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Chapter 3

Age-dependent effects of methylphenidate on emotion regulation and impulsivity: a randomized controlled trial in stimulant treatment-naive patients with ADHD

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Abstract

Background: Emotion dysregulation and impulsivity are key features of Attention-Deficit/Hyperactivity Disorder (ADHD), that significantly impair daily functioning of patients. However, it is unknown to what extent these features are affected by medications like methylphenidate (MPH), and whether they are modulated by age.

Methods: In a randomized controlled trial (ePOD-MPH), 99 stimulant treatment-naive patients (50 children, 49 adults) with ADHD (DSM-IV, all subtypes) were randomly assigned to either MPH or matched placebo for 16 weeks. Changes in activity and connectivity of the amygdala during emotion regulation, and in (para)cingulate gyrus during response inhibition, were assessed with functional Magnetic Resonance Imaging (fMRI) before, during and one week after trial end. Data were analyzed as intention-to-treat.

Findings: In children, MPH improved emotion dysregulation during the trial, whereas it negatively affected right amygdala reactivity one week after trial end. In contrast, in adults, MPH had no effect over placebo on behavioral measures, nor on amygdala reactivity. MPH treatment decreased amygdala-cortico functional connectivity, but less so in adults at trial end, than in children. As for response inhibition, MPH did not affect (para)cingulate reactivity in children nor adults, but did induce paracingulate-cortical hyperconnectivity at trial end in adults.

Interpretation: Four months of treatment with MPH affected right amygdala reactivity and connectivity, as well as paracingulate connectivity and emotion dysregulation in an age-dependent manner. These prolonged, positive effects of MPH on emotion dysregulation should reassure parents and clinicians when considering MPH prescription for ADHD in children, at least on the short term.

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is one of the most common neurodevelopmental disorders with a lifetime prevalence of 7.2% worldwide[1]. First symptoms often appear at a pre-school age and can persist throughout adulthood. The main pharmacological treatment for ADHD in both children and adults is treatment with stimulants like methylphenidate (MPH), that effectively alleviate symptoms of inattention and hyperactivity. Two other important symptom clusters in ADHD are emotional dysregulation and impulsivity. In ADHD, emotional dysregulation is typically defined as being easily angered and easily annoyed. Impulsivity as psychological construct, encompasses domains of delay impulsivity, reflection impulsivity and response inhibition[2]. Both clusters of symptoms often seriously impair daily life functioning for children and adults with ADHD[3].

The amygdala has been associated with aberrant emotional processing in various affective disorders[4] and evidence is accumulating that it plays an important role in emotional dysregulation in ADHD as well. For instance, a recent mega-analysis in 3242 subjects found that of many brain regions studied, the largest volume reduction in ADHD patients was present in the amygdala[5]. Also, ADHD patients often show a heightened amygdala reactivity to negative emotional stimuli[6]. These deficits are thought to involve a dysfunctional striato-amygdalo-medial prefrontal cortical network[6].

Studies on amygdala connectivity in relation to emotion are only beginning to emerge. Hulvershorn et al. e.g. showed that a higher emotional dysregulation was associated with hyper-connectivity of a cortico-amygdalar network, including the anterior cingulate cortex, in children aged 6-13, who were mostly treatment naive[7]. The authors concluded that amygdala-prefrontal cortex hyper-connectivity is associated with difficulty in regulating the expression of negative emotions. Furthermore, in adolescents with ADHD (aged 11-16 years), a hyper-connectivity of the amygdala with the lateral prefrontal cortex was reported in response to fearful faces. This was aggravated after MPH abstinence, along with a hyper-reactivity of the amygdala[8]. Thus, amygdala-prefrontal cortex connectivity appears to be altered in ADHD patients, specifically in relation to aspects of negative affect and fearful stimuli.

As for impulsivity, patients with ADHD in particular show abnormalities in response inhibition[9,10], which has been associated with an aberrant activity and connectivity in fronto-striatal circuits[11]. A combined magnetoencephalography and functional Magnetic Resonance Imaging (fMRI) study has shown a prefrontal

underactivation during an inhibition task in adults with persistent ADHD, relative to adults with remitted ADHD[12]. Single doses of stimulants have been found to normalize fronto-striatal activity and connectivity during response inhibition[11,13–15], and a recent meta-regression analysis reported moderate and consistent treatment effects of the stimulant methylphenidate (MPH) on executive function, including response inhibition[16]. However, to our knowledge, there are so far no studies that investigated effects of treatment with stimulants on fronto-striatal activity.

Also, most studies had been conducted in previously medicated patients, but it remains unknown to what extent the above mentioned observations are affected by stimulant medication, or whether they represent an intrinsic feature of ADHD. This is highly relevant, as treatment with stimulants, such as MPH, effectively reduce symptoms of inattention and hyperactivity in ADHD patients[17]. Preclinical data however, suggest that MPH can induce anxiety and depressive-like behavior[18] and also in a rat model for ADHD, stimulant treatment during adolescence increased impulsivity during adulthood[19]. Furthermore, the most comprehensive study on long-term effects of ADHD medication to date, i.e. the Multimodal Treatment Study of ADHD (MTA), found that children treated with ADHD medication had higher rates of anxiety and depression (19.1%) than children receiving behavioral therapy (4.3%), as measured six years after treatment[20].

