Effect modification by age of methylphenidate on the development of the dopaminergic system in the brain

"ePOD-MPH"

RESEARCH PROTOCOL

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PROTOCOL TITLE Effect modification by age of methylphenidate on the outgrowth of the dopaminergic system in the brain

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form (General Assessment and Registration form) is the application form that is required for submission to the accredited Ethics Committee (ABR = Algemene Beoordeling en Registratie)
ADHD	Attention-Deficit/Hyperactivity Disorder
AE	Adverse Event
AR	Adverse Reaction
ASL	Arterial Spin Labelling
BOLD	Blood Oxygen Level-Dependent
CA	Competent Authority
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
CCMO	Central Committee on Research Involving Human Subjects
CV	Curriculum Vitae
DA	Dopamine
DAT	Dopamine Transporter
DBD	Disruptive Behavioral Disorder scale
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4th Edition
DSMB	Data Safety Monitoring Board
DTI	Diffusion Tensor Imaging
ELIT	Evaluation List Insomnia Therapy
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials GCP Good Clinical Practice
FA	Fractional Anisotropy
(rs)-fMRI	(Resting State) Functional Magnetic Resonance Imaging
HSDL	Holland Sleep Diagnostic List
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MPH	Methylphenidate
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
phMRI	Pharmaceutical Magnetic Resonance Imaging
PPAA	alpha-phenyl-2-piperidine acetic acid (ritalinic acid)
RLS	Restless Legs Syndrome
(S)AE	Serious Adverse Event
SPC	Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)
SPECT	Single Photon Emission Computed Tomography
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WISC-R	Wechsler Intelligence Scale for children-Revised
WMO	Medical Research Involving Human Subjects Act (Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale:

50-90% of prescribed pediatric drugs have never been tested or licensed in children, only in adults. Approximately 100 million children in the European Union are prescribed off-label or unauthorized drugs and in doing so risk adverse reactions or do not respond to treatment at all. In fact, medication doses used in children are no more than 'guestimates'. Clearly, there are potential dangers in assuming that children will have the same response to therapy as adults. Methylphenidate (MPH) is primarily used as treatment for attention deficit hyperactivity disorder (ADHD), effectively reducing symptoms of inattention, hyperactivity, and impulsivity in up to 70% of children. It is assumed that MPH does this by blocking the DA transporter (DAT) thus increasing extracellular DA in the brain. Its efficacy and safety has been documented in many studies. However, there is still a gap of knowledge concerning the influence of MPH on brain development and its effect on brain structure and function. Studies in animals and humans raise serious concerns and call for further investigation of possible short and long-term effects on brain structure and function.

Hypothesis:
Administration of MPH during brain development, but not in adulthood, results in an altered outgrowth of the DA system. This long-lasting disturbance of the DA system may result in behavioral abnormalities, such as anxiety and depression.

Objectives:
Primary objective of the study:

1. To report on the effect modification by age of MPH treatment on the outgrowth of the DA system using state-of-the-art Magnetic Resonance Imaging (MRI) techniques

Secondary objectives:

1. To report on the effect modification by age of MPH on the outgrowth of the DA system using several functional outcome measures (functional MRI (fMRI), neuropsychological test battery)
2. To report on the effects of MPH on restless legs syndrome (RLS) symptoms and insomnia.

Study design:
A pharmacological MRI (phMRI) study for assessment of dopaminergic function and connectivity in a 16-week multicenter randomized, double-blind, placebo-controlled trial with MPH in 100 children-, and adult male ADHD patients in which the effect modification by age is investigated before and after treatment. Patients will be stratified into two age groups: adolescents (10-12 years of age) and adults (23-40 years of age) and randomly assigned to receive a flexible dose of either MPH or placebo, resulting in four groups consisting of 25 subjects each.

Study population:
50 children (10-12 years of age) and 50 adult (23-40 years of age) male outpatients diagnosed with combined type ADHD as defined in the DSM-IV, and in need of pharmacotherapy according to existing guidelines.

Interventions:
- Random assignment to a flexible dose of MPH or placebo drug treatment for a period of 16 weeks with a 1 week washout period.
- 3.0 Tesla MR imaging including pharmacological phMRI following a DA challenge with oral MPH (0.5 mg/kg: approximately 15 mg for children, 35 mg for adults), diffusion tensor imaging (DTI) and resting state and task-related fMRI scans.
- Assessment of a neuropsychological test battery.
- Assessment of sleep timing using an actigraph, sleep log, and questionnaire.

All MRI imaging and the neuropsychological tests will take place at the AMC twice: once before start of study medication (baseline) and 18 weeks later. These assessments will last 5 hours total. During treatment a small assessment (short MRI scan and neuropsychological assessment) will take place (1 hour total). The assessment of sleep timing will take 75 minutes total.

Main study parameters/endpoints:

Primary study parameters/endpoints:

- phMRI: % change in ASL signal from baseline in response to acute oral MPH challenge before and after 16 weeks of MPH treatment
- DTI: % change in FA and MD values from baseline after 16 weeks of MPH treatment
- Resting state fMRI (rs-fMRI): % change in functional connectivity (FC) within specific (DA) neuronal networks
- % change of above mentioned outcome parameters during treatment vs. baseline and post-treatment

Secondary study parameters/endpoints:

- fMRI: % change in task related BOLD signal from baseline
- Neuropsychological functioning: change in outcome of several well-validated neuropsychological (computer) tasks addressing emotional processing and impulsivity/behavioral inhibition compared to baseline measurements.
- Sleep log and actigraphy: % change from baseline

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

Risks and burden

Pharmacotherapy, MPH and placebo: Subjects are randomly assigned to either MPH or placebo treatment for 16 weeks. The pharmacotherapy with MPH as proposed in this study does not differ from current standards of treatment for ADHD. MPH is registered in children aged 6 years and older for treatment of ADHD in the Netherlands and abroad. Since all patients are in need of pharmacotherapy according to existing medical guidelines, treatment with MPH should be considered part of standard clinical practice and poses no extra risk or burden. Side effects, however, are mild and concern appetite suppression. In the Netherlands a waiting list of 16 weeks is very common before (child- and adolescent) psychiatric evaluation can take place and treatment can be commenced. It is within this timeframe that the current study will be executed. All patients will start pharmacotherapy within 2 weeks after study enrollment. Patients can start or continue active treatment as soon as the second assessment of DA function is finished, which will be within 17 weeks after study enrollment. Thus, there will be only a very small or no delay in the placebo group before active treatment will take place. Following the treatment period, subjects will be drug free for 1 week. A drug free period is common in standard practice today (e.g., ‘drug free holidays’). The wash-out period therefore poses no extra risk and the burden of re-emerging symptoms is considered minimal. Taking all this into consideration we consider the risks

associated with study participation negligible, and the burden minimal with respect to pharmacotherapy in the MPH and placebo groups.

MR imaging: MRI is a non-invasive imaging modality. MRI studies in children were earlier approved by the medical ethical evaluation board (METC) of the Academic Medical Centre (AMC) Amsterdam in children from 8 years of age and older (e.g. MEC 06.053). Extensive coaching and preparation of the children to prevent stress and anxiety will be part of the MRI scanning procedure. Every possible step will be undertaken to make sure the burden experienced by the MRI scans is kept to a minimum. The risks associated with study participation involving MRI scanning are negligible, and the burden is considered minimal.

Oral challenge with MPH: In order to evaluate the DA system with MRI we need to administer a DA challenge. The use of phMRI is the least invasive tool available at this moment for determining DA function, as all other methods available to assess DA function involve more invasive techniques (e.g., PET/SPECT involving radiation exposure) and are therefore objectionable, especially in children and adolescents. We will use a single dose of MPH: 0.5 mg/kg (approximately 15 mg for children, 35 mg for adults). MPH (0.5 mg/kg) has been administered as an oral bolus in previous MRI studies up to 50 mg, which was well tolerated (Rao 2000, Mehta 2000, Silveri 2004). This dose may cause an increase in heart rate and systolic blood pressure, but has no significant side effects. We feel that administration of oral MPH to ADHD patients in this study is justifiable for a number of reasons: a) the challenge will be done with the same medication they would normally be treated with, only in a slightly higher dosage b) the placebo group would have normally received MPH and will, in all likelihood, receive MPH treatment after their study participation ends c) A similar dose (0.6 mg/kg), at commencement of treatment has been successfully given at times in order to convince parents of the diagnosis of ADHD and of the benefits of MPH treatment (Kent 1999). This dose was well tolerated in the treatment group of children and adolescents aged 4-14 years. Thus, also with respect to the oral challenge, we classify the risks associated with study participation as negligible, and the burden as minimal.

Other interventions: There are no risks associated with the assessment of a neuropsychological test battery, the sleep log and questionnaires nor collecting saliva samples. The burden associated with the neuropsychological tests is considered minimal. Actigraphy (five days on three occasions), sleep log (five days on three occasions) and completion of questionnaires (three times) will pose a minimal burden on the subjects.

Benefit

There is a group benefit associated in participating in this study. This study will increase our understanding of the safety of MPH in young children and adolescents, as more information about the effects of MPH on the maturing brain will be available. The safety of prescribing MPH to children and adolescents has been the subject of increasing concern in the community. The combined results will increase our understanding of the safety of MPH in children and adolescents. This is particularly important because a lot of children treated with MPH for ADHD do not meet the criteria for this disorder, or are treated under pressure of school (Zembla 2010). In addition, we will gain insight into basal neurocognitive and neuroadaptive processes in the DA system of the developing brain, as well as the pathophysiology of ADHD. Furthermore, these studies serve to emphasize the importance of ensuring a proper diagnosis when prescribing psychotropic medications. It may be concluded that it is beneficial to treat children and adolescents with MPH as early as possible in life, for a brief period of time, thereby normalizing a malfunctioning DA system. This is also of considerable interest because of increased depression and anxiety following MPH treatment (Molina 2009). Eventually, these data will result in a better healthcare of children and adolescents. Validating the phMRI response as an indicator of treatment effect may help in identifying new effective and efficient pharmacological interventions. Finally, but most importantly, there is also an individual benefit associated with study participation: the

subjects in the MPH group will start pharmacological treatment within 2 weeks after study enrolment, whereas in standard practice this can mount up to 4 months, due to long waiting lists at departments of (child- and adolescent) psychiatry.

Group relatedness:
Since we study the effect of MPH on outgrowth of the DA system in the maturing brain, it is essential to include individuals in which brain development is still ongoing, thus minors. The cut-off point for children of 10-12 years of age is chosen because several MRI parameters greatly change until 8-10 years of age (Ben Bashat 2005), whereas the rate of increase of grey and white matter maturation reduces after 10 years of age. Peak prevalence of ADHD is 10 years of age (Burd 2003). Only male subjects are included in the present study, since ADHD is more prevalent in boys than in girls and because the maturation of the brain greatly differs between boys and girls (Giedd 2004). Although there is already much known about the effects of MPH on dopaminergic function, we need to include adult patients (23-40 years of age) in this study to compare with the children, because the state-of-the art imaging techniques we are using in the current study have not been used in this context before. We also need to include adult subjects to be able to study the effects of age (they are thus essential in meeting our study objectives), and be able to refer our data to. This study will not only provide new insights into the modulating effect of age on MPH response, but will also enhance our understanding of ADHD in the pediatric population, of and the role of DA and dopaminergic interventions upon brain development. Most importantly, this study serves to investigate the relevance of ensuring a proper diagnosis when prescribing stimulants such as MPH.

