Pharmacological MRI in the assessment of monoaminergic function
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CHAPTER 10

THE EFFECTS OF PSYCHOTROPIC DRUGS ON DEVELOPING BRAIN (EPOD) STUDY; OBJECTIVES AND METHODS OF A NEUROIMAGING STUDY INVESTIGATING THE EFFECTS OF FLUOXETINE AND METHYLPHENIDATE

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ABSTRACT

Animal studies have shown that methylphenidate (MPH) and fluoxetine (FLX) have different effects on dopamine and serotonin in the developing brain compared to the developed brain. The effects of Psychotropic drugs On Developing brain (ePOD) study is a combination of different approaches to determine whether there are related findings in humans. Animal studies were carried out to observe age-related effects of psychotropic drugs and to validate different new neuroimaging techniques. We have set up two double-blind placebo controlled clinical trials with MPH in 50 boys (10-12 years) and 50 young men (23-30 years) suffering from ADHD (ePOD-MPH) and with FLX in 40 girls (12-14 years) and 40 young women (23-30 years) suffering from depression and anxiety disorders (ePOD-SSRI) next to a cross-sectional study on age-related effects of these psychotropic medications in patients who were treated previously with MPH or FLX (ePOD-Pharmo). The effects of psychotropic drugs on the developing brain are studied using neuroimaging techniques together with neuropsychological and psychiatric assessments of cognition, behavior and emotion. All assessments take place before, during (only in case of MPH) and after chronic treatment. The combined results of the three different approaches will provide new insight into the modulating effect of MPH and FLX and age-at-treatment during brain development.
INTRODUCTION

Brain development during adolescence is a vulnerable and critical process (i.e. synaptogenesis, (Swaab and Boer, 2001), and therefore sensitive to pharmacological interventions. Treating children and adolescents during this period with serotonergic (5-HTergic) or dopaminergic (DAergic) drugs like fluoxetine (FLX) and methylphenidate (MPH), is likely to have influence on maturation of the brain. For instance, FLX (a selective serotonin reuptake inhibitor (SSRI) is known to increase extracellular levels of 5-HT by blocking the serotonin transporter (SERT) and animal studies have demonstrated that periadolescent 5-HT pharmacological manipulations can lead to abnormal outgrowth of the 5-HT system (Iñiguez et al., 2010; Karanges et al., 2011). Recently, pilot experiments by our group have shown that chronic treatment with FLX results in a significant increase in prefrontal and hypothalamic SERT (+30%, p< 0.01) in juvenile-treated rats, but not in adult treated rats (Bouet et al., 2012). These findings are in accordance with Wegerer et al. and Bock et al., who have also shown that this effect persists into adulthood, long after discontinuation of treatment with SSRIs (Bock et al., 2005; Wegerer et al., 1999). These studies suggest that 5-HT manipulations may have an impact on the regulation of 5-HT outgrowth which is dependent on the age of exposure.

Also for the DAergic system, recent animal studies with MPH have demonstrated that the effect on DAergic functioning in the brain differs between children and adults, suggesting an age effect of treatment. In rats for instance, early treatment with MPH led to a considerable (-50%) reduction of dopamine transport density (DAT) in the striatum and other DA-rich brain regions when compared to non-treated animals, whereas no effects have been observed in adult animals (Grund et al., 2006; Kirchheiner et al., 2001). These alterations in the DA system have been shown to relate to behavioral abnormalities. For example, young rats treated with MPH show after long-term follow-up, more anxiety- and depression-related behavior than adult rats treated with MPH (Bolanos et al., 2003). There is some clinical evidence for related findings in humans, for example in the NIMH Collaborative Multisite Multimodal Treatment Study of Children With Attention-Deficit/Hyperactivity Disorder (MTA) where children who received behavioral therapy had a lower rate of diagnoses of anxiety or depression (4.3%) after treatment than the children who were treated with methylphenidate (19.1%) thus indicating an increase of emotional disorders six to eight years after treatment with methylphenidate (Molina et al., 2009). Differences have also been found between adolescent and adult patients treated with MPH on fMRI studies, with adolescent patients treated with MPH showing more activity in the prefrontal cortex after treatment than adult patients (Epstein et al., 2007).