Increasing evidence now suggests that effects of ADHD medication may depend on the age of first exposure. For instance, young rats treated with MPH showed more anxiety and depression-related behavior in adulthood than rats that were first treated with MPH as adult[18]. These differences might be explained by age-dependent effects of stimulants on the dopamine (DA) system, as e.g. early treatment with MPH led to a 50% reduction in DA transporter (DAT) density in the rat striatum when compared to non-treated animals, whereas no such effects were observed in adult animals[21]. Furthermore, in a randomized clinical trial (RCT) in medication-naive patients with ADHD, we found that four months of MPH treatment resulted in increased DA reactivity only in children, but not in adult patients[22], further supporting the concept that effects of MPH on the human brain may be modulated by age.

Therefore, the aims of the current study were twofold: a) to investigate whether treatment with MPH influences emotional processing and response inhibition in stimulant-naive patients with ADHD, and b) whether this effect is modulated by age. Based on the above mentioned literature, we expected that MPH would increase fMRI brain activity in the amygdala during emotion

recognition and fronto-striatal circuitry during response inhibition in children, but not-, or less so, in adults. We also hypothesized that an increase would be found in amygdala and fronto-striatal connectivity with prefrontal regions in children, but not -, or less so, in adults.

Methods

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[23]. The primary objective of the ePOD-MPH RCT was to study the age-dependency of the effects of MPH on the outgrowth of the DA system, the results of which are published elsewhere[22]. Our secondary outcome measures are functional measures underlying these changes: emotional processing and impulsivity. The reward processing task turned out to be too long for the children and was eventually omitted from our scan protocol. We measured fMRI activity on emotion recognition and response inhibition tasks at three times points (baseline (BL), 8 weeks during treatment (DT) and 1 week after trial end (post treatment (PT). Furthermore, functional connectivity was assessed, as well as behavioral measures of anxiety, depression, emotional dysregulation and inhibition. 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 RCT 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 (Alkmaar), the department of Child and Adolescent Psychiatry at the Bascule/AMC (Amsterdam), and the 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), as confirmed by a structured interview, i.e. the Diagnostic Interview Schedule for Children (NIMH-DISC-IV: authorized Dutch translation[24] and the Diagnostic Interview for ADHD (DIVA) for adults[25]. Patients with co-morbid axis I psychiatric disorders requiring treatment with medication at study entry, a history of major neurological or medical illness as well as clinical treatment with drugs influencing the DA system (for adults before 23 years of age), such as stimulants, neuroleptics, antipsychotics, and D2/3 agonists were excluded. More detailed inclusion and exclusion criteria are listed in the study protocol[23]. 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 assessments, 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. Participants as well as care providers and research personnel were blinded[23]. The hospital pharmacy of the Medical Centre Alkmaar assigned participants to a specific allocation, using sequentially numbered containers. 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.

fMRI

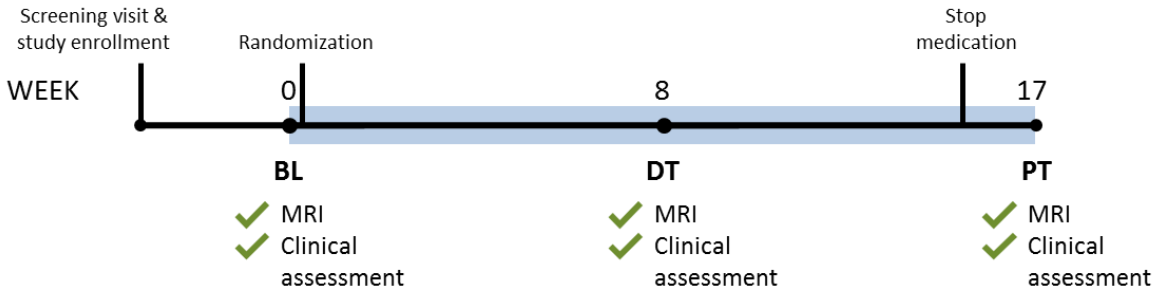
Subjects performed an emotion recognition and response inhibition fMRI paradigm at 3 different time points during the trial (Figure 1). To further minimize learning effects, a practice run was presented prior to the first MRI scan. Two versions of the tasks were used to overcome learning effects. The emotion recognition paradigm consisted of a blocked design and was adapted from a task previously used to assess drug effects on amygdala reactivity[26]. The emotional stimuli consisted of angry and fearful faces whereas the neutral stimuli consisted

of ellipses assembled from scrambled faces. The response inhibition paradigm consisted of an event-related design and was adapted from a go-no/go task previously used in ADHD patients[27]. For a detailed description of the task, please see the Supplementary Material.

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 (parameters in Supplementary Material). Data were preprocessed and analyzed using in-house MATLAB scripts (Massachusetts: The Matworks Inc.) and FSL 5.0 (FMRIB's Software Library)(for details see Supplementary Materials).

For our regions of interest (ROI) analyses for the emotion recognition task, mean signal intensity for the left and right amygdala was extracted from the first level contrasts using masks from the Harvard-Oxford atlas. For the response inhibition task, we used the (para)cingulate gyrus as a ROI, based on previous literature showing acute effects of stimulants on this brain region[11,14,15]. Because the striatum was not activated during response inhibition, we omitted this ROI from our analyses. Mean signal intensities were extracted from a seed in the (para)cingulate gyrus using mean activation values of all subjects during correct response inhibition.

Figure 1. Timeline 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).



