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

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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 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 min 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 age*diagnosis effect approaching significance (p = 0.05) in the right amygdala due to lower reactivity in children with Attention Deficit/Hyperactivity Disorder (ADHD) vs. controls (−31%), but higher reactivity in adults with ADHD vs. controls (+31%). MPH significantly 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 decrease of amygdala reactivity in adults might be a promising avenue for managing emotional dysregulation when replicated for chronic MPH treatment.

1. 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; 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 with emotional dysregulation show higher rates of anxiety disorders and disruptive behavior 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). Also in adults with ADHD emotional dysregulation seems to play a role since depression and anxiety are 5–10 times more prevalent in patients 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 (Reimherr et al., 2005).

The amygdala is one of the hallmark regions for emotional

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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 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, 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 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). 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 and Rakic, 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 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 (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 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.

2. Method

2.1. Participants

The data we presented are the baseline data of the effects of Psychotropic Drugs on brain Development (ePOD-MPH) study (Bottelier et al., 2014), a 16 week double-blind randomized placebo controlled multicenter trial of the use of MPH in stimulant naive patients with ADHD with DA function as primary outcome measure (Schrantee et al., 2016). 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 Center (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 validated Diagnostic Interview for ADHD (DIVA 2.0) (Kooij, 2013; 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 2.0 for adults retrospectively in childhood. For current symptoms in adults a cutoff of 6 of 9 criteria was used on the DIVA 2.0. Patients were excluded if they were diagnosed on the Mini International Neuropsychiatric Interview (M.I.N.I.-plus) (Sheehan et al., 1998) with a comorbid 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.

2.2. Clinical ratings

Authorized 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(Rösler 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 Sobanski et al. 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.

2.3. fMRI procedures, task paradigm and acquisition parameters

2.3.1. Procedure

ADHD patients underwent two MRI scans, one before and one 90 min after an oral challenge with short acting MPH (0.5 mg/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. 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.

2.3.2. fMRI task paradigm

The experimental paradigm consisted of a blocked design and has

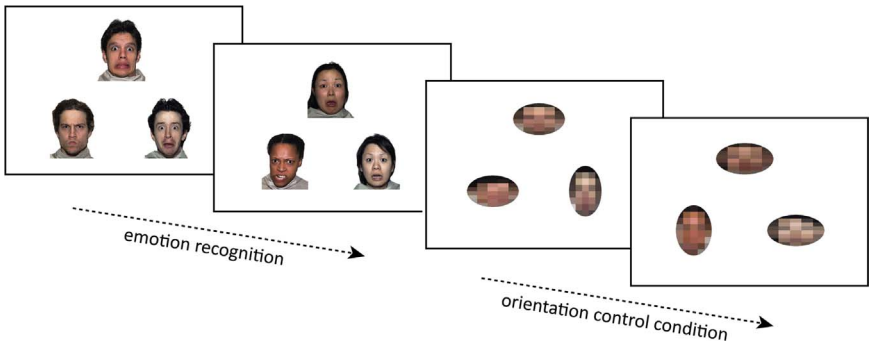

Fig. 1. fMRI task paradigm. Two blocks of emotional stimuli were interleaved with three neutral blocks, each 30-s block containing six 5-s trial. Emotional stimuli consist of angry and fearful faces. Neutral stimuli consist of ellipses assembled from scrambled faces. For each trial, subjects have to 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.

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 (Fig. 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.

2.3.3. fMRI acquisition parameters

The MRI study was performed on a 3.0 T 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 min.

2.4. 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.

2.5. 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) 2 mm template. fMRI time series were high-pass filtered with a cutoff of 0.1 Hz. 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 Euclydian 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 et al. (2012) and 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*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 $p < 0.05$).

3. Results

3.1. Group characteristics

Characteristics of the patients and controls are displayed in Table 1. Adult patients were older than adult controls ($t_{56} = 2.49$; $p = 0.02$). The majority of the adult patients (75%) was under 30 years of age. 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 (0.5 mg/kg with a maximum of 20 mg in children and 40 mg in adults). For this single administration, the mean dose was 18.71 ± 2.53 mg MPH in children and 38.26 ± 2.63 mg MPH in adults.

3.2. 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 artefacts 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 (Fig. 2).

3.3. ROI analysis

We observed a strong hemisphere × group interaction ($F = 7.647$;

Table 1
Demographics and patient characteristics.

