease. The dAMPH/METH model induces more subtle changes as the damage is confined to nerve terminals, and thus provides a better model for DA abnormalities in neuropsychiatric disorders, such as ADHD.

In **Chapters 3 and 4**, we therefore investigated the potential of phMRI to measure DAergic changes in both a rat and human model of dAMPH-induced DA dysfunction. We chose a dAMPH rat model that had previously been shown to reduce DA transporter (DAT) binding in the striatum (Belcher *et al*, 2005), a finding that was corroborated in our study. The acute MPH challenge elicited an increased phMRI response in the dAMPH-treated rats, which we attributed to possible increases in DA release in combination with a reduced clearance of DA from the synaptic cleft, as supported by our findings of lower DAT and increased homovanillic acid levels. These findings are in contrast to previous phMRI studies showing a consistently blunted – rather than exaggerated – effect in 6-OHDA/MPTP treated animals. This discrepancy is probably due to the complete ablation of DA cell bodies in the SN in the latter studies, whereas in the dAMPH model we induced rather subtle imbalances in DA receptor dynamics.

Ablation of nerve terminals automatically implies reduced DAT and reduced DA release, and in that case the phMRI response generally shows a good correlation with PET/SPECT, autoradiography and microdialysis measurements (Chen *et al*, 1997; Jenkins *et al*, 2004). In the case of more subtle receptor remodeling, the phMRI response likely is a combination of the response of the entire DAergic synapse, including pre- and post-synaptic receptor binding, DA synthesis and activity of metabolizing enzymes. While the advantage of phMRI is that the hemodynamic response reflects the complete sum of excitatory and inhibitory activity, it appears to be less suited to study individual receptor alterations compared to PET and SPECT, at least in dAMPH models affecting the whole DA system.

When we translate this to a human model of recreational dAMPH use reported in **Chapter 4**, we can draw more or less similar conclusions. We observed a blunted DA release in dAMPH users, both using SPECT and ASL phMRI, which is in agreement with previous findings from our group (Schouw *et al*, 2013). In that study, however, also no correlation was found between the phMRI response and DAT binding, nor did we observe an association between D₂/D₃ receptor levels and the phMRI hemodynamic response. Nevertheless, there was a weak association between the phMRI response and DA release following a dAMPH challenge assessed using SPECT.

The reason that invasive methods, such as microdialysis and autoradiography, and phMRI correlated much better in preclinical studies with 6-OHDA and MPTP lesion models is probably a combination of both the lesion model and the homogeneity and experimental control in those studies. So, while our results suggests that the phMRI response to dAMPH in humans can be explained at least partially by DA release, it can probably also be attributed to the ratio of pre- and post-synaptic receptors.

The origins of the phMRI signal

Evidence is accumulating that the change in phMRI response can be largely explained in terms of receptor occupancies. Landmark studies about the origin of the BOLD response have demonstrated that hemodynamic changes are more associated with local field potentials, comprising pre- and postsynaptic signals from synapses and dendrites, than with action potentials (Logothetis, 2002). Intuitively, these findings make sense, as the lion's share of the energy expenditure is employed for pre- and post-synaptic signaling, which thus require more oxygen and glucose from the vascular system (Attwell and Laughlin, 2001). This suggests that although DA release is correlated with the phMRI response in some situations (e.g. in the case of potent neurotoxins as explained above), most of the phMRI response reflects pre- and postsynaptic receptor dynamics.

