The Neural Correlated of Working Memory in Children and Adolescents with ASD and the Effects of Cognitive Load

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Arts

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Abstract

Research on the neural bases of cognitive deficits in autism spectrum disorder (ASD) has shown that working memory (WM) difficulties are associated with abnormalities in the prefrontal cortex. However, few studies have examined the impact of cognitive load on WM and its neural underpinnings in children and adolescents. We used fMRI and an n-back task with four levels of difficulty to compare the cortical activation patterns associated with WM in children with and without ASD across cognitive load. Findings revealed impaired modulated activity as a function of cognitive load in prefrontal and parietal cortices in children with ASD relative to typical controls. Results suggest that children with ASD rely mainly on posterior brain regions associated with lower-level visual processing, whereas controls showed activity in frontal lobes related to the classic WM network. Findings will help guide future longitudinal work by localizing areas of vulnerability to developmental disturbances.
Acknowledgments

First, I would like to express my sincere thanks to all of the families and children who participated in this project and made it possible.

I would like to express my appreciation and thanks to my supervisors Dr. Margot Taylor and Dr. Mary Lou Smith who help foster my growth as a researcher and professional. Also, many thanks to Dr. Mary Pat McAndrews for her support and guidance during this year.

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Most of all, thank you to my family who continue to motivate me and be a constant source of encouragement.
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Chapter 1
Introduction

1 Introduction

Approximately 1 in 88 children have an Autism Spectrum Disorder (ASD) and the proportion of children being diagnosed is rising [1]. ASD is a neurodevelopmental disorder classically characterized by the triad of social deficits, communicative difficulties and repetitive behaviours [2], with evidence of cognitive and executive function impairment [2-9]. The impaired executive processes may account for many of the profound behavioural manifestations in ASD, and contribute to autistic symptomology [6-7]. Emerging literature on cognitive difficulties in ASD, as well as their neural underpinnings, provides evidence for working memory (WM) deficits that are associated with frontal lobe abnormalities, particularly in prefrontal cortex activity [4,10-14].

Previous neuroimaging, electrophysiology and neurochemical studies in ASD have identified atypical white and gray matter volumes [15-18], functional connectivity [10,19], cortical sulcal and gyral anatomy [20], brain lateralization [21], neural perfusion [22] and serotonin synthesis capacity [23] compared to typically developing (TD) individuals, with the frontal cortex implicated in a number of these differences [24]. Although there is substantial evidence for developmental anatomical abnormalities of the frontal lobes in ASD, associations with cognitive performance are lacking [25]. This suggests that executive dysfunction in ASD may be related to functional, rather than anatomical frontal lobe aberrations. Further, protracted maturation of the frontal lobes makes the functions they support, such as WM, susceptible to developmental disturbances [26-27].

WM is important for learning, social ability [28], academic achievement [29] and many complex cognitive operations [30-31]. Impairments in WM have been reported in ASD, yet our understanding of its development and neural correlates is still very limited in this population. We investigated the neural systems underlying visuospatial WM capacity using functional magnetic resonance imaging (fMRI) to determine the role of WM load on possible functional differences between children with and without ASD.
Extant research on the behavioural characterization of WM function in ASD suggests both impaired [32-36] and intact [32-34,36-37] WM performance relative to TD individuals. Discrepancies in the literature may partly be due to methodological inconsistencies regarding task choice, comparison groups or population age. The majority of studies that found significant group differences assessed WM using tasks that systematically increased in complexity and/or cognitive load, and thereby imposed heavier demands on WM and executive functions [9,33,35]. Further, there is evidence of primarily visuospatial WM impairment, whereas verbal WM appears relatively intact in individuals with ASD [37,34,36].