In conclusion
The overall nature and extent of the risks associated with participation in the current study are to be classified as negligible and the burden minimal. There is an individual-, as well as a group benefit associated with study participation.

1. INTRODUCTION AND RATIONALE

ADHD is a condition in which children can display any of the following behavior: increased distractibility, heightened impulsivity, problems concentrating or hyperactivity (DSM-IV 2002). This can lead to problems in academic and social functioning and increases the risk of developing behavioral problems or substance use disorders (Rapport 2002, Brown 2005). Since the 1950's the symptoms of ADHD in children have been successfully treated with stimulants. A growing number of children are being diagnosed with ADHD and subsequently treated with stimulants, mainly methylphenidate (MPH). Over the last decades not only the amount of children diagnosed and treated has increased considerably, children are also being diagnosed and treated at younger ages (Zito 2000; Van Dijk et al. 2008). Methylphenidate (MPH) has been extensively tested on adults and the effectiveness of the drug on ADHD symptoms in school-aged children has been found to be significantly better than behavioral treatment (anon. 1999). However, aside from behavioral measures, little is known about the short and long-term effects of MPH on neural functioning and brain development. This lack of information on the effects on brain development causes unease with parents concerning the drug's safety and as a consequence often leads to poorer compliance with treatment. The lack of scientific knowledge leads to a lot of speculations, and a true feast for the media (e.g. ADHD is in Argos juni 20010, de ADHD hype Zembla september 2010).

MPH blocks the DA transporters (DAT) in the brain. This causes greater amounts of extracellular in dopaminergic brain regions such as the striatum and prefrontal cortex (Volkow 1998, Castellanos 2002). Recent animal studies have demonstrated that the effect of MPH on dopaminergic functioning in the brain differs between children and adults, indicative of an age effect of treatment. For instance, early treatment with MPH leads to a considerable (-50%) reduction of DAT in the striatum and other dopamine-rich regions of rats when compared to non-treated animals, whereas in adult animals consistently no effects have been observed (Moll 2001, Grund 2006). Also, in young animals MPH induces immediate early gene activation consistent with long-term brain plasticity and reorganization (Adriani 2006, Penner 2002, Chase 2003, 2005, Brandon 2003), and it has been shown that MPH produces oxidative damage in the frontal cortex of young-, but not adult rats (Martins 2006), attenuates adult hippocampal neurogenesis only in young rats (Lagace 2006) and increases rCBV (Anderson 2008) See 4.4. for more details on some of these studies, and in addition studies conducted by our own group.

These alterations in the DA system have been shown to relate to behavioral abnormalities. For example, young treated rats with MPH show more anxiety and depression related behavior than adult rats (Gray 2006, Bolanos 2003)). Also in children randomly assigned to 14-month treatments in the NIMH Collaborative Multisite Multimodal Treatment Study of Children With Attention-Deficit/Hyperactivity Disorder (MTA; N = 436) 6 to 8 years previously, the children who received behavioral therapy had a lower rate of diagnoses of anxiety or depression (4.3%) than the children in the combined (17.7%) or medication treatment group (19.1%) (Molina 2009).Pre-clinical and clinical studies show a decreased sensitivity to the development of substance abuse disorders when exposed to MPH during a period of brain development, when compared to adult life (Anderson 2001, Bolanos 2003).

There are thus solid indications that the developing brain responds differently to psychotropic drugs such as MPH when compared to the adult brain (Andersen & Navalta 2004; Bachrach 2004). Despite the fact that these alterations in brain responses are poorly understood, children receive psychotropic medication such as MPH on a large scale, and this has doubled in the Netherlands over the past five years. Last year the number of prescriptions for MPH increased from 506.000 to 581.000 (NRC Handelsblad augustus 2010). Strikingly, up to 66% of those treated with stimulants for ADHD do not meet the criteria for this disorder (Rey 2003). At school teachers may even require that a child is being treated to improve the performance at school (Zembla september 2010). The above mentioned findings not only implicate the critical role DA plays in the maturation of the brain, but also raise serious concern and call for further investigation of possible short and ong-term effects of MPH on brain structure and function in the human brain. Based on these

findings and observations, the main purpose of this study is to investigate the effect of age on the DA outgrowth after MPH treatment in patients suffering from ADHD and to link these outcomes to findings in animal studies, using non-invasive MRI techniques.

Hypothesis:
Administration of MPH during brain development, but not in adulthood, results in an altered outgrowth of the DA system. This long-lasting disturbance of the DA system may result in behavioral abnormalities, such as anxiety and depression.

2. OBJECTIVES

Objectives:
Primary objective of the study:

1. To report on the effect modification by age MPH treatment on the outgrowth of the DA system using state-of-the-art Magnetic Resonance Imaging (MRI) techniques

Primary outcome measures:
phMRI:
With phMRI, changes in cerebral hemodynamics following a DA challenge can be measured and are expected to reflect the functional activity of DAT (Jenkins et al. 2004). It differs from functional *f*MRI, in that the neuronal system is not activated by a motor or cognitive task, but pharmacologically. Neuronal activity is measured by an increase in blood oxygenation-level dependent (BOLD) signal or an increase in cerebral blood flow (CBF) as measured with arterial spin labelling (ASL), both of which closely correlate with neuronal activity and integrity of the DA system (Sanchez-Pernaute 2005, Chen 1999, Zhang 2006, Hewitt 2005, Shah 2004). For instance, in DA-lesioned animals, intravenous amphetamine challenge of the DA system results in a reduced phMRI response which strongly correlates with PET measures of DAT (see figure below from Jenkins 2004).

SPECT and PET are not suitable for children because of the invasive nature of radioactively labelled contrast agents. This makes phMRI an ideal imaging modality to determine any differences in the DA system. Based on the literature and experiments from our own group (see section 4.4 on power sample calculation for more detail) we expect an increased CBF in specific brain areas (e.g. thalamus and frontal cortex) evoked by DA challenge with MPH in treated children when compared to pre-treatment baseline scans and untreated subjects, reflecting changes in DA neuronal activity.

DTI:
In the current study we will be using diffusion tensor imaging (DTI). DTI is a technique which measures the micro-structural features of white matter (WM) and microfiber pathways by measuring the diffusional motion of water molecules. Fractional anisotropy (FA) is a normalized measure that provides information about the degree of fibre organization and integrity. Any process that results in alterations in axonal architecture, such as altered axonal outgrowth, can result in changes in FA and ADC (Reneman et al., 2001; Moeller et al., 2005; de Win et al., 2007). A previous study observed an increase, or rather or normalization, of white matter volume in ADHD medicated children compared to unmedicated children (Castellanos 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 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.
Again, no effect of treatment on these scan parameters are expected in adults.

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 (for review see Fox and Raichle 2007). In a recent study conducted in rats, we observed a significant age*treatment effect in functional connectivity between the interhemispheric functional connectivity (FC) within the hippocampal-substantia nigra network in rats chronically treated with MPH (see 4.4 for more detail).

Secondary objectives:

1. To report on the effect modification by age of MPH on the outgrowth of the DA system using several functional outcome measures (functional MRI (fMRI), neuropsychological test battery)
2. To report on the effects of MPH on restless legs syndrome (RLS) symptoms and insomnia.

fMRI & neuropsychological tests:
Based on observations in animals (reduced sensitivity to drug abuse and increases in depressive-and anxiety like symptoms) a neuropsychological test battery and fMRI tasks will be administered, addressing emotional processing and impulsivity/behavioral inhibition in particular. In turn this information can be linked to results from phMRI and DTI imaging in order to determine any links between behavioral and fMRI data and changes in the dopaminergic system. We will look for correlations between altered behavioral responses, fMRI responses, phMRI responses, DTI measures and rs-fMRI response (see below).

Restless legs (RLS) and insomnia
RLS is a chronic progressive neurological disorder that has a greater incidence in ADHD children, adolescents and adults than in the general population. It is possible that RLS is co-morbid with ADHD or that they share a common DA deficit. Also, ADHD and RLS have been found to be associated with sleep disorders such as insomnia and a common genetic polymorphism (E.Fliers, personal communication, submitted). In a recent study, 64% of children with ADHD were estimated to suffer from RLS judged by their nocturnal periodic limb movement (Pinchietti 2009). It has been shown that MPH reduces total sleep time but improves sleep quality by consolidating sleep in adults. 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 (see above), 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. The hypothesis is that due to an expected reduction in DA turnover rate after early MPH treatment (see above), there will be long lasting positive effects on RLS symptoms and sleep disorders only in children, but not adults. 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 timing using questionnaires, sleep log and actigraph 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. A small actigraph unit, also called an actimetry sensor, is worn by a patient to measure gross motor activity.

3. STUDY DESIGN

A (pharmacological) MR imaging study combined with a 16-week multicenter randomized, double-blind, placebo controlled trial with MPH in 100 male ADHD patients. Subjects will be stratified into two age categories: children (10-12 years of age) and adults (23-40 years of age) and randomly assigned to either placebo or MPH treatment. This will result in 4 groups of 25 subjects each. To assess the effects of MPH on the outgrowth of the DA system state-of-the art MR imaging studies will be performed, which have been shown to assess the DA system non-invasively. In addition, a neuropsychological test battery will be conducted, sleep timing and presence of RLS will be determined and subjects will be genotyped. Assessments

will take place before treatment (baseline), during (week 8) and 1 week following end of trial (see flowchart below). Baseline measurements will be compared to the results during the trial and trial end. Subsequently, differences in treatment-effect will be compared between the two age categories (children vs. adult). In view of our hypothesis that MPH results in long-lasting or even permanent changes in the developing DA system, we expect no differences between the two age-groups during treatment (due to the presence of MPH), only after stopping the treatment (because the effect of MPH has subsided in adults, but enduring changes in the paediatric brain have taken place). Total burden associated with study participation is 10 hours of assessments at the AMC. In addition, the 3 weeks prior to the assessments we ask the participants to wear an actigraph (wrist-watch) and keep a short sleep log (total burden is 75 minutes for filling in the sleep logs and questionnaires) at home.