Further evidence is provided by the fact that large increases in DA release can still result in inhibition of the hemodynamic response (Mandeville *et al*, 2013), implying that other factors, such as pre- and postsynaptic receptor activation, dominate the hemodynamic response. Indeed, a computational model based on D₁ and D₂ receptor activation was able to predict the hemodynamic response to cocaine and amphetamine administration, and could explain prior findings and discrepancies in literature (Mandeville *et al*, 2013). For example, rats have a higher ratio of excitatory (D₁) versus inhibitory (D₂) DA receptors than primates, but both species have a higher affinity for D₂ than for D₁ receptors. Combining this knowledge allows us to predict that with a comparable high dose of dAMPH, rats will show a larger positive response than primates: the D₁ occupation will dominate in higher doses, whereas the high affinity of D₂ is expected to drive the response at lower doses. This is also in line with a study showing negative cerebral blood volume (CBV) with low doses, but an increasingly higher positive CBV with higher dAMPH doses (Ren *et al*, 2009). This is interesting in light of the difference we find between MPH and dAMPH administration (**Chapter 4 and 9** respectively). Indeed, MPH decreases cerebral blood flow (CBF) in the striatum, whereas dAMPH, which induces much higher DA release, increases CBF. However, this conclusion has to be treated with caution, as the administration and analysis method also differed between these studies.

Neurovascular coupling

We cannot interpret the phMRI signal exclusively in terms of neuronal activation, but have to take into account neurovascular coupling as well, a process that tightly links blood flow and neuronal activation. Following neuronal activation, be it task- or drug-induced, signaling proteins activate the vasculature both directly and/or through activation of astrocytes, although the exact mechanisms are poorly understood (Attwell and Iadecola, 2002).

A large part of the phMRI signal is accounted for by the activation of pre- and post-synaptic receptors, which gives rise to a hemodynamic response. Nevertheless, capillaries in the brain contain D₅ receptors that, after activation by DA, can dilate these vessels and increase the hemodynamic response. Conversely, astroglial cells contain D₃ receptors that can induce vasoconstriction and decrease the hemodynamic response (Choi *et al*, 2006). Clearly, the effects of psychostimulants on the hemodynamic response are also partially mediated by these non-neuronal processes.

Neurovascular coupling represents a complex process modulated by a wide range of molecules and despite many efforts to elucidate its neurobiological basis, many questions remain (Buxton, 2012). One common feature that has been established is that neuronal activation increases intracellular calcium in neurons and astrocytes, which will activate vasoactive substances that in turn, will induce changes in CBF. The advancement of combined BOLD/ASL MRI over the recent years will hopefully provide us with answers to a number of these questions, as this offers information about metabolic processes in addition to blood flow measurements. This might be especially helpful to study the possible interactions of certain compounds with neurovascular coupling. Furthermore, it may help to unravel the effects of drug on neurovascular coupling, as there is no reason to believe that different neurotransmitter systems induce vascular responses in the same way (Jenkins, 2012).

Systemic effects

Psychotropic drugs are chemical substances that alter brain function, but the effects are often not limited to the brain. Outside the brain, DA receptors can also be found in the pancreas, kidneys, immune system and blood vessels. In the blood vessels, DA acts as a vasodilator and a number of DA receptor subtypes have also been identified on blood vessels and in the heart (Missale *et al*, 1998). In accordance with previous literature, we also observed that dAMPH and MPH administration increased heart rate (HR). As phMRI per se measures a hemodynamic response, changes in HR, but also in blood pressure and blood flow, could in principle affect the MR signal. Unlike phMRI signal changes resulting from neuronal activation, systemic effects on the vasculature are not region-specific and therefore often dubbed 'global signal changes'. These confounding effects need to be taken into consideration in the analysis and interpretation of the data, e.g. by means of modeling the HR throughout the experiment as an additional regressor.

In this thesis (**Chapter 4**), we show that intravenous dAMPH administration is indeed associated with an increased HR as well as with a decreased global CBF. As we also demonstrated that these global effects were associated with the HR changes directly after infusion of dAMPH (i.e. before HR stabilized), we decided to correct for the global signal by means of proportional scaling. In the same study (**Chapter 5**), we used independent component analysis (ICA) to regress physiological confounds, such as HR, out of the BOLD response. This was also successful as we showed no residual effects of HR on resting state network connectivity.