Thus, evidence is slowly emerging that the long-term effects of drug exposure are delayed and come to expression once the vulnerable system reaches maturation
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(i.e., typically during adulthood). This phenomenon is known as neuronal imprinting and occurs when the effects of drug exposure outlast the drug itself (Andersen and Navalta, 2004). Still, very little is known on exposure during later brain development. Most (clinical) studies are hampered by the fact that they are retrospective in design, and therefore the findings could be caused by other factors on which the groups differed. As pointed out by Shaw and colleagues: ‘….the ideal study design for this question would be a randomized trial comparing cortical growth in children on psychostimulants against an unmedicated comparison group—but this would be both logistically and ethically challenging’ (Shaw et al., 2009). Notwithstanding this challenge, we have set up three studies: two randomized controlled trials (RCTs) and a retrospective cohort study, investigating the possibility of the existence of ‘neuronal imprinting’ in children medicated with these drugs while using several modalities to assess neurocognitive development. Here we report on the objectives and methods of these studies.

OBJECTIVES

Primary objective:
The primary objectives of the ePOD randomized clinical trials (RCTs) are to report on the short-term age-dependency of the effect(s) of MPH treatment on the developing DA system and on the age-dependency of the effect(s) of FLX on the developing 5-HTergic system, using pharmacological MRI (phMRI). Furthermore, we aim to study the long-term effects of these drugs in a cohort study based on medical prescription data.

Secondary objectives:
1. To report on the age-dependency of MPH and FLX on the outgrowth of the DA system and the 5-HT system using functional outcome measures (DTI, fMRI, rs-fMRI and neuropsychological assessments (NPA)).
2. To report on the age-dependency of the effects of FLX on 5-HT driven HPA axis activity using cortisol measures.
3. To report on the role of the 5-HTTLPR polymorphism upon the age-dependency of FLX on the outgrowth of the 5-HTergic system.
4. To report on the effects of MPH on restless legs (RLS) symptoms and insomnia.
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METHODS

General design of the ePOD project

As only a long-term prospective study in patients randomly assigned to MPH or SSRIs and placebo conditions can determine unequivocally whether the (adverse) effects of these medications on the neurotransmitter systems interact with the age when these drugs are prescribed. However, it would not be ethical to deprive subjects in a placebo setting from treatment for extensive periods of time. Therefore, in addition to the RCTs, which will last 4-5 months, we investigate the long-term effects in a cohort study based on medical prescription (the Pharmo study). The three sub-studies of the ePOD project include:

· ePOD-MPH: The first RCT with medication naïve ADHD patients will involve three NPA and MRI scanning sessions: before starting with the study medication (baseline session), during treatment with MPH or placebo (week 8) and after trial end following a 1-week washout period (week 17).

· ePOD-SSRI: The second RCT involving medication naïve MDD and anxiety disorder (AD) patients receive NPA and MRI sessions: before starting with the study medication (baseline session) and after treatment with FLX (week 19) including a 3-week washout period.

· ePOD-Pharmo: This will be a cross-sectional study with a cohort of subjects suffering from ADHD or MDD or AD now or in the past. They will receive the same assessments as in the first two sub-studies but only once.

The outcome measures in the ePOD-MPH, ePOD-SSRI and ePOD-Pharmo studies. In addition, currently known potential confounders like age in months, ratings of symptom severity and in the SSRI trial, the 5-HTTLPR polymorphism, are registered. Both clinical trials are approved by the Central Committee on Human Research in the Netherlands (CCMO).

Randomized Controlled Trial design and study samples

The RCTs consist of a 16-week multicenter randomized, double blind, placebo-controlled trial with a washout period of one week (MPH) or three weeks (FLX). Subjects are stratified into two age categories: children (MPH), 10-12 years/adolescents (FLX), 12-14 years and adults (MPH and FLX), 23-30 years. These two groups are randomly assigned to either placebo or active treatment. NeuroPsychological Assessment (NPA) and Magnetic Resonance Imaging (MRI) assessment days will take place before treatment (baseline), during treatment (only in the MPH trial) and following the washout period (see Figure 1 for the timeline for ePOD-SSRI). Baseline measurements are compared with the results during the trial and at endpoint. Differences in treatment-effect are compared between the two age categories (children vs. adult) and with healthy controls. In view of our hypothesis that the active
treatment results in long lasting or even permanent changes in the developing monoamine system, we expect no differences between the two age-groups during treatment (due to the presence of active treatment: MPH trial only), but only after stopping the treatment (because the effect of active treatment has subsided in adults, but enduring changes in the pediatric brain have taken place). Washout periods were chosen based on chemical properties (rate of elimination based on five half–live times) and ethical considerations (time without treatment).

