Affect modulation of methylphenidate in patients with Attention Deficit Hyperactivity Disorder

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Affect modulation of methylphenidate in patients with Attention Deficit Hyperactivity Disorder

Marco Bottelier
Affect modulation of methylphenidate in patients with Attention Deficit Hyperactivity Disorder
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Affect modulation of methylphenidate in patients with Attention Deficit Hyperactivity Disorder

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CHAPTER 1

General introduction and thesis outline
Attention deficit hyperactivity disorder (ADHD) is the most commonly diagnosed neurodevelopmental disorder, affecting 8-12% of children worldwide (Biederman and Faraone, 2004; Thomas et al., 2015) and 2.5% of adults (Simon et al., 2009). Relatively little is known about the prevalence and correlates of this disorder and the exact pathophysiology of ADHD is still unclear, although many studies suggest that abnormalities of the dopamine system are involved (Fusar-Poli et al., 2012). Methylphenidate (MPH), an amphetamine-like psychostimulant drug which enhances dopaminergic (DA) and noradrenergic (NA) functioning by increasing extracellular DA and NA, is licensed for use in children over 6 years and often prescribed in children and adolescents (Hodgkins et al., 2013). MPH has been shown to be very effective in alleviating symptoms of inattention, hyperactivity and impulsivity in 70% of patients (Schacter et al., 2001; Spencer et al., 2005). There are concerns however regarding the long term consequences of its potential interference with brain development (Andersen and Navalta, 2004). The Health Council of the Netherlands, in 2014 expressed its concern particularly regarding the lack of knowledge on the long-term effects of the drug, while prescription rates are increasing in children and adults (McCarthy et al., 2012) (the Health Council of the Netherlands 2014). Only recently, a decrease in prescription rates was noted in the Netherlands in children under fourteen years of age (Stichting Farmaceutische Kengetallen 2016).

Emotion dysregulation in ADHD
For a long time it has been noted that emotion regulation, an individual’s ability to modify an emotional state to promote adaptive, goal-oriented behaviors (Shaw et al., 2014), is impaired in patients suffering from ADHD. Emotion dysregulation is defined as being easily angered and easily annoyed in children with ADHD, and is found in some 25-45% of children and 30-70% of adults with ADHD (Shaw 2014). Anxiety and depression are not only found more often in children with ADHD than typically developing controls in the last 12 months (9-14% in ADHD vs 1-2% in healthy controls in a community sample), but this effect seems to be more extensive in adult patients with ADHD, were 38,3% comorbid MDD was found in the ADHD group compared to 11,1% in the control group and 47,1% comorbidity with anxiety compared to 19,5% in the control group (Kessler et al., 2006). Emotion dysregulation represents an important feature leading to serious impairment (Wehmeier et al., 2010a), although not regarded a core clinical feature of ADHD in DSM 5. For instance, a substantial proportion of children with ADHD manifest difficulties regulating negative affect (Anastopoulos et al., 2011; Sobanski et al., 2010). Moreover, patients with ADHD and emotion dysregulation are significantly more impaired in peer-relationships, family life, occupational attainment and academic performance than those with ADHD alone (Wehmeier et al., 2010b). Different concepts such as emotion-lability, irritability and emotion dysregulation have been used in the literature to operationalize problems with emotion regulation in ADHD. In 2014 Shaw and Stringaris (Shaw et al., 2014) conceptualized this domain. They stated that emotion dysregulation in ADHD may arise from problems in orienting toward, recognizing and/or allocating attention to emotional stimuli. These deficits may arise from dysfunction within the striato-amygdalo-medio prefrontal cortical network (see also Figure 1).

Shaw proposed three models to explain the overlap between ADHD and emotion dysregulation (1). Emotion dysregulation may be considered a core diagnostic feature of ADHD, with high correlation and a shared neuropsychological, neurobiological end genetic basis (2), the combination of ADHD and emotion dysregulation forms a nosologic new entity, with a distinct neuropsychological, and genetic basis for ADHD and (3) emotion dysregulation and ADHD alone, or symptoms of emotion dysregulation and ADHD are correlated but distinct dimensions where deficits in emotion processing mediates dysregulation and correlates with deficits mediating core ADHD symptoms (Shaw, et al., 2014).
Furthermore, emotion dysregulation is also considered a phenotype for the occurrence of depression and anxiety disorders later in life (Ambrosini et al., 2013). For instance, children with ADHD matching a dysregulation profile (one or two standard deviations above the mean on the combined subscale for attention problems, aggressive behavior and anxious/depressed behavior) have higher rates of anxiety disorders later in life compared to those with ADHD who did not match this dysregulation profile (Althoff et al., 2010). Likewise, children with a comorbid major depressive disorder next to their ADHD have a high risk for suicide attempts (Chronis-Tuscan et al., 2012). Indeed, emotion dysregulation (or irritability) in children with ADHD is predictive of a concurrent affective disorder and complements the predictive validity of this symptom to identify later affective disorders in young adulthood (Ambrosini et al., 2013; Copeland et al., 2009). Furthermore, studies following children with ADHD into adulthood have found elevated rates of adult mood disorders (Klein et al., 2012).

However, although emotional dysregulation is prevalent in ADHD throughout the lifespan and is a major contributor to impairment, it is a much neglected research area (Shaw et al., 2014). This is remarkable given that emotional dysregulation and the treatment thereof can influence the course and outcome of ADHD (Barkley and Fischer, 2010; Marc et al., 2000).

Stimulant treatment and emotion dysregulation in ADHD

Until now, the role that stimulant treatment plays in the occurrence of emotional problems in ADHD still has not been established. Rates of mood and anxiety disorders in children and adults with ADHD are beyond those that would be expected by chance alone (Kessler et al., 2005; Larson et al., 2011; Meinzer et al., 2014).

On the one hand, ADHD and anxiety or depressive disorders coexist and are unrelated to ADHD medications, as suggested by one study in which a significant prevalence of comorbid depression and anxiety was found in medication-naïve adolescents with ADHD (Smalley et al., 2007). Also, there is growing evidence that with ADHD stimulant treatment is associated with normalization of enhanced emotional reactivity (Conzelmann et al., 2009; Daviss et al., 2008; Posner et al., 2011a), suggesting a positive effect of ADHD medications on emotional dysfunction, as well as a protective effect on the development of depressive disorders in ADHD; individuals without a history of Major Depressive Disorder (MDD) reported earlier initiation of stimulant treatment than patients with a history of comorbid MDD (Daviss et al., 2008).

On the other hand, there are preclinical and clinical data suggesting that having a history of stimulant medication is associated with the development of emotional problems later in life. Exposing preadolescent rats to a DA-ergic agent like MPH results in profound changes associated with a depression-like state later in life (Bolaños et al., 2008; Carlezon et al., 2003; Wiley et al., 2009). However, these studies were conducted in normal developing rats which make it to extend these conclusions to clinical practice. But also in humans there are some indications that treatment with ADHD medications at younger age may induce long term effects on emotional state. For instance, children initially randomized to pharmacological treatment in the Multimodal Study of children with ADHD (MTA) had higher rates of anxiety and depression diagnoses at 6 years follow-up, as compared to children initially receiving behavioral treatment and community care (Molina et al., 2013). Perhaps more convincing, is the finding that children who had received pharmacological treatment for ADHD were retrospectively more likely to meet criteria for a depression (MDD) than children with ADHD who had not received pharmacological treatment for ADHD (Jerrell et al., 2015). In this study, duration of treatment was also associated with increased risk for depression (Jerrell et al., 2015). These conflicting findings stress the need for a better understanding of the mechanism of emotion regulation in ADHD and the effect of ADHD medications hereupon.

Neurobiological substrates for emotional functioning in ADHD

Amygdala function measured with functional Magnetic Resonance Imaging (fMRI) is a potential biomarker for emotional processing in adult patients with anxiety disorders and depression (Lau et al., 2010) as well as in adolescent patients with these disorders (Tao et al., 2012). The amygdala is activated most strongly during the processing of emotional faces (Hariri et al., 2002). Therefore, assessment of emotional (dys)regulation in ADHD, increased amygdala reactivity may serve as a premorbid neural biomarker of risk, has been shown to be the case in depression, observable in at-risk individuals before the onset of clinical symptoms or disorder (Swartz et al., 2015). It has been suggested that ADHD patients are more susceptible to negative stimuli following amygdala activation, due to deficits in early processing of visual emotional stimuli and in the modulation of the startle reflex (Shaw et al., 2014). For instance, in ADHD patients, amygdala hyper-activation has been found both during subliminal perception of fearful faces, and during active rating of fear while viewing neutral faces (Brotman et al., 2010; Malisz et al., 2011; Marsh et al., 2008). These functional deficits align with reports of amygdala structural abnormalities in ADHD patients, including surface morphology, volume, as well as dopamine receptor density (Hoogman et al., 2017; Plessen et al., 2006). Not only amygdalar reactivity but also connectivity of the amygdala with areas in the prefrontal and cingulate cortex is involved in emotional processing. In healthy young adults, less anxiety and a more positive daily emotion was found to be related to less connectivity between the amygdala and prefrontal and posterior cingulate cortices (Uchida et al., 2014). In adolescents diagnosed with ADHD, an atypical connectivity with prefrontal areas was found (Posner et al., 2011b). Emotional dysregulation was related to functional connectivity of a cortico-amygdalar...
network and this enhanced amygdala-LPFC connectivity in ADHD patients may suggest an amplification of the negative affect associated with fearful faces (Hulvershorn et al., 2014).

Dopaminergic modulation of amygdala functioning

Although emotional function is typically thought to involve the serotonergic system, several experimental studies support the idea of a DA-ergic contribution to an emotional response (Delaveau et al., 2009; Volkow et al., 2007) but it is unclear whether dopamine activity is enhanced or depressed. Although much less studied, clinical studies now also support DA disruption in emotional processing. For instance, several studies have found positive effects of drugs that increase DA concentrations in the brain, such as the DA reuptake inhibitor MPH: this drug stabilized mood in patients suffering from MDD (El-Mallakh, 2000). Likewise, in healthy controls, the DA releaser and reuptake inhibitor dextroamphetamine (dAMPH), potentiated the response of the amygdala during the perceptual processing of angry and fearful facial expressions (Hariri et al., 2002). Also, in adolescents suffering from ADHD, MPH normalized increased activity of the right amygdala (Posner et al., 2011b). The role of DA in amygdala activity was also suggested by the contribution of a SLC6A3 transporter gene polymorphism to individual variability in amygdala reactivity (Bergman et al., 2014). In fact, it has been shown that DA in the basolateral amygdala is critical for fear processing (Fadok et al., 2009).

As Schultz already pointed out in 1994, the DA-ergic system thus seems to play an important role in emotional processing and this may explain ADHD-related emotional dysfunctions in ADHD but also a positive impact of MPH on emotional dysfunction in ADHD (Schultz 1994). Most of the previous studies however were done in patients not naïve to medication, therefore still raising the possibility that either ADHD itself is associated with emotional dysfunction, or that emotional dysfunction is induced by treatment with psychostimulants.

Neurochemical imprinting

There is some evidence for age dependent effects of emotional dysregulation in ADHD and the effect of ADHD medications thereupon, as there are solid preclinical indications that the developing brain responds differently to MPH when compared to the adult brain (for review see Andersen and Navalta, 2004). For instance, early treatment with MPH leads to a considerable reduction of the DA transporter (DAT) in the striatum and other DA rich regions of adolescent rats when compared to non-treated animals, whereas in adult animals there have consistently been no effects (Grund et al., 2006; Moll, 2001). Also, in young animals MPH induces immediate early gene activation consistent with long-term brain plasticity and reorganization (Adriani et al., 2006). MPH produces oxidative damage in the frontal cortex of young but not adult rats (Martins et al., 2006) and MPH attenuates adult hippocampal neurogenesis only in young rats (Lagace et al., 2006; van der Marel et al., 2014). But also on a behavioral level these age-dependent effects have been noted. Several preclinical studies have demonstrated that exposing pre-adolescent rats to a DA-ergic agents like MPH results in profound changes associated with a depression-like state later in life (Bañados et al., 2003; Carlezon et al., 2003; Wiley et al., 2009).

Evidence is slowly emerging that the long-term effects of drug exposure are delayed and come to expression once the vulnerable system reaches maturation. This phenomenon is known as ‘neuronal imprinting’ and occurs when the effect of drug exposure outlasts the drug itself (Andersen and Navalta, 2004) ‘as brain plasticity permits experiences to shape the immature brain to meet the demands of the environment. Change occurs at various levels – from neuroanatomy, including within a given region and its connectivity to other regions, to the function of neurotransmitter systems and their reactivity to pharmacological agents in the short- and long-term. The nature and degree to which drug exposure influences the final adult topography is influenced greatly by the maturational phase of these critical factors. Moreover, evidence is slowly emerging that suggests that the long-term effects of drug exposure are delayed and expressed once the vulnerable system reaches maturation (i.e., typically during adulthood.’

Still, very little is known on exposure of MPH during later brain development. Most (clinical) studies are hampered by the fact that they are retrospective in design, and therefore the findings could be caused by other factors on which the groups differed. As pointed out by Shaw and colleagues: ‘...the ideal study design for this question would be a randomized trial comparing cortical growth in children on psychostimulants against an unmedicated comparison group—but this would be both logistically and ethically challenging’ (Shaw et al., 2009).

Thesis outline

The aim of this thesis was to explore the occurrence of affective problems in ADHD, to disentangle the effects of MPH on affective problems in ADHD, and to consider age effects on these neurobiological substrates of treatment with MPH. This work is part of a larger research project entitled “effects of Psychotropic drugs On the Developing brain (ePOD)”, in which the effects of MPH, as well as fluoxetine on brain development and behavior are investigated.

General introduction

Chapter 1 offers a short introduction on the significance and relevance of studying affect modulation in ADHD and the effects of DA-ergic agents thereupon. Preclinical findings and the sharp rise in prescriptions of stimulants in the first decade of this century underpin the relevance of studying the imprinting effects of MPH on the developing brain.
Part I – Affect regulation in children with ADHD
First we disentangled the role of stimulant use in the co-occurrence of anxiety and depression with ADHD. In chapter 2 self-reported depressive and anxiety symptoms in stimulant naïve boys with and without ADHD were assessed and the age-trajectory of comorbid symptoms in naïve and prior medicated boys with ADHD were compared.

Part II – Effects of DA modulation on amygdala reactivity in subjects with dysfunctional DA system
To explore the role of the DA system in emotional processing and more specifically the modulation of the amygdala in subjects with documented DA dysfunction, in chapter 3 we examined emotional function in a group of dAMPH users, using task related fMRI before and after oral administration of MPH. We included dAMPH users, because of preliminary evidence that users of this drug suffer from a dysfunctional DA system.

In chapter 4 we extrapolated our findings to patients with ADHD, and investigated the effect of an acute challenge of MPH on amygdala reactivity in medication naïve children and adults with ADHD, using a similar fMRI task as in chapter 3, before and after oral administration of MPH.

Part III – Affect modulation of MPH in ADHD and age dependent effects; the ePOD study
To disentangle the effects of chronic treatment with MPH on affective problems in ADHD and the modulating effects of age, we set up two projects, including a randomized clinical trial (RCT) with MPH. In chapter 5 we describe the objectives and methods of this RCT entitled ‘the effects of Psychototropic drugs On the Developing brain (ePOD-MPH)’, which included medication naïve patients, directly comparing children and adults, in order to investigate its age-dependency (i.e. to investigate the effects on human brain development). In addition, in a retrospective cohort study (ePOD-Pharmo), we investigated the long-term effects of MPH in adults diagnosed with MPH, stratified for age of first exposure.

Chapter 6 describes the results of the ePOD-MPH RCT on emotional dysregulation. In this RCT, medication naïve children and adults were randomly assigned to 4 months of treatment with MPH or placebo. Amygdala reactivity, connectivity and clinical symptoms were measured using fMRI at three time points; before, during and one week after treatment.

As in ePOD-MPH the results are limited to 4 months of ADHD treatment, we explored the long-term effects of chronic stimulant exposure on emotional behavior and in chapter 7, we present the results of ePOD-Pharmo in which the long term modifying effects of age on anxiety and depression of age-of-first-stimulant-
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General introduction and thesis outline

Chapter 1

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CHAPTER 2

Comorbid depression and anxiety symptoms in children and adolescents with ADHD

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Submitted
Abstract

Children with ADHD are at increased risk of developing depression and anxiety. However, previous studies on this co-occurrence have not accounted for stimulant use, while animal and human studies suggest a role of stimulants in the development of depression and anxiety.

In the present study we first assessed self-reported depressive and anxiety symptoms in stimulant naïve boys (10 - 17 years) with (n = 74) and without ADHD (n = 58), and, second, compared the age-trajectory of comorbid symptoms in naïve (n = 30) and prior medicated (n = 51) boys with ADHD (12 - 17 years). Effects of age, ADHD diagnosis, and medication use were analyzed cross-sectionally, in regression analyses with self-reported depressive symptoms on the Children’s Depression Inventory (CDI) and anxiety symptoms on the Screen for Child Anxiety Related Emotional Disorders (SCARED) rating scale as dependent variables.

The ADHD group reported more depressive symptoms than controls, but groups were similar on anxiety symptoms. Furthermore, prior stimulant use was not associated with comorbid symptoms of depression and anxiety.

The results support both a genetic and early developmental explanation of depressive comorbidity in ADHD. When replicated, these findings could be reassuring to therapists, parents and clients who are deciding on the use of stimulant medication for ADHD.

Introduction

ADHD is a neurodevelopmental disorder affecting approximately 5 to 10% of children (Faraone et al., 2003; Polanczyk et al., 2007). In addition to impairment caused by core symptoms of inattention, and/or hyperactivity and impulsivity, individuals with ADHD often have comorbid disorders. In ADHD, levels of depression and anxiety and the risk of a lifetime comorbid diagnosis are significantly higher as compared to ‘typically developing’ (TD) individuals (Angold et al., 1999; Biederman et al., 2006; Blackman et al., 2005; Chronis-Tuscano et al., 2012; Guttmann-Steinmetz et al., 2010; Larson et al., 2011; Lavigne et al., 2009; Ostrander and Herman, 2006; Roy et al., 2014) In a childhood study with a clinically referred sample, comorbid Major Depression Disorder (MDD) was present in 24% of children with ADHD (Spencer, et al., 2000). Although studies with community samples show lower rates than studies with clinical samples, rates of comorbidity are still higher in ADHD as compared to TD (depression: 9 - 14% in ADHD as opposed to 1 - 2% in TD, anxiety: 15 - 35% in ADHD as opposed to 2 - 15% in TD; Blackman et al., 2005; Larson et al., 2011)(Pliszka, et al., 1999).

In late childhood and early adolescence, levels of depression and anxiety are age-dependent, with increasing age being associated with an overall increase in depression (Costello et al., 2011, 2003; Saluja et al., 2004) anxiety (increase of panic disorder and agoraphobia, decrease of separation anxiety disorder, specific phobias, and social phobia; (Copeland et al., 2014; Costello et al., 2011). Differences in development of (comorbid) depression and anxiety between ADHD and TD already occur at a young age (Lavigne et al., 2009). As onset of comorbid depression and anxiety generally follows onset of ADHD, co-occurrence has been suggested to be related to both negative environmental interactions (after a history of failure and punishment, more depressive symptoms develop(Ostrander and Herman, 2006; Schatz and Rostain, 2006) and to shared genetic components (Biederman et al., 1992; Cole et al., 2009). In adults with ADHD, comorbidity seems more extensive than in children with ADHD. For example, a cohort study revealed that 38.3% of an ADHD group met criteria for MDD and 47.1% met criteria for any anxiety disorder in the last 12-months, as opposed to 11.1% and 19.5% of a control group(Kessler et al., 2006). Knowledge regarding comorbid depression and anxiety in ADHD is of importance, as these comorbidities may influence the course of ADHD, as well as the outcome and treatment response (March et al., 2000), (Spencer, 2006b). For example, children with multiple comorbid anxiety disorders have poorer daily functioning and parent-reported quality of life as compared to children with ADHD only (Sciberras and Lycett, 2015) and children with ADHD and MDD are at a high risk of suicide attempts (Chronis-Tuscano et al., 2012). As such, it is important to know how ADHD and depression and anxiety are related.
Children and adolescents with ADHD are often treated with psychostimulants (Hodgkins et al., 2013). However, previous studies on the co-occurrence of ADHD and depressive or anxiety symptoms have not considered the potential confounding effects of medication history on comorbidity. This is surprising, as both animal and human studies suggest that having a history of stimulant medication is associated with the development of a depression- and anxiety-like state, presumably due to cellular alterations in reward pathways in the brain (Bolanos et al., 2008; Carlezon William A et al., 2003; Wiley et al., 2009). For example, (normal) Sprague-Dawley rats treated with MPH during adolescence showed more depressive- and anxiety-like behaviour in adulthood than saline vehicle-treated control rats. In line with this, although not controlled for baseline levels of ADHD, children who had received pharmacological treatment for ADHD were more likely to meet MDD criteria than children with ADHD who had not received pharmacological treatment, and duration of treatment was associated with this increased risk (Jerrell et al., 2014). Furthermore, the children initially randomized to pharmacological treatment in the Multimodal Treatment of Attention Deficit Hyperactivity Disorder (MTA) study study had higher rates of anxiety or depression diagnosis at six year follow-up, as compared to children initially receiving behavioral treatment and community care, although this effect was not observed at later follow-up (Molina et al., 2009). On the other hand, Smalley and colleagues (Smalley et al., 2007) showed significant comorbid depression and anxiety in stimulant naïve adolescents with ADHD in a cohort study, suggesting that these disorders also co-exist without the influence of stimulants. Some studies take this even further by suggesting a protective effect of stimulant use. A retrospective study (Daviss et al., 2008) of ADHD revealed that individuals without a history of comorbid MDD reported earlier initiation of stimulant treatment than individuals with a history of comorbid MDD, which authors interpret as a potential protective effect of stimulant use. In addition, a cohort study, monitoring children newly diagnosed with ADHD, demonstrated that duration of stimulant treatment in children was related to depression, with a reduced comorbidity risk with longer treatment duration (Lee et al., 2015). Although contrasting, these findings emphasize the importance of taking medication status into consideration in ADHD comorbidity research, as it could well be that previous findings on comorbidity have been affected by medication history.

Therefore, the present cross-sectional study aims to determine the relationship between ADHD diagnosis, (prior) stimulant use, age, and depressive and anxiety symptoms in boys between the ages of 10 to 17 years. Two studies were executed. Study 1 evaluated whether having an ADHD diagnosis moderates the relationship between age and depressive/anxiety symptoms between the ages of 10 to 17 years. In order to exclude possible (short- or long-term) influences of pharmacotherapy, this study included stimulant naïve boys between the ages of 10 to 17 years with a clinical diagnosis of ADHD, and a TD group of a similar age.

The second study examined whether having a history of stimulant use moderates the relationship between age and depressive and anxiety symptoms, comparing a stimulant naïve and a medicated sample of male adolescents aged 12 to 17 years with ADHD (ADHD- and ADHD+).

Following the hypothesis that both shared genetic components and negative environmental interactions (Daviss et al., 2008) contribute to the development of depressive and anxiety comorbidity in stimulant naïve boys with ADHD, we expected increased depression and anxiety in the ADHD group as compared to the TD group. In addition, we expected an interaction between age and ADHD diagnosis for depressive symptoms (a stronger increase in depressive symptoms with increasing age for the ADHD group in comparison to the TD group), and an interaction between age and ADHD diagnosis for anxiety symptoms (showing stable or increasing anxiety symptoms in the TD group, and a stronger increase in anxious symptoms with increasing age for the ADHD group). Given the conflicting evidence in the literature, we did not formulate specific expectations regarding the relationship between stimulant use and comorbidity, however, a different trajectory of anxiety and depression in ADHD- and ADHD+ adolescents could imply a role of stimulant medication in the development of comorbid depression and anxiety in ADHD.

Methods

Participants

In Study 1, we included a male stimulant naïve ADHD group (n = 74) and TD group (n = 58) aged 10 to 17 years. In Study 2, we included boys aged 12 to 17 years who were stimulant naïve (ADHD-; n = 30), or had a history of stimulant treatment (ADHD+; n = 51). For both studies, participants with an estimated IQ < 80 and/or a history of major medical or neurological trauma or illness were excluded. Participants with ADHD were recruited through child and adolescent psychiatry outpatient clinics. They either had been diagnosed previously, or were diagnosed at the intake of the study with ADHD (IT, HI or CT) by experienced clinicians, based on the DSM-IV-TR (APA, 2000). Children and adolescents with ADHD were included only when their clinical diagnosis was confirmed with a structured interview (Diagnostic Interview Schedule for Children [NIMH DISC-IV; Ferdinand & Van der Ende, 1998] parent- or self-report). In addition, the ADHD- group had never used stimulants and was seeking treatment for ADHD related impairment. ADHD+ participants were eligible when parents reported that children used stimulant medication currently and had taken medication compliantly in the past four weeks. Treatment was discontinued 24 hours before the assessment of depressive and anxiety symptoms, as the adolescents also participated in neuropsychological
testing for which they needed to be stimulant free at the time of testing (Boyer et al., 2014). The TD group was recruited through typical schools. Exclusion criteria were a clinical psychiatric diagnosis of ADHD or ASD, and scoring above the 90th percentile on the parent DBD (Oosterlaan et al., 2000). For participants aged 17 years, DBD norms for 16-year olds were applied, as norms for 17 year olds were not available.

**Group selection**

**ADHD** - Parents of the ADHD groups were approached through their child's clinician at child and adolescent psychiatry outpatient clinics. Before entering the studies, caregivers and participants aged 12 years or older gave written informed consent, and children younger than 12 years gave verbal informed consent. All children and adolescents filled out the SCARED and CDI, and were tested with the Block Design and Vocabulary subtests of the WISC-III-R (Kort et al., 2002), in order to estimate IQ. Parents were interviewed using the DISC-IV and filled out the DBD rating scale.

**TD** - Fifty-eight TD children were recruited through regular schools throughout the Netherlands. Parents of TD children and adolescents received a letter through school asking for their cooperation. After giving informed consent, procedures were the same as described for the ADHD group, except no DISC-IV interview was administered.

**4.2.3 Inclusion materials**

The DISC-IV (Ferdinand & Van der Ende, 1998) is a structured interview based on DSM-IV (APA, 1994) and ICD-10 (World Health Organization, 1993) criteria. The ADHD section of the DISC-IV was administered by one of the researchers or a trained research assistant in order to verify the clinical diagnosis and determine the ADHD subtype.

The DBD (Oosterlaan et al., 2000) is a questionnaire assessing parent-reported externalizing symptoms in children aged 6 to 16 years. The DBD comprises 42 items reflecting core symptoms of inattention (9 items), hyperactivity/impulsivity (9 items), oppositional defiant disorder (ODD; 8 items), and conduct disorder (CD; 16 items). Parents indicate the frequency of the child’s externalizing behavior. Responses range from not at all, a little, pretty much to very much and are scored with 0, 1, 2, or 3 respectively. Good internal consistency was shown for the subscales inattention, hyperactivity/impulsivity, and oppositional defiant disorder with a non-clinical, normative sample of n = 1607 (α = .88 to .89). The DBD is commonly used to exclude TD children scoring above the 90th percentile (Boyer et al., 2014; Luman et al., 2012; Wiersema et al., 2006).

**Dependent measures**

The Children’s Depression Inventory (Kovacs, 1985; Timbremont and Braet, 2001) is a self-report measure of childhood depression. The CDI was designed for children aged 7 to 17 years and comprises 27 items. Each item is formulated as three statements, from which the respondent chooses the one that best reflects his/her thoughts and feelings in the last two weeks. The total score ranges between 0 and 54, as statements are scored as 0, 1, or 2, with a higher total score indicating more depressive symptoms. Internal consistencies of α = .85 and α = .86 have been found in nonclinical and clinical samples respectively (Timbremont and Braet, 2001).

The Screen for Child Anxiety Related Emotional Disorders (Muris et al., 2007) is a self-report questionnaire, screening for symptoms of childhood anxiety. It was designed for children aged 7 to 19 years and gives an indication of the presence of the following anxiety disorders: panic (13 items), social phobia (7 items), obsessive-compulsive (9 items), posttraumatic stress (4 items), generalized (9 items), separation anxiety (12 items), and specific phobias [i.e. animal (3 items), medical (7 items), and situational (5 items)]. Children and adolescents indicate whether they experience the symptom never or seldom, sometimes, or often (scored 0, 1, or 2 respectively). Good internal consistency (α = .92) was shown with a non-clinical, normative sample of n = 1011 (Muris et al., 2007).

**Statistical analyses**

In both Study 1 and Study 2, we conducted two linear, forced-entry regression analyses, one with the raw total CDI score and one with the raw total SCARED score as the dependent variable. Predictor variables in Study 1 analyses were age (continuous), ADHD diagnosis (dichotomous: yes/no) and the interaction between age and ADHD diagnosis. In Study 2, age (continuous), prior stimulant use (dichotomous: yes/no), and the interaction between age and prior stimulant use were entered as predictors. In order to determine whether an additive differential effect of age and ADHD diagnosis was present, and to account for multicollinearity, the predictors age and ADHD diagnosis were entered in the first model, and age x ADHD diagnosis was added in the second model. Given the number of comparisons and the conservativeness of a Bonferroni correction, we used the Hochberg (1988) modification of the Bonferroni correction to account for multiple comparisons. This resulted in alphas ranging from .004 to .05 for observed p-values ordered sequentially from smallest to largest. We looked for outliers and adjusted these values to the next highest score plus one (Field, 2009) in order to reduce their influence on the regression model (for SCARED n = 1 outlier with value 100

---

1The majority of the children (aged 9-12 years) were asked to participate in an RCT with medication treatment (Bottelier et al., 2015) Some of the children, and all of the adolescents (aged 12-17 years), were included in an RCT with cognitive behavioral therapy (Boyer et al., 2015) Questionnaires were administered before the start of treatment.
was adjusted to a value of 79). Assumptions of linearity and multicollinearity were met. In order to meet the assumption of normality of the standardized residuals, data was square root transformed (Study 1) and log transformed (Study 2) for the depression analysis, and log transformed (Study 1) for the anxiety analysis, which normalized the skew (D = 0.062, \( p = .200 \), D = 0.081, \( p = .200 \), and D = 0.061, \( p = .200 \) respectively). All statistical analyses were done with Statistical Package for the Social Sciences version 20 (SPSS/IBM 2011).

