Chapter 7

Age-dependent effects of methylphenidate on the human dopaminergic system in young vs adult patients with attention-deficit/hyperactivity disorder


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ABSTRACT

Importance: Although numerous children receive methylphenidate (MPH) for treatment of attention-deficit/hyperactivity disorder (ADHD), little is known about age-dependent and possibly lasting effects of MPH on the human dopaminergic (DA) system.

Objective: To determine whether effects of MPH on the DA system are modified by age and to test the hypothesis that MPH treatment of young but not adult patients with ADHD induces lasting effects on the cerebral blood flow (CBF) response to a DA challenge, a noninvasive probe for DA function.

Design, setting and participants: A randomized, double-blind, placebo-controlled trial (ePOD-MPH study) among ADHD referral centers in the greater Amsterdam area in the Netherlands, between June 1, 2011, and June 15, 2015. Additional inclusion criteria were male sex, age 10 to 12 years or 23 to 40 years, and stimulant treatment-naive status.

Intervention: Treatment with either MPH or a matched placebo for 16 weeks.

Main outcome measure: Change in CBF response to an acute challenge with MPH, noninvasively assessed using pharmacological magnetic resonance imaging, between baseline and 1 week post-treatment. Data were analysed using intent-to-treat analyses.

Results: Among 131 individuals screened for eligibility, 99 patients met DSM-IV criteria for ADHD. Sixteen weeks of MPH treatment increased the CBF response to MPH within the thalamus (mean difference = 6.50; 95% CI 0.4 to 12.6; p = 0.04) of children aged 10 to 12 years old but not in adults or in the placebo group. In the striatum, the MPH condition differed significantly from placebo in children, but not in adults (mean difference = 7.73; 95% CI 0.7 to 14.8; p = 0.03).

Conclusion and relevance: We confirm pre-clinical data and demonstrate age-dependent effects of MPH treatment on human extracellular DA-ergic striatal-thalamic circuitry. Given its societal relevance, these data warrant replication in larger groups with longer follow-up.

Trial registration: CCMO identifier:(NL34509.000.10); trialregister.nl identifier: (NTR3103).
INTRODUCTION

Methylphenidate (MPH) is the most frequently prescribed medication for the treatment of attention-deficit/hyperactivity disorder (ADHD). MPH effectively reduces symptoms of inattention, hyperactivity, and impulsivity in up to 80% of children with ADHD (MTA group, 1999). MPH increases extracellular dopamine (DA) levels in the brain, by blocking the DA transporters in the synapse (DAT) (Volkow et al, 2012). Its short-term safety has been documented in many studies, and efficacy is amongst the highest of all psychiatric medications (van de Loo-Neus et al, 2011). However, despite its prevalent use in children and adolescents, very little is known about lasting effects of MPH on the developing DA system (Andersen and Navalta, 2004; van de Loo-Neus et al, 2011; Volkow and Insel, 2003).

The adolescent brain is a rapidly developing system that maintains high levels of plasticity. As such, the brain during this unique timeframe may be particularly vulnerable to drugs that interfere with these processes or modify the specific transmitter systems involved. Effects of MPH on brain development have so far only been studied in healthy male animals (Urban et al, 2012; Volkow and Insel, 2003), with short wash-out periods (Bourgeois et al, 2014). More recent evidence now indicates that psychotropic drugs affect the brain in a differential manner that depends on the age of exposure (Andersen and Navalta, 2004). Whereas long-term stimulant exposure in adult animals results in a temporary adaptation to the drug effects, more lasting (and sometimes permanent) alterations are seen when MPH is administered to juvenile animals, a process referred to as ‘neurochemical imprinting’ (Andersen and Navalta, 2004).

Surprisingly, safety studies on the effects of MPH on DA function in the developing brain are scarce in children (Bourgeois et al, 2014). Regardless of this rather alarming paucity of findings, increasingly greater numbers of children and young adolescents are exposed to MPH, many of whom likely do not meet the criteria for ADHD (Elder, 2010). This heightened use has led to considerable debate and concern (e.g., amongst parents) about the long-term consequences, or possible side effects, of MPH in children. Such knowledge is urgently needed as recently emphasized by a number of entities, including the U.S. Food and Drug Administration (2004), National Institutes of Health (1998), and the European Committee for Medicinal Products for Human Use (2008).