	Children			Adults		
Demographics	ADHD (N = 35) *mean ± SD*	Control (N = 11) *mean ± SD*		ADHD (N = 46) *mean ± SD*	Control (N = 12) *mean ± SD*	
Age (y)	11.4 ± 0.9	11.4 ± 0.8	$p = 0.98$	28.7 ± 4.7	25.2 ± 1.9	$p = 0.02$
Estimated IQ[a]	105.4 ± 19.1	121.6 ± 10.9	$p < 0.02$	107.0 ± 5.1	107.9 ± 7.7	$p = 0.74$
ADHD subtype[b]						
Inattentive	60.0%			34.8%		$p = 0.02$
Hyperactive	0%			0%		
Combined	40.0%			65.2%		$p = 0.02$
Baseline symptom severity						
DBD-RS	36.2 ± 7.0	7.3 ± 4.1		–		$p < 0.01$
ADHD-SR	–			31.2 ± 9.8	12.1 ± 5.7	$p < 0.01$
Clinical rating scales						
CDI	7.9 ± 4.8	3.0 ± 3.2	$p < 0.01$	–		
SCARED	28.2 ± 18.2	11.1 ± 6.6	$p = 0.01$	–		
BDI	–			7.3 ± 5.8	2.0 ± 1.7	$p < 0.01$
BAI	–			8.9 ± 7.0	2.6 ± 2.0	$p < 0.01$
Emotional lability[c]	3.7 ± 2.9	0.9 ± 1.2	$p < 0.01$	5.2 ± 2.5	1.5 ± 1.7	$p < 0.01$
Co-morbidity						
Depressive episode(s) in the past[d]				6/46 = 13.0%		
Anxiety disorder in the past[d]				2/46 = 4.3%		
ODD/CD[e]	2/35 = 6%					

DBD-RS = Disruptive Behavior Disorder –Rating Scale; ADHD-SR = Attention-Deficit/Hyperactivity Disorder – Self Rating; CDI = Child Depressive Inventory; SCARED = Screen for Child Anxiety Related Disorders; BDI = Beck's Depression Inventory; BAI = Beck's Anxiety Inventory.

[a] For children: WISC, for adults, NART.

[b] For children: DBD-SR, for adults ADHD-SR, χ^2 test.

[c] 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.

[d] For adults: MINI Plus 5.0.

[e] For children: NIMH DISC-IV.

$p < 0.01$). We therefore analyzed the left and the right amygdala separately.

3.3.1. Left amygdala

In the left amygdala we found no age*diagnosis effect ($F_{1,79} = 2.67$; $p = 0.11$).

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 $t_{56} = -1.04$; $p = 0.31$) (Fig. 3). After the challenge with MPH, adult patients showed reduced amygdala reactivity (−36.3%, $F_{1,45} = 6.71$; $p = 0.01$). In contrast, no change was found in children ($F_{1,34} < 0.01$; $p = 0.98$) (Fig. 3).

3.3.2. Right amygdala

The age*diagnosis effect we found at baseline in the right amygdala was approaching significance ($F_{1100} = 3.88$; p = 0.05). No significant baseline differences between young and adult patients were found in the right amygdala ($t_{79} = 1.48$; $p = 0.14$).

