Structural and functional connectivity in children and adolescents with and without attention deficit/hyperactivity disorder

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Background: Attention deficit/hyperactivity disorder (ADHD) has frequently been associated with changes in resting-state functional connectivity, and decreased white matter (WM) integrity. In the current study, we investigated functional connectivity within Default Mode and frontal control resting-state networks (RSNs) in children with and without ADHD. We hypothesized the RSNs of interest would show a pattern of impaired functional integration and segregation and corresponding changes in WM structure. Methods: Resting-state fMRI and diffusion-weighted imaging data were acquired from 35 participants with ADHD and 36 matched typically developing peers, aged 6 through 18 years. Functional connectivity was assessed using independent component analysis. Network topology and WM connectivity were further investigated using graph theoretical measures and tract-based spatial statistics (TBSS). Results: Resting-state fMRI analyses showed increased functional connectivity in right inferior frontal gyrus (IFG), and bilateral medial prefrontal cortex (mPFC) within the Default Mode and frontal control networks. Furthermore, a more diffuse spatial pattern of functional connectivity was found in children with ADHD. We found no group differences in structural connectivity as assessed with TBSS or graph theoretical measures. Conclusions: Resting-state networks show a more diffuse pattern of connectivity in children with ADHD. The increases in functional connectivity in right IFG and bilateral mPFC in children with ADHD may reflect reduced or delayed functional segregation of prefrontal brain regions. As these functional changes were not accompanied by changes in WM, they may precede the development of the frequently reported changes in WM structure. Keywords: Attention deficit/hyperactivity disorder; functional connectivity; structural connectivity; Default Mode Network; developmental delay.

Introduction

Attention deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, characterized by hyperactive, impulsive, and/or inattentive behaviors that impair the individual’s performance in multiple settings (American Psychiatric Association, 2013).

In the past decade, ADHD research has increasingly focused on brain connectivity and has yielded heterogeneous results: during rest, reduced functional connectivity of the Default Mode Network (DMN) has often been reported (Fair et al., 2010; Posner, Park, & Wang, 2014; Qiu et al., 2011), as well as more complicated patterns of increased and decreased connectivity between the DMN and executive control networks (Cao et al., 2009; Fair et al., 2010, 2012; Qiu et al., 2011; Sun et al., 2012) and within limbic and affective systems (Cao et al., 2009; Castellanos, Kelly, & Milham, 2013; Posner et al., 2013; Tian et al., 2006). Studies of the white matter (WM) structure underlying these functional networks have shown similar heterogeneous findings: global WM volume has most often been reported to be decreased, but regional increases and decreases in fractional anisotropy (FA), a commonly used measure of WM microstructural organization or integrity, have been observed throughout the brain (reviewed in van Ewijk, Heslenfeld, Zwiers, Buiterlaar, & Oosterlaan, 2012). Most region-of-interest studies have focused on changes in frontostriatal systems because of their role in cognitive control and reward processing (Castellanos & Proal, 2012; Durston, van Belle, & de Zeeuw, 2011). Yet reports of changes in other tracts again suggest there may be more widespread network changes in ADHD.

Many of these findings have been interpreted to reflect an immature pattern of functional connectivity, in line with the maturational delay hypothesis of ADHD (El-Sayed, Larsson, Persson, Santosh, & Rydelius, 2003; Rubia, 2007; Shaw et al., 2007). One of the most notable studies to date showed that ADHD was associated with a trajectory of cortical maturation seemingly similar to controls, but that peak cortical thickness in areas associated with cognitive control and attention was reached up to 5 years later in children with ADHD (Shaw et al., 2007). In terms of connectivity, it has been hypothesized that ADHD is associated with impairments in functional network segregation (as local connections...
Defiant disorder; ADHD, attention deficit/hyperactivity disorder; CBCL, Child Behavior Checklist; IQ, intelligence quotient; ODD, oppositional–compliance disorder.

Use of psychostimulant medication 27/35
Comorbid disruptive disorders (ODD) 4/35
- CBCL externalizing problems
- CBCL internalizing problems
- Costello, Edelbrock, & Costello, 1985). The children with ADHD that were on stimulant medication were instructed not to take their medication 24 hr prior to the MRI scan.
- No other forms of psychoactive medication (see Table 1). No other forms of psychoactive medication were included in this study. The children with ADHD that were on stimulant medication were instructed not to take their medication 24 hr prior to the MRI scan.

