Neural Correlates of Visuospatial Working Memory in ADHD and Controls

Under review as:

ABSTRACT

BACKGROUND Impaired visuospatial working memory (VSWM) is suggested to be a core neurocognitive deficit in Attention-Deficit/Hyperactivity Disorder (ADHD), yet the underlying neural activation patterns are poorly understood. Furthermore, it is unclear to what extent age and gender effects may play a role in VSWM-related brain abnormalities in ADHD.

METHODS Functional magnetic resonance imaging (fMRI) data were collected from 109 individuals with ADHD (60% male) and 103 controls (53% male), aged 8-25 years, during a spatial span VSWM task.

RESULTS VSWM-related brain activation was found in a widespread network, which was more widespread compared with N-back tasks used in the previous literature. Higher brain activation was associated with higher age and male gender. Compared with controls, individuals with ADHD showed greater activation in the left inferior frontal gyrus (IFG) and lateral frontal pole during memory load increase, explained by reduced activation during the low memory load in the IFG pars triangularis, and increased activation during high load in the IFG pars opercularis. Age and gender effects did not differ between subjects with and without ADHD.

CONCLUSION Results indicate that individuals with ADHD have difficulty to efficiently and sufficiently recruit left inferior frontal brain regions with increasing task difficulty.
INTRODUCTION

Impaired working memory is suggested to be a core neurocognitive deficit in Attention-Deficit/Hyperactivity Disorder (ADHD) (Martinussen, Hayden, Hogg-Johnson, & Tannock, 2005; Willcutt et al., 2005), which is most pronounced on tasks that tap into the spatial domain (Martinussen et al., 2005). While several studies have investigated the neural correlates of working memory in ADHD using functional magnetic resonance imaging (fMRI) (Cortese et al., 2012), these studies typically used non-spatial working memory tasks. In contrast, the literature on the neural correlates of visuospatial working memory (VSWM) in ADHD is surprisingly scarce.

In healthy controls, VSWM is reported to be controlled by several (pre)frontal and parietal brain regions, including the dorso- and ventrolateral prefrontal cortex, premotor cortex, posterior parietal cortex, cingulate cortex, and intra-parietal sulcus (Klingberg, 2006; Kwon, Reiss, & Menon, 2002; Nelson et al., 2000; Wager & Smith, 2003). To our knowledge, only two fMRI studies of VSWM compared individuals with ADHD to controls. One study in ADHD showed higher activation for adults with ADHD in the left supplementary motor area in a low memory load condition (Ko et al., 2013), while the other reported higher activation in children with ADHD in the dorsolateral prefrontal cortex and posterior cingulate cortex during high, but not low, memory load (Bédard et al., 2014). Both studies reported no group differences in activation during memory load increase (high versus low memory load) and no differences on any of the behavioural measures. Together, these findings suggest that individuals with ADHD may have difficulty recruiting prefrontal regions efficiently to obtain performance levels similar to controls.

Importantly, both fMRI VSWM studies in ADHD used a visuospatial N-back task, while most behavioural studies of VSWM use spatial span tasks. At the behavioural level, performance on N-back and span tasks is only weakly correlated, and the construct validity of the N-back task as a measure of working memory has not conclusively been established, as opposed to span tasks. Researchers have been discouraged to treat the two tasks as measuring the same construct, or to generalize results on underlying brain circuitries from N-back studies to the concept of working memory span (Kane, Conway, Miura, & Colflesh, 2007; Redick & Lindsey, 2013). To our knowledge, only one study has described neural activation patterns associated with spatial span performance. The authors reported activation in the superior and middle frontal gyri, inferior and superior frontal sulci, cingulate cortex, and large parts of the parietal and occipital cortices, in a small sample of 13 typically developing children (Klingberg,
Forssberg, & Westerberg, 2002). Given that only two fMRI studies investigated VSWM in ADHD, and both studies used relatively small samples (20-30 subjects per group) and used N-back tasks and not spatial span tasks, the current literature lacks a clear investigation of the neural correlates of spatial span VSWM in ADHD.