In the subsequent chapters, we employed oral rather than intravenous administration, which has previously been shown to be associated with slower changes in HR (Heal *et al*, 2013). There are multiple ways to deal with global effects on CBF, but the choice of the method is dependent on the problem or question. First of all, regional CBF (rCBF) is typically obtained by dividing the CBF map or ROI value by the mean grey matter (GM) CBF. However, this can be problematic when drugs have a widespread neuronal effect, because a large part of the mean grey matter CBF is then determined by the drug effects and dividing by GM CBF will remove some of the true variance associated with the neuronal effects. Nevertheless, when the systemic vascular effect is larger than the expected neuronal changes, not correcting for GM CBF might lead to wrong conclusions about the directionality of the result (Figure 2 in **Chapter 4**).

A second, and related issue arises when using phMRI in longitudinal studies; baseline CBF has been shown to be sensitive to a number of factors, including caffeine intake, sleep and time of day (Clement *et al*, 2014). In order to compare subjects over time it might be possible to control for some, but not all of these confounders. Intuitively, it would make sense to look at the percentage change in CBF rather than absolute change in CBF to control for variations in baseline CBF. However, consensus has not been reached as to whether absolute or relative CBF changes best reflect neuronal activity.

In theory, autoregulatory processes in the brain would keep CBF stable in times of blood pressure changes, at least within a certain normal physiological range (Zaharchuk *et al*, 1999). Studies have indeed shown that changes in CBF in response to neuronal activation are additive to global CBF changes under certain conditions (Brown *et al*, 2003; Whittaker *et al*, 2015). The BOLD response on the other hand does seem to depend on baseline CBF levels (Brown *et al*, 2003). This again suggests that BOLD MRI may be less suited for phMRI studies as a stand-alone technique, but might provide complementary information when combined with perfusion measurements. For example, in **Chapter 10** we showed that nor baseline CBF, nor changes in CBF in the amygdala after MPH correlated with changes in the BOLD signal in an emotional processing task, suggesting BOLD-based task phMRI adds additional functional information to ASL-based challenge phMRI.

Interactions between neurotransmitter systems

Neuroreceptor stimulation by psychotropic compounds can also produce 'non-local activation' and hemodynamic responses downstream of the actual receptor binding. For example, activation of DA receptors in the striatum can activate prefrontal areas through fronto-striatal-thalamic loops, which can also be assessed with resting-state connectivity (**Chapter 5**). Furthermore, DA receptors can be found on GABAergic, serotonergic and noradrenergic (NA) neurons as well, potentially inducing a neural response in those downstream areas. In **Chapter 10** we discuss possible downstream effects of MPH on amygdala reactivity, either through direct effects of DA on the limbic system or through interaction with the serotonin system.

There is also increasing evidence for the role of the NA system in the subjective and neuronal effects of amphetamine (Ventura *et al*, 2003). Amphetamine also binds to the NA transporters, which far outnumber DATs in the prefrontal cortex. Furthermore, DA receptors are located on NA neurons in the PFC (Devoto and Flore, 2006). This suggests a large influence of the NA system on the hemodynamic response in this brain area.

These examples demonstrate that the phMRI response will rarely display receptor activation of a single neurotransmitter, but rather that it represents a network of brain areas that together contribute to the neuronal response. In that sense, phMRI provides a complementary tool to receptor PET and SPECT, which often focus on specific receptor subtypes. Especially for drug discovery studies this is a very interesting additional measure, as the phMRI profile can be compared to that of other types of drugs in order to get an idea of the neuronal targets and as such, possible therapeutic or side effects (Bruns *et al*, 2015).

Subjective effects

In **Chapter 3** we demonstrated an exaggerated phMRI response to MPH following repeated dAMPH administration in rats. In **Chapter 4**, however, we showed that recreational users of dAMPH display a blunted response to a dAMPH challenge. This could perhaps be explained by the type of challenge (MPH vs. dAMPH) or the route of administration (i.p. vs i.v.). Yet, if that would be the case, we would rather expect that dAMPH would elicit a larger positive response, as it is a much more potent DA releaser. An alternative explanation could be that because rats have a different DA system than humans, they would rather show an exaggerated response to dAMPH than exhibit a blunted response. However, non-human primates have also been shown to sensitize to DAergic drugs, such as cocaine (Narendran and Martinez, 2008). And despite some negative results in humans, only one study has shown a sensitized response to dAMPH 14 days and 1 year after the first administration (Boileau *et al*, 2006). The discrepancy in our results is therefore more likely explained by the context of drug administration and the extent of previous exposure. It has been suggested that sensitization may occur early in the course of drug abuse, and that after more repeated exposure DA synaptic remodeling takes place, resulting in a blunted response to subsequent DAergic stimuli (Narendran and Martinez, 2008). In addition, the contextual environment and expectancy of the reward could play an important role here too. For example, administering amphetamine in a social versus isolated environment has shown to alter the physiological response to the drug (Mitchell *et al*, 1996).