Results

Descriptives

Even though age was entered as a continuous variable in the main analyses, we compared the younger (aged 10 - 11 years, \( n = 28 \)) and older stimulant naïve ADHD groups (aged 12 - 17 years, \( n = 46 \)) on ADHD symptoms and diagnostic subtypes, to determine whether our sample characteristics were in line with the literature. We assigned children from the age of twelve to the older group, as children in the Netherlands generally make the transition from middle to high school at the age of twelve. We applied independent samples t-tests to normally distributed data and Mann-Whitney tests to not normally distributed data. The younger and older children differed significantly in the type of ADHD diagnosis on the DISC, with relatively more children in the older group meeting criteria for the IT (\( \chi^2 = 11.146, df = 2, p = .004 \)), as can be seen in Table 5.1. This is consistent with the development of ADHD symptoms over time, with a decline in inattentive and especially hyperactive/impulsive symptoms during adolescence (Biederman et al., 2000) However, younger and older children did not differ significantly in parent-reported inattentive (U = 558.0, \( z = -0.036, p = .72 \)), hyperactive/impulsive (t(68) = 1.83, \( p = .072 \)), ODD (U = 590.0, \( z = 0.024, p = .98 \)) and CD symptoms (U = 547.50, \( z = -0.61 \)) on the DBD. In addition, the age groups did not differ in CDI Total Score (t(71) = -1.228, \( p = .23 \)) and IQ (U = 747.00, \( z = 1.713, p = .09 \)), but the younger age group did show a higher SCARED Total Score as compared to the older age group (U = 435.5, \( z = -2.208, p = .03 \)).

The stimulant naïve ADHD and TD group were comparable in mean age and IQ. As expected, the ADHD group scored higher on DBD symptoms of inattention, hyperactivity/impulsivity, ODD and CD (see Table 4.2).

<table>
<thead>
<tr>
<th>w</th>
<th>Aged 9 - 11</th>
<th>Aged 12 - 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISC-IV IT</td>
<td>12</td>
<td>43%</td>
</tr>
<tr>
<td>DISC-IV HI</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>DISC-IV CT</td>
<td>15</td>
<td>53%</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>100%</td>
</tr>
</tbody>
</table>

Note: DISC=Diagnostic Interview Schedule for Children; IT=inattentive subtype; HI=hyperactive/impulsive subtype; CT=combined subtype.

Table 2.2 | Characteristics of the stimulant naïve ADHD and TD children and adolescents.

<table>
<thead>
<tr>
<th>ADHD-</th>
<th>TD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (SD)</td>
<td>n</td>
</tr>
<tr>
<td>Age</td>
<td>11.9 (1.5)</td>
</tr>
<tr>
<td>IQ</td>
<td>105.0 (16.0)</td>
</tr>
<tr>
<td>DBD(^a)</td>
<td>Inatt</td>
</tr>
<tr>
<td></td>
<td>Hyp/Imp</td>
</tr>
<tr>
<td></td>
<td>ODD</td>
</tr>
<tr>
<td></td>
<td>CD</td>
</tr>
<tr>
<td></td>
<td>CDI</td>
</tr>
<tr>
<td></td>
<td>SCARED</td>
</tr>
</tbody>
</table>

Note: ADHD=Attention Deficit Hyperactivity Disorder; TD=Typically Developing children; IQ=estimated IQ; DBD=Disruptive Behavior Disorders scale; Inatt=inattention; Hyp/Imp=hyperactive/impulsive; ODD=Oppositional Defiant Disorder; CD=Conduct Disorder; CDI=Children’s Depression Inventory Total Score; SCARED=Screen for Child Anxiety Related Disorders Total Score.

\(^a\)raw score, \(^b\)Mann-Whitney Test, \(^*p<.05\), \(^{**}p<.01\), \(^{***}p<.001\)
The ADHD- and ADHD+ group were comparable in IQ and DBD symptoms of hyperactivity/impulsivity, ODD and CD (see Table 5.3). However, the ADHD+ group was older, and their parents reported less DBD symptoms of inattention. In the ADHD+ group, 13 participants used short-acting MPH (n = 1 once daily, n = 9 twice daily, n = 3 three times daily), 36 participants used long-acting MPH, and 2 used dexamphetamine (n = 2 twice daily). The daily dosage of MPH ranged between 10 and 90 mg, with a mean dosage of 33.41 mg (SD = 17.04, n = 41). Dosage was not significantly associated with the number of depressive and anxiety symptoms (r = -.06, p = .73 and r = .16, p = .33 respectively). No information was available about the duration of stimulant treatment.

Table 2.3 | Characteristics of the prior medicated and stimulant naïve adolescent Comorbidity in stimulant naïve boys (Study 1)

|          | ADHD+  | ADHD-  | Statistics  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>M (SD)</td>
<td>n M (SD)</td>
<td>n</td>
</tr>
<tr>
<td>IQ</td>
<td>105.9 (18.2)</td>
<td>51</td>
<td>106.0 (14.5)</td>
</tr>
<tr>
<td>DBD*Inatt</td>
<td>16.4 (5.1)</td>
<td>50</td>
<td>19.8 (5.2)</td>
</tr>
<tr>
<td>Hyp/Imp</td>
<td>8.7 (6.5)</td>
<td>50</td>
<td>10.1 (6.7)</td>
</tr>
<tr>
<td>ODD</td>
<td>5.8 (4.3)</td>
<td>50</td>
<td>6.2 (5.2)</td>
</tr>
<tr>
<td>CD</td>
<td>0.8 (1.6)</td>
<td>48</td>
<td>1.4 (1.9)</td>
</tr>
<tr>
<td>CDI</td>
<td>10.2 (5.8)</td>
<td>51</td>
<td>9.3 (4.0)</td>
</tr>
<tr>
<td>SCARED</td>
<td>20.8 (14.1)</td>
<td>50</td>
<td>20.9 (10.2)</td>
</tr>
</tbody>
</table>

Note: ADHD=Attention Deficit Hyperactivity Disorder; ADHD+=prior medicated ADHD group; ADHD-=stimulant naïve ADHD group; IQ=estimated IQ; DBD=Disruptive Behavior Disorders scale; Inatt=inattention; Hyp/Imp=hyperactive/impulsive; ODD=Oppositional Defiant Disorder; CD=Conduct Disorder; CDI=Children’s Depression Inventory Total Score; SCARED=Screen for Child Anxiety Related Emotional Disorders Total Score. *raw score, †Mann-Whitney U or independent samples t-test, *p<.05, **p<.01, ***p<.001

Comorbidity in stimulant naïve boys (Study 1)
Depressive symptoms in ADHD- and TD boys aged 10 to 17 years

The total number of children that completed the CDI was 131. The linear regression analysis on square root transformed data revealed that in the first model, age and ADHD diagnosis together explained a significant proportion of variance in CDI scores (F(2, 128) =12.661, p < .001, R² = .165). ADHD diagnosis significantly predicted CDI scores (β = .379, t (130) = 4.662, p < .001 respectively); having an ADHD diagnosis was associated with an increase in depressive symptoms. Following correction for multiple comparisons (α = .005), age was not a significant predictor (β = .198, t (130) = 2.433, p = .016). The second model, incorporating the interaction term age x ADHD diagnosis, did not add to the first model (ΔF(1, 127) = 0.051, p = .821, ΔR² = .001). Table 5.4 and Figure 5.1 present the relationship between age, ADHD diagnosis, and depressive symptoms.

Table 2.4 | Regression analysis with age and ADHD diagnosis predicting depressive symptoms

<table>
<thead>
<tr>
<th></th>
<th>CDIa,b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>Variable</td>
<td>B</td>
</tr>
<tr>
<td>Age</td>
<td>0.10</td>
</tr>
<tr>
<td>ADHD diagnosis</td>
<td>0.65</td>
</tr>
<tr>
<td>Age * ADHD</td>
<td>0.02</td>
</tr>
<tr>
<td>R²</td>
<td>.17</td>
</tr>
<tr>
<td>F ΔR²</td>
<td>12.66*</td>
</tr>
</tbody>
</table>

Note: CDI=Children’s Depression Inventory; ADHD=Attention-Deficit Hyperactivity Disorder

*a square root transformed dependent variable, b n=131.

*significant value (predictor specific thresholds after Hochberg correction: α=.04 for the interaction term, α=.008 for age, and α=.004 for ADHD diagnosis).
**Figure 2.1** | The relationship between age, ADHD diagnosis and depressive symptoms

![Graph showing the relationship between age, ADHD diagnosis, and depressive symptoms.](image)

Note: ADHD=Attention-Deficit Hyperactivity Disorder, TD=Typically Developing children, CDI=Children’s Depression Inventory Total Score.

Anxiety symptoms in ADHD- and TD boys aged 10 to 17 years

A total number of 132 children completed the SCARED rating scale. The linear regression analysis on log-transformed data revealed that the first model with age and ADHD diagnosis did not explain a significant proportion of variance in SCARED scores ($F(2, 129) = 2.529, p = .084, R^2 = .038$). The second model, adding the interaction term age x ADHD diagnosis, did not add to the first model ($\Delta F(1, 128) = 1.168, p = .282, \Delta R^2 = .009$). Although the Mann-Whitney test showed that the younger ADHD group had more anxiety symptoms than the older ADHD group, the regression analysis showed that age and ADHD diagnosis, and the interaction between age and ADHD diagnosis, did not predict anxiety symptoms. Figure 4.2 and Table 4.5 present the relationship between age, ADHD diagnosis, and anxiety symptoms.

**Table 2.5** | Regression analysis with age and ADHD diagnosis predicting anxiety symptoms

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE B</th>
<th>B</th>
<th>SE B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.02</td>
<td>0.02</td>
<td>-0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>ADHD diagnosis</td>
<td>0.09</td>
<td>0.06</td>
<td>0.52</td>
<td>0.41</td>
</tr>
<tr>
<td>Age * ADHD</td>
<td>-0.04</td>
<td>0.03</td>
<td>-0.67</td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td>.04</td>
<td></td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>$F \Delta R^2$</td>
<td>2.53</td>
<td></td>
<td>1.17</td>
<td></td>
</tr>
</tbody>
</table>

Note: SCARED= Screen for Child Anxiety Related Emotional Disorders, ADHD=Attention-Deficit Hyperactivity Disorder.

$log$ transformed dependent variable, $n=132$.

*significant value (predictor specific thresholds after Hochberg correction: $\alpha=.03$ for the interaction term, $\alpha=.02$ for age, and $\alpha=.01$ for ADHD diagnosis).
Comorbidity in medicated boys (Study 2)

Depressive symptoms in ADHD+ and ADHD- boys aged 12 to 17 years

The total number of adolescents completing the CDI was 81. A linear regression analysis on log transformed data from an ADHD+ and ADHD- group revealed that the first model with age and medication group did not explain a significant proportion of variance in CDI scores (F(2, 78) = 1.274, \( p = .285 \), R\(^2\) = .032). The incorporation of the interaction-term in the second model did not add to the first model (AF(1, 77) = .781, \( p = .380 \), \( \Delta R^2 \) = .010; see Appendix 5.1). Thus, age and medication use did not predict the number of depressive symptoms.

Anxiety symptoms ADHD+ and ADHD- adolescents aged 12 to 17 years

The total number of adolescents completing the SCARED rating scale was 80. A linear regression analysis on normal data from an ADHD+ and ADHD- group revealed that the first model with age and group did not explain a significant proportion of variance in SCARED scores (F(2, 77) = .051, \( p = .950 \), R\(^2\) = .001). Incorporating the interaction-term in the second model did not add to the first model (\( \Delta F(1, 76) = .517 \), \( p = .474 \), \( \Delta R^2 \) = .007; see Appendix 4.2). Age and medication use did not predict the number of anxiety symptoms.

Exploratory analyses

As some anxiety disorders (separation anxiety disorder, specific phobias, social phobia) have been reported to decrease, and others (panic disorder and agoraphobia) have been reported to increase with increasing age (Costello et al., 2011), these effects might have counteracted each other. Thus, we created a dependent variable omitting the SCARED items regarding anxiety disorders that decrease with increasing age in the general population (35 items remaining) and tested the predictive value of age and ADHD diagnosis or medication group in the first model and of the interaction term in the second model. Neither the models in Study 1 predicted anxiety (F(2, 129) = 1.919, \( p = .15 \), and F(3, 128) = 1.611, \( p = .19 \)), nor the models in Study 2 (F(2, 77) = 0.214, \( p = .81 \), and F(3, 76) = 0.358, \( p = .78 \)) predicted anxiety.

Discussion

The aim of the two present studies was to determine the relationship between depressive and anxiety symptoms, ADHD diagnosis, and prior stimulant use in child and adolescent boys. In the primary study we incorporated a unique sample of stimulant naïve child and adolescent boys with ADHD (aged 10-17 years). The results showed more depressive symptoms in the stimulant naïve ADHD group as compared to the TD group. As reported previously for boys (Costello et al., 2011) an age effect was not evident for depressive symptoms, thus, older boys generally did not show more depressive symptoms than younger boys. With reference to the other group, neither group demonstrated an increase of depression with increasing age. Furthermore, levels of anxiety were similar in the stimulant naïve ADHD and TD group, and no age effects or differences in age effects between groups were observed. In the second study, we compared stimulant naïve to prior medicated boys with ADHD (aged 12 - 17 years). Importantly, depressive or anxiety symptoms were not related to prior stimulant use. To sum up, the present study shows increased levels of self-reported depressive symptoms, but not anxiety symptoms, in stimulant naïve boys aged 10 to 17 years with ADHD. In addition, comparable levels of depression and anxiety were observed in boys aged 12 to 17 years with and without a history of stimulant use.

The association between ADHD diagnosis and depressive symptoms in stimulant naïve participants, observed in Study 1, combined with the lack of a relationship between stimulant use and depression, observed in Study 2, suggest that having a history of stimulant use is not associated with the development of depressive comorbidity in ADHD. This extends previous research showing co-morbidity in a cohort study with stimulant naïve adolescents with ADHD (Smalley et al., 2007) Yet, it contrasts work indicating either detrimental or protective effects of stimulants on comorbid depressive symptoms (Daviss et al., 2008; Jerrell et al., 2014; Lee et al., 2015). Furthermore, we did not observe accumulation of comorbid symptoms with increasing age in ADHD as compared to TD. This finding was coherent with a study showing comparable comorbidity levels in medicated school-aged boys and adolescents with ADHD (Biederman et al., 1998). However, accumulation of comorbid symptoms could be expected if depression would (in part) follow from ongoing negative environmental interactions, which are more prevalent in ADHD (Ostrander and Herman, 2006). Thus, the results are in line with hypotheses of shared genetic components for ADHD and depression or early environmental influences (Biederman et al., 1992; Cole et al., 2009), but not with ongoing effects of environmental interactions or prior use of stimulant medication. As for anxiety, we observed a comparable level of symptoms across ages, diagnostic status, and medication status, suggesting that ADHD and prior stimulant use do not affect anxiety, even though elevated anxiety levels have consistently been described in male medicated ADHD groups as compared to TD groups (Angold et al., 1999; Biederman et al., 2006; Guttmann-Steinmetz et al., 2010; Larson et al., 2011). In part, this could be in line with the fact that we studied the present occurrence of anxiety symptoms, as opposed to lifetime diagnosis of anxiety disorders, which is evaluated in the majority of studies. Symptoms of specific disorders, such as separation anxiety, decrease drastically from childhood to early adolescence, and the normal levels of anxiety in the present study could be in line with the fact that we focused on current symptoms. As for depression, the lack of age-depend-
Comorbid depression and anxiety symptoms in children and adolescents with ADHD

Chapter 2

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ency might be explained by the inclusion of boys only, while the age-dependency of anxiety is more consistent in girls than in boys (Copeland et al., 2014).

Several alternative interpretations for the present findings were explored. For example, the lack of a relationship between anxiety and age could be due to counteracting of separate anxiety disorders decreasing and increasing with increasing age (Costello et al., 2011). Furthermore, one might argue that comorbid disruptive disorders explain comorbid depressive symptoms (Nigg et al., 2004) Also, an overlap between ADHD and depression on items of the CDI (e.g. concentration problems; Hoza et al., 1993) could explain the difference in depressive symptoms between the stimulant naïve ADHD and TD group. We therefore conducted separate analyses, omitting SCARED items that decrease with increasing age, incorporating ODD symptoms into the predictive model, and omitting CDI items regarding concentration, school accomplishment and finishing homework. None of these approaches altered the present conclusions (ODD and CDI analyses available from first author upon request).

Remaining caveats could well be that we, first of all, combined participants recruited for two different types of treatment studies within a cross-sectional design. However, the young group (mainly consisting of participants from the medication trial) and old group (mainly consisting of participants from the trial with behavioural treatment) in Study 1 were comparable in parent reported age of ADHD symptom onset, in ODD and CD symptoms, and the observed shift in diagnostic subtypes (from combined to inattentive) with increasing age is in line with the literature (Biederman et al., 2000). Nevertheless, recruitment bias cannot be ruled out as the treatment goals of the original children and adolescent studies differed (respectively medication and cognitive behavioral therapy). Second, several factors limit the findings of Study 2. The time off-medication at assessment was 24 hours only, while questions in the depression questionnaire apply to the past two weeks. Thus, a longer period of time off medication would be optimal in determining potentially lasting effects of MPH, however, this is ethically and practically challenging. Furthermore, we did not document the duration of stimulant use in the ADHD+ group, hence, we only know they received stimulant treatment for at least four weeks prior to discontinuation for assessment. Also, the current dosage was quite low (mean dosage of 33.41 mg), however, a study demonstrated that the additional effect of increasing the MPH dosage over 10 mg was marginal in children aged 12-17 years (Smith et al., 1998). Even though this indicates that sensitivity to stimulants might be higher during adolescence as compared to childhood, the effects of higher dosing on comorbidity need to be determined, preferably in a study in which treatment duration is documented and, if ethically possible, following a longer off-medication interval. A third limitation is that we included a clinical sample of boys, limiting the generalizability of the conclusions to girls and non-clinical samples, and evaluated current symptoms instead of the lifetime occurrence of comorbid disorders. Studies with TD children reveal higher levels of depression and anxiety and an (steeper) age-related increase in girls as compared to boys (Costello et al., 2011, 2003). Regarding comorbidity in ADHD, results are less consistent, but most reveal a higher risk of internalizing problems in girls as compared to boys (Gershon, 2002; Romano et al., 2005; Yoshimasu et al., 2012), for null findings see (Abikoff et al., 2002; Gaub and Carlson, 1997). Nevertheless, many studies have revealed increased mood comorbidity in boys with ADHD, which underlines the importance of the current study. Finally, we observed only small effects and large variance, suggesting that other predictors, not included in our model, contribute to comorbid levels of depression and anxiety in ADHD. Other factors associated with the level of comorbidity in ADHD might be, for example, parenting behaviour and psychopathology, social functioning, and emotional lability (Karustis et al., 2000; Ostrander and Herman, 2006; Seymour et al., 2012; Sobanski et al., 2010). Unfortunately, we did not assess all of these factors in the present study, but we did take the age trajectories of different anxiety disorders, the role of oppositional behavior, and overlap between the CDI and ADHD symptoms into account.

In summary, the present study shows increased levels of self-reported depressive symptoms, but not anxiety symptoms, in stimulant naive boys aged 10 to 17 years with ADHD, and comparable levels of depression and anxiety in stimulant naïve and prior medicated boys aged 12 to 17 years with ADHD. These findings are in line with hypotheses of shared genetic components for ADHD and depression, or early environmental influences, and do not support a role of stimulant medication in the development of depression and anxiety. Thus far, the role of stimulant use on mood and affect in humans has received little attention, while it is relevant to know whether stimulants affect the development of comorbidity, as scientific consensus and guidelines on the treatment of ADHD depend on such knowledge. Hence, limitations of the present study should be kept in mind and the findings should be replicated in order to adequately conclude a lack of stimulant effects on the development of depression and anxiety. For future research, it would be of interest to prospectively determine the effect of stimulants on the development of depression and anxiety, preferably in a randomized, placebo-controlled trial.
Appendices chapter 2

Appendix 2.1 | Regression analysis with age and stimulant use predicting depressive symptoms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE B</td>
<td>β</td>
<td>B</td>
</tr>
<tr>
<td>Age</td>
<td>0.03</td>
<td>0.02</td>
<td>.19</td>
<td>0.06</td>
</tr>
<tr>
<td>Stimulant use</td>
<td>-0.02</td>
<td>0.06</td>
<td>-.03</td>
<td>0.54</td>
</tr>
<tr>
<td>Age * stimulant use</td>
<td>-.04</td>
<td>0.05</td>
<td>.05</td>
<td>-1.18</td>
</tr>
<tr>
<td>R²</td>
<td>.03</td>
<td></td>
<td>.04</td>
<td></td>
</tr>
<tr>
<td>F ∆R²</td>
<td>1.27</td>
<td>0.78</td>
<td></td>
<td></td>
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</tbody>
</table>

*log transformed dependent variable, a, n=81

*significant value (predictor specific thresholds after Hochberg correction: α=.04 for stimulant use, α=.03 for the interaction term, and α=.02 for age).

Appendix 2.2 | Regression analysis with age and stimulant use predicting anxiety symptoms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE B</td>
<td>β</td>
<td>B</td>
</tr>
<tr>
<td>Age</td>
<td>-0.36</td>
<td>1.14</td>
<td>-.04</td>
<td>1.54</td>
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<tr>
<td>Stimulant use</td>
<td>0.28</td>
<td>3.21</td>
<td>.01</td>
<td>-23.80</td>
</tr>
<tr>
<td>Age * stimulant use</td>
<td>1.75</td>
<td>2.43</td>
<td>.98</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>&lt;.01</td>
<td></td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>F ∆R²</td>
<td>0.05</td>
<td>.52</td>
<td></td>
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</tr>
</tbody>
</table>

*a, n=80

*significant value (predictor specific thresholds after Hochberg correction: α=.05 for stimulant use, α=.04 for age, and α=.03 for the interaction term).

References


References


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Comorbid depression and anxiety symptoms in children and adolescents with ADHD


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CHAPTER 3

Effects of methylphenidate during emotional processing in amphetamine users: preliminary findings

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Marieke L.J. Schouw — Michiel B. de Ruiter
Henricus G Ruhé — Ramón J.L. Lindauer
Liesbeth Reneman

Brain Imaging and Behavior, 2015; 9: 878-886
Abstract

D-amphetamine (dAMPH) and methylphenidate (MPH) are stimulants used in the treatment of Attention Deficit Hyperactivity Disorder (ADHD). Preclinical studies have shown that in healthy animals, dAMPH induces dopamine (DA) dysfunction, as evidenced for instance by loss of DA and its transporters. It has also been suggested that DA plays an important role in emotional processing, and that altered DA-ergic intervention may modulate amygdala function. To explore the role of the DA system in emotional processing we examined emotional processing using functional magnetic resonance imaging (fMRI) in eight male recreational users of dAMPH and eight male healthy controls. We compared brain activation between both groups during an emotional face-processing task with and without an oral MPH challenge. All subjects were abstinence for at least 2 weeks during the baseline scan. The second scan was performed on the same day 1½ hours after receiving an oral dose of 35 mg MPH.

We observed a significant Valence*Group interaction indicated that only for fearful faces, significant group differences were present ($p = .037$) that were robust against adjustment for age ($p = .015$) Furthermore, duration of amphetamine use in years was positively correlated with amygdala reactivity in dAMPH ($r = .76; p = .029$). These exploratory findings are in line with previous findings suggesting that DA plays a role in emotional processing.

Introduction

Although emotional function is typically thought to involve the serotonergic system, several experimental studies support the idea of a dopamine (DA)-ergic contribution to an emotional response, as suggested by biochemical, pharmacological and lesion experiments (for review see (Bolaños et al., 2003; Carlezon William A et al., 2003; Delaveau et al., 2005)).

Clinical studies now also support DA disruption in emotional processing. For instance, several studies have found positive effect of the drugs that increase DA concentrations in the brain: such as the DA reuptake inhibitor methylphenidate (MPH), as this drug stabilizes mood in patients suffering from major depression (El-Mallakh, 2000). Likewise, in healthy controls, the DA releaser and reuptake inhibitor d-amphetamine (dAMPH), potentiacted the response of the amygdala during the perceptual processing of angry and fearful facial expressions (Hariri et al., 2002). In adolescents suffering from attention deficit hyperactivity disorder (ADHD), fear processing is associated with amygdalar hyper activation (Brotman et al., 2010; Posner et al., 2011b) and MPH normalized increased activity of the right amygdala (Posner et al., 2011a). Hence, there seems to be a role for DA disruption in emotional processing.

Emotional dysregulation has been described in children with ADHD 6 years but not 8 years after stimulant treatment (Molina et al., 2009). In line with this, several preclinical studies have demonstrated that exposing preadolescents rats to a DA-ergic agents like MPH results in profound changes associated with a depression-like state later in life (Bolaños et al., 2003; Carlezon William A et al., 2003). It has been shown in animals that these emotional deficits can be reversed by antidepressant treatment in adulthood with a serotonine reuptake inhibitor, such as fluoxetine (Bolaños et al., 2008). The amygdala is a brain structure critical for emotional processing and activates most strongly during the processing of emotional faces (Tessitore et al., 2002a). Amygdala function measured with functional Magnetic Resonance Imaging (fMRI) is a well-known biomarker of emotional dysregulation, i.e depression (Tao et al., 2012). For instance, increased amygdala activity assessed with fMRI has been found in patients suffering from major depressive disorder (MDD), which decreased after successful treatment with paroxetine (Ruhé et al., 2011).

To further explore the role of the DA system in emotional processing, we examined emotional function in a group of dAMPH users, using task related fMRI before and after oral administration of MPH. We included dAMPH users, because there is preliminary evidence that users of this drug suffer from a dysfunctional DA system. We set out to answer the following questions: 1) Does amygdala function differ between recreational dAMPH users and healthy control subjects? 2) Does a DA-ergic challenge with MPH modulate amygdala function? 3) If so, does it affect amygdala function differently in recreational dAMPH users when compared to control subjects?
If indeed DA plays a role in emotional processing, as suggested in the literature described above, we hypothesized that in recreational dAMPH users we would observe an increased responsiveness of the amygdala to negative or fearful faces, which would decrease following a challenge with MPH, presumably due to enhanced DA transmission.

**Methods**

**Subjects**

Subjects were recruited by posting advertisements around the medical campus, on websites and in regional newspapers. A total of eight male, recreational amphetamine users and eight male, healthy control subjects were recruited. The eligibility criterion for the dAMPH group was previous use of dAMPH on more than 40 occasions. This threshold was chosen based on the work of Reneman and co-workers (Reneman et al., 2001) who found lower DAT binding in ecstasy users with an average dAMPH use on more than 45 occasions. The eight control subjects were healthy subjects with no self-reported use of amphetamines.

Subjects were asked to refrain from using caffeine-containing products on assessment days. Both controls and dAMPH users agreed to abstain from all psychoactive drugs for at least two weeks before scanning and therefore dAMPH dependence was reason for exclusion. All subjects indicated being able to abstain without external help during this two-week period and were asked to comply with urine drug screening on the day they were scanned (with an enzyme-multiplied immunoassay for amphetamines, cocaine, cannabis, alcohol, opiates and benzodiazepines). Exclusion criteria for all participants were: any neuropsychiatric diagnosis or history of brain disease or injury, use of medication with affinity for DA (e.g., MPH), a positive urine-screen for any DA-ergic drugs or any contra-indication to MRI such as metallic implants or claustrophobia. Subjects received a small financial compensation for their participation.

**Procedure**

The tasks were presented in the same order for every subject; first a go-no-go task, then a reward task and then the emotional face recognition task. Results of the go-no-go task (in preparation) and the reward task (Schouw et al. 2013) are reported elsewhere. To minimize learning effects, a practice run for each task was presented outside of the scanner. After the first scanning session, subjects received 35 mg MPH immediate release formulation (approximately 0.5 mg per kg body weight) orally. Subjects were then free to relax for 1½ hours until peak plasma levels were expected (Demenescu et al., 2011; Hysek et al., 2014) and then re-entered the MRI scanner for the second session that was identical to the first. MPH was obtained from Sandoz B.V. (Weesp, the Netherlands).

**Imaging**

All MR imaging was performed using a 3.0 Tesla Philips MR scanner equipped with an SENSE 8-channel head coil and body coil transmission (Philips Medical Systems, Best, The Netherlands). The session protocol consisted of a high-resolution 3D T1-weighted anatomical scan for registration and segmentation purposes and a fast single shot echo planar image (EPI) sequence for BOLD analysis. The BOLD acquisition imaging parameters were: TR/TE 2300/30 ms; FOV 220×220 mm²; 40 slices; voxel size 3 x 3 x 3 mm; no gap; 80° flip angle, SENSE 2.0.

**Emotion processing task**

The implicit emotion processing task was presented by a video projection system onto a white screen using E-prime software (Psychological Software Tools, USA). Subjects saw the screen via a mirror attached to the head coil. Responses were logged via a response box attached to the computer presenting the stimuli.

All subjects performed a modified version of the event-related implicit emotion processing task (Demenescu et al., 2011). Color photos of fearful, happy and neutral facial expressions were presented. The stimuli were selected from the Karolinska Directed Emotional Faces (KDEF) stimulus set and consisted of standardized facial expressions of emotions expressed by amateur actors. Twenty-four stimuli (twelve male and twelve female faces) were presented for each of the three facial expressions. In addition, two control stimuli consisting of an arrow pointing to the left or right, overlaid on a scrambled face, were presented for each of the three facial expressions. Each stimulus type was presented 80 times (40 with an arrow pointing to the left, 40 with an arrow pointing to the right). Each stimulus type was not presented more than twice in a row. Each stimulus was shown on the screen for 2.5 s with an interstimulus interval (black screen) varying between 0.5 and 1.5 s. Participants were instructed to indicate each face’s gender by pressing one of two buttons with the index finger of the left or right hand on two button boxes (left for a male face and right for a female face). For the control stimuli, participants had to push a button according to the direction the arrow was pointing in (left button for left direction and right button for right direction), the direction of the arrows and correct faces were counterbalanced.

**Analysis**

Continuous variables of group characteristics were analyzed using unpaired two-tailed student’s t-tests (log transformed if necessary) and Mann-Whitney tests for drug history variables. All demographic and behavioral data were analyzed in SPSS version 18.0 (SPSS Inc, Chicago, Ill) and are presented as mean ± standard deviation unless indicated otherwise. Reaction times were entered into a mixed model ANOVA in SPSS with the factors Group (2 levels: healthy controls and dAMPH), Drug challenge (2 levels: pre and post) and Stimulus type (4 levels: fearful, happy, neutral and baseline). MRI scans were analyzed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/
software/spm8) implemented in Matlab version 7.13. Images were first manually reoriented to the anterior commissure. Subsequently, fMRI images were realigned to the first volume, corrected for differences in slice acquisition time, co-registered to the anatomical scan, segmented into grey matter, white matter and cerebrospinal fluid, spatially (non-linearly) normalized to the Montreal Neurological Institute (MNI) T1 template, resampled into 2x2x2 mm voxels and spatially smoothed using a 6 mm full width at half maximum Gaussian kernel. Statistical analysis was performed within the framework of the general linear model. To determine BOLD activation in response to different facial expressions, box-car regressors convolved with a canonical hemodynamic response function were used to model responses to each facial expression. Data were high-pass filtered at 128 s and temporal autocorrelation was modeled with an AR(1) process provided within SPM8. To assure that the faces paradigm elicited reliable activations, a whole brain second level fMRI analysis across groups and sessions was performed for each facial expression (random effects analysis). The resulting statistical parametric maps were initially thresholded at p < 0.001. Clusters significant at the p < 0.05 level family-wise error (FWE) corrected for multiple comparisons were considered statistically significant. Next, a region of interest (ROI) analysis was performed by extracting mean BOLD activation in the bilateral amygdala with the MarsBaR toolbox (Matthew Brett, Jean-Luc Anton, Romain Valabregue, Jean-Baptiste Poline. Region of interest analysis using an SPM toolbox [abstract] Presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2-6, 2002, Sendai, Japan. Available on CD-ROM in NeuroImage, Vol 16, No 2.) using a mask of the bilateral amygdala as defined by the Automated Anatomical Labeling atlas (Tzourio-Mazoyer et al., 2002). Because no statistically significant differences were found for the left and right amygdala the mean amygdala response across hemispheres was used. Mean amygdala activations for the left and right amygdala were analysed with mixed models in SPSS with the factors Group (2 levels: healthy controls and dAMPH), Hemisphere (2 levels: left and right), Drug challenge (2 levels: pre and post) and Affective Valence (3 levels: fearful, happy and neutral). Additional analyses were run with age as a covariate. The association between the extent of dAMPH use and amygdala reactivity was studied with Pearson product-moment correlation coefficient provided within SPSS (two-tailed).