A total of 100 children (10-12 years of age) and adult (23-30 years of age) male outpatients diagnosed with ADHD (all subtypes) and in need of pharmacological therapy will be included in ePOD-MPH and total of 80 adolescent (12-14 years of age) and adult (23-25 years of age) female outpatients with moderate to severe MDD or an anxiety disorder in need of pharmacological treatment will be included in ePOD-SSRI. Patients that have used medications or drugs that influence the monoamine systems involved before age 23 will not be eligible. Patients are being recruited from clinical programs at the Child and Adolescent Psychiatry Center Triversum (Alkmaar) and from the department of (Child and Adolescent) Psychiatry of the Bascule/AMC (Amsterdam), and from PsyQ mental health facility in The Hague. The diagnosis will be made by an experienced psychiatrist based on the Diagnostic and Statistical Manual of Mental Disorders, (DSM-IV), Fourth Edition, (American Psychiatric Association, 1994) and confirmed by a structured interview: Diagnostic Interview Schedule for Children (National Institute of Mental Health Diagnostic Interview Schedule for Children Version IV (NIMH-DISC-IV, (Shaffer et al., 2000)), Dutch translation Ferdinand RF, van der Ende J, 1998, Rotterdam, the Netherlands), in children or in parents and Diagnostic Interview for Adult ADHD (DIVA), (Kooij, 2012), in the MPH trial and the Diagnostic Interview Schedule for Children in children and the Composite International Diagnostic Interview (CIDI; lifetime version 2.1 (World Health Organization, 1990), Dutch translation by Smitten, Smeets and van den Brink, Amsterdam 1998) and Hamilton Anxiety Scale (HAM-A) (Hamilton, 1969) in adults. Patients with co-morbid axis I psychiatric disorders requiring treatment with medication at study entry, with IQ lower than 80 (as measured by a subtest of the Wechsler Intelligence Scale for children-Revised (WISC-R), National Adult Reading Test (NART) (Willshire et al., 1991), Dutch translation (Schmand et al., 1991) and MDD patients with current risk of suicide attempt are excluded.

We chose to include male patients in ePOD-MPH to limit subject variation and because ADHD is most prevalent in males (Boyle et al., 2011). The cut-off point of 10-12 years of age is chosen because peak prevalence of ADHD is 10 years of age (Burd et al., 2003) and also because several MRI parameters greatly change until 8-10 years of age (Ben et al., 2005), whereas the rate of increase of neuronal growth and pruning reduces after 10 years of age. The age range of the adults is chosen in line with previous studies involving a comparison between matured versus immature brain (Sowell et al., 1999). Only female subjects were chosen in ePOD-SSRI based on the higher prevalence of MDD and AD in this
population (Hasin et al., 2005). For the adolescent group we chose a cut-off point of 12-14 years of age because the risk of MDD and AD onset increases approximately 8 fold at this age compared to children younger than 10 years of age (Birmaher et al., 1996; Merikangas et al., 2010).

**Figure 1. Timeline study procedures SSRI trial; *only in adolescents.**

**Cohort study design and study sample**

In the ePOD-Phamo study, early- and late SSRI- and MPH exposed subjects are recruited through the Pharmo database, in addition to unexposed subjects. The PHARMO Institute (Utrecht, the Netherlands) collects, maintains and performs research on patient-centric data to derive real-life insights for tailoring of medicines, in order to improve the effectiveness and risk management of drugs as used in daily practice. A major characteristic of the PHARMO Institute is that it focuses on geographically and demographically defined areas where complete coverage of the population is established (denominator). This results in a unique database with a large quantity of anonymized patients enabling follow-up of exposure and medical events for more than 20 years. In the ePOD-Phamo project subjects will be invited to participate in a single assessment day (cross-sectional design) with similar NPA and MRI investigations as in the ePOD RCTs. The outcome measures will be compared to adult controls that have not been previously treated with these drugs and with healthy controls (not part of the current trial).

**Study sample of the ePOD-Phamo trial**

Subjects eligible for study participation are 23-30 years of age and preferably diagnosed with ADHD or MDD/anxiety disorder. The first two groups will have a history of MPH or SSRI (preferably FLX) treatment before the age of 16. Groups three and four are defined as with a history of treatment only in adulthood (between 23 and 30 years of age). Age-and
sex matched control groups will be formed consisting of medication naive subjects suffering from ADHD or MDD/anxiety disorder. All six groups will contain 25 subjects.

