Detecting dopamine dysfunction with pharmacological MRI

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Chapter 1

General introduction and thesis outline
THE DOPAMINE SYSTEM

The dopamine (DA) system is a neurotransmitter system that plays a role in a wide variety of cognitive and behavioral processes, such as motivation, reward, motor control, attention and executive functions. Abnormalities of the DA system have been observed in a number of neurological and psychiatric disorders, including Parkinson’s disease, schizophrenia, substance dependence and attention-deficit/hyperactivity disorder (ADHD). Dopaminergic neurons reside primarily in the substantia nigra and the ventral tegmental area, from where they project to subcortical and cortical areas via mesolimbic, mesocortical, nigrostriatal and tuberoinfundibular pathways (Figure 1). DA is synthesized from its precursor L-DOPA and stored in vesicles in the pre-synaptic terminal. DA neurotransmission is regulated by a number of processes in the presynaptic terminal, such as the DA transporter (DAT), involved in re-uptake of DA from the synaptic cleft, and the vesicular mono-amine transporter 2 (VMAT2), for re-uptake of DA from the cytosol into synaptic vesicles. In addition, excess extracellular DA can be cleared by mere diffusion or metabolized by enzymes such as monoamine oxidase (MAO) and Catechol-O-methyltransferase (COMT). Moreover, presynaptic D2/D3 autoreceptors can influence DA neurotransmission by reducing neuronal firing. At the post-synaptic terminal, DA can influence signal transduction by binding to both excitatory and inhibitory receptors, i.e. the D1- (D1, D5) and D2-types (D2, D3, D4) respectively.

Figure 1. a) Illustration of several aspects of DA synthesis, recycling and degradation within the dopaminergic synapse b) Illustration of dopaminergic projections from the substantia nigra (SN), ventral tegmental area (VTA) and pituitary gland (PG).
STIMULANTS

There are many drugs that can influence the DA system, including stimulants. Stimulants are psychotropic drugs that can enhance arousal and alertness. They are prescribed by clinicians for treatment of ADHD and narcolepsy, and also frequently used in a recreational manner as an illicit substance. The most commonly prescribed stimulants are methylphenidate and dexamphetamine. Methylphenidate binds to the DAT and noradrenaline transporters (NAT), thereby blocking reuptake of these neurotransmitters from the synaptic cleft, which results in increased levels of extracellular DA (Volkow et al., 2001). Dexamphetamine is also a DA and noradrenalin (NA) reuptake inhibitor, but additionally causes reverse transport at the vesicular and membrane DA transporter, therefore leading to even higher levels of extracellular DA.

Recreational drug use

Behaviorally, stimulants are associated with increased energy, impulsivity, alertness, and, in higher doses, they cause a ‘high’. Its energizing properties make dexamphetamine a popular drug in the rave scene. The use of dexamphetamine, commonly referred to as ‘speed’, has a prevalence of 3.6 % in youth and young adults (EMCDDA, 2015). MPH, on the other hand, is not used much as a recreational drug, yet is becoming more popular amongst students as a cognitive enhancer. Speed is typically snorted or dissolved in a drink, whereas MPH is almost always taken orally. Both drugs have been associated with cardiovascular problems, ranging from increased blood pressure to sudden cardiac death. In the brain, both preclinical and clinical research has demonstrated significant alterations in the DA system that were associated with amphetamine, particularly after chronic high dose administration (Advokat, 2007). For instance, amphetamine causes degeneration of DA nerve terminals and receptor up- and down-regulation, and has been linked to cognitive problems in the domains of memory and executive function (Schouw et al., 2013).