Results

Sample characteristics

The dAMPH group used dAMPH for a mean of 13.9 (±8.7) years on a mean of 27.8 (±17.1) occasions/year and a usual dose of 0.8 (±1.2) grams/occasion. The mean cumulative lifetime exposure to dAMPH was 352.6 (465.3) grams and mean time since the last dose was 1.1 (±1.3) month. Table 1 shows that the dAMPH group was slightly older and had a normal but slightly lower pre-morbid IQ than the control group although years of education did not differ significantly. In addition, dAMPH users had used significantly more tobacco, cannabis and cocaine.

Table 1 | Demographics for dAMPH users and controls with standard deviation (±) and p-values for t-test (Age, IQ and Years of education) or Mann-Whitney test.

<table>
<thead>
<tr>
<th></th>
<th>dAMPH</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=8</td>
<td></td>
<td>n=8</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>26.0 (± 4.0)</td>
<td>22.0 (± 3.0)</td>
<td>0.04</td>
</tr>
<tr>
<td>DART-IQ</td>
<td>104.5 (± 3.0)</td>
<td>110.4 (± 4.2)</td>
<td>0.007</td>
</tr>
<tr>
<td>Years of education</td>
<td>15.1 (±3.6)</td>
<td>16.4 (±2.9)</td>
<td>0.46</td>
</tr>
<tr>
<td>dAMPH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average dAMPH use (occasions/year)</td>
<td>27.8 (±17.1)</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Duration of dAMPH use (years)</td>
<td>13.9 (± 8.7)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Usual dose (grams/occasion)</td>
<td>0.8 (± 1.2)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Total exposure (grams)</td>
<td>352.6 (± 465.3)</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Time since last exposure (months)</td>
<td>1.1 (± 1.3)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Other substances</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average tobacco use (cigarettes/month)</td>
<td>261.0 (±279.8)</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Average alcohol use (units/month)</td>
<td>103.5 (±146.6)</td>
<td>104.5 (±83.5)</td>
<td>0.49</td>
</tr>
<tr>
<td>Average cannabis use (joints/year)</td>
<td>410.3 (±480.5)</td>
<td>19.4 (±31.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Average MDMA use (pills/year)</td>
<td>3.8 (±10.6)</td>
<td>0</td>
<td>0.32</td>
</tr>
<tr>
<td>Average cocaine use (occasions/year)</td>
<td>5.0 (±5.2)</td>
<td>0.1 (±0.4)</td>
<td>0.009</td>
</tr>
<tr>
<td>NA = Not applicable</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 | Mean reaction time RT (SD)

<table>
<thead>
<tr>
<th></th>
<th>dAMPH</th>
<th>Controls</th>
<th>post</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=8</td>
<td></td>
<td>n=8</td>
<td></td>
</tr>
<tr>
<td>Happy</td>
<td>798</td>
<td>(125)</td>
<td>726</td>
</tr>
<tr>
<td></td>
<td>749</td>
<td>(91)</td>
<td>654</td>
</tr>
<tr>
<td>Neutral</td>
<td>799</td>
<td>(129)</td>
<td>651</td>
</tr>
<tr>
<td></td>
<td>599</td>
<td>(66)</td>
<td>531</td>
</tr>
<tr>
<td>Baseline</td>
<td>575</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 3
Effects of methylphenidate during emotional processing in amphetamine users: preliminary findings

Figure 1 | Mask used to extract mean BOLD activation for region of interest (ROI) analysis

Figure 2 | Main effects of participants during the task and session.

Table 3 | Whole brain fMRI analyses for fearful, happy and neutral faces averaged across all participants and sessions

<table>
<thead>
<tr>
<th></th>
<th>Fearful MNI</th>
<th>Z</th>
<th>k</th>
<th>Happy MNI</th>
<th>Z</th>
<th>k</th>
<th>Neutral MNI</th>
<th>Z</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala (l)</td>
<td>-20 -12 -16</td>
<td>3.30</td>
<td>10</td>
<td>-18 -20 -16</td>
<td>3.71</td>
<td>16</td>
<td>-22 2 -14</td>
<td>3.21</td>
<td>3</td>
</tr>
<tr>
<td>Amygdala (r)</td>
<td>20 -10 -10</td>
<td>4.46</td>
<td>118</td>
<td>18 -12 -10</td>
<td>4.98</td>
<td>49</td>
<td>18 -10 -10</td>
<td>3.21</td>
<td>5</td>
</tr>
<tr>
<td>PFC BA 44/45 (r)</td>
<td>50 34 24</td>
<td>4.67</td>
<td>320 *</td>
<td>50 -74 16</td>
<td>4.21</td>
<td>246 *</td>
<td>50 -34 24</td>
<td>4.67</td>
<td>320 *</td>
</tr>
</tbody>
</table>
ROI analyses
The omnibus ANOVA at amygdala ROIs did not show significant differences between the left and right amygdala so this factor was dropped from the analyses. Although the Session*Valence*Group interaction was not significant ($p = .73$), a significant Valence*Group interaction indicated that only for fearful faces, significant group differences were present ($p = .037$) that were robust against adjustment for age ($p = .015$) (Figure 3).

Figure 3 | Amygdala activation to fearful, happy and neutral facial expressions for amphetamine users and controls.

For fearful faces, a trend significant Group*Session interaction ($p = .073$, $p = .26$ after adjustment for age indicated that before the oral MPH challenge, dAMPH users showed more activation to fearful faces than the control group, and that this difference disappeared after the MPH challenge (Figure 4).

Correlation analysis
Before the challenge, duration of amphetamine use in years was positively correlated with amygdala reactivity in dAMPH ($r = .76; p = .029$; Figure 5). This correlation disappeared after the challenge ($r = .07$, ns).

This was confirmed by post-hoc analyses: before the oral MPH challenge, the two groups differed significantly in amygdala activation ($p = .027$, $p = .05$ after adjustment for age). This difference disappeared after the challenge ($p = .94$). The MPH challenge was associated with a marginally significant increase in amygdala reactivity in the controls ($p = .075$) whereas the challenge was associated with a nonsignificant decrease in reactivity in the amphetamine users ($p = .51$). No significant Group*Session interactions were present for happy faces ($p = .27$) or neutral faces ($p = .35$).
Figure 5 | Correlation of years of amphetamine use with amygdala reactivity in dAMPH users.

Discussion

In this study of amygdala activation before and after MPH challenge in dAMPH users and controls, we found that only presentation of fearful faces was associated with abnormal, increased, activation of the amygdala. However, the modulating effect of MPH was not significant, although we observed a trend that MPH increased amygdala activity in the control group, without such an effect in the dAMPH group. Interestingly, in the dAMPH users the extent of dAMPH exposure was positively correlated with amygdala reactivity.

Our experimental and control group were not homogenous; our dAMPH group was slightly older, and we therefore corrected our statistical analysis for age. The dAMPH users also had a normal but lower premorbid IQ than the control group, although the years of education did not differ significantly. In addition, the dAMPH group had used significant more tobacco, cannabis and cocaine (table 1). Unfortunately, the groups were to small to also correct for IQ and drug use.

Our findings of abnormal baseline amygdala activation in recreational dAMPH users most likely reflect an abnormal DA transmission in this brain region, as dAMPH has been previously found to affect the DA system and the amygdala seems to be under modulatory influence of DA. For instance, preclinical studies have shown that even relatively low doses of dAMPH (equivalent to the doses used in clinical practice) induce DA dysfunction in rodents and non-human primates (Ricaurte, 2005) as evidenced by for instance reductions in DA, the DA transporter (DAT) and an increase in D1 receptor density (Bonhomme et al., 1995). In line with this, in humans, Reneman and co-workers have shown that recreational dAMPH use is linked with reduced striatal DA transporter (DAT) availability (Reneman et al., 2001). Because the DAT is a structural component of the DA-axon, loss in DAT likely indicates DA-ergic neurotoxicity. In addition, in recreational users of dAMPH, we previously found a blunted hemodynamic response to a challenge with MPH (Schouw et al., 2010), as well as blunted DA release induced by an i.v. challenge with dAMPH (Schrantee et al., 2015). These pre-clinical findings in the literature provide additional evidence that our results of abnormal emotional processing in dAMPH users likely underlie abnormal DA neurotransmission.

Indeed, DA has been found to modulate the human amygdala response in anxious patients (Bergman et al., 2014), but also in patients suffering from Parkinson’s disease (Tessitore et al., 2002b) as well as ADHD (Posner et al., 2011a). The dose-response correlation between lifetime exposure of dAMP use and amygdala activity in the present study further supports a relationship between dAMPH use and amygdala hyperactivity and is in line with previous findings (Hariri et al., 2002).

We did not find amygdala (or other brain region activity) with the neutral and happy faces. This might be surprising in relation to our DA hypothesis and the role of DA in rewarding processes. We think this is related due to the overall weak responses to our stimuli. Since aversive stimuli are known to give the strongest activation this might be the reason that other stimuli used did not threshold for brain activity. Furthermore, in healthy controls there may also be a greater top down regulatory activity from the ventrolateral and medial prefrontal cortex when stimuli are negative rather than positive (Musser et al., 2013). This top down regulation of emotional responses might be more disturbed in the dAMPH users, like in patients with DA-ergic dysfunction such as ADHD, than in the control group, giving less strong emotional responses in the control group (Shaw et al., 2014).

We observed a trend that MPH may induce a different (opposite) effect in dAMPH users as in healthy volunteers: MPH seems to increase in amygdala activation in healthy controls, whereas no effect in dAMPH users. Studies with larger sample sizes are needed to replicate these findings. Increased amygdala activation induced by a DA-ergic challenge in healthy volunteers has been reported in other studies using dAMPH as a challenge (Delaveau et al., 2005; Hariri et al., 2002). Interestingly, in patients suffering from ADHD, stimulants had a normalizing effect on the activity of the right amygdala (Posner et al., 2011b; Shaw et al., 2014; Volkow et al., 2007) as well as emotional processing (Conzelmann et al., 2011) and emotional lability, although negative mood persisted after treatment with MPH (Williams et al., 2008). Studies with similar results as ours, in subjects...
with dysfunctional DA systems, lend further support to our hypothesis that DA dysfunction, or DA deficiency, most likely underlie the abnormal fear processing in dAMPH users. Also structural effects have been found after prolonged MPH use, for example in a study on patients with a bipolar I disorder, a decrease in amygdala volume was found in adolescents using MPH (Geller et al., 2009).

In fact, it has been shown that DA in the basolateral amygdala is critical for fear-processing (Fadok et al., 2009). Additionally, some midbrain DA neurons increase their firing rates to aversive stimuli and predictive cues (Guarraci et al., 1999; Horvitz, 2000). DA levels in the ventral midbrain increase during aversive events and DA neurons of these brain areas project to limbic brain areas important for fear learning (Abercrombie and Zigmond, 1989; Kalivas and Duffy, 1995). In these areas, DA facilitates long term potentiation, an important neural correlate of memory (Lemon and Manahan-Vaughan, 2006). Finally, in a recent human study dimensional scores on a fear subscale and not on a depressive subscale were found to be predictive for right amygdala activity in human adolescents when processing fearful, happy and neutral faces (Van Den Bulk et al., 2014) and higher amygdala activity is displayed by a common polymorphism in a region of the DAT gene (SL-C6A3), during the processing of aversive emotional stimuli in humans (Bergman et al., 2014).

Besides this, not only DA but also norepinephrine (NE) may play a role since NE is known for its relevance in the treatment of depression (Blair, 2013). Both MPH and dAMP activate the NE system. Previously, NE has been implicated in the therapeutic action of MPH as it significantly occupied the NE transporter at clinically significant doses (Hannestad et al., 2013; Wright et al., 2001). Alternatively, our findings may be explained by an increased sensitivity to corticotropin-releasing factor, as chronic amphetamine treatment has been shown to enhance corticotropin-releasing factor-induced serotonin release in the amygdala of rats (Scholl et al., 2010). The corticotropin-releasing factor increases serotonin release in the central nucleus of the amygdala, and this neurochemical circuitry has been shown to mediate fear processing as well.

Our findings may, when replicated in a larger sample, have clinical implications. But what happens if DA-acting agents are administered to healthy subjects (e.g., to improve scholarly achievements), or in ADHD patients that have not been correctly diagnosed as suffering from ADHD? We would like to argue that in these cases, MPH administration has a detrimental effect, as in our study MPH seems to induce an increase in amygdala activity in healthy subjects. Indeed, in animal studies MPH has been shown to induce anxiety like behavior. For example, Bolaños et al. found that in normal Wistar rats prolonged treatment with MPH during adolescence induced anxiety like behavior, in which the animals were significantly more sensitive to stressful situations and had enhanced levels of corticosterone (Bolaños et al., 2003). Additionally, MPH has been shown to enhance decoding of negative emotions including fear in healthy human subjects (Hysek et al., 2014).

A limitation of the current study is the study design, because it lacks a placebo-controlled condition. Therefore, we cannot exclude that the effects we measured were indeed induced by MPH or due to sensitization or habituation. However, as mentioned previously, the dose-response correlation further supports a relationship between dAMPH use and amygdala hyperactivity. Another limitation of this study is the possibility that pre-existing differences between dAMPH users and healthy controls underlie differences in amygdala function. People with a dysfunctional DA system with low response on an acute DA-ergic challenge, may be predisposed to use dAMPH. Our findings in this relatively small sample size in this explorative study, should be replicated in a larger study population.

Conclusion

During fear processing, we observed increased amygdala activity in a group of recreational dAMPH users compared to healthy control subjects, which disappeared following oral MPH administration. MPH induced a marginally significant increase in amygdala activity in healthy controls subjects. These finding are in line with the literature, underpinning the modulatory influence of DA on amygdala function.
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Effects of methylphenidate during emotional processing in amphetamine users: preliminary findings

Chapter 3 Effects of methylphenidate during emotional processing in amphetamine users: preliminary findings


CHAPTER 4

Age-dependent effects
of acute methylphenidate
on amygdala reactivity in
stimulant treatment-naive
patients with Attention
Deficit Hyperactivity Disorder

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Hyke G.H. Tamminga — Cheima Bouziane
J.J. Sandra Kooij — Michiel B. de Ruiter
Liesbeth Reneman

Under review
Chapter 4  

Abstract

In the present study, we investigate whether methylphenidate (MPH) affects emotional processing and whether this effect is modulated by age. We measured amygdala reactivity with functional Magnetic Resonance Imaging (fMRI) during processing of angry and fearful facial expressions in male stimulant treatment-naive patients with Attention Deficit Hyperactivity Disorder (ADHD) (N=35 boys; N=46 men) and 23 healthy control subjects (N=11 boys; N=12 men). In ADHD patients, we also measured amygdala reactivity 90 minutes after an acute oral challenge with MPH (0.5 mg/kg). Mean amygdala reactivity was analyzed for all subjects using a repeated measures analysis of variance (ANOVA). Whole-brain maps were analyzed for the patients only. At baseline, we found a trend significant age*diagnosis effect in the right amygdala due to lower reactivity in children with ADHD vs. controls (-31%), but higher reactivity in adults with ADHD vs. controls (+31%). MPH reduced right amygdala reactivity in all patients, resulting in further reductions in children. In the left amygdala, reduction of amygdala reactivity was confined to adult ADHD patients whereas there was no change in children with ADHD. MPH-induced reduction of amygdala reactivity in adults might be a promising avenue for managing emotional dysregulation when replicated for chronic MPH treatment.

Introduction

Emotional dysregulation has recently gained recognition as an important feature of Attention-Deficit/Hyperactivity Disorder (ADHD). (Barkley and Fischer, 2010; Wehmeier et al, 2010; Shaw et al., 2014). In clinical samples as well as in population based studies 24-50 % of the children with ADHD manifest difficulties regulating negative affect (Sobanski et al., 2010; Stringaris and Goodman, 2009). This often leads to serious impairment; in longitudinal studies, children who display emotional dysregulation show higher rates of anxiety disorders and disruptive behaviour disorders after 14years follow-up (Althoff et al., 2010; Karalunas et al., 2014). Likewise, children with comorbid anxiety disorder have poorer daily functioning (March et al., 2000, Spencer et al., 2013) and parents report lower quality of life as compared to children without comorbid anxiety (Sciberras and Lycett, 2015). Children with a major depression in addition to their ADHD have a high risk for suicide attempts (Chronis-Tuscano et al., 2012). Emotional dysregulation also seems to play a role in adult ADHD, since depression and anxiety are 5-10 times more prevalent in adults with ADHD than in the general population. (Kessler et al, 2006; Klein et al., 2012). Furthermore, emotional problems influence the course and outcome of ADHD (March et al., 2000; Spencer et al 2013; Wehmeier et al., 2010), are associated with persistence of ADHD into adulthood (Barkley and Fischer, 2010) and predict lower quality of life in young adults with ADHD (Reimherr et al, 2005).

The amygdala is one of the hallmark regions for emotional processing (Ledoux, 2000). In patients suffering from major depressive disorder for example, heightened amygdala reactivity to negative emotional stimuli is commonly observed in functional imaging studies of amygdala reactivity during emotional processing in patients with ADHD. Although results are mixed, a recent review by Shaw (Shaw et al., 2014) showed that in larger studies (predominantly left) amygdala reactivity to negative emotional stimuli was heightened in ADHD patients, whereas no changes in amygdala reactivity were found in other studies. In this review, it was also suggested that treatment with psychostimulants is often linked to a beneficial effect on emotion dysregulation. Indeed, in adolescent patients with ADHD, acute administration of psychostimulants normalizes reactivity in the amygdala during emotional processing (Posner et al., 2011). However, previous (stimulant) treatment often interferes with the interpretation of these effects (Manos et al., 2011). Therefore, it is presently unknown whether amygdala reactivity, and thus emotional dysregulation, in ADHD reflects disorder-, or treatment-related functioning.

In normal development, amygdala reactivity steadily decreases from early childhood to young adulthood (Gee et al., 2013). However, in subjects with familial risk for depression or a history of stressful life events, heightened amygdala reac-
Age-dependent effects of acute methylphenidate on amygdala reactivity in stimulant treatment-naive patients with Attention Deficit Hyperactivity Disorder.

Chapter 4

Activity emerges during adolescence and increases with age, prior to the emergence of clinical depressive symptoms (Schwartz et al., 2015). The developmental emergence of atypical development of amygdala reactivity has not yet been determined in stimulant treatment-naive ADHD subjects, but is critical in advancing our ability to predict, and ultimately prevent, the emergence of emotional dysregulation in ADHD patients.

It is also known that not only serotonin but also dopamine (DA) signaling plays a role in modulating amygdala activity during emotional processing. For instance, amygdala reactivity is attenuated by acute treatment with DA D2 receptor antagonists (Takahashi et al., 2005). However, these findings in adults might not extrapolate to children, as the DA system undergoes significant alterations during adolescence. For instance, DA concentrations as well as DA-ergic innervation of the frontal cortex peaks during adolescence (Rosenberg et al., 1994), whereas the density of D1 and D2 receptors peaks during childhood and declines between childhood and adulthood (Lidow et al., 1991, Lidow et al., 1992). Therefore, it is conceivable that the effects of MPH on amygdala reactivity are modulated by age.

To investigate age-dependent effects and effects of MPH, we measured amygdala reactivity during processing of emotional faces with functional Magnetic Resonance Imaging (fMRI) in stimulant treatment-naive paediatric (aged 10-12 years of age) and stimulant treatment-naive adult (aged 23-40 years of age) male patients with ADHD. Moreover, we also investigated whether the effects of MPH the effects of MPH differ between children and adults. We hypothesized that because of the increasing amygdala reactivity with age in patients with a depressive disorder (Swartz et al., 2015) and because of the high comorbidity of ADHD with depression (Kessler et al., 2006), we would observe increased amygdala reactivity in adult ADHD patients compared to children with ADHD. We also hypothesized that because of the ontogeny of DA systems, the effects of MPH on amygdala reactivity vary with age.

Method

Participants

The data we present are the baseline data of the effects of Psychotropic Drugs on brain Development (ePOD-MPH) study (Bottelier et al., 2014), which was a 16 week double-blind randomized placebo controlled multicenter trial on the use of MPH in stimulant naïve patients with ADHD with DA function as the primary outcome measure (Schrantee et al., 2016). Here we report the amygdala reactivity before and after an acute challenge with MPH in 99 stimulant treatment-naive ADHD patients (all subtypes) stratified for age: 50 boys (aged 10-12 years) and 49 adult males (aged 23-40 years). In addition, as a comparison group, we included 11

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children (aged 10-12 years) and 12 adults (aged 23-40 years) as non-ADHD control subjects. Patients were recruited from clinical programs at the Child and Adolescent Psychiatry Center Triversum (Alkmaar) and from the department of (Child and Adolescent) Psychiatry at the Bascula/Academic Medical Centre (AMC, Amsterdam). Adult patients were recruited from clinical programs at the PsyQ mental health facility (The Hague) and from the department of Psychiatry of the AMC (Amsterdam). All patients were diagnosed by an experienced psychiatrist based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 4th edition; APA 1994) and the diagnosis was subsequently confirmed with a structured interview: Diagnostic Interview Schedule for Children (National Institute of Mental Health Diagnostic Interview Schedule for Children Version IV (NIMH-DISC-IV, authorized Dutch translation) (Ferdinand and van der Ende, 2002) for children and the Diagnostic Interview for ADHD (DIVA 2.0) (Kooij, 2013, Ramos Quiroga et al., in press) in adults. Inclusion criteria for the patients were at least 6 of 9 symptoms of inattention or hyperactivity/impulsivity on the DISC-IV (for children) and on the DIVA for adults retrospectively in childhood. For current symptoms in adults a cutoff of 6 of 9 criteria was used on the DIVA. Patients were excluded if they were diagnosed on the Mini International Neuropsychiatric Interview (M.I.N.I.-plus) (Sheehan et al., 1998) with a co-morbid axis I psychiatric disorder requiring pharmacological treatment at study entry. Additional exclusion criteria were a history of neuropsychiatric disease, current DA-ergic medication and MRI contraindications.

This study was approved by the Central Committee on Research Involving Human Subjects (CCMO, the Netherlands). All subjects (and for children, their parents or legal representatives) gave written informed consent.

Clinical Ratings

Approved Dutch translations of the Disruptive Behavior Disorders Rating Scale (DBD-RS) (Oosterlaan et al., 1998) rated by the parents were used to examine the ADHD symptoms in children. In adults, the ADHD Self Report Scale (Rösl er et al., 2006) was used. To measure emotional dysregulation in children the items ‘is often angry and resentful’, ‘often loses temper’ and ‘is often touchy or easily annoyed by others’ from the DBD-RS were used in accordance to the items from the Conners Global Index (GSOBI) used (Sobanski et al., 2010); and for adults the items that suggest emotional dysregulation ‘overly active and compelled to do things’, ‘difficulty unwinding’ and ‘restless and fidgety’ from the ADHD-SR were used. In addition, we screened for anxiety and depressive symptoms using the Child Depression Inventory (CDI) (Kovacs, 1992) and the Screen for Child Anxiety Related Disorders (SCARED) (Muris et al., 2007) for children and the Beck’s Depression Inventory (BDI) (Beck et al., 1961) and Beck’s Anxiety Inventory (BAI) (Beck et al., 1988) for adults.
fMRI procedures, task paradigm and acquisition parameters

Procedure
ADHD patients underwent two MRI scans, one before and one 90 minutes after an oral challenge with short acting MPH (0.5mg/kg with a maximum of 20 mg in children and 40 mg in adults). MPH was obtained from Sandoz B.V. (Weesp, the Netherlands). Control subjects underwent one MRI scan without an oral challenge with MPH. To minimize learning effects, a practice run was presented outside of the scanner.

fMRI task paradigm
The experimental paradigm consisted of a blocked design and has been previously used to assess drug effects on amygdala reactivity (van Wingen et al., 2008). The emotional stimuli consisted of angry and fearful faces whereas the neutral stimuli consisted of ellipses assembled from scrambled faces (Figure 1). Two blocks of emotional stimuli were interleaved with three neutral blocks, each 30-s block containing six 5-s trials. For each emotional trial, three stimuli were presented simultaneously, and subjects had to decide which one of the lower two stimuli expressed the same emotion as the target stimuli presented above. Similarly, for each neutral trial, three stimuli were presented, but subjects had to decide which of the bottom two ellipses was identically oriented to the target ellipse. Two versions of the task were used to overcome learning effects.

Figure 1 | fMRI task paradigm

Two blocks of emotional stimuli were interleaved with three neutral blocks, each 30-s block containing six 5-s trial. Emotional stimuli consist of angry and fearful faces. Neutral stimuli consist of ellipses assembled from scrambled faces. For each trial, subjects have to decide for which one of the lower two stimuli expressed the same emotion as the targeted stimuli presented above, or, for each neutral trial, which of the bottom two ellipses were identically orientated to the target ellipse.

fMRI acquisition parameters
The MRI study was performed on a 3.0T Philips scanner (Philips Healthcare, Best, The Netherlands) using an 8-channel receive-only head coil. A high-resolution 3D T1-weighted anatomical scan was acquired for registration purposes and fMRI data were acquired using a single shot echo planar imaging sequence with parameters: TR/TE=2300/30ms, resolution=2.3×2.3×3 mm, 39 sequential slices, FOV=220x220x117 mm, GE-EPI read-out, 70 dynamics, no gap, 80° flip angle, total duration 2:42 minutes.

Cerebral blood flow in the amygdala
It has previously been suggested that amygdala activation in task-related fMRI could be explained, in part, by non-neural signals (Plichta et al., 2014). To assess whether MPH induced hemodynamic changes in addition to neuronal activity, we measured cerebral blood flow (CBF) using arterial spin labeling (ASL) MRI in the amygdala in both sessions for the patients. We calculated mean CBF in the amygdala to compare the effect of MPH in both age groups.

Data analysis
Behavioral response data were extracted from E-prime and analyzed using IBM SPSS version 22. Functional image analysis was performed with in-house MATLAB scripts (MATLAB version 2013a Natick, Massachusetts: The Mathworks Inc.) and FEAT (FMRI Expert Analysis Tool) in FSL 5.0 (FMRIB’s Software Library (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/)). The first volume of the fMRI series was discarded to allow for T1 equilibration. Images were skull stripped, analyzed for motion artifacts, spatially smoothed with a FWHM Gaussian kernel of 5 mm and spatially normalized and resampled to Montreal Neurological Institute (MNI) 2mm template. fMRI time series were high-pass filtered with a cutoff of 0.1Hz. First-level analyses were performed by modeling the signal changes using the stimulation paradigm (faces versus shapes), convolved with canonical hemodynamic response function. The six standard rigid-body motion parameters and a confound matrix of volumes that were corrupted by large motion were added to the model (Lemieux et al., 2007). The confounded time points were determined using a net displacement vector according to Euclidian root mean square (RMS) (Power et al., 2012). Data from subjects with extreme motion (frame wise displacement > mean + 2*standard deviation using both the method by Power (Power et al., 2012) and van Dijk (van Dijk et al., 2012) were removed from the analysis.

For our regions of interest (ROI) analyses, mean signal intensity for the left and right amygdala was extracted from the first level contrasts using masks from the Harvard-Oxford atlas provided within FSL. Mean signal intensities were analyzed with IBM SPSS version 22 and entered in a repeated measures ANOVA to assess baseline differences, response to the challenge and the interaction age*chall-
Results

Group characteristics
Characteristics of the patients and controls are displayed in Table 1. Adult patients were older than adult controls ($t_{56}=2.49; p=0.02$). Children in the control group had a higher IQ than the patient group ($t_{45}=2.72; p<0.01$). The adult patient group had predominantly the combined type of ADHD ($p=0.02$) and the children group had predominantly inattentive type of ADHD, ($\chi^2=3.12; p=0.02$). Patients and controls differed on baseline symptom severity (children: $t_{48}=13.04; p<0.01$; adults $t_{56}=6.44; p<0.01$). Children and adult patients scored significantly higher on the clinical rating scales measuring depressive and anxiety symptoms than their control groups (Table 1). In children, 7.7% had a comorbid oppositional defiant disorder or a conduct disorder. In adult patients, 13% had a depressive disorder and 4.3% an anxiety disorder in the past.

Directly after the first MRI scan patients, but not controls, received a challenge with short acting MPH (0.5mg/kg with a maximum of 20 mg in children and 40 mg in adults). For this single administration, the mean dose was $18.71 \pm 2.53$ mg MPH in children and $38.26 \pm 2.63$ mg MPH in adults.

fMRI task
Accuracy across groups was comparable, but did not increase after MPH (Supplementary Figure 1). Data from 3 adults and 6 children was incomplete, 2 MRI scans from children contained coil sensitivity artifacts and data from 7 children was excluded because of excessive motion. Presentation of negative emotional faces elicited activation of the bilateral amygdala, bilateral and medial prefrontal cortex, and bilateral occipital and parietal areas including the fusiform gyrus at baseline, before administration of MPH (Figure 2).