The primary aim of the "Effects of Psychotropic medication On brain Development - Methylphenidate (ePOD-MPH)" study was to assess the effects of long-term MPH treatment on DA function in children and adult patients with ADHD. We probed DA function using MPH-based pharmacological magnetic resonance imaging (phMRI), a powerful non-invasive technique to investigate DA function in vivo (Jenkins et al, 2004; Schrantee et al, 2015). We hypothesized increased cerebral blood flow (CBF) response to MPH in children treated with MPH for four months (with a one-week washout) due to increased DA levels (Andersen et al, 2008), but no such lasting effects in MPH-treated adults.

METHODS

Trial design

The ePOD-MPH trial is a 16-week double-blind, randomized, placebo-controlled, multicentre trial (RCT) with MPH and a blinded end-point evaluation in stimulant treatment-naive patients with ADHD (Figure 1). The effect of age on CBF response to a DA challenge and overall clinical
Figure 1. CONSORT flow diagram
outcome was assessed using phMRI in children and adults with ADHD, randomly assigned to either placebo or active treatment with MPH, at baseline and following a one-week washout (Figure 2). The study protocol was approved and registered by the Central Committee on Research Involving Human Subjects (an independent registry) on March 24, 2011, and subsequently at the Netherlands Trial register (NTR3103) during enrolment of the first patient on October 13, 2011. The trial ended on June 17, 2015 and was monitored by the Clinical Research Unit of the Academic Medical Center (AMC).

**Participants**

Participants were 50 stimulant treatment-naive boys (10-12 years of age) and 49 stimulant treatment-naive men (23-40 years of age) diagnosed with ADHD and recruited through clinical programs at the Child and Adolescent Psychiatry Center Triversum (Alkmaar), Department of Child and Adolescent Psychiatry at the Bascule/AMC (Amsterdam), and PsyQ Mental Health Facility (The Hague). All children and adults who were included met strict criteria for ADHD according to the Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) (American Psychiatric Association, 1994), and were diagnosed by an experienced psychiatrist which was confirmed with a structured interview (i.e. Diagnostic Interview Schedule for Children (NIMH-DISC-IV authorized Dutch translation) (Ferdinand and van der Ende, 1998) and the Diagnostic Interview for ADHD (DIVA) for adults (Kooij, 2012). Patients with co-morbid axis I psychiatric disorders requiring treatment with medication at study entry; a history of major neurological or medical illness; as well as a history of clinical treatment with drugs influencing the DA system (for adults before 23 years of age), such as stimulants, neuroleptics, antipsychotics, and D2/3 agonists, were excluded. More detailed inclusion and exclusion criteria are listed in the study protocol. All patients and parents or legal representatives of the children provided written informed consent.

**Intervention, randomization and blinding**

Patients were randomly assigned to either MPH or placebo treatment (for details, see Supplementary Materials). The treating physician prescribed the study medication under double-blind conditions on clinical guidance (i.e. reduction of ADHD symptoms) in accordance with Dutch treatment guidelines. Adult participants received coaching sessions and parents of children received psycho-education. Adherence to the study medication was monitored at each of the control visits (week 1, 3, 5, 8 and 12 for children and week 1, 2, 4 and 8 for adults).

**Primary outcome measure: DA function**

We used phMRI to assess CBF response to the DA challenge MPH. PhMRI is based on the principle that neurotransmitter-specific drug challenges evoke changes in neurovascular coupling and resultant changes in brain hemodynamics, such as CBF (Jenkins, 2012). phMRI has been shown to indirectly assess DA (dys)function in a non-invasive manner similar to positron emission computed tomography (PET) and single photon emission computed tomography (SPECT) studies (Chen et al, 1997; Jenkins et al, 2004; Schouw et al, 2013; Schrantee et al, 2015).

The phMRI scan consisted of two sessions, one before and one 90 minutes after oral administration of 0.5mg/kg MPH (with a maximum dose of 20 mg for children and 40 mg for
adults) acquired on a 3.0T Philips MR scanner. Heart rate (HR) was determined using a peripheral pulse unit and carotid flow was measured using 2D-phase-contrast MRI. phMRI assessment took place at baseline (week 0) and post-treatment following a one-week wash-out (week 17) to ascertain drug clearance (MPH has a half-life of 2-3 hours (Swanson and Volkow, 2003)). Arterial spin labelling (ASL) phMRI was used to assess CBF. The mean of CBF values in the grey matter (GM) of three a priori selected regions-of-interest (ROIs (Figure 3); i.e. striatum, thalamus, and anterior cingulate cortex (ACC)), was used for statistical analysis. These ROIs were selected because the striatum is rich in DAT (the primary target of MPH), and because animal literature has demonstrated large phMRI effects of early MPH treatment in the thalamus and ACC (Andersen et al, 2008). Data acquisition, post-processing, absolute CBF values, exploratory voxel-based analyses of CBF maps, HR and carotid flow are described in detail in the Supplementary Materials and Supplementary Figure 1, 2, 3 and 4 respectively).