Method

Participants

Thirty-five right-handed children and young adolescents with a primary diagnosis of ADHD were recruited through the Department of Psychiatry at the University Medical Center in Utrecht, and through advertising. The clinical diagnosis was confirmed by a qualified researcher using the Diagnostic Interview Schedule for Children – Parent Version (DISC-P; Costello, Edelbrock, & Costello, 1985). The children with ADHD were either medication naïve or using psychostimulant medication (see Table 1). No other forms of psychoactive medication were included in this study. The children with ADHD that were on stimulant medication were instructed not to take their medication 24 hr prior to the MRI scan.

Thirty-six typically developing right-handed subjects were recruited through local primary schools (Bos et al., 2014). All subjects were screened by phone interview, to confirm the absence of major neurological or psychiatric disorders, as well as the absence of psychiatric conditions in their first-degree relatives. None of the control subjects were using any form of psychoactive medication. Parents of the typically developing subjects also participated in a DISC-P interview session to confirm the absence of any psychiatric condition in the subject.

Subjects were matched for age, gender, Tanner stage, and socioeconomic status (see Table 1). Tanner stage was assessed in order to preclude group differences in pubertal development. Intelligence quotient was assessed for all participants using the abbreviated version of the Wechsler Intelligence Scale for Children (WISC-III; Kort et al., 2005), using four subtests. Finally, the parent-rated Child Behavior Checklist (CBCL) was collected for all participants to measure the severity of ADHD symptoms. Differences on demographic variables were assessed using the appropriate Students’ t- or chi-square tests.

The study was approved by the Ethics Committee of the University Medical Centre Utrecht, The Netherlands, and took into account the ethical principles for medical research involving human subjects as stated in the declaration of Helsinki (amendment of Washington, 2002). Written and oral information was provided, after which written informed consent was obtained from all parents. All children and adolescents provided written and/or verbal assent.

MRI acquisition

All participants aged 12 years or under participated in a mock MRI-scanner practice session in our laboratory, in order to reduce potential anxiety and the risk of motion artifacts (Durston et al., 2009). Children only participated in the actual MRI scan after a successful practice session.

Data were acquired using a 3.0T Philips Achieva MR scanner (Philips Medical Systems, Best, The Netherlands). First, a high-resolution (T1)-weighted image was acquired (TR = TE = 10/4.6, flip angle = 8°, matrix 304 × 309, voxel size 0.75 × 0.75 × 0.8). Furthermore, two diffusion weighted imaging (DWI) sets of 30 weighted diffusion scans (b = 1,000 s/mm²) and five unweighted B0 scans (b = 0 s/mm²) were acquired, of which the second diffusion set was acquired with a reversed k-space read-out direction (DWI-MR using parallel imaging SENSE p-reduction 3, TR/TE = 7,035/68, EPI factor 35; FOV 240 × 240 mm, voxel size 2 mm isotropic, no gap, 75 slices). During the acquisition of the T1- and diffusion-weighted images, all participants watched a movie.

Finally, resting-state functional images were collected in a single block of 294 dynamics with a 2D-EPI SENSE sequence (TR/TE = 2,000/35, flip angle = 70°, matrix 68 × 66, voxel size 3 × 3 × 3.5), with a total duration of 10 min. Participants were instructed to focus on a fixation cross during the procedure.

Resting-state analyses: independent component analysis

Resting-state fMRI data were preprocessed using SPM8 (Wellcome Dept. of Cognitive Neurology, www.fil.ion.ucl.ac.uk) using a standard preprocessing pipeline (Bos et al., 2014).

Table 1 Demographic and clinical characteristics of the sample

<table>
<thead>
<tr>
<th></th>
<th>ADHD (N = 35)</th>
<th>TDC (N = 36)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age, mean ± SD (range)</td>
<td>11.2 ± 2.6 (7.5–16.4)</td>
<td>12.1 ± 2.2 (6.4–15.4)</td>
<td>.111</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>26/9</td>
<td>29/7</td>
<td>.527</td>
</tr>
<tr>
<td>Total IQ, mean ± SD (range)</td>
<td>105.5 ± 15.9 (83–141)</td>
<td>109.6 ± 16.4 (80–145)</td>
<td>.285</td>
</tr>
<tr>
<td>Parental education (years), mean ± SD</td>
<td>13.1 ± 4.0</td>
<td>14.3 ± 2.0</td>
<td>.131</td>
</tr>
<tr>
<td>Tanner stage, mean ± SD</td>
<td>1.8 ± 1.3</td>
<td>2.2 ± 1.4</td>
<td>.148</td>
</tr>
<tr>
<td>CBCL attention problems T-score, mean ± SD</td>
<td>64.7 ± 6.7</td>
<td>52.4 ± 2.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CBCL internalizing problems T-score, mean ± SD</td>
<td>54.6 ± 9.1</td>
<td>45.8 ± 8.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CBCL externalizing problems T-score, mean ± SD</td>
<td>58.6 ± 9.5</td>
<td>41.5 ± 7.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Comorbid disruptive disorders (ODD)</td>
<td>4/35</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Use of psychostimulant medication</td>
<td>27/35</td>
<td>–</td>
<td></td>
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ADHD, attention deficit/hyperactivity disorder; CBCL, Child Behavior Checklist; IQ, intelligence quotient; ODD, oppositional defiant disorder; SD, standard deviation; TDC, typically developing children.