Table 1 | Demographics and patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th>ADHD (N=35)</th>
<th>Control (N=11)</th>
<th>Adults</th>
<th>ADHD (N=46)</th>
<th>Control (N=12)</th>
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<tr>
<td>Demographics</td>
<td></td>
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<tr>
<td>Age (y)</td>
<td>mean ± SD</td>
<td>11.4 ± 0.9</td>
<td>11.4 ± 0.8</td>
<td>28.7 ± 4.7</td>
<td>25.2 ± 1.9</td>
<td>107.9 ± 7.7</td>
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<tr>
<td>Estimated IQ</td>
<td>mean ± SD</td>
<td>110.4 ± 19.1</td>
<td>121.6 ± 10.9</td>
<td>102.0 ± 5.1</td>
<td>107.0 ± 5.1</td>
<td>101.9 ± 5.5</td>
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<td>ADHD subtype</td>
<td></td>
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<td></td>
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<tr>
<td>Inattentive</td>
<td>60.0%</td>
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<td></td>
<td>0%</td>
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<td>Hyperactive</td>
<td>40.0%</td>
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<td></td>
<td>65.2%</td>
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<tr>
<td>Combined</td>
<td>60.0%</td>
<td></td>
<td></td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline symptom severity</td>
<td>mean ± SD</td>
<td>36.2 ± 7.0</td>
<td>126.4 ± 23.1</td>
<td>7.1 ± 4.8</td>
<td>7.0 ± 2.5</td>
<td>31.2 ± 9.8</td>
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<td>CDI</td>
<td>mean ± SD</td>
<td>7.9 ± 4.8</td>
<td>7.1 ± 4.8</td>
<td>3.7 ± 2.9</td>
<td>3.7 ± 2.9</td>
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<td>SCARED</td>
<td>mean ± SD</td>
<td>10.1 ± 6.6</td>
<td>11.1 ± 6.6</td>
<td>0.9 ± 1.2</td>
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<tr>
<td>BAI</td>
<td>mean ± SD</td>
<td>8.9 ± 7.0</td>
<td>8.9 ± 7.0</td>
<td>5.2 ± 2.5</td>
<td>5.2 ± 2.5</td>
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<td>Emotional lability</td>
<td>mean ± SD</td>
<td>7.3 ± 4.8</td>
<td>7.3 ± 4.8</td>
<td>2.0 ± 1.7</td>
<td>2.0 ± 1.7</td>
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<tr>
<td>Co-morbidity</td>
<td>mean ± SD</td>
<td>3.7 ± 2.9</td>
<td>3.7 ± 2.9</td>
<td>1.5 ± 1.7</td>
<td>1.5 ± 1.7</td>
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</tr>
<tr>
<td>Depressive episode(s) in the past</td>
<td>mean ± SD</td>
<td>6.46 ± 13.0%</td>
<td>6.46 ± 13.0%</td>
<td>2.46 ± 4.3%</td>
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<td>2.46 ± 4.3%</td>
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<tr>
<td>Anxiety disorder in the past</td>
<td>mean ± SD</td>
<td>2.35 ± 6%</td>
<td>2.35 ± 6%</td>
<td>2.35 ± 6%</td>
<td>2.35 ± 6%</td>
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Table 1 | Demographics and patient characteristics (Part II)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>DBD-RS</td>
<td>DBD-SR</td>
<td>Disruptive Behavior Disorder – Rating Scale</td>
</tr>
<tr>
<td>ADHD-SR</td>
<td>ADHD-SR</td>
<td>Attention-Deficit Hyperactivity Disorder – Self Rating</td>
</tr>
<tr>
<td>CDI</td>
<td>CDI</td>
<td>Child Depressive Inventory</td>
</tr>
<tr>
<td>SCARED</td>
<td>SCARED</td>
<td>Screen for Child Anxiety Related Disorders</td>
</tr>
<tr>
<td>Beck’s Depression Inventory</td>
<td>BDI</td>
<td></td>
</tr>
<tr>
<td>Beck’s Anxiety Inventory</td>
<td>BAI</td>
<td></td>
</tr>
</tbody>
</table>

For children: WISC, for adults, NART
For children: DBD-SR, for adults ADHD-SR, \( \chi^2 \) test
For children the items ‘is often angry and resentful’, ‘often loses temper’ and ‘is often touchy or easily annoyed by others’ from the DBD-RS were used; and for adults the items ‘overly active and compelled to do things’, ‘difficulty unwinding’ and ‘restless and fidgety’ from the ADHD-SR were used.
For adults: MINI Plus 5.0
For children: NIMH DISC-IV

ROI analysis
We observed a strong hemisphere \( \times \) group interaction (\( F = 7.647; p < 0.01 \)). We therefore analyzed the left and the right amygdala separately.

Left amygdala
At baseline, before the challenge with MPH, no significant differences between young and adult patients were found in the left amygdala (\( t_{59} = 0.65; p = 0.52 \)). In addition, no significant differences were found between ADHD patients and controls (children \( t_{59} = 0.97; p = 0.34 \); adults \( t_{56} = 1.04; p = 0.31 \)) (Figure 2). After the challenge with MPH, adult patients showed reduced amygdala reactivity (-36.3\%, \( F_{1,45} = 6.71; p = 0.01 \)), in the direction of the healthy controls from which they did not differ anymore (post-challenge compared to controls: \( t_{56} = 1.48; p = 0.14 \)). The age*diagnosis effect we found at this extented below control values (\( t_{56} = 2.42; p = 0.02 \)).

![Figure 2](image)

Figure 2 | ROI analysis

- a) amygdala ROI used for the analysis. b) left and c) right amygdala activity before and after acute MPH administration in patients compared to healthy controls (HC). * \( p < 0.05 \) significant reduction in adults ADHD patients following MPH administration in left amygdala; \# \( p < 0.05 \) significant reduction (main effect) of MPH in ADHD patients; \$ \( p < 0.05 \) significantly lower amygdala reactivity in children with ADHD post-MPH compared to control children.

Right amygdala
No significant baseline differences between young and adult patients were found in the right amygdala either (\( t_{59} = 1.48; p = 0.14 \)). The age*diagnosis effect we found at baseline in the right amygdala was borderline significant (\( p = 0.05 \)). As shown in Figure 2 amygdala reactivity in children with ADHD was lower when compared to adults with ADHD (-31\%). And right amygdala reactivity of adult patients higher when compared to control subjects (+ 31 \%). However, as these post hoc tests were not significant, they should be interpreted with caution as we cannot rule out a type I error.

After the challenge with MPH, we observed a main effect of challenge, showing a reduction in amygdala reactivity after the MPH challenge (\( F_{1,39} = 4.29; p = 0.042 \)) in both children and adults (-28\%, and -18\% respectively). In adults, amygdala reactivity did not differ anymore from adult control values (\( t_{56} = -0.89, p = 0.38 \)), whereas in children with ADHD, MPH did induce a significant reduction that extented below control values (\( t_{56} = 2.42; p = 0.02 \)).

CBF and correlation with clinical scales
Children showed higher baseline amygdala CBF than adults (\( p < 0.01 \)). However, MPH did not affect CBF in the amygdala (left: \( F_{1,49} = 2.48; p = 0.12 \); right: \( F_{1,49} = 1.32; p = 0.25 \)) nor was there an age*challenge interaction (left: \( F_{1,49} = 0.42; p = 0.52 \); right: \( F_{1,49} = 2.43; p = 0.12 \)). We did not find a correlation between amygdala reactivity and the clinically rated emotional dysregulation in children (left: \( r = 0.17 \) \( p = 0.26 \); right \( r = 0.04 \) \( p = 0.98 \)) or adults (left: \( r = 0.20 \) \( p = 0.15 \); right \( r = 0.21 \) \( p = 0.12 \)).

Whole brain exploratory analyses
At baseline, children showed less activation in medial and inferior lateral prefrontal and thalamic areas while viewing negative emotional faces, but more activation in the precuneus and the posterior cingulate areas, compared to adults. These results show robust task effects including amygdala activation. Because we had strong a priori hypothesis about the anatomical location of the effects (i.e., the amygdala) we constrained our primary analysis to the amygdala. Furthermore, MPH induced an increase in ACC activity extending into medial prefrontal cortex in children, while in adults a more widespread increase in activation was seen in the cortical areas including the bilateral insula (Figure 3).
Discussion

Here we compared neural correlates of emotional processing in stimulant treatment-naive children and adults with ADHD compared to healthy controls and the effects of MPH thereupon. We demonstrate that both emotional processing and the effects of MPH on emotional processing are modulated by age. In adults, amygdala reactivity normalized towards control levels following MPH, whereas in children MPH further reduced right amygdala reactivity, whereas MPH had no effect on the left amygdala. However, amygdala reactivity did not correlate with emotional dysregulation measured with clinical rating scales.

Our findings suggest that increased amygdala reactivity in ADHD patients is dependent on age. In normal developing subjects, amygdala reactivity steadily decreases from early childhood through young adulthood (Gee et al., 2013), which is consistent with the (non-significant) difference between young and adult controls. In contrast, in ADHD patients, we observed a borderline significant difference in the opposite direction. Thus, it is possible that in ADHD, the course of amygdala activity is altered.

Our finding that acute administration of MPH normalizes amygdala reactivity in (adult) ADHD patients, is in line with previous findings (Posner et al., 2011). Our data suggest that as far as the left amygdala is concerned, there is an age effect; whereas MPH induced a significant reduction in left amygdala reactivity in adult ADHD patients, it had no effect on left amygdala reactivity in children. Our data suggest that as far as the left amygdala is concerned, there may be an age effect as well.

Contrary to previous studies (Hariri et al., 2002; Kienast et al., 2008; Tessitore et al., 2002) we observed that administration of stimulants decreased rather than increased amygdala reactivity. However, these studies were done in patients with Parkinson disease or in healthy control subjects. In patients with ADHD and recreational amphetamine use, MPH reduced and in doing so normalized amygdala reactivity (Bottelier et al., 2015; Posner et al., 2011) which is in line with our findings. An alternative less direct explanation for our findings of reduction of amygdala reactivity to negative valenced facial expression after administration of MPH in adults but not children, is that of increased top down modulation of the amygdala activity rather than a direct effect to the amygdala. Indeed, adults have a more intact and salient architecture in the top down control network as suggested in the literature (Lidow et al., 1991; Lidow and Rakic, 1992; Rosenberg and Lewis, 1994). These studies showed ongoing maturation of the DAergic system in the frontal cortex but not in the amygdala. Taken together, MPH may increase top down control over the amygdala including prefrontal cortex and parietal cortex networks only in adults, but not children because adults have more intact and salient architecture in the top down control network (Gee et al., 2013). This is further supported by our whole brain analysis where we found that MPH induced a more widespread increase in activation in the cortical areas including the bilateral insula.

On the other hand, more and more evidence is emerging that the DA system plays an important role in emotional processing, whereas it undergoes profound changes between childhood and adulthood. Amygdala reactivity is attenuated by acute treatment with DA D2 receptor antagonists (Takahashi et al., 2005) and as we have demonstrated recently, recreational dexamphetamine users have increased amygdala activation to angry and fearful faces compared to a control group, which reduced after acute administration of MPH (Bottelier et al., 2015). The age-dependent effects of MPH on amygdala reactivity that we report here are probably the result of significant alterations in expression of DA between childhood and adulthood. There is, for example, a difference in expression of the DA D1 and D2 receptors with age; the expression of D2...
is higher during juvenile period, and the expression of D1 higher in adulthood (Rosenberg and Lewis, 1994).

In our sample, children and adults with ADHD had higher scores on depression and anxiety symptoms compared to healthy controls. However, only the SCARED score in children was above the clinical cut-off score; the scores on the CDI in children, and on the BDI and BAI in adults were below the cut-off values. The scores on the subset of the DBD-RS and the ADHD-SR, measuring emotional dysregulation, were higher in patients than in controls ($p< 0.01$ for both children and adults). We did not find a correlation between amygdala reactivity with the ‘emotional dysregulation’ score. Previous literature is ambiguous on this topic; in some studies, emotional dysregulation was related to amygdala reactivity where in others it was not (Shaw et al., 2014). The items we used to measure emotional dysregulation in children and adults are extracted from the DBD-RS and the ADHD-SR and measure emotional dysregulation by approach. From a developmental perspective, we considered the items extracted from the ADHD-SR to extend the items we used in children. The lack of correlation we found in amygdala reactivity to clinical symptoms of emotional dysregulation however might be due to the lack of specificity of our clinical symptom scale measuring emotional dysregulation.

Other limitations of our study are that the results cannot be extrapolated to all children and adults with ADHD, because we only studied male subjects with restricted age ranges. Additional studies are needed in female patients, since female sex hormones modulate DAT expression (Wagner et al., 2007), and in multiple age categories. Furthermore, we included relatively small controls groups, which precluded whole brain comparisons with the ADHD groups. Although we controlled for practice effects, the MPH scan was always performed after the baseline scan, which could possibly induce an order effect. In addition, although previous studies have attributed the BOLD signal to vascular rather than neuronal changes (Plichta et al., 2014), our CBF measurements show that this is not the case in our sample. Although we made the subjects familiar with the task outside the scanner so to make them less anxious inside the MR scanner, we cannot exclude that the decrease in amygdala activity we found is due to habituation effects rather than the MPH challenge. Indeed, previous studies have reported habituation effects due to reduced anxiety because of familiarity with scanning procedures (Breiter et al., 1996; Fischer et al., 2003; Wright et al., 2001). However, habituation effects have been reported in the right amygdala (Wright et al., 2001). The fact that we found a decrease of amygdala reactivity in both the right and the left amygdala in adults makes it more plausible that the decrease of amygdala reactivity we found was more likely a challenge effect than a habituation effect.

Moreover, the clinical ratings used in this study were measurements that only assess a prolonged state of depression and anxiety. Other more suitable rating scales (such as the Conners Global Index: Lability Scale (Sobanski 2010) should be used in future studies to measure acute effects of MPH on emotional lability. Stimulants like MPH are short acting, and the patient’s emotionality can rapidly change both during and at the end of the effect period, when the effect of the stimulant is winding down (Kollins et al., 1998).

In our sample, children with ADHD had predominantly the inattentive subtype of ADHD (60 % inattentive, 40 % combined type) while in the adult ADHD group the combined type was dominant (65.2% combined type, 34.8 % inattentive type). This is indeed contrary to what is expected within developmental trajectories in ADHD and might be due to the age of inclusion of medication naïve children with ADHD. Children with ADHD are usually diagnosed and treated at younger age, thus biasing for selection of predominantly inattentive type at 10-12 years, the age we chose for inclusion. The increased prevalence of the combined type of ADHD in adults compared to children may be due to a selection bias: adult patients could refer themselves to the clinic instead of being referred by their general practitioner (as was the case for the children), a normal procedure in the Netherlands. Nevertheless, in comparing amygdala activity between children and adult patients with ADHD this difference in subtype might be a confounding factor given the potential role of the amygdala in mood and behavioral issues. On the other hand, follow-up of clinical childhood ADHD samples have not yielded many participants who meet adult ADHD criteria (Klein 2012, Biedermann 2006) and evidence is emerging that adults presenting with ADHD symptoms do not suffer a childhood onset neurodevelopmental disorder (Moffitt et al., 2015). Finally, the effects of chronic treatment may differ from an acute challenge as has been shown to be the case in animal studies (Fagundes et al., 2014). Our findings thus stress the need for additional studies in children and adults to investigate the effect of chronic treatment on amygdala reactivity.

These limitations notwithstanding, our findings demonstrate age-dependent differences in emotional processing as well as effects of MPH on emotional processing in ADHD patients. Whereas in adults acute MPH administration seems to normalize increased levels of amygdala reactivity, in children it may further reduce (right) amygdala reactivity. The finding that MPH does not increase amygdala reactivity in children may be reassuring for clinicians treating paediatric ADHD patients, as emotional dysregulation of MPH is an often assumed side effect of MPH. Moreover, in adults MPH-induced reduction towards normal values of amygdala reactivity might be a promising avenue for managing emotional dysregulation problems, when replicated for chronic MPH treatment.
Supplementary Figure 1 | Accuracy across groups was comparable, but did not increase after MPH.

References


CHAPTER 5

The effects of Psychotropic Drugs on the developing brain: methods and design

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Chapter 5 The effects of Psychotropic Drugs on the developing brain: methods and design

Abstract

Background: Animal studies have shown that methylphenidate (MPH) and fluoxetine (FLX) have different effects on dopaminergic and serotonergic system in the developing brain compared to the developed brain. The effects of Psychotropic drugs On the Developing brain (ePOD) study is a combination of different approaches to determine whether there are related findings in humans. Methods/Design: Animal studies were carried out to investigate age-related effects of psychotropic drugs and to validate new neuroimaging techniques. In addition, we set up two double-blind placebo controlled clinical trials with MPH in 50 boys (10–12 years) and 50 young men (23–40 years) suffering from ADHD (ePOD-MPH) and with FLX in 40 girls (12–14 years) and 40 young women (23–40 years) suffering from depression and anxiety disorders (ePOD-SSRI). Trial registration numbers are: Nederlands Trial Register NTR3103 and NTR2111. A cross-sectional cohort study on age-related effects of these psychotropic medications in patients who have been treated previously with MPH or FLX (ePOD-Pharmo) is also ongoing. The effects of psychotropic drugs on the developing brain are studied using neuroimaging techniques together with neuropsychological and psychiatric assessments of cognition, behavior and emotion. All assessments take place before, during (only in case of MPH) and after chronic treatment. Discussion: The combined results of these approaches will provide new insight into the modulating effect of MPH and FLX on brain development.

Background

The brain in development is dependent on the emergence of critical developmental processes (i.e. synaptogenesis, (Swaab DF, Boer K., 2001) and therefore sensitive to pharmacological interventions. Treating children and adolescents with serotonergic (5-HTergic) or dopaminergic (DAergic) drugs like fluoxetine (FLX) and methylphenidate (MPH), is therefore likely to have influence on the maturation of the brain.

For the 5-HTergic system, FLX (a selective serotonin reuptake inhibitor (SSRI), registered for the treatment of depression in children aged 8 years and older, is known to increase extracellular levels of 5-HT by blocking the serotonin transporter (SERT). However, animal studies have demonstrated that periadolescent 5-HT pharmacological manipulations can lead to abnormal outgrowth of the 5-HT system (Iriguez et al., 2014; Karanges et al., 2011). Experiments by our group have shown that chronic treatment with FLX results in a significant increase in prefrontal and hypothalamic 5-HT transporter (SERT; +30%, p < 0.01) in juvenile-treated rats, but not in adult treated rats(Bouet et al., 2012). These findings are in accordance with Wegerer and Bock who have also shown that this effect persists into adulthood, long after discontinuation of treatment with SSRIs (Bock et al., 2005; Wegerer et al., 1999). Recently it was confirmed that FLX administration upregulates SERT long-lastingly, also in non-human primates (Kirchheiner et al., 2001). These preclinical studies suggest that 5-HT manipulations have an impact on the regulation of 5-HT outgrowth which is dependent on the age of exposure.

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

There is some clinical evidence for related findings in humans. For example, after concerns about increased suicide risk among children and adolescents treated with SSRIs, the Food and Drug Administration and European Medicines Agency (EMEA) stated in 2003–2004 that SSRIs were contraindicated for treating depression in children and adolescents. Furthermore, in the NIMH Collaborative Multisite Multimodal Treatment Study of Children With Attention-Deficit Hyperactivity Disorder (MTA) children who received behavioral therapy had a lower rate of diagnoses of anxiety or depression (4.3%) than the children who were treated with MPH (19.1%) thus indicating a (transient) increase in the occurrence of emotional disorders six to eight years after treatment with MPH (Molina et al., 2009a). Age-related differences have also been found between adolescent and adult patients on fMRI studies, with adolescent patients treated with MPH showing more activity in the prefrontal cortex after treatment than adult patients (Epstein et al., 2007).

Thus, evidence is slowly emerging that the long-term effects of drug exposure are delayed and come to expression once the vulnerable system reaches maturation (i.e., typically during adulthood). This phenomenon is known as ‘neuronal imprinting’ and occurs when the effects of drug exposure outlast the drug itself (Andersen and Navalta, 2004). Still, very little is known on exposure during later brain development. Most (clinical) studies are hampered by the fact that they are retrospective in design, and therefore the findings could be caused by other factors on which the groups differed. As pointed out by Shaw and colleagues: ‘...the ideal study design for this question would be a randomized trial comparing cortical growth in children on psychostimulants against an unmedicated comparison group—but this would be both logistically and ethically challenging’ (Shaw et al., 2009).

Notwithstanding this challenge, we have set up three studies (the effects of Psychotropic drugs On the Developing brain ‘ePOD’ project): two randomized controlled trials (RCTs) and a retrospective cohort study, investigating the pos-
sibility of the existence of neuronal imprinting in children medicated with these drugs while using several modalities to assess neurocognitive development. Here we report on the objectives and methods of these studies.

**Objectives**

**Primary objectives**
1. The primary objective of the ePOD studies is to report on the short-term age-dependency of the effect(s) of MPH treatment on the developing DA system and on the age-dependency of the effect(s) of FLX on the developing 5-HTergic system, using pharmacological MRI (phMRI) as our main outcome measure.
2. Furthermore, we aim to study the long-term effects of these drugs in a cohort study based on medical prescription data.

**Secondary objectives**
1. To report on the age-dependency of MPH and FLX on the outgrowth of the DA system and the 5-HT system using functional outcome measures (diffusion tensor imaging [DTI], functional MRI [fMRI], resting-state fMRI [rs-fMRI] and neuropsychological assessment [NPA]).
2. To report on the age-dependency of the effects of FLX on 5-HT driven HPA axis activity using cortisol measures.
3. To report on the role of the 5-HTTLPR polymorphism upon the age-dependency of FLX on the outgrowth of the 5-HT-ergic system
4. To report on the effects of MPH on restless legs (RLS) symptoms and insomnia.

**Methods/design General design of the ePOD project**

Only a long-term prospective study in patients randomly assigned to MPH or SS-RRIs and placebo conditions can determine unequivocally whether the (adverse) effects of these medications on the neurotransmitter systems interact with the age when these drugs are prescribed. To this purpose we designed two RCTs, one with MPH and one with FLX. However, it would not be ethical to deprive subjects in a placebo setting from treatment for extensive periods of time. Therefore, in addition to the RCTs, which will last 4 months, we investigate the long-term effects (at least 7 years) in a cohort study based on medical prescription data. The three sub-studies of the ePOD project include:

- **ePOD-MPH**: A 16 week RCT with MPH in 100 medication naïve ADHD patients. This RCT involves three separate NPA and MRI assessments: the first before starting with the study medication (baseline session), the second during treatment with MPH or placebo (week 8) and the final assessment after trial end following a 1-week washout period (week 17).
- **ePOD-SSRI**: A 16 week RCT with FLX in 80 medication naïve patients suffering from MDD and anxiety disorders (AD). It involves two separate NPA and MRI assessments: before starting with the study medication (baseline session) and after treatment with FLX following a 3-week washout period (week 19).
- **ePOD-Pharmo**: A cohort study based on medical prescription data. One hundred and fifty subjects will be recruited through a database containing prescription data on MPH or FLX (and other antidepressants) Subjects in this cohort based study will receive the same assessments as in the RCTs but only once.

Randomized controlled trials: design and study samples

The two RCTs consist of 16-week multicenter randomized, double blind, placebo-controlled trials with a washout period of one week (MPH) or three weeks (FLX). Subjects are stratified into two age categories: MPH: boys aged 10–12 years, and adults aged 23–40 years. FLX: girls aged 12–14 years and adults aged 23–40 years. These two age groups are randomly assigned to either placebo or active treatment. MRI and NPA assessments will take place before treatment (baseline), during treatment (only in the MPH trial) and following the washout period (see Figure 1 for the timeline for ePOD-SSRI RCT). Baseline measurements will be compared with the results obtained at trial end, and for the ePOD-MPH RCT also during the trial. Differences in outcome measures will be compared between the two age categories (children vs. adults), in addition to healthy controls (separate study). In view of our hypothesis that the active treatment results in long lasting or even permanent changes in the developing brain, we expect no or a small change in change scores between baseline- and post-treatment assessments, whereas in children we expect to find larger changes, as enduring changes will have taken place in the developing brain, but only transient accommodation in the developed brain. Washout periods were chosen based on chemical properties (rate of elimination based on five half–live times) and ethical considerations (time without treatment).

**Figure 1 | Timeline study procedures SSRI trial; *only in adolescents.**
A total of 50 children (10–12 years of age) and 50 adult (23–40 years of age) male outpatients diagnosed with ADHD (all subtypes) and in need of pharmacological therapy will be included in ePOD-MPH RCT. A total of 40 adolescent (12–14 years of age) and 40 adult (23–40 years of age) female outpatients with moderate to severe MDD or an anxiety disorder in need of pharmacological treatment will be included in ePOD-SSRI RCT. Patients that have used medications or drugs that influence the monoamine systems before age 23 are not eligible.

Patients are recruited from clinical programs at the Child and Adolescent Psychiatry Center Triversum (Alkmaar), from the department of (Child and Adolescent) Psychiatry of the Bascule/AMC (Amsterdam), and from PsyQ mental health facility in The Hague. The diagnosis is made by an experienced psychiatrist based on the Diagnostic and Statistical Manual of Mental Disorders, (DSM-IV), Fourth Edition, (APA, 1994) and confirmed by a structured interview: Diagnostic Interview Schedule for Children (National Institute of Mental Health Diagnostic Interview Schedule for Children Version IV (NIMH-DISC-IV, authorized Dutch Translation) (Shaffer et al., 2000), in children or in parents and the Diagnostic Interview for Adult ADHD (DIVA) (Kooij, 2012) in adults in the RCT with MPH. For the ePOD-SSRI trial we use the Diagnostic Interview Schedule for Children in children and in adults the Composite International Diagnostic Interview (CIDI; lifetime version 2.1 authorized Dutch translation) (WHO, 1990). In addition, children must have a Children’s Depression Rating scale-Revised (CDRS-R) (Poznanski et al., 1979) score of > 45, and a Children’s Global Assessment Scale (CGAS)(Shaffer et al., 1983) score < 50. In adults, a Hamilton Rating Scale for Depression (HRSD-(Bech, 1986)≥18, a Clinical Global Impression scale (CGI) (Guy, 1976)> 4, and or Hamilton anxiety scale (HAM-A)(Hamilton, 1959) > 20 are required for study inclusion. Subjects must exhibit stable dysphoria/depressed mood and/or anhedonia for at least 2 weeks prior to enrollment and mood should be pervasive (defined as present most of the time in at least two of three contexts: at home, at school or with friends). In both RCTs, patients with co-morbid axis I psychiatric disorders requiring treatment with medication at study entry, with IQ lower than 80 (as measured by a subtest of the Wechsler Intelligence Scale for children-Revised (WISC-R), National Adult Reading Test (NART), authorized Dutch translation (Schmand et al., 1991) and MDD patients with current risk of suicide attempt are excluded.

We chose to include only male patients in ePOD-MPH to limit subject variation and because ADHD is most prevalent in males (Boyle et al., 2011). Thus, to keep our sample as homogeneously as possible and prevent inclusion problems, only male subjects are included in the ePOD-MPH study. The cut-off point of 10–12 years of age was chosen because peak prevalence of ADHD is 10 years of age (Burd et al., 2003) and also because several MRI parameters greatly change until 8–10 years of age (Bashat et al., 2005) whereas the rate of increase of neuronal growth and pruning reduces after 10 years of age. The age range of the adults is chosen in line with previous studies involving a comparison between matured versus immature brain (Sowell et al., 1999).

Only female subjects are included in ePOD-SSRI based on the higher prevalence of MDD and AD in this population (Hasin et al., 2005). Thus, to keep our sample as homogeneously as possible and prevent inclusion problems, only female subjects are included in the ePOD-SSRI study. For the adolescent group we chose a cut-off point of 12–14 years of age because the risk of MDD and AD onset increases approximately 8 fold at this age compared to children younger than 10 years of age (Birmaher et al., 1996; Merikangas et al., 2010).

Cohort based study: design and study sample

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

Assessments

Clinical rating scales

For both RCTs we use a set of clinical rating scales to assess symptom severity and functioning at baseline and after treatment. For each study separately we have some additional, disorder-specific rating scales. In the ePOD-MPH RCT, an authorized Dutch translation of the Disruptive Behavior Disorders Rating Scale (DBD-RS) (Pelham et al., 1992) will be used in children and in adults the ADHD-SR(M. Rösler et al., 2006) Clinical improvement will be rated in both RCTs by the
clinician using CGAS (Shaffer et al., 1983) and CGI (Guy, 1976) scales in children, and in adults using the Global Assessment of Function (Endicott et al., 1976). In both RCTs, the Children’s Depression Inventory (CDI) (Saylor et al., 1984) and the Screen for Child Anxiety Related Emotional Disorders (SCARED) (Birmaher et al., 1997) will be administered to children, and the Beck Depression Inventory (BDI) (Beck et al., 1961) and the Beck Anxiety Index (BAI) (Beck AT, Epstein N, Brown G, 1988) to adults. These rating scales are also administered in the ePOD-Pharmo study.

Imaging parameters

Imaging parameters are directed towards the DAergic and 5-HTergic system. DAergic and 5-HTergic brain activity will be assessed using phMRI, which is the primary outcome measure of the ePOD project. In addition, DA connectivity will be assessed using rs-fMRI and DTI, and functional brain activity using DA-related (motor inhibition) or 5-HT-related (emotional processing) fMRI tasks. Due to time restrictions of the ePOD-SSRI scan protocol, 5-HT connectivity will only be assessed using DTI.

phMRI

Application of fMRI in combination with a pharmacological challenge (phMRI) has the potential to provide an index of changes in neurotransmitter function. With phMRI a neurotransmitter specific pharmacological challenge is given, which causes changes in neurovascular coupling and subsequent region-specific changes in brain hemodynamics. It differs from fMRI, in that the neuronal system is not activated by a motor or cognitive task, but pharmacologically. phMRI has been shown to adequately assess the DA integrity and functionality, as DA-lesioned primates showed a blunted hemodynamic response to a d-amphetamine challenge, following DA lesionsioning, which correlated strongly with DA transporter availability and motor function (Jenkins et al., 2004). During the phMRI scan, after several minutes of baseline scanning, subjects will receive an oral dose of MPH (0.5 mg/kg with a maximum dose of 20 mg in children and 40 mg in adults). This challenge dose was chosen as it induces maximum blockade of the DAT (80% occupancy), which occurs at serum concentrations of about 8–10 ng/ml. Higher concentrations are not likely to be very effective in further blocking DAT (Moll et al., 2001). After 90 minutes, subjects will undergo a second MRI session, and the same MRI sequences are repeated, now under the influence of MPH. The 90 minute time window was chosen, because DAT occupancy is significantly correlated with plasma concentration of MPH, which peaks between 1 and 2 hours following ingestion of MPH (Andersen and Teicher, 2008; Schouw et al., 2012). DAT occupancy has also been shown to be relatively stable between 1 and 2 hours after ingestion of MPH (Schouw et al., 2012). Based on the literature (reduction in DAT densities in young, but not adult treated animals) (Swanson and Volkow, 2002) and experiments from our own group in d-amphetamine users with phMRI and a MPH challenge (Spencer et al., 2006), we expect that treatment with MPH will induce a long-lasting changes in the brain hemodynamic phMRI response in DA rich brain areas (e.g. striatum) in children, but not adults. We expect that in adult patients MPH will be accommodated by a series of transient compensatory reactions. However, in children MPH will induce changes in the form of long-lasting developmental alterations of the system, reflecting existence of ‘neuronal imprinting’ in the human brain (Silveri et al., 2004).

