Detecting dopamine dysfunction with pharmacological MRI

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Chapter 10

Age-dependent effects of acute MPH on amygdala reactivity in stimulant treatment-naive patients with ADHD

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Submitted
Chapter 10

ABSTRACT

Objective: The purpose of the present study was to investigate whether methylphenidate (MPH) affects emotional processing in patients with attention-deficit/hyperactivity disorder (ADHD), and whether this effect is modulated by age, as the brain undergoes profound changes between childhood and adulthood.

Method: We measured amygdala reactivity with functional Magnetic Resonance Imaging (fMRI) during processing of angry and fearful facial expressions before and 90 minutes after an acute oral challenge with MPH (0.5mg/kg) in 81 male stimulant treatment-naive patients with ADHD (N=35 boys; N=46 men) and 23 healthy control subjects (N=11 boys; N=12 men). 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.

Results: At baseline, we found a trend significant age*diagnosis effect in the right amygdala (F1,100=3.88; p=0.05) due to lower reactivity in ADHD vs. control children (-31%), but higher reactivity in ADHD vs. control adults (+31%). MPH reduced right amygdala reactivity in all patients (F1,79=4.09; p=0.045), normalizing to control levels in adults, but resulting in further reductions in children. In the left amygdala, reduction (and normalization) of amygdala reactivity was confined to adult ADHD patients (-36.3%, F1,45=6.71; p=0.01) whereas there was no change in children with ADHD (F1,38=0.33; p=0.57).

Conclusions: MPH-induced normalization of amygdala reactivity in adults might be a promising avenue for managing emotional dysregulation, when replicated for chronic MPH treatment. Moreover, the finding that MPH does not increase amygdala reactivity in children may be reassuring for clinicians, as emotional dysregulation of MPH is an often assumed side effect of MPH.
Age-dependent acute effects of MPH on amygdala reactivity in ADHD

INTRODUCTION

Emotional dysregulation has recently received attention as an important feature in children and adults with attention-deficit/hyperactivity Disorder (ADHD) (Barkley and Fischer, 2010; Wehmeier et al, 2010). In clinical samples as well as in population based studies 24-50 % of the children with ADHD manifest difficulties regulating negative affect (Stringaris and Goodman, 2009). This often leads to serious impairment; in longitudinal studies, children with emotional dysregulation show higher rates of anxiety disorders and disruptive behaviour disorders after 14 years follow-up (Althoff et al, 2010). Furthermore, children with comorbid anxiety disorder have poorer daily functioning and parents report lower quality of life as compared to children without comorbid anxiety (Sciberras et al, 2014). Also in adults with ADHD emotional dysregulation seems to play a role because depression and anxiety are 5-10 times more prevalent in patients with ADHD than in the general population (Kessler et al, 2006). Furthermore, emotional problems are associated with persistence of ADHD into adulthood (Barkley and Fischer, 2010) and predict lower quality of life in young adults (Reimherr et al, 2005).

The amygdala is one of the hallmark regions for emotional processing (Le Doux, 2000). In patients suffering from major depressive disorder for example, heightened amygdala reactivity to negative emotional stimuli is commonly observed in functional imaging studies (Groenewold et al, 2013; Hamilton et al, 2012). So far, only a few studies have examined amygdala reactivity during emotional processing in patients with ADHD. Although results are mixed, a recent review by 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, treatment with psychostimulants was also 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). For example, in rats, treatment with the stimulant methylphenidate (MPH) during adolescence induced depressive-like symptoms in adulthood (Bolaños et al, 2008). In recreational stimulant (speed) users we found increased amygdala reactivity during presentation of angry and fearful faces, which disappeared after acute administration with MPH (Bottelier et al, 2015). Therefore, it is presently unknown whether amygdala reactivity, and thus emotional dysregulation, in ADHD reflects disorder-, or treatment-related functioning.

During 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 reactivity emerges during adolescence and increases with age, prior to the emergence of clinical depressive symptoms (Swartz 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) signalling 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). Attenuated release of DA in the amygdala of patients with ADHD has been suggested to normalize amygdala functioning (Volkow et al, 2007). 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 and Lewis, 1994), whereas the density of D₁ and D₂ receptors peaks during childhood and declines between childhood and adulthood (Lidow and Rakic, 1992; Lidow et al., 1991). 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 pediatric (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 are modulated by age (differ between children and adults). We hypothesized that because of the increasing amygdala reactivity with age found in patients with a depressive disorder and because of the high comorbidity of ADHD with depression, we would observe increased amygdala reactivity in adult ADHD patients when compared to children with ADHD. We also hypothesized that because of the ontogeny of DA system, the effects of MPH on amygdala reactivity are modulated by age.

