Detecting dopamine dysfunction with pharmacological MRI

Schrantee, A.G.M.

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Chapter 6

The effects of Psychotropic drugs On the Developing brain (ePOD) study: methods and design

Marco A Bottelier, Marieke LJ Schouw, Anne Klomp, Hyke GH Tamminga, Anouk Schrantee, Cheima Bouziane, Michiel B de Ruiter, Frits Boer, Henricus G Ruhé , Damiaan Denys, Roselyne Rijsman, Ramon JL Lindauer, Hans B Reitsma, Hilde M Geurts, Liesbeth Reneman

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ABSTRACT

Background: Animal studies have shown that methylphenidate (MPH) has different effects on the dopaminergic system in the developing brain compared to the developed brain. The effects of Psychotropic drugs On the Developing brain (ePOD) study is a combination of different approaches to determine whether there are related findings in humans.

Design: Animal studies were carried out to investigate age-related effects of psychotropic drugs and to validate new neuroimaging techniques. In addition, we set up a double-blind placebo controlled clinical trial with MPH in 50 boys (10–12 years) and 50 young men (23–40 years) suffering from attention-deficit/hyperactivity disorder (ADHD) (ePOD-MPH). Trial registration number: Nederlands Trial Register NTR3103. A cross-sectional cohort study on age-related effects of these psychotropic medications in patients who had been treated previously with MPH (ePOD-Pharmo) is also ongoing. The effects of psychotropic drugs on the developing brain are studied using neuroimaging techniques together with neuro-psychological and psychiatric assessments of cognition, behavior and emotion. All assessments take place before, during and after chronic treatment.

Discussion: The combined results of these approaches will provide new insight into the modulating effect of MPH on brain development.

INTRODUCTION

The brain in development is dependent on the emergence of critical developmental processes (i.e. synaptogenesis (Swaab and Mirmiran, 1986)), and therefore sensitive to pharmacological interventions. Treating children and adolescents with dopaminergic (DAergic) drugs like methylphenidate (MPH), is therefore likely to have influence on the maturation of the brain.

Recent animal studies with MPH, a DA reuptake inhibitor and stimulant drug frequently prescribed in the treatment of attention-deficit/hyperactivity disorder (ADHD), have demonstrated that these effects are also age-dependent. For instance, early treatment with MPH led to a considerable (-50%) reduction of dopamine transport density (DAT) in rat striatum when compared to non-treated animals, whereas no effects were observed in adult animals (Grund *et al*, 2006). These alterations in the DA system have been shown to result in behavioral abnormalities. For example, young rats treated with MPH show more anxiety- and depression-related behavior in adulthood than adult rats treated with MPH (Bolaños *et al*, 2003).

There is some clinical evidence for related findings in humans. For example, in the National Institute of Mental Health (NIMH) Collaborative Multisite Multimodal Treatment Study of Children With ADHD (MTA) children who received behavioral therapy had a lower rate of diagnoses of anxiety or depression (4.3%) than the children who were treated with MPH (19.1%), thus indicating a (transient) increase in the occurrence of emotional disorders six to eight years after treatment with MPH (Molina *et al*, 2009). Age-related differences have also been found between adolescent and adult patients on in functional magnetic resonance imaging (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 (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 two studies (the effects of Psychotropic drugs On the Developing brain 'ePOD' project): a randomized controlled trial (RCT) 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 objectives

1. The primary objective of the ePOD study is to report on the short-term age-dependency of the effect(s) of MPH treatment on the developing DA system using pharmacological MRI (phMRI) as our main outcome measure.
2. Furthermore, we aim to study the long-term effects of these drugs in a cohort study based on medical prescription data.

Secondary objectives

Our secondary objectives are:

1. To report on the age-dependency of MPH on the outgrowth of the DA system using functional outcome measures (diffusion tensor imaging [DTI], fMRI, resting-state-fMRI [rs-fMRI] and neuropsychological assessment (NPA)).
2. To report on the effects of MPH on restless legs (RLS) symptoms and insomnia.

METHODS

General design of the ePOD project

Only a long-term prospective study in patients randomly assigned to MPH and placebo conditions can determine unequivocally whether the (adverse) effects of this medication on the neurotransmitter systems interact with the age when they are prescribed. To this purpose we designed an RCT with MPH. 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 months, we investigate the long-term effects (at least 7 years) in a cohort study based on medical prescription (the ePOD-Pharmo study). The two sub-studies of the ePOD project include:

- ePOD-MPH: A 16 week RCT with MPH in 100 medication naive ADHD patients. This RCT involves three separate NPA and MRI assessments: the first before starting with the study medication (baseline session), the second during treatment with MPH or placebo (week 8) and the final assessment after trial end following a 1-week washout period (week 17).
- ePOD-Pharmo: A cohort study based on medical prescription data. Seventy-five subjects will be recruited through a database containing prescription data on MPH. Subjects in this cohort based study will receive the same assessments as in the RCT but only once.