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Twelve children with ADHD and two typically developing children (TDC) were excluded due to excessive motion (Table S1). There were no between-group differences in absolute subject motion \(F(53, 3) = 0.777, p = .512\), framewise displacement or the number of frames repaired (Table S2). Resting-state preprocessing and motion correction are described in detail in the Appendix S1.

Functional connectivity was assessed in FSL (Smith et al., 2004) using independent component analysis by means of the multivariate exploratory linear decomposition into a fixed set of 25 independent components (MELODIC; Beckmann, DeLuca, Devlin, & Smith, 2005; Beckmann & Smith, 2004), followed by a dual regression approach (Filippini et al., 2009).

**Selection of networks.** Five components were discarded from statistical analysis as they represented nonneuronal noise, such as cerebrospinal fluid and WM. The remaining 20 components were classified as containing BOLD connectivity of interest (Figures S1 and S2). For the within-network statistical analyses, we selected nine components (Figure 1A) that showed spatial similarity to previously described resting-state networks (RSNs; Laird et al., 2011) including frontal and striatal regions of interest, and the DMN.

Between-group differences in within-network functional connectivity in the nine selected RSNs were tested using a general linear model with 5,000 permutations in Randomise (FMRIB Software Library Randomise v2.9), with age, gender, and...
voxelwise gray matter density as covariates in all initial analyses. As there was no significant effect of gender, this covariate was consequently removed from the design. By including the individual gray matter density maps as a covariate, the results were corrected for differences in gray matter density and possible misregistrations (Oakes et al., 2007). If a between-group effect was found, the relation between functional connectivity and age was further explored within that network, with gender and voxelwise gray matter density as covariates. All results were corrected for multiple comparisons, using familywise error (FWE) at \( p < .05 \) and threshold-free cluster enhancement (TFCE; Smith & Nichols, 2009). All group comparisons were masked by group main effects (i.e. those voxels that fell within the TDC and/or ADHD group-map), to restrict analyses to connectivity within the RSNs of interest. Group main effects were obtained by means of nonparametric estimation of the group means – again with age and voxelwise gray matter density as nuisance variables, using 5,000 permutations in Randomise at a FWE-threshold of \( p < .05 \), using TFCE. Exploratory analysis of between-group differences in the size of the RSNs of interest were performed by calculating the average number of voxels with a \( Z > 3.1 \), corresponding to \( p = .001 \) uncorrected.

**Diffusion-weighted analyses**

Preprocessing steps for graph theoretical analysis of the diffusion-weighted data are described in detail in Appendix S1. Clustering coefficient \( (\mathcal{C}) \) and characteristic path length \( (\mathcal{L}) \) of the FA-weighted matrices were calculated using the Brain Connectivity Toolbox (Rubinov & Sporns, 2010). To correct for differences in topology based on the number of edges in the connectivity matrix, a randomization procedure was performed. For each subject, 1,000 random matrices with preserved degree sequence and an identical collection of connection weights were formed. From these randomized matrices, individual \( \mathcal{L}_{\text{random}} \) and \( \mathcal{C}_{\text{random}} \) values were computed by averaging over the trials. The normalized characteristic path length lambda \( (\lambda) \) was calculated as: \( \lambda = \mathcal{L}/\mathcal{L}_{\text{random}} \). The normalized clustering coefficient gamma \( (\gamma) \) was calculated as: \( \gamma = \mathcal{C}/\mathcal{C}_{\text{random}} \).

Statistical analyses were performed using the R statistical software package (www.r-project.org). Averages and between-group differences in mean and regional FA and mean diffusivity (MD), and (node-specific) graph theoretical measures were analyzed using a linear regression model with age as covariate.