ADHD medication

Clinically, stimulants have been used as a pharmacological treatment for ADHD, a neurodevelopmental disorder that is characterized by symptoms of inattention, hyperactivity and impulsivity. Although ADHD symptoms often occur already at preschool age, particularly the symptoms of inattention can persist into adulthood (Biederman et al., 2011). Although the exact pathophysiology of the disorder is still unclear, many studies suggest abnormalities of the DA system are involved. Molecular neuroimaging studies using techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been used to directly evaluate the effects of stimulants on DA receptor abnormalities in ADHD patients. For example, a meta-analysis demonstrated that stimulant treatment-naive ADHD patients showed lower DAT density than controls, and that stimulant-treated patients had a higher DAT density (Fusar-Poli et al., 2012). In addition, structural and functional magnetic resonance imaging (MRI) studies have found normalizing effects of stimulants on grey matter volumes (Nakao et al., 2011) and activation during tasks of inhibition, timing and attention (Rubia et al., 2013). This is in accordance with the finding that stimulants can alleviate ADHD symptoms in up to 70% of patients (Spencer et al., 2005).
A DEVELOPMENTAL PERSPECTIVE

Development of the dopamine system

During early embryonic development, DA plays an important role in neuronal proliferation and the migration of interneurons. In late-gestation, DA receptors contribute to shaping the network of cortical pyramidal cells and striatal medium spiny neurons (Money and Stanwood, 2013). The DA system continues to develop postnatally, throughout childhood and adolescence, and reaches maturity in young adulthood (Wahlstrom et al, 2010). Studies in rodents have demonstrated extensive remodeling during adolescence with a peak in DA receptor expression in subcortical areas, whereas in the cortex a steady increase from childhood to adulthood is observed. The development of the DA system in primates is studied less intensively, but it is thought that phylogenetic differences exist between species. For example, in non-human primates and humans, D1 and D2 receptor expression appears to peak prior to adolescence and decline slowly thereafter. On the other hand, DA tissue concentrations peak in adolescence and then follow a similar pattern (Wahlstrom et al, 2010). At the behavioral level, DA plays a key role in the maturation of cognitive capacities during adolescence (Nieoullon, 2002). Thus, the continuous shaping of the DA system is tightly linked to brain maturation, but also renders it vulnerable for external perturbations during these sensitive periods.

Neuronal imprinting

Despite the high efficacy of stimulants in patients with ADHD, pharmacotherapy is not curative and concerns have been raised about the explosion of stimulant use over the past 10 years. In 2014 the Health Council of the Netherlands presented a report expressing their concern about the increased number of ADHD diagnoses and its concomitant rise in prescription rates (Health Council of the Netherlands, 2014). This is in spite of guidelines establishing behavioral treatment as first line of treatment in mild to moderate ADHD. Moreover, knowledge about the long-term effects of stimulants on the developing brain is scarce.

Although short-term safety has been established in a large number of clinical trials, consequences of long-term exposure to stimulants in childhood or adolescence are not well studied in patients with ADHD. This is surprising to say the least, as animal studies have shown that stimulants can induce long-lasting changes in DA function and behavior when administered early in life. For example, early MPH exposure in rats reduced sensitivity to reward in place-conditioning tests and increased depressive-like behavior in the forced swim test (Carlezon et al, 2003). Moreover, stimulant treatment in juvenile rats decreased DAT expression with 25%, an effect that doubled when these animals were assessed in adulthood. Importantly, these effects were absent when the animals were treated with MPH in adulthood (Moll et al, 2001).

These findings suggest that stimulant treatment in childhood or adolescence can have effects that outlast those of the drug itself and become more pronounced when the brain has matured. The idea that psychotropic drugs can induce persistent changes in the development of neurotransmitter systems has been coined the ‘neuronal imprinting hypothesis’. To test this hypothesis, the ‘effects of Psychotropic drugs On the Developing brain’ (ePOD) study was set up. To our knowledge, this was the first prospective study investigating the age-dependent effects of MPH treatment on the DA system.
IMAGING OF THE DOPAMINE SYSTEM

The earliest studies on the DA system were conducted using ex vivo techniques, such as immunohistochemistry and autoradiography. Later, to study the brain in vivo, molecular imaging techniques were developed. First, positron emission tomography (PET) and single photon emission computed tomography (SPECT) were used to assess the structure and function of the DA system. However, as these neuroimaging techniques require the use of radioligands, magnetic resonance imaging (MRI) methods have been developed to provide non-invasive alternatives.