Randomized controlled trial: design and study sample

The RCT consist of 16-week multicenter randomized, double blind, placebo-controlled trial with a washout period of one week (Figure 1). Subjects are stratified into two age categories: boys aged 10–12 years, and men aged 23–40 years. These two age groups are randomly assigned to either placebo or active treatment. MRI and NPA assessments will take place before treatment (baseline), during treatment and following the washout period. Baseline measurements will be compared with the results obtained at trial end. Differences in outcome measures will be compared between the two age categories (children vs. adult), in addition to healthy controls (separate study). In view of our hypothesis that the active treatment results in long lasting or

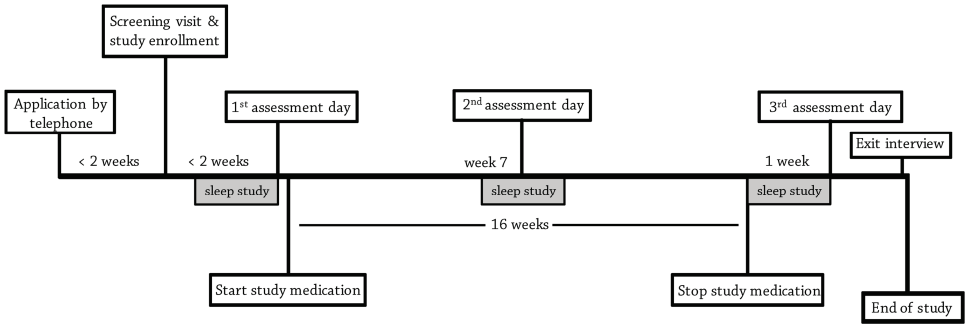

Figure 1. Timeline study procedures ePOD-MPH trial

even permanent changes in the developing brain, we expect no or a small change in difference scores between baseline- and post-treatment assessments in adults, whereas in children we expect to find larger changes, as enduring changes will have taken place in the developing brain, but only transient accommodation in the developed brain. The washout period was chosen based on chemical properties (rate of elimination based on five half-life times) and ethical considerations (time without treatment).

A total of 50 children (10–12 years of age) and 50 adult (23–40 years of age) male outpatients diagnosed with ADHD (all subtypes) and in need of pharmacological therapy will be included in ePOD-MPH RCT. Patients that have used medications or are dependent on drugs that influence the monoamine systems before age 23 are not eligible.

Patients are recruited from clinical programs at the Child and Adolescent Psychiatry Center Triversum (Alkmaar), from the department of (Child and Adolescent) Psychiatry of the Bascule/AMC (Amsterdam), and from PsyQ mental health facility in The Hague. The diagnosis is 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, authorized Dutch Translation) (Ferdinand and van der Ende, 1998), in children or in parents and the Diagnostic Interview for Adult ADHD (DIVA) (Kooij, 2012) in adults in the RCT with MPH. In both RCTs, 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), authorized Dutch translation (Schmand *et al*, 1998) and MDD patients with current risk of suicide attempt are excluded.

We chose to include only male patients in ePOD-MPH to limit subject variation and because ADHD is most prevalent in males (Boyle *et al*, 2011). Thus, to keep our sample as homogenous as possible and prevent inclusion problems, only male subjects are included in the ePOD-MPH study. The cut-off point of 10–12 years of age was 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 (Bashat *et al*, 2005), whereas the rate of increase of neuronal growth and pruning reduces after 10 years of age. The cut-off point of 23 years for matured

brain in the adults is chosen in line with previous studies involving a comparison between matured versus immature brain (Sowell *et al*, 1999).

Cohort based study: design and study sample

In the ePOD-Pharmo study, we investigate the long-term effects of age following MPH treatment on our main outcome parameter (phMRI). Exposed subjects are stratified into two age groups: one group that has been prescribed early in life with these medications, and another group late in life. Subjects are recruited through a medical prescription database from the Pharmo Institute (Utrecht, the Netherlands). This out-patient pharmacy database is a database that contains drug dispensing data since 1986 from over 3 million residents in the Netherlands, corresponding to approximately 20% of the Dutch population. The dispensing date, prescriber, prescribed dosage regimen, and duration are known. Subjects participate in a single assessment day (cross-sectional design) with similar NPA and MRI investigations as in the ePOD RCTs, mentioned above. Subjects eligible for study participation are 23–40 years of age and presumably diagnosed with ADHD. The early exposed group contains subjects with a history of MPH (male subjects) before the age of 16 (thus at least 7 years ago). The late exposed group contains subjects treated between 23 and 40 years of age. The early-, and late exposed groups will be compared to an age-, and gender matched unexposed control group, consisting of medication naive subjects suffering from ADHD. Every group (three in total) will contain 25 subjects.