FLOWCHART PARTICIPATION

Week **-2**	**-1**	**0**	**7**	**8**	**17**	**18**
		Start trial Baseline			**End trial** Washout	
Screening visit	Actigraphy& sleep log & question-naires	**1st assessment**	Actigraphy & Sleep log & question-naires	**2nd assessment**	Actigraphy & Sleep log & question-naires	**3rd assessment**
Study inclusion		Turn in Actigraph & Sleep log		Turn in Actigraph & Sleep log		Turn in Actigraph & Sleep log
				Neuropsy-chological testing (30')		Neuropsy-chological testing (1 hr)
		Neuropsy-chological testing (1 hr)		MRI scan (1 x 30')		
		MRI scan (2 x 30' with 90' wait) (total 2.5 hrs)				MRI scan (2 x 30' with 90' wait) (total 2.5 hrs)
Total = 0.5 hr (rest is part of standard practice)	**Total= 25'** (5 x 5' daily)	**Total = 4 hrs AMC**	**Total= 25'** (5 x 5' daily)	**Total = 1.5 hr AMC**	**Total= 25'** (5 x 5' daily)	**Total = 4 hrs AMC**

4. STUDY POPULATION

4.1 Population (base)

A total of 100 children (10-12 years of age) and adult (23-40 years of age) male outpatients diagnosed with ADHD (combined type) and in need of pharmacological therapy will be included. Patients will be recruited from clinical programs at the department of (Child and Adolescent) Psychiatry of the Bascule/AMC de Meren (Amsterdam), ADHD_Behandelcentrum (Capelle a/d Ijssel), PsyQ/PBG, Prezens/GGZinGeest and Child and Adolescent Psychiatry Centre Triversum (Alkmaar), as well as 1st lijn (most probably

physicians). Adult medication naïve ADHD patients will also be recruited through advertisement. All subjects will participate on a voluntary base. Since we are studying the effect of MPH on development of the DA system in the maturing brain, it is essential to include individuals in which brain development is still ongoing versus subjects with matured brains. The cut-off point of 10-12 years of age is chosen because peak prevalence of ADHD is 10 years of age (Burd 2003) and also because several MRI parameters greatly change until 8-10 years of age (Ben Bashat 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 1999). The rate of increase in white matter has more or less stabilized, a decrease in grey matter in the striatum and other DA regions has begun, and age effects on DAT densities are not very large within this age-range. Although there is already much known about the effects of MPH on dopaminergic function, we need to include adult subjects in this study, because the state-of-the art imaging techniques we are using in the current study have not been used in this context before. We also need to include adult subjects to be able to study the effects of age (they are thus essential in meeting our study objectives) and to be able to interpret our data (as a reference).

4.2 Inclusion criteria

Male outpatients newly diagnosed with ADHD all subtypes as defined in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV, American Psychiatric Association 1994) and as determined by a structured interview (Diagnostic Interview Schedule for Children fourth edition, DISC-IV; Ferdinand et al., 1998) in parents or Diagnostic Interview voor ADHD bij volwassenen (DIVA; Kooij and Francken, 2010) in adults.

Clinical rating scales:

- Children: Swanson, Nolan and Pelham (SNAP) Questionnaire (Swanson et al, 1982) (by both parent and teacher), Clinical Global Impression scale (CGI), Global Assessment Scale (CGAS Shaffer et al., 1983), Children's Depression Inventory (CDI), and an anxiety scale (SCARED Birmaher 1989)
- Adults: ADHD-SR, Clinical Global Impression scale (CGI), Global Assessment of Function (GAF), Beck Depression Inventory (BDI), Hamilton anxiety scale (HAM-A Hamilton 1959), and Beck Anxiety Inventory (BAI).

Based on the above mentioned criteria, subjects will be enrolled in the study that are suffering from ADHD and are in need of pharmacotherapy with MPH and therefore are eligible for participation in this study. Age range is 10-12 or 23-40 years of age at the time of study entry. Patients are only included after giving informed consent and, in case of minors, informed consent by their legal guardians. A (child and adolescent) psychiatrist will review every patient's ability to give a valid informed consent.

4.3 Exclusion criteria

-Co-morbid Axis I psychiatric disorders requiring treatment with medication at study entry, and a history of major neurological or medical illness (including epilepsy, traumatic brain injury and chronic severe tics or Tourette syndrome).
-IQ < 80 (subtest Wechsler Intelligence Scale for children-Revised (WISC-R; Wechsler 1981) or National Adult Reading Test (NART; Nelson 1991, Dutch translation Schmand et al. 1991)
- Current or previous treatment with medications that influence the DA system (for adults before 23 years of age) such as: neuroleptics, antipsychotics, D2/D3 agonists (pramipexole and ropinirole)
-Current or previous dependency of drugs that influence the DA system (for adults before 23 years of age), such as: MDMA, amphetamine, methamphetamine, cocaine, heroin and LSD

-Contraindications to MPH treatment: cardiovascular diseases such as hypertension, arrhythmia, hyperthyroidism, glaucoma, suicidality, psychosis, Tourette disorder.
-Prenatal use of MPH by mother of the patients.
-Contraindications to MRI (metal implants, pacemakers, claustrophobia, etc.)

4.4 Sample size calculation

Our trial is the first study that examines DA functioning after MPH treatment in children and young adults using MR imaging. This means that there is only limited and indirect data available to perform a sample size calculation. Our goal for this research is 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. Several pieces of evidence support the view that the expected differences and the methods we use in our study are indicative for our primary outcome measures and will lead to standardized effect sizes of at least 1.25. These include the following:

A. With respect to our primary outcome measure (phMRI: % change in ASL signal from baseline in response to acute oral MPH challenge before and after 16 weeks of MPH treatment):

DAT binding studies after MPH treatment in rats

In rats a persistent and significant decrease in DAT density has been observed in the striatum (and hippocampus in our own studies) after treatment with MPH in young rats by on average 1840 fmol/mg protein [SD 263] whereas in adult animals no significant decrease (445; SD 943) was observed (Moll 2001). Based on these data and this technique, the standardized effect size of adult vs. young following MPH treatment was 2.31.

We and others have shown in previous studies in users of amphetamine that since DAT is a structural component of the DA axon, an increase or decrease in DAT reflects the innervation-, and/or outgrowth of the system (Reneman 2002). Since it is not possible to conduct ex-vivo binding studies in human subjects, and since conventional methods that assess DAT density or function involve more invasive techniques (e.g., PET/SPECT involving radiation exposure), the non-invasive method that best resembles the above mentioned technique is phMRI (with a DA challenge), as pointed out by the following studies:

Jenkins (2004) investigated the DA system in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioned monkeys with phMRI using in cerebral blood volume (rCBV) as hemodynamic outcome measure. They observed a profound and significant reduction in rCBV evoked by i.v. amphetamine in MPTP treated monkeys (see figure 1 from Jenkins et al., below), which correlated with DAT densities. The authors concluded that phMRI is a powerful new tool for assessment of normal and dysfunctional DA circuits.

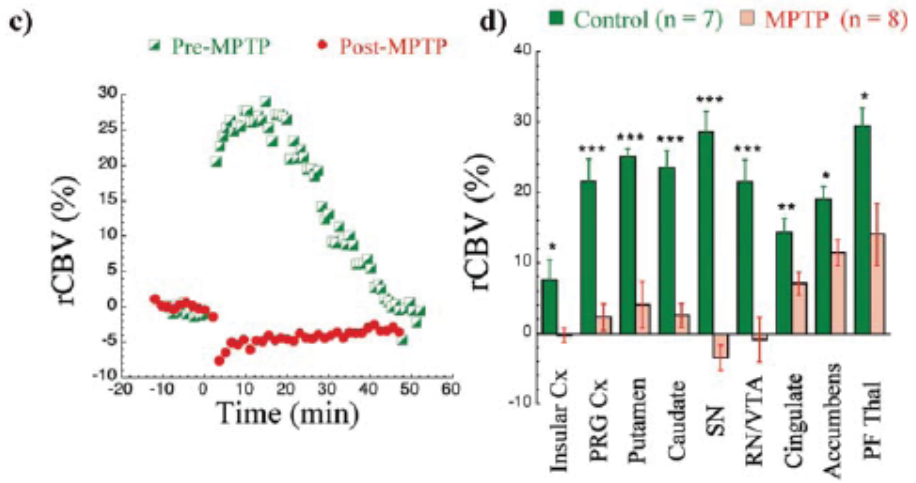


Figure 1 Changes in the response to amphetamine before and after MPTP treatment. Shown are an animal before MPTP treatment (*a*) and 4 months after cessation of MPTP treatment (*b*) in which some signal change is noted in the accumbens. The time course of rCBV changes in putamen in this representative study is shown before and after MPTP (*c*). The average changes in the regional rCBV measurements before and after MPTP are shown in parkinsonian primates (*d*). $*p < 0.05$; $**p < 0.01$; $***p < 0.005$. Insular Cx, Insular cortex; PRG Cx, PRG cortex; SN, substantia nigra; RN/VTA, red nuclei/ventral tegmental area; PF Thal, parafascicular thalamus.

To determine the usefulness of phMRI in assessing DA dysfunction using arterial spin labelling (ASL) as hemodynamic outcome measure, we examined the hemodynamic response (CBF) evoked by MPH (35 mg, orally) combined with SPECT imaging to assess the degree of DAT in amphetamine abusers and control subjects. Lasting reductions in striatal DAT following d-amphetamine treatment have been found in animals and non-human primates treated with this drug (Ricaurte 2005). In line with this, we observed a 12% reduction in striatal DAT densities in users compared to non-users (p<0.04). More interestingly, whereas both groups did not differ in CBF before MPH administration at baseline, MPH evoked a reduction in CBF in control subjects in the thalamus (-36%, p=<0.01) and cerebellum (-45%, p=0.015), whereas we observed a blunted hemodynamic response (CBF) to MPH in amphetamine abuser in these brain regions (respectively +9 and +1%). MPH evoked no significant changes in the striatum. The effect size in the thalamus with this technique is in the order of 4.0 in control subjects, compared to -3.16 in amphetamine users.

In an experimental study it was found that chronic MPH treatment caused a profound and significant increase in rCBV (or CBF) in the frontal cortex of 106%, cingulated gyrus of 185% and thalamus of 101% in rats, whereas no significant changes were observed in the striatum (see figure 2 below from Andersen 2008). The standardized effect size in this study was 5.6 in the thalamus (estimated from figure below).

The large expected effect size of chronic MPH treatment on brain hemodynamics (in the order of 5.0) and the ability to assess these hemodynamic changes in subjects with known DAT reductions (as pointed out above), support the view that the expected differences and the methods we use in our study will lead to standardized effect sizes of at least 1.25.