Connectivity

For the functional connectivity analyses, ROI time courses for each respective task were extracted. For the ER paradigm, left and right amygdala time-course were separately entered into two first-level models. Connectivity of the ROIs with other brain regions was obtained and entered into subsequent random-effects analyses to assess changes in 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 and behavioral variables

In children, we assessed anxiety and depressive symptoms using the Child Depression Inventory (CDI)[28] and the Screen for Child Anxiety Related Disorders (SCARED)[29]. In adults, we used the Beck's Depression Inventory (BDI)[30] and Beck's Anxiety Inventory (BAI)[31]. All clinical scales were assessed at BL, DT and PT. Emotional 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.[32] 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[33] assessing emotional dysregulation in adults.

Behavioral measures of impulsivity were assessed using the button-responses on the response inhibition task. The number of commission errors was used to reflect impulse control.

Statistical analysis

Data were processed using SPSS v22 (IBM Corp., Armonk, USA). fMRI activity and clinical and behavioral 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 for fMRI activity and clinical and behavioral variables to investigate the main effect of time point, medication and age group, and its corresponding interaction effects. An unstructured covariance matrix was assumed, with a fixed intercept and the model was estimated using maximum likelihood. Follow-up pairwise comparisons were corrected for multiple testing using a Sidak correction.

Behavioral response data of the fMRI task were extracted from E-prime. Whole brain connectivity analyses were analyzed per protocol.

Results

Clinical characteristics and randomization

Between June 1, 2011, and February 6, 2015, a total of 99 patients with ADHD were randomized to MPH or placebo. No serious adverse events were reported in any of the subjects. Treatment groups did not differ in age, clinical impairment nor in ADHD severity (Table I). Children diagnosed with ADHD had predominantly the inattentive subtype of ADHD (60 % inattentive, 40 % combined type), while in the adult ADHD group, the combined type was more prominent (65.2% combined type, 34.8 % inattentive type).

Table I. Characteristics of the study groups

	Children		**Adults**	
	MPH n=25	**placebo** n=25	**MPH** n=24	**placebo** n=24
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (y)	11.4 (0.8)	11.3 (0.9)	28.6 (4.6)	29.0 (4.9)
Estimated IQ[1]	104.8 (21.0)	103.4 (15.1)	107.9 (8.8)	107.9 (6.4)
ADHD subtype				
Inattentive	14	14	11	5
Hyperactive/impulsive	0	1	0	2
Combined	11	10	13	19
ADHD symptoms				
DBD-RS Inattention	21.7 (3.2)	22.8 (3.4)	-	-
DBD-RS Hyperactivity	15.0 (5.0)	16.4 (6.3)	-	-
ADHD-SR	-	-	31.8 (9.9)	31.1 (9.7)
Adherence	84% (15%)	80% (18%)	90% (8%)	86 (8%)

[1]For 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

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, the following were missing: for the ER paradigm, 37 were missing due to dropout, missing a session, motion artifacts in MRI data or incomplete understanding of the task (12.6%); for the RI paradigm, 50 scans were missing due to dropout, missing a session, or missing behavioral data, motion artifacts in MRI data or incomplete understanding of the task (17.0%).

fMRI results

Emotion Recognition paradigm

Linear mixed model analyses did not show a significant age x medication x time interaction, neither for left nor for right amygdala reactivity (left: F(2,89)=0.21, p=0.81; right: F(2,91)=0.08, p=0.92), nor a significant time x medication interaction in children (F(2,43)=0.50, p=0.61) or 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). Post-hoc tests revealed decreased right amygdala reactivity in children in the placebo condition from 8 weeks of treatment (DT) to 1 week post treatment (PT) (F(2,1,24)=7.72 p=0.01) that was absent in the MPH group. In contrast, no significant change over time was found in adults (all p>0.05).

Response Inhibition paradigm

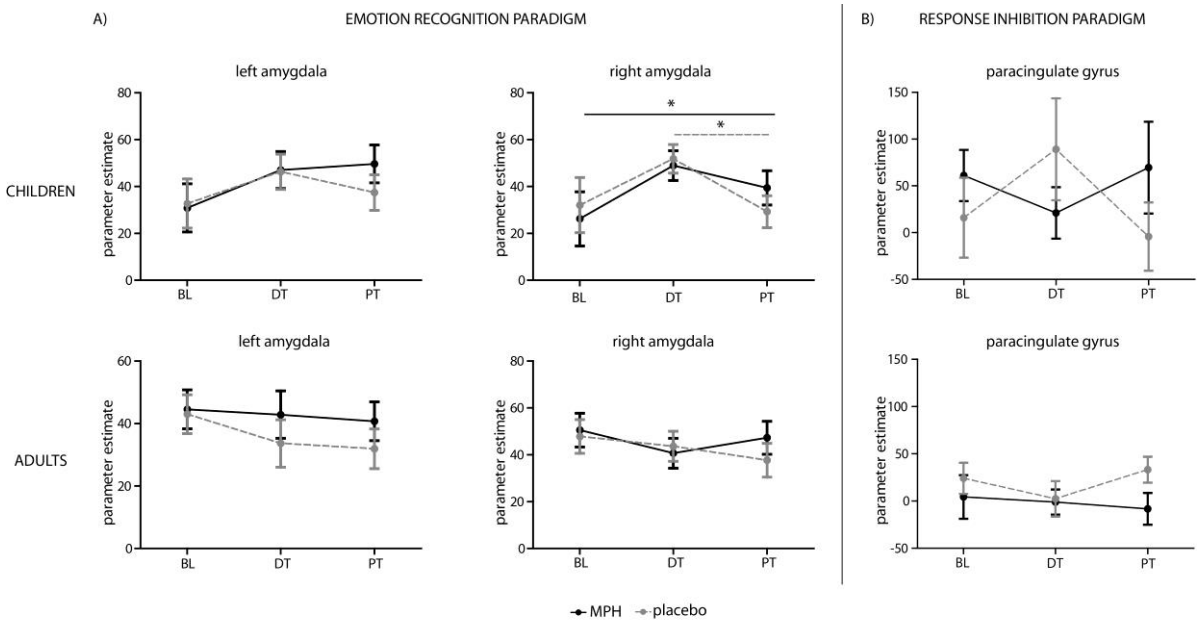
Linear mixed model analysis did not show a significant age x medication x time interaction for the paracingulate ROI reactivity (F(2,87)=2.15, p=0.12), nor significant time x medication interactions in the children (F(2,44)=1.56, p=0.22) or adults (F(2,47)=1.13, p=0.33). No significant two-way interaction effects were observed for time and age (F(2,87)=0.28, p=0.756), nor for time and medication (F(2,87)=0.79, p=0.46). A main effect of age was observed (F(1,87)=4.13, p=0.045), indicating lower reactivity in adults compared to children (Figure 2). Post-hoc tests revealed a non-significant lower paracingulate reactivity in adults PT in the MPH group compared to the adult placebo condition (t(41)=-1.9, p=0.06). No significant change over time was found for adults nor children (all p>0.05).