phMRI

phMRI is a collective term for a number of MRI techniques that have one common characteristic: the administration of a drug challenge to assess its effects on brain hemodynamics (Jenkins, 2012). In brief, it assesses the hemodynamic response of the brain to a particular psychotropic drug. It can broadly be divided into two main categories: task phMRI and challenge phMRI. In task phMRI, the activity of the brain during a particular cognitive task is compared between a drug-condition and a control-condition. Its advantage is that one often has a clear hypothesis of the location of expected task activation, but the downside is that you cannot disentangle potential interaction effects between the task and the drug. Alternatively, challenge phMRI assesses the effect of a drug on brain activation when no task is present, i.e. in rest. As the drug binds to its target neurotransmitter system, it induces neurotransmission, which in turn elicits a hemodynamic response via neurovascular coupling (Figure 2). Challenge phMRI can therefore provide an overview of neurotransmitter distribution and functionality, and is, as such, ideally suited for drug discovery studies.

The disadvantage of both task and challenge phMRI is that not only the targeted neurotransmitter system is activated, but also downstream regions can receive increased neuronal input and resultant hemodynamic activation. PhMRI is therefore less specific to receptor subtypes than PET or SPECT. However, it is a non-invasive technique that can be used for longitudinal designs and vulnerable patient populations, such as children.

Figure 2. Schematic representation of the phMRI response
Chapter 1

**phMRI contrasts**

In humans, two main MRI methods to assess changes in hemodynamic response as a result of neuronal activation are Blood Oxygen Level-Dependent (BOLD) imaging and Arterial Spin Labeling (ASL), the latter providing a perfusion contrast (Wang et al, 2011). BOLD MRI makes use of the difference in magnetic susceptibility between oxygenated and deoxygenated blood, detectable by T2*-weighted sequences (Ogawa, 1992). Neuronal activation increases oxygen consumption, resulting in more deoxygenated hemoglobin. As deoxygenated blood has lower T2* (i.e. produces more distortions in the magnetic field), it reduces the BOLD signal. However, neuronal activation also increases local blood flow and thus the inflow of oxygenated blood. Because oxygenated blood is diamagnetic, it increases the BOLD signal. As the increased blood flow far outweighs the production of deoxygenated hemoglobin, the net BOLD signal increases during neural activation. BOLD imaging is however not quantitative and therefore primarily used for task-fMRI, in which the stimulus condition can be compared to a control condition, or for resting-state fMRI, to assess correlations between relative BOLD signal time series in spatially distinct brain areas. For task based phMRI, the BOLD contrast is therefore most used.

For longitudinal comparisons, ASL is preferred, as this is a semi-quantitative technique (Wang et al, 2011). It makes use of blood as an endogenous contrast agent. Blood passing into the brain is labeled at the main feeding arteries in the neck by applying an inversion pulse (Figure 3). The labeled blood travels to the brain and creates small decreases in longitudinal magnetization in the imaging plane. The perfusion contrast is then generated by subtracting two acquired images, one with label and one without (control). Because only 4% of an imaging voxel consists of blood, ASL suffers from low signal to noise ratio (SNR) and therefore signal averaging is necessary. In addition to being a semi-quantitative technique, ASL is a more direct technique than BOLD because it measures cerebral blood flow, whereas the BOLD signal is a mix of blood flow, blood volume and oxygen consumption.
**Figure 3.** BOLD and ASL MRI. **a)** A simplified schematic representation of the BOLD response. At the onset of neuronal activity, deoxyhemoglobin outnumbers the oxyhemoglobin, resulting in the characteristic initial dip. However, soon thereafter, the blood flow response increases the oxyhemoglobin levels inducing an increase in the BOLD signal. **b)** A simplified schematic explanation of ASL-MRI. *(Left)* Arterial blood is inverted as it passes through the labeling slab, and as it reaches the tissue, the MR signal is acquired in the imaging slab. *(Right)* A control and label image are subtracted to obtain a perfusion-weighted image, which can be quantified to obtain cerebral blood flow in mL/min/100g tissue.
Chapter 1

**THESIS OUTLINE**

The goal of this thesis was twofold: first, to gain more insight into the neurobiological processes underlying dopamine phMRI. Second, to apply dopamine phMRI as a non-invasive tool in a clinical research setting to study the effects of stimulants on brain development. Although dopamine dysfunction is widespread in a number of neurological and psychiatric disorders, currently used imaging techniques to visualize the DA system all require the use of radioactive tracers. In contrast, phMRI is a non-invasive technique that can provide us with a proxy of DA function, but can also be assessed repeatedly in most clinical populations. Here, we first further validated phMRI as a non-invasive technique to detect DA function and dysfunction by comparing it to other, ‘gold-standard’, imaging tools in subjects with known dopamine dysfunction (PART I). Then, in PART II, we apply phMRI to a clinical population of children and adults diagnosed with ADHD to test the age-dependent effects of stimulant treatment on the dopamine system in these patients.