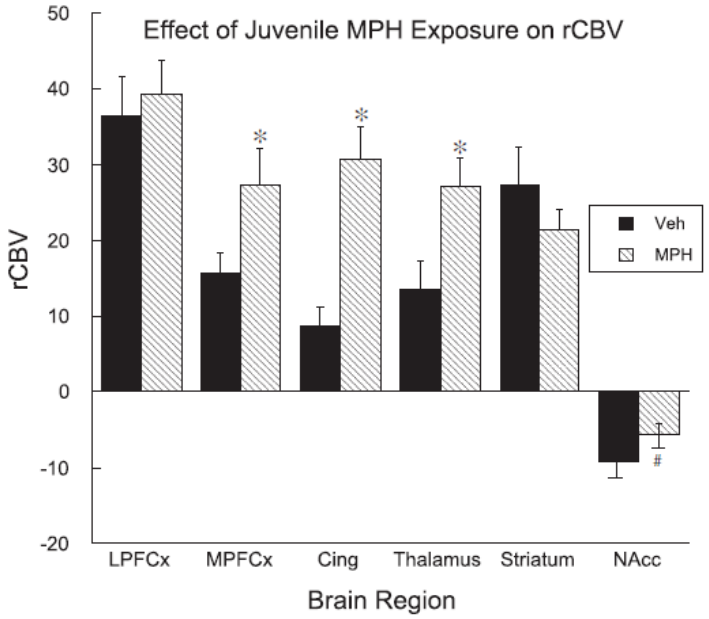


FIG. 2. Graphic comparison of regional cerebral blood volume (rCBV) maps as shown in Fig. 1 of adult animals exposed to either methylphenidate (MPH; 2 mg/kg, twice daily) or vehicle (VEH) between P20 and P35 following MPH challenge. Means ± SEM for $n = 7$ rats are presented. $^{*}P < 0.01$, $^{\#}P < 0.08$. Abbreviations as in Fig. 1.

B. With respect to our second primary outcome measure: DTI: % change in FA and MD values from baseline after 16 weeks of MPH treatment:

Effects of MPH on white matter volume and cortical thinning

A few studies have focused on the structural correlates of psychostimulant treatment. Two cross-sectional studies found a 'neuroprotective' effect of psychostimulants. Treatment with psychostimulants was associated with an increase in white matter volume, moving the volume of these areas to more normative values (average total white matter 336.2 [SD=41.9] in unmedicated ADHD patients and 369.1 [SD=55.3]) (Castellanos 2002). The standardized effect size is 0.68 in this study. Likewise, in a longitudinal study it was shown that stimulants normalized the rate of cortical thinning in the right motor strip, the left middle/inferior frontal gyrus, and the right parieto-occipital region (mean cortical thinning of 0.16 mm/year [SD=0.17], compared with 0.03 mm/year [SD=0.11] in the group taking psychostimulants), since the rate of cortical thinning in the group not taking stimulants (0.16 mm/year) was in excess of age-approriate rates (Shaw 2009). The standardized effect size is 0.93 in this study.

In the current study we will be using diffusion tensor imaging (DTI). DTI is a technique which measures the microstructural features of white matter (WM) and microfiber pathways by measuring the diffusional motion of water molecules. Fractional anisotropy (FA) is a normalized measure that provides information about the degree of fibre organization and integrity. Any process that results in alterations in axonal architecture, such reduced axonal outgrowth, can result in changes in FA and ADC (Reneman et al., 2001; Moeller et al., 2005; de Win et al., 2007). Because DTI is a much more sophisticated technique to investigate changes in white matter following chronic MPH treatment than just measuring white matter volume (as done in the Castellanos paper with a standardized effect size of 'just' 0.68) we expect that the DTI method we use in our study will lead to standardized effect size of at least 1.25.

rs-fMRI

Resting-state fMRI (rs-fMRI) is a relatively new neuroimaging technique that allows extensive assessment of (changes in) organization of whole functional networks (Fox and Raichle, 2007). 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. In a recent study conducted in rats, we observed a significant age*treatment effect in functional connectivity (FC) within the hippocampal-substantia nigra network in rats chronically treated with MPH (see figure below) in which the FC was increased in adolescent treated rats chronically treated with MPH. The FC increased with 0.162 (SD 0.16) in adolescent rats, whereas this decreased with 0.06 (SD 0.08) in adult rats. The standardized effect size of age following MPH treatment in this study was 1.85 with this technique.

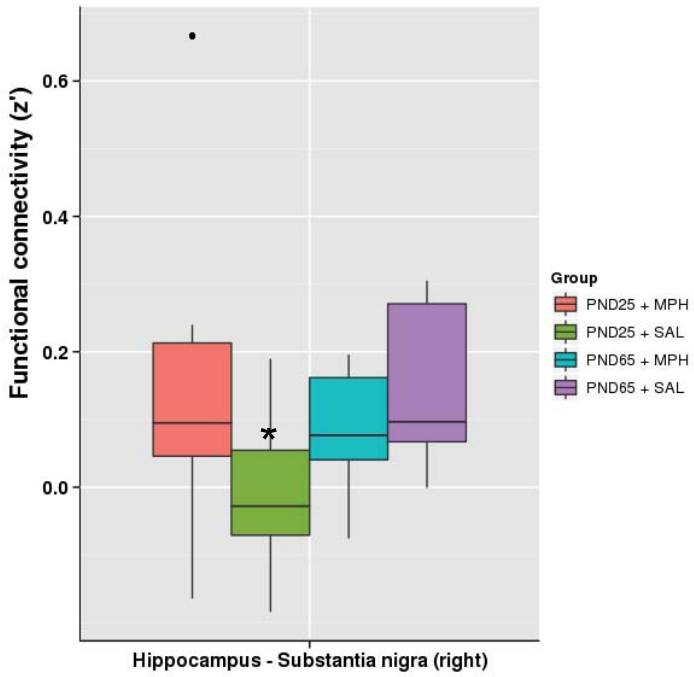


Figure 3. Increased FC between right hippocampus and substantia nigra of adolescent male Wistar rats (PND25) following chronic treatment with MPH (0.5 mg/kg orally, 3 weeks), but not adult rats (PND 65), suggesting an increase in functional DA connectivity only in young treated animals.

Compared to most of the above mentioned studies, the current trial will have the benefit of having before and after treatment measurements data from each patient. Having this paired data will reduce between subject variability. This will then increase the power of our trial to detect differences between groups. Thus, based on above observations, it is likely that the standardized effect sizes in our study will be around 1.25 or higher. A sample size of 15 patients in each treatment*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 investigate the effect modification by age of the effects of methylphenidate. To account for an expected drop-out of 25%, we will include 25 patients in each treatment*age group, meaning 25 children randomized to placebo, 25 children to methylphenidate, 25 adults to placebo, and 25 adults to methylphenidate.

Data management and analysis will be conducted in close collaboration with the division of Clinical Epidemiology, Biostatistics and Bioinformatics (KEBB) of the AMC.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

There are five interventions in this study that can not be considered part of standard practice:

1) Double blind assignment to placebo or MPH treatment
Patients are treated with either the psychostimulant MPH or with a placebo. Patients are randomly assigned to one of the treatments. After 16 weeks of treatment, there is a medication-free period of 1 week, in order assure that the body is cleared of all active ingredients of the study medication. T ½ of MPH is 2-4 hours, so 1 week is more than sufficient for MPH to be metabolized and be removed from the DA transporter. See section 6 for more information about the investigational medicinal products.

2) Neuropsychological test battery
On the assessment days, prior to the MRI scan, a neuropsychological test battery will be conducted. This will last one hour and will consist of the following (computer) tasks:
- Standard reaction time task
- Rey Auditory Verbal Learning Task (verbal memory)
- Sustained Attention to Response Task (SART)
- N-back (working memory task)

3) MRI scan
Patients will undergo three MRI scanning sessions: before starting with the study medication (baseline session), during treatment (week 8) and after trial end following a 1 week medication-free period. On baseline and trial end subjects will be scanned twice, before and after the oral challenge with MPH. The MRI scanning sessions consist of the following sequences:

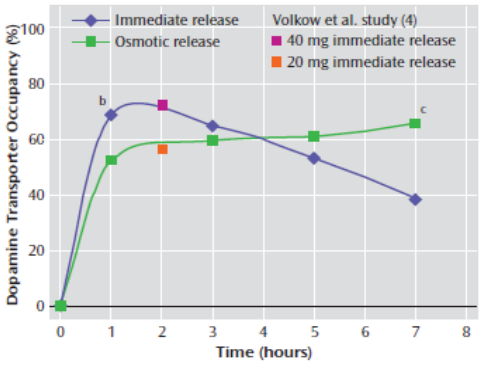
1) Anatomical 3D T1-weighted scan
2) Baseline phMRI scan
3) Baseline rs-fMRI
4) Task-related fMRI scan (emotional processing, motor inhibition and reward)
   Total scanning time: 30'

   Exit from scanner and ingestion of oral MPH. 90 minutes rest outside of the scanner during which subjects are free to play and have a non-caffeinated drink.

4) DTI scan
5) Post MPH phMRI scan
6) Post MPH rs-fMRI
4) Post MPH Task-related fMRI scan (motor inhibition and reward)
   Total scanning time: 30'

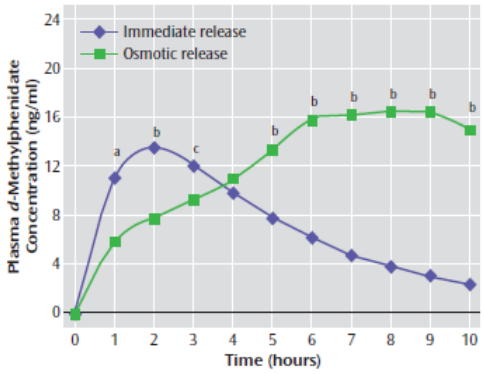
The first four sequences will be made consecutively (lasting a total of approximately 30 minutes) after which subjects are removed from the scanner. At this point they will receive an oral dose of MPH (0.5 mg/kg, children ± 15 mg; adults ± 35 mg). DAT occupancy is significantly correlated with plasma concentration of MPH which peaks between 1 and 2 hours following MPH ingestion (Spencer 2006, Silveri 2004). DAT occupancy has also been shown to be relatively stable between 1 and 2 hours post ingestion of 0.5 mg/kg MPH and in the order of 60-70% (see Figure 4 below from Spencer 2006).

Figure 4 Mean Striatal Dopamine Transporter Receptor Occupancy in Healthy Subjects After Receipt of a Single Dose of Immediate-Release (N=6) or Osmotic-Release (N=6) Methylphenidate[a]



[a] Mean dopamine transporter occupancy values obtained in healthy subjects 2 hours after receipt of 40 mg and 20 mg of immediate-release methylphenidate in a study by Volkow et al. (4) are shown for comparison.
[b] Significant difference between groups (F=5.19, df=1, 10, p<0.05).
[c] Significant difference between groups (F=57.01, df=1, 10, p<0.001).

Figure 4 Mean Plasma *d*-Methylphenidate Concentrations in Healthy Subjects After Receipt of a Single Dose of Immediate-Release (N=6) or Osmotic-Release (N=6) Methylphenidate



[a] Significant difference between groups (F=8.62, df=1, 22, p<0.01).
[b] Significant difference between groups (F=16.3–198, df=1, 20–22, p<0.001).
[c] Significant difference between groups (F=6.27, df=1, 22, p<0.05).

For these reasons, subjects will be put back into the scanner after 90 minutes and the same sequences will be assessed, now under the influence of MPH (again 30 minutes).