**Secondary outcome measure: clinical assessment**

Clinical change was rated using the Clinical Global Impression Severity (CGI-S) and Improvement (CGI-I) (Guy, 1976) scores, both on a 7-point Likert scale. Response to treatment was defined as a score of 1 or 2 on the CGI-I (indicating “very much improved” or “much improved”) and compared between groups at trial-end.

**Figure 3.** Representative T1-weighted image with subject-specific grey matter ROIs superimposed. Red = striatum; green = thalamus blue = anterior cingulate cortex;
**Statistical analysis**

All primary analyses are intent-to-treat with the significance level set at \( P<0.05 \) (2-sided). To evaluate the effect of MPH on the development of the DA system, paired t-tests were used to assess individual change in acute CBF response following an MPH challenge from baseline to post-treatment (\( \Delta i \) CBF) for all four groups separately (Figure 4). The effect of treatment on \( \Delta i \) CBF within both age groups was assessed using independent Student’s t-tests. To test the interaction between age and MPH on \( \Delta i \) CBF, a two-way analysis-of-variance (ANOVA) was performed with age and medication group as factors. Missing values of CBF (4% due to drop-out, 9% in total) and clinical assessments (4% due to drop-out and 19% in total) were replaced using nearest-neighbor interpolation within age and medication group. For baseline characteristics and clinical outcome, a Fisher’s exact test was used for the analysis of categorical data with odds ratio (OR) as effect size estimate (CGI-I), and student’s t-test and ANOVA were used for continuous variables (CGI-S and CBF) with normal distributions and partial eta squared (\( \eta^2 \)) as effect size estimate. Statistical analyses were conducted with IBM SPSS version 22. Sample size calculations are presented in the Supplementary Materials.

**Table 1. Demographics and characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th></th>
<th>Adults</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>MPH</td>
<td>placebo</td>
<td>MPH</td>
<td>placebo</td>
</tr>
<tr>
<td></td>
<td>n=25</td>
<td>n=25</td>
<td>n=24</td>
<td>n=24</td>
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<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>11.4 (0.8)</td>
<td>11.3 (0.9)</td>
<td>28.6 (4.6)</td>
<td>29.0 (4.9)</td>
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<td>Estimated IQ *</td>
<td>104.8 (21.0)</td>
<td>103.4 (15.1)</td>
<td>107.9 (8.8)</td>
<td>107.9 (6.4)</td>
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<td>ADHD subtype</td>
<td></td>
<td></td>
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<tr>
<td>Inattentive</td>
<td>14</td>
<td>14</td>
<td>11</td>
<td>5</td>
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<tr>
<td>Hyperactive/impulsive</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Combined</td>
<td>11</td>
<td>10</td>
<td>13</td>
<td>19</td>
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<tr>
<td>ADHD symptoms</td>
<td></td>
<td></td>
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<tr>
<td>DBD-RS Inattention</td>
<td>21.7 (3.2)</td>
<td>22.8 (3.4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DBD-RS Hyperactivity</td>
<td>15.0 (5.0)</td>
<td>16.4 (6.3)</td>
<td>-</td>
<td>-</td>
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<td>ADHD-RS</td>
<td>-</td>
<td>-</td>
<td>30.6 (10.0)</td>
<td>30.4 (9.3)</td>
</tr>
<tr>
<td>Clinical impairment</td>
<td>4.0 (0.2)</td>
<td>4.0 (0.3)</td>
<td>3.8 (0.5)</td>
<td>3.8 (0.5)</td>
</tr>
<tr>
<td>Adherence</td>
<td>84% (15)</td>
<td>80% (18)</td>
<td>90% (8)</td>
<td>86% (8)</td>
</tr>
</tbody>
</table>

* For children: Wechsler Intelligence Scale for Children (WISC); for adults, National Adult Reading Test (NART); DBD-RS=disruptive behavior disorder rating scale; ADHD-RS=Attention Deficit Hyperactivity Disorder - Rating Scale
RESULTS

Randomization and baseline characteristics
Between June 1, 2011 and February 6, 2015, a total of 99 patients with ADHD in three Dutch centers were randomized to MPH or placebo treatment. After randomization, one subject disclosed that he had been treated for ADHD with MPH before and was therefore excluded from the statistical analyses. Fifty children and 48 adults were included in the primary analysis (Figure 1), although one subject was included at 22 years and 5 months of age. Treatment groups did not differ in age, ADHD symptom severity, and clinical impairment (Table 1). No serious adverse events were noted in any of the subjects studied.