Only a few studies have examined the neural correlates of WM function in ASD, and surprisingly, no neuroimaging studies of pre-adolescent children exist. Overall, WM processes are largely subserved by the prefrontal and parietal cortices [4,38-42]. Converging literature identifies a broad system of prefrontal, premotor, dorsal cingulate and posterior parietal activation in visual WM tasks [see 42], and neuroimaging studies in ASD provide evidence for atypical activity in these regions [10,12,14]. Using a visuospatial WM task, Luna et al. (2002) found behavioural impairments in WM, as well as reduced activation in the dorsolateral prefrontal cortex (DLPFC) and posterior cingulate cortex in individuals with ASD compared to controls. Interestingly, there was no evidence of impaired activation in other crucial areas known to support WM neural circuitry, such as the anterior cingulate cortex. A study by Koshino et al. (2005), demonstrated bilateral activation in the DLPFC in adult controls, whereas individuals with ASD showed limited activation in the left, and more right hemisphere recruitment of prefrontal regions, despite the absence of differences in performance accuracy on an n-back letter task. The authors suggested that while typical adults processed letter stimuli using verbal codes, those with ASD employed visual strategies [10], supporting the idea that WM deficits in ASD may be attributed to their inability to select appropriate processing strategies. In the only study of adolescents, Silk et al. (2006), using a visuospatial WM mental rotation task where behavioural performance was similar for ASD and control groups, observed impaired cortical activation in the frontal lobes in the ASD group, including the anterior cingulate, DLPFC and caudate nucleus, but normal activation in the parietal cortices relative to controls. These findings suggest dysfunctional frontostriatal networks in ASD. More work is needed to understand the neural correlates and developmental trajectory of WM dysfunction in ASD, as limited research is available reporting on pre-adolescent children.
One of the most common experimental paradigms used to manipulate cognitive load in studying WM is the ‘n-back’ protocol [10,39,41-45]. In a typical n-back task, participants view a series of stimuli and indicate whether the currently presented stimulus matches one presented ‘n’ (e.g. 0, 1, 2 or 3) trials prior. As difficulty level increases, the number of interfering stimuli between the target and relevant stimulus increases; this consequently increases both memory load and executive function demand in a non-linear fashion. As such, it is difficult to quantify WM capacity ability and identify relevant brain areas that activate exclusively in response to WM processing independent of other cognitive demands. Therefore, in the present study we used an n-back task which had developmentally-graded increases in memory load while keeping executive function constant across all difficulty levels—a 1-back colour matching task (CMT) [46]—to allow for a direct investigation of the influence of cognitive load on WM. Normal adults showed positive linear relations between cortical activity and CMT task difficulty in areas involved in WM function [47]. Negative linear relations were found in areas typically associated with the default mode network (DMN). The DMN is a network of brain regions that are found in a wide range of neuroimaging studies, including the medial prefrontal cortex, posterior cingulate and inferior parietal lobules, characterized by decreased activation during goal-oriented or attention-demanding tasks [48]. Neurodevelopmental disorders, including ASD, have been associated with abnormal function [49-50] and structural anatomy [51] of the DMN that may interfere with cognitive function, and thereby contribute to observed WM impairments.
Chapter 2
Objectives, Hypothesis and Rationale

2 Objectives, Hypothesis and Rationale

The aim of the present study was to identify and compare the neural systems underlying visuospatial WM capacity in children and young adolescents with and without ASD using fMRI, and understand the effect of cognitive load. We hypothesized that although children with ASD may perform similarly to matched controls on behavioural measures of WM, frontal cortical areas related to WM capacity would be under-recruited in children with ASD relative to controls. Specifically, we expected activity to be linearly modulated (positively for WM areas, negatively in default-mode areas) by task difficulty in TD children (comparable to previous work with adults), but not in children with ASD.

With the protracted maturation of the frontal lobes, and their susceptibility to developmental anomalies, understanding development in these regions is crucial, particularly in populations with frontal lobe abnormalities, as seen in ASD. Given the links between social function, school success and executive function ability, investigating the neural bases of WM deficits in children with ASD will contribute to our knowledge of the underlying causes of ASD-related behaviour. This will help to identify the nature of atypical brain development with future expectations of establishing age-appropriate interventions that can effectively target WM function and, in turn, other symptoms of ASD.
Chapter 3
Methods

3 Methods

3.1 Participants

Participants recruited for this study included 42 children aged 7-13 years with high functioning ASD, and 31 healthy TD control children. After sex-, IQ- and age-matching, and excluding children from the analyses based on excessive movement, and inadequate task performance or protocol completion, the study sample consisted of 19 children with ASD (3 girls and 16 boys) and 17 controls (4 girls and 13 boys). The groups were matched for age (ASD $M=11.21$, $SD=1.23$; TD $M=11.12$, $SD=2.00$; $t_{(34)}=.17$, ns), sex ($\chi^2_{(1)}=.34$, ns.), and Full Scale IQ as determined by the Wechsler Abbreviated Scale of Intelligence-II [52] (ASD $M=108.21$, $SD=14.77$; TD $M=116.18$, $SD=9.34$; $t_{(34)}=1.95$, ns.). We substituted the group average IQ for one TD child whose data was missing.