During the trial (week 8) subjects will be scanned once (duration 30 minutes), without the challenge. We will compare anatomical, rs-fMRI, DTI and task related fMRI datasets (and the neuropsychological testing) to a group of age and gender matched healthy controls (not part of the current trial).

We have selected three task related fMRI scans either based upon their involvement of the DA system and/or the known interaction with MPH. In view of our hypothesis (persistent changes in DA system in children following MPH treatment, but not adults) we expect to find in children a normalized pattern of activation on these tasks during treatment, which persist after trial end. In contrast, the activation pattern in adult ADHD subjects will normalize during the trial and fall back to pre-treatment (hypoactivation) values after trial end.

The fMRI tasks consist of the following:

- Emotional processing: The response to emotional faces (e.g. happy, angry, sad, fear) are measured, either by correct response, event related potential (ERP) or by signal changes in fMRI. Emotional responses are elicited in many different brain regions, where the amygdala seems to be a relay between visual systems en modulatory responses. ERP studies in response to MPH have been performed in adolescents with ADHD (Williams 2008). They showed an improvement in the recognition of fear and anger after MPH administration which correlated with ERP signal modulations.

- Motor inhibition: Frontal–striatal function and its modulation by MPH will be assed using a motor inhibition task: the go/no-go task. MPH has been shown to normalize striatal hypoactivation in ADHD subjects (Vaidya 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 methylphenidate. Two versions of the response inhibition task will be used to control for response and stimulus characteristics of the go and no-go trial blocks. Go and no-go blocks will be matched for the number of motor responses in the response-controlled version and for the number of stimuli in the stimulus-controlled version. The effects of MPH on frontal and striatal activation during response inhibition will be compared within and between groups.Administration of MPH during brain development, but not in adulthood, results

in an altered outgrowth of the DA system. This long-lasting disturbance of the DA system may result in behavioral abnormalities, such as anxiety and depression.

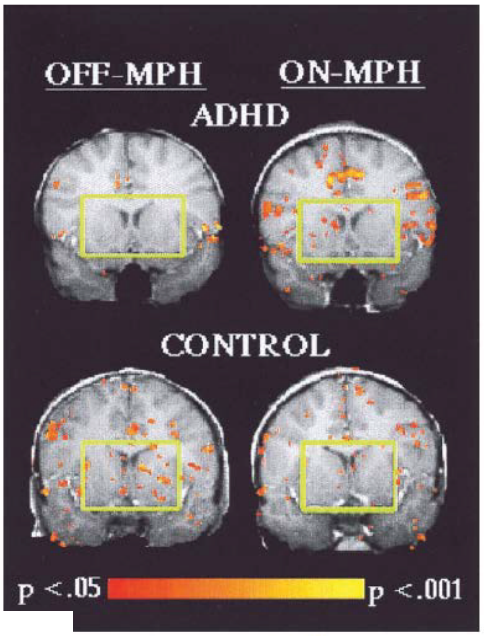


Figure 5 Activation during response inhibition on the stimulus-controlled task in a coronal slice located 12 mm anterior to the anterior commissure for an ADHD and a control child. Green squares highlight the opposite effect of MPH in the head of the caudate and putamen in the ADHD and control child.

- Reward: Reward processing will be investigated because reward deficiency, involving hyporesponsiveness of dopamine-innervated mesolimbic regions plays a central role in psychobiological theories of ADHD and addiction. Subjects are shown cues with different outcome possibilities. Cues can predict whether subject has a good chance of winning a small, medium or large monetary reward when responding or whether subject has a good chance of losing said sum. Participants will be explained that they will have to pretend to be actually winning or losing, as they will not actually receive the money “won” in the task. This task investigates incentive processing. Subjects receiving MPH are expected to have a greater anticipatory response and to experience greater feelings of loss when “losing” the large amount (Knutson 2004). The effects of MPH on the nucleus accumbens activation during incentive processing will be compared within and between groups.

4) Actigraphy and sleep log

To report on the age-dependency of the effects of MPH on sleep timing, RLS questionnaires (the Holland Sleep Diagnostic List (HSDL) and Evaluation List Insomnia Therapy (ELIT), sleep log and actigraph will be administered at three time points. This will be done before the trial starts, during the trial (week 7) and in the washout period. Subjects will be provided with a small diary with four questions to be filled out before sleep and four when waking up. The actigraph measures movements. It is a wristwatch that is worn 24 hours a day for 5 days with a marker to be pressed when going to bed and when waking up. During the intake it will be determined whether any subjects fulfil the criteria for Restless Legs Syndrome (RLS) or periodic limb movement disorder. Subjects suffering from RLS will be given an extra questionnaire with 10 questions on RLS severity at the start of the three actigraphy periods.

5.2 Use of co-intervention (if applicable)

Patients are asked to postpone surgery, placing of dental braces, piercings or tattoos until after the study, if possible. They will be requested not to use caffeinated products on the assessment days.

6. INVESTIGATIONAL MEDICINAL PRODUCT

6.1 Name and description of investigational medicinal product

MPH (methylphenidatehydrochloride): an indirect sympathicomimetic similar in pharmacological properties to amphetamine. It is routinely prescribed for the treatment of ADHD and occasionally for narcolepsy. It is assumed to function by blocking DA uptake, resulting in a greater availability of extracellular dopamine. MPH is successful in alleviating ADHD symptoms in about 70% of individuals (Greenhill 2002).

Placebo: The placebo tablet matches the MPH tablet with respect to appearance, size, shape and presence of scoring line, and will be manufactured according to GMP guidelines.

6.2 Summary of findings from non-clinical studies

See the Summary of Product Characteristics (SPC) of MPH.

6.3 Summary of findings from clinical studies

When treating ADHD, MPH is the first treatment of choice. The most comprehensive study on MPH's effectiveness was the MTA (multimodal treatment of children with ADHD) study from 1999 (published anonymously). This was a 14-month randomized controlled trial where 579 subjects were randomized over four treatment groups: (1) medication (2) psychosocial treatment (3) combined treatment (medication and psychosocial treatment) (4) community-treatment/assessment and referral. Both the medication and combined treatment group scored significantly better than psychosocial treatment alone on ADHD symptoms. Brown et al (2005) have reviewed studies with several different treatment options and share the conclusion that so far stimulants, preferably in combination with behavioral therapy are the preferred method of treatment in ADHD.

6.4 Summary of known and potential risks and benefits

MPH

A great amount of clinical studies evaluating stimulant treatment for ADHD exist. Review articles consider common occurring adverse effects from stimulant medication to be appetite suppression, sleep disturbances, headaches, motor tics, abdominal pain, irritability, nausea, and fatigue. Approximately 70% of children with ADHD will respond to treatment with MPH without serious side effects in long-term treatment (Goldman 1998, Brown 2005). In the other 30%, medication has no effect or side-effects are too severe to continue treatment. Regarding the prevalence and severity of side-effects, Efron et al. published a study in 1997 on medication side-effects for MPH and dexamphetamine in the treatment of ADHD in 115 children between 5 and 15 years of age who received treatment with MPH (0.3 mg/kg) for 2 weeks. It was concluded that many symptoms commonly attributed to stimulant medication are actually pre-existing characteristics of children with ADHD and improve with stimulant treatment (see also table 2 and 3 below). Appetite suppression was the only substantial side effect on MPH. This was the only side effect of MPH that was reported with significantly greater frequency and severity on the drug compared with baseline. We can therefore conclude that MPH is very effective in treating ADHD with only mild side effects (appetite

suppression). Appetite suppression can be diverted by taking medication after meals, rather than before.

TABLE 2. Prevalence of 17 "Side Effects" of Stimulants on DEX and MPH, Compared With Baseline

Side Effect	Baseline No (%)	DEX No (%)	MPH No (%)	$P < .01$*
Trouble sleeping	67 (54)	88 (70)	79 (64)	BD
Poor appetite	43 (34)	74 (59)	69 (56)	BD, BM
Irritable	102 (82)	102 (82)	100 (80)	
Proneness to crying	87 (70)	95 (76)	89 (71)	
Anxiousness	96 (77)	85 (68)	76 (61)	BM
Sadness/unhappiness	85 (68)	74 (59)	69 (56)	
Headaches	51 (41)	38 (30)	30 (24)	BM
Stomachaches	56 (45)	50 (40)	40 (32)	
Nightmares	49 (39)	35 (28)	26 (21)	BM
Daydreams	94 (75)	78 (62)	77 (62)	
Talking little with others	41 (33)	37 (30)	35 (28)	
Uninterested in others	47 (38)	43 (34)	39 (31)	
Drowsiness	31 (25)	23 (18)	22 (18)	
Biting fingernails	63 (50)	50 (40)	56 (45)	BD
Unusually happy	52 (42)	33 (26)	35 (28)	BD
Dizziness	16 (13)	18 (14)	15 (12)	
Tics or nervous movements	44 (35)	32 (26)	35 (28)	

* BD indicates baseline vs DEX; BM, baseline vs MPH.

TABLE 3. Mean Severity of 17 "Side Effects" of Stimulants on DEX and MPH, Compared With Baseline

Side Effect	Baseline	DEX	MPH	F*	P*	Contrast†
Trouble sleeping	2.21	3.61	2.69	12.9	<.01	BD, DM
Poor appetite	1.22	2.74	2.12	19.9	<.01	BD, BM
Irritable	4.57	3.65	2.94	21.0	<.01	BD, BM, DM
Proneness to crying	3.35	3.4	2.7	4.9	<.01	DM
Anxiousness	3.94	2.71	2.07	30.9	<.01	BD, BM, DM
Sadness/unhappiness	2.86	2.43	1.69	15.5	<.01	BM, DM
Headaches	1.25	.83	.65	4.8	.01	BM
Stomachaches	1.59	1.42	1.14	1.8	.16	
Nightmares	1.09	.79	.48	10.6	<.01	BM, DM
Daydreams	3.13	1.76	1.94	19.2	<.01	BD, BM
Talking little with others	1.00	1.15	.77	2.1	.13	
Uninterested in others	1.25	1.08	.99	.8	.47	
Drowsiness	.72	.64	.45	1.4	.24	
Biting fingernails	2.87	1.84	2.02	12.9	<.01	BD, BM
Unusually happy	1.35	.83	.94	2.9	.06	
Dizziness	.34	.36	.26	.61	.54	
Tics or nervous movements	1.64	.83	.81	6.9	<.01	BD, BM

* Analysis of variance with repeated measures (df 2,122). P values refer to the F statistics.
† Statistically significant pairwise contrasts ($P < .01$). BD indicates baseline vs DEX; BM, baseline vs MPH; DM, DEX vs MPH.

It is recommended by the 'Nederlands Kenniscentrum Farmacotherapie bij Kinderen' (NKFK) that before starting treatment with MPH pulse, blood pressure, length and weight should be measured. Also, one should exclude the presence of tics, stereotype behavior (borderline) psychotic behavior or epilepsy, because MPH may worsen these symptoms. Although the

FDA in the US has found no robust links between sudden cardiac death and the use of stimulants, it is the general consensus that in case of a positive family history for sudden cardiac death or other indications of cardiac problems an ECG must be made before starting treatment with MPH. No risks are foreseen with respect to the oral challenge with MPH for the phMRI study.