In the ePOD-SSRI study, an intravenous (i.v.) challenge with citalopram (5 mg in adolescents and 7.5 mg in adults) will be administered during a single scanning session. A 5-HT challenge is subject to more variability and therefore needs to enter the brain in a rapid and consistent manner over the time course of a single scan session, which requires intravenous administration (Anderson et al., 2008). Citalopram is currently the only SSRI registered for i.v. administration. When used for therapeutic purposes, intravenous citalopram is given at the same dose as the oral route of administration and it is well within the therapeutic range even for children (Guelfi et al., 2000). Citalopram increases 5-HT release by inhibiting the reuptake of 5-HT by SERT. It has been used previously in phMRI studies and has been proven an adequate probe of 5-HT function (Anderson et al., 2008; McKie et al., 2005). We have previously shown that phMRI is able to detect 5-HT neuronal imprinting effects: in young rats chronic FLX treatment resulted in an increased 5-HT reactivity as measured with phMRI, whereas in adult animals FLX it reduced 5-HT brain activity (Klomp et al., 2012). We expect an increased signal in 5-HT rich brain areas (e.g. prefrontal cortex, hippocampus and hypothalamus) after 5-HT challenge only in FLX treated adolescents when compared to pretreatment baseline scans, in line with our previous findings in rats (Klomp et al., 2012).

rs-fMRI

A relatively new fMRI approach (i.e., resting-state fMRI (rs-fMRI)) allows assessment of changes in organization of whole functional networks, including DAergic and 5-HTergic networks. Rs-fMRI detects baseline brain activity related to ongoing neuronal signaling at “rest” and is performed by low-pass filtering of spontaneous blood oxygenation level-dependent (BOLD) fMRI signals. A decreased functional connectivity between anterior cingulated cortex and precuneus has been found using this technique in adult ADHD patients (Castellanos et al., 2008). There are a number of studies that have investigated the effects of MPH or SSRIs on this parameter (Posner et al., 2013; Rubia et al., 2009; Shin et al., 2013; Wong CG and MC, 2012; Zhu et al., 2013) which found that these drugs normalize brain activation and functional connectivity abnormalities in patients suffering from ADHD or MDD. In accordance with this literature, we expect to find age-dependent normalization of functional connectivity abnormalities.
DTI
With diffusion tensor imaging (DTI), the micro-structural organization of white matter (WM) can be visualized. By measuring the diffusion motion of water molecules, and the fact that this motion is restricted by myelin sheaths, an impression of axonal direction and integrity can be obtained (Mori and Zhang, 2006). Fractional anisotropy (FA) is the most commonly used readout marker in DTI and provides information about the degree of fiber organization and integrity. Any process that results in alterations in axonal architecture, such as decreased axonal outgrowth, can result in decrease in FA (de Win et al., 2007; Moeller et al., 2005; Reneman et al., 2001). A previous DTI study in children suffering from ADHD, observed an increase, or rather normalization, of white matter volume in ADHD medicated children compared to unmedicated children (Castellanos et al., 2002). In line with this, chronic treatment with MPH in pre-adolescent rats was found to increase (fold change >1.5) genes involved in striatal growth of novel axons (Adriani et al., 2006). Furthermore, in a recent study in rats we observed opposite effects of MPH on FA measures: MPH induced an increase in FA in the corpus callosum of adolescent rats, whereas a slight reduction in adult animals (van der Marel et al., 2014). Therefore, we also expect to find age-related findings in the current RCT with MPH: an increase in FA in MPH treated children when compared to pre-treatment baseline scans, and no effect or a small effect in adult patients.

Considering the 5-HTergic system, we have previously shown that alterations in axonal integrity linked to the 5-HTergic system can be adequately assessed using DTI (de Win et al., 2007). We hypothesize that chronic treatment with SSRIs leads to increased outgrowth of the 5-HT system, since 5-HT acts as a growth factor in the maturing brain (Whitaker-Azmitia et al., 1995). Therefore we expect an increase in FA (reflecting 5-HT neuronal growth) in 5-HT rich brain areas only in FLX treated adolescents when compared to pretreatment baseline scans. Like for MPH, no effect of treatment on these scan parameters are expected in adults.

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

An emotional processing task (MPH and SSRI trials, and ePOD-Pharmo study): The BOLD response to negative emotional faces (angry and fearful faces) is measured in a block-design fMRI task (Hariri et al., 2002). Emotional respons-
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(Picchietti et al., 1999). It has been shown that MPH reduces total sleep time but improves sleep quality by consolidating sleep in adults (Huang et al., 2011). However, the effect of MPH on RLS in ADHD children has never been investigated. In view of the expected inhibitory effect of MPH on DA metabolism it is important to investigate the occurrence and severity of RLS and sleep disorders in children and compare these to adults, and the effect of MPH thereupon. Sleep disorders and RLS are effective and non-invasive outcome measures to evaluate the effect of age following MPH treatment in the human brain. Therefore, we will assess RLS severity and sleep quality in the ePOD-MPH trial using questionnaires (Cambridge-Hopkins RLS questionnaire (CH-RLSq), International RLS severity scale (iRLSS)), John Hopkins RLS severity scale (JH-RLS-ss), Epworth sleepiness scale (ESS) and the the Holland Sleep Diagnostic List (HSDL) (Kerkhof et al., 2013) and sleep log and actigraphy at three time points during the study: the week prior to the trial, during the trial, and during the washout period. Actigraphy is a non-invasive method of monitoring human rest/activity cycles. To measure gross motor activity, each patient will wear a small actigraphy sensor, also called an actimetry sensor, for five consecutive days. We hypothesize that due to an expected long-term reduction in DA turnover rate after early MPH treatment, there will be long lasting positive effects on RLS symptoms and sleep disorders only in children, but not adults.

Cortisol measurements

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

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

Power analysis

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

Statistical analysis

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

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

Ethical considerations

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

The RCTs have been approved by the Central Committee on Human Research in the Netherlands (CCMO), the Pharmo cohort study has been approved by the local medical ethics committee (METC) of the Academic Medical Center Amsterdam (AMC). All subjects participate on a voluntary base and receive a small financial compensation (50 euro and travel expenses). Written and informed consent from both patients and legal caregivers will be obtained in all cases.

Discussion

In the ePOD project we propose a set of neuroimaging studies and neuropsychological assessments in which we examine the neural circuitry in adolescents with depression or anxiety and ADHD before and after treatment. As pointed out recently in an editorial from the American Journal of Psychiatry (Cullen, 2012) this type of research is greatly needed in a field in which most imaging studies have been conducted in adults. Because of ongoing brain development during adolescence, the neuropathophysiology, let alone the treatment, that underlie these disorders could be distinct. ‘Slowly emerging evidence suggests that the long-term effects of drug exposure are delayed and expressed once the vulnerable system reaches maturation (i.e., typically during adulthood). This phenomenon, known as neuronal imprinting, occurs when the effects of drug exposure outlast the drug itself (Silveri et al., 2004). Thus, understanding the persistent effects critically depends on the window of observation.’ (Andersen and Navalta, 2004). Therefore, ePOD is a unique clinical study in children and adults which will exactly grab this window of opportunity to measure age related effects of psychotrophic drugs with sophisticated neuroimaging techniques. Embracing this concept should influence how we conduct preclinical assessments of developmental drug exposure, and ultimately how we conduct clinical assessments of drug efficacy, effectiveness, and safety for the treatment of childhood psychiatric disorders (Andersen and Navalta, 2011).

As the safety of antidepressants to children still is a subject of concern, particularly since FLX is now licensed for the treatment of MDD in children of 8 years and older, information about the safety of FLX in treating childhood depression...
is needed. Especially the potential for an increased suicide risk in association with SSRIs in general has led to much debate (Whittington et al., 2004), as has also been pointed out by the Medicines Evaluation Board of the Netherlands (Wohlforth et al., 2006) and several comments in the Lancet in response to an article by Ebmeier and colleagues (Ebmeier et al., 2006).

The neurotransmitter 5-HT plays a crucial role in axonal outgrowth of 5-HT projections during brain development (Whitaker-Azmitia et al., 1995) Earlier animal work demonstrated that postnatal 5-HT pharmacological manipulations can lead to abnormal outgrowth of the 5-HT system (Azmitia et al., 1990; Shemer et al., 1991; Won et al., 2002). As an SSRI, FLX increases extracellular 5-HT concentrations by blocking SERT. Recently, studies in non-human primates have shown that FLX persistently upregulates SERT, but not 5-HT1A receptors, in the neocortex and the hippocampus of non-human primates (Shrestha et al., 2014). These findings are in line with pilot experiments of our group and findings of Wegener et al. and Bock et al., in rats, which also indicated that this effect persists into adulthood, long after discontinuation of treatment with SSRIs (Bock et al., 2005; Wegener et al., 1999). Also, we showed with phMRI that juvenile-treated rats respond more strongly to a 5-HT challenge than same-age untreated rats, while adult-treated rats show a diminished response after previous chronic treatment (Klomp et al., 2012)). Homberg et al., 2011; Iñiguez et al., 2014; Mason et al., 2009) This study showed that the phMRI technique is very well suited to address the primary objective of the ePOD-MPH studies: investigating whether the effect(s) of FLX on serotonin depend upon age. As may be expected, on a behavioural level, results are less consistent, although age-dependent responses to SSRIs on depression-like behaviour are described in both rats and mice. All these findings most likely reflect the earlier described neuronal imprinting effects.

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

However, there are also some limitations of the present study designs that need to be mentioned. One limitation is that the treatment provided to adolescents and adults is not the same in the ePOD-SSRI RCT. Adolescent subjects will receive CBT, whereas adult patients will not. As mentioned previously, CBT is not part of standard clinical practice in the adult MDD population and will therefore not be provided to the adult patients. From a methodological point of view it would have been ideal to isolate the effect of FLX and add no other treatment than this one in both age groups. However, this is not ethical as CBT is always part of standard clinical practice in the Netherlands in adolescent patients suffering from MDD. However, since change scores from baseline to post-treatment will be determined for each patient (Δi), the potential effect of (the lack) of CBT will be minimized. Another limitation is that no conclusions from the ePOD-SSRI and ePOD-MPH RCTs can be made on the long-term effects of these medications on brain development. The RCTs last for ‘only’ 4 months, and the washout period is 3 weeks maximum. For that reason, we designed the ePOD-Pharmo study, in which subjects are screened at least 7 years later following early FLX or MPH exposure. In addition, all participants in the RCTs are asked if they are willing to participate in a follow up study, scheduled in 3–5 years, and most are willing to participate. Thus, by combining the RCTs in which we investigate the causality of the age-dependency of FLX and MPH, together with the ePOD-Pharmo study which is directed towards the long-term effects of these medicines, will ultimately provide missing knowledge.

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

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

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

References


Chapter 5

The effects of Psychotropic Drugs on the developing brain: methods and design


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Chapter 5: The effects of Psychotropic Drugs on the developing brain: methods and design


CHAPTER 6

Age-dependent effects of methylphenidate on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive patients with ADHD

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Submitted
Abstract

Background: Emotion dysregulation is a key feature of attention deficit hyperactivity disorder (ADHD) and causes serious impairment. However, it is currently unclear whether this is a consequence of stimulant medication or an intrinsic feature of ADHD, and whether its modulated by age.

Methods: In the ePOD-MPH randomized controlled trial 99 stimulant treatment-naive patients with ADHD (DSM-IV) were randomly assigned to either MPH or matched placebo treatment for 16 weeks. Emotion regulation was assessed using amygdala reactivity with Magnetic Resonance Imaging (MRI). Processing of angry and fearful facial expressions was assessed at three time points in the RCT: before, during and one week after trial end. Secondary outcome measures were amygdala connectivity and clinical symptoms. Data were analysed intention to treat.

Findings: MPH over placebo positively influenced emotion dysregulation during the trial but negatively affected right amygdala reactivity in children at trial end. Although in adults MPH had no effect over placebo on behavioral measures nor amygdala reactivity, it induced cortical-amygdala hyper connectivity one week after trial end.

Interpretation: Four months of treatment with MPH affected right amygdala reactivity, amygdala connectivity and emotion dysregulation age-dependently. The lasting positive effects of MPH on emotion dysregulation should comfort parents and clinicians in prescribing MPH for ADHD in children, at least on the short term. Longer follow up studies are needed to investigate the clinical significance of our neuroimaging findings which have been suggested to precede emotional problems later in life.

Introduction

Emotional dysregulation defined as being easily angered and easily annoyed in children with ADHD, often leads to serious impairment in children and adults with Attention-Deficit Hyperactivity Disorder (ADHD) (Barkley and Fischer, 2010; Wehmeier et al., 2010), but so far has received relatively little attention. This is remarkable given that emotional problems influence the course and outcome of ADHD (Qian et al., 2016), have been associated with persistence of ADHD into adulthood (Barkley and Fischer, 2010) and predict lower quality of life in young adults (Reimherr et al., 2005). Furthermore, surprisingly little is known about the neural substrates of emotion dysregulation in ADHD.

The amygdala has been associated with aberrant emotional processing in affective disorders (Hamilton et al., 2012; Tranter et al., 2009). Accumulating evidence implicates the amygdala as an important area underlying emotion dysregulation in ADHD. For instance, a recent mega-analyses in 3242 subjects found largest volume reductions in the amygdala of ADHD patients (Hoogman et al., 2017). ADHD patients also show predominantly heightened amygdala reactivity to negative emotional stimuli as a literature review reported (Shaw et al., 2014). These deficits are thought to arise from a dysfunction within the striato-amygdalo-medial prefrontal cortical network (Shaw et al., 2014). However, studies on abnormalities in amygdala connectivity are only beginning to emerge. For instance, Hulvershorn et al. showed that more severe emotional dysregulation was associated with hyperconnectivity of a corticoamygdalar network including the anterior cingulate cortex and frontal pole in children aged 6-13 who were mostly treatment naïve (Hulvershorn et al., 2014). Furthermore, in adolescents with ADHD (aged 11-16 years) hyperconnectivity of the amygdala with lateral prefrontal cortex to fearful faces was aggravated after MPH abstinence, along with amygdala hyper reactivity (Posner et al., 2011). Thus, amygdala-prefrontal cortex hyperconnectivity in ADHD patients may reflect an overrepresentation of the negative affect associated with fearful faces.

However, most studies so far were conducted in previously medicated patients, and none of the studies were placebo-controlled, thus complicating whether to attribute these amygdala abnormalities to ADHD, or to stimulant treatment per se. In other words, it is unknown whether the abovementioned observations represent an intrinsic feature of ADHD or a consequence of stimulant treatment. This is highly relevant, because although treatment with stimulants, such as methylphenidate (MPH), effectively reduce symptoms of inattention and hyperactivity in ADHD patients (The MTA group, 1999), preclinical data suggest that MPH does induce anxiety and depressive-like behavior (Bolaños et al., 2008; Carlezon William A et al., 2003). Also, the most comprehensive study on the long-term effects of ADHD medications to date, the Multimodal Treatment Study of
ADHD (MTA) found that children treated with ADHD medications had a higher rate of clinical diagnoses of anxiety and depression (19.1%) than children receiving behavioral therapy (4.3%) six-, but not eight years, after treatment (Molina et al., 2009).

There is increasing evidence suggesting that the effects of ADHD medications on emotional processing are dependent on the age of exposure. For instance, young rats treated with MPH showed more anxiety and depression-related behavior in adulthood than adult treated rats (Bolaños et al., 2003). These effects might be mediated through age-dependent effects of stimulants on the dopamine (DA) system. For instance, early treatment with MPH led to a considerable (-50%) reduction of DA transporter (DAT) density in rat striatum when compared to non-treated animals, whereas no such effects were observed in adult animals (Moll et al., 2001). Moreover, in a randomized clinical trial (RCT) in patients with ADHD we found that four months of MPH treatment resulted in increased DA functionality only in children, but not in adult patients (Schran- tee et al., 2016), providing further evidence that the effects of MPH on the human brain may also be modulated by age.

Therefore, the aims of this study were twofold: a) to investigate whether MPH treatment affects amygdala reactivity in patients with ADHD and 2) whether this effect is modulated by age. To this end, we measured amygdala reactivity during an emotion recognition task using functional magnetic resonance imaging (fMRI) at three time points (baseline, during treatment and 1 week after trial end) in a RCT involving 99 stimulant treatment-naive children and adults with ADHD randomly assigned to 16 weeks of MPH or placebo treatment. Furthermore, functional connectivity of the amygdala was assessed, as well as clinical symptoms of emotional dysregulation. Based on strong age-dependent effects reported in the preclinical literature (Bolaños et al., 2008; Carlezon William A et al., 2003) and emerging clinical literature (Schran- tee et al., 2016), and the (transient) increase in diagnoses of anxiety and depression in the MTA study following pharmacological treatment in medication naïve children with ADHD (Molina et al., 2009), as well as aggravated amygdala hyper reactivity after MPH abstinence (Posner et al., 2011), we expected that MPH would increase amygdala reactivity in children but not -, or less so in adults. We also hypothesized an increase in amygdala connectivity with prefrontal regions as well as an increase in emotional dysregulation in children but not -, or less so in adults.

Method

The ‘effects of Psychotropic drugs On Developing brain-MPH’ (ePOD-MPH) RCT was a 16-week double-blind, randomized, placebo-controlled, multicenter trial with MPH and a blinded end point evaluation in stimulant treatment-naive patients with ADHD (Bottelier et al., 2014). The primary objective of the ePOD-MPH RCT was to study the age-dependency of the effects of MPH on the developing DA system, the results of which are published elsewhere (Schran- tee et al., 2016). Our secondary outcome measures, amygdala reactivity-, connectivity and emotional dysregulation, was assessed using fMRI at three different intervals: at baseline (BL), during the trial (DT), and after a 1-week washout (PT). The trial started on June 1, 2011 and ended on June 15, 2015, and was monitored by the Clinical Research Unit of the Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands. The ePOD-MPH study was registered by the Dutch National Competent Authority on March 28 2011 (NL34509.000.10) and subsequently at the Netherlands Trial Register on October 13 2011 (NTR3103).

Participants

Participants were 50 stimulant treatment-naive boys (10-12 years of age) and 49 stimulant-treatment naïve men (23-40 years of age) that participated in the ePOD-MPH trial and were diagnosed with ADHD and recruited through clinical programs at the Child and Adolescent Psychiatry Center Triversum (Amster- mar), department of Child and Adolescent Psychiatry at the Bascele/AMC (Amsterdam), and PsyQ Mental Health Facility (The Hague). All children and adults were diagnosed by an experienced psychiatrist and met strict criteria for ADHD according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 4th edition), confirmed by a structured interview, i.e. the Diagnostic Interview Schedule for Children (NIMH-DISC-IV: authorized Dutch translation (Ferdinand and van der Ende, 1998) and the Diagnostic Interview for ADHD (DIVA) for adults (Koolj, 2012). Patients with co-morbid axis I psychiatric disorders requiring treatment with medication at study entry, a history of major neurological or medical illness as well as clinical treatment with drugs influencing the DA system (for adults before 23 years of age), such as stimulants, neuroleptics, antipsychotics, and D2 agonists were excluded. All patients and parents or legal representatives of the children provided written informed consent after receiving a complete description of the study.
Intervention, randomization and blinding

After baseline MRI assessment, patients were stratified by age and randomized to either placebo or MPH treatment (1:1) using a permuted block randomization scheme generated by the local Clinical Research Unit. The treating physician prescribed the study medication under double-blind conditions on clinical guidance (reduction of ADHD symptoms) in accordance with Dutch treatment guidelines. The hospital pharmacy of the Medical Centre Alkmaar assigned participants to a specific allocation, using sequentially numbered containers. Participants as well as care providers and research personnel were blinded. The placebo tablet was identical to the MPH tablet with respect to appearance and was manufactured and labelled according to GMP guidelines (2003/94/EG). Adherence to the study medication was monitored at each of the control visits.

Primary outcome measure: Amygdala reactivity

Subjects performed an emotion recognition fMRI paradigm at 3 different time points during the trial: BL, DT and PT (Figure 1).

Figure 1 | Time line ePOD study.

Stimulant treatment-naive patients with ADHD were randomized to treatment condition (MPH) or placebo condition. After 16 weeks treatment was discontinued and followed by a wash out period of one week. fMRI scans were made at baseline (BL), after 8 weeks of treatment (DT), and one week after discontinuation (PT).

The experimental paradigm consisted of a block design and was adapted from a task previously used to assess drug effects on amygdala reactivity (Hariri et al., 2002; van Wingen et al., 2008). The emotional stimuli consisted of angry and fearful faces whereas the neutral stimuli consisted of ellipses assembled from scrambled faces. Two blocks of emotional stimuli were interleaved with three neutral blocks, each 30-s block containing six 5 s trials. For each emotional trial, three stimuli were presented simultaneously, and subjects had to decide which one of the lower two stimuli expressed the same emotion as the target stimuli presented above. Similarly, for each neutral trial, three stimuli were presented, but subjects had to decide which of the bottom two ellipses was identically oriented to the target ellipse. Two versions of the task were used to overcome learning effects. To further minimize learning effects, a practice run was presented prior to the first MRI scan.

The MRI study was performed on a 3.0T Philips scanner (Philips Healthcare, Best, The Netherlands) using an 8-channel receive-only head coil. A high-resolution 3D T1-weighted anatomical scan was acquired for registration purposes and fMRI data were acquired using a single shot echo planar imaging sequence with parameters: TR/TE=2300/30 ms, resolution=2.3×2.3×3 mm, 39 sequential slices, FOV=220×220×117 mm, GE-EPI read-out, 70 dynamics, no gap, 80° flip angle, total duration 2:42 minutes. Data were analyzed using in-house MATLAB scripts (MATLAB version 2013a, Natick, Massachusetts: The Matworks Inc.) and FEAT (FMRI Expert Analysis Tool) in FSL 5.0 (FMRIB’s Software Library) (http://fsl.fmrib.ox.ac.uk/fsl). The first volume of the fMRI series was discarded to allow for T1 equilibration. Images were skull stripped, motion-corrected, spatially smoothed with a FWHM Gaussian kernel of 5 mm and spatially normalized and resampled to Montreal Neurological Institute (MNI) 2mm template. fMRI time series were high-pass filtered with a cutoff of 0.01 Hz. First-level analyses were performed by modeling the signal changes using the stimulation paradigm (faces versus shapes), convolved with a canonical hemodynamic response function. The six-standard rigid-body motion parameters and a confound matrix of volumes that were corrupted by large motion were added to the model. Confounded time points were determined using a net displacement vector according to Euclidian root mean square (RMS). Data from subjects with extreme motion (frame wise displacement > mean + 2*standard deviation using both the method by Power and van Dijk (Power et al., 2012; van Dijk et al., 2012) were removed from the analysis. For our regions of interest (ROI) analyses, mean signal intensity for the left and right amygdala was extracted from the first level contrasts using masks from the Harvard-Oxford atlas provided within FSL.

Secondary outcome measures

Connectivity

For the functional connectivity analyses, amygdala time courses were extracted using the Harvard-Oxford masks. To obtain connectivity measures we separately added the left and right amygdala time-course to the first-level model. Connectivity was obtained and entered into subsequent random-effects analyses.
to assess changes in amygdala connectivity over time. Statistical parametric maps were masked with a gray matter mask, thresholded at a Z-value > 2.3 with a cluster-based FWE correction at p<0.05 and a minimum cluster size of 100 voxels.

Clinical variables
In children we assessed anxiety and depressive symptoms using the Child Depression Inventory (CDI) (Kovacs, 1985) and the Screen for Child Anxiety Related Disorders (SCARED) (Muris P, Bodden D, Hale W, 2007). In adults, we used the Beck’s Depression Inventory (BDI) (Beck et al., 1961) and Beck’s Anxiety Inventory (BAI) (Beck AT, Epstein N, Brown G, 1988). All clinical scales were administered at BL, DT and PT, at the same time points of the MR scanning. Emotion dysregulation was measured by distracting the items ‘is often angry and resentful’, ‘often loses temper’ and ‘is often touchy or easily annoyed by others’ from the DBD-RS in children, in accordance to the items Sobanski et al. (Sobanski et al., 2010) distilled from the Child Behavior Checklist (CBCL) and the items ‘overly active and compelled to do things’, ‘difficulty unwinding’ and ‘restless and fidgety’ from the ADHD-SR (Rösler et al., 2006) suggesting emotion dysregulation in adults.

Statistical analysis
Data were processed using SPSS version 22 (IBM Corp., Armonk, USA). Amygdala reactivity and clinical variables were analyzed based on intention-to-treat, with the significance level set at p<0.05 (2-sided). A linear mixed model was used on amygdala reactivity and clinical variables to investigate the main effect of time point, medication and age group, and its corresponding interaction effects. An unstructured covariance matrix was assumed, with a fixed intercept and the model was estimated using maximum likelihood. Follow-up pairwise comparisons were corrected for multiple testing using a Sidak correction. Behavioral response data of the fMRI task were extracted from E-prime. Whole brain connectivity analyses were analyzed per protocol.

Results
Clinical characteristics and randomization
Between June 1 2011 and June 15, 2015, a total of 99 patients with ADHD were randomized to MPH or placebo whereof 50 children and 49 adults were included. No serious adverse events were reported in any of the subjects. Treatment groups did not differ in age, clinical impairment and ADHD severity (Table 1). However, children with ADHD had predominantly the inattentive subtype of ADHD (60 % inattentive, 40 % combined type) while in the adult ADHD group the combined type was dominant (65.2% combined type, 34.8 % inattentive type).

Table 1. Characteristics of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Children MPH</th>
<th>placebo</th>
<th>Adults MPH</th>
<th>placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=25</td>
<td>n=25</td>
<td>n=24</td>
<td>n=24</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>11.4±0.8</td>
<td>11.3±0.9</td>
<td>28.6±4.6</td>
<td>29.0±4.9</td>
</tr>
<tr>
<td>Estimated IQ1</td>
<td>104.8±21.0</td>
<td>103.4±15.1</td>
<td>107.9±8.8</td>
<td>107.9±6.4</td>
</tr>
<tr>
<td>ADHD subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inattentive</td>
<td>14</td>
<td>14</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Hyperactive/impulsive</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Combined ADHD symptoms</td>
<td>11</td>
<td>10</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>DBD-RS Inattention</td>
<td>21.7±3.2</td>
<td>22.8±3.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DBD-RS Hyperactivity</td>
<td>15.0±5.0</td>
<td>16.4±6.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ADHD-SR</td>
<td>-</td>
<td>-</td>
<td>31.8±9.9</td>
<td>31.1±9.7</td>
</tr>
<tr>
<td>Adherence</td>
<td>84%±15</td>
<td>80%±18</td>
<td>90%±8</td>
<td>86±8</td>
</tr>
</tbody>
</table>

1For children: Wechsler Intelligence Scale for Children (WISC); for adults: National Adults Reading Test(NART); DBD-RS=disruptive behavior disorder rating scale; ADHD-SR=Attention Deficit Hyperactivity Disorder-Self Report

Treatment assignment and details
In Supplementary Figure 1, treatment allocation and drop-out rates are reported according to CONSORT standards. One adult was excluded from the analysis due to undisclosed prior stimulant treatment. Eight adults underwent the PT scan at 8 weeks instead of at 17 weeks of the trial due to significant technical changes (scanner upgrade) to the MRI scanner. The mean treatment duration did not differ between both treatment groups in adults (p=0.68) nor children (p=0.73). From the total of 294 MRI scans 37 were missing due to dropout, a missing session, motion, artifacts in MRI data or incomplete understanding of the task (12.6%). Missing data for the clinical measures were 6.1% (18/294) for the CDI and BDI in children and adults, 6.1% (18/294) for the SCARED and BAI and 10.9% (32/294) for the emotion dysregulation rating.
Age-dependent effects of methylphenidate on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive patients with ADHD

Chapter 6

Amygdala reactivity
Linear mixed model analyses did not show a significant age x medication x time interaction for either left or right amygdala reactivity (left: F(2,89)=0.21, p=0.81; right: F(2,91)=0.08, p=0.92), nor significant time x medication interaction in the children (F(2,43)=0.50, p=0.61) nor adults (F(2,43)=0.68, p=0.51). However, a two-way interaction between time and age was observed in the right amygdala (F(2,91)=4.82, p=0.01), but no significant main effect (time: F(1,89)=0.38, p=0.69). No such effect was observed in the left amygdala (time x age: F(2,89)=2.35, p=0.10) (Figure 2).

Connectivity
From baseline to eight weeks of treatment, longitudinal analyses per group indicated that MPH decreased connectivity between the amygdala and various brain regions in both age groups (Figure 3 and Supplementary Table 1).
Age-dependent effects of methylphenidate on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive patients with ADHD

Figure 3 | Whole-brain connectivity with left and right amygdala.

Maps were thresholded at $z=2.3$, gray matter masked and cluster with voxel sizes > 100 are displayed. BL = baseline; DT = during treatment; PT post-treatment.

In children, this reduction DT in connectivity was widespread including cortical and subcortical areas, whereas in adults MPH decreased the connectivity mainly in frontal regions. Subsequently, PT, both MPH groups showed a return to BL connectivity patterns with persisting increased connectivity compared to DT in adults, but not children. Additionally, in MPH-treated adults, connectivity was more widespread and stronger PT than at BL and in children. Remarkably, children in the placebo group showed an increase in connectivity during treatment that returned to baseline after discontinuation of the 16 weeks trial.

Direct statistical comparisons within the two age groups confirmed these observations: children treated with MPH showed a greater reduction in cortical and subcortical regions compared to children in the placebo group and compared to BL. After discontinuation, children treated with MPH showed a greater increase of connectivity (indicating a return to BL levels) than children treated with placebo. Similarly, adults treated with MPH showed a greater reduction in cortical (and some subcortical) regions compared to placebo and compared to BL. After discontinuation adults treated with MPH showed a greater increase of connectivity (indicating a return to BL levels) than adults treated with placebo. Additionally, in MPH-treated adults, connectivity was stronger PT compared to placebo and BL. Finally, direct comparisons between children and adults treated with MPH confirmed that the pattern of amygdala connectivity with other brain regions differed for children and adults with more subcortical regions involved in MPH-induced connectivity changes in children than adults.

Clinical Outcome

Linear mixed model analyses did not show a significant age x medication x time interaction for anxiety ($F(2,93)=0.67$, $p=0.52$) or depressive symptoms ($F(2,92)=2.64$, $p=0.08$), nor for emotion dysregulation symptoms ($F(2,89)=0.58$, $p=0.08$). Moreover, no medication x time effects were found for either children or adults for any of the clinical outcomes. However, we found a main effect of time for the CDI ($F(2,49)=20.62$, p<0.001), SCARED ($F(2,49)=12.73$, p<0.001) and emotion dysregulation ($F(2,44)=5.53$, p<0.007) in children, and for the BDI ($F(2,44)=5.44$, p<0.008) and emotional dysregulation ($F(2,45)=13.18$, p<0.001) in adults (Figure 4).

For emotional dysregulation, this effect was mainly driven by a decrease in the MPH-treated children from baseline to post-treatment ($F(1,22)=12.83$, p<0.002) but not the placebo condition ($F(1,23)=1.36$, p=0.26). In adults, both medication conditions showed a decline from BL to one week PT (MPH $F(1,21)=8.76$, p<0.007; placebo $F(1,21)=6.71$, p<0.02). For symptoms of depression and anxiety both the MPH and placebo conditions in children showed improvement from BL to one week PT (for depression: MPH $F(1,24)=14.89$, p<0.001; placebo $F(1,24)=14.19$, p<0.001; for anxiety: MPH $F(1,25)=5.92$, p<0.02; placebo $F(1,24)=17.21$, p<0.001). However, in adults, no treatment effects were found on depressive symptoms (MPH $F(1,24)=3.61$, p=0.07; placebo $F(1,24)=0.16$, p=0.69) nor anxiety symptoms (MPH $F(1,20)=1.19$, p=0.29; placebo $F(1,20)=0.48$, p=0.50) from BL to PT (Figure 4). No association between amygdala reactivity and clinical symptoms was found in children nor adults in any of the treatment conditions.
Figure 4 | Symptom scores on clinical rating scales for each timepoint.