Treatment assignment and details
Treatment allocation and drop-out rates are reported in Figure 1. Due to unforeseen technical changes to the MRI scanner, eight adults underwent the post-treatment scan at eight weeks instead of 17 weeks of the trial. Average treatment duration did not differ between treatment groups in adults (p=0.68) and children (p=0.73).

Main outcome: CBF response to MPH challenge
Paired t-tests indicate a significant increase (mean difference= 6.50, p=0.04; Figure 2) in CBF change from pre-treatment to post-treatment in the thalamus of children treated with MPH and non-significant differences in the striatum and ACC (5.70, p=0.07 and 5.47, p=0.06, respectively, Figure 4), presumably reflecting increased DA levels. As hypothesized, treatment of adults with MPH did not induce such an effect, nor did placebo treatment in either age group. Furthermore, striatal CBF values were also significantly higher (7.73, p=0.03) in children treated with MPH when compared to placebo, whereas no such treatment effect was observed in adults (Table 2). Finally, two-way ANOVA showed a non-significant age*treatment interaction in the striatum (95% CI -0.3 to 16.3; p=0.06), ηp²=0.04. The difference in response to MPH/placebo treatment in children can also be observed in the difference maps (Supplementary Figure 2).

Table 2. CBF response to DA challenge. Differences between treatment groups in individual CBF (ml/100g/min) response to MPH from pre- to post-treatment (Δi CBF)

<table>
<thead>
<tr>
<th>Δi CBF</th>
<th>Children (n=50)</th>
<th>Adults (n=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean [95% CI]</td>
<td>P-value*</td>
</tr>
<tr>
<td>Striatum</td>
<td>7.7 [0.7 to 14.8]</td>
<td>0.03</td>
</tr>
<tr>
<td>Thalamus</td>
<td>7.5 [-2.1 to 17.1]</td>
<td>0.12</td>
</tr>
<tr>
<td>ACC</td>
<td>4.9 [-2.9 to 12.7]</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Independent Student t-tests

CBF = cerebral blood flow; ACC = anterior cingulate cortex; CI = confidence interval
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Figure 4. Treatment effects on CBF response to DA challenge
Means ± SEM for the change in CBF response to MPH from pre- to post-treatment ($\Delta$ CBF in ml/100g/min). * $p < 0.05$ Independent t-test comparing $\Delta$ CBF between the two treatment groups in the striatum: 95% CI 0.7 to 14.8; $p=0.03$, $\eta^2=0.09$ (see also table 2). † $p<0.05$ Paired t-test comparing pre- to post-treatment change in CBF ($\Delta$ CBF) within each group in the thalamus 95% CI 0.4 to 12.6; $p=0.04$, $\eta^2=0.17$

Clinical assessment
A repeated-measures ANOVA showed a significant treatment*time interaction in both children ($p=0.01$, $\eta^2=0.21$) and adults ($p=0.02$, $\eta^2=0.20$) on the CGI-S (Figure 5), with the MPH groups reporting lower global clinical impairment compared to placebo groups. The MPH group in children showed more improvement than the placebo group at week 3 ($p=0.03$, $\eta^2=0.09$) and 8 ($P<0.01$, $\eta^2=0.15$), whereas in adults this was only significant at week 3 ($p=0.01$ $\eta^2=0.14$) and week 17 ($p=0.01$, $\eta^2=0.14$). On the CGI-I, the proportion of patients who reported to feel “much improved” or “very much improved” (compared to baseline) one week after trial-end was significantly higher for the MPH condition (62.5%) relative to placebo (8.3%) ($P<0.001$, OR=18.33) in adult patients, but not in children (MPH group=12% vs placebo group=0% ; $p=0.24$, OR=7.93).

Figure 5. Treatment effects on global clinical impairment.
Means ± standard error of the mean (SEM) for Clinical Global Impression (CGI) Score at baseline, week 3, week 8 and at post-treatment (week 17). * $p<0.05$ comparing treatment groups on individual time points (Student’s t-test)
DISCUSSION

We studied whether age modulates the effects of prolonged MPH treatment on the human DA system. Following four months of MPH treatment, we found significant increases in CBF response to a DA challenge in the striatum and thalamus, one week after trial-end in treatment-naive children, suggesting lasting changes in the DA system. This effect was specific for children as placebo treatment failed to show such an effect in either age group and active treatment with MPH in adults had no effect either. In contrast, MPH induced persistent clinical improvement in adults, but not children.