Literature documenting the developmental trajectory of VSWM-related brain activation patterns in ADHD is rather limited, though this issue is highly important in light of the discussion whether brain abnormalities in ADHD may catch up during adolescence or represent a more persistent deficit (Rubia, 2007; Shaw et al., 2007; Silk et al., 2009). Evidence from studies in healthy controls suggests that children and adults recruit similar brain networks, including the bilateral prefrontal cortex and posterior parietal cortex, but that brain activation in these regions increases over age (Kwon et al., 2002; Thomas et al., 1999). To our knowledge, no studies have investigated age effects on VSWM-related brain activation in ADHD. At the behavioural level, some evidence exists for different developmental trajectories, with VSWM impairment in ADHD becoming more pronounced during adolescence (Westerberg, Hirvikoski, Forssberg, & Klingberg, 2004), but others have reported similar levels of impairment for children, adolescents and young adults with ADHD (Van Ewijk et al., 2014). Studying age effects on VSWM-related brain activation may shed more light on the persistence of these deficits.

Gender differences in VSWM-related brain activation are another relatively unexplored topic. In healthy controls, differences in cognition and brain structure and function between males and females are well documented (Bell et al., 2006; Goldstein et al., 2005; Gong, He, & Evans, 2011; Hsu et al., 2008; Speck et al., 2000), underlining the importance of taking gender effects into account when studying the neural correlates of cognitive tasks (Goldstein et al., 2005). In ADHD, gender effects have been reported on neurocognitive performance (Balint et al., 2009; Gaub & Carlson, 1997; Gershon & Gershon, 2002), though no studies specifically investigated VSWM. The presence of gender effects in ADHD at the behavioural level raises the question whether this may be due to differences in the task-related recruitment of brain regions. One fMRI study that specifically addressed this issue, indeed found underactivation for male, but not female subjects with ADHD (Valera et al., 2010). This study, however, used a verbal N-back task, and a sample of adult subjects. It is important to replicate these findings with a spatial span task, and investigate whether possible gender differences in VSWM also exist in children and adolescents with ADHD.
The current study set out to map spatial span-related brain activation patterns in a large sample of children, adolescents and young adults with and without ADHD, to further unravel the neurophysiological underpinnings of VSWM impairment in ADHD. To the best of our knowledge, we are the first fMRI VSWM study in ADHD using a spatial span task, which is more comparable with the current literature on VSWM deficits in ADHD at the behavioural level. Moreover, considering our large gender-balanced sample with a broad age range, we were able to extend the existing literature by investigating the role of age and gender on neural correlates of VSWM, and examine whether these effects differ between individuals with and without ADHD. Based on previous results by Klingberg and colleagues (Klingberg et al., 2002) we expected to find VSWM-related brain activation in inferior and superior frontal and parietal regions, the occipital cortex, and the cingulate. We hypothesized that individuals with ADHD would show underactivation in these regions compared to controls and that impairment was similar for all ages, with stronger abnormalities for males than females.

METHODS

Participants

Participants were part of the NeuroIMAGE cohort (Von Rhein et al., 2014), including both ADHD and healthy control families. Inclusion criteria for the current study were: age between 8-30 years, European Caucasian descent, IQ≥70, and no known neurological or genetic disorder. Comorbid psychiatric disorders reported by parents were excluded, except oppositional defiant disorder (ODD), conduct disorder (CD), and pervasive developmental disorder not otherwise specified (PDD-NOS), given their high co-occurrence in ADHD. Furthermore, participants were excluded if they had any contraindication to MRI scanning (e.g. implanted metal or medical devices). Data were initially available of 281 participants who met inclusion criteria, aged between 8-25 years, including 156 ADHD subjects (all subtypes) and 125 healthy controls.

Diagnostic procedure

To determine ADHD diagnoses, all participants were assessed with the Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997) and Conners’ ADHD questionnaires from multiple informants.
A comprehensive diagnostic algorithm was employed to determine combined symptom counts for inattentive and hyperactive/impulsive behaviour, derived from both measures. Participants with a combined symptom count of ≥6 symptoms on one or both of the dimensions were diagnosed with ADHD, provided they: a) met the DSM-IV and 5 (American Psychiatric Association, 2000, 2013) criteria for pervasiveness and impact of the disorder (measures derived from the K-SADS), b) showed an age of onset before 12, in accordance with the DSM-5 (derived from the K-SADS; Polanczyk et al., 2010), and c) the child received a T score ≥63 on at least one of the DSM ADHD scales on either one of the Conners’ ADHD questionnaires. Controls were required to have a T score <63 on the Conners’ ADHD questionnaires, and have ≤3 symptoms on the combined symptom count, to ensure they did not show signs of (subthreshold) ADHD. Criteria were slightly adapted for young adults (≥18 years), such that a combined symptom count of 5 symptoms was sufficient for a diagnosis (Kooij et al., 2005). Young adults were included as controls when they had ≤2 symptoms on both combined symptom counts. A more detailed description of diagnostic procedures has been published elsewhere (Von Rhein et al., 2014).