Exclusion criteria for all participants were the presence of any current significant Axis I psychiatric comorbidities, neurological disorders, medical illnesses, prematurity, uncorrected vision, colour blindness, IQ<70, as well as standard MRI contraindicators (e.g., ferromagnetic implants). A history of developmental delay, learning disability, and ADHD was used to exclude TD children only; however, these factors were also not current primary diagnoses in the ASD group. Six children with ASD were each on one psychotropic medication (Strattera, Biphentin, Fluoxetine, Concerta, Abilify and Atomoxetine). Their fMRI data were examined in comparison to children with ASD who were not taking medication, and the data did not differ between these subgroups (see Additional File 1). Children were recruited through community support centers, parent support groups, email lists, hospital ads and private schools. Informed consent, clinical and cognitive testing, and MRI scanning were performed at the Hospital for Sick Children in Toronto. Experimental procedures were approved by the hospital Research Ethics Board. All children gave informed assent and the parents provided informed written consent.
Clinical diagnoses of ASD were confirmed in all cases with a combination of expert clinical judgment and the Autism Diagnostic Observation Schedule-General (ADOS-G) [53]. All children completed the Backwards Digit Recall, Listening Recall, Digit Recall, Mazes Memory and Block Recall subscales of the Working Memory Test Battery for Children (WMTB-C) [54] to supplement behavioural data collected during fMRI tasks. See Table 1 for demographic and neuropsychological test characteristics.

**Table 1. Demographic and Neuropsychological Test Characteristics of Study Sample**

<table>
<thead>
<tr>
<th>Variable</th>
<th>ASD (N = 19)</th>
<th>TD (N = 17)</th>
<th>Significance Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>84.21</td>
<td>76.47</td>
<td>$\chi^2_{(1)} = 0.56$, ns.</td>
</tr>
<tr>
<td>Age</td>
<td>11.21 (1.23)</td>
<td>11.12 (2.00)</td>
<td>$t_{(34)} = 0.17$, ns.</td>
</tr>
<tr>
<td>IQ</td>
<td>108.21 (14.77)</td>
<td>116.18 (9.45)</td>
<td>$t_{(34)} = 1.95$, ns.</td>
</tr>
<tr>
<td><strong>Neuropsychological Test Data (WMTB-C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Recall</td>
<td>108.79 (19.97)</td>
<td>111.06 (25.68)</td>
<td>$t_{(34)} = 0.30$, ns.</td>
</tr>
<tr>
<td>Block Recall</td>
<td>93.84 (19.93)</td>
<td>98.18 (28.67)</td>
<td>$t_{(34)} = 0.53$, ns.</td>
</tr>
<tr>
<td>Mazes Memory</td>
<td>94.11 (17.78)</td>
<td>91.00 (28.35)</td>
<td>$t_{(34)} = 0.40$, ns.</td>
</tr>
<tr>
<td>Listening Recall</td>
<td>101.63 (16.78)</td>
<td>110.94 (30.20)</td>
<td>$t_{(34)} = 1.16$, ns.</td>
</tr>
<tr>
<td>Backward Digit Recall</td>
<td>91.95 (17.65)</td>
<td>102.53 (30.85)</td>
<td>$t_{(34)} = 1.28$, ns.</td>
</tr>
</tbody>
</table>

### 3.2 The Colour Matching Task (CMT)

Children were required to attend to coloured figures of a clown presented one at a time in sequence. Children were taught to ignore the clown’s face and irrelevant colours (blue and green), and focus on other, relevant colours (yellow, purple, pink, red, orange, brown and grey). The number of relevant colours (capacity) in each figure increased by one to increase difficulty levels (Fig. 1a). Children indicated after each item whether the relevant colours of the current figure matched those from the immediately preceding figure, disregarding colour location and repetition (i.e. 1-back). Using a keypad with the right hand, children responded in the scanner by pushing a button for ‘same’ when the clown figure was wearing the same colours as the previous
clown, and ‘different’ when the clown figure was wearing different colours. All children successfully completed practice trials on a computer outside the scanner with accuracy of 80% or greater.