Voxelwise statistical analysis was performed using tract-based spatial statistics (TBSS) within FSL (www.fmrib.ox.ac.uk/fsl; Smith et al., 2004; Appendix S1). Group differences in voxelwise structural connectivity (FA and MD) were tested using a general linear model with 5,000 permutations in Randomise, with age as covariate in all analyses. Results were corrected for multiple comparisons, using FWE at \( p < .05 \) and TFCE (Smith & Nichols, 2009).

**Results**

**Group functional network characteristics**

There were no between-group differences in between-network correlations (Figure S2). Nine components were selected for analysis of within-network connectivity. For both groups, these components included core regions of: the DMN (IC1, IC5, IC9, and IC19), the right- (IC4), left- (IC11), bilateral- (IC18), and frontal- (IC17) executive networks, and a striatal network (IC22; Figure 2A). More elaborate slice intersections are shown in Figure S1.

**Within-network functional connectivity in ADHD**

In ADHD, connectivity within the DMN (IC5) was increased in the right inferior frontal gyrus (IFG; Figure 1B: peak cluster \( T \text{value} = 5.67, \ p_{FWE} = .002 \), MNI-coordinates: \( x = 33, y = 23, z = -4.5, k = 28 \)). These results persisted after correction for gray matter density (peak cluster \( T \text{value} = 6.29, \ p_{FWE} = .001, k = 27 \)). When inspecting the group main effects, the right IFG cluster was not present in the TDC, whereas it was in the children with ADHD.

Furthermore, connectivity was increased in ADHD in an anterior DMN network (IC19) in the bilateral, but mostly right medial prefrontal cortex (mPFC; Figure 1C: peak cluster \( T \text{value} = 4.60, \ p_{FWE} = .004 \), MNI-coordinates: \( x = -6, y = -31, z = 41, k = 4 \)), which also did survive correction for gray matter density (IC1: peak cluster \( T \text{value} = 5.00, \ p_{FWE} = .02, k = 9 \)). There were no between-group differences in connectivity in any of the other selected RSNs of interest. Functional connectivity in the significant clusters in IC1, IC5, and IC19 was not correlated with measures of attention as assessed by the CBCL.

**Structural connectivity in ADHD**

Analysis of regional differences in FA or MD using TBSS yielded no significant results after FWE-correction for multiple comparisons. The graph theory approach similarly returned no differences in average FA or MD that survived false discovery rate (FDR)-correction for multiple comparisons. Furthermore, there were no between-group differences in average (normalized) clustering coefficient \( C/\gamma \) and (normalized) path length \( L/\lambda \), or node-specific differences that survived FDR-correction. Explorative correlations between the extracted Z-values of functional connectivity in the rIFG and bilateral mPFC and structural measures of node-specific normalized clustering coefficient \( \gamma \) in the right pars opercularis and pars triangularis, and bilateral medial orbitofrontal cortex returned no significant results.

**Effects of age on connectivity in ADHD**

Within IC5, functional connectivity showed a negative relationship with age \( (r = -.527, \ p = .001) \) in two small clusters in the bilateral posterior cingulate cortex (PCC) in all children (Figure 2A,B: peak cluster \( T \text{value} = 4.45, \ p_{FWE} = .006 \), MNI-coordinates: \( x = -12, y = -49, z = 9.5, k = 85 \)). Furthermore, functional connectivity of bilateral mPFC was negatively correlated with age in children.
with ADHD \( (r = -0.529, p = 0.009) \) and TDC \( (r = -0.379, p = 0.027) \); Figure 2C,D: peak cluster \( T \) value \( =6.20, \) \( p_{\text{FWE}} = <0.001, \) MNI-coordinates: \( x = -6, y = 2, z = -4.5, k = 2,436 \) in an area that largely overlapped with the group main effect in IC19.

Analyses of changes with age showed an increase in FA with age \( [\text{TBSS: } F(1, 70) = 36.5, p < 0.001; \text{Graph: } F(1, 70) = 9.4, p = 0.003] \), and decreases in MD \( [\text{TBSS: } F(1, 70) = 19.1, p < 0.001; \text{Graph: } F(1, 70) = 24.1, p < 0.001] \) (Figure 3). The mean values for FA and MD that were derived from the two different analysis techniques (i.e. TBSS and graph analysis) were highly correlated \( (r = 0.743, p < 0.001 \text{ and } r = 0.884, p < 0.001, \) respectively).

**Spatial characteristics of functional networks in ADHD**

While core regions of all networks of interest overlapped between children with and without ADHD, exploration of between-group differences showed a significantly higher number of voxels included in the network in two frontal RSNs in children with ADHD:

- IC17 \( [F(55, 1) = 5.02, p = 0.029] \) and IC19 \( [F(55, 1) = 4.28, p = 0.043] \) (Figure 1D).