Additional sections of the K-SADS-PL were administered to assess the presence of comorbid ODD, CD, mood and anxiety disorders. A custom questionnaire filled in by parents assessed the presence of an official diagnosis of PDD-NOS.

Procedure

The current study was part of a comprehensive assessment protocol encompassing phenotypic assessment, neurocognitive measures, and an MRI protocol including an fMRI scan (Von Rhein et al., 2014). Participants were asked to withhold the use of psychoactive medication for 48 hours before measurement. Those who were not able to comply were excluded from the analyses (n=13 ADHD subjects), given the reports on the normalizing effects of ADHD medication on working memory performance and brain activation (e.g. Cubillo et al., 2013). Before the scan, participants were familiarized with the scanning procedure and sounds using a mock-scanner to reduce anxiety and head movements, and a practice version of the VSWM task was administered outside the scanner shortly before going into the scanner. During the testing day, participants were motivated with short breaks and at the end of the day, participants received a reward of €50 and a copy of their MRI scan. Full-scale IQ was estimated by the Vocabulary and Block Design subtests of the Wechsler Intelligence
Scale for Children-III (WISC-III) or Wechsler Adult Intelligence Scale III (WAIS-III; for participants ≥17 years). Data acquisition was carried out in the Netherlands, either in Amsterdam at the VU University Amsterdam and VU University Medical Centre, or in Nijmegen at the Donders Centre for Cognitive Neuroimaging at the Radboud University Nijmegen. The study was approved by the Dutch local medical ethics committees. Informed consent was signed by all participants (parents signed informed consent for participants under 12 years of age).

Spatial span task

The spatial span task used to measure VSWM is an adapted version of a task developed by Klingberg and colleagues (Klingberg et al., 2002; McNab et al., 2008; Van Ewijk et al., 2014). Two trial types (baseline and working memory) and two memory loads (low and high) were implemented in the task. Each trial consisted of a sequence of either three or six yellow circles (low and high memory load, respectively), sequentially displayed on a 4x4 grid for 500 ms each, with a 500 ms inter-stimulus interval in between. Subsequently, during a 2000 ms response window, a probe consisting of a number with a question mark was presented in one of the 16 locations. During working memory trials, participants were asked to remember the spatial location and temporal order of the presentation of cues, and indicate with a ‘yes’ or ‘no’ response (right or left button, respectively) whether the location of the probe was stimulated before, at the indicated temporal position. During baseline trials, red circles followed by the probe (always the number 8) were presented sequentially in the four corners of the grid in a predictive manner, and participants were required to pay attention but not to try to remember the sequence, and always had to press the ‘no’ button. During both conditions, feedback was presented after the response in the form of a green or red coloured bar below the probe (for correct and incorrect responses, respectively), for the remainder of the response window. Accuracy on both conditions was determined in terms of the percentage of correct responses. The task was administered in 4 blocks of 24 trials each (presented in fixed random order), with a short break in between blocks to motivate participants and to avoid fatigue effects, with a total task duration of approximately 16 minutes.

Behavioural data analysis

Possible group differences in sample characteristics and behavioural data of the spatial span task were analysed using SPSS (version 21, IBM, Chicago, IL, USA). Behavioural data were analysed using Linear Mixed Models with a random intercept per family, to account
for correlated data within families. A repeated measures model examined group differences on working memory performance, with memory load as within-subject factor, and group (ADHD versus controls), age and gender as predictors. Furthermore, scan site was included as a covariate to control for possible site differences, and performance on baseline trials was included to control for basic processing or motivational effects. Note that results on behavioural performance on the spatial span task in a largely overlapping sample have been described in detail elsewhere (Van Ewijk et al., 2014).