Our finding that MPH treatment induces persistent increases in the CBF response to an acute challenge with MPH in children is in line with preclinical phMRI studies, that report long-lasting increases in regional CBV in several DA-rich brain regions in juvenile rats treated with MPH (Andersen et al., 2008). Similarly, more invasive techniques have demonstrated that long-term MPH treatment with clinically relevant doses causes long-lasting reductions in striatal DAT (Moll et al., 2001), expression of D₃ receptors in the prefrontal cortex (PFC) (Andersen et al., 2008), increased DA levels (Jeziorski et al., 2007) and a reduction in prefrontal neuronal excitability and synaptic transmission (Urban et al., 2012) in juvenile, but not adult, rats. In humans, structural MRI studies have shown that stimulant treatment affects brain maturation, as untreated children with ADHD show more rapid cortical thinning and smaller white matter volumes than children with ADHD on stimulant medication (Castellanos et al., 2002; Shaw et al., 2009). A number of PET studies compared on- and off-medication conditions in children and adults with ADHD, showing mostly reductions in CBF when off-medication, in motor- and anterior cingulate cortices (Langleben et al., 2002), somatosensory cortices, striatum (Lee et al., 2005) and parietal areas (Szobot et al., 2003) in children, whereas in adults decreases in precentral gyri and striatum, but increased CBF in the vermis (Schweitzer et al., 2003). In addition, an ASL-based study reported reduced frontal and striatal perfusion in adults with ADHD medication (O’Gorman et al., 2008). These findings are in line with our exploratory voxel-wise analyses showing reduced CBF following MPH challenge (Supplementary Figure 2).

Preclinical evidence suggests that our current findings are mediated, in part, by changes in the expression of cortical D₃ receptors; for instance, juvenile exposure to MPH induced a long-lasting decrease in D₃ mRNA in the medial PFC (Andersen and Sonntag, 2014). In rats, D₃ expression is high during early adolescence, then wanes until becoming absent in adulthood. Reduced D₃ autoregulation following early MPH treatment causes DA levels to rise, which subsequently increases activity at other DA receptors. D₂/D₃ receptors have the highest affinity for DA. However, as extracellular DA levels increase, more binding to D₁ occurs, inducing increases in hemodynamic response (Andersen et al., 2008).

Because ADHD is associated with DA hypofunction (Volkow et al., 2012), a lasting increase in DA neurotransmission, as evidenced by increased CBF values in response to an acute challenge with MPH (which we found in MPH-treated children only), will likely result in positive effects on the clinical condition. However, this was not the case in the children in our cohort as the positive effect of MPH on clinical assessment during the trial waned after drug clearance. In contrast, adults in the MPH group showed clinical improvement after wash-out, but no difference with the response in the placebo group at week 8, which is probably due to the large placebo response as a result of coaching. Although there is limited evidence for cognitive interventions in children (Sonuga-Barke et al., 2013), coaching has shown to be beneficial in
Adult ADHD both with and without additional pharmacological treatment (Emilsson et al., 2011; Philipsen et al., 2015; Safren et al., 2010; Weiss et al., 2012). Nevertheless, it cannot be excluded that pharmacological treatment is less robust in adults than in children and this warrants further research.

As maturation of several brain regions is not complete until adolescence (Giedd et al., 1999), drugs given during the sensitive early phases of life, may affect neurodevelopmental trajectories that can have more profound effects later in life (Moll et al., 2001). Indeed, the most comprehensive trial on the long-term effects of ADHD, the MTA study (MTA group, 1999), reported that six years after enrollment medication management was associated with a transient increase in prevalence of anxiety and depression (Molina et al., 2009). This finding is in line with animal studies that reported increased anxiety and depression scores in juvenile MPH-treated rats (Carlezon et al., 2003) as well as memory impairments (LeBlanc-Duchin and Taukulis, 2007). In addition, cohort studies have provided evidence for age-dependent effects. For example, adult ADHD is associated with a high rate of substance abuse (Dalsgaard et al., 2014), but ADHD stimulant medication use in childhood does not increase this risk (Humphreys et al., 2013; Molina et al., 2013) and/or may even decrease such vulnerability (Spencer et al., 2006).