**Clinical rating scales**

For both RCTs we use one set of clinical rating scales to assess symptom severity and functioning at baseline and after treatment. For each study separately we have some additional, disorder-specific rating scales. The basic set includes the Clinical Global Impression scale (CGI), (Guy, 1976), the Clinical Global Assessment Scale (CGAS) (Shaffer et al., 1983) in children and the Global Assessment of Function (Endicott et al., 1976) in adults. In both groups the Children’s Depression Inventory (CDI) (Saylor et al., 1984) and an anxiety scale, the Screen for Child Anxiety Related Emotional Disorders (SCARED) (Birmaher et al., 1997), will be administered to children and the Beck Depression Inventory (BDI) (Beck et al., 1961) and the Beck Anxiety Index (BAI) (Beck et al., 1988) to adults. To measure disorder specific symptoms, a translation of the Disruptive Behavior Disorders Rating Scale (DBD-RS) (Pelham, Jr. et al., 1992) is used in children and the ADHD-SR (Rosler et al., 2006) in adults in the ePOD-MPH study. To measure depressive symptoms in children the Childhood Depression Rating Scale (CDRS) (Poznanski et al., 1979) will be completed in the ePOD-SSRI study.

**Imaging parameters**

During the ePOD-MPH study two scanning sessions will take place at baseline and after the washout period. Imaging parameters are directed towards the DAergic and 5-HTergic system: DAergic and 5-HTergic brain activity will be assessed using phMRI, which is the primary outcome measure of the study. DA and 5-HT connectivity will be assessed using rs-fMRI and DTI, and task related brain activity during 5-HT (emotional processing) and DA (motor inhibition) fMRI tasks.

**phMRI**

With phMRI, a neurotransmitter specific pharmacological challenge is given, causing changes in neurovascular coupling and subsequent region-specific hemodynamic changes. DAergic (dys)function has been previously assessed by imaging changes in cerebral hemodynamics following a DAergic challenge (Jenkins et al., 2004). Based on the literature and experiments from our own group (in submission), we expect in the MPH study an increased CBF in specific brain areas (e.g. thalamus and frontal cortex) evoked by the DA challenge with MPH in treated children when compared to pre-treatment baseline scans and untreated subjects, reflecting changes in DA neuronal activity (Andersen and Teicher, 2008).
After the baseline session subjects will receive an oral dose of MPH (0.5 mg/kg with a maximum dose of 20 mg in children and 40 mg in adults) for the phMRI scan. Subjects will be put back into the scanner after 90 minutes and the same sequences will be repeated, now under the influence of MPH. This time window was chosen, because DAT occupancy is significantly correlated with plasma concentration of MPH, which peaks between 1 and 2 hours following ingestion of MPH (Silveri et al., 2004; Spencer et al., 2006). DAT occupancy has also been shown to be relatively stable between 1 and 2 hours after ingestion of MPH (Spencer et al., 2006).

In the ePOD-SSRI study, an intravenous (i.v.) challenge with citalopram (5 mg in adolescents and 7.5 mg in adults) will be administered during a single scanning session. This 5-HT challenge is subject to more variability and therefore needs to enter the brain in a rapid and consistent manner over the time course of a single scan session which requires intravenous administration (Anderson et al., 2008). Citalopram is currently the only SSRI registered for i.v. administration. When used for therapeutic purposes, intravenous citalopram is given at the same dose as the oral route of administration and it is well within the therapeutic range even for children (Guelfi et al., 2000). Although posing an extra burden, especially to the adolescent group, i.v. administration of the challenge is necessary to ensure reliable data collection. Citalopram increases 5-HT release by inhibiting the reuptake of 5-HT by SERT. It has been used previously in phMRI studies and has been proven an adequate probe of 5-HT function (Anderson et al., 2008; McKie et al., 2005), as well the interaction effect of age by SSRI treatment in rats (Klomp et al., 2012). We expect an increased signal in 5-HT rich brain areas (e.g. prefrontal cortex, hippocampus and hypothalamus) after 5-HT challenge only in FLX treated adolescents when compared to pretreatment baseline scans, due to increased SERT densities (Bouet et al., 2012) and based upon previous phMRI results in rats (Klomp et al., 2012).

rs-fMRI

A relatively new fMRI approach (i.e., resting-state fMRI (rs-fMRI)) allows extensive assessment of changes in organization of whole functional networks. Rs-fMRI aims to detect baseline brain activity related to ongoing neuronal signaling at “rest” and is performed by low-pass filtering of spontaneous blood oxygenation level-dependent (BOLD) fMRI signals (Fox and Raichle, 2007). In adult ADHD a decreased functional connectivity between anterior cingulated cortex and precuneus has been found using this technique (Castellanos et al., 2008). However, the effect of MPH or SSRI treatment on functional connectivity either in adults or children is unknown.
DTI