In addition, expectancy effects were demonstrated in a study showing strong DA release when subjects were told they would receive dAMPH, but actually received placebo (Boileau *et al*, 2007). In line with preclinical literature on context dependency of drug rewards, it has been suggested that the blunted response to cocaine in cocaine-abusers could have been due to the clinical hospital environment in which they received the drug. Nevertheless, in **Chapter 4** we still observed subjective effects of dAMPH in the recreational users and thus a slight divergence between the subjective and neurochemical measures.

Together, this shows that the response to DAergic drugs can be complex and is dependent on a multitude of factors affecting both the subjective and physiological response. Therefore, when designing a phMRI study, it is important to acknowledge these effects and control for them by counterbalancing drug and placebo conditions or including control conditions.

The age effect

As a non-invasive imaging technique, phMRI is especially valuable for research in children. In the second half of this thesis we show that phMRI with an oral MPH challenge can provide us with information about the function of the DA system and that the phMRI response differs

across different ages (**Chapter 9**). As phMRI is non-invasive, it is an ideal technique to directly compare pediatric and adult patients. In addition, ASL as a phMRI contrast is ideally suited for use in children, because children have high basal levels of CBF and thus higher SNR. Nevertheless, it is also important to keep in mind the challenges of comparing adults and children, because it is currently still under investigation how baseline CBF levels affect the phMRI response. The BOLD response in elderly subjects has been shown to be altered, possibly as a result of age-related changes to the vascular system (D'Esposito *et al.*, 2003). Indeed, Riecker *et al.* (2003) demonstrated lower CBF responses to hypercapnia in elderly, indicating reduced vascular reactivity, which might again affect neurovascular coupling. In order to design a phMRI protocol suited for the age group of interest, it is important to recognize that higher CBF means faster arrival times, and thus to choose the post-labeling delay in such a way that the labeled blood has arrived in the tissue at the time of measurement.

Recreational dAMPH use

In line with animal studies on chronic amphetamine exposure, we report DA abnormalities in recreational users of dAMPH in **Chapter 4 and 5**. We show changes in D_2/D_3 receptors as well as changes in DA release. Contrary to earlier studies investigating DA neurotoxicity, the sample in our study consisted of moderate recreational users, indicating that already a relatively low number of dAMPH exposures can result in significant changes in the DA system. This is interesting as treatment with daily oral MPH does not appear to induce such DA changes in young adults with ADHD (**Chapter 8**) and suggests that higher doses, via intranasal administration, can induce more toxicity to the DA system, which would result in long-term adaptations. However, future studies are needed to assess whether these effects are transient. Furthermore, as the study we reported in PART I (**Chapter 4 and 5**) was a cross-sectional one, we cannot exclude that the effects we found are at least partly due to pre-existing differences between drug users and controls.

In addition to changes in the brain, we also found differences between amphetamine users and controls in the cardiovascular system (data not shown). Interestingly, one study found that subjects having high subclinical scores on inattention/hyperactivity questionnaires show more increase in HR and BP following dAMPH than subjects with low scores (Sevak *et al.*, 2010). One could speculate that this suggests fundamental individual differences in the DA system, affecting both the cardiovascular system and the brain, predisposing subjects to drug use because of the physiological and subjective effects. Nevertheless, many (case) studies have demonstrated that dAMPH and METH dependence are associated with cardiovascular problems and reductions in DAergic markers, and the current evidence therefore gives stronger support to the toxicity hypothesis of dAMPH. Yet, these two explanations are of course not mutually exclusive.