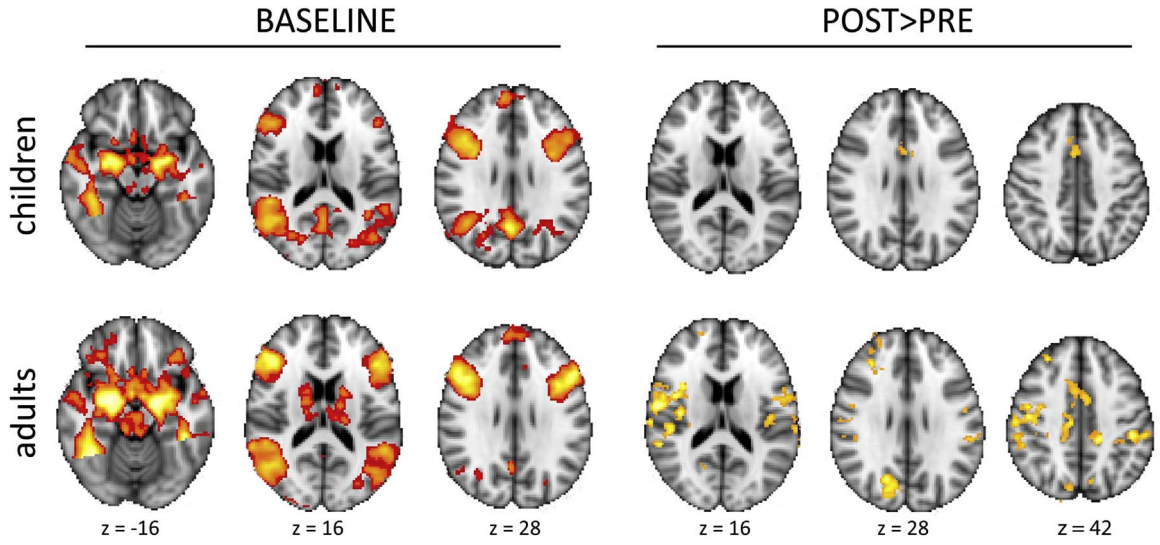

Fig. 2. Whole brain analysis. Whole brain activation in children and adults, at baseline and after a single dose of methylphenidate (0.5 mg/kg with a maximum of 20 mg in children and 40 mg in adults). At baseline, presentation of negative emotional faces elicited activation of the bilateral amygdala, 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.

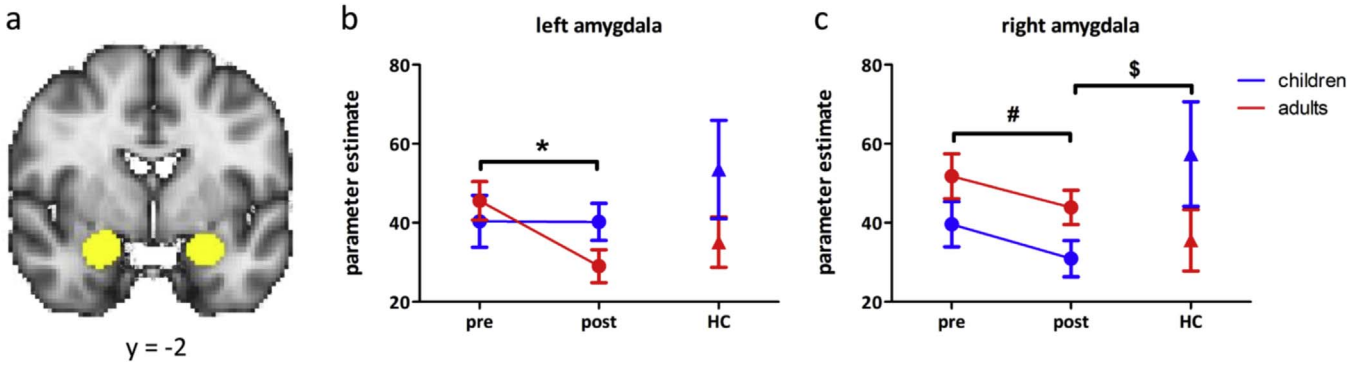

Fig. 3. ROI analysis. a) amygdala ROI used for the analysis. b) left and c) right amygdala activity before and after acute MPH administration in patients compared to healthy controls (HC). * $p < 0.05$ significant reduction in adults ADHD patients following MPH administration in left amygdala; # $p < 0.05$ 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.

After the challenge with MPH, we observed a main effect of 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). In adults, amygdala reactivity did not differ from adult control values (t_{56} = −0.89, p = 0.38), whereas in children with ADHD, MPH did induce a significant reduction that extent below control values (t_{44} = 2.42; p = 0.02).

3.4. 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 $r = 0.21$ $p = 0.12$).

3.5. 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. 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 (Fig. 2).

4. 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. Our results provide preliminary evidence that both emotional processing and the effects of MPH on emotional processing are modulated by age. In adults, we found no differences in amygdala reactivity between patients and controls before and after the challenge, whereas in children MPH further reduced right amygdala reactivity. MPH had no effect on the left amygdala in children. 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 nonsignificant difference in the opposite direction. Thus, it is possible that in ADHD, the course of amygdala reactivity is altered.

The notion that acute administration of MPH decreases 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.

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 dopaminergic 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 in adults, whereas in children MPH induced an increase in ACC activity extending into the medial prefrontal cortex.

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; 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 were 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. Future studies should use validated instruments for this purpose like the Conners Global Index Lability Scale (Sobanski, 2010).

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. 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 reactivity we found is due to habituation/expectation effects rather than the MPH challenge. To rule these effects out we ideally would also have administered a placebo condition. Therefore, our results need to be interpreted with caution. Because this is a study involving minors, we were restricted in our possibilities by the medical ethical committee in doing so. 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.