Top row shows data for the children and bottom row for the adults. BL = baseline; DT = during treatment; PT = post-treatment. Data are expressed as mean and standard error of the mean. *p<0.05

Discussion

In this 4 month RCT with MPH in stimulant treatment-naive children and adults with ADHD, right amygdala reactivity changed significantly over time in children, but not in adults, regardless of treatment group. One week after discontinuation, we observed a significant reduction in right amygdala reactivity in children in the placebo condition, whereas in the MPH condition such an effect was absent. Whereas in both children and adults MPH treatment decreased functional connectivity of the amygdala with other cortical and subcortical brain regions, after treatment discontinuation connectivity returned to baseline levels, but in adults less so than in children. In children, more subcortical regions were involved in MPH-induced connectivity changes than in adults. Finally, although symptoms of anxiety and depression did improve during the trial, they did not differ between both medication groups in children, although the MPH condition appeared to drive the main effect of emotional liability. In adults, we observed improvement on depressive and emotion dysregulation symptoms, but in both conditions. In sum, in this RCT we found that MPH modulated right amygdala reactivity, amygdala connectivity and emotion dysregulation age-dependently.

MPH might result in lasting increases in amygdala reactivity for children with ADHD, as we found a significant decrease in amygdala reactivity from 8 weeks of treatment to posttreatment only in the placebo group. This is worrisome, as heightened amygdala activation has previously been associated with emotion dysregulation and increased symptoms of anxiety and depression (Swartz et al., 2015; Uchida et al., 2014). However, although the one-week washout ensures clearance of MPH, it is unclear whether these changes in amygdala reactivity persist beyond this week. These findings on amygdala reactivity contrast our findings on functional amygdala connectivity in children. Because increased connectivity of amygdala with prefrontal regions has been associated with higher levels of emotion dysregulation (Uchida et al., 2014), the transient reductions we observed in children and adults in the MPH condition suggest positive effects during treatment that disappear after treatment cessation.

Clinical symptoms associated with depression, anxiety and emotion dysregulation showed persistent improvement when compared to baseline in our sample of ADHD children. This is in line with previous studies in children and adolescents with ADHD (Hulvershorn et al., 2014; Posner et al., 2011). One explanation could be that this is a result of MPH-induced enhanced DA function in the amygdala, as we previously found to be the case in the children that were studied here (Schransee et al., 2016). Indeed, DA signaling is thought to be attenuated in the amygdala of patients with ADHD (Volkow et al., 2007). However, the fact that both MPH and placebo condition showed improvement in children on most of these behavioral measures suggests that this effect is more related with being in treatment. At first sight our findings on fMRI amygdala reactivity and clinical symptoms may seem inconsistent in children (positive effects on emotion dysregulation, anxiety and depressive symptoms, lasting increased right amygdala reactivity and transient positive effects on amygdala connectivity). It has recently been shown that in subjects with familial risk or stressful life events, heightened amygdala activation has previously been associated with emotion dysregulation during adolescence prior to the emergence of clinical depressive symptoms (Swartz et al., 2015). So although treatment decreased clinical symptoms and amygdala connectivity, the increased right amygdala reactivity could be a precursor for long term effects. Indeed, in the MTA trial increased occurrence of anxiety and depression was only observed 6 years after enrollment, not before (Molina et al., 2009). Therefore, even though the neurobiological and clinical evidence is mixed in our MPH-treated children at PT with a one week wash-out, it needs to be established what the long-term effects of MPH treatment are on the development of emotional regulation problems later in life. Nevertheless, our findings bear considerable clinical relevance, because clinicians tend to delay prescribing MPH or titrating an adequate dose fearing to induce depressive symptoms (Jerrell et al., 2014). Here, we show that there is no reason to withhold treatment at least on the short-term, as MPH does not exert negative effects on symptoms of...
emotion regulation, anxiety and depression after 4 months of treatment, either in adults or children.

The strength of the present study is its design. To rule out the influence of a history of medication use we only included stimulant treatment-naïve patients. For ethical reasons, we could not extend the follow-up period to more than 4 months. A limitation of our RCT is that we could not include healthy controls so we do not know if the amygdala reactivity we found at baseline was heightened in our sample compared to the general population. Further limitations of our study are that the results cannot be extrapolated to all children and adults with ADHD, because we only studied male subjects in a specific age range. In addition, the children we included predominantly had the inattentive subtype of ADHD and adults the combined type. This is contrary to what is expected within developmental trajectories in ADHD and might be due to the age of inclusion. Children with ADHD are usually diagnosed and treated at younger age, thus biasing for selection of predominantly inattentive type at 10-12 years, the age we chose for inclusion. The increased prevalence of the combined type of ADHD in adults may reflect a selection bias: adult patients could refer themselves to the clinic instead of being referred by their general practitioner (as was the case for the children), a normal procedure in the Netherlands. Nevertheless, in comparing amygdala reactivity between children and adult patients with ADHD, the differences in subtype might be a confounding factor given the potential role of the amygdala in mood and behavioral issues, which we cannot rule out. On the other hand, follow-up of clinical childhood ADHD samples have not yielded many participants who meet adult ADHD criteria and indeed evidence is emerging that adults presenting with ADHD symptoms do not suffer a childhood onset neurodevelopmental disorder (Moffitt et al., 2015).

Conclusion

Four months of treatment with MPH affected right amygdala reactivity, amygdala connectivity and emotion dysregulation age-dependently. In children, MPH lastingly and positively influenced emotion dysregulation scores, but on the other hand negatively affected right amygdala reactivity (no return to baseline levels). Although in adults MPH had no effect over placebo on behavioral measures nor amygdala reactivity, it did induce cortical-amygdala hyper connectivity. Although we here demonstrate mixed results for neurobiological vs. clinical findings, the lasting positive effects of MPH on emotion dysregulation scores should comfort parents and clinicians in prescribing MPH for ADHD in children. Longer follow up studies are needed to investigate the clinical significance of our neuroimaging findings which have been suggested to precede emotional problems later in life.

References


Hoogman, M., Bralten, J., Hibr, D.P., Mennes, M., Zwiers, M.P., Schweren, L.S.J., et al., 2017. Sub-


Age-dependent effects of methylphenidate on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive patients with ADHD
Age-dependent effects of methylphenidate on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive patients with ADHD

### Supplementary Table 1 Connectivity of amygdala with different brain areas and their coordinates at different time points; BL: baseline, DT: During Treatment, PT: Post-Treatment

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>BL &gt; DT</th>
<th>DT &gt; BL</th>
<th>DT &gt; PT</th>
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<tbody>
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<tr>
<td>Left amygdala</td>
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<tr>
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<tr>
<td>Parsiagulate gyrus</td>
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<td><strong>Adults MPH</strong></td>
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<td>Parsiagulate gyrus</td>
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**MNI coordinates**

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<td>Parsiagulate gyrus</td>
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**Seed brain area**

- **Left amygdala**
- **Right amygdala**

**No of voxels**

- **BL > DT**: 3663
- **DT > BL**: 1359
- **DT > PT**: 1399

**Z-value**

- **BL > DT**: 4.59
- **DT > BL**: 4.33
- **DT > PT**: 4.18

**References**

Age-dependent effects of methylphenidate on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive patients with ADHD

<table>
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<th>Region</th>
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<th>PT &gt; BL children MPH</th>
<th>PT &gt; DT adults MPH</th>
<th>PT &gt; BL adults MPH</th>
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MNI = Montreal Neurological Institute brain coordinates

Left and right amygdala time-course was separately to the first-level model. Connectivity was obtained and entered into subsequent random-effects analyses to assess changes in amygdala connectivity over time. Statistical parametric maps were thresholded at a Z-value > 2.3 with a cluster-based FWE correction at p < 0.05 and a minimum cluster size of 100 voxels.
CHAPTER 7

Long-term effects of stimulant exposure on cerebral blood flow response to methylphenidate and behavior in attention deficit hyperactivity disorder

Anouk Schrantee — Cheima Bouziane
Esther E. Bron — Stefan Klein — Marco A. Bottelier
J.J.Sandra Kooij — Serge A.R.B. Rombouts
Liesbeth Reneman

Brain Imaging and Behavior (in press)
Abstract

Stimulant prescription rates for attention deficit hyperactivity disorder (ADHD) are increasing, even though potential long-term effects on the developing brain have not been well-studied. A previous randomized clinical trial showed short-term age-dependent effects of stimulants on the DA system. We here assessed the long-term modifying effects of age-of-first-stimulant treatment on the human brain and behavior. 81 male adult ADHD patients were stratified into three groups: 1) early stimulant treatment (EST; <16 years of age) 2) late stimulant treatment (LST: ≥23 years of age) and 3) stimulant treatment naive (STN; no history of stimulant treatment). We used pharmacological magnetic resonance imaging (phMRI) to assess the cerebral blood flow (CBF) response to an oral methylphenidate challenge (MPH, 0.5 mg/kg), as an indirect measure of dopamine function in fronto-striatal areas. In addition, mood and anxiety scores, and recreational drug use were assessed. Baseline ACC CBF was lower in the EST than the STN group (p=0.03), although CBF response to MPH was similar between the three groups (p=0.01). ADHD symptom severity was higher in the STN group compared to the other groups (p=0.01). In addition, the EST group reported more depressive symptoms (p=0.04), but not anxiety (p=0.26), and less recreational drug use (p=0.04). In line with extensive pre-clinical data, our data suggest that early, but not late, stimulant treatment long-lastingly affects the human brain and behavior, possibly indicating fundamental changes in the dopamine system.

Introduction

Attention-deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders diagnosed in children and adolescents (Thomas et al., 2015), and also has a high prevalence in adults (approximately 2.5%) (Simon et al., 2009). The most prescribed treatment for ADHD is stimulant medication, such as methylphenidate (MPH) and dexamphetamine (dAMPH). Stimulants act upon the dopamine (DA) neurotransmitter system by increasing extracellular DA, and have been shown to be very effective in reducing behavioral symptoms in ADHD (van de Loo-Noe-Neus et al., 2011). However, as prescription rates of stimulants are rising (McCarthy et al., 2012), concern about potential long-term consequences of stimulants on the developing DA system is increasingly being voiced by a number of entities. These include parents of patients, healthcare professionals, the Food and Drug Administration (FDA) and National Institutes of Health (NIH).

Prospective studies are the ideal study design to investigate the long-term effects of stimulants on the development of the DA system. A prospective study in non-human primates assessed the effects of 1-year treatment with MPH or placebo during adolescence. This positron emission tomography (PET) study found that the MPH group lacked the expected age-related decline observed in the placebo group, suggestive of increased D2/D3 receptors (Gill et al., 2012). In rats, juvenile treatment with MPH reduced DA transporter (DAT) densities immediately after treatment. When these rats were assessed in adulthood, DAT densities were even further reduced. In adult-treated rats, no such effect was observed (Moll et al., 2001). Another prospective study in rats showed that early MPH treatment persistently increased MPH-induced change in cerebral blood volume (CBV) in the thalamus, cingulate and medial prefrontal cortex (mPFC) later in life (Andersen et al., 2008). Behaviorally, juvenile MPH exposure reduced cocaine self-administration (Andersen et al., 2001), but increased anxiety and depressive-like behaviors in rats (Bolaños et al., 2003; Carlezon et al., 2003).

Human prospective studies are limited to short-term effects (and treatment durations) for ethical reasons. Data from a recent randomized controlled trial (RCT) from our group were in line with that preclinical work, showing that four months of MPH treatment increased cerebral blood flow (CBF) response to acute MPH, in children, but not in adults with ADHD using phMRI (Schrantee et al., 2016). However, to study the lasting effects of early stimulant treatment, retrospective cohort studies can provide important information. For example, studies have shown both positive (Biederman et al., 2009; Mannuzza et al., 2008; Wilens et al., 2003) and negative (Molina et al., 2009) associations between stimulant treatment in adolescence and occurrence of substance-use disorders (SUDs), as well as anxiety and depressive disorders. However, the long-term effects of age-of-first stimulant treatment on DA development have not yet been studied in this context.
Long-term effects of stimulant exposure on cerebral blood flow response to methylphenidate and behavior in attention-deficit hyperactivity disorder

Taken together, the available evidence suggests that the effects of stimulants on the DA system are dependent on age-of-first-treatment, possibly reflecting ‘neuro-chemical imprinting’ (Andersen and Navalta, 2004). This theory also predicts that these effects are only fully expressed when the system reaches maturation (e.g., typically during adulthood). Using a cohort-study, we here studied the relation between age-of-first-stimulant-exposure and the DA system using pharmacological magnetic resonance imaging (phMRI). phMRI is a non-invasive imaging technique to indirectly assess DA function, which indirectly measures DA neurotransmitter function by assessing hemodynamic changes induced by a dopaminergic drug challenge. These phMRI signal changes strongly correlate with DA release and DA transporter availability in preclinical and clinical studies (Chen et al., 1997; Schrantee et al., 2015).

We included three groups of adult ADHD patients: those that had either been exposed to stimulants early in life (before the age of 16), later in life (after the age of 23), or were naive to stimulant treatment. Based on the literature, we hypothesized a higher CBF response to an MPH challenge in early exposed individuals compared to late exposed, or stimulant treatment-naive individuals; higher anxiety and depression scores in early but not late exposed, or stimulant treatment-naive individuals; but less use of recreational drugs use in early, but not late- or stimulant treatment-naive individuals.

Methods and Materials

Participants
81 male ADHD patients (23-40 years) were recruited via outpatient clinics, newspaper advertisements, databases containing prescription data (Pharmo Institute Utrecht) and the ePOD-MPH RCT (Schrantee et al., 2016). All subjects had a clinical diagnosis of ADHD requiring pharmacological treatment with a stimulant (diagnosed by psychiatrist, psychologist or pediatrician (primarily) or GP according to DSM-III or DSM-IV criteria according to Dutch treatment guidelines). Exclusion criteria were: IQ < 80, history of brain trauma or neurological disease, MRI contra-indications and substance use (including cocaine, heroin, synthetic drugs, or alcohol) meeting diagnostic criteria for abuse/dependence. Subjects were stratified into three exposure groups: 1) early stimulant treatment (EST) group: subjects treated with stimulants for at least four months before the age of 16 years 2) a late stimulant treatment (LST) group: subjects treated with stimulants for at least four months after the age of 23 years and 3) a stimulant treatment-naive (STN) group: containing subjects with no history of stimulant medication. Four months of treatment was chosen in line with effects found on CBF in MPH-treated children in a prospective study (Schrantee et al., 2016). The age limit of the EST group was chosen because this coincides with the end of puberty in boys, and because preclinical studies have reported effects of treatment in early adolescence (Bottelier et al., 2014). Self-reported prescription history was verified with available prescription data from pharmacies and treating physicians. The study was carried out in accordance with the Declaration of Helsinki (2012) and was approved by the Medical Ethical Committee. All subjects gave written informed consent.

Procedures
Subjects underwent two phMRI scan sessions, in which we assessed the CBF response to an acute challenge to MPH, as a proxy for DA functionality (Chen et al., 1997; Schrantee and Reneman, 2014). The first phMRI scan session was immediately followed by an oral challenge of MPH of 0.5 mg/kg MPH (with a maximum dose of 40mg). The second scan session was conducted after 90 minutes, which is the time after which peak plasma levels of MPH are reached (Swanson and Volkow, 2003). In both sessions an arterial spin labeling (ASL) scan was obtained to assess CBF in the fronto-striatal circuitry. All subjects were medication-free for at least a week before the scan, to prevent acute effects of stimulant treatment on CBF. In addition, subjects were instructed to abstain from drugs of abuse at least one week before the study, alcohol at least 24 hours before the study and not to use caffeine or tobacco on the study day.

MRI acquisition and image analysis
Data were acquired using a 3.0T Philips MR Scanner (Philips Medical Systems, Best, The Netherlands). First, an anatomical 3D-FFE T1-weighted scan was obtained with the following scan parameters: TR/TE=9.8/4.6; FOV=256x256x120; voxel size=0.875x0.875x1.2mm. CBF images were acquired using a pseudo continuous arterial spin labeling (pCASL) sequence with the following parameters: TR/TE=4000/14ms; post-labeling delay=1650ms; label duration=1525ms; FOV=240x240x119; 75 dynamics; voxel size=3x3x7mm, GE-EPI, SENSE=2.5, no background suppression, scan time=10 minutes. Heart rate (HR) was monitored using a peripheral pulse unit.

Data were processed using the Iris pipeline for CBF quantification and multi-atlas region segmentation (Bron et al., 2014). All image registrations were performed using Elastix registration software (Klein et al., 2010). For the ASL data, motion estimation was performed using rigid registration with a group-wise method that uses a similarity metric based on principal component analysis. Then, outlier rejection was performed to correct for sudden head movements. Outlier rejection was based on the Msk images, the subtractions of all pairs of control (M) and label images (M). For each pair of Msk images, we computed the sum of squared differences (SSD) which is the sum of all squared voxel-wise differences between the two images. As such, for each of the 75 time points, we obtained 74 SSD values.
over which we computed the median and SD. To obtain a more robust estimate of the SD, we computed this based on only the SSD values that were lower than the median. If more than 50% of the SSD values were larger than the median + (3*SD) this timepoint was considered an outlier. Subsequently, motion correction was performed on the remaining timepoints, and the resulting motion-compensated MRI images were averaged to obtain a perfusion-weighted image (ΔM). Motion was quantified as the mean framewise displacement. Quantification of CBF was performed using the single-compartment model (Buxton et al., 1998), which is the recommended approach for pCASL (Alsop et al., 2015). The following parameters were used: labeling efficiency GM = 0.85, T1GM = 1.6ms, blood-brain partition coefficient GM = 0.95mL/g. The average of M0 images was used as a proton-density normalization image (M0) for the CBF quantification. Differences in post-labeling delays between slices (due to the 2D read-out) were accounted for. CBF was quantified in GM only, with a 3D method for partial volume correction based on local linear regression using the tissue probability maps (Asllani et al., 2008; Oliver et al., 2012). For each subject, probabilistic GM segmentations based on the T1-weighted scan (SPM8, Statistical Parametric Mapping, UCL, London, UK) were rigidly registered to the ΔM images by maximizing mutual information. For further analysis, CBF maps were transformed to the space of the T1-weighted scan. An example of a representative perfusion-weighted image can be observed in Figure 1.

Fig. 1 | Axial view of a perfusion-weighted ASL scan from a representative subject.

For each participant, we defined three regions of interest (ROIs) using a multi-atlas approach, registering 30 labeled T1-weighted images (Gousias et al., 2008; Hammers et al., 2003) with the participants’ T1-weighted images. The labels of the 30 atlas images were fused using a majority voting algorithm to obtain a final ROI labeling (Heckemann et al., 2006) time and expertise requirements make this approach impractical. To achieve automation, an individual segmentation can be propagated to another individual using an anatomical correspondence estimate relating the atlas image to the target image. The accuracy of the resulting target labeling has been limited but can potentially be improved by combining multiple segmentations using decision fusion. We studied segmentation propagation and decision fusion on 30 normal brain MR images, which had been manually segmented into 67 structures. Correspondence estimates were established by nonrigid registration using free-form deformations. Both direct label propagation and an indirect approach were tested. Individual propagations showed an average similarity index (SI). For three pre-defined ROIs, comprising the striatum, thalamus and anterior cingulate cortex (ACC), CBF mean values were extracted (Figure 2). The striatum was selected because it is rich in DAT (the primary target of action of MPH) and the thalamus and prefrontal cortex were chosen because the animal literature demonstrated largest effects of early MPH treatment using phMRI in these two important neuronal projections from the striatum (Andersen et al., 2008).

Rating scales and questionnaires
Premorbid intellectual function was estimated using the National Adult Reading test (Dutch version). Current ADHD symptom severity was assessed using the ADHD-Rating Scale (ADHD-RS) (Kooij et al., 2008). Current mood and anxiety symptoms were evaluated using the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI), respectively. In addition, lifetime recreational drug use was assessed using a drug history questionnaire.

Statistical analyses
SPSS version 22.0 (IBM, Armonk, NY, USA) was used for all statistical testing. Data were assessed for normality. To assess the effect of age-of-first-exposure on CBF response to MPH we used a repeated-measures analysis of variance (ANOVA) for each ROI separately with group (EST, LST or STN) as a between-subjects factor and MPH-challenge (pre- or post-MPH) as a within-subjects factor, with subsequent post-hoc Sidak’s tests. Baseline differences in CBF were assessed using an univariate ANOVA. To further examine this age-dependency, we correlated age-of-first-exposure with CBF response and mood symptoms in the LST and EST group. In additional exploratory analyses, we assessed the effect of treatment duration and time-since-last-use. Differences in recreational drug use were assessed for cannabis, 3,4-methylenedioxy-methamphetamine (MDMA), cocaine and amphetamine using a χ2 test. To this end, subjects were divided in users (for cannabis >1x per week, for other drugs >10x lifetime) and non-users.
Results

Patient characteristics
Age and estimated IQ differed statistically significantly between the three groups of ADHD subjects, with the EST group being slightly younger and having a lower IQ than the STN and LST group (Table 1). In addition, current symptom severity was significantly higher in the STN group compared to the EST and LST group. Inherent to the design of the study, the EST group started medication treatment at a younger age and was treated for a much longer period of time (94.9 vs 11.8 months) than the LST group.

Baseline CBF and MPH-induced changes in CBF
One patient did not complete the second ASL scan and was removed from the analysis. Motion during the MRI scan did not differ between the three groups (baseline: F(2,78)=1.13, p=0.33; change: F(2,78)=0.75 p=0.48). The MPH challenge increased HR (p<0.01), but this effect did not differ between the three groups (F(2,75)=1.51 p=0.23).

ANOVA revealed a significant effect of group on baseline CBF in the ACC (F(2,78)=3.62, p=0.03), but not in the striatum (F(2,78)=2.07, p=0.13) or thalamus (F(2,78)=1.51, p=0.23). Post-hoc tests showed that the STN group had a higher ACC CBF than the EST group (p=0.03), but not compared to the LST group (p=0.36) (Figure 2). The acute MPH challenge reduced CBF (ΔCBF) in the striatum (F(80,1)=6.69, p<0.01) and ACC (F(80,1)=20.28, p<0.01), but not the thalamus (F(80,1)=0.12, p=0.73) (Figure 2). However, no significant interaction effects were observed between group and ΔCBF in the ROIs studied, nor did we find a significant correlation between ΔCBF and age-of-first-exposure (r=0.2 for all ROIs), treatment duration (r=0.1 for all ROIs) or time-since-last-treatment (r=0.1 for all ROIs). None of the results were affected by adding age, ADHD symptom severity or baseline CBF values as covariates to the model.

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<th>Table 1: Participant characteristics</th>
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<th>LST</th>
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<tr>
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<td>Age first stimulant treatment (years)</td>
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<td>8.4</td>
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<td>Treatment duration (months)</td>
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<td>Time since last treatment (years)</td>
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<td>BAI</td>
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<td>19%</td>
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<td>Amphetamine (% of subjects &gt; cutoff)</td>
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<td>26%</td>
<td>19%</td>
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</table>

* p <0.05 
1 more than once a week 
2 more than 10 x lifetime
Chapter 7

Long-term effects of stimulant exposure on cerebral blood flow response to methylphenidate and behavior in attention-deficit hyperactivity disorder

Depression, anxiety and recreational drug use

We found a significant overall effect of group on depressive symptoms, (F(75,2)=4.57, p<0.01), but not on symptoms of anxiety (F(76,2)=1.38, p=0.26). Post hoc analyses revealed higher BDI scores in the EST than the LST group (p<0.01). The EST individuals indicated using less cannabis, MDMA, cocaine as well as amphetamine than the LST and STN individuals, although this was only statistically significant for cannabis and cocaine (Table 1).

Discussion

Here we investigated if age of first stimulant exposure modulates the effect CBF response to MPH, mood and anxiety symptoms as well as recreational drug use. We did not find a different CBF response to MPH between groups, but the EST group showed lower baseline ACC CBF than the STN group, which could be a result of early-induced changes by stimulants to the developing DA system. In line with this, and as hypothesized, the EST group showed higher depression- but not anxiety-levels and reported less recreational drug use.

Long-term effects of stimulants on CBF baseline

To our knowledge, this is the first study investigating the long-term effects of age-of-first-exposure on the DA system in humans. The DA system is in development all throughout childhood and adolescence. For example, cortical D₂/D₃ expression peaks in early childhood, followed by a sharp decline during adolescence (Seeman et al., 1987), whereas dopamine transporter (DAT) density peaks mid-adolescence while slowly declining thereafter (Meng et al., 1999). In non-human primates MPH treatment during adolescence resulted in less decline of striatal D₂/D₃ receptor binding following one year of MPH treatment compared to the placebo group, suggesting halted development of these receptors (Gill et al., 2012) few studies have addressed their long-term effects on the developing brain or susceptibility to drug use in adolescence. Here, we determined the effects of chronic methylphenidate (MPH). In line with that study, lower CBF in the ACC in the EST group compared to the STN group, as we observed here, might reflect higher density of D₂/D₃ receptors induced by early treatment, because experimental phMRI studies in rats have shown that negative rCBV responses reflect agonism of D₂/D₃ receptors, whereas positive rCBV changes are associated with agonism of D₁/D₅ receptors (Chen 2010). However, this interpretation is speculative; Gill et al. (2012) found changes in the striatum, whereas we reported changes in the ACC. Future studies will need to study the changes in DA function and connectivity within all areas of the fronto-thalamo-striatal loops in more detail. Moreover, we did not find significant differences in ACC CBF between the EST and LST group and therefore

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**Figuur 2.** Regions of interest used for analyses.

A

B) change in CBF (ml/100g/min) following acute MPH challenge (oral, 0.5 mg/kg) in the striatum, thalamus and anterior cingulate cortex (ACC). There was a main effect of challenge in the striatum and ACC, but not the thalamus. We found no group*time interaction in any of the ROIs. Mean and standard error of the mean are displayed. C) scatter dot plot of CBF baseline values (ml/100g/min) for all subjects. The EST group demonstrated significantly lower CBF than the STN group in the ACC only *p<0.05. red = STN; green = EST; blue=LST

Blue = striatum; green = anterior cingulate cortex; yellow = thalamus. B) change in CBF (ml/100g/min) following acute MPH challenge (oral, 0.5 mg/kg) in the striatum, thalamus and anterior cingulate cortex (ACC). There was a main effect of challenge in the striatum and ACC, but not the thalamus. We found no group*time interaction in any of the ROIs. Mean and standard error of the mean are displayed. C) scatter dot plot of CBF baseline values (ml/100g/min) for all subjects. The EST group demonstrated significantly lower CBF than the STN group in the ACC only *p<0.05. red = STN; green = EST; blue=LST
caution is needed in interpreting the age-dependency of this effect.

In accordance with predictions from the neuronal imprinting theory, we did not find differences in baseline CBF between the LST and STN group. This is in contrast with a study in adult ADHD patients, showing increased DAT following one year of stimulant medication (Wang et al., 2013). Increased DAT availability could result in lower CBF because of less availability of extracellular DA, because less DA release results in relatively more $D_1/D_2$ receptor stimulation. However, they measured DAT 24 hours after the last clinical dose of MPH (Wang et al., 2013), whereas we conducted our phMRI scans at least one week after treatment cessation. Although 24 hours should ensure dissipation of acute MPH effects, transient up-regulation of DAT cannot be excluded in that study.

Modulation by age of stimulant exposure: MPH challenge

Our findings of reductions in CBF in the fronto-striatal circuitry after an acute challenge with MPH are in agreement with studies comparing on/off medication periods in adult ADHD patients (O’Gorman et al., 2008; Schweitzer et al., 2002). Studies in healthy volunteers report more mixed results. In adult volunteers, MPH induced increased CBF in striatum and thalamus in an ASL study (Marquand et al., 2012), but increased CBF in the anterior cingulate, supplementary motor areas and temporal poles in a $^{15}$O PET study (Udo de Haes et al., 2007). Decreased CBF was reported in lateral frontal, rostral cingulate and sensorimotor areas, amygdala, parahippocampal gyrus and in multiple regions of the occipital and temporal cortices for the ASL study, but in superior temporal gyr, right medial frontal gyrus, and right inferior parietal cortex for the PET study. One reason for the discrepancy between studies in volunteers and ADHD patients might be altered DA release in ADHD patients (Cherkasova et al., 2014; Volkow et al., 2007). We found that in EST individuals with a mean treatment duration of eight years, CBF response to MPH was similar to that of LST and STN subjects. This finding was in contrast to our hypothesis, as preclinical studies have suggested that juvenile administration will result in DA changes that will last and possibly even expand as the brain matures (Moll et al., 2001). This hypothesis was supported by our RCT showing that four months of MPH treatment induced increased striatal and thalamic CBF response to a MPH challenge in stimulant-treatment naive children, but not adults with ADHD (Schrantee et al., 2016). The current results suggest that at least a part of this effect on the developing DA system is transient or compensated. Interestingly, we did not find a difference in CBF response to MPH between LST and STN subjects, suggesting an absence of tolerance to MPH following long-term treatment in adulthood. Interestingly, Volkow et al. (2012), found reduced striatal, but also no increased extra-striatal DA release to a DA challenge after 12-month MPH treatment in adults ADHD patients. Also in recreational dexamphetamine (dAMPH) users we observed a blunted striatal CBF response to dAMPH (Schrantee et al., 2015). However, recreational use of stimulants is usually associated with high dose binges, whereas much lower doses are used for stimulant treatment of ADHD.

Long term modulating effect of age of stimulant exposure on behavior

Although the short-term benefits of stimulants on ADHD symptoms are well-established, studies on long-term efficacy are inconclusive (van de Loo-Neus et al., 2011). Here, we observed lower ADHD symptom severity in the EST and LST group compared to the STN group, whereas the stimulant-treated groups did not differ, despite the long time since last exposure in the EST group. Our findings not only suggest that MPH is useful in reducing symptoms in adult ADHD (the LST group), but also suggests that the effects of treatment in the EST group are long-lasting.

Animal studies have suggested an increased risk for depressive symptoms following MPH exposure early in life (Bolaños et al., 2008; Carlezon William A et al., 2003), but results from human studies are equivocal (Biederman et al., 2009; Mannuzza et al., 2008; Brooke S.G. Molina et al., 2009; Wilens et al., 2003). One limitation of our study is that, as a result of the study design, children with both ADHD and depressive symptoms could have been more likely to receive treatment at young age and thus end up in our EST group. In the current study, we observed that EST subjects have more depressive symptoms (~mild-moderate depression) than the other groups. This is in line with a transient increased anxiety and depression in the MTA trial (Brooke S.G. Molina et al., 2009), but in contrast with studies reporting protective effects of stimulant use on symptoms of anxiety and depression (Biederman et al., 2009; Daviss et al., 2008; Lee et al., 2015).