6.5 Description and justification of route of administration and dosage

MPH will be administered in tablet form and has to be taken orally. Placebo will be in the same form as the MPH and dosage and administration will be similar as well. Starting dose is 0.3 mg/kg day in 1-2 doses for the children and 0.5 mg/day in 1-2 doses for the adult patients. Dosage can be increased weekly with 5-10 mg/day to a maximum of 60 mg daily in the absence of dose limiting effects and until a satisfactory clinical response is achieved. This is in accordance with the SPC guidelines and also in accordance with Nederlands Kenniscentrum Farmacotherapie bij Kinderen (NKFK). There may thus be a (small) difference in dosage between children and adults. This is inevitable, since this difference is the resultant of a difference in pharmacokinetics, and other effects of MPH on the body of children versus adults. Because it is not possible to correct statistically for this difference in dosage, it is all the more important that we base the dosage on clinical response (and not a fixed dosage).

For the phMRI study all subjects will receive an oral challenge with MPH of 0.5 mg/kg. The rationale for a challenge with 0.5 mg/kg dose is that it is sufficient to produce a signal change on the phMRI (pilot data) and is well tolerated in previous studies by the study participants (Rao 2000, Silveri 2004). Also DAT occupancy has also been shown to be relatively stable between 1 and 2 hours post ingestion of 0.5 mg/kg MPH (Spencer 2006) and in the order of 60-70%.

6.6 Dosages, dosage modifications and method of administration

Trial protocol follows the standards of treatment currently used in the Netherlands by child psychiatrists. Subjects will receive oral dosages of MPH starting with 0.3 mg/kg day in 1-2 doses for the children and 0.5 mg/day in 1-2 doses for the adult patients. Dosage can be increased weekly with 5-10 mg/day to a maximum of 60 mg daily. Dosage will be increased weekly by 5-10 mg until target clinical dosage is reached with a maximum of 60 mg per day. Clinical dosage is dependent on the reduction of symptoms as assessed by the treating physician. Because placebo medication is not expected to influence symptoms, it is expected that placebo tablets will be increased to the maximum (equal to 60mg of placebo). If, after in- or decreasing the dosage, serious side effects occur, the patient will return to the previous dosage and dosage modifications will be more gradual thereafter. Decisions about dosage modifications are always and only taken by the treating psychiatrist. Study medication has to be taken daily at regular intervals (at a fixed time during the day, e.g. in the morning) and should be swallowed with a half glass of water or dissolved in a half glass of water and swallowed immediately and completely (See Patient Information Leaflet). Compliance will be assessed by counting of the returned medication.

6.7 Preparation and labelling of Investigational Medicinal Product

The hospital pharmacy of the Slotervaart hospital (Amsterdam) will supply the investigational sites with the double-blinded study medication, according to Good Manufacturing Practice (GMP)-criteria, annex 13. MPH will be provided in identical blinded form in 10 mg tablets packed per container with similar type of container, dispensing aids and formulation number. Blinding is done by the CRU of the AMC. Labelling will be according to European standards, as defined in the guideline Good Manufacturing Practice (2003/94/EG).

6.8 Drug accountability

The study medication will be used only as directed in the protocol. Subjects must return all unused medication and empty medication packaging. A record, of overall dispensing, all returns and any study treatments not dispensed, will be kept and their destruction will be arranged by the hospital pharmacy. Accurate records accounting for the receipt of the investigational products and for the disposition of the material will be maintained.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

- phMRI: % change in ASL signal from baseline in response to acute oral MPH challenge before and after 16 week MPH treatment
- DTI: % change in FA and MD values from baseline after 16 week MPH treatment
- rs-fMRI: % change in functional connectivity parameters from baseline
- % change of above mentioned outcome parameters during treatment vs. baseline and post-treatment

7.1.2 Secondary study parameters/endpoints (if applicable)

- fMRI: % change in task related BOLD signal from baseline
- Neuropsychological functioning: change in outcome of several well-validated neuropsychological (computer) tasks addressing emotional processing and impulsivity/behavioral inhibition compared to baseline measurements.
- Sleep log and actigraph: % change from baseline

7.1.3 Other study parameters (if applicable)

Possible confounding parameters that can intervene with the main study parameters and which are therefore included as study parameter are:

- Age in months
- Symptom severity

Safety monitoring requires inclusion of the following parameters:
Response rate:
Ratings on ADHD severity and improvement from baseline; change on DBD (by both parent and teacher) in children and ADHD-SR in adults. In addition: CDI, CGI and CGAS in children and BDI, CGI and GAF in adults. In addition, the SCARED and BAI will be assessed at 3 fixed intervals as pointed out in more detail under 7.3. Response to treatment is defined as a score of 1 or 2 on the CGI improvement item (indicating “very much improved ”or “much improved”) at trial end (week 16) and again 1 weeks later (week 18).
Blood pressure: measured at 5 fixed intervals during one of the control visits because of cardiovascular action of MPH.
Length, body weight, BMI measured at 5 fixed intervals during one of the control visits because of appetite suppression of MPH.

7.2 Randomisation, blinding and treatment allocation

Randomization will be performed on a central computer using a specialized computer program developed by the Clinical Research Unit of the AMC. Patients will be stratified by age and then randomized to either placebo or MPH treatment (1:1) using a permuted block randomization scheme. Allocation will be concealed for all parties. Subjects will be randomized after the MRI scan on the 1st assessment day. When ending the study, blinding is checked with the patient and his psychiatrist as well as the study investigators.

7.3 Study procedures

A flow chart of the study procedures is given on page 16. Study procedures involve: a) inclusion; b) actigraphy c) 1st assessment day; d) treatment and monitoring; e) Short assessment f) 2nd assessment day; g) exit-interview.

a) Inclusion

(Child- and adolescent) psychologists and psychiatrists at the AMC/de Bascule (Amsterdam), ADHD_Behandelcentrum (Capelle a/d IJssel), PsyQ/PBG, Prezens/GGZinGeest and Triversum (Alkmaar) involved in treatment of patients suffering from ADHD along with regional physicians will be informed about the study a few months prior to the start of the study. Study inclusion-, and exclusion criteria will be given to them (brochure). The brochure will also be available in the waiting room. Subjects can be included either via a) primary care (most probably physicians) or b) from a (child- and adolescent) psychologist, or psychiatrist who encounters a patient that might be eligible for study inclusion. Adult medication naïve ADHD patients will also be recruited through advertisement.Patients that are eligible for study inclusion will be given the patient information letter (enclosed) by the physician, (child- and adolescent) psychologist, or psychiatrist in which detailed information on the study is given. In the following week, the suitability of the patient for study participation will be discussed in the respective treatment teams and the patient will be informed by telephone on the outcome of that meeting by one of the (child- and adolescent) psychiatrists, or trial nurse/research assistant. If the patient is still willing to participate, any additional information about the study procedures will be discussed and a screening visit (with a trial nurse/research assistant) and appointment with the (child-and adolescent) psychiatrist (Drs. M. Bottelier or Dr. S. Kooij) will be scheduled within 10 working days. Adult subjects recruited through advertisement, with a positive ADHD self questionnaire (‘ADHD zelf-rapportage’ Kooij and Buitelaar 1994), and willing to participate will be scheduled an appointment for the screening visit directly. The screening visit includes a clinical interview including clinical rating scales for ADHD in order to see if the patient meets most of the previously described inclusion criteria for ADHD (see section 4.3) and does not meet any of the previously described exclusion criteria (see section 4.4). In addition, blood pressure, length, body weight will be assessed. Study data of the patient will be stored at the AMC and the location site. At the beginning of the screening visit, patients (and their legal guardian(s) in case of under-aged patients) will be asked for written informed consent after thorough explanation of the study procedures. The trained staff will review every patient’s ability to give a ‘valid’ informed consent. Following the screening visit in which most in- and exclusion criteria have been checked by a trial nurse/research assistant, and the patient is eligible for study inclusion, a (child-and adolescent) psychiatrist (drs. M. Bottelier or Dr. S. Kooij) will conduct a global assessment/impression and a physical examination to formally include the patient in the study. An appointment for the first assessment at the AMC will be made, which will take place within two weeks after the screening visit. In addition, an actigraph and a sleep log will be given to them. All patients will be treated by drs. M. Bottelier or Dr. S. Kooij, or under his/her supervision by a research physician. Subjects that have been discussed in the respective treatment teams and were found ineligible for study participation will be treated irrespectively at location site.

b) Actigraphy

At three time points subjects will be asked to wear an actigraph and fill in a sleep log for 5 days to objectively register their sleep timing. This will be done before the trial starts (week 0), during de trial (week 7) and in the washout period (week 17) in order to determine the effects of MPH treatment on sleep timing. Subjects will be provided with a

small diary with four questions to be filled out before sleep and four when waking up. Movements will be measured by a wristwatch (actigraph) that is worn on the non-dominant wrist 24 hours a day for five days, with a marker to be pressed before going to bed and when waking up. During intake it will be determined whether subjects fulfil the criteria for Restless Legs Syndrom (RLS). Subjects suffering from RLS will be given an extra questionnaire with 10 questions on RLS severity at the start of the three actigraphy periods.

c) 1st assessment day:
A timeline of an assessment day is given in Figure below. Before starting the study medication, an assessment will be made at the AMC. Participants will be welcomed and the program of that day will be explained in detail. The actigraph, questionnaire and completed sleep log are turned in. Subjects are then asked to fill out several questionnaires (addressing sleep, substance (ab)use in the last two weeks including smoking and drinking coffee and other possible confounding parameters (see section 7.1.3)).Next, the children will go to the MRI scanner room in order to get acquainted with the scanning procedures and the MRI scanner. Extensive coaching and preparation of the children to prevent stress and anxiety will be part of the procedure (see attachment). After this, the neuropsychological test battery will be conducted. This will take one hour. For a description of the different tests, see section 5.1. Hereafter, patients will go (back) to the MRI scanner. Prior to MR imaging, articles or clothing that contain metal pieces, jewellery and make-up should be taken off. All subjects will be provided with metal free hospital clothing if necessary and have to wear ear plugs. Before the MRI scan a photoplethysmograph will be placed on the left hand index finger and a pneumatic respiratory belt strapped around the upper abdomen. The subjects will be placed in the MRI scanner in the supine position and lie down on a table that slides into the tunnel. During the entire MRI session, an open transmit/receive head coil is placed over the head of the subject. The subject will be able to see and to breathe normally. The subject will be asked not to move the head during MR scanning.