Image acquisition and preprocessing

MRI scanning was carried out on either a 1.5 Tesla Sonata or a 1.5 Tesla Avanto MRI scanner (Siemens, Erlangen, Germany), using the same Siemens 8-channel head coil and closely matched pulse sequence parameters. Whole-brain, high-resolution T1-weighted anatomical images were acquired in the sagittal plane (MP-RAGE, 176 slices, acquisition matrix 256x256, voxel size 1x1x1 mm; TE/TR/T1=2.95/2730/1000 ms, FA=7°, GRAPPA-acceleration 2). Four functional runs were acquired (GE-EPI, TR=2340 ms, TE=40 ms, FOV=224 mm, voxel size=3.5x3.5x3.0 mm, slice gap 0.5 mm, 38 slices). Functional images of each subject were realigned using rigid body transformations, from which 3 translation and 3 rotation parameters were estimated, and subsequently images were slice time corrected using standard SPM8 routines (Wellcome Trust Centre for Neuroimaging, London, UK).

Subsequent fMRI data processing was carried out using FSL FEAT (FMRI Expert Analysis Tool; FMRIB Analysis group, Oxford, UK), including removal of the first three volumes, spatial smoothing (6mm FWHM Gaussian kernel) and highpass filtering (100 seconds). Subsequently, each participant’s functional image was registered to their T1 image using linear registration in FLIRT (12 DOF), followed by nonlinear registration of the images to the MNI152 template using FNIRT. Runs with >3 mm motion in any direction (as determined by the maximum absolute displacement throughout the entire run) were excluded from analysis. Data of 23 runs of ADHD subjects and 8 runs of control subjects were discarded. Next, FSL Motion Outliers was run to detect time points with large motion and model these as a confound matrix, thereby regressing the effect of these time points out of the analysis.

Fifty-six participants were excluded from analysis due to incidental findings after visual inspection [e.g. enlarged ventricles or unexpected hypo-intensities; 2 ADHD, 4 controls], scan quality [e.g. artefacts, missing volumes, insufficient coverage of the entire brain; 12 ADHD,
Accordingly, the final dataset included 109 ADHD subjects and 103 controls.

**FMRI analysis**

Four contrasts of interest were set up: low memory load, high memory load, mean working memory (both loads averaged), and load difference (high minus low memory load). Each contrast consisted of activation during working memory trials corrected for activation during baseline trials of the same load(s), modelled from the start of the trial to the onset of the probe. Using FSL FEAT, each participant’s activation map was estimated for each contrast for each run, while including confound regressors for motion (3 translation and 3 rotation parameters) and time points with extreme motion outliers (derived from FSL Motion Outliers). Second, for each participant, data from all runs were concatenated using a single-subject fixed effects model, resulting in four contrast maps (low load, high load, mean working memory, and load difference) per participant, reflecting contrast-specific activity across all available runs.

Whole-brain analyses were conducted in FSL FEAT using a mixed effects model (FLAME1). Z statistic images were thresholded using a cluster forming threshold of \( Z > 2.3 \) and a (corrected) cluster significance threshold of \( p < .05 \) (Worsley, 2001). Automatic outlier de-weighting (Woolrich, 2008) was used to reduce the impact of remaining outlier data points on subsequent analyses. Whole-brain analyses examined brain activation on the load difference and mean working memory contrasts. A General Linear Model (GLM) was set up to examine a) spatial span-related brain activation (across all subjects), b) group differences (ADHD versus controls), c) age- and gender effects (across all subjects), and d) group-by-age and group-by-gender interactions. In all analyses, scan site was included as a covariate to control for possible site effects. In case of significant effects on the load difference contrast, follow-up analyses were conducted to further investigate the nature of this effect. To this end, the mean activation parameter (beta value) was extracted from the significant cluster for each participant for the low and high memory load contrasts separately, and analyses of variance were conducted in SPSS to examine group differences on both loads, while including the same predictors and covariates as the whole-brain analysis. To ensure that group differences were not driven by comorbid disorders in ADHD, post-hoc analyses on group differences were repeated while excluding participants with ODD, CD, affect or anxiety disorders, and PDD-NOS.
RESULTS

Behavioural results

Sample characteristics are described in Table 1. The Linear Mixed Model for VSWM performance showed a main effect of memory load, with better performance on the low compared with the high memory load across all subjects (Table 2). A significant group difference was found with lower accuracy for ADHD compared with controls, which did not interact with memory load. Age was significantly associated with VSWM performance (better performance with increasing age), and did not interact with group. Furthermore, better performance on baseline trials was associated with better VSWM performance. No significant effect of gender and no group-by-gender interaction were found.