With diffusion tensor imaging (DTI), the micro-structural organization of white matter (WM) can be imaged. By measuring the diffusion motion of water molecules, and the fact that this motion is restricted by myelin sheaths, an impression of axonal direction and integrity can be obtained (Mori and Zhang, 2006). Fractional anisotropy (FA) is the most commonly used unit in DTI and provides information about the degree of fiber organization and integrity. Any process that results in alterations in axonal architecture, such as decreased axonal outgrowth, can result in decrease in FA (de Win et al., 2007; Moeller et al., 2005; Reneman et al., 2001). A previous study with MPH observed an increase, or rather normalization, of white matter volume in ADHD medicated children compared to unmedicated children (Castellanos and Tannock, 2002). Chronic treatment with MPH in pre-adolescent rats was found to increase (fold change >1.5) genes involved in striatal growth of novel axons (Adriani et al., 2006). Therefore we expect an increase in FA in MPH treated children when compared to pre-treatment baseline scans and when compared to non-treated subjects.

Considering the 5-HTergic system we have previously shown that alterations in axonal integrity linked to the 5-HTergic system can be adequately assessed using DTI (de Win et al., 2007). We hypothesize that chronic treatment with SSRIs leads to increased outgrowth of the 5-HT system, since 5-HT acts as a growth factor in the maturing brain (Whitaker-Azmitia et al., 1996). Therefore we expect an increase in FA (reflecting 5-HT neuronal growth) in 5-HT rich brain areas only in FLX treated adolescents when compared to pretreatment baseline scans. No effect of treatment on these scan parameters are expected in adults.

fMRI

We have selected two task-related fMRI scans either based upon their involvement of the DA system and/or the 5-HT system and the known interaction with MPH or FLX and treatment response in anxiety and depressive disorders. In view of our hypothesis we expect to find a normalized pattern of activation on these tasks in children during treatment, which will persist after the end of the trial. In contrast, the activation pattern in adult subjects will normalize during the trial and fall back to pre-treatment (hypoactivation) values after the end of the trial. The fMRI tasks consist of the following:

Emotional processing (MPH and SSRI trials): The BOLD response to negative emotional faces (angry and fearful faces) is measured in a block-design fMRI task (Hariri et al., 2002). Emotional responses are elicited in many different brain regions, where the amygdala seems to be a relay between visual systems and modulatory responses. Emotional processing is known to be regulated by 5-HT and to be affected in mood disorder (Surguladze et al., 2004).
MDD patients are believed to express a heightened responsiveness to negative emotional stimuli and a reduced detection of positive affect (Anderson et al., 2011b; Harmer et al., 2009), which is explained by hyperactivity of the affective neurocircuitry, including the amygdala (Drevets et al., 2008). SSRIs have been found to decrease amygdala activity in response to negative affect in both healthy subjects and MDD patients, which might (partially) symptom remission following antidepressant treatment in MDD (Anderson et al., 2011a; Delaveau et al., 2011; Murphy et al., 2009b).

Motor inhibition (only MPH trial): Frontal–striatal function and its modulation by MPH will be assessed using a motor inhibition task: the go/no-go task (Durston et al., 2003). MPH has been shown to normalize striatal hypoactivation in ADHD subjects (Vaidya et al., 1998). Specifically, fronto–striatal activation during response inhibition will be measured on two versions of a go/no-go task, each with and without administration of MPH. The effects of MPH on frontal and striatal activation during response inhibition will be compared within and between groups.

**Neuropsychological tests**
A neuropsychological test battery (Standard Reaction Time Task, Rey Auditory Verbal Learning Task, Sustained Attention to Response Task (SART) (Johnson et al., 2007), N-back (working memory task) (Smith and Jonides, 1999), Maudsley Index of Delay Aversion (MIDA) (Kuntsi et al., 2001)) will be administered, addressing reaction time, verbal memory, sustained attention, working memory and delay aversion in particular. This information can be linked to results from imaging in order to determine any links between behavioral and fMRI data and changes in the monoamine systems. We will look for correlations between altered behavioral responses and fMRI responses, phMRI responses, DTI measures and rs-fMRI response.