Assessments

Clinical rating scales

We use a set of clinical rating scales to assess symptom severity and functioning at baseline and after treatment. An authorized Dutch translation of the Disruptive Behavior Disorders Rating Scale (DBD-RS) (Pelham *et al*, 1992) will be used in children and in adults the ADHD-SR (Kooij *et al*, 2008). Clinical improvement will be rated in both RCTs by the clinician using CGAS (Shaffer *et al*, 1983) and CGI (Guy, 1976) scales in children, and in adults using the Global Assessment of Function (Endicott *et al*, 1976). The Children's Depression Inventory (CDI) (Saylor *et al*, 1984) and 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. These rating scales are also administered in the ePOD-Pharmo study.

Imaging parameters

DAergic brain activity will be assessed using phMRI, which is the primary outcome measure of the ePOD project. In addition, DA connectivity will be assessed using rs-fMRI and DTI, and functional brain activity using inhibition and emotional processing fMRI tasks.

phMRI - Application of fMRI in combination with a pharmacological challenge (phMRI) has the potential to provide an index of changes in neurotransmitter function. With phMRI a neurotransmitter specific pharmacological challenge is given, which causes changes in neurovascular coupling and subsequent region-specific changes in brain hemodynamics. It differs from fMRI, in that the neuronal system is not activated by a motor or cognitive task, but

pharmacologically. phMRI has been shown to adequately assess the DA integrity and functionality, as DA-lesioned primates showed a blunted hemodynamic response to a d-amphetamine challenge, following DA lesioning, which correlated strongly with DA transporter availability and motor function (Jenkins *et al*, 2004). During the phMRI scan, after several minutes of baseline scanning, 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). This challenge dose was chosen as it induces maximum blockade of the DAT (80% occupancy), which occurs at serum concentrations of about 8–10 ng/ml. Higher concentrations are not likely to be very effective in further blocking DAT (Swanson and Volkow, 2003). After 90 minutes, subjects will undergo a second MRI session, and the same MRI sequences are repeated, now under the influence of MPH. The 90 minute 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). Based on the literature (reduction in DAT densities in young, but not adult treated animals) (Moll *et al*, 2001) and experiments from our own group in d-amphetamine users with phMRI and a MPH challenge (Schouw *et al*, 2013), we expect that treatment with MPH will induce a long-lasting changes in the brain hemodynamic phMRI response in DA rich brain areas (e.g. striatum) in children, but not adults. We expect that in adult patients MPH will be accommodated by a series of transient compensatory reactions. However, in children MPH will induce changes in the form of long-lasting developmental alterations of the system, reflecting existence of 'neuronal imprinting' in the human brain (Andersen and Navalta, 2004).

rs-fMRI - A relatively new fMRI approach (i.e., resting-state fMRI (rs-fMRI)) allows assessment of changes in organization of whole functional networks, including DAergic networks. Rs-fMRI detects 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). A decreased functional connectivity between anterior cingulate cortex and precuneus has been found using this technique in adult ADHD patients (Castellanos et al, 2008). There are a number of studies that have investigated the effects of MPH on this parameter (Rubia et al, 2009; Wong and Stevens, 2012; Zhu et al, 2013), which found that these drugs normalize brain activation and functional connectivity abnormalities in patients suffering from ADHD. In accordance with this literature, we expect to find age-dependent normalization of functional connectivity abnormalities.

DTI - With diffusion tensor imaging (DTI), the micro-structural organization of white matter (WM) can be visualized. 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 readout marker 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 a decrease in FA (Moeller *et al*, 2015; Reneman *et al*, 2001; de Win *et al*, 2007). A previous DTI study in children suffering from ADHD, observed an increase, or rather normalization, of white matter volume in ADHD medicated children compared to unmedicated

children (Castellanos *et al*, 2002). In line with this, 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). Furthermore, in a recent study in rats we observed opposite effects of MPH on FA measures: MPH induced an increase in FA in the corpus callosum of adolescent rats, whereas a slight reduction in adult animals (van der Marel *et al*, 2014). Therefore, we also expect to find age-related changes in the current RCT with MPH: an increase in FA in MPH treated children when compared to pre-treatment baseline scans, and no effect or a small effect in adult patients.

fMRI - We have selected two fMRI task paradigms. 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:

- An emotional processing task: 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 en modulatory responses. Emotion dysregulation is an important feature of ADHD and can affect the course and outcome of the disease (Barkley and Fischer, 2010).
- A motor inhibition task: 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 assessment

A neuropsychological test battery (Standard Reaction Time Task, Rey Auditory Verbal Learning Task (Van der Elst *et al*, 2005), 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 cognitive 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 trial using questionnaires (Cambridge-Hopkins RLS questionnaire (CH-RLSq, International RLS severity scale (iRLSS), John Hopkins RLS severity scale (JH-RLS-ss), Epworth sleepiness scale (ESS) and the the Holland Sleep Diagnostic List (HSDL) (Kerkhof *et al*, 2013)) 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 to monitor 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.

Potential confounders

The study is designed to limit several important possible confounding parameters, such as gender effects 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 in the RCTs. Because of the design of the study, we have limited power and can correct for a maximum of 2 or 3 confounders. Therefore, age (in months) and ratings of symptom severity will be taken into account as covariates.

Power analysis

Since these trials are the first to examine DA functioning following 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 MPH on the outgrowth of the DA-ergic system if these differences are in the magnitude of a standardized effect size of 1.25. From pilot experiments in rats and studies in humans with known alterations of DA (e.g. d-amphetamine users) 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. These paired data 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. To account for an expected drop-out of 25% and motion artifacts in MRI scanning, we will include 25 patients in each treatment-by-age

group. Because of slightly higher subject variability (but less motion artifacts in adults) 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 on the outgrowth of the DA-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 intention-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 analysis of variance (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 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 sleep-log actigraph for the trial.

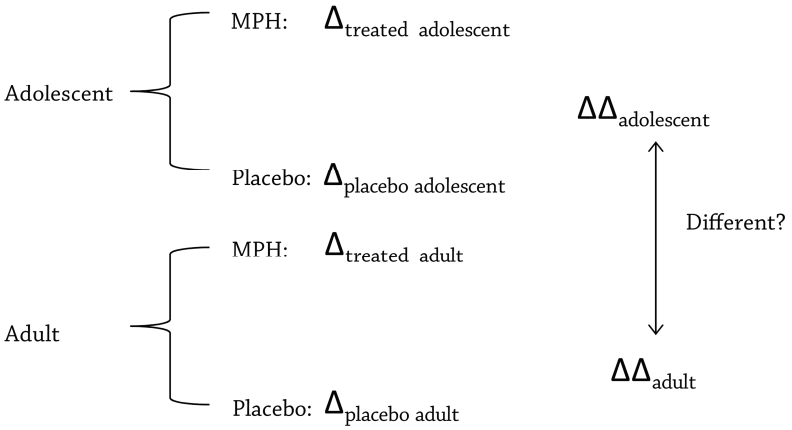

Figure 2. Statistical analysis age*treatment effect

Ethical considerations

Evidently, there are important ethical considerations that need to be taken into account for 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 trial we overcome the treatment delay by including patients from the waiting list and offering psycho-education when necessary.

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 travel expenses). Written and informed consent from both patients and legal caregivers will be obtained in all cases.

DISCUSSION

In the ePOD project we propose a set of neuroimaging studies and neuropsychological assessments in which we examine the neural circuitry in adolescents with 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 pathophysiology that underlies these disorders, let alone the treatment, could be distinct. Slowly emerging evidence suggests that the long-term effects of drug exposure are delayed and only 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 (Andersen and Navalta, 2004). Thus, understanding the persistent effects critically depends on the window of observation. Therefore, ePOD is a unique clinical study in children and 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).

MPH is being prescribed to increasingly younger children (van Dijk *et al*, 2008). 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 for 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 (Bolaños *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.

However, there are also some limitations of the present study designs that need to be mentioned. For example, no conclusions from the ePOD-MPH RCTs can be made on the long-term effects of medication on brain development. The RCTs last for 'only' 4 months, and the washout period is one week maximum. For that reason, we designed the ePOD-Pharmo study, in which subjects are screened at least 7 years later following early MPH exposure. In addition, all participants in the RCTs are asked if they are willing to participate in a follow up study, scheduled in 3–5 years. Thus, by combining the RCTs in which we investigate the causality of the age-dependency of MPH, together with the ePOD-Pharmo study which is directed towards the long-term effects of these medicines, will ultimately provide missing knowledge.

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. 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 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 distinct the neural mechanisms that underlie psychiatric disorders like 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 ADHD before and after treatment. The combination of prospective studies with a cross-sectional cohort study, using the same outcome measures, will increase our understanding not only of the working mechanisms of MPH in children and adolescents, but also provide more information about the safety of these substances in the maturing brain.

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