Table 1. Sample characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=103)</th>
<th>ADHD (n=109)</th>
<th>Test statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (M, SD)</td>
<td>16.8 (2.9)</td>
<td>17.1 (3.3)</td>
<td>$F_{1,210} = 0.39$</td>
<td>.532</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>53.4%</td>
<td>59.6%</td>
<td>$\chi^2_{1,210} = 0.84$</td>
<td>.406</td>
</tr>
<tr>
<td>IQ (M, SD)</td>
<td>104.0 (13.5)</td>
<td>99.1 (15.6)</td>
<td>$F_{1,210} = 6.10$</td>
<td>.014</td>
</tr>
<tr>
<td>Scan site (% scanned in Amsterdam)</td>
<td>73.8%</td>
<td>46.8%</td>
<td>$\chi^2_{1,210} = 16.07$</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Number of ADHD symptoms (M, SD)</td>
<td>0.7 (1.3)</td>
<td>13.4 (3.0)</td>
<td>$F_{1,210} = 1512.70$</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hand preference (% right-handed)</td>
<td>86.1%</td>
<td>88.0%</td>
<td>$\chi^2_{2,210} = 0.83$</td>
<td>.660</td>
</tr>
<tr>
<td>ADHD medication (% with a history of medication use)</td>
<td>0.0%</td>
<td>82.9%</td>
<td>$\chi^2_{2,210} = 135.58$</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Socio-economic status (M, SD)</td>
<td>13.2 (2.6)</td>
<td>11.7 (2.2)</td>
<td>$F_{1,203} = 21.51$</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ODD/CD (%)</td>
<td>0.0%</td>
<td>24.8%</td>
<td>$\chi^2_{1,210} = 29.24$</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Internalizing disorder (%)</td>
<td>1.0%</td>
<td>7.7%</td>
<td>$\chi^2_{1,210} = 5.62$</td>
<td>.035</td>
</tr>
<tr>
<td>PDD-NOS (%)</td>
<td>0.0%</td>
<td>15.6%</td>
<td>$\chi^2_{1,210} = 22.03$</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Total number of symptoms, derived from the diagnostic algorithm (see Methods).*

FMRI results

Whole-brain analyses were conducted on both the mean working memory and load difference contrasts. Anatomical labels of all fMRI results are summarized in Supplementary Table S1.

First, to estimate spatial span-related brain activation, mean activation patterns for each contrast were estimated across all participants. For the mean working memory contrast, a
widespread pattern of brain activation was found. To aid interpretation, activation patterns were evaluated at a more conservative threshold (Z>8) than the one used in the whole-brain analysis, to obtain regions showing the strongest effect. Significant clusters were bilaterally located in the middle frontal gyrus, precentral gyrus, superior parietal cortex, insula, thalamus, bilateral occipital cortex, and cerebellum (Figure 1, left panel). Activation associated with load difference was found bilaterally in the lateral frontal pole, paracingulate gyrus, the inferior, middle and superior frontal gyri, middle temporal gyrus, and occipital and posterior parietal regions (Figure 1, right panel).

Second, group differences between ADHD and controls were investigated. No significant group differences were found on the mean working memory contrast. On the load difference contrast, ADHD showed higher activation compared with controls in the left inferior frontal cortex, more specifically in the left inferior frontal gyrus (IFG; Brodmann Areas (BA) 44/45), extending to the lateral frontal pole (BA 10) (see Figure 2). For follow-up analyses, the significant cluster was evaluated at a more conservative threshold (Z>2.5, minimum cluster size=100 voxels) compared with the one used in the whole-brain analysis to subdivide it into more meaningful regions. This threshold yielded three separate clusters: the IFG - pars opercularis, IFG - pars triangularis, and the lateral frontal pole (Table 3). Follow-up analyses examined group differences on the low and the high memory load separately in each of the three clusters. Results revealed significant group differences in the IFG pars opercularis on the high memory load (F(1,207)=11.08, p=.001) but not the low load (F(1,207)=0.503, p=.479), and the IFG pars triangularis on the low memory load (F(1,207)=24.77, p<.001) but not the high load (F(1,207)=0.373, p=.542) (see Figure 3). No group differences were found in the lateral frontal pole (all p>.05).
Figure 1. Mean activation patterns across all participants for the mean working memory contrast (average of both loads, corrected for baseline trials) and the load difference contrast (high minus low memory load, corrected for baseline trials).
Figure 2. Higher activation for ADHD compared with controls on the load difference contrast (high minus low memory load, corrected for baseline trials). The significant cluster was located in the left inferior frontal gyrus (pars opercularis and pars triangularis; Brodmann areas 44/45), and extended to the lateral frontal pole (Brodmann area 10).