A total of 24 task blocks (168 task trials) and 24 baseline blocks were presented across 4 runs. Each run consisted of six 32-second blocks for each difficulty level (6 levels in total); within each block there was a constant difficulty level, and all difficulty levels were randomized within each run (Fig. 1b). The same 4 runs were presented to all children in the same order. Task blocks consisted of 8 stimuli of the same difficulty, and alternated with 20-second baseline blocks, where clowns were coloured only in blue and green, and children were instructed to look at the figures but not respond. Participants had 3 seconds to view a stimulus and respond, followed by a 1-second inter-stimulus interval where a fixation cross was presented (Fig. 1c). The fMRI task took approximately 22 minutes of scan time.
A) There were 6 levels of difficulty where the number of relevant colours (yellow, purple, pink, red, orange, brown and grey) increased by one to increase the difficulty level. Difficulty = (# of colours) + 2. Children were taught to ignore the clown’s face, colour location, colour repetition and irrelevant colours (blue and green);

B) It was a block design task, where each run consisted of six 32 second task blocks (for each difficulty) followed by 20 second baseline blocks where clowns are presented in only blue and green (ignore). Task blocks were presented pseudo-randomly within each run;

C) Example of part of a sequence in a block; children indicated if the current clown was wearing the same or different colours as the previous clown. Stimuli were presented for 3 seconds followed by a 1 second inter-stimulus fixation cross.

Performance data were recorded for both accuracy and reaction time; items were correct if responded to correctly within 3 seconds of stimulus presentation. To ensure adequate task completion, children were excluded if they did not achieve at least 60% accuracy (averaged across 4 runs) on the easiest 2 difficulty levels, and also excluded if they did not have at least 2
out of 4 runs where 50% or more of the blocks were acceptable in terms of performance (60% accuracy) and motion. Motion was deemed acceptable if children moved less than 1.5mm from their median head position in at least 60% of the volumes within a task block. Motion was also accounted for in the fMRI preprocessing.

### 3.3 Image Acquisition

All imaging data were acquired using a 3T Siemens Trio MRI scanner with a 12-channel head coil. Head stabilization and motion restriction were achieved with foam padding. The structural scan was a high-resolution T1-weighted 3D MP-RAGE image (Sagittal; FOV=192x240x256mm; 1mm isometric voxels; TR/TE/TI/FA= 2300/2.96/900/9), which was also used as an individual anatomical reference for the functional images. During structural image acquisition, children watched a movie of their choice using MR-compatible goggles and earphones. Functional images were acquired with single-shot echo planar imaging sequence (Axial; FOV = 192x192; Res = 64x64; 30 slices 5mm thick; 3x3x5mm voxels; TR/TE/FA = 2000/30/70). Visual stimuli for the functional tasks (CMT) were displayed on MR-compatible goggles. Children responded to trials using a dual button MR-compatible keypad. Stimuli were displayed and performance was recorded using the software Presentation (Neurobehavioral Systems Inc.).

### 3.4 Behavioural Data Analyses

**CMT.** The majority of children had poor accuracy on the two most difficult levels—levels 7 and 8 (D7 and D8); thus analyses of only the first four difficulty levels (D3 to D6) are presented. Accuracy (proportion correct) and response times were calculated for each difficulty level by averaging across runs for each group. Data were analyzed using repeated measures factorial ANOVAs, with group (ASD and TD) as a between subject-factor and difficulty level (D3, D4, D5, and D6) as a within subject-factor.

**WMTB-C.** Standardized scores on the subscales were compared across group using t-tests, to determine if there were the differences between ASD and TD children on these neuropsychological measures of WM.
3.5 fMRI Data Analyses

Image preprocessing of functional data was performed using a combination of standard AFNI [55] and FMRIB’s Software Library (FSL) [56] tools. The first 3 volumes of each run were discarded for scanner stabilization. After slice timing and motion correction, data were smoothed using a 6mm FWHM Gaussian kernel, temporally filtered (lower and upper cut-off frequencies of 0.01 Hz and 0.2 Hz, respectively), and converted to percent signal change. Before group-level analyses, images were registered to the Montreal Neurological Institute (MNI) 152 template. A motion signal generated from maximum displacement (MD) was regressed out of the data. MD data were then used to examine group differences in motion. The mean displacement for both groups was less than 0.6 mm.