**METHODS**

**Participants**

Here we report on 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 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 Bascule/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) (American Psychiatric Association, 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, 1998) for children and the Diagnostic Interview for ADHD (DIVA 2.0) (Kooij, 2012; Ramos-Quiroga et al, 2016) 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, 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.
Table 1. Demographics and patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th></th>
<th>Adults</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADHD</td>
<td>Control</td>
<td>ADHD</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>(N=35)</td>
<td>(N=11)</td>
<td>(N=46)</td>
<td>(N=12)</td>
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<td></td>
<td>mean (SD)</td>
<td>mean (SD)</td>
<td>mean (SD)</td>
<td>mean (SD)</td>
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<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age (y)</td>
<td>11.4 (0.9)</td>
<td>11.4 (0.8)</td>
<td>p=0.98</td>
<td>28.7 (4.7)</td>
</tr>
<tr>
<td>Estimated IQ *</td>
<td>105.4 (19.1)</td>
<td>121.6 (10.9)</td>
<td>p&lt;0.02</td>
<td>107.0 (5.1)</td>
</tr>
<tr>
<td>ADHD subtype b</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Inattentive</td>
<td>60.0%</td>
<td>-</td>
<td>34.8%</td>
<td>-</td>
</tr>
<tr>
<td>Hyperactive</td>
<td>0%</td>
<td>-</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>Combined</td>
<td>40.0%</td>
<td>-</td>
<td>65.2%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Symptom severity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBD-RS</td>
<td>36.2 (7.0)</td>
<td>7.3 (4.1)</td>
<td>-</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>ADHD-RS</td>
<td>-</td>
<td>31.2 (9.8)</td>
<td>12.1 (5.7)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>Clinical rating scales</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CDI</td>
<td>7.9 (4.8)</td>
<td>3.0 (3.2)</td>
<td>p&lt;0.01</td>
<td>-</td>
</tr>
<tr>
<td>SCARED</td>
<td>28.2 (18.2)</td>
<td>11.1 (6.6)</td>
<td>p=0.01</td>
<td>-</td>
</tr>
<tr>
<td>BDI</td>
<td>-</td>
<td>7.3 (5.8)</td>
<td>2.0 (1.7)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>BAI</td>
<td>-</td>
<td>8.9 (7.0)</td>
<td>2.6 (2.0)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Emotional lability c</td>
<td>3.7 (2.9)</td>
<td>0.9 (1.2)</td>
<td>p&lt;0.01</td>
<td>5.2 (2.5)</td>
</tr>
<tr>
<td><strong>Comorbidity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression d</td>
<td></td>
<td></td>
<td>6/46=13.0%</td>
<td></td>
</tr>
<tr>
<td>Anxiety disorder d</td>
<td></td>
<td></td>
<td>2/46= 4.3%</td>
<td></td>
</tr>
<tr>
<td>ODD/CD e</td>
<td>2/35= 6%</td>
<td></td>
<td>2/46=3.5%</td>
<td></td>
</tr>
</tbody>
</table>

DBD-RS = Disruptive Behavior Disorder –Rating Scale; ADHD-RS=Attention-Deficit/Hyperactivity Disorder – Rating Scale; CDI= Child Depressive Inventory; SCARED= Screen for Child Anxiety Related Disorders; BDI = Beck’s Depression Inventory; BAI=Beck’s Anxiety Inventory * For children: WISC, for adults, NART b For children: DBD-SR, for adults ADHD-RS, χ² 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-RS were used  d Depressive episode in the past | Anxiety disorder in the past (adults: MINI Plus 5.0) +NIMH DISC-IV

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

Authorized Dutch translations of the Disruptive Behavior Disorders Rating Scale (DBD-RS) (Pelham et al, 1992) rated by the parents were used to examine the ADHD symptoms in children. In adults, the ADHD Rating Scale (ADHD-RS) (Kooij et al, 2008) 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 with the items Sobanski et al. (Sobanski et al, 2010) distillated from the Child Behavior Check List.
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-RS were used. In addition, we screened for anxiety and depressive symptoms using the Child Depression Inventory (CDI) (Kovacs, 1985) and the Screen for Child Anxiety Related Disorders (SCARED) (Birmaher et al., 1997) 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.