Table 3. Significant clusters of higher activation for ADHD versus controls on the load difference contrast.

<table>
<thead>
<tr>
<th>Cluster #</th>
<th>Anatomical label</th>
<th>Hemisphere</th>
<th>n voxels</th>
<th>MNI coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inferior frontal gyrus, pars triangularis</td>
<td>Left</td>
<td>413</td>
<td>-36 36 6</td>
</tr>
<tr>
<td>2</td>
<td>Lateral frontal pole</td>
<td>Left</td>
<td>159</td>
<td>-34 56 10</td>
</tr>
<tr>
<td>3</td>
<td>Inferior frontal gyrus, pars opercularis</td>
<td>Left</td>
<td>125</td>
<td>-52 20 4</td>
</tr>
</tbody>
</table>

Note: Results were evaluated at a more conservative threshold (Z>2.5) than in the whole-brain analysis to obtain meaningful clusters. MNI coordinates represent the location of the maximum intensity voxel (maximum z-statistic).
Figure 3. Regions of interest (ROIs) used for follow-up analyses. ROIs were derived from the significant cluster from the group analysis in which ADHD showed higher activation compared with controls on the load difference contrast (see Figure 2). This cluster was thresholded at a more conservative threshold (Z>2.5) than in the whole-brain analysis to obtain separate clusters with unique anatomical labels. Resulting clusters were located in the inferior frontal gyrus, pars triangularis (a) and pars opercularis (b), and the lateral frontal pole (c). The right panel shows extracted activation parameters in each cluster on both memory loads, showing a significant difference (marked with an asterisk) in the IFG pars opercularis on the high memory load (ADHD>controls), and in the IFG pars triangularis on the low memory load (ADHD<controls).
Figure 4. Effects of age and gender on spatial span-related brain activation, across all participants. Upper panel: age was positively associated with activation on the mean working memory contrast (green) and negatively with activation on the load difference contrast (yellow). Post-hoc analyses of the load difference contrast revealed that this was due to significantly higher activation for older subjects on the low load (marked with an asterisk), but not the high load (graph shown on the right). Lower panel: Gender effects represented higher activation for males compared with females on the mean working memory contrast (red), and higher activation for females compared with males associated with the load difference contrast (orange). Post-hoc analyses of the load difference contrast showed that this was due to higher activation for males compared with females on the low load (marked with an asterisk), but not the high load (graph shown on the right).
Excluding subjects with comorbid disorders did not change the results. Activation in none of the three clusters showed a significant correlation with behavioural performance on the task (represented by mean accuracy across both memory loads, corrected for baseline; all \(p>.05\)).

Third, age and gender effects were examined. Age-related increases were found on mean working memory in the bilateral precentral gyrus, middle and superior frontal gyrus, frontal operculum, cingulate and paracingulate gyrus, superior parietal cortex, pallidum, occipital cortex and cerebellum, and in the right supramarginal gyrus, putamen, and orbitofrontal cortex. Activation associated with load difference significantly decreased with age in the posterior division of the cingulate gyrus and the bilateral supplementary motor cortex. To further illustrate the nature of this age-by-memory load interaction, follow-up analyses were conducted while splitting the sample at the mean age (17.0 years), in order to compare activation in the younger (\(n=106\)) compared with the older (\(n=106\)) subjects. Analyses showed that the age-by-memory load interaction was due to higher activation on the low load for the older compared with the younger age group (\(F_{1,207}=7.541, p=.007\)), while activation on the high memory load was similar for both age groups (\(F_{1,207}=0.070, p=.791\)) (Figure 4, upper panel).

Gender effects were found for mean working memory, with higher activation for males than females in the bilateral frontal pole, paracingulate gyrus, superior frontal gyrus, posterior parietal regions (precuneus), and the right angular gyrus. Group differences for load difference were located in partly the same regions, including the bilateral frontal pole, paracingulate gyrus and superior frontal gyrus, with higher activation for females compared with males. Follow-up analyses showed that this was due to higher activation for males compared with females on the low memory load (\(F_{1,207}=25.275, p<.001\)), but similar activation on the high memory load (\(F_{1,207}=0.012, p=.911\)) (Figure 4, lower panel).