PART II - AGE-DEPENDENT EFFECTS OF MPH ON THE DA SYSTEM IN ADHD PATIENTS*ADHD*

In this thesis, we report on the first randomized clinical trial that investigated the age-dependent effects of stimulants on the DA system (PART II). We still lack sufficient knowledge about the effect of psychotropic drugs on the developing brain, despite the fact that many children are often treated for years. Our study design was unique in many respects (**Chapter 6**); for example, it is one of the first clinical trials to directly compare adults and children using the same MR methods. Next, and most importantly, we assessed the age-dependent effects of both MPH and placebo treatment for 4 months. The trial was conducted in the time that children were on the waiting list to see a psychiatrist, and as the waiting list was 4 months at the time, our trial did not cause a treatment delay for the children in the placebo group and therefore allowed us to also investigate the placebo effect.

In **Chapter 8**, we showed that MPH treatment for 4 months can induce persistent effects on the CBF response after washout in children, but not in adults with ADHD. This is the first translation of the ‘neuronal imprinting theory’ from pre-clinical work to a clinical study. It demonstrates that despite many studies into the safety of MPH, stimulant medication could actually have an effect on brain development. However, we remain uncertain about the influence of such changes in brain development on ADHD symptoms, comorbidity and daily functioning. In our trial, we did not find persistent positive effects on global clinical impression in children, nor did we observe a lasting improvement or deterioration on neuropsychological tests of attention, working memory and impulsivity following the wash-out period.

Notwithstanding, the ‘neuronal imprinting theory’ predicts that drug-induced changes in neurotransmitter systems are more pronounced once the brain has fully matured. Therefore, we included a retrospective study assessing the effects of early stimulant treatment on both neurobiological and clinical outcomes (**Chapter 9**). Contrary to the results of the clinical trial, no effects of early stimulant treatment on the pHMRI response were found, but baseline CBF differed in the anterior cingulate cortex (ACC) compared to patients that were stimulant-treated later in life or not at all.

This raises the question what better reflects DA signaling: CBF with or without a DA-ergic challenge? Although one study suggested that ACC CBF (without challenge) reflects striatal DAT expression (da Silva *et al*, 2011), most preclinical studies found a high correlation between the DA pHMRI and DA structure and function (e.g. receptor densities, extracellular DA). Indeed, in **Chapter 5** we show a correlation between striatal DA release and change in front-striatal connectivity in recreational ddAMPH users, but not between baseline D₂/D₃ receptors and baseline fronto-striatal connectivity. It appears the jury is still out, and further studies are needed to address the relationship between CBF and neurotransmitter signaling.

In our studies, we frequently opted for an ROI approach, as we had clear hypotheses from animal studies about affected regions and because we wanted to look as selectively as possible at DA-ergic effects, and not per se at the downstream effects of stimulant medication. Surprisingly, we find the largest effects in the thalamus (**Chapter 7 and 8**), an area not that rich in DA receptors, instead of the striatum, the main target of stimulant medication. However, the thalamus is a major output area of the striatum, which could explain these results. Alternatively, the thalamus is rich in NA innervation, the second major target of MPH, which, as stated above, suggests that we should consider multiple neurotransmitters when interpreting

phMRI findings. Moreover, it is important to acknowledge that the striatum is not the only area affected by ADHD medication – stimulants are administered systemically and will affect the entire brain. To illustrate this with a metaphor (David Anderson, Ted Talk): “It’s a little like trying to fix your car by pouring oil over the engine – some of it may dribble into the right place, but a lot of it will do more harm than good” – which brings us to the question of the clinical implications of our findings.