Connectivity

Emotion Recognition paradigm

Longitudinal analyses per group indicated that MPH decreased connectivity between the amygdala and various brain regions in both age groups in the period from BL to 8 weeks of treatment (DT) (Figure 3 and Supplementary Table I). In children, this reduction in connectivity at DT was widespread including cortical and subcortical areas, whereas in adults, MPH decreased the connectivity mainly in various frontal regions. Subsequent analysis showed that at PT, children treated with MPH showed a return to BL connectivity patterns, whereas MPH-treated adults, connectivity was more widespread and stronger at PT than at BL. Remarkably, children in the placebo group showed an increase in connectivity during treatment that returned to baseline 1 week after discontinuation of the 16 weeks trial.

Figure 2. Activity values for the left and right amygdala during emotion recognition and paracingulate gyrus during response inhibition, for each time point. 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.



Direct statistical comparisons within the two age groups confirmed these observations: children treated with MPH showed a greater reduction in connectivity in the 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 (reaching a return to BL levels) than adults treated with placebo. Additionally, in MPH-treated adults, connectivity was stronger at 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 being involved in MPH-induced connectivity changes in children than adults.

Response Inhibition paradigm

From BL to PT, longitudinal analyses per group indicated an increased connectivity between the (para)cingulate cortex and the right lateral occipital cortex in adults in the MPH, but not placebo condition, nor children (Figure 4 and Supplementary Table I). In children, the interaction between treatment and DT<BL was significant, reflecting a non-significant decrease in (para)cingulate-right insular cortex and (para)cingulate-cortical connectivity in the MPH group, whereas a non-significant increase was present in the placebo condition. In addition, a significant interaction between treatment and DT>PT was observed, due to a non-significant decrease in paracingulate-right putamen and paracingulate-right insular cortex connectivity in the MPH condition, whereas a non-significant increase in connectivity was present in the placebo condition (Figure 4). These effects were not present in adults.

Behavioral symptoms

Linear mixed model analyses failed to reveal 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 emotional 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 for 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 emotion dysregulation ($F(2,45)=13.18$, $p<0.001$) in adults (Figure 5). For emotion 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 in 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 $F(1,24)=25.19$, $p<0.001$; for anxiety: MPH $F(1,25)=5.92$, $p=0.02$; placebo $F(1,24)=17.21$, $p<0.001$). However, in adults, no treatment effects were found for depressive symptoms (MPH $F(1,21)=3.61$, $p=0.07$; placebo $F(1,22)=0.16$, $p=0.69$) nor for anxiety symptoms (MPH $F(1,20)=1.19$, $p=0.29$; placebo $F(1,20)=0.48$, $p=0.50$) from BL to PT (Figure 5). Also, no association between amygdala reactivity and clinical symptoms was found in children nor adults in any of the treatment conditions.

No significant age x medication x time interaction was found for the number of commission errors during the response inhibition paradigm ($F(2,89)=1.64$, $p=0.200$). Furthermore, no medication x time effects were found for either children ($F(2,45)=2.25$, $p=0.117$) or adults ($F(2,43)=0.02$, $p=0.977$). However, a main effect of time was found in both the children ($F(2,45)=4.03$, $p=0.024$) and adults ($F(2,43)=30.03$), $p<0.001$). Main effect of medication in the adults was non-significant ($F(1,48)=3.20$, $p=0.080$) (Figure 6). Post-hoc tests revealed a significant decrease in commission errors in the children treated with MPH from BL to DT ($p=0.014$), but not in the placebo children. In addition, a non-significant increase in commission errors was found for MPH treated children from DT to PT ($p=0.058$). In both the adult treatment groups, the commission errors reduced from BL to DT (both $p<0.001$), but increased from DT to PT (MPH $p=0.005$, placebo $p=0.003$).

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.