Data were analyzed with the FSL fMRI Expert Analysis Tool (FEAT) [56]. Data were fit first to a block-design general linear model convolved with a gamma function to model haemodynamic response, using the task parameters (D3 to D6). To examine areas that linearly modulate as a function of difficulty, linear trend analyses were conducted from D3 to D6 using fixed-effects higher level modelling. Individual results were then averaged across runs for each subject in a second level analysis. Between-group comparisons were carried out using FMRIB’s Local Analysis of Mixed Effects (FLAME) [56] to obtain an accurate between-subject variance estimation, which increased our ability to detect real activation [57]. Significant activations were reported using cluster-based thresholding determined by \( Z > 2.3 \), and a corrected cluster significance threshold of \( p < 0.05 \). Regions of interest (ROIs) were selected from areas showing significant group differences between TD and ASD groups in the linear trend analyses. Average percent signal change and standard error scores were extracted from spherical ROIs (6 mm radius) centered about the local maxima, and plotted as a function of difficulty to further examine activation patterns across cognitive load.
Chapter 4
Behavioural Data

4 Results

4.1 Behavioural Data

There was a significant main effect of group on accuracy, $F_{(1, 34)} = 5.15$, $p=0.03$, $\eta^2 = 0.13$, which was driven solely by TD children ($M=0.80$, $SD=0.12$) performing more accurately than ASD children ($M=.71$, $SD=0.12$) only at D5 ($t_{(34)}=2.30$, $p=0.03$). As such, comparisons of brain activity between control and ASD groups were made under comparable accuracy scores across most levels. There was a significant main effect of difficulty on accuracy, $F_{(3, 34)} = 53.87$, $p<0.001$, $\eta^2 = 0.61$, with performance accuracy decreasing as a function of difficulty level in both groups (Fig. 2a). Post hoc pairwise comparisons, adjusted for multiple comparisons, revealed that accuracy on most difficulty levels were significantly different from each other at $p<0.05$, the exceptions being between D5 and D6 in the ASD group (Table 2a), and between D3 and D4, and between D4 and D5 in TD children (Table 2b). Although we established criteria (see methods) to eliminate performance as a confounding factor, we also ran a supplementary analysis, inputting performance as a covariate. There were no areas of activation that correlated with performance in either group, suggesting that findings were not confounded by performance per se.

Overall, response times increased with increasing difficulty in TD children, but only increased up until D5 in children with ASD (Fig. 2b). There was no main effect of group on response times ($F_{(1, 34)} = 2.33$, $p=0.14$), but there was a main effect of difficulty level, $F_{(3, 34)} = 54.60$, $p<0.001$, $\eta^2 = 0.62$. Post hoc comparisons in the ASD group showed that response times differed between difficulty levels, except between D4 and D6, and between D5 and D6 (Table 3a). In TD children, response times significantly differed between difficulty levels, except between D4 and D6, and D5 and D6 (Table 3b).

In terms of the WMTB-C, there were no between group differences across all subscales.
Table 2. Differences in CMT accuracy (proportion correct) across difficulty levels.

A. ASD children

<table>
<thead>
<tr>
<th></th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>SE</td>
<td>MD</td>
<td>SE</td>
</tr>
<tr>
<td>D4</td>
<td>0.06**</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>D5</td>
<td>0.17***</td>
<td>0.03</td>
<td>0.11**</td>
</tr>
<tr>
<td>D6</td>
<td>0.23***</td>
<td>0.03</td>
<td>0.17***</td>
</tr>
</tbody>
</table>

***p<0.001, ** p<0.01, *p<0.05

B. TD children

<table>
<thead>
<tr>
<th></th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>SE</td>
<td>MD</td>
<td>SE</td>
</tr>
<tr>
<td>D4</td>
<td>0.05</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>D5</td>
<td>0.11*</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>D6</td>
<td>0.22***</td>
<td>0.03</td>
<td>0.17***</td>
</tr>
</tbody>
</table>

***p<0.001, ** p<0.01, *p<0.05, corrected for multiple comparisons using Bonferroni

Notes: Post hoc ANOVA tests using accuracy scores for (A) children with ASD and (B) TD children. D3-D6= Difficulty levels 3 to 6; MD = Mean difference; SE: Standard error.