In the current study, we could not assess the effect of treatment on SUDs, as this was an exclusion criterion; however a large number of subjects in this sample were recreational drug users. Interestingly, we found lower drug use in the EST group compared to the STN and LST group, especially regarding MDMA and cocaine use. This is in line with literature showing that whereas adult ADHD is associated with a high rate of substance abuse (Dalsgaard et al., 2014), childhood treatment does not increase (Humphreys et al., 2013; Molina et al., 2013), and may even decrease this risk (Spencer et al., 2006). Our findings are also consistent with the literature on self-medication in ADHD (Wilens, 2004), suggesting that ADHD patients not taking stimulants are more likely to use drugs to alleviate behavioral symptoms. An alternative explanation, and not necessarily incompatible, is that lower vulnerability for SUDs may be due to changes in the dopaminergic reward system following early stimulant treatment.
Methodological considerations
The cohort we studied was heterogeneous in terms of symptom onset, treatment duration, symptom severity and probably also the course of the disorder. Furthermore, the interaction between development, age-of-first stimulant treatment, and duration of treatment is likely not linear, which needs to be taken into account for future studies. As a long-term RCT would not be ethical, we have to rely on pre-clinical studies and retrospective cross-sectional studies to inform us about possible long-term effects of stimulants on the DA system. Currently, many imaging initiatives are established to share clinical and imaging data, which could facilitate replication of small hypothesis-driven studies, such as this one, in larger samples.

ASL-phMRI is an indirect method to measure neurotransmitter function. Previous studies have shown that phMRI closely parallels DA function (Chen et al., 1997). Nevertheless, as we measure a vascular response to neuronal function, it is possible that the CBF changes are caused by alterations in other neurotransmitter systems, such as the noradrenalin system, or mediated in part by cardiovascular effects. However, even though HR increased following MPH, we did not find differences between the groups and therefore cardiovascular effects are unlikely to explain our results. We used a fixed-order, open-label design for two reasons; first, CBF varies considerably across days and therefore a baseline scan followed by an MPH scan was preferred. Second participants can easily discriminate between MPH and placebo and therefore blinding was not possible in this study. Poly-drug use is a limitation in this study and because of the high association between ADHD and drug (ab)use it is difficult to correct for or quantify the possible effect on our results. In addition, we cannot exclude that increased depressive symptoms in the EST group are a pre-existing vulnerability, instead of consequence of early stimulant treatment.

Conclusion
Our results suggest long-lasting effects of early stimulant treatment on baseline CBF, ADHD symptoms, mood as well as recreational drug use. Nevertheless, we did not find lasting effects of stimulant exposure on the phMRI response, suggesting that at least some effects on the developing DA system are transient or compensated for. It is likely that the neurochemical imprinting effect of stimulant treatment on the DA system is a dynamic process. Our data thus stress the need for prospective follow-up studies including assessment at multiple ages to completely characterize the long-term effects of ADHD medication on the human brain.

References
Chapter 7

Function in Treatment-Naive Adults

2014. Amphetamine-Induced Dopamine Release and Neurocognitive Function in Treatment-Naive Adults

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CHAPTER 8

A power analysis for future clinical trials on the potential adverse effects of SSRIIs on amygdala reactivity

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Anouk Schrantee — Guido van Wingen
Henricus G. Ruhé — Michiel B. de Ruiter
Liesbeth Reneman

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Abstract

Treatment of adolescents with antidepressants may induce an increased risk for suicidality in this population. The activity of the amygdala during processing of emotional faces with functional Magnetic Resonance Imaging (fMRI) is a well-known measure of emotional dysregulation. Based upon data of our prematurely ended randomized clinical trial with fluoxetine (NTR2111) in anxious and or depressed girls (12-14 years of age) we calculated that with the found effect size of $r=0.66$, compared to placebo, only 8 subjects are needed to demonstrate increased amygdala activity following 16 weeks of treatment with fluoxetine.

Introduction

The safety of antidepressants to children and adolescents is a subject of concern, since in 2004 the Food and Drug Administration issued a black box warning regarding the use of antidepressants in children and adolescents (Hammad, 2004). Indeed, the neurobiological effects of antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), on the developing neuronal circuitry of the human brain are largely unknown, as most imaging studies have been conducted in adults so far. However, SSRI studies in periadolescent animals, including non-human primates, show long-lasting effects on serotonergic outcome measures, such as an increase in the density of the serotonin (5-HT) transporters (Bouet et al., 2012, Wegerer et al., 1999, Shrestha et al., 2014) as well as anxious and depressive like behavior (Ansorge et al., 2004). In line with this, in adolescents SSRI prescription is associated with transient, age-delimited effects on suicidal ideation. To investigate the effects of SSRIs on brain regions and circuits critical to anxiety and depression in children, we designed a 16 week randomized clinical trial (ePOD-SSRI) with fluoxetine in medication naive girls suffering from major depressive disorder (MDD) and/or anxiety disorder (AD). We measured the activity of the amygdala during processing of emotional faces with functional Magnetic Resonance Imaging (fMRI) as it is a well-known measure of emotional dysregulation, i.e., depression. For instance, in adults fMRI has shown increased amygdala activity in patients suffering from MDD, which decreases after successful treatment with paroxetine (Ruhé et al., 2014). Only one previous study has been conducted with this technique in adolescents, and reported normalization of amygdala activity following 8 week treatment with fluoxetine (Tao et al., 2012). However, subjects were scanned during treatment, making it impossible to distinguish between effects of acute fluoxetine and the lasting effects of prolonged fluoxetine exposure on brain development. Here we report our preliminary findings on 7 patients who were included in the ePOD-SSRI trial with a wash out period of 3 weeks. Unfortunately, because of insufficient study inclusion (dental braces, insufficient study participation of primary care facilities) we had to end our clinical trial prematurely. Based on the findings of early SSRI treatment in animals discussed above, we hypothesized to find an increased responsiveness of the amygdala to fearful faces following chronic treatment with fluoxetine, when compared to placebo, reflecting hyperactivity of the affective neurocircuitry. The purpose of this brief report is to provide a power analysis for future clinical trials on the potential adverse effects of SSRIs on human brain development.
Chapter 8

Materials and Methods

ePOD-SSRI was a 16-week multicenter double blind, placebo-controlled trial (NTR2111) with a blinded end-point evaluation with fluoxetine in antidepressant naïve subjects suffering from MDD, and described in greater detail elsewhere (Bottelier et al., 201). It was reviewed and approved by the Central Committee on Human Research in the Netherlands (CCMO) in the Hague. Written informed consent was obtained from patients and legal guardians before randomization. The effect of fluoxetine on emotional processing was assessed using fMRI in 7 girls suffering from MDD, before random assignment to either placebo or active treatment with fluoxetine (using a permuted block randomization scheme 1:1), and again 3 weeks after trial end (in week 19). Inclusion criteria were: female outpatients aged 12-14 years of age with a history of at least 2 weeks of moderate or severe MDD and or anxiety disorder (AD), as defined in the DSM-IV and as determined by a structured interview (Diagnostic Interview Schedule for Children fourth edition, DISC-IV) in need of pharmacotherapy with a score of > 3 on the Clinical Global Impressions severity subscale (CGI-S), and a total score of > 45 on the Depression Rating scale-Revised (CDRS-R). Exclusion criteria were co-morbid axis I psychiatric disorders requiring pharmacological treatment; IQ < 80; current risk of suicide attempt and previous antidepressant use. According to standard criteria response to treatment was defined as a score of 1 or 2 on the CGI-S improvement item (indicating “very much improved” or “much improved”) at trial end (week 19). Partial response was defined as a score of 3 and 4, and worsening of depression as a CGI-S improvement item score of 5, 6, or 7. The fMRI paradigm we used was a modified version of the event-related implicit emotion processing task (Demescu et al., 2011) in which subjects viewed negative emotional faces and neutral stimuli (ellipses consisting of scrambled faces) alternated in a block design. fMRI data were acquired on a 3T Philips scanner (Best, the Netherlands) fitted with an 8-channel head-coil using the following scan parameters: TR/TE = 2300/30ms; GE-EPI readout; voxel size = 2.3x2.3x3mm; flip angle 80°. Data were preprocessed using FSL and a region of interest (ROI) analysis of amygdala activation was conducted, as the amygdala is an important relay for emotional processing between visual systems and modulatory responses. As there were no significant differences between the left and right amygdala at baseline and post-treatment, bilateral amygdala was used in subsequent analyses. One subject was excluded due to excessive motion. Due to the small sample size non-parametric statistical tests were used.

Table 1

<table>
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<th>CDRS* children Baseline</th>
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</table>

* adjusted score

MDD = Major Depressive Disorder; SoP = Social Phobia; GAD = Generalized Anxiety Disorder; PD = Panic Disorder; OCD = Obsessive Compulsive Disorder; SpP = Specific Phobia
Chapter 8

A power analysis for future clinical trials on the potential adverse effects of SSRIs on amygdala reactivity

Results

Prior to randomization, the fluoxetine and placebo group did not differ on demographics or clinical characteristics (table 1).

Baseline amygdala activation also did not differ between the two groups. As shown in figure 1, fluoxetine treatment increased amygdala activation to threatening faces compared to baseline (mean=11.67 sd=14.47 p=0.14, +40.94%), whereas placebo treatment resulted in equal or less amygdala activation (mean=-5.88 sd=8.37 p=0.11, -36.36%). The interaction term between treatment x time showed a trend (U=1.0, p=0.11) and an effect size of r = 0.66. A power calculation indicated that in future clinical trials only 8 subjects are needed, 4 in each arm, to detect a significant effect (with 80% power and α=0.05) of fluoxetine treatment on amygdala activation. At trial end, all girls in the placebo group had shown a ‘partial response’ or ‘much improved’ (CGI-S improvement score <4), whereas in the 3 girls that had received active treatment with fluoxetine a mixed response was observed (CGI –S improvement was 2, 4 or 6).

Figure 1

Amygdala activation before and after randomization for 16-week treatment with placebo or fluoxetine in girls suffering from severe MDD and/or AD. Y axis shows mean BOLD response in bilateral amygdala (anatomical ROI). Fluoxetine treatment increases amygdala activation to threatening faces compared to baseline (on average +40.94%), whereas placebo treatment resulted in equal or less amygdala activation (on average -36.36%).

Discussion

In this first RCT on the effects of SSRIs on brain development, we found a trend of increased amygdala activity for fluoxetine-, vs placebo treated girls suffering from MDD. As the FMRI paradigm we used is strongly associated with emotional dysregulation, our preliminary findings suggest that treatment with fluoxetine may aggravate emotional dysregulation in these young/adolescent girls with MDD.

Our findings are in concordance with preclinical studies measuring the effect of fluoxetine on the developing brain. In fact, in juvenile rats chronically treated with fluoxetine we also observed an increased brain response to an acute challenge with fluoxetine, whereas this response was decreased in adult treated rats (Klomp et al., 2012). An increase in 5-HT transporter density following SSRI treatment in early, but not in adult, rats and non-human primates, may underlie this increased reactivity of the juvenile brain following antidepressant treatment. Indeed, evidence is gradually emerging that the long-term effects of drug exposure are delayed and come to expression once the vulnerable system reaches maturation (i.e., typically during adulthood). This phenomenon is known as neuronal imprinting and occurs when the effects of drug exposure outlast the drug itself (Andersen and Navalta, 2011). As the prefrontal cortex is still developing in adolescents, we hypothesize that children and adolescents lack the ‘regulatory’ prefrontal decreasing activity of antidepressant action to negative stimuli (Ma, 2015) thereby inducing different effects in children and adolescents vs. adults.

Our results are in contrast with a previous clinical study in depressed girls which found a decrease in amygdala activity after 8 weeks of open label treatment with fluoxetine (Tao et al., 2012). However, subjects in that study were scanned during treatment, thereby making it difficult to disentangle acute and long-term effects and together with the lack of a placebo group this likely explains the discrepancy between this study and ours.

In sum, in view of the trend we observed on adverse effects of fluoxetine treatment on amygdala activity in female adolescents, we concluded that: 1) it is important to replicate this highly relevant finding in a larger sample, as this may underlie the 2-fold increased risk for suicidal behavior in children and adolescents treated with antidepressants, 2) we have shown that such a study does not need to be very large, as the effect size of 0.66 is large.
References


Summary, general discussion and conclusions
9.1 Summary

Attention deficit hyperactivity disorder (ADHD) is the most commonly diagnosed neurodevelopmental disorder, affecting 8-12% of children worldwide (Biederman and Faraone, 2004; Thomas et al., 2015) and 2.5% of adults (Simon et al., 2009). In addition to impairment caused by core symptoms of inattention, and/or hyperactivity and impulsivity, individuals with ADHD often have comorbid disorders. Emotional dysregulation in children with attention deficit hyperactivity disorder (ADHD) is defined as being easily angered and easily annoyed, which may arise from problems orienting toward, recognizing and/or allocating attention to emotional stimuli and from a dysfunctional striato-amygdala-medio prefrontal cortical network. For a long time, clinicians and parents have noted that emotional dysregulation is an important feature of ADHD leading to serious impairment. A substantial proportion of children with ADHD manifest difficulties regulating negative affect. Emotion dysregulation in ADHD may even be considered to be a phenotype for developing depression later in life (Ambrosini et al., 2013). In children and adults, rates of mood and anxiety disorders are beyond those that would be expected by chance alone (Kessler et al., 2006; Meinzer et al., 2014). However, until recently, not much attention has been given to emotional problems in children and adults with ADHD. This is remarkable given that these emotional problems influence the course, treatment response and outcome of ADHD (Biederman et al., 1998; March et al., 2000).

Conflicting findings on the role of pharmacological treatment with dopaminergic agents such as methylphenidate (MPH) on the prevalence of emotional problems in ADHD, stress the need for a better understanding of the mechanisms underlying emotion regulation in ADHD and the effect of MPH thereupon. The amygdala is a brain nucleus that is crucial for emotional processing and is most active during the perception of emotional faces. Amygdala function measured with functional Magnetic Resonance Imaging (fMRI) is a potential biomarker for emotional processing in patients with anxiety disorders and in patients with depression (Lau et al., 2010; Tao et al., 2012).

In this dissertation, we aimed to explore the occurrence of affective problems in ADHD, disentangled the effects of MPH on affective problems in ADHD, and considered age effects on these neurobiological substrates of treatment with MPH.

To this purpose, we determined in Chapter 2 the relationship between ADHD diagnosis, (prior) stimulant use, age, and depressive and anxiety symptoms in boys between the ages of 10 to 17 years. In two cross-sectional studies, comorbidity of anxiety and depression in ADHD and the effect of stimulants on the development of these comorbid disorders was addressed. More depressive symptoms were found in boys and adolescents with ADHD compared to typical developing children. Medication history did not predict the number of depressive symptoms, nor the number of anxiety symptoms although there was no information about the duration of treatment available.

To explore the role of the DA in emotional processing and more specifically the modulation of the amygdala in subjects with documented DA dysfunction, in chapter 3 we explored the effects of the DA-ergic agent MPH on amygdala function in eight male recreational users of dAMPH and eight healthy controls. As hypothesized, we found a higher amygdala reactivity to fearful faces in the dAMPH users compared to healthy controls at baseline, which correlated positively to extent of dAMPH use. These findings of abnormal baseline amygdala activation in recreational dAMPH users most likely reflect an abnormal DA transmission in this brain region and lend further support to our hypothesis that the amygdala seems to be under modulatory influence of DA, and that DA dysfunction underlies abnormal emotion processing. Our trend significant finding that MPH induced increased amygdala reactivity after the challenge with MPH in control subjects (compared to a blunted response in dAMPH users), further triggered us to investigate the role of MPH role in modulating emotional processing in patients with ADHD treated with this drug. We found that prior DA-ergic status was important for the fMRI response to MPH in the amygdala (presumably due to de-sensitization), hence we included medication naïve ADHD patients in chapter 4. We included both children as adults in order to further investigate the age-dependency of the effect.

In chapter 4 we extrapolated our findings of chapter 3 to patients with ADHD, and investigated the effect of an acute challenge of MPH on amygdala reactivity in medication naïve boys and adult patients with ADHD. In line with the literature, and previous studies in recreational users of dAMPH with documented DA dysfunction (chapter 3), we found that also in ADHD fear processing is associated with amygdala hyperactivation. We also found that both fear processing as well as the acute effects of MPH on emotional processing are modulated by age. In adults, MPH reduced amygdala reactivity towards control levels, whereas in children MPH further reduced normal levels of right amygdala reactivity. Although children and adult patients scored significantly higher on clinical rating scales measuring depressive and anxiety symptoms, these measures did not correlate with amygdala reactivity, likely because they only assess a prolonged state of emotional lability. Other than a direct effect of MPH on the amygdala, our age-dependent findings may underlie age-dependent top down control of the amygdala, as DA-ergic maturation of the frontal cortex is still ongoing in childhood whereas it is already matured in the amygdala.

Although this study suggests a positive effect of MPH on emotional processing in both children and adults with ADHD (i.e. a reduction in amygdala reactivity), these findings are limited to an acute challenge with MPH. In chapter 6...
we therefore report the age-dependent effects of chronic treatment with MPH in a clinical trial, which is described in greater detail in chapter 5, which describes the objectives and methods of the effects of Psychotropic drugs On the Developing brain (ePOD) project including the ePOD-MPH trial.

Thus, to address the effects of chronic treatment with MPH in ADHD patients on emotional functioning, the effects of 4 months treatment were investigated in the ePOD-MPH RCT in chapter 6. Based upon the hypothesis that was generated in chapter 4 (more normalizing effects of MPH in children in reducing amygdala hyper-reactivity due to more intact and salient architecture in the top down control network in adults), we added functional connectivity of the amygdala with other brain regions to our original outcome measures (apart from amygdala reactivity and clinical symptoms). One week after 4 months of treatment, MPH still positively influenced emotional lability, but negatively affected right amygdala reactivity in children. Although in adults MPH had no effect over placebo on behavioral measures nor amygdala reactivity, it induced lasting cortical-amygdala hyper-connectivity. Furthermore, although symptoms of anxiety and depression did improve during the trial, they did not differ between both medication groups in children nor adults. From this we can conclude that a) in line with the literature, MPH lastingly reduced emotional dysregulation in children, but not adults; b) that this is unrelated to lasting attenuation of anomalous amygdalar-cortical connections, as we observed no lasting effects in children, and hyper-connectivity in adults. Rather, these age-dependent findings underlie persistent increases in DA activity in the children treated MPH, as reported elsewhere (Schrantee et al., 2016). Taken together, the two studies suggest that our findings are mediated in part by developmental changes in the ontogeny of the DA system (i.e., expression of cortical D3 receptors, which in rats is high during early adolescence and then wanes until becoming absent in adulthood). This is also in line with our observations on the importance of DA in emotional processing (chapters 3 and 4).

Our findings on chronic MPH treatment contrast those obtained following a single acute MPH administration, presumably underlying sensitization effects in adults, and/or ‘neurochemical imprinting’ effects in children: whereas children in chapter 4 demonstrated a reduction in right amygdala reactivity, we found that chronic treatment induced opposite effects, and increased right amygdala reactivity. Similarly, in adults a single dose of MPH reduced amygdala hyper-reactivity, whereas chronic treatment did not result in any lasting effects (blunted response, similar to that observed in dAMPH users in chapter 3). Taken together with our findings of increased cortical-amygdala connectivity in adults, but not children, we provide evidence that the effects of chronic treatment with MPH affect amygdala reactivity, amygdala connectivity and emotion dysregulation age-dependently, i.e. MPH affects development of brain regions involved in emotional processing. Because amygdala hyper-reactivity and connectivity has been shown to precede emotional problems later in life, our findings urge for longer follow up studies to investigate the clinical significance of our neuroimaging findings in children and adults. Meanwhile, the lasting positive effects of MPH on emotion dysregulation in children should comfort parents and clinicians in prescribing MPH for ADHD in children, at least on the short term.

In chapter 7 we explore the long-term effects of MPH treatment, and found that adult patients treated with ADHD medications before the age of 16 years had higher depression rates (moderate to mild depression) compared to late exposed patients (start of pharmacological treatment after the age of 23), as well as less use of cannabis and cocaine when compared to late exposed individuals and drug naïve individuals. Interestingly, ADHD symptom severity was higher in the unexposed individuals compared to either medicated group. In line with extensive pre-clinical data, these data suggest that pharmacological treatment with ADHD medications have a positive effect on the long-term, which is in contrast to current views that there is limited and inconsistent evidence for long-term advantage of medication treatment beyond symptom control (Van de Loo-Neus et al., 2011). Our findings also indicate that early treatment does not increase the risk for developing a drug abuse disorder, but rather protects for such an effect, as previously suggested in the literature. Finally, our findings suggest that on the long term early-, and not late exposure to ADHD medications induce depressive symptoms. Together with the results from chapter 6, we demonstrate the potential of fMRI as a biomarker to assess amygdala reactivity in ADHD. Identification of children at risk of developing depressive symptoms later in life can be done using a combination of parameters; amygdala reactivity from fMRI, eicosapentaenoic acid/arachnoid acid ratio (Mocking et al., 2017), connectivity parameters, and machine learning techniques (Jentsch et al., 2015). Its role in clinical treatment of patients with ADHD should therefore be further explored.

While the neurobiological effects of antidepressants on the developing brain are largely unknown and most of the imaging studies included adult patients, we conducted a power analysis in chapter 8 for future clinical trials on the adverse effects of SSRI’s. We chose fluoxetine because the Food and Drug Administration issued a black box warning in 2004 regarding the use of antidepressants in children and adolescents. Since then, its use in children has been an issue of strong public debate, also here in the Netherlands (Dehue, 2014). And not without reason, as we found that fluoxetine treatment increased amygdala reactivity, with opposite effects in the placebo condition. Our calculations suggest that in future studies ‘only’ 8 patients are needed to demonstrate such an effect of fluoxetine on emotional processing.
In conclusion, in this dissertation the main findings were:
- Anxiety and depression commonly co-occur with ADHD.
- DA plays an important role in emotional processing.
- During treatment, there is no added value of MPH over placebo on positive effects on anxiety and depression scores in children and adults.
- Only in children there is a lasting positive effect of MPH on emotion dysregulation, at least for 1 week after treatment end.
- MPH induces increased amygdala reactivity in children (but not adults), a potential biomarker for depressive symptoms later in life.
- The age-dependent effects of MPH on amygdala reactivity likely underlie age-dependent effects of MPH on the DA system.
- On the long-term, ADHD medications seem to induce depression in children treated with the drug years ago.
- In combination with other parameters and techniques like machine learning, amygdala reactivity may be a useful biomarker during treatment to select children at risk of developing depression later in life.
- The functional significance of the MPH-induced hyper-connectivity in adults still needs to be established.
- ADHD medications have long-term positive effects on ADHD symptom severity as well as on drug abuse in subjects treated with these drugs early in life (before age 16).

These findings were obtained in relatively small samples, with restricted age ranges and involved subjects of the male gender. Also, our RCT had a limited follow up and/or treatment period. Our findings therefore need to be replicated in larger cohorts with longer follow-up periods. However, for now they may have the following clinical implications:

Clinical implications of our main findings

**Emotion dysregulation commonly co-occurs with ADHD and influences treatment outcome.** For a long time children with ADHD and emotion dysregulation were considered to have a bipolar disease and were therefore treated with anti-epileptics, at least in the US. Nowadays, in cases of uncertainty regarding the origin of affective problems in ADHD, clinicians tend to delay prescribing MPH or titrate a dose that is inadequate to relieve ADHD symptoms. Also, some clinicians first start treatment with antidepressive medications i.e. SSRIs to reduce symptoms of anxiety and depression, before starting treatment of ADHD (in both children and adults). Interestingly, with the introduction of the Disruptive Mood Dysregulation Disorder (DMDD) in DSM 5 as a separate disorder the debate on treatment strategies of that disorder still have to start. Children with ADHD and emotion dysregulation may be diagnosed as having DMDD hence being withheld from an adequate treatment strategy for emotional dysregulation in ADHD. Or they may be treated with agents like antipsychotics or anti-epileptics with unknown long-term effects. In any case, our data support the notion that evidence based treatment recommendations for management of affective problems in ADHD are urgently needed.

**DA plays an important role in emotional processing.** Now that we know that DA plays an important role in emotion regulation in ADHD, we do not only better understand emotion dysregulation and comorbidity of anxiety and depression in ADHD, but also have arguments to treat emotional dysregulation with adequate doses of MPH, rather than SSRIs. This is clinically relevant since positive results of treatment with MPH on affective problems (only in children!) in ADHD are usually within one or two weeks (Coghill and Marcovitch, 2004) and treatment with SSRIs’s takes up to 12 weeks to optimize the effects (Ruhé et al., 2006).

**During treatment, there is no added value of MPH over placebo on positive effects on anxiety and depression scores in children nor adults.** Although symptoms of anxiety and depression did improve during the trial, they did not differ between both medication groups in children, although the MPH condition appeared to drive the main effect of emotional liability. In adults, we observed improvement on depressive and emotion dysregulation symptoms, but also in both conditions. Other explanations could be that education about the disease itself and its treatment and having an explanation for the behavioral problems helped to increase self-esteem. Another explanation could be that the high frequency of the structured control visits made the treatment predictable for the children and thus lesser anxiety was experienced during treatment.

**The age-dependent effects of MPH on amygdala reactivity likely underlie age-dependent effects of MPH on the DA system.** Now that we know that DA plays an important role in emotion regulation in ADHD, we do not only better understand emotion dysregulation and comorbidity of anxiety and depression in ADHD, but also have arguments to treat emotional dysregulation with adequate doses of MPH, rather than SSRIs. This is clinically relevant since positive results of treatment with MPH on affective problems (only in children!) in ADHD are usually within one or two weeks (Coghill and Marcovitch, 2004) and treatment with SSRIs’s takes up to 12 weeks to optimize the effects (Ruhé et al., 2006).

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was symptom improvement over baseline (Molina et al., 2007). But are in line with clinical guidelines and conclusions from reviews suggesting ADHD is a chronic condition with a strong persistence over time and long during symptom control by extended use of medication is necessary to provide long term benefits (Van de Loo-Neus et al., 2011).

ADHD medications induce depression in subjects treated with these drugs during childhood or adolescence, in line with a large body of preclinical and emerging clinical literature. Clinicians sometimes tend to delay prescribing MPH or titrating an inadequate dose in the fear of inducing depressive symptoms. Our data suggest that there is no reason to withhold treatment to children at least on the short-term. On the long-term, deciding whether or not to treat children with ADHD the clinician should consider whether treatment with MPH does more good than harm. MPH is highly effective, and the good of the treatment (in terms of higher life expectancy, less accidents, higher education levels and income) (Klein et al., 2012; Moffitt et al., 2015) should be balanced against the undesirable consequences of MPH treatment. As these long-term undesirable consequences are still largely unknown, more insight into the long-term consequences of this drug on the developing brain are urgently needed, as concluded also by the Gezondheidsraad in 2014. When replicated, our findings may be used to facilitate evidence based treatment recommendations for management of affective problems in ADHD. In adults, on the other hand, we found no lasting effect of MPH on affective problems (other than that it induced amygdala hyper-connectivity, see below).

Amygdala reactivity may be a useful biomarker in combination with other parameters and techniques like machine learning, during treatment to select children at increased risk of developing depression later in life. Identifying this group of patients and consequently follow up and treat them will not only reduce harm at an individual level but will also be more cost effective, since it is no longer necessary to follow up all patients with ADHD. In combination with identifying more predictive features like a strong response to medication in the first two years (Van de Loo-Neus et al., 2011) we will be able to improve personalized medicine and save costs to better treat patients in need for treatment.

The functional significance of the MPH-induced hyper-connectivity in adults still needs to be established and follow up is needed. Although medication trials have shown MPH to be effective in controlling ADHD symptoms in adults (Medori et al., 2008), hyper-connectivity with the amygdala could be an indication for vulnerability to depression. More research on safety and efficacy of MPH in adults is needed, in light of recent discussions in the Netherlands (Geneesmiddelenbulletin 2016) regarding off-label treatment with MPH in adulthood.

In sum, we here add further evidence to the management of affective problems in ADHD: our data suggest that there is no need to withhold treatment with MPH in ADHD patients with emotion dysregulation as MPH seems to be effective in reducing emotion dysregulation, at least in children. Although these findings need to be replicated in larger cohorts with wider ranges of ages and including including men and women, we did not find evidence that MPH induced affective problems at least on the short-term. On the long-term, treatment of children and adolescents with ADHD medications seems to have predominantly positive effects (reduction in ADHD symptoms as well as drug abuse). However, we also found an increased occurrence of depressive symptoms in subjects treated with ADHD medications early in life. Future studies should find out whether the long-term undesirable effects outweigh its positive effects. In addition, patient stratification using the amygdala reactivity fMRI biomarker may be used to identify those patients at increased risk for developing a clinical depression later in life, which seems to be already evident short after treatment. Our ongoing follow-up study in the subjects that participated in the ePOD-MPH trial will at least provide evidence whether such an approach may be beneficial.

Methodological considerations
In fMRI, the BOLD response to negative emotional faces (angry and fearful faces) is measured in a block-design fMRI task (Hariri et al., 2002). Emotional responses are elicited in many different brain regions, where the amygdala seems to be a relay between visual systems and modulatory responses. Blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) is an effective and reliable tool for measuring stable individual differences in amygdala function (Manuck et al., 2007). Repeatability of the BOLD response amplitude to emotional faces is discussed in some studies (Lipp et al., 2014). While in other studies good reliability of BOLD signal in emotion processing areas (Mende-Siedlecki P et al., 2013) including the amygdala was demonstrated (Gee et al., 2015). Test-retest reliability with the same paradigm we used in our study are highest in studies with shorter time intervals (Plichta et al., 2014) and considered fair (Sauder et al., 2013) to strong (Intra Class Coefficients >.60) for both left and right amygdala to angry and fearful faces (Plichta et al., 2014). However, without a placebo challenge in chapter 4 we cannot rule out the possibility that changes in amygdala reactivity we found are unrelated to habituation effects; a second time in the scanner could have made patients less anxious and subsequently showing less amygdala reactivity.

Motion
In fMRI studies in general, and in fMRI studies in children with ADHD in particular, correction for motion is requested. BOLD signal acquisition depends upon precise spatial and temporal placement of magnetic gradients. We realigned our data as part of the fMRI preprocessing. However, spatial realignment corrects motion-induced shifts in space but does not correct intensity changes resulting from
disruption of the physical principles underlying MRI. Therefore, we performed extra motion control by adding six standard rigid-body motion parameters and a confound matrix to volumes that were corrupted by large motion (Lemieux et al., 2007). To control for effects of transient subject movements the confounded time points were determined using a net displacement vector according to Euclidian root mean square (RMS) (Power et al., 2012). We demonstrate that subject motion produces substantial changes in the timecourses of resting state functional connectivity MRI (rs-fcMRI). Data from subjects with extreme motion were removed from the analysis using the method by Power (Power et al., 2014) and van Dijk (van Dijk et al., 2012).