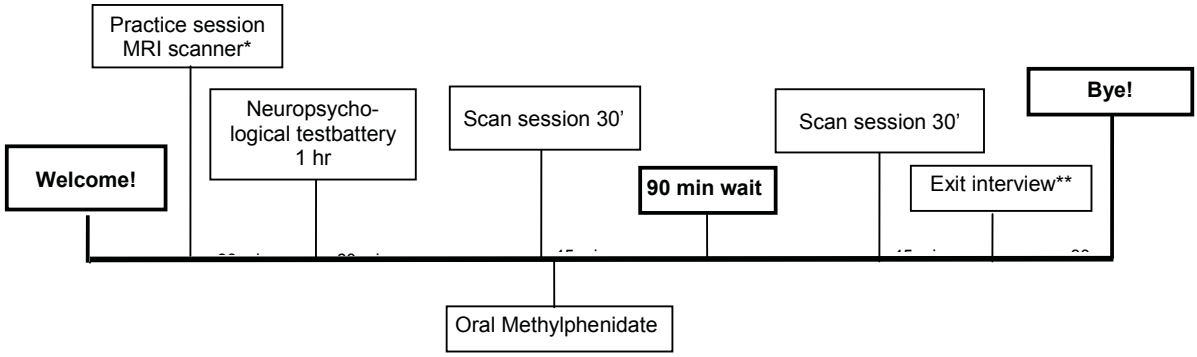


Figure 6 Timeline assessment day at AMC (*only during 1st assessment, **only during 2nd assessment). Total duration 2.5-3 hours and 1.5 hour waiting.

For a description of the different scans that will be performed, see section 5.1.

During the anatomical and DTI scan, it is possible for the subject to listen to music or to watch a DVD. The fMRI scan will include an emotional face recognition task, reward task and a go-no go task. In between scans, subjects will be removed from the scanner and given an oral dose of MPH. Subjects are free to spend their time until the second scan. Total scan

duration is approximately 30 minutes for the 1st MRI session, and 30 minutes for the 2nd MRI scan.

d) Treatment and monitoring
Following the first assessment, patients will start with their study medication as soon as possible (preferably the next day). Treatment is then continued for the next 16 weeks followed by a 1 week wash-out period. See section 6 for information about the investigational medicinal product(s) and dosage (modifications). During these 16 weeks, patients are monitored by or under supervision of Drs M. Bottelier as is part of standard clinical practice. Treatment monitoring will take place after 1 week, 3 weeks 5 weeks and at the assessment visits at 8 and 16 weeks, in accordance with current guidelines. During these visits, safety data (blood pressure and pulse, all adverse events reported by patient and their guardians or observed by the investigators and/or psychiatrist) will be collected, as well as assessments of ADHD symptoms (DBD and ADHD-SR) (all visits) and depressive symptoms (CDI and BDI) (week 3, 8 and 16) and anxiety (SCARED, and BAI) (week 3, 8 and 16). In addition the Hamilton anxiety and depression scale will be administered at inclusion- and exit-interviews. These control visits are part of standard treatment guidelines, but will be partially standardized by the study investigators. Patients are able to contact their physicians concerning dosage adjustments at all times during the study.

e) Short assessment
During the trial in week 8 subjects will be asked to come to the AMC, to repeat a part of the MRI study and neuropsychological test battery. No challenge with MPH will be given. Total scan duration will be 30' and 30' on neuropsychological test battery. Total duration will be 1 hour. The actigraph, completed sleep log and questionnaire are turned in, which were sent to them the week before (week 7).

f) 2nd assessment day:
The 16 weeks of medication use are followed by a 1 week medication-free period, after which patients return to the AMC for a second assessment day (week 18). All procedures (including neuropsychological testing, questionnaires, MRI scan session with oral MPH challenge; see above) will be repeated.

g) Exit-interview: The study ends with an exit interview by one of the study investigators. This exit-interview is designed to evaluate the patient's participation in the study (to check blinding of the study, drug and therapy compliance, etc.), as well as re-assessment of the rating scales (DBD, SCARED and CDI in children and ADHD-RS, HAM-A/D, BAI and BDI in adults), and response to treatment (CGI and CGAS). The 2nd assessment day ends with a short exit interview by one of the study investigators. After this, the patient's psychiatrist (Drs. M. Bottelier, or under his supervision) will be informed about the type of study medication the patient received and can decide at that point to terminate, continue or switch medication, whatever is judged to be in the best clinical interest of the patient.

7.4 Withdrawal of individual subjects

Patients can leave the study at any time, without consequences for their treatment. Patients who drop out of the study will continue to be cared for by the (child-and adolescent) psychiatrist that had been treating them, or a colleague of him. The psychiatrist will be informed about the type of study medication the patient received and can decide at that point to terminate, continue or switch medication, whatever is judged to be in the best clinical interest of the patient. The treating psychiatrist or study investigators can decide at any time to withdraw a patient from the study for urgent medical reasons. Patients who no longer wish to participate in the study will be asked about the reason(s) for their discontinuation and about the presence of any adverse events. If the patients have been treated for 6 weeks or more, but wish to withdraw their informed consent, they will be asked for their willingness to

participate in a 2nd assessment day (or part of it) and an exit-interview. Adverse events will be followed up until abated and any remaining study material and investigational products should be returned by the patient.

7.4.1 Specific criteria for withdrawal

The reasons for discontinuation will be recorded as well as other variables that might be relevant. Subjects can be withdrawn from participation for the following reasons:

- Withdrawal of informed consent by patient or legal guardian(s)
- Serious adverse event
- Patient does not comply with the study directives (e.g. non-compliant on medication intake)
- Patient needs medication mentioned in the exclusion criteria of this study
- Patient is not capable to finish the study for any reason
- Patient needs to be hospitalized
- Patient meets one of the other exclusion criteria during the study

7.5 Replacement of individual subjects after withdrawal

If a subject chooses to withdraw from further participation following randomization or has to withdraw for other reasons at any time during the study, permission will be asked from the patient to use the data obtained so far. In case a patient has been treated for 6 weeks or more, and has not withdrawn her informed consent, she will be asked to participate in the 2nd assessment and an exit interview (or part of it). Withdrawn subjects will be replaced, as long as they have not yet been randomized.

7.6 Follow-up of subjects withdrawn from treatment

Patients that withdraw from the study remain cared for by their psychiatrist (Drs. M. Bottelier) or by a colleague of him. Subjects will always be asked about the presence of any adverse events when withdrawing from the study. Adverse events will be followed up, even after discontinuation of the study.

7.7 Premature termination of the study

Patients can leave the study at any time, without consequences for their treatment. Patients who drop out of the study will continue to be cared for by the (child-and adolescent) psychiatrist that had been treating them, or a colleague of him. The psychiatrist will be informed about the type of study medication the patient received and can decide at that point to terminate, continue or switch medication, whatever is judged to be in the best clinical interest of the patient. The treating psychiatrist or study investigators can decide at any time to withdraw a patient from the study for urgent medical reasons.

8. SAFETY REPORTING

8.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subject's health. The investigator will take care that all subjects are kept informed.

Adverse events (AE) are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational drug. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

A serious adverse event (SAE) is any untoward medical occurrence or effect that at any dose results in death;

- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, lack of efficacy of an IMP used for the treatment of a life threatening disease, major safety finding from a newly completed animal study, etc.

All SAEs will be reported to the accredited METC that approved the protocol, according to the requirements of that METC. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment, will be classified as other significant adverse events (OAE). Examples of these are marked hematological and other laboratory abnormalities or abnormalities on the MR images, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment. All OAEs will be recorded.

8.2 Follow-up of adverse events

All adverse events (AEs, SAEs and OAEs) will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general psychiatrist or a medical specialist.

9. STATISTICAL ANALYSIS

9.1 Descriptive statistics

To evaluate the effect modification by age of the effect of MPH on the development the dopaminergic system, the change in our primary outcome measures (ASL signal, FA and FC values) from baseline to post-treatment will be determined for each patient (Δ_i) per brain region of interest (ROI). There are six DA brain regions of interest, namely anterior cingulate cortex, hippocampus, frontal cortex, striatum, thalamus and cerebellum. These individual changes (Δ_i) will be used to estimate the treatment effect in children (mean Δ in MPH treated patients minus mean Δ in placebo treated patients) and in adults (see Figure 7, below). The central analysis is whether this treatment effect is different in children compared to adults at various time points: different in children compared to adults after 8 weeks of therapy and 1 week after the end of treatment. This hypothesis will be formally examined using a repeated-measures mixed-model analysis.

The model includes brain region (6 levels), treatment group (2 categories), age group (2 categories), and time (3 categories) and the interaction between treatment and age, and time since treatment to examine whether the impact of MPH treatment differs by age and time since treatment. 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 sleep timing).
This is a randomized clinical trial, so the influence of confounding is expected to be low (they can only be the result of chance). The study is designed such that several important possible confounding parameters are already equal (differences small), such as that only males are included and a there is a small age range. Furthermore, because of the design of the study, we have, from a statistical point of view, limited power to perform a lot of correcting analyses. We 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.

Figure 7 Statistical analysis

All analyses will initially be conducted using the intended-to-treat principle, but for the imaging outcomes a per-protocol analysis will also be performed. All statistical analyses will be supervised by a senior member of the dept. of Epidemiology & Biostatistics of the AMC.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version of 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

10.2 Recruitment and consent

(Child- and adolescent) psychologists and psychiatrists at the AMC/de Bascule (Amsterdam), ADHD-Behandelcentrum (Capelle a/d IJssel), PsyQ/PBG, Prezens/GGZinGeest and Triversum (Alkmaar) involved in treatment of patients suffering from ADHD along with regional physicians will be informed about the study a few months prior to the start of the study. Study inclusion-, and exclusion criteria will be given to them (brochure). The brochure will also be available in the waiting room. Subjects can be included either via a) primary care (most probably physicians) or b) from a (child- and adolescent) psychologist, or psychiatrist who encounters a patient that might be eligible for study inclusion. Adult medication naïve ADHD patients will also be recruited through advertisement. Patients that are eligible for study inclusion will be given the patient information letter (enclosed) by the physician, (child- and adolescent) psychologist, or psychiatrist in which detailed information on the study is given. In the following week, the suitability of the patient for study participation will be discussed in the respective treatment teams and the patient will be informed by telephone on the outcome of that meeting by one of the (child- and adolescent) psychiatrists, or trial nurse/research assistant. If the patient is still willing to participate, any additional information about the study procedures will be discussed and a screening visit (with a trial nurse/research assistant) and appointment with the (child-and adolescent) psychiatrist (Drs. M. Bottelier

or Dr. S. Kooij) will be scheduled within 10 working days. Adult subjects recruited through advertisement, with a positive ADHD self questionnaire ('ADHD zelf-rapportage' Kooij and Buitelaar 1994), and willing to participate will be scheduled an appointment for the screening visit directly. The screening visit includes a clinical interview including clinical rating scales for ADHD in order to see if the patient meets most of the previously described inclusion criteria for ADHD (see section 4.3) and does not meet any of the previously described exclusion criteria (see section 4.4). In addition, blood pressure, length, body weight will be assessed. At the beginning of the screening visit, patients (and their legal guardian(s) in case of under-aged patients) will be asked for written informed consent after thorough explanation of the study procedures. The trained staff will review every patient's ability to give a 'valid' informed consent. Study data of the patient will be stored at the AMC and the location site. Following the screening visit in which most in- and exclusion criteria have been checked by a trial nurse/research assistant, and the patient is eligible for study inclusion, a (child-and adolescent) psychiatrist (drs. M. Bottelier or Dr. S. Kooij) will conduct a global assessment/impression and a physical examination to formally include the patient in the study. An appointment for the first assessment at the AMC will be made, which will take place within two weeks after the screening visit. In addition, an actigraph and a sleep log will be given to them. All patients will be treated by drs. M. Bottelier or Dr. S. Kooij, or under his/her supervision by a research physician. Subjects that have been discussed in the respective treatment teams and were found ineligible for study participation will be treated irrespectively at location site.