**Actigraphy and sleep log**
Restless Legs Syndrome is a chronic progressive neurological disorder that has a greater incidence in ADHD children, adolescents and adults than in the general population (Cortese et al., 2005). It is possible that RLS is co-morbid with ADHD or that they share a common DAergic deficit. Also, ADHD separately and ADHD together with RLS have been found to be associated with sleep disorders such as insomnia and a common genetic polymorphism (Fliers et al., 2012; Imeraj et al., 2012; Yoon et al., 2012). In a recent study, 64% of children with ADHD were estimated to suffer from RLS judged by their nocturnal periodic limb movement (Picchietti et al., 1999). It has been shown that MPH reduces total sleep time but improves sleep quality by consolidating sleep in adults (Huang et al., 2011). However, the effect of MPH on RLS in ADHD children has never been investigated. In view of the expected inhibitory effect of MPH on DA metabolism it is important to investigate the occurrence and
Severity of RLS and sleep disorders in children and compare these to adults, and the effect of MPH thereupon. Sleep disorders and RLS are effective and non-invasive outcome measures to evaluate the effect of age following MPH treatment in the human brain. Therefore, we will assess RLS severity and sleep quality in the ePOD-MPH study using questionnaires (the Holland Sleep Diagnostic List (HSDL) (Kerkhof et al., 2012) and sleep log and actigraphy at three time points during the study: the week prior to the trial, during the trial, and during the washout period. Actigraphy is a non-invasive method of monitoring human rest/activity cycles. To measure gross motor activity, each patient will wear a small actigraph unit, also called an actimetry sensor, for five consecutive days. We hypothesize that due to an expected long-term reduction in DA turnover rate after early MPH treatment, there will be long lasting positive effects on RLS symptoms and sleep disorders only in children, but not adults.

**Cortisol measurements**

In the ePOD-SSRI study salivary cortisol levels will be determined in salivary samples taken at home on a 'normal' weekday in the week before baseline and washout assessment days in order to determine the cortical awakenings response (CAR) and the diurnal cortisol cycle. Samples will be collected at 5 different moments: 1) directly after waking up, 2) 30 minutes after waking up, 3) 4 hours after waking up, 4) 8 hours after waking up, and 5) 12 hours after waking up. To determine the peak after a 5-HT challenge, one salivary sample will be collected before the MRI scan session (baseline measure) and a second sample 30 minutes after the 5-HT challenge (directly after the MRI scan) on the day of both the MRI scan sessions.

**Potential confounders:**

The study is designed to limit several important possible confounding parameters, such as gender effects (only women are included in the FLX trial and only men in the MPH trial) and aging effect (small age range, only young adults included). A within subject approach (pre- and post-treatment measurement in every subject) is used to rule out most between subject differences. Because of the design of the study, we have limited power and can correct for a maximum of 2 or 3 confounders. Therefore, first age in months and ratings of symptom severity will be taken into account as covariates. In addition in the ePOD-SSRI study, the 5-HTTLPR polymorphism will be determined. The long allele of this SERT polymorphism in the promoter region (5-HTTLPR) has an activity twice that of the short allele (Lesch et al., 1996), resulting in higher densities of SERT. It is expected to be an important confounder to take into account when measuring SERT functioning. Also, significant associations between the long variant and a favorable treatment response have been repeatedly reported (Serretti et al., 2007).
Power analysis

Since these trials are the first to examine 5-HT and DA functioning following FLX and MPH treatment in children and young adults using MR imaging, there is only limited and indirect data available to perform a sample size calculation. The goal of our research is to detect differences in the age-dependency effect of FLX and MPH on the outgrowth of the DA-ergic and 5-HT-ergic system if these differences are in the magnitude of a standardized effect size of 1.25. From pilot experiments in rats and humans we presume that the expected differences with our methods will lead to standardized effect sizes of at least 1.25. Both current trials will have the benefit of having before and after treatment measurements data from each patient. This paired data will reduce the between subject variability. This will increase the power of our trial to detect differences between groups. A sample size of 15 patients in each treatment-by-age group (4 groups) will be sufficient to detect standardized effect size of 1.25 with a two-sided significance level of 5% and a power of 90% to demonstrate age-dependency of the effects of MPH and FLX. To account for an expected drop-out of 25%, we will include 20 patients in each group for the FLX trial. Because the expected drop-out in the MPH trial is probably higher, due to motion artifacts in MRI scanning, we will include 25 patients in each treatment-by-age group. Because of slightly higher subject variability in the ePOD-Pharmo study (age and duration of treatment) again a sample size of 25 was chosen.

Statistical analysis

To evaluate the age-dependency of the effect of MPH and FLX on the outgrowth of the DA-ergic and 5-HT-ergic system, the change in our primary outcome measures (CBF) from baseline to post-treatment will be determined for each patient (Δi). These individual changes (Δi) will be used to estimate the treatment effect in adolescents (mean Δ in treated patients minus mean Δ in placebo treated patients) and in adults, which will be compared, as shown also in Figure 2. All analysis will initially be conducted using the intension-to-treat principle, but for the imaging outcomes a per-protocol analysis will also be performed.