Table 3. Differences in CMT response times (in seconds) across difficulty levels.

A. Children with ASD

<table>
<thead>
<tr>
<th></th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>SE</td>
<td>MD</td>
<td>SE</td>
</tr>
<tr>
<td>D4</td>
<td>-0.25***</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>D5</td>
<td>-0.38***</td>
<td>0.05</td>
<td>-0.13**</td>
</tr>
<tr>
<td>D6</td>
<td>-0.33**</td>
<td>0.07</td>
<td>-0.08</td>
</tr>
</tbody>
</table>

***p<0.001, ** p<0.01, *p<0.05, corrected for multiple comparisons using Bonferroni

B. TD children

<table>
<thead>
<tr>
<th></th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
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</thead>
<tbody>
<tr>
<td>MD</td>
<td>SE</td>
<td>MD</td>
<td>SE</td>
</tr>
<tr>
<td>D4</td>
<td>-0.22***</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>D5</td>
<td>-0.41***</td>
<td>0.04</td>
<td>-0.19***</td>
</tr>
<tr>
<td>D6</td>
<td>-0.47***</td>
<td>0.07</td>
<td>-0.25**</td>
</tr>
</tbody>
</table>

***p<0.001, ** p<0.01, *p<0.05, corrected for multiple comparisons using Bonferroni

Notes: Post hoc ANOVA tests using response times for (A) children with ASD and (B) TD children. D3-D6= Difficulty levels 3 to 6; MD = Mean difference; SE: Standard error.
4.2 fMRI Data

**Task-related activation within groups.** The primary objectives of the fMRI analysis were to investigate the pattern of brain activity exhibited as a function of difficulty level (WM capacity) and examine if this differed in children with and without ASD. As expected, linear trend analyses (D3 – D6) showed that while some brain areas linearly increased in activity as a function of difficulty, others decreased. As shown in Figure 3, TD children showed significant bilateral activation that increased linearly as a function of difficulty in the bilateral middle occipital gyrus (BA19), fusiform gyrus (BA37), precuneus (BA7), inferior frontal gyrus (BA45), right DLPFC (BA9), and dorsal cingulate/dorsal medial prefrontal cortex (BA 32/8) extending to the anterior cingulate (B33/24; Table 4a). TD children also showed activation that decreased linearly in the posterior cingulate (BA23/31) and anterior medial prefrontal gyrus (BA10) as a function of difficulty (Table 4b). In contrast, children with ASD demonstrated bilateral activation in the middle occipital gyrus (BA19) and fusiform gyrus (BA37) that increased linearly with increasing difficulty (Table 5a), and activation that decreased linearly in the posterior cingulate (BA23/31) and anterior medial prefrontal gyrus (BA10/32), as a function of difficulty (Table 5b).
Table 4. Linear trend analyses across difficulty levels: TD group

A. Regions increasing in activation

<table>
<thead>
<tr>
<th>MNI Coordinates</th>
<th>Voxels</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z value</th>
<th>P-value</th>
<th>Hem.</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-26</td>
<td>-90</td>
<td>16</td>
<td>5.04</td>
<td>1.88x10-34</td>
<td>L</td>
<td>Middle occipital gyrus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>-82</td>
<td>-10</td>
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B. Regions decreasing in activation

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<th>P-value</th>
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Notes. Results from linear trend analyses from D3-D6 for TD children. Areas that increased as a function of difficulty level (A) are associated with WM and visuospatial processing, whereas areas that decreased as a function of difficulty level (B) are associated with the default mode network. MNI coordinates represent the peak Z value of the cluster; X= peak local maxima within cluster.