In BOLD fMRI studies, the sequence is vulnerable to motion artifacts within the imaging volume due to a longer read out. In our study, motion differed between adults and children and despite advanced imaging techniques and exclusion of the worst cases, residual effects could have remained. The balance between reducing the total imaging protocol time and gathering enough data per scan is a delicate one, especially in fMRI studies in children. Hopefully fast imaging techniques can increase the amount of information obtained in a limited amount of time. These techniques are now included in new imaging initiatives in ADHD (Silk et al., 2016).

Clinical sample
The children we included in our RCT had predominantly the inattentive subtype of ADHD. This is contrary to what is expected within developmental trajectories in ADHD and might be due to the age of inclusion. Children with hyperactive and combined type of ADHD are usually diagnosed and treated at younger age, which led to a selection bias of predominantly inattentive type in a small window of 10-12 years, the age we chose for inclusion. Furthermore, long term follow up studies in ADHD have questioned the continuity of childhood ADHD in adults (Moffitt et al., 2015) and subtyping ADHD in adulthood is not considered clinically meaningful (Klein et al., 2012).

Recruitment
In performing the ePOD RCT we met the ethical and logistic challenges already mentioned by Shaw and colleagues (Shaw et al., 2009). Unfortunately, we had to prematurely end the fluoxetine RCT because of limited inclusion of children and no inclusion in adults at all, despite huge efforts with multiple centers for child and adolescent psychiatry and multiple centers for adult psychiatry and general practitioners in the Amsterdam area. Also, serious comorbidity with suicidality and subsequent violation of protocol and hospitalizations prevented us from targeting our study population. However, due to the strong effect size we found, we calculated that for future studies only 8 patients are needed to demonstrate the effects of fluoxetine on amygdala reactivity. Notwithstanding the obstacles we had with our inclusion, we hope our data will support future studies on the (adverse) effects of SSRIs since they are highly relevant and needed.

The inclusion of children and adults with ADHD for the ePOD –MPH trial was less of a problem thanks to the collaboration with several centers of child and adolescent psychiatry like Triversum, De Bascule, UVA-minds and Kenter and thanks to PsyQ in the Haque, where most adult patients were recruited. We completed the inclusion of adult patients with fathers of children already included in the study, meeting the criteria of ADHD. This is a promising avenue for further studies since they were highly motivated and had less comorbidity, especially less frequent a history of drug abuse.

This is relevant because although we excluded adult patients with drug dependency in our RCT, we could not exclude all patients with former drug abuse due to high comorbidity rates between ADHD and drug abuse (Sobanski, 2006). We did not correct our findings of emotion dysregulation, anxiety and depression in adults for former drug abuse, however due to randomization these effects should be equally distributed. Besides, in the same population we found cognitive deficits in children with ADHD comparable to cognitive deficits in adults with ADHD regardless of former history of recreational drug abuse in adults (Tamminga, 2016).

Future directions
Follow up studies in this patient group and prospective studies in other age groups and female patients with ADHD are needed to establish more definitive conclusions on the effects of MPH on emotion regulation in ADHD on the long term. As long term placebo controlled trials are not feasible we will have to look at longitudinal observational studies. Using pharmacological MRI we can assess the effects of MPH on brain chemistry (Schrantee et al., 2016). Structural MRI can provide us with information on the long-term effects of DA on micro and macro structure of the brain.

Next to MPH, other treatment strategies for emotion dysregulation have to be explored. Not only SSRI’s to treat comorbid anxiety and depression in ADHD, but also for example postsynaptic alpha 2 adrenoreceptor agonists like guanfacine emerges as a potential emotion regulator, by boosting activation of the left dorsolateral prefrontal cortex (Schultz 2013).

We are now conducting the follow-up study of the ePOD-MPH trial, on average three years after the enrollment of the first study. This is a natural follow up study in which we quantify the extent of MPH use by means of prescription data form the pharmacies. Hopefully these data and data from long term follow up neuroimaging studies (Silk et al., 2016) and hypotheses driven studies like ours, in addition to collected data from several MRI studies in international initiatives (Neu-
roimage, ENIGMA e.g.), will provide more information on the long-term effects of stimulant treatment on emotion dysregulation, anxiety and depression. With more data, stratifying ADHD patients on basis of their vulnerability for developing anxiety and depression with fMRI for specific treatment will become scientific and no longer science fiction.

References


Chapter 9


CHAPTER 10
Dutch summary
Nederlandse samenvatting
ADHD is een neurobiologische ontwikkelingsstoornis die bij ongeveer 8 tot 12% van de kinderen voorkomt en 2,5% van de volwassenen. Naast de beperkingen die worden veroorzaakt door kern symptomen als onoplettendheid, hyperactiviteit en impulsiviteit, hebben patiënten met ADHD vaak co-morbide stoornissen. Dokters en ouders weten al lange tijd dat emotionele dysregulatie in ADHD, gedefinieerd als gemakkelijk geprikkeld en boos raken, een belangrijk onderdeel van ADHD vormt en leidt tot een aanzienlijke ziektebelast. Emotionele dysregulatie komt voort uit problemen met het richten vande aandacht op emoties, het herkennen van emoties en het inschatten van emoties en hangt neurobiologisch samen met disfuncties van het striato-amygdaalo-prefrontale netwerk. Een aanmerkelijk deel van de kinderen met ADHD heeft dan ook problemen met het reguleren van emoties. Emotionele dysregulatie kan worden beschouwd als een fenotype van depressie later in het leven. Zo komen bij kinderen en volwassenen stemmingsstoornissen en angststoornissen vaker voor dan op basis van toeval verwacht kan worden. Tot voor kort werd echter niet veel aandacht besteed aan emotionele dysregulatie bij kinderen en volwassenen met ADHD. Dit is opmerkelijk omdat emotionele dysregulatie invloed heeft op het beloop, de respons op behandeling en de uitkomst van ADHD.

Tegenstrijdige bevindingen over de rol van farmacologische behandeling met dopaminerger werkende middelen zoals methylfenidaat (MPH) op de prevalentie van emotionele problemen bij ADHD, benadrukken de noodzaak om beter te begrijpen wat de mechanismen van emotie regulering in ADHD zijn en wat het effect van MPH daarop is. De amygdala is een hersenstructuur die essentieel is voor het verwerken van emoties en die het sterkst activeert tijdens het zien van emotioneel beladen gezichten. Amygdala-functie gemeten met fMRI is een mogelijke biomarker voor emotionele verwerking bij jonge en volwassen patiënten met angststoornissen depressies.

In dit proefschrift beoog ik emotionele dysregulatie bij ADHD verder te exploreren, de effecten van MPH op affectieve problemen bij ADHD te ontrafelen en de leeftijd-effecten te onderzoeken van behandeling met MPH op deze neurobiologische substraten.

Als eerste stap om dit verder te onderzoeken bepaalden we in hoofdstuk 2 de relatie tussen de diagnose ADHD, (voormalig) gebruik van stimulantia, leeftijd en symptomen van angst en depressie in ADHD. Vervolgens onderzochten we de effecten van stimulantia op het ontwikkelen van angst en depressie. Er werden meer depressieve symptomen gevonden bij kinderen met ADHD ten opzichte van kinderen met een normaal ontwikkelingspatroon. Het gebruik van stimulantia was niet gerelateerd aan de mate van depressieve symptomen, noch aan angst symptomen. Gegevens over de behandelduur waren echter niet voorhanden waardoor een eventueel effect van MPH op het ontstaan van depressie bij ADHD in deze groep niet uitgesloten kon worden.

Om de rol van dopamine in emotieregulatie en meer specifiek, het moduleren van de amygdala functie, te onderzoeken in patiënten met een dysfunctionerend dopaminesysteem, onderzochten we in hoofdstuk 3 de effecten van het dopaminerg werkende middel MPH op het functioneren van de amygdala in een groep van acht amfetamine (speed) gebruikers en acht gezonde controles. Zoals we vooraf veronderstelden, wonden we op baseline een hogere activiteit van de amygdala in reactie op angstige gezichten bij amfetamine gebruikers vergeleken met gezonde controles en was de duur van het amfetamine gebruik gecorreleerd aan de mate van amygdala reactiviteit. De bevinding, dat op baseline de amygdala reactiviteit bij amfetamine gebruikers verschilt van gezonde controles, is waarschijnlijk het gevolg van een afwijkende dopaminerge beïnvloeding staat en dat dopamine mogelijk een rol speelt bij emotieregulatie. De trend significante bevinding dat in gezonde controles, na een eenmalige gift MPH, de amygdala reactiviteit toeneemt (terwijl in amfetamine gebruikers de respons na MPH afvlakt), was voor ons aanleiding voor verder onderzoek naar de rol van MPH in het moduleren van emotionele processen bij patiënten met ADHD die met MPH behandeld worden. Omdat we in deze studie constateerden dat voormalig gebruik van dopaminerg werkende middelen invloed heeft op de amygdala fMRI-respons (vermoedelijk ten gevolge van desensitisatie), hebben we medicatie naïeve ADHD-patiënten geïncludeerd voor de studie die we beschrijven in hoofdstuk 4. Voor deze studie hebben we zowel kinderen als volwassenen met ADHD geïncludeerd om zo de leeftijdseffecten verder te onderzoeken.

In hoofdstuk 4 extrapoleren we onze bevindingen van hoofdstuk 3 naar patiënten met ADHD, en onderzochten we het effect van een eenmalige toediening van MPH op amygdala reactiviteit bij medicatie naïeve jongens en mannen met ADHD. Overeenkomstig de literatuur en eerdere studies in recreatieve dAMP gebruikers met een dysfunctie van het dopaminesysteem (hoofdstuk 3), vonden we dat ook in patiënten met ADHD het zien van emotionele gezichtsuitdrukkingen geassocieerd was met amygdala hyperreactiviteit. We stelden verder vast dat zowel de neurobiologische verwerking van emotionele stimulati als de acute effecten van MPH daarop, leeftijdshandelijk zijn. Bij volwassenen verminderde MPH de amygdala-reactiviteit richting het niveau van de gezonde controles, terwijl bij kinderen MPH amygdala-reactiviteit verder verminderde; tot onder het niveau van de gezonde controles. Kinderen en volwassen patiënten scoorden verder aanzienlijk hoger op klinische beoordelingschalen die depressieve en angst symptomen meten, dan de gezonde controles. Deze scores correleerden echter niet met de reactiviteit van de amygdala bij het zien van emotionele gezichtsuitdrukkingen. Een mogelijke verklaring hiervoor is dat deze beoordelingschalen alleen een langdurige staat van emotionele labiliteit meten. Een andere verklaring kan zijn dat onze leeftijdshandelijke bevindingen niet alleen een gevolg zijn van het directe effect van MPH op de amygdala, maar berusten op een leeftijdshandelijke
top-downcontrole van de amygdala reactiviteit. Dit is mogelijk omdat dopaminer-
ge uitrijping van de frontale cortex voortduurt tot na de adolescentie, terwijl de dopaminerger uitrijping in de amygdala al eerder is voltooid.

Hoewel deze studie een positief effect suggereert van MPH op emotie verwerking bij zowel kinderen als volwassenen met ADHD (dat wil zeggen een ver-
mindering van amygdala-reactiviteit liet zien), zijn deze bevindingen beperkt tot een eenmalige behandeling met MPH. In hoofdstuk 6 beschrijven we daarom de leeftijdafhankelijke effecten van langdurige (vier maanden) behandeling met MPH. Deze klinische studie wordt meer in detail beschreven in hoofdstuk 5, waarin we de opzet en methoden van de ePOD trial (effects of Psychotropic drugs On the Deve-
loping brain-studie) uiteenzetten, waar de ePOD-MPH-trial deel van uitmaakt.

Het doel van de ePOD MPH-trial in hoofdstuk 6 was om bij patiënten met ADHD de effecten van vier maanden behandeling met MPH op het emotioneel functioneren nader te bestuderen. Deze periode kwam destijds overeen met de wachtlijst en maakte het daardoor aanvaardbaar om ook wilsonbekwame patiënten (de kinderen) gedurende die periode met een placebo te behandelen. Op basis van onze bevindingen in hoofdstuk 4 (meer normaliserende effecten van MPH bij kinderen dan bij volwassenen door het verminderen van amygdala reactiviteit), hebben we functionele connectiviteit van de amygdala met andere hersengebie-
den, toegevoegd aan onze oorspronkelijke uitkomstmaten (amygdala-reactiviteit en klinische symptomen). We stelden vast dat een week na het staken van vier maanden behandeling, MPH nog steeds (net als tijdens de behandeling) een posi-
tieve invloed heeft op emotionele dysregulatie, maar dat de amygdala reactiviteit gemeten met fMRI bij kinderen negatief wordt beïnvloed door MPH. Ofschoon bij volwassenen MPH een week na het staken niet meer effect had op gedragsma-
ten en amygdala reactiviteit dan de placebo behandeling, induceerde MPH wel een versterkte connectiviteit tussen de amygdala en gebieden in de cortex. Hoe-
wel symptomen van angst en depressie tijdens de studie verbeterden, verschil-
den die niet tussen MPH en placebo conditie, zowel in kinderen als volwassenen. Hieruit kunnen we concluderen dat: a) overeenkomstig de literatuur, MPH bij kinderen, maar niet bij volwassenen, emotionele dysregulatie langdurig vermin-
derde; b) dat dit niet verband houdt met langdurige verbetering in afwijkende amygdala-corticale verbindingen, aangezien we bij kinderen geen blijvende effec-
ten op de connectiviteit hebben waargenomen (maar wel bij volwassen patienten). Deze leeftijdafhankelijke bevindingen lijken eerder verband te houden met een toegenomen dopamine activiteit na behandeling met MPH in deze kinderen, zoals we elders rapporteerden (Schartee et al., 2016). Al met al suggereerden deze stud-
dies dat onze bevindingen gedeeltelijk gemedieerd worden door ontwikkelings-
veranderingen in het dopamine systeem (bijvoorbeeld in de expressie van corticale D3-receptoren, die in elk geval in ratten tijdens de vroege adolescentie hoog is, vervolgens vermindert en in de volwassenheid afwezig is). Deze laatste veronder-

stelling komt ook overeen met onze observaties over de rol die dopamine speelt in de neurobiologische verwerking van emoties (hoofdstukken 3 en 4).

Onze bevindingen bij chronische MPH-behandeling in hoofdstuk 6 lijken in tegenspraak met de resultaten die we vonden bij eenmalige toediening van MPH in hoofdstuk 4. Terwijl kinderen in hoofdstuk 4 een vermindering van de rechter amygdala-reactiviteit lieten zien, vonden we hier in hoofdstuk 6 dat chronische behandeling tegengestelde effecten veroorzaakte en juist amygdala-reactiviteit in de rechter amygdala verhoogde. Dit is waarschijnlijk het gevolg is van sensitisatie effecten in volwassenen en ‘chemical imprinting’ effecten in kinderen. In overeen-
stemming hiermee, vermindering van enkele procenten van de amygdala-hyperreactivi-
titeit in MPH-nàive volwassenen, terwijl chronische behandeling niet resulteerde in enige effecten (een afgevlakte respons, overeenkomstig de respons die we waargenamen bij AMPH-behandelingen in hoofdstuk 3). Samen met onze bevindingen van verhoogde connectiviteit tussen de amygdala en de cortex bij volwassenen, maar niet bij kinderen, tonen wij hier aan dat vier maanden behandeling met MPH de amygdala-reactiviteit, amygdala-connectiviteit en emotionele dysregulatie, leef-
tijdafhankelijk beïnvloedt; MPH beïnvloedt de ontwikkeling van hersengebie-
den die betrokken zijn bij het verwerken van emoties. Omdat het niet aangetoond dat amygdala hyperreactiviteit en -connectiviteit vooraf kunnen gaan aan emotionele problemen op latere leeftijd, zoals depressies, benadrukken onze bevindingen de noodzaak van langdurige vervolgstudies om de klinische betekenis van onze bevin-
dingen bij kinderen en volwassenen verder te onderzoeken. Tot die tijd zijn de blij-
vende positieve effecten van MPH op emotionele dysregulatie bij kinderen, zowel voor ouders als voor de dokters die MPH voorschrijven, geruststellend te noemen, in ieder geval op de korte termijn.

In hoofdstuk 7 hebben we de lange termijn effecten van MPH-behandeling onderzocht en vonden wij dat volwassen patiënten die behandeld werden met AD-
HD-medicijnen vóór de leeftijd van 16 jaar, meer depressieve symptomen hadden dan patiënten die op latere leeftijd met medicatie werden behandeld (begin van de farmacologische behandeling na de leeftijd van 23 jaar). Wij vonden eveneens minder gebruik van cannabis en cocaïne in deze groep vroeg behandelden in ver-
gelijking tot de groep die op latere leeftijd met MPH werden behandeld maar ook in vergelijking tot de groep die nooit eerder met stimulantia waren behandeld. Interessant was dat de ernst van de ADHD-symptomen in de groep die niet eerder met medicatie was behandeld hoger was in vergelijking tot de ernst van de ADHD symptomen in beide medicatiegroepen. Overeenkomstig de preklinische litera-
tuur, suggereerden deze bevindingen dat behandeling met medicatie voor ADHD op

het lange termijn voordeel van behandeling met medicatie (Van de Loo-Neus et al., 2011). Uit onze bevindingen blijkt ook dat behandeling voor het zestiende jaar
het risico op het ontwikkelen van middelenmisbruik niet verhoogt, maar eerder beschermt werkt voor een dergelijk effect, zoals in de literatuur gesuggereerd. Hiernaast vonden we echter dat behandeling met medicatie voor ADHD op jonge leeftijd en niet op latere leeftijd, depressieve symptomen veroorzaakt op latere leeftijd. Het zou kunnen zijn dat we met de fMRI biomarker die we in hoofdstuk 6 hebben geïdentificeerd (amygdala reactiviteit), in combinatie met andere parameters zoals bijvoorbeeld de verhouding eicosapentaenzuur / arachniodezuur op de celmembranaan de amygdala connectivity gecombineerd met gevanceerde multivariate analyse methodes zoals ‘Machine Learning’ technieken, op termijn een methode wordt gevonden die kinderen kan identificeren met een verhoogd risico op depressieve symptomen later in het leven. Dit vraagt uiteraard om nader onderzoek.

Omdat de neurobiologische effecten van antidepressiva, zoals selectieve serotonine heropname remmers (SSRI’s), op het ontwikkelen menselijke brein ook grotendeels onbekend zijn, aangezien de meeste beeldvormende studies tot nu toe bij volwassenen zijn uitgevoerd, hebben we in hoofdstuk 8 een poweranalyse uitgevoerd voor toekomstige klinische trials naar mogelijke nadelige effecten van SSRI’s op emotie verwerking. In 2004 gaf de Food and Drug Administration een black box waarschuwing af over het gebruik van antidepressiva bij kinderen en adolescenten. Sindsdien is het gebruik ervan bij kinderen een belangrijk onderwerp in het publieke debat, ook hier in Nederland. In onze voortijdig beëindigde RCT (ePOD-SSRI) ontdekten we dat de behandeling met fluoxetine de reactiviteit amygdala verhoogde, met tegenovergestelde effecten in de placebo conditie. We vonden dat in toekomstige studies ‘slechts’ 8 patiënten nodig om zo’n effect van fluoxetine op emotie verwerking gemeten met fMRI aan te tonen.

Concluderend waren de belangrijkste bevindingen in dit proefschrift:

- Angst en depressie komen vaak voor bij ADHD.
- Dopamine speelt een belangrijke rol in emotie verwerking gemeten met fMRI.
- Tijdens de behandeling is er geen toegevoegde waarde van MPH boven placebo op het verbeteren van angst- en depressie scores bij kinderen en volwassenen.
- Alleen bij kinderen is er een blijvend positief effect van MPH op emotionele dysregulatie, in ieder geval één week na het einde van de behandeling.
- MPH veroorzaakt verhoogde amygdala reactiviteit bij kinderen (maar niet bij volwassenen); een potentiele biomarker voor depressieve symptomen later in het leven.
- De leeftijd-afhankelijke effecten van MPH op het DA-systeem liggen waarschijnlijk ten grondslag aan de leeftijd afhankelijke effecten van MPH op de amygdala-reactiviteit.

- Op de lange termijn lijken ADHD-medicijnen depressie te kunnen veroorzaken bij kinderen die jarenlang met het medicijn zijn behandeld.
- In combinatie met andere parameters en technieken zoals Machine Learning, kan amygdala-reactiviteit een mogelijke biomarker zijn om voorafgaande aan de behandeling met MPH kinderen te selecteren die een verhoogd risico hebben op het ontwikkelen van depressie op latere leeftijd.
- De functionele betekenis van de MPH-geïnduceerde hyper-connectiviteit bij volwassenen moet nog worden vastgesteld.
- ADHD-medicijnen hebben op de lange termijn positieve effecten op de ernst van ADHD-symptomen en op het verminderen van misbruik van drugs bij patiënten die op jongere leeftijd behandeld werden met deze drugs (vóór het 16e jaar).

Echter, deze bevindingen werden verkregen in relatief kleine studiegroepen met beperkte variatie in leeftijd. Bovendien waren de proefpersonen uitsluitend van het mannelijke geslacht. Ook had onze RCT een beperkte behandelduur en een korte follow up periode. Onze bevindingen moeten daarom worden herhaald in grotere cohorten met een langere follow up duur.

References


Marco Bottelier was born on 29 June 1967 in Haarlem. In 1986, he obtained his VWO diploma at the Hermann Wesselink College in Amstelveen. After completing a year of General Literature at the University of Utrecht, he studied Medicine at the University of Amsterdam. During his Master studies, he was a lecturer in Medical Psychology, and followed courses at the faculty of Philosophy. There he wrote a small thesis on the history of emotions under supervision of professor H. Pott. Also, he conducted research at the Royal Tropical Institute and in Bangkok for antigen detection in tuberculosis under supervision of dr. A. Kok.

After the basic training for psychiatrist at GGZ Centraal, Prof. P. Van Harten, he graduated as a child and youth psychiatrist at UMC Utrecht with Prof. Herman van Engeland and subsequently worked as a staff member. In 2006 he became a child and youth psychiatrist at the acute ward for youth at Triversum, Alkmaar. From 2009 he has been in charge of clinical care and since 2014, he is chief psychiatrist at Triversum. Since 2008, he has been involved as a researcher in the effects of Psychotropic Drugs on Developing brain (ePOD) project led by Prof. L. Reneman. The research results described in this thesis are derived from this. Since 2017 he is applying the Topclass degree at Erasmus University in Rotterdam.

He is married to theater director Eva Midelhoff and is the proud father of two high school students, Jonas and Julia.
PhD Portfolio

List of publications


Bottelier MA, Schrantee A, Ruiter de MB, Ruhé HG, Lindauer RJL, Reneman L. Age-dependent effects of acute MPH on affect modulation in medication naïve ADHD patients. Under review


Submitted


Dankwoord

Mijn eerste en grootste dank gaat uit naar de kinderen, de ouders van de kinderen en de volwassen patiënten die hebben deelgenomen aan de ePOD studie en aan de kinderen die aan de controlegroep van ePOD wilden meewerken. Zij waren vaak bereid om de hele provincie Noord-Holland door te reizen, uren door te brengen in het AMC of op het Roetersseiland, vragenlijsten in te vullen en te wachten op de volgende scan. Zonder jullie bereidheid om dit voor andere kinderen en volwassenen met ADHD te doen, was dit project nooit geslaagd, alle dank en respect daarvoor! Met velen van jullie heb ik gelukkig nog contact en een groot aantal is ook weer bereid om aan ePOD 2.0 mee te werken, geweldig!

Ik ben heel trots dat ik deel mocht uitmaken van het ePOD team in het AMC. Inmiddels is bijna de tweede lichting promovendi afgezwaaid van dit prachtige project uit de koker van professor Liesbeth Reneman, met in de beginfase input van onder andere professor Dick Swaab, professor Hilde Geurts, professor Wim van de Brink, dr Eric Ruhé en professor Frits Boer.

Ik ben zeer veel dank verschuldigd aan mijn promotor Liesbeth Reneman; lieve Liesbeth, jij bent niet alleen geniaal maar ook een geniaal leider; je bent wat Jim Collins in zijn boek ‘Good to great’ beschrijft, een echte ‘type 5 leider’; je bent bescheiden, stelt je dienstbaar op maar hebt glashelder voor ogen waar je naar toe wilt en bent ongelofelijk volhardend om dat doel te bereiken. Het is niet voor niets dat bedrijven in de VS die de stap naar ‘zeer succesvol’ hebben gemaakt, allen geleid worden door dit type leaders. Natuurlijk had ik me kunnen realiseren toen jij een jaar of negen geleden, na een gezamenlijk ski-weekeinde aangaf dat je op zoek was naar een ingang in de kinderpsychiatrie en ik voor mezelf toegaf wetenschappelijke ambities te hebben, dat onze gezamenlijke reis geen vlakke etappe in de Tour de France zou worden maar één met meerdere ‘cols van de buitencategorie’. De uitspraak van Albert Einstein dat ‘Imagination more important is than knowledge’, die je aan je promovendi bij aanvang van het promotietraject meegaf, was in mijn ogen aanvankelijk een geruststellende uitspraak, ik dacht van mezelf te weten dat ik over voldoende voorstellingsvermogen beschikte, me niet realiserend hoeveel ‘imagination’ er nodig is om een promotietraject te volbrengen. Gelukkig bleef bij de verbeelding die nodig was om het grotere geheel voor ogen te houden voeden, ook na stagnerende inclusies of meerdere afwijzingen van een artikel dat aanvankelijk bijna door de American Journal of Psychiatry geaccepteerd was. Ik vond het een eer om deel uit te mogen maken van je ePOD team en hoop dat we in de toekomst ePOD 2.0 kunnen volbrengen, al wens ik ons ook weer eens een ski-weekeindje toe, want dat is er de afgelopen jaren bij ingeschoten.

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Chapter 10

PhD training

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<td>2010 Training of trainees in child psychiatry</td>
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Daarnaast dank ik mijn tweede promotor Ramon Lindauer; beste Ramon, kersverse professor in de Kinder en Jeugdpsychiatrie, ‘less is more’ is van toepassing op de keren dat wij elkaar gesproken hebben. Hoewel een paar keer per jaar gezien de lengte van mijn promotietraject bij elkaar opgeteld toch wel weer een aanzienlijk aantal is, zat de kracht hem in de structuur die jij op volkomen natuurlijke wijze aanbracht. De focus op de ePOD-studie die je me voorhield is essentieel voor mij geweest!

Zeer veel dank aan mijn co-promotor Michiel de Ruiter; beste Michiel, zonder jouw geduldige precisie was dit project mij niet gelukt. Het spijt me dat ik weinig op een bevestiging van het vooroordeel moest zijn dat medici in het algemeen veel te weinig statistisch onderlegd zijn maar dankzij jouw geduld en bereidheid om keer op keer de stappen toe te lichten in de ingewikkelde analyses van de MRI-data, leerde ik mijn staat van ‘bewust onbekwaam’ beter te verdragen en te begrijpen wat de ‘pittfalls’ in de analyses waren. Dank daarvoor!

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Anouk, de fonkeling van een ‘bright young scientist’ is soms verblinding en vaak moest ik mezelf voorhouden dat het op mijn leeftijd echt geen schande was dat ik jouw snelheid van denken niet kan bijbenen. Ik ben zeer dankbaar voor de enorme energie die jij in het ePOD project hebt gestopt en onze intensieve en langdurige samenwerking, van de gezamenlijke inclusies en wervingsbijeenkomsten in de SSRI-studie, het vele scanwerk dat jij vaak in de weekenden verrichte, tot jouw krachtige denkwerk bij het analyseren en interpreteren van de data. Ik ben zeer onder de indruk van jouw steile leercurve!

Lieve Hyke, 75 kinderen hebben we samen voor intake gezien waarvan er uiteindelijk 50 aan de studie deelnamen. Dat betekende de 75 dagdelen kriskraad door Noord-Holland en evenzovele DISC’s, SCARED’s CDI’s en NPO’s. Terugkerend onderwerp in de gesprekken in de auto op weg naar Hoofddorp of Den Haag was, met hoe weinig gestandaardiseerde middelen/ vragenlijsten we in de kinderpsychiatrie diagnostiek verrichten. Des te opvallender hoe vaak we hetzelfde hadden gezien in de nabespreking van de gedragsobservaties in de verrichtte onderzoeken. Jouw wetenschappelijke integriteit en mijn pragmatische insteek hielden elkaar goed in evenwicht. Dank ook voor je heldere inbreng bij het schrijven van de artikelen. Ik hoop dat we elkaar in de kliniek als collega’s zullen tegenkomen.

Cheima, nadat we in de SSRI-studie geen volwassenen wisten te includeren leek me de kans dat we 50 stimulantia-naïve volwassen mannen met ADHD zouden weten te vinden nihil. De voortvarendheid waarmee je deze taak op je nam en de vanzelfsprekendheid die je uitstraalde hebben er ongetwijfeld bij bijgedragen dat het niet alleen lukte deze 50 deelnemers te vinden maar ze grotendeels ook nog binnen de studie hebt weten te houden.

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Verder bijzondere dank aan dr. Eric Ruhe, niet alleen betrokken in de ontwerp fase van het ePOD project maar ook nauw betrokken bij de uitvoering van de SSRI-studie. Beste Eric, jouw inzet voor het SSRI-project had een beter lot verdient, helaas haalden we ondanks alle actieve werving (met taart!) in jouw huisartsen netwerk de inclusie niet, maar dank voor je immers positieve support en je snelle en kritische commentaar bij het schrijven.


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het UMC Utrecht liet weten, dat het ‘not done’ was om naar de periferie te gaan tenzij je naar Triversum ging. Dank aan de medewerkers van de poliklinieken voor het aanbrengen van mogelijke deelnemers, aan bestuursvoorzitter Guy Berden; Guy, dank dat je binnen Triversum een onderzoek klimaat wist te creëren waarin een project als ePOD mogelijk was, aan Noor Tromp en de wetenschapscommissie voor jullie kritisch meedenken, aan Jan Lont voor je, letterlijke, hulp achter de schermen bij de werving en aan Roy de Vos en Femke Frech; jullie hebben ervoor gezorgd dat de polikliniek Den Helder de hofleverancier van ePOD werd.

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Eef, liefje, toen je me 8 jaar geleden voorhield dat wanneer ik echt een uitdaging zocht, een PhD veel meer was dan een marathon lopen, moest ik je daar gelijk in geven. Al heb ik me vaak afgevraagd waarom ik niet ‘gewoon’ een marathon ben gaan lopen en jij ondertussen een master Bedrijfskunde en twee directeurschappen verder bent, ben ik heel blij dat we elkaar stimuleren en uitdagen en hoop ik dat we dat nog lang kunnen blijven doen!

Tot slot dank ik mijn paranimfen Vladan Ilic en David Middelho. Het is heel steunend om straks niet als enige vijftiger voor de promotiecommissie te hoeven staan!
Marlene Dumas, *Rejects*, work in progress, 1994 – present, ink was hand watercolor on paper, 60 x 50 cm each. © Marlene Dumas

foto: Peter Cox