Chapter 1 introduces the phMRI imaging technique used in this thesis. In addition, we provide an introduction into the function and dysfunction of the dopamine system, with a focus on recreational drug use and ADHD. Recreational amphetamine use was chosen as a model of subtle DA dysfunction, because it bridges the gap between phMRI studies in animals and the clinical phMRI studies described in PART 2. ADHD is a neurodevelopmental disorder in which DA abnormalities are thought to play an important role and is therefore a disorder that could benefit a great deal from a non-invasive imaging technique like phMRI. In Chapter 2, we reviewed the ability of phMRI, as compared to conventional neurochemical imaging techniques such as PET and SPECT, to detect dopamine dysfunction (neurotoxicity). In addition, we discuss the strengths and weaknesses of phMRI and the steps that need to be taken to ensure application in the clinical (research) setting.

**PART I**

*Evaluating DA dysfunction using phMRI: comparison to the gold-standard*

First, in Chapter 3, we investigated a) whether chronic administration of dexamphetamine to rats altered the phMRI response to a dopamine challenge and b) what neurobiological changes in monoaminergic systems underlie this response. In order to model subtle changes in the DAergic nerve terminals, we chose an AMPH regime that is known to induce mild neurotoxicity. These experiments were conducted in rats, as this species has been mostly used to validate DA phMRI in lesion models. To compare phMRI to other imaging techniques in humans, we chose recreational AMPH use in humans to investigate dopamine dysfunction as repeated AMPH treatment in rodents is known to be associated with subtle DA nerve terminal changes. In Chapter 4 we used ASL-based phMRI and SPECT to study the dopamine response to an intravenous dexamphetamine challenge in both recreational amphetamine users and control subjects. We hypothesized that both imaging techniques would demonstrate a blunted dopamine response in amphetamine users compared to controls, due to DA abnormalities in the users. In the same sample, we measured resting-state functional connectivity in functional networks previously found to be influenced by the dopamine system (Chapter 5), again before and after a dexamphetamine challenge, to assess whether changes in DA neurotransmission in the users group also resulted in changes in connectivity. Additionally, we examined whether changes in fronto-striatal connectivity reflect DA function as measured with SPECT, as we expected that altered DA release in the striatum would affect proper functioning of this circuitry.
PART II

phMRI as a tool to assess long-term effects of stimulants on the DA system in ADHD

Chapter 6 describes the study procedures of the randomized controlled (ePOD-MPH) trial that we conducted in patients with ADHD to test the hypothesis that stimulants, such as MPH, have lasting effects on the development of the dopamine system. In Chapter 7 we describe the results of this trial regarding the main outcome measure of ePOD-MPH: ASL-based phMRI. We measured CBF before and after a single dose of MPH to obtain a phMRI response, as a proxy of DA function. We hypothesized that 4 months of MPH treatment would increase this phMRI response to MPH in children, but not in adults with ADHD. Then, in the same sample, we take a closer look at age differences in the DA response to acute administration of MPH in stimulant treatment-naive patients (Chapter 8). Based on developmental differences in the DA system, we expected divergent responses between children and adults. Eventually we were interested in the long-term effects of stimulants on the DAergic system and whether this resulted in changes in behavior and symptomatology. This topic was investigated using a retrospective cohort study design in Chapter 9. To assess whether changes in DA neurotransmission also affect other important symptoms in ADHD, such as emotional dysregulation, we used task-based phMRI to investigate baseline differences in the response to MPH between children and adults during an emotion recognition task (Chapter 10). Finally, in Chapter 11 we provide a summary and general discussion of the findings in this thesis.
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