Another limitation is that our whole brain findings are assessed using a cluster-forming threshold of $Z > 2.3$. This threshold might be too lenient leading to more false positives as was shown in a recent study (Eklund et al., 2016). However, since the whole brain analyses were only explanatory analyses the consequences are limited.

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 for medication naive 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, 2010) 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., 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 suggest 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.

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Appendix A. Supporting information

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References

Althoff, R.R., Verhulst, F.C., Rettew, D.C., Hudziak, J.J., Van Der Ende, J., 2010. Adult

outcomes of childhood dysregulation: a 14-year follow-up study. J. Am. Acad. Child Adolesc. Psychiatry 49.
Barkley, R.A., Fischer, M., 2010. The unique contribution of emotional impulsiveness to impairment in major life activities in hyperactive children as adults. J. Am. Acad. Child Adolesc. Psychiatry 49, 503–513.
Beck, A.T., Epstein, N., Brown, G., Steer, R., 1988. An inventory for measuring clinical anxiety; psychometric properties. J. Consult. Clin. Psychol. 56, 893–897.
Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., Erbaugh, J., 1961. An inventory for measuring depression. Arch. Gen. Psychiatry 4, 561–571.
Biedermann, J., 2010. Young adult outcome of attention deficit hyperactivity disorder: a controlled 10-year follow-up study. Psych. Med. 36, 167–179.
Bottelier, M.A., Schouw, M.L.J., Klomp, A., Tamminga, H.G.H., Schrantee, A.G.M., Bouziane, et al., 2014. The effects of Psychotropic drugs On Developing brain (ePOD) study: methods and design. BMC Psychiatry 14, 48.
Bottelier, M.A., Schouw, M.L.J., de Ruiter, M.B., Ruhe, H.G., Lindauer, R.J.L., Reneman, L., 2015. Effects of methylphenidate during emotional processing in amphetamine users: preliminary findings. Brain Imaging Behav. http://dx.doi.org/10.1007/s11682-014-9350-8.
Breiter, H.C., Buckner, L., Etcoff, R., Whalen, N.L., Kennedy, P.J., Rauch, W.A., S.L, et al., 1996. Response and habituation of the human amygdala during visual processing of facial expression. Neuron 17, 875–887.
Chronis-Tuscano, A., Molina, S., Pelham, W., 2012. NIHPublicAccess 67, 1044–1051.
Eklund, A., Nichols, T.E., Knutsson, H., 2016. Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. Proc. Natl. Acad. Sci. USA 113, 7900–7905.
Fagundes, A.O., Aguiar, M.R., Aguiar, C.S., Scaini, G., Sachet, M.U., Bernhardt, N.M., 2010. Effect of acute and chronic administration of methylphenidate on mitochondrial respiratory Chain in the brain of young rats. Neurochem. Res. 35, 1675–1680.
Ferdinand, R.F., van der Ende, J., 2002. NIMH- DISC Diagnostic Interview Schedule for Children. Authorized Dutch Translation. Erasmus MC-Sophia, Rotterdam, the Netherlands.
Fischer, H., Wright, C.I., Whalen, P.J., McInerney, S.C., Shin, L.M., Rauch, S.L., 2003. Brain habituation during repeated exposure to fearful and neutral faces: a functional MRI study. Brain Res. Bull. 59, 387–392.
Gee, D.G., Humphreys, K.L., Flannery, J., Goff, B., Telzer, E.H., Shapiro, M., Hare, T.A., Bookheimer, S.Y., Tottenham, N., 2013. A developmental shift from positive to negative connectivity in human amygdala – prefrontal circuitry. J. Neurosci. 33. pp. 4584–4593.
Hariri, A.R., Tessitore, A., Mattay, V.S., Fera, F., Weinberger, D.R., 2002. The amygdala response to emotional stimuli: a comparison of faces and scenes. Neuroimage 17, 317–323.
Karalunas, S.L., Fair, D., Musser, E.D., Aykes, K., Iyer, S.P., Nigg, J.T., 2014. Subtyping attention-deficit/hyperactivity disorder using temperament dimensions. JAMA Psychiatry 71, 1015.