The central analysis examines whether this treatment effect is different in adolescents compared to adults (effect modification or interaction by age). This hypothesis will be formally examined using ANOVA. The model includes treatment group (2 categories), age group (2 categories), and the interaction between treatment and age to examine whether the impact of MPH and FLX treatment differs by age. Depending on the imaging modality we will use a whole brain voxel based analysis or an ROI analysis. The same approach can be used for explorative analysis on the age-dependency of the effects on secondary outcome measures such as behavioral outcome (fMRI, neuropsychological assessment) and behavioral measures, and cortisol response for the FLX trial and sleep-log actigraph for the MPH trial.
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Ethical considerations

Evidently, there are important ethical considerations that need to be taken into account with medication studies in children. In our case, the most important restriction is the duration of the clinical trial, or the time that a child would not receive adequate treatment (placebo condition). The duration of the RCT could not be longer than the time a child would otherwise also not receive adequate treatment, due to (relatively) long waiting lists in the Netherlands: typically 4 months at the time these studies were being evaluated by the Central Committee on Human Research in the Netherlands (CCMO). In the MPH study we overcome the treatment delay by including patients from the waiting list and offering psycho-education when necessary. In addition, in the ePOD-SSRI trial we give at least 18 sessions Cognitive Behavioral Therapy (CBT) to all adolescent participants. Therapy will be in accordance with the ‘Doepressie’ protocol, a psychotherapeutic program which is a Dutch translation of the internationally well-used program ‘Coping with Depression Course for Adolescents’ (Clarke and Lewinsohn, 1989). CBT is not part of standard clinical practice in the adult MDD population and will therefore not be provided to the adult patients. Adult MDD patients, who already receive some form of behavioral therapy at the start of the study, may continue this if they wish, but adult MDD patients cannot start a new therapy. Moreover, studies with SSRI’s, especially in children have shown a placebo response up to 40% making treatment with placebo more ethically acceptable [Kennard 2009].

The RCTs have been approved by the Central Committee on Human Research in the Netherlands (CCMO), the Pharmo cohort study has been approved by the local medical ethics committee (METC) of the Academic Medical Center Amsterdam (AMC). All subjects participate on a voluntary base and receive a small financial compensation (50 euro and

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**Figure 2. Comparison of mean Δ in treated patients minus mean Δ in placebo treated patients.**
travel expenses). Written and informed consent from both patients and legal caregivers will be obtained in all cases.

DISCUSSION

In the ePOD studies we propose a set of neuroimaging studies and neuropsychological assessments in which we examine the neural circuitry in adolescents with depression or anxiety and ADHD before and after treatment. As pointed out recently in an editorial from the American Journal of Psychiatry (Cullen, 2012), this type of research is greatly needed in a field in which most imaging studies have been conducted in adults. Because of ongoing brain development during adolescence, the neuropathophysiology, let alone the treatment, that underlie these disorders could be distinct. Slowly emerging evidence suggests that the long-term effects of drug exposure are delayed and expressed once the vulnerable system reaches maturation (i.e., typically during adulthood). This phenomenon, known as neuronal imprinting, occurs when the effects of drug exposure outlast the drug itself. Thus, understanding the persistent effects critically depends on the window of observation. Therefore, ePOD is a unique clinical study in children and (young) adults which will exactly grab this window of opportunity to measure age related effects of psychotropic drugs with sophisticated neuroimaging techniques. Embracing this concept should influence how we conduct preclinical assessments of developmental drug exposure, and ultimately how we conduct clinical assessments of drug efficacy, effectiveness, and safety for the treatment of childhood psychiatric disorders (Andersen and Navalta, 2004).

As the safety of antidepressants to children still is a subject of concern, particularly since FLX is now licensed for the treatment of MDD in children of 8 years and older, information about the safety of FLX in treating childhood depression is needed. Especially the potential for an increased suicide risk in association with SSRIs in general has led to much debate, as has also been pointed out by the Medicines Evaluation Board of the Netherlands (Wohlfarth et al., 2006) and several comments in the Lancet in response to an article by Ebmeier and colleagues (Ebmeier et al., 2006).