**Discussion**

In this study, we investigated both functional and structural connectivity of frontal control and DMNs in young children and adolescents with ADHD, compared to their typically developing peers. We found that there were increases in functional connectivity in prefrontal regions, and more widespread connectivity throughout the brain, in keeping with the delayed maturation hypothesis of ADHD. These changes in functional connectivity were not accompanied by changes in the underlying WM structure.

We found an increase in connectivity of right IFG and mPFC in ADHD within the DMN. IFG has been frequently implicated in ADHD and is a key region involved in cognitive and behavioral control (Aron & Poldrack, 2005). Increased nodal efficiency of (right) IFG has previously been reported in children with ADHD and it has been suggested that this may...
reflect greater effort in this region to achieve the same level of performance as typically developing controls (Wang et al., 2009). In addition, ADHD is associated with ‘attentional lapses’ related to inadequate suppression (i.e. interference) of the DMN during goal-directed behavior (Castellanos et al., 2008; Kelly, Uddin, Biswal, Castellanos, & Milham, 2008; Sonuga-Barke & Castellanos, 2007). However, while the recruitment of right IFG into DMN may point toward inadequate modulation between task-positive and task-negative networks, we did not find any evidence for such changes in between-network connectivity in the current study.

Furthermore, the recruitment of right IFG and increased connectivity of mPFC in DMN may reflect reduced functional network segregation in ADHD. In line with previous studies, we also found that mPFC connectivity decreased with age in all children (Betzel et al., 2014; Fair et al., 2009; Van Duijvenvoorde, Achterberg, Braams, Peters, & Crone, 2015). Nevertheless, on average, connectivity in mPFC remained higher in children with ADHD. Such an increase in connectivity in a region where typically a decrease with age would be expected could again be a sign of reduced functional network segregation (Fair et al., 2010; Sripada, Kessler, Fang, et al., 2014). However, whether the differences in functional connectivity observed here indeed point toward a maturational delay in prefrontal brain regions during childhood in ADHD remains to be determined as recent findings have suggested that increased connectivity in prefrontal areas may persist into adulthood (Mostert et al., 2016).

There may be multiple explanations for the observed dissociation between structural and functional network organization in this study. For one, our findings may be limited by the methods applied. At a more lenient statistical threshold, we did find differences in WM structure in regions frequently implicated in ADHD. However, these results did not survive correction for multiple comparisons and were therefore not reported. In this case, our sample size may have been too small to detect the subtle developmental differences that are associated with ADHD. During childhood and adolescence, the brain is exposed to a dynamically changing environment. While brain development is highly coordinated, great variability between individuals is still found (Walhovd, Tamnes, & Fjell, 2014). Subsequently, between-group differences in structural connectivity may have been obscured by the many developmental processes at play.

Furthermore, even though brain structure is thought to be highly predictive of function, there is not a one-to-one relationship between them (Honey, Thivierge, & Sporns, 2010; Honey et al., 2009; van den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009). During development, the relation between structure

Figure 3 For the entire sample, fractional anisotropy (FA) (panel A and C) showed an increase with age, whereas MD (panel B and D) decreased with age. There were no between-group differences in mean FA or MD between children with attention deficit/hyperactivity disorder (ADHD) and typically developing children, as assessed with tract-based spatial statistics (TBSS) or graph theoretical measures (Jolles, van Buchem, Crone, & Rombouts, 2011). As such, the more widespread pattern of functional connectivity in ADHD could indeed reflect more immature functional network development (Sato, Hoexter, Castellanos, & Rohde, 2012; Sripada, Kessler, Fang, et al., 2014). However, whether the differences in functional connectivity observed here indeed point toward a maturational delay in prefrontal brain regions during childhood in ADHD remains to be determined as recent findings have suggested that increased connectivity in prefrontal areas may persist into adulthood (Mostert et al., 2016).
Attention deficit/hyperactivity disorder (ADHD) has frequently been associated with changes in resting-state network connectivity, as well as decreased white matter (WM) integrity. We found increased connectivity in right inferior frontal gyrus and bilateral medial prefrontal cortex in children and adolescents with ADHD, without corresponding group differences in WM structure. Resting-state networks showed a more diffuse pattern of connectivity in children with ADHD, which together with increases in prefrontal functional connectivity in ADHD may reflect reduced or delayed functional network segregation of prefrontal brain regions. The delay in functional network development may be associated with heightened Default Mode Network interference and attentional lapses in ADHD.
References


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