Kessler, R.C.P.D., Adler, L.M.D., Barkley, R.P.D., Biederman, J.M.D., Conners, C.K.P.D., Demler, Olga, M.A., et al., 2006. The Prevalence and Correlates of Adult ADHD in the United States: results from the national comorbidity survey replication. Am. J. Psychiatry 163, 716–723.
Kienast, T., Hariri, A.R., Schlagenhauf, F., Wrase, J., Sterzer, P., Buchholz, H.G., et al., 2008. Dopamine in amygdala gates limbic processing of aversive stimuli in humans. Nat. Neurosci. 11, 1381–1382.
Klein, R.G., Mannuzza, S., Olazagasti, M.A., Roizen, E., Hutchison, J.A., Lashua, E.C., et al., 2012. Clinical and functional outcome of childhood attention-deficit/hyperactivity disorder 33 years later. Arch. Gen. Psychiatry 69, 1295–1303.
Kollins, S.H., Rush, C.R., Pazzaglia, P.J., Ali, J.A., 1998. Comparison of acute behavioral effects of sustained-release and immediate-release methylphenidate. Exp. Clin. Psychopharmacol. 6, 367–374.
Kooij, J.J.S., 2013. Adult ADHD:Diagnostic Assesment and treatment, 3rd ed. Springer, Amsterdam.
Kovacs, M., 1992. Kovacs M: the children's depression inventory (CDI). Psychopharmacol. Bull. 1985 (21), 995–998.
Ledoux, J.E., 2000. Emotion circuits in the brain. Annu. Rev. Neurosci. 23, 155–184.
Lemieux, L., Salek-Haddadi, A., Lund, T.E., Laufs, H., Carmichael, D., 2007. Modelling large motion events in fMRI studies of patients with epilepsy. Magn. Reson. Imaging 25, 894–901.
Lidow, M.S., Goldman-Rakic, P.S., Gallager, D.W., Rakic, P., 1991. Distribution of dopaminergic receptors in the primate cerebral cortex: quantitative autoradiographic analysis using [3H]raclopride, [3H]spiperone and [3H]SCH23390. Neuroscience 40, 657–671.
Lidow, M.S., Rakic, P., 1992. Scheduling of monoaminergic neurotransmitter receptor expression in the primate neocortex during postnatal development. Cereb. Cortex 2, 401–416.
Manos, M.J., Brams, M., Childress, A.C., Findling, R.L., López, F.A., Jensen, P.S., 2011. Changes in emotions related to medication used to treat ADHD. Part I: literature review. J. Atten. Disord. 15, 101–112.
March, J.S., Swanson, J.M., Arnold, L.E., et al., 2000. Anxiety as a predictor and outcome variable in the Multimodal study of children with ADHD. J. Abnorm Child Psychol. 28, 527.
Moffitt, T.E., Houts, R., Asherson, P., Belsky, D.W., Corcoran, D.L., Hammerle, M., et al., 2015. Is adult ADHD a childhood-onset neurodevelopmental disorder? Evidence from a four-decade longitudinal cohort study. Am. J. Psychiatry 172, 967–977.
Muris P., Bodden D., Hale W., 2007. SCARED-NL. Vragenlijst over angst en bang-zijn bij kinderen en adolescenten. Handleiding bij de gereviseerde Nederlandse versie van de Screen for Child Anxiety Related Emotional Disorders.
Oosterlaan, J., Scheres, A., Antrop, I., Roeyers, H., Sergeant, J.A., 1998. Vragenlijst voor Gedragsproblemen bij Kinderen (VvGK). [Dutch Translation of the Disruptive Behavior Disorders Rating Scale]. Harcourt Test Publishers, Amsterdam.
Plichta, M.M., Grimm, O., Morgen, K., Mier, D., Sauer, C., Haddad, L., Tost, H., Esslinger, C., Kirsch, P., Schwarz, A.J., Meyer-Lindenberg, A., 2014. Amygdala habituation: a reliable fMRI phenotype. Neuroimage 103, 383–390.
Posner, J., Nagel, B.J., Maia, T.V., Mechling, A., Oh, M., Wang, Z., Peterson, B.S., 2011. Abnormal amygdalar activation and connectivity in adolescents with attention-deficit/hyperactivity disorder. J. Am. Acad. Child Adolesc. Psychiatry 50, 828–837.
Power, J.D., Barnes, K.A., Snyder, A.Z., Schlaggar, B.L., Petersen, S.E., 2012. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 59, 2142–2154.
Ramos-Quiroga, J.A., Nasillo, V., Richarte, V., Corrales, M., Palma, F., Ibáñez, P., Michelsen, M., Van de Glind, G., Casas, M., Kooij, J.J., 2016. Criteria and concurrent validity of DIVA 2.0: a semi-structured diagnostic interview for adult ADHD. J. Atten. Disord.
