Affect modulation of methylphenidate in patients with Attention Deficit Hyperactivity Disorder

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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 it is 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 hyperconnectivity 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-amygdala-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 hyperreactivity (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 (Schranette 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 (Schranette 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 (Schranette 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 naive 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 (Amsterdam), department of Child and Adolescent Psychiatry at the Basculis/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 (Kooij, 2012). Patients with co-morbid axis I psychiatric disorders requiring treatment with medication at study entry, a history of major neurological or medical illness as well as clinical treatment with drugs influencing the DA system (for adults before 23 years of age), as 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=3000/30 ms, resolution=2.3x2.3x3 mm, 39 sequential slices, FOV=220x220x117 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 Mathworks 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 MRI 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</th>
<th>placebo</th>
<th>Adults</th>
<th>placebo</th>
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<tr>
<td></td>
<td>MPH</td>
<td>MPH</td>
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<td>MPH</td>
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<tr>
<td>n=25</td>
<td>n=25</td>
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<tr>
<td>mean±SD</td>
<td>mean±SD</td>
<td>mean±SD</td>
<td>mean±SD</td>
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</tr>
<tr>
<td>Age (y)</td>
<td>11.4±0.8</td>
<td>11.3±0.9</td>
<td>28.6±4.6</td>
<td>29.0±4.9</td>
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<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>
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<td>ADHD subtype</td>
<td></td>
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<td></td>
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<td>Inattentive</td>
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<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</td>
<td>11</td>
<td>10</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>ADHD symptoms</td>
<td></td>
<td></td>
<td></td>
<td></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.
**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).*
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 $F(1,24)=17.21$, $p<0.001$). However, in adults, no treatment effects were found on depressive 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.
Age-dependent effects of methylphenidate on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive patients with ADHD

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 (Schran- tee 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 reactivity emerges 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

**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.
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-naive 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.

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Supplementary table 1: connectivity of amygdala with different brain areas and their coordinates at different time points; BL = baseline, DT = During Treatment.

<table>
<thead>
<tr>
<th>seed</th>
<th>brain area</th>
<th># of voxels</th>
<th>max Z-value</th>
<th>MNI coordinates</th>
</tr>
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<tbody>
<tr>
<td>BL &gt; DT</td>
<td>left amygdala</td>
<td>precentral gyrus</td>
<td>3643</td>
<td>4.59</td>
</tr>
<tr>
<td></td>
<td>left amygdala</td>
<td>caudate nucleus</td>
<td>1782</td>
<td>4.31</td>
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<tr>
<td></td>
<td>left amygdala</td>
<td>occipital pole</td>
<td>957</td>
<td>4.11</td>
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<tr>
<td></td>
<td>left amygdala</td>
<td>superior frontal gyrus</td>
<td>804</td>
<td>4.56</td>
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<td></td>
<td>left amygdala</td>
<td>planum temporale</td>
<td>670</td>
<td>4.4</td>
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<tr>
<td></td>
<td>left amygdala</td>
<td>inferior frontal gyrus</td>
<td>549</td>
<td>3.91</td>
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<tr>
<td></td>
<td>left amygdala</td>
<td>putamen</td>
<td>219</td>
<td>3.97</td>
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<td></td>
<td>left amygdala</td>
<td>thalamus</td>
<td>167</td>
<td>3.85</td>
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<td>adults MPH</td>
<td>right amygdala</td>
<td>superior frontal gyrus</td>
<td>581</td>
<td>3.6</td>
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<tr>
<td></td>
<td>left amygdala</td>
<td>frontal pole</td>
<td>135</td>
<td>3.4</td>
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<tr>
<td></td>
<td>right amygdala</td>
<td>anterior cingulate cortex</td>
<td>101</td>
<td>3.55</td>
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<tr>
<td></td>
<td>left amygdala</td>
<td>superior temporal gyrus</td>
<td>312</td>
<td>3.76</td>
</tr>
<tr>
<td>DT &gt; BL</td>
<td>children placebo</td>
<td>left amygdala</td>
<td>insular cortex</td>
<td>487</td>
</tr>
<tr>
<td></td>
<td>left amygdala</td>
<td>paracingulate gyrus</td>
<td>130</td>
<td>3.62</td>
</tr>
<tr>
<td>DT &gt; PT</td>
<td>children placebo</td>
<td>left amygdala</td>
<td>central opercular cortex</td>
<td>1399</td>
</tr>
</tbody>
</table>
### Age-dependent effects of methylphenidate on amygdala reactivity and connectivity: a randomized controlled trial in stimulant treatment-naive patients with ADHD

| PT > DT | children MPH | left amygdala | 573 | 3.9 | 4 | -6 | 22 | 3 | 18 | 20 | 0 | -8 | -10 | 4 |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| --- | adults MPH | left amygdala | 4876 | 4.66 | 0 | -28 | 1069 | 3.84 | -8 | 2 | 34 | 46 | -24 | 22 |
| --- | --- | right amygdala | 4876 | 4.66 | 0 | -28 | 1069 | 3.84 | -8 | 2 | 34 | 46 | -24 | 22 |
| --- | --- | left amygdala | 4876 | 4.66 | 0 | -28 | 1069 | 3.84 | -8 | 2 | 34 | 46 | -24 | 22 |
| --- | --- | right amygdala | 4876 | 4.66 | 0 | -28 | 1069 | 3.84 | -8 | 2 | 34 | 46 | -24 | 22 |
| --- | --- | left amygdala | 241 | 3.92 | 10 | -42 | 22 | 3 | 18 | 20 | 0 | -8 | -10 | 4 |
| --- | adults MPH | left amygdala | 746 | 4.63 | 62 | 2 | 6 | 62 | 38 | 2 | 2 | 62 | 38 | 2 |
| --- | --- | left amygdala | 746 | 4.63 | 62 | 2 | 6 | 62 | 38 | 2 | 2 | 62 | 38 | 2 |
| --- | --- | left amygdala | 746 | 4.63 | 62 | 2 | 6 | 62 | 38 | 2 | 2 | 62 | 38 | 2 |
| --- | --- | left amygdala | 746 | 4.63 | 62 | 2 | 6 | 62 | 38 | 2 | 2 | 62 | 38 | 2 |

PT = post treatment
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.