10.3 Objection by minors or incapacitated subjects (if applicable)

The code of conduct involving minors ('Gedragscode bij verzet minderjarigen', CCMO) is known to all involved investigators and will be followed at all times. Specifically, prior to study inclusion the child and his parent(s)/legal guardian(s) will be informed extensively, both with written information and during a screening visit. During these screening visits the parents will be informed by the trial nurse on the nature of the study procedures. An agreement will be made with the parents to determine which behavior as displayed by the child will be regarded as an unwillingness to participate. In case of objection to participate, the informed consent will be automatically withdrawn. During the assessment days of the study, but also during screening visits the behavior of the child will be assessed by the trial coordinator or trial nurse. Willingness to participate will also be objectively measured by a short rating scale to be completed by the child, the parent(s) present and the researcher present. This rating scale will consist of a 10-point visual analogue of feeling good or bad. The scale will be presented at start of the assessment day, after being introduced to the MRI scanner and just before entering the MRI scanner. If any of the individuals checks the last rating as < 7, procedures will be halted immediately. After determining the reason for the low score and addressing any issues causing anxiety, the rating may be offered one more time to see if scoring has improved. Of each patient a file will be made which includes all aspects outlined under 9.0 of the Gedragscode bij verzet minderjarigen', CCMO, which at the end of the trial will be included in the medical file of the patient.

10.4 Benefits and risks assessment, group relatedness

10.4.1 Group relatedness

We study the effect of MPH treatment on the developing DA system. It is therefore essential to include individuals in which brain development is still ongoing, thus minors. The cut-off point for children of 10-12 years of age is chosen because several MRI parameters greatly change until 8-10 years of age (Ben Bashat 2005), whereas the rate of increase reduces after 10 years of age. Peak prevalence of ADHD is 10

years of age (Burd 2003). Because brain maturation is not complete until 18-20 years of age (Sowell 1999) and because we want to exclude any aging effects, cut off-point for the adult patient group is chosen at 23-40 years of age.

10.4.2 Benefit

The safety of prescribing MPH to children has been subject of increasing concern in the community. Information about the safety of MPH in treating ADHD is greatly needed, particularly since MPH is now being subscribed to more and increasingly younger children (Zito 2000; Van Dijk 2008). This study will increase our understanding of the safety of MPH in children, as more information about the safety of MPH on the maturing brain will be available. In addition, we will gain insight into basal neurocognitive and neuroadaptive processes in the developing brain, as well as more knowledge on the pathophysiology of ADHD. Eventually, these data could be used to better protect the health of children and adolescents by establishing the safety of MPH for treatment of ADHD, and ultimately help identify new effective and efficient pharmacological interventions. The use of phMRI in assessing DAT functionality may have important prognostic factors, for instance in predicting responsiveness to MPH medication in the near future. In the meantime, this study serves to further emphasize the importance of ensuring a proper diagnosis when prescribing MPH. The impact is thus not only on a medical level, but on a society level as well. In the meantime, these studies serve to further emphasize the importance of ensuring a proper diagnosis when prescribing psychotropic medications.

Finally, but importantly, there is also an individual benefit associated with study participation: all subjects will start pharmacological treatment within 2 weeks after study enrollment, whereas in standard practice this can mount up to 3 months, due to long waiting lists at departments of (child- and adolescent) psychiatry ('Wachtlijst bij de Jeugdpsychiater? Dan maar een pil' NRC Handelsblad 15 december 2006). Lucertis publishes monthly their waiting lists, and the waiting period until treatment range from 12 to 24 weeks in the Randstad
http://www.lucertis.nl/26378/Wachttijden.html

All patients, including the placebo group will (continue to) receive all other active components of treatment, including a thorough evaluation, psycho education about ADHD, access to a treatment provider, supportive care and the expectation of improvement.

10.4.3 Risk assessment

General risks (part of standard practice)

In general, specific risks applicable to the current study are not different from those that are normally seen in standard clinical practice such as adverse effects of medication, inadequate response to treatment and the risk of violation of confidentiality. Risks associated with clinical assessment procedures could include mild to moderate emotional discomfort or frustration associated with completing diagnostic interviews or questionnaires. The patient's condition may not improve or may even worsen while participating in this study. These risks and burdens would also occur in standard practice and can therefore be considered negligible.

Chronic MPH treatment (part of standard practice)

A great amount of clinical studies evaluating stimulant treatment for ADHD exist. Approximately 70% of children with ADHD will respond to treatment with MPH without serious side effects in long-term treatment (Goldman 1998, Brown 2005). Review articles consider common occurring adverse effects from stimulant medication to be appetite suppression, sleep disturbances, headaches, motor tics, abdominal pain,

irritability, nausea, and fatigue. However, it has been shown that many symptoms commonly attributed to stimulant medication are actually pre-existing characteristics of children with ADHD and improve with stimulant treatment. Appetite suppression was the only substantial side effect on MPH (Efron 1997); in clinical practice this is circumvented by taking medication after meals. All patients will be monitored for this and other adverse events during the entire trial with study medication and until three weeks after, both by their psychiatrist and the study investigators, as is standard clinical practice. The aforementioned side effects of MPH treatment would also occur without participating in the study, as they are part of standard practice. Therefore, there is no added risk or burden of this study with respect to MPH treatment.

Chronic placebo treatment (not part of standard practice)
In the Netherlands a waiting list of 17 weeks is very common before (child-and adolescent) psychiatric evaluation can take place and treatment can be commenced (see above). It is in this timeframe that the current study will be executed. All patients will start pharmacotherapy within 2 weeks after study enrollment. Patients can start or continue active treatment as soon as the second assessment of DA function is finished, which will be within 18 weeks after study enrollment. There is therefore only a very small or no delay in the placebo group before active treatment will take place. Therefore we consider the risks associated with study participation negligible, and the burden minimal with respect to pharmacotherapy in the MPH and placebo groups. If needed, the placebo group can start treatment with MPH at the end of the trial. Therefore, we conclude that patients treated with placebo are not harmed by study participation. In sum, we consider the risks associated with study participation involving placebo treatment as negligible, and the burden minimal.

MR imaging (not part of standard practice)
MRI itself is a non-invasive imaging modality; MRI studies in children are earlier approved by the METC of the AMC in children from 8 years of age and older (e.g. MEC 06.053). All patients will receive extensive information about the MRI procedures beforehand. Extensive coaching and preparation of the children to prevent stress and anxiety will be part of the standard procedures: the children get the opportunity to get acquainted with the MRI scanner and its procedures before the real scan session starts, in order to prevent stress and anxiety. Scan sessions will start with scans during which the patient can watch a DVD or listen to music. There will be minor discomfort caused by having to lie absolutely still for several minutes and being fixed in a small space. Subjects suffering from claustrophobia are therefore excluded from participation. Considering the coaching and preparation of the children beforehand, we consider the burden of this procedure to be minimal. Since MR imaging is considered a safe standard medical procedure, also in children and adolescents, we evaluate the risks associated with MR scanning to be negligible.

MPH challenge (not part of standard practice)
In order to evaluate the DA system with MRI we need to administer a DA challenge. The use of phMRI is the least invasive tool available at this moment for determining DA function, as all other methods to challenge or assess DA function involve more invasive techniques (e.g., PET/SPECT involving radiation exposure) and are therefore objectionable, especially in children. We will use a single oral dose of MPH: 0.5 mg/kg (approximately 15 mg for children, 35 mg for adults). MPH (0.5 mg/kg) has been administered as an oral bolus in previous MRI studies up to 50 mg, which was well tolerated. This dose may cause an increase in heart rate and systolic blood pressure, but has no significant side effects. We feel that administration of oral MPH to ADHD patients in this study is justifiable for a number of reasons: a) the challenge will be done with the same medication they would normally be treated with, b) the placebo group would have normally received MPH and will, in all likelihood, receive

active treatment immediately after trial, without a significant treatment delay c) A similar dose (0.6 mg/kg), at commencement of treatment has been successfully given at times in order to convince parents of the diagnosis of ADHD and of the benefits of MPH treatment (Kent 1999). This dose was well tolerated in the treatment group of children and adolescents aged 4-14 years. Thus, also with respect to the oral challenge, we classify the risks associated with study participation as negligible, and the burden as minimal.

In conclusion
The overall nature and extent of the burden associated with participation in the current study are to be classified as minimal and the risk negligible. There is an important individual-, as well as a group benefit associated with study participation.

10.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.6 Incentives (if applicable)

Adult participants will be paid 200 euro's at completion of study, in addition to the so called 'eigen bijdrage' if applicable (for adult subjects). Parents of the children will receive 100 Euro at study completion. If there are costs to the participants for participation in this research project, such as travel expenses, those costs will be paid for. I addition, a small gift will be given to the subjects at the end of each assessment day. If they like, subjects can receive a copy of their MRI on CD-ROM.

11. ADMINISTRATIVE ASPECTS AND PUBLICATION

11.1 Handling and storage of data and documents

Data management will be implemented according to Good Clinical Practice (GCP)-guidelines and supported by the independent Clinical Research Unit (CRU) of the AMC. Source data is only accessible for the coordinating investigator, the study monitor and the patient's psychiatrist (not involved in the study). All other data concerning the study will only be accessible for the investigators. Data will be digitally stored on non-rewritable CD's. The data of all subjects will be coded and this coding will not be retraceable to the individual patient. The key to this coding is safeguarded by the coordinating investigator and the CRU of the AMC. Non-digital data will be stored in a locked closet in a locked room and all data will be saved for a maximum fifteen years. All persons involved in the study agree to keep confidential any information pertaining to the subject's identity, which becomes known to

them in the course of the study. All outcome data beside treatment outcome (answers given on the questionnaires and during the interviews, results on the neuropsychological tests, etc.) will be kept private. This type of data (unrelated to treatment) is only accessible by the study investigators, thus parents or legal guardians and treating physicians/psychiatrists/psychologists will not be informed about these outcomes.

11.2 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

11.3 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.4 End of study report

The investigator will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. In case the study is ended prematurely, the investigator will notify the accredited METC, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.5 Public disclosure and publication policy

Information about this study is available at the database of EudraCT and the website of the Dutch National Competent Authority, the 'Centrale Commissie Mensgebonden Onderzoek' (CCMO). Research data will be published in international scientific papers.

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