Reimherr, F.W., Marchant, B.K., Strong, R.E., Hedges, D.W., Adler, L., Spencer, T.J., West, S.A., Soni, P., 2005. Emotional dysregulation in adult ADHD and response to atomoxetine. Biol. Psychiatry 58, 125–131.
Rosenberg, D.R., Lewis, D.A., 1994. Changes in the dopaminergic innervation of monkey prefrontal cortex during late postnatal development: a tyrosine hydroxylase immunohistochemical study. Biol. Psychiatry 36, 272–277.
Rösler, M., Retz, W., Thome, J., Schneider, M., Stieglitz, R.D., Falkai, P., 2006. Psychopathological rating scales for diagnostic use in adults with attention-deficit/hyperactivity disorder (ADHD). Eur. Arch. Psychiatry Clin. Neurosci. 256, 3–11.
Schrantee, A., Tamminga, H.G.H., Bouziane, C., Bottelier, M.A., Bron, E.E., Mutsaerts, H.-J.M.M., et al., 2016. Age-dependent effects of methylphenidate on the human dopaminergic system in young vs adult patients with attention-deficit/hyperactivity disorder. JAMA Psychiatry 73, 955.
Sciberras, A.E., Lycett, K., 2015. Anxiety in children with attention-deficit / hyperactivity disorder. Pediatrics 133, 801–808.
Shaw, P., Stringaris, A., Nigg, J., Leibenluft, E., 2014. Emotion dysregulation in attention deficit hyperactivity disorder. Am. J. Psychiatry 171, 276–293.
Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., W.E., et al., 1998. The Mini-international neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59, 22–33.
Sobanski, E., Banaschewski, T., Asherson, P., Buitelaar, J., Chen, W., Franke, B., et al., 2010. Emotional lability in children and adolescents with attention deficit/hyperactivity disorder (ADHD): clinical correlates and familial prevalence. J. Child Psychol. Psychiatry Allied Discip. 51, 915–923.
Spencer, T.J., Faraone, S. V., Surman, C.B.H., Petty, C., Clarke, A., Batchelder, H., et al, 2013. NIH public access towards defining deficient emotional self regulation in youth with attention deficit hyperactivity disorder using the child behavior check list : a controlled study 123, 50–59.
Stringaris, A., Goodman, R., 2009. Mood lability and psychopathology in youth. Psychol. Med. 39, 1237–1245.
Swartz, J.R., Williamson, D.E., Hariri, A.R., 2015. Developmental change in amygdala reactivity during adolescence: effects of family history of depression and stressful life events. Am. J. Psychiatry 172, 276–283.
Takahashi, H., Yahata, N., Koeda, M., Takano, A., Asai, K., Suhara, T., et al., 2005. Effects of dopaminergic and serotonergic manipulation on emotional processing: a pharmacological fMRI study. Neuroimage 27, 991–1001.
Tessitore, A., Hariri, A.R., Fera, F., Smith, W.G., Chase, T.N., Hyde, et al., 2002. Dopamine modulates the response of the human amygdala: a study in Parkinson's disease. J. Neurosci. 22, 9099–9103.
van Dijk, K.R.A., Sabuncu, M.R., Buckner, R.L., 2012. The influence of head motion on intrinsic functional connectivity MRI. Neuroimage 59, 431–438.
van Wingen, G., van Broekhoven, F., Verkes, R.J., Petersson, K.M., Bäckström, T., Buitelaar, J., et al., 2008. Progesterone selectively increases amygdala reactivity in women. Mol. Psychiatry 13, 325–333.
Wagner, A.K., Kline, A.E., Ren, D., Willard, L.A., Wenger, M.K., Zafonte, R.D., 2007. Gender associations with chronic methylphenidate treatment and behavioral performance following experimental traumatic brain injury. Behav. Brain Res. 181, 200–209.
Wehmeier, P.M., Schacht, A., Barkley, R.A., 2010. Social and emotional impairment in children and adolescents with ADHD and the impact on quality of life. J. Adolesc. Health 46, 209–217.
Wright, C.I., Fischer, H., Whalen, P.J., McInerney, S.C., Shin, L.M., Rauch, S.L., 2001. Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli. Neuroreport 12, 379–383.