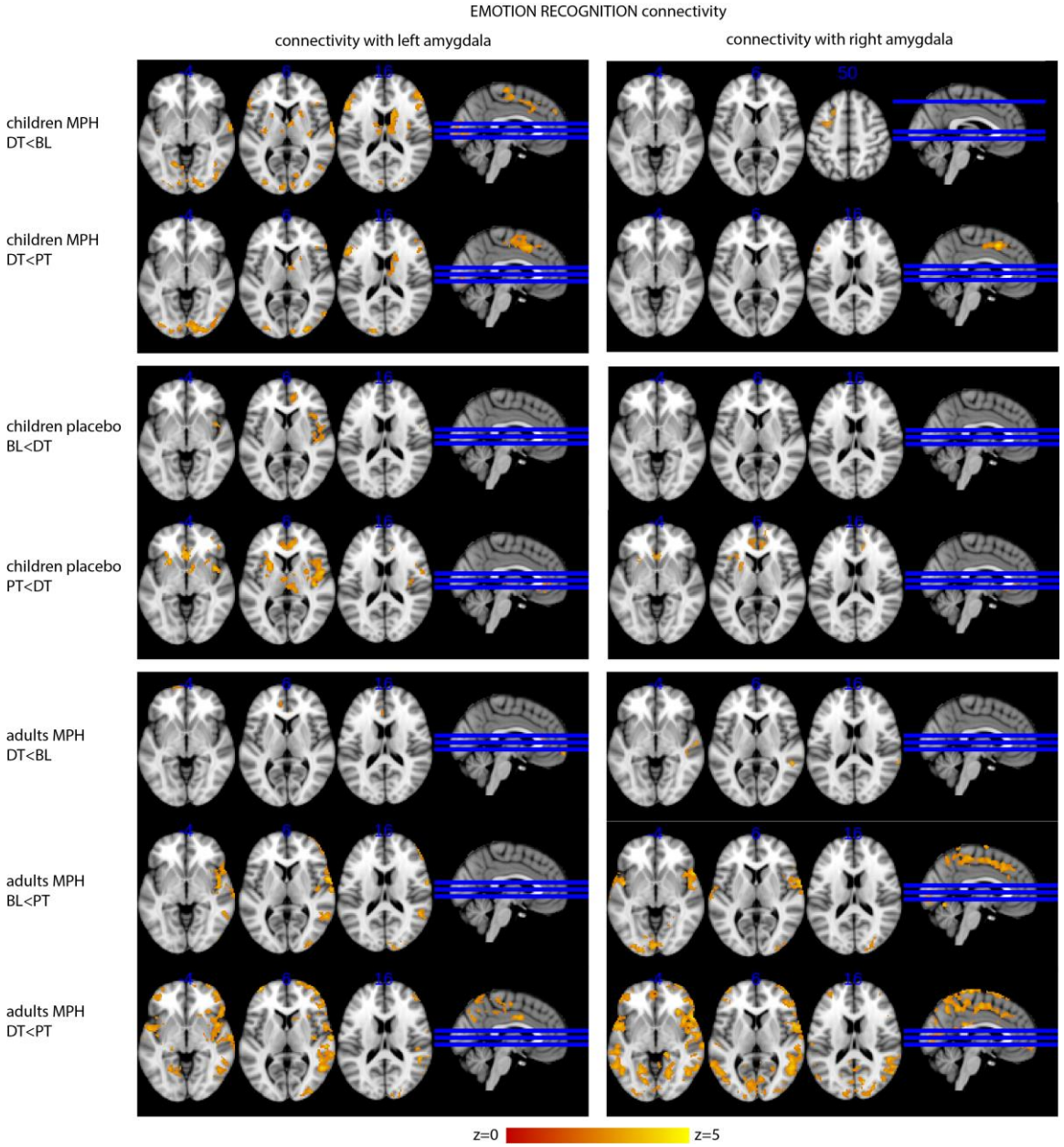


Figure 4. Whole-brain connectivity with the paracingulate gyrus.
Maps were thresholded at z=2.3, gray matter masked and cluster with voxel sizes > 100 are displayed.
BL = baseline; DT = during treatment; PT post-treatment.

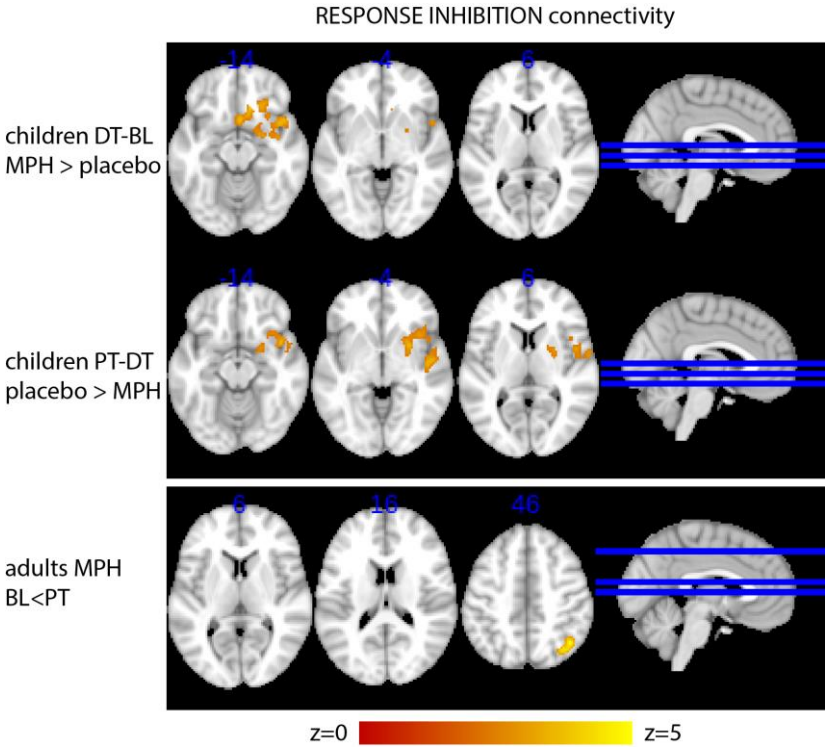


Figure 5. Symptom scores on clinical rating scales for each timepoint.
Top row shows data for the children and bottom row for the adults. BL = baseline; DT = during treatment; PT post-treatment. Data are expressed as mean and standard error of the mean. *p<0.05