A major strength of our current study is its design, in which effects of confounders, such as age and gender are very small. We chose to include only male patients to limit subject variation as girls and boys differ considerably in brain growth patterns (Giedd et al., 1999), and because ADHD is most prevalent in males (Bottelier et al., 2014). The selective inclusion of stimulant treatment-naive patients was also critical for addressing our objective. Ideally, we would have used a longer wash-out period as it the effects of drug exposure on the developing brain are hypothesized to be only fully expressed during early adulthood (Andersen and Navalta, 2004; Moll et al., 2001) and our current results indicate that such follow-up studies deserve to be conducted. However, for ethical reasons, the time that a child would not receive adequate treatment (placebo condition) dictated the length of this RCT as the waiting list for treatment in the Netherlands is typically four months.

Limitations
Due to its complexity, the power of the study was limited, especially because we examined three different brain regions, which could have increased the risk of a Type I error. Hence, our findings need to be replicated using a larger sample size with more statistical power. In addition, it is likely that the effects of MPH are not confined to the ROIs studied, but likely affect DA-ergic projections throughout the brain, including other cortical regions. Another potential weakness is that despite its advantages and sensitivity as discussed above, phMRI remains an indirect measure of DA function, that specifically assesses the hemodynamic response as a proxy of neurotransmission and physiological effects could affect the hemodynamic response. For example, HR differs between children and adults (Christou and Seals, 2008). Although acute MPH administration increased HR, it occurred in both children and adults. Moreover, we found no age*MPH interaction at baseline nor did MPH treatment significantly alter HR in either age group (Supplementary Figure 3). Thus, although we did not find evidence for systemic vascular effects (see also Supplementary Figure 4), we cannot fully disentangle DA neurotransmission from direct effects of DA on the microvasculature (Choi et al., 2006). Another potential
limitation is baseline differences in CBF between children and adults. However, additional analyses assessing changes in relative CBF provided similar results (Supplementary Materials). Thus, it is unlikely that the differences between children and adults are attributable to differences in HR or global CBF. Furthermore, previous studies have shown that time-course changes in the phMRI signal closely parallel microdialysis measurements of striatal DA release (Chen et al., 1997), and also correlate well with PET and SPECT measurements of DAT availability (Jenkins et al., 2004), DA release (Schrantee et al., 2015), and behavioral measures of DA dysfunction (Jenkins et al., 2004; Schrantee et al., 2015). PhMRI studies in rats (Andersen et al., 2008) further report data similar to our current findings. This collective evidence indicates that phMRI is ideally suited to non-invasively study MPH effects in children.

Conclusion
In line with extensive preclinical data, we provide the first evidence that MPH treatment during a specific period of maturation alters CBF response, likely reflecting increased DA neurotransmission due to neurochemical imprinting by MPH. On the short-term, these alterations do not induce major benefits or harm regarding clinical improvement, but the long-term consequences remain to be established. Our data thus stress the need for longer follow-up studies that address possibly progressive disturbances of the DA system and associated behavioral abnormalities.
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Chapter 7

SUPPLEMENTARY METHODS

Trial design and randomization
After baseline MRI assessment, patients were stratified by age and randomized to either placebo or MPH treatment (1:1), using a permuted block randomization scheme generated by the local Clinical Research Unit. The hospital pharmacy (Alkmaar) assigned participants to a specific allocation, using sequentially numbered containers. Participants as well as care providers and research personnel were blinded. The placebo tablet was identical to the MPH tablet with respect to appearance and was manufactured and labeled according to GMP guidelines (2003/94/EG). After trial commencement no significant changes were introduced to the study protocol, other than that the age-range of the adult participants was expanded from 23-30 years to 23-40 years due to inadequate inclusion rate in this age group in March 2014.

Sample size calculation
Our trial is the first study that examines DA functioning after MPH treatment in children and adults using phMRI. This means that there was only limited and indirect data available to perform a sample size calculation. Our goal for this research was to be able to detect differences in the age-dependent effect of MPH on the outgrowth of the dopaminergic system if these differences are in the magnitude of a standardized effect size of 1.25, as explained in more detail in the study protocol. To detect a standardized effect size of 1.25, a sample size of 15 patients per age group would be sufficient (two-sided significance level of 5% and a power of 90%), but to account for drop-out (including drop-out due to motion artifacts on MRI) 25 subjects in each group were included in the ePOD project. If participants dropped out of the study after randomization, they were not replaced.

phMRI acquisition and processing
phMRI data were acquired using two 3.0 T Philips scanners (Achieva / Intera, Philips Medical Systems, Best, The Netherlands). CBF was measured with arterial spin labeling (ASL) MRI. A 2D gradient-echo echo-planar imaging pseudo-continuous ASL (pCASL) sequence with the following parameters was used: repetition time (TR)/echo time (TE) = 4000/14 ms; post-labeling delay = 1650 ms; label duration = 1525 ms; field-of-view (FOV) = 240 x 240 mm; 17 contiguous slices; 75 control-label pairs (Mcontrol and Mlabel); voxel size = 3 x 3 x 7 mm, no background suppression. In addition, an anatomical 3D fast field echo (FFE) T1-weighted (T1w) scan was obtained with the following scan parameters: TR/TE = 9.8/4.6; FOV = 256 x 256 x 120; voxel size = 0.875 x 0.875 x 1.2 mm.