Clinical implications

With regards to the clinical outcome, we observe a clear pattern of differences between early and late stimulant treatment in **Chapter 9**. Although having been treated with ADHD medication in general was associated with less ADHD symptoms at time of assessment, early stimulant treatment was also associated with more depressive symptoms and less recreational drug use. Of course, this is a cross-sectional study and therefore pre-existing differences could explain the results as well. For the ePOD study we are still analyzing the longitudinal data of the fMRI tasks, but the baseline data of the emotion recognition task (**Chapter 10**) already show the age-dependence of the MPH response. This is important given that a substantial portion of patients suffers from problems regulation their emotions, which has been shown to influence the course and outcome of ADHD.

Of course, longitudinal follow-up studies are necessary to examine whether these DAergic changes persist and whether those are associated with changes in behavioral outcome and symptomatology. Should follow-up studies find such an association, whether positive or negative, the responsible authorities will need to take a close look at the prescription guidelines, in order to decide whether revisions are necessary. This links in with other evidence suggesting that long-term treatment of ADHD does not necessarily improve outcomes later in life. The largest randomized controlled trial, the NIMH Collaborative Multisite Multimodal Treatment Study of Children With ADHD (MTA), showed that despite beneficial outcomes in the pharmacological treatment groups after 14 months of follow-up, the initial group randomization did not influence outcome 8 years later. Only a positive response to any of the treatments, in combination with less ADHD symptoms at trial onset and a higher socioeconomic status were associated with a good long-term prognosis. Overall, the ADHD group fared worse compared to a control group on most of the measures (Molina *et al*, 2009). In addition to that, a systematic review demonstrated that indeed there is no evidence of improved clinical outcome, beyond mere symptom control, after sustained pharmacological treatment (>2 years) (van de Loo-Neus *et al*, 2011).

Adult ADHD

In addition to the question about the long-term outcome of pediatric ADHD, it is also still unknown what the effect of stimulants is on patients that present with ADHD in adulthood. ADHD has generally been assumed to be a neurodevelopmental disorder, with symptoms sometimes presenting as early as preschool age. Following from that, it has also been assumed that adult ADHD is a result of persisting symptoms from childhood. However, recently this idea was challenged by a large population-based study in New Zealand showing that adult ADHD patients did not fulfill the childhood criteria (Moffitt *et al*, 2015). This suggests that pediatric ADHD and adult ADHD are possibly different entities. Despite comparable symptoms, they

might have a different course of disease and different neurobiological underpinnings, which will again have implications for treatment guidelines of pediatric and adult ADHD. This paradigm shift might be an important one, and past studies, when viewed in this light, can help to explain some of the discrepancies in current ADHD literature, and future studies will be designed in a different way to investigate this largely unexplored field. The implications for the findings in this thesis might be that the age-dependent differences of pharmacological treatment with MPH that we found may not be accounted for by age alone, but can also partially be explained by different pathophysiology. Nevertheless, neurobiological abnormalities (similar to the phenotype) likely overlap between the two ADHD groups, which is supported by findings of DA dysfunction in both groups.

Challenges in pediatric imaging

As mentioned above, one of the strengths of the study is the fact that we directly compared children and adults. However, this also presented a number of challenges. Clearly, the brain of children and adults is different. First of all, there are volumetric differences between children and adults. A landmark study by Gogtay et al. (2004) showed the pruning and maturation of brain areas in an posterior-to-anterior gradient from childhood to adolescence. Therefore, some studies in children have suggested the use of specific pediatric templates (Sanchez et al. 2012). We chose not to do this, foremost because we wanted to make a direct comparison between children and adults and for voxel-wise analyses we therefore needed to register all data to one template. In addition, the main outcome, the mean CBF response to MPH in striatum, thalamus and ACC, was analyzed in subject space. Second, it has already been mentioned a number of times throughout this thesis that baseline CBF differs between children and adults. As we used within-subject analysis to obtain the final outcome measure (see **Chapter 7**, statistical analysis), this would have mitigated the baseline differences to a large extent. Nevertheless, if baseline CBF indeed influences the magnitude of the CBF response to MPH, this could have introduced some bias in the results.