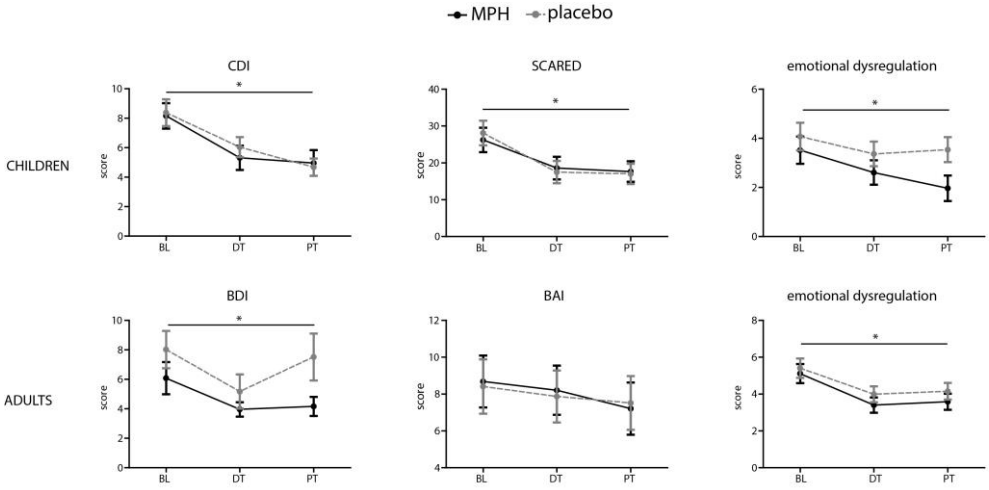
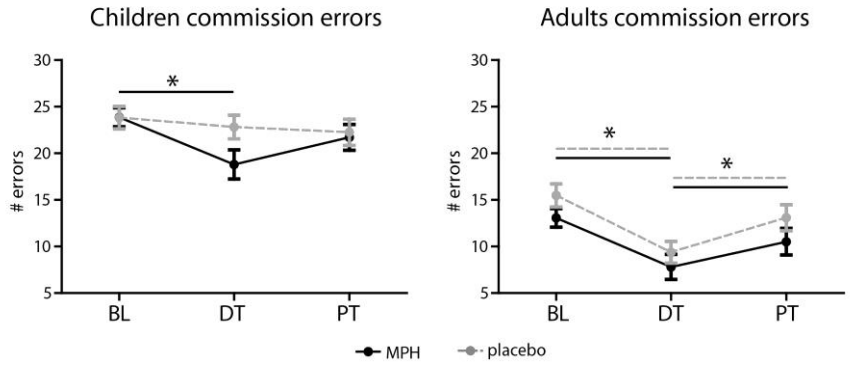


Figure 6. Number of commission errors during the response inhibition paradigm for both the children and adults. BL = baseline; DT = during treatment; PT post-treatment. Data are expressed as mean and standard error of the mean. *p<0.05



Discussion

In this 4 month RCT in stimulant treatment-naive children and adults with ADHD, treatment with MPH affected right amygdala reactivity and connectivity, as well as paracingulate connectivity and emotion dysregulation, in an age-dependent manner.

The significant decrease in amygdala reactivity during emotional processing from 8 weeks of treatment to end of treatment, only in the placebo condition, indicates that MPH treatment might result in lasting increases in amygdala reactivity in children with ADHD. This could be a concern, as a heightened amygdala activation has previously been associated with emotion dysregulation and increased symptoms of anxiety and depression[34,35]. Also, increased connectivity of the amygdala with prefrontal regions has been associated with higher levels of emotion dysregulation[34]. The transient reductions we observed in children and adults therefore suggest positive effects of MPH on functional connectivity during treatment, that disappear after treatment cessation in children. In adults, however, these positive effects persisted at least one week after trial end. Overall, these neurobiological data suggest potentially long-lasting, negative effects of MPH on amygdala reactivity in children, as well as on functional connectivity in adults.

MPH treatment did not affect (para)cingulate reactivity in children nor adults. The literature reports reduced fronto-striatal functional activity in children with ADHD during response inhibition, which normalizes following an acute dose of MPH[11,14,15]. Only one study investigated the effect of MPH on fronto-striatal functional connectivity[14]. In line with this, we also found that four months of

treatment with MPH increased functional connectivity during response inhibition, only in adults. In children, on the other hand, we observed a transient reduction in functional connectivity during MPH treatment, in addition to a reduction in commission errors in week 8. The most likely explanation for the discrepancy, is that in this trial we treated patients, whereas in these prior studies, MRI scans were made 1-2.5 hours after the MPH administration. In a recent meta-regression analysis we also did not find an effect of MPH on executive functioning (during neuropsychological assessment) in studies with medication-naive participants[16].

The effects of MPH on behavioral measures were rather modest: MPH reduced emotion dysregulation in children at trial end, which was absent in the placebo condition and in both adult conditions. Most behavioral assessments improved during the trial when compared to baseline in children, and to a lesser extent also in adults. While this is in line with previous studies on children and adolescents with ADHD[7,8], the fact that both the MPH and placebo condition showed improvement on most of these behavioral measures suggests that this effect is more related with being in treatment per se and not drug dependent.