Fourth, whole-brain analyses were conducted to determine whether age and gender effects were similar for subjects with and without ADHD. No group-by-age or group-by-gender interactions were found on either the mean working memory or the load difference contrast, indicating that group differences were similar for males and females, and across the whole age range investigated.
DISCUSSION

The current study set out to investigate the neural correlates of VSWM impairment in ADHD. Using a spatial span task, we examined VSWM-related brain activation patterns in a large sample of individuals with and without ADHD, and additionally investigated the effects of age and gender as well as group-by-age and group-by-gender interactions. Behavioural results showed impaired performance for individuals with ADHD on both the high and the low memory load, with similar decreases in performance during memory load increase. fMRI results showed higher brain activation for ADHD compared with controls during memory load increase in left inferior frontal regions, which was similar for males and females, and similar across development from childhood to young adulthood. VSWM-related age and gender effects were found across all participants and both memory loads in widespread regions, with higher activation with increasing age, and higher activation in males compared with females.

Across all participants (ADHD and controls alike), a widespread pattern of spatial span-related brain activation was found in all four lobes in cortical as well as subcortical regions (Figure 1). Regions of spatial span-related activation are consistent with previous findings in a small sample of typically developing children, using a similar task (Klingberg et al., 2002), and largely coincide with activation patterns associated with executive control (Dosenbach et al., 2007; Gilbert et al., 2006; Matthews, Nigg, & Fair, 2014), an important component of working memory (Baddeley, 2003). Considering our large sample size, broad age range and even distribution of gender, we here provide a robust estimate of spatial span-related brain activation patterns in children, adolescents and young adults, with and without ADHD. Compared with previously obtained results with spatial N-back tasks, which elicit activation in frontal and parietal regions (Kwon et al., 2002; Nelson et al., 2000; Wager & Smith, 2003), our spatial span task yielded a more widespread network of activation that also extends to occipital, subcortical and cerebellar regions. This discrepancy indicates that, as previously suggested (Kane et al., 2007; Redick & Lindsey, 2013), spatial span and N-back tasks may indeed be controlled by (partly) different brain networks, and results from one working memory task should not automatically be generalized to the other. Given that at the behavioural level, VSWM impairments in ADHD have mainly been demonstrated using spatial span tasks, current results underline the importance of adopting similar paradigms in future fMRI research in ADHD, to be able to compare and integrate behavioural and fMRI findings more reliably.
During memory load increase, individuals with ADHD showed higher brain activation compared with controls in regions of the left inferior frontal cortex, more specifically in regions of the left IFG, pars opercularis and triangularis, extending to the lateral frontal pole (Figures 2, 3). Abnormal brain activation in the left IFG in ADHD concurs with previous literature on working memory-related brain activation in ADHD (Cortese et al., 2012), though most of these studies used non-spatial tasks. Notably, brain activation associated with the spatial domain of working memory is typically reported to be more specific to the right hemisphere, while left hemispheric activity is more strongly associated with verbal working memory and more general phonological processes (e.g. d’Esposito et al., 1998; Smith, Jonides, & Koeppe, 1996). More specifically, the left IFG corresponds to Broca’s area, strongly implicated in speech production as well as internal speech (Bookheimer, 2002; Hinke et al., 1993). The spatial span task used in the current study requires active rehearsal of cues and is correlated with complex reasoning skills (Klingberg, 2006), and as with most spatial span tasks, participants are likely to rehearse the items verbally. Consequently, it is possible that our finding of higher IFG activation in ADHD during memory load increase represents greater difficulty or effort within this group to internally verbalise the location of the cues, when the task becomes more complex.

Follow-up analyses determining the nature of the group-by-memory load interaction (see Figure 3) indicated underactivation in ADHD during low memory load in the IFG pars triangularis. It is possible that during low memory load, a relatively simple condition, ADHD subjects failed to recruit this region due to a lack of arousal or attention, which improved when the task became more complex. Conversely, in the IFG pars opercularis, individuals with ADHD showed higher activation compared with controls on the high memory load. However, higher activation did not transfer to better behavioural performance. This discrepancy points towards reduced efficiency of prefrontal brain regions in ADHD during VSTM, in line with previous findings (Bédard et al., 2014). For the lateral frontal pole cluster, no group differences were found on either the low or the high memory load.