Data were processed using the Iris pipeline for CBF quantification and multi-atlas region segmentation (Bron et al, 2014). All image registrations were performed using Elastix registration software (Klein et al, 2010).

CBF was analyzed in GM only. To correct for patient motion, the time series were rigidly registered using a group-wise method that uses a similarity metric based on principal component analysis (Huizinga et al, 2014). After motion correction, all pairs of Mcontrol and Mlabel images were subtracted (Mdiff). As large motion influences the ASL signal quality, outlier rejection was performed for the Mdiff images. For every ASL scan, we have 75 time points, and therefore 75 Mdiff images. For each pair of Mdiff images, we computed the sum of squared differences (SSD) which is the sum of all squared voxel-wise differences between the two images.
As such, for each of the 75 time points, we obtained 74 SSD values over which we computed the median and SD. To obtain a more robust estimate of the SD, we computed this based on only the SSD values that were lower than the median. If more than 50% of the SSD values were larger than the median + (3*SD) this timepoint was considered an outlier. If more than 15 out of 75 timepoints were outliers the complete timeseries was removed from the analysis (5% of scans). After removal of the outliers motion correction was performed on the remaining timepoints, and the resulting motion-compensated Mdiff images were averaged to obtain a perfusion-weighted image (ΔM). The average of Mcontrol images was used as a proton-density normalization image (M0) for the CBF quantification. For each subject, probabilistic GM segmentations (SPM8, Statistical Parametric Mapping, UCL, London, UK) were rigidly registered to the ΔM images by maximizing mutual information. CBF was quantified using the single-compartment model proposed by Buxton et al. (1998) which is the recommended approach for pCASL (Alsop et al., 2014). The quantification accounted for post-labeling delay differences between slices due to the 2D read-out. The following parameters were used: labeling efficiency αGM = 0.85, T1GM = 1.6ms, blood-brain partition coefficient λGM = 0.95mL/g. CBF was quantified in GM only using a 3D method for partial volume correction based on local linear regression using the tissue probability maps (Asllani et al., 2008; Oliver et al., 2012).

**ROI-based analysis**

For each participant, CBF maps were transformed to T1w space and regions of interest (ROIs) were defined using a multi-atlas approach. This involved the registration of 30 labeled T1w images (Gousias et al., 2008; Hammers et al., 2003), each containing 83 ROIs, with the participants’ T1w images. Registration with the participants’ nonuniformity-corrected T1w images (Tustison et al., 2010) were performed with a rigid, affine, and a non-rigid B-spline transformation model consecutively. For this registration, both the participants’ and the labeled T1w images were masked using the Brain Extraction Tool (Smith, 2002), these masks were also used for initialization of the registration. The labels of the 30 atlas images were fused using a majority voting algorithm to obtain a final ROI labeling (Heckemann et al., 2006). For these ROIs, mean CBF values within GM were computed, which are used in our study.

**Exploratory voxel-based analysis**

A group template space was constructed based on the T1w images of all subjects using a procedure that avoids bias towards any of the individual T1w images (Bron et al., 2014). In this approach, the coordinate transformations from the template space to the subject’s T1w space were derived from pairwise image registrations of all pairs of T1w images. For these pairwise image registrations, we used T1w images that were non-uniformity corrected and skull-stripped using the multi-atlas brain mask explained above. The pairwise registrations were performed using a similarity, affine, and non-rigid B-spline transformation model consecutively. A similarity transformation is a rigid transformation including isotropic scaling. CBF maps were transformed to template space in one pass by concatenating the template-T1w transformation and the inverted ASL-T1w transformation and subsequently smoothed with an 8 mm FWHM kernel. As these were exploratory analyses, complete-case analysis was used. Voxel-wise changes in CBF difference maps per session (baseline or post-treatment) were assessed non-parametrically using the Randomise toolbox in the Functional...
SUPPLEMENTARY RESULTS

Additional statistics Figure 4

Children:
MPH group. Striatum 95% CI -0.4 to 11.8; p=0.07, η²=0.13. ACC 95% CI -0.1 to 11.1; p=0.06, η²=0.15
Placebo group. Striatum 95% CI -1.9 to 6.0; p=0.30 η²= 0.04. Thalamus 95% CI -6.7 to 8.7; p=0.79, η²= 0.003. ACC: 95% CI -6.4 to 5.2; p=0.83, η²= 0.002.