This opens up a whole different area of research into the neurovascular coupling differences between children and adults which would be very interesting to pursue. Another difficulty in MR acquisitions of pediatric patients is motion. Although we used a 2D readout and no background suppression for our ASL acquisition, rendering the images less sensitive to motion artifacts, motion across imaging volumes was extensive and required advanced motion correction. In addition, for the BOLD fMRI studies (such as **Chapter 10**) the readout is typically longer, making the sequence more vulnerable to motion artifacts within the imaging volume. Motion differed between adults and children in our study and despite advanced correction techniques and exclusion of the worst cases, residual effects could still remain. For pediatric studies, the balance between reducing the total imaging protocol time and gathering enough data per scan is a delicate one. Hopefully, fast acquisition techniques, such as multi-band, can increase the amount of information obtained in a limited amount of time and are now being included in new imaging initiatives in ADHD (Silk *et al*, 2016).

FUTURE PERSPECTIVES

phMRI

As relatively novel technique, ASL has advanced fast and is currently probably the best technique to do *phMRI* research in humans. In this thesis, ASL is better able to distinguish between dAMPH users and controls (**Chapter 4 and 5**) and we expect that subsequent analyses of longitudinal BOLD data in the ePOD study will also show higher sensitivity of ASL *phMRI*. This is probably due to the fact that ASL is a quantitative technique and a more direct measure than BOLD. In addition, ASL measurements are currently also preferred over CBV measurements, despite increased sensitivity to pharmacological challenges because of increased SNR. Particularly in subcortical areas CBV could have a potential benefit, especially compared to BOLD, but perhaps also in ASL (Mandeville and Jenkins, 2001; Wang *et al*, 2011), suggesting a potential benefit of CBV measurements also in humans. Yet, although CBV measurements are currently valuable for pre-clinical studies, for example in drug development, they are less suited for clinical studies as they involve iron oxide contrast agent administration. Although some of these contrast agents are available in humans, they are not FDA approved for use as a contrast agent and it is unclear whether repeated measurements can be performed because of contrast agent deposition.

Nevertheless, as mentioned above, ASL suffers from poor SNR, and technological advancements are needed in order to improve its sensitivity. If we want to move towards personalized medicine, ASL *phMRI* reproducibility rates need to be improved. Reproducibility studies exist for ASL, but so far only one study has attempted to study this using a pharmacological challenge. Using an oral citalopram challenge, reproducibility was poor, which could partially be due to the PASL (instead of pCASL) acquisition and the small effects induced by an oral compared to intravenous challenge (Klomp *et al*, 2012). In addition, one study observed a reasonable reliability using BOLD *phMRI* with an intravenous ketamine challenge (De Simoni *et al*, 2013).

Obtaining better CBF estimates could be accomplished by improving both the acquisition and post-processing methods. Acquisition in clinical population could potentially be improved by prospective motion correction, a technique in which head motion is measured in real time and the new head position is automatically updated by the scanner. In addition, ASL could benefit from acquisitions with multiple post-labeling delays (Mezue *et al*, 2014). First, it accounts for the variation in arrival time of the bolus (tagged spins) across vascular territories in the brain. Second, and perhaps more important for *phMRI*, DAergic agents can reduce global flow, which could mean that the label has not had time to diffuse in the tissue yet and will still reside in the intravascular space, resulting in incorrect inferences, particularly in voxel-wise analyses. Another method that has received attention to increase the information obtained from a *phMRI* scan is calibrated BOLD.

In a typical calibrated BOLD experiment, BOLD and ASL are measured at the same time using a dual-echo sequence during a hypercapnia experiment, in which CO₂ levels are varied. In this way, multiple variables that add information about the underlying physiology can be estimated, such as baseline CBF, cerebral metabolic rate of oxygen (CMRO₂) and the oxygen extraction fraction (OEF) (Blockley *et al*, 2013). Moreover, the possibility for simultaneous PET/MRI measurements offers the unique opportunity to investigate neurovascular coupling after drug administration. For example, the effect of D₂/D₃ receptor antagonism, DA receptor

sensitization and opioid system activation on neurovascular coupling has been investigated using these tools (Sander *et al*, 2013, 2015; Wey *et al*, 2014) and those studies show that, using adequate modeling, the hemodynamic response can provide a good estimate of neurotransmission. On the post-processing side, ASL could benefit from improved motion correction using noise estimate weighting tagging (Tanenbaum *et al*, 2015), from 2D-flow measurements to correct for systemic effects on global CBF, from improved partial voluming correction (Mutsaerts *et al*, 2014) and finally from estimation of transit time variation.