At first sight, our post-treatment findings in children may seem inconsistent (i.e. an enduring positive effect on emotional dysregulation, and increases in right amygdala reactivity). However, as recently shown in subjects at risk, or after stressful early life events, a heightened amygdala reactivity emerges during adolescence, and generally prior to the emergence of clinical depressive symptoms[35]. Thus, although MPH treatment improved emotional dysregulation, the increase in right amygdala reactivity could be a predictor for long term effects. Indeed, in the MTA trial, an increased occurrence of anxiety and depression was not observed until 6 years after enrolment, but not earlier[20].

Our findings bear considerable clinical relevance, because clinicians tend to delay the prescription of MPH, or hesitate to titrate an adequate dose, as they may fear that MPH may induce depressive symptoms[36]. Here, we show that there is no reason to withhold treatment for this reason, at least on the short-term, as MPH does not exert negative effects on symptoms of emotion regulation, anxiety and depression after 4 months of treatment, neither in children nor in adults. Our findings provide further evidence that the effects of MPH on the human brain are modulated by age: in addition to age-dependent effects of MPH on the dopaminergic system[22], we found that the effects of MPH on functional readouts are also modulated by age. However, it remains to be established what the long-term effects of MPH treatment are on children regarding the development of emotional behavior and impulsivity. Follow up studies of this RCT will have to

point out whether unwanted side-effects of MPH treatment on emotional behavior on the long-term are indeed more pronounced in children than adults, which the amygdala reactivity fMRI data predict[37].

A strength of the current study is its design. To rule out the influence of earlier medication, we only included stimulant treatment-naive patients. For ethical reasons, we could not extend the follow-up period to more than 4 months. Another limitation of our RCT is that we did not include healthy controls, and it is e.g. unknown whether the amygdala reactivity we found at baseline, was heightened in our sample compared to the general population. Other limitations of our study are that the results cannot be extrapolated to all children and adults with ADHD, because we only studied male subjects within a restricted age range. In addition, the (ratio between the) subtypes of ADHD differed slightly between our cohorts of children and adults, which might be due to the developmental trajectories in ADHD and the current age of inclusion; children with ADHD are usually diagnosed and treated at a younger age, and this may bias towards a selection of more inattentive types of ADHD at the age of 10-12 years, i.e. the age we selected for inclusion. The increased prevalence of the combined type of ADHD in adults may possibly reflect a selection bias, as adult patients could refer themselves to the clinic instead of being referred by their general practitioner, as is often the case for children; this is a normal procedure in the Netherlands. Nevertheless, by comparing amygdala reactivity between children and adult patients with ADHD, the differences in subtype might be a confounding factor, which cannot be ruled out. On the other hand, studies of clinical childhood ADHD samples have not yielded many participants who met adult ADHD criteria, and evidence is emerging to suggest that also adults presenting with ADHD symptoms do not suffer from a childhood onset neurodevelopmental disorder, but may rather represent a different subtype of the disorder[37]. This could be accounted for in future studies.

Conclusion

Four months of treatment with MPH affected right amygdala reactivity, amygdala and paracingulate connectivity and emotion dysregulation in an age-dependent manner. Longer follow up studies are needed to investigate whether unwanted side-effects of MPH treatment on emotional behavior will persist in children, and to a lesser extent also in adults. Such studies should also investigate in adults whether the lasting positive effects of MPH treatment on functional connectivity during response inhibition persist. The lasting positive effects of MPH

on emotion dysregulation scores should reassure parents and clinicians when discussing the prescription of MPH to children, at least on the short term.

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Supplementary Material

fMRI paradigm

Subjects performed an emotion recognition and response inhibition fMRI paradigm at 3 different time points during the trial (Figure 1). To further minimize learning effects, a practice run was presented prior to the first MRI scan. Two versions of the tasks were used to overcome learning effects.

The emotion recognition paradigm consisted of a blocked design and was adapted from a task previously used to assess drug effects on amygdala reactivity. 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.

The response inhibition paradigm consisted of an event-related design and was adapted from a go-no/go task previously used in ADHD patients. In each run, 57 trials were presented of which 25% no-go trials in a pseudo-random order. Stimulus duration was 500 ms with an interstimulus interval of 3500 ms. Patients were asked to respond as fast as possible to visual stimuli using a button-press, but to withhold their response to a specific stimulus. Characters of the Pokémon cartoon series were used as stimuli in order to make the task more accessible for children. The task consisted of 3 runs, each lasting 3 min and 56 s.