The neurotransmitter 5-HT plays a crucial role in axonal outgrowth of 5-HT projections during brain development (Whitaker-Azmitia et al., 1996). Earlier animal work demonstrated that postnatal 5-HT pharmacological manipulations can lead to abnormal outgrowth of the 5-HT system (Azmitia et al., 1990; Shemer et al., 1991; Won et al., 2002). As an SSRI, FLX increases extracellular 5-HT concentrations by blocking SERT. Recently, pilot experiments of our group have shown that chronic treatment with FLX results in a significant increase in prefrontal and hypothalamic SERT (+30%, p< 0.01) in juvenile-treated rats, but not in adult-treated rats. These findings are in line with findings of Wegerer et al.
and Bock et al., which also have shown that this effect persists into adulthood, long after discontinuation of treatment with SSRIs (Bock et al., 2005; Wegerer et al., 1999). Also, we showed with phMRI that juvenile-treated rats respond more strongly to a 5-HT challenge than same-age untreated rats, while adult-treated rats show a diminished response after previous chronic treatment (Klomp et al., 2012). The phMRI technique is very well suited to address the primary objective of the ePOD-MPH studies: investigating whether the effect(s) of FLX on serotonin depend upon age. As may be expected, on a behavioural level, results are less consistent, although age-dependent responses to SSRIs on depression-like behaviour are described in both rats and mice (Homberg et al., 2011; Iñiguez et al., 2010; Mason et al., 2009). All these findings most likely reflect the earlier described neuronal imprinting effects.

MPH is being prescribed to increasingly younger children (van Dijk et al., 2008; Zito et al., 2002). A meta-analysis has shown that in the USA and Australia up to 18 – 66% of those treated with stimulants do not meet the criteria for ADHD (Rey and Sawyer, 2003). The increased prescription rates and concerns about proper diagnostic protocols have led to much public debate on the safety of MPH in the treatment of children. Indeed, a meta-analysis has shown that non-compliance is estimated at 20-65% and is attributed in part to apprehension about the safety of psychostimulants (Swanson, 2003). Recent work on the effects of MPH has shown that it may indeed normalize rates of cortical thinning, especially that of the prefrontal cortex (Shaw et al., 2009). In addition, in adult ADHD several reports on grey matter reductions were not able to distinguish between ADHD and psychostimulant effects (Amico et al., 2011; Seidman et al., 2011). However, reports on greater rates of depression and anxiety in the treated groups of the MTA study sample and in several studies involving rats indicate that effects of MPH treatment may have mixed positive and negative effects (Bolanos et al., 2003; Gray et al., 2007; Molina et al., 2009). Our main outcome parameter phMRI may be able to shed more light on the effects of MPH on the development of the DAergic system. This will increase our understanding of the safety and working mechanisms of MPH in a vulnerable population. In addition, we will gain insight into basal neurocognitive and neuroadaptive processes in the developing brain, as well as increasing our knowledge on the pathophysiology of ADHD.

As recently indicated by Tao and colleagues, studies are needed that use the same methodology simultaneously in both adolescents and adults, to overcome methodological differences, and correct interpretation of the age-dependency of results (Tao et al., 2012). Sample differences in age and illness status or differences in the image acquisition/analysis approach may obscure the age-dependency of the findings. These issues are overcome by the current study design. Since this study employs randomized controlled trials and has the benefit of having before and after treatment measurements from each patient, we will be able to reduce subject variability. This increases the ability of our trial to detect differences between groups. Moreover, this study employs novel non-invasive MRI techniques in children
and adolescents, which provide new insights into the effects of psychotropic drugs on the developing brain. The use of phMRI in assessing DAergic and 5-HTergic functionality may have important prognostic factors, for instance in predicting responsiveness to psychostimulants or antidepressant medication in the near future.

Conclusion

So far, most imaging studies have been conducted in adults. Ongoing brain development during adolescence may distinguish the neural mechanisms that underlie psychiatric disorders like depression, anxiety, and ADHD. Examination of these mechanisms during early phases of the disorder provides the opportunity to avoid confounds due to complex treatment histories or potential scarring from years of disease. A better understanding of adolescent-specific mechanisms will be “a critical foundation for the advancement of early treatment interventions, which could significantly affect public health” (Cullen, 2012).

In the ePOD studies we propose a set of neuroimaging studies and neuropsychological assessments in which we examine the neural circuitry in adolescents with depression or anxiety and adolescents with ADHD before and after treatment. The combination of prospective studies with a cross-sectional cohort, using the same outcome measures, will increase our understanding not only of the working mechanisms of both FLX and MPH in children and adolescents, but also provide more information about the safety of these substances in the maturing brain.