Group differences did not interact with age, suggesting parallel delayed developmental trajectories of VSTM-related brain activation for individuals with ADHD compared with their typically developing peers, between childhood and young adulthood. This finding is consistent with our previous report on behavioural VSTM performance in ADHD in a partially overlapping sample (Van Ewijk et al., 2014), and implies that abnormalities in VSTM-related brain activation do not catch up, but also do not worsen when children with ADHD grow into
adulthood (in contrast to one other study; Westerberg et al., 2004). The absence of a gender interaction indicates that abnormal brain activation is present in males as well as females with ADHD. This finding is consistent with the absence of a group-by-gender interaction at the behavioural level, but is in contrast to our hypothesis based on a previous report, which suggested that only males with ADHD show abnormal VSWM-related brain activation patterns (Valera et al., 2010). It is possible that our large sample size allowed us to detect group differences in females, which were not captured by previous, smaller, studies. Another explanation may lie in the age range investigated. While our sample consisted of mostly adolescents and young adults, the study by Valera and colleagues included adults with mean ages between 32-36 years. It is possible that observed brain abnormalities in females in our sample reflect a developmental delay in girls with ADHD, which may normalize in their late twenties or early thirties. Longitudinal studies, with large numbers of both male and female participants, could provide more insight.

Across all participants, age-related increases in VSWM-related brain activation were found in regions that largely overlap with our general VSWM-related activation patterns (Figures 1, 4). This indicates that children recruit similar brain regions as young adults, but start to activate these regions more intensively throughout their development, consistent with a previous report using a spatial N-back task (Kwon et al., 2002). Additionally, on the low memory load, we found higher activation in the supplementary motor cortex and posterior cingulate gyrus for older compared with younger participants. Given the role of the posterior cingulate gyrus in memory functioning (Kim, 2011; Sutherland & Hoesing, 1993), this finding could signify a developmental effect, representing an increase in recruitment of memory-related brain regions over age.

Gender effects were mainly found in frontal brain regions, with higher activation for males compared with females (Figure 4), consistent with previous research using N-back or spatial attention tasks (Goldstein et al., 2005; Gur et al., 2000). In the lateral frontal pole and paracingulate and superior frontal gyri, the gender effect was only present at the low, but not the high memory load. Importantly, behavioural performance was similar between male and female subjects at both memory loads, indicating a discrepancy between brain activation and behavioural performance. These results may indicate that males recruit more brain resources than females to reach similar levels of VSWM performance. In sum, these findings underline the importance of taking age and gender into account when studying VSWM-
related brain activation, especially when dealing with psychiatric disorders that are subject to uneven gender distributions.

The current results should be considered in light of some limitations. First, it should be noted that the developmental trajectory of VSWM should ideally be investigated in longitudinal samples, following children with ADHD into young adulthood with assessment at multiple timepoints. However, our large sample size with broad age range did allow us to provide exploratory insights into the developmental trajectory of VSWM. Second, generalization of results is restricted to clinically referred samples of European-Caucasian descent.

In conclusion, we here show spatial span-related brain activation patterns in more widespread regions than have been reported with N-back tasks, indicating that these tasks may not measure exactly the same construct and should not be used interchangeably. Individuals with ADHD showed underactivation during low memory load in the IFG pars triangularis, and greater activation compared with controls during high memory load in the IFG pars opercularis. Abnormal brain functioning in ADHD was present in males as well as females, and developmental trajectories were similar for ADHD and controls. Furthermore, we report effects of age and gender on the location and intensity of these activation patterns across all participants, underlining the importance of taking these factors into account in future studies.
### SUPPLEMENTARY TABLE

Table S1. Significant clusters of activation associated with working memory, age and gender on the mean working memory and load difference contrasts.

<table>
<thead>
<tr>
<th>Cluster #</th>
<th>Hemisphere</th>
<th>N voxels</th>
<th>MNI coordinates</th>
</tr>
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<tbody>
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<td></td>
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</table>

*Note:* Working memory activation for the mean working memory contrast was evaluated at Z>8 to obtain peak activation clusters. MNI coordinates represent the location of the maximum intensity voxel (maximum z-statistic).
REFERENCES