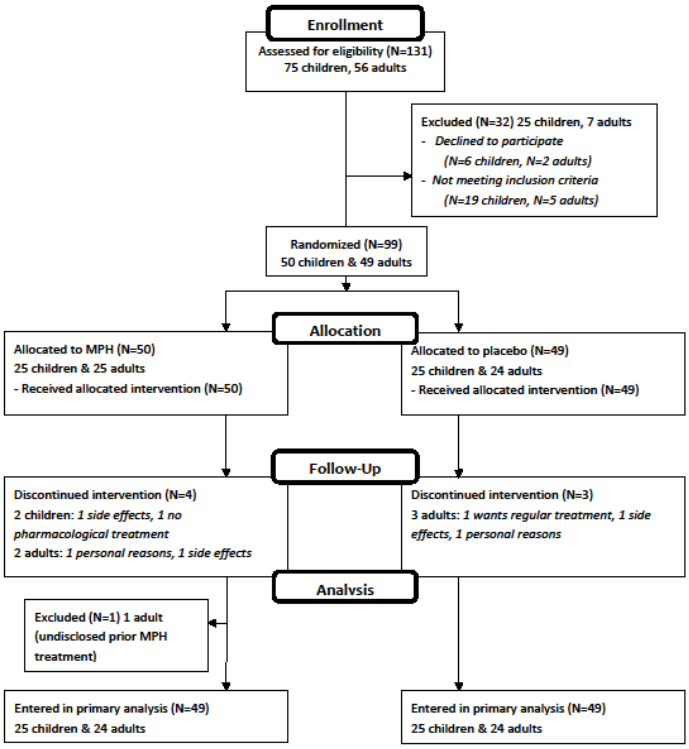
MRI acquisition

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 Parameters were: TR/TE=2300/30 ms, resolution=2.3×2.3×3 mm, 39 sequential slices, FOV=220x220x117 mm, GE-EPI read-out, no gap, 80° flip angle. For the emotion recognition task, 70 dynamics were used, and for the response inhibition task, 263 dynamics were used.

MRI preprocessing

Data were preprocessed and analyzed using in-house MATLAB scripts (Massachusetts: The Matworks Inc.) and FSL 5.0 (FMRIB's Software Library) 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 [38,39]) were removed from the analysis.

Supplementary Figure 1. Consortium figure.



Supplementary Table I.

	seed	brain area	# of voxels	max Z-value	MNI coordinates		
					X	Y	Z
EMOTION RECOGNITION							
BL > DT							
children MPH	left amygdala	precentral gyrus	3643	4.59	-32	-10	66
		caudate nucleus	1782	4.31	20	16	10
		occipital pole	957	4.11	20	-90	-4
		superior frontal gyrus	804	4.56	20	2	56
		planum temporale	670	4.4	66	-6	4
		inferior frontal gyrus	549	3.91	-52	22	12
		putamen	219	3.97	-24	-2	12
		thalamus	167	3.55	-10	-4	10
	right amygdala	superior frontal gyrus	581	3.6	-24	8	56
adults MPH	left amygdala	frontal pole	135	3.4	-14	64	-12
		anterior cingulate cortex	101	3.55	-10	36	12
	right amygdala	superior temporal gyrus	312	3.76	60	-14	-8
DT > BL							
children placebo	left amygdala	insular cortex	487	3.4	44	6	-6
		paracingulate gyrus	130	3.62	14	45	2
TT > PT							
children placebo	left amygdala	central opercular cortex	1399	4.18	50	-2	2
		subcallosal cortex	573	3.9	-4	22	-6
		putamen	512	4.36	-24	18	0
		thalamus	408	3.89	8	-2	2
		frontal orbital cortex	211	3.42	22	20	-10
		precuneus cortex	164	3.07	22	-58	24
		subcallosal cortex	615	3.82	-4	22	-6
		putamen	252	3.21	-24	20	0
PT > TT							
children MPH	left amygdala	superior frontal gyrus	4876	4.66	20	0	54
		occipital pole	1069	3.84	28	-90	8
		inferior frontal gyrus	503	4.08	-54	20	22
		caudate nucleus	299	3.39	14	20	22
	right amygdala	paracingulate gyrus	2391	4.89	-4	12	46
		precentral gyrus	649	4.04	44	-6	60
adults MPH	left amygdala	middle temporal gyrus	4413	4.6	50	-56	8
		anterior cingulate cortex	1805	3.82	2	-2	34
		insular cortex	700	4.36	-38	-10	-8
		angular gyrus	352	3.55	-46	-54	44
		lingual gyrus	292	4.18	-14	-62	-6
		frontal pole	291	4.41	-38	60	4
		precentral gyrus	188	3.24	42	0	4

Supplementary Table I (continued).

	seed	brain area	# of voxels	max Z-value	MNI coordinates X	Y	Z
	right amygdala	occipital fusiform gyrus	8650	5.04	-32	-72	-12
		lateral occipital cortex	7791	4.94	-6	-66	64
		frontal pole	897	4.91	-40	46	-10
		temporal pole	490	3.83	-56	12	-10
		posterior cingulate cortex	241	3.92	10	-42	28
		frontal medial cortex	217	3.49	12	46	-10
		superior frontal gyrus	161	3.91	-24	8	68
PT > BL							
adults MPH	left amygdala	central opercular cortex	746	4.43	62	2	6
		superior parietal lobe	382	3.45	32	38	62
		middle temporal gyrus	365	4.07	52	-46	12
		lateral occipital cortex	299	3.91	14	-62	56
		middle frontal gyrus	296	3.87	30	2	54
		lateral occipital cortex	286	4.06	42	-84	2
		precentral gyrus	204	3.77	28	-22	66
		frontal pole	169	3.43	52	40	18
	right amygdala	occipital fusiform gyrus	8650	5.04	-32	-72	-12
		lateral occipital cortex	7791	4.94	-6	-66	64
		frontal pole	897	4.91	-40	46	-10
		temporal pole	490	3.83	-56	12	-10
		posterior cingulate cortex	241	3.92	10	-42	28
		frontal medial cortex	217	3.49	12	46	-10
		superior frontal gyrus	161	3.91	-24	8	68
RESPONSE INHIBITION							
DT > BL							
children MPH > children placebo	paracingulate qyrus	cerebral cortex	497	4.11	46	12	-18
		subcallosal cortex	196	4.7	12	24	-12
PT > DT							
children placebo> children MPH	paracingulate qyrus	cerebral cortex	1374	3.69	46	12	-18
PT > BL							
adults MPH	paracingulate gyrus	cerebral cortex	223	5.22	42	-62	46