Adults:
MPH group. Striatum 95% CI -2.2 to 3.6; p=0.62, η²= 0.011. Thalamus 95% CI -4.8 to 8.2, p=0.59, η²= 0.013. ACC 95% CI -3.1 to 3.8; p=0.85, η²= 0.002
Placebo group: Striatum 95% CI -2.9 to 3.9; p=0.78, η²= 0.004. Thalamus 95% CI -5.5 to 4.7; p=0.87, η²= 0.001. ACC: 95% CI -6.0 to 2.1, p=0.34, η²= 0.04.

Relative CBF changes

Previous studies have investigated whether absolute or relative changes in CBF best represent underlying neuronal activity, but no consensus has been reached. Therefore, we here show the statistical analyses of the relative CBF, calculated as (post-MPH – pre-MPH)/pre-MPH from baseline to post-treatment. The results are comparable to those reported in the main manuscript, with the child MPH group showing persistent changes in CBF response to MPH, whereas the other groups do not. Hence, these results do not change our conclusions.

- Children, paired t-tests:
MPH group. Striatum p=0.06. Thalamus p=0.04. ACC p=0.05
Placebo group. Striatum p=0.39 Thalamus p=0.97 ACC: p=0.92
- Adults, paired t-tests:
MPH group. Striatum p=0.65. Thalamus p=0.57. ACC p=0.81
Placebo group. Striatum p=0.81 Thalamus p=0.92 ACC: p=0.43

Additional outcome measures

During each of the ASL scans, heart rate (HR) was monitored using a peripheral pulse unit (PPU). Average HR per scan was calculated (results are displayed in eFigure 4). HR differed between children and adults at baseline (p<0.01) and we found increased HR after acute MPH administration (children p<0.01; adults p<0.01). However, we found no age*MPH interaction at baseline (p=0.11), nor did MPH treatment significantly alter HR over the trial (children p=0.25 ; adults p=0.43).

In addition, blood flow in the internal carotid arteries (ICAs) was determined using 2D phase-contrast MRI at an imaging slice placed perpendicular to the ICA’s with unidirectional velocity encoding (venc 80 cm/s), TE/TR/FA = 5.68 ms/15 ms/15°, NSA= 2, section thickness 4 mm, in-plane resolution = 0.45x0.45mm. ROI’s were drawn on the ICA’s in GTflow software and
net flow (ml/s) was extracted (results are displayed in eFigure 5). The MPH challenge did not affect flow (p=0.11), nor was the response to MPH significantly different after MPH treatment (p=0.45).

Together, these results suggest that despite increased HR, MPH did not induce large changes in the blood flow to the brain, nor did these effects change after treatment. Therefore, systemic effects of MPH do not influence our results and conclusions of persistent changes in CBF response to MPH in the child MPH group.

SUPPLEMENTARY REFERENCES
Supplementary Figure 1. Absolute CBF response to MPH.
Shown are the absolute CBF values in ml/100g/min for each group at each time point. On the x-axis
1 = pre-MPH, 2 = post-MPH. Data are displayed as mean + SEM.
Supplementary Figure 2. Voxelwise difference maps of the change in CBF response to acute MPH challenge. Voxel-wise difference maps of reduction in CBF from baseline and post-treatment for all groups (complete cases) of the phMRI response to MPH. Uncorrected t-stat maps are displayed thresholded at t=1.96 visualizing the difference in response to MPH and placebo treatment in children and adults.
Supplementary Figure 3. Heart rate response to MPH. Heart rate (HR) in beats per minute (bpm) before and after MPH at baseline (BL1, B2) and post-treatment (PT1, PT2); HR differed between children and adults at baseline (p<0.01) and we found increased HR after acute MPH administration (children p<0.01; adults p<0.01). However, we found no age*MPH interaction at baseline (p=0.11), nor did MPH treatment significantly alter HR over the trial (children p=0.25; adults p=0.43).

Supplementary Figure 4. ICA flow response to MPH. Internal carotid artery (ICA) net flow before and after MPH at baseline (BL1, B2) and post-treatment (PT1, PT2) in a subset of adult patients (N=22). The MPH challenge did not affect flow (p=0.11), nor was the response to MPH significantly different after MPH treatment (p=0.45).