Procedure
Subjects 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). 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 orientated 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 second block containing six 5 second trials. Emotional stimuli consist of angry and fearful faces. Neutral stimuli consists 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 vascular changes in addition to neuronal activity induced hemodynamic changes, 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. For a more detailed description of the ASL methods, please see the Supplementary Methods.

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 Matworks 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 x standard deviation using both the method by Power (2012) and van Dijk (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 x challenge. For the exploratory whole brain analyses in patients, first-level contrasts were entered into higher-level mixed effects analyses (initial cluster-forming threshold Z>2.3; cluster significance threshold of p<0.05).
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 scale 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 4 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).

ROI analysis
We observed a strong hemisphere x 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 ($t_{79}=0.65; p=0.52$). In addition, no significant differences were found between ADHD patients and controls (children $t_{44}=0.97; p=0.34$; adults $t_{56}=1.04; p=0.31$) (Figure 3). After the challenge with MPH, adult patients showed reduced amygdala reactivity (-36.3%, $F_{1,45}=6.71; p=0.01$), normalizing towards controls (post-challenge compared to controls: $t_{56}=0.68, p=0.50$). In contrast, no change was found in children ($F_{1,34}<0.01; p=0.98$) (Figure 3). However, we did not find a significant interaction effect ($F_{1,79}=2.67; p=0.11$).

Right amygdala - No significant baseline differences between young and adult patients were found in the right amygdala either ($t_{79}=1.48; p=0.14$). Right amygdala reactivity in ADHD children was slightly lower when compared to control children (-31%, $p=0.34$), but slightly higher (+31%, $p=0.18$) in ADHD adults when compared to control adults, resulting in a trend significant age*diagnosis effect ($F_{1,100}=3.88; p=0.05$) (Figure 3). We observed a main effect of
Age-dependent acute effects of MPH on amygdala reactivity in ADHD

challenge, showing a reduction in amygdala reactivity after the MPH challenge ($F_{1,79}=4.29; p=0.042$) in both children and adults (-28%, and -18% respectively). This resulted in a normalized effect in adults ($t_{56}=-0.89, p=0.38$), but MPH further reduced amygdala reactivity compared to controls in children with ADHD ($t_{44}=2.42; p=0.02$).

**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 adult 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.

**Figure 3.** Whole brain analysis
Whole brain activation in children and adults, at baseline and after a single doses methylphenidate (0.5 mg/kg with a maximum of 20 mg in children and 40 mg in adults). At baseline, 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. Children showed less activation in medial and inferior lateral prefrontal and thalamic areas, but more activation in the precuneus and the posterior cingulate areas compared to adults. MPH induced an increase in ACC activity extending into medial prefrontal cortex in children. In adults, MPH induced a more widespread increase in activation in the cortical areas, including the bilateral insula.
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\(_{1,82}=2.48\); p=0.12; right: F\(_{1,82}=1.32\); p=0.25) nor was there an age\(^{*}\)challenge interaction (left: F\(_{1,82}=0.42\); p=0.52; right: F\(_{1,82}=2.43\); 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. 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 2).

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 trend 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 (Brotman et al, 2010; Takahashi et al, 2005; Volkow et al, 2007). However, our data suggest that these effects are dependent on age: whereas MPH induced a significant reduction in left amygdala reactivity in adult ADHD patients, it had no-, or an opposite effect in children when compared to controls (in the left and right amygdala, respectively) in children. Future longitudinal studies should investigate whether these age effects sustain after chronic treatment with MPH. If so, these results contribute to the discussion whether or not emotional dysregulation in adults with ADHD should be treated with both MPH and Selective Serotonin Reuptake Inhibitors (SSRI’s) or, when our results are replicated, whether MPH alone suffices as a first step. For children with ADHD, our data lack evidence for such an approach.

As pointed out previously, 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 D\(_{2}\)
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; 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-RS, 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 were in others it was not (Shaw et al, 2014).

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. 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, 2010). 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 normalization of amygdala reactivity might be a promising avenue for managing emotional dysregulation problems, when replicated for chronic MPH treatment.
REFERENCES


Supplementary Figure 1. a) Reaction time (RT) for shapes b) RT for shapes c) %accuracy for shapes d) % accuracy for faces; left: session 1, right: session 2; MPH dashed: adults, blank: children