Stimulant use in ADHD

Stimulant treatment is used widely for the treatment of ADHD in children, even though a systematic review showed that there is no evidence that stimulant treatment improves functioning in the long run, beyond mere symptom control (van de Loo-Neus *et al*, 2011). In addition, stimulants are increasingly being prescribed to children despite limited knowledge about the long-term effects on DA development. Moreover, there is even evidence that children without ADHD are being prescribed stimulants to improve school performance and there has been a surge in misuse of stimulant in college students (Lakhan and Kirchgessner, 2012). Yet, the negative effects of stimulants on the normal brain during development are elusive. As long-term placebo controlled studies are not feasible we will have to look at longitudinal observational studies, with pHMRI to assess the effects on brain neurochemistry. In our clinical trial we provide evidence for age-dependent changes, but it is unclear what the clinical consequences are; how do DA changes link to function, cognitively and behaviorally? Using functional MRI we can assess how DA differences can influence behavior and cognition, whereas structural MRI (volumetric measurements and diffusion tensor imaging) can provide us with information of the long-term effects of DA changes on the micro- and macro structure of the brain. Currently, we are conducting the follow-up of the ePOD trial, on average three years after the enrollment in the first study. This is an observational follow-up in which we quantify the extent of MPH use by means of prescription data from the pharmacies. Hopefully, these data will shed some light on the long-term effects of stimulant treatment on DA function and behavioral and neurocognitive outcome in the developing brain.

But what about an alternative to stimulant treatment? Possible new pharmaceutical targets, cognitive training, diet, exercise and more targeted psychosocial interventions might improve daily functioning of ADHD patients. The focus of ADHD treatment has long been on the striatal DA system, but more specific and patient-targeted pharmaceuticals could be beneficial and reduce side effects. PhMRI could play an important role in the testing of drug targets, and stratification of patients, in order to provide the right patient with the right drug at the right dose and at the right time. The focus in this thesis was to evaluate the effect of medication on the neurochemistry in the brain. Nevertheless, for optimal treatment results we would have to zoom out and take a look at the complete picture of the patient, beyond the 'ADHD brain', and examine environmental factors as well. However, the current scientific evidence for psychosocial interventions is less strong than for pharmaceutical treatment, which is reflected in the variation in treatment guidelines across the world (Fabiano *et al*, 2015; Sonuga-Barke *et al*, 2013). Nevertheless, there might be potential for psychosocial interventions, such as psycho-education for parents and coaching for adult patients (currently used in the Netherlands), to improve overall outcome in patients with ADHD.

CONCLUSIONS

Over the past decade, phMRI has been developed as the first non-invasive technique to assess drug-induced changes in neurotransmitter function. Preclinical studies have provided us with increased knowledge of neurotransmitter fluctuations after drug challenges that have been translated to human studies. Although our initial results from studies in dAMPH users and ADHD patients are promising as they show that phMRI can detect DA abnormalities in the human brain as well, technological improvements are necessary to allow advancement of this field. The requirement to monitor drug treatment over time urges for high within-subject reproducibility, whereas the wish to predict treatment response requires high between-subject specificity.

In this thesis, we showed for the first time that stimulants have differential effects on the developing compared to the matured brain in humans. Although this area of research is still in its infancy, public awareness of the possible long term consequences of the current increase in prescription rates is rising, which hopefully allows more longitudinal studies into the effects of stimulants as well as other psychotropic drugs on the developing brain. The importance of developmental biology has, after all, long been acknowledged, as Rousseau (1712-1778) once quoted: 'A child is not just a miniature adult'; but will require even more emphasis in the future.

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