Table 5. Linear trend analyses across difficulty levels: ASD group

A. Regions increasing in activation

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<tr>
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<th>z</th>
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<th>P-value</th>
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### B. Regions decreasing in activation

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</table>

Notes. Results from linear trend analyses from D3-D6 for children with ASD. Areas that increased as a function of difficulty level (A) are associated with WM and visuospatial processing, whereas areas that decreased as a function of difficulty level (B) are associated with the default mode network. MNI coordinates represent the peak Z value of the cluster; X= peak local maxima within cluster.
Figure 3. Group activation maps for the linear trend analyses in ASD and TD groups during CMT.
Significant activations using cluster-based thresholding determined by Z>|2.3|, and a corrected cluster significance threshold of \( p=0.05 \). Areas in red depict regions of increasing activation as a function of difficulty, and areas in blue depict regions of decreasing activation. dmPFC= dorsal medial prefrontal cortex; Post Cing= posterior cingulate cortex; Prec= precuneus; DLPFC= dorsolateral prefrontal cortex; ACC= anterior cingulate cortex; IFG= inferior frontal gyrus; MidOcG= middle occipital gyrus; FusG= fusiform gyrus; amPFG= anterior medial prefrontal gyrus.
Between-group differences in task-related activation. We examined whether there were significant group effects in increasing or decreasing activation as a function of difficulty. As shown in Figure 4, three regions demonstrated group differences in activation. These areas included the bilateral precuneus (BA7), right DLPFC (BA9) and left dorsal medial premotor cortex (BA8; Table 6). In these regions, TD children showed greater increasing activation with increasing difficulty relative to children with ASD. See Figure 5 for graphs of percent signal change for ROIs in these areas.

**Table 6.** Regions of significant differences between TD and ASD groups

<table>
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<tr>
<th>Voxels</th>
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<th>y</th>
<th>z</th>
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<th>P-value</th>
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<td>Dorsal medial premotor cortex</td>
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</table>

Notes. Results from between group comparisons of the linear trend analyses from D3-D6. All regions reported are areas in which TD children showed greater increasing activation with increasing difficulty level than children with ASD. There were no areas where children with ASD showed greater increasing or decreasing activity across difficulty than TD children. MNI coordinates represent the peak Z value of the cluster; X= peak local maxima within cluster.
Figure 4. Results from between-group comparisons. Significant activations using cluster-based thresholding determined by $Z>2.3$, and a corrected cluster significance threshold of $p=0.05$. Areas in red depict regions where the control children showed significantly more increasing activation as a function of difficulty level than the ASD group. There were no areas where the ASD group showed more activation or deactivation than controls. Prec = precuneus; DLPFC = dorsolateral prefrontal cortex; dmPMC = dorsal medial premotor cortex.

Figure 5. Percent signal changes as a function of difficulty between task difficulty and baseline conditions in areas where children with ASD significantly differed from TD children in the linear trend analyses. DLPFC = dorsolateral prefrontal cortex.
Chapter 5
Discussion and Conclusions

5 Discussion and Conclusions

Using a task that isolated cognitive processes of WM, we observed a significantly reduced linear increase in prefrontal and parietal activations as a function of WM load in children with ASD relative to TD controls. These areas included bilateral precuneus, right DLPFC and left medial premotor cortices. Other areas known to underlie WM function, including the anterior cingulate and inferior frontal gyri, did not differ in activation between TD and ASD subjects. Overall, TD children demonstrated an opposing system of cognitive processes where areas related to task difficulty (frontal regions) increased in activity, and areas associated with the brain’s DMN (posterior cingulate and anterior medial frontal gyrus) decreased in activity with increasing cognitive demand. This coordinated function appeared to be absent in children with ASD due to impaired modulated activity in both the prefrontal cortex and occipital-parietal network. There were no significant differences in performance on any subtests of the WMTB-C, as well as on the CMT, between ASD and TD groups (except at D5), suggesting that the findings are not confounded by behavioural differences.

TD children showed both increasing frontal and posterior/parietal activation as a function of cognitive load, whereas the ASD group showed increasing activation in only the posterior visual regions, including the left and right fusiform and middle occipital gyri. This pattern is consistent with findings of Koshino and colleagues (2005), who concluded that during WM function, individuals with ASD rely on posterior regions related to low-level cognition, rather than areas of high level-cognition, such as in the prefrontal cortices. More activation in occipital-temporal areas in ASD has been proposed to reflect visually based processing styles and a tendency to rely primarily on visual features and details of objects, rather than on WM [58]. Less extensive use of prefrontal areas may be the result of abnormal prefrontal development [15-16] and connectivity [10,19] reported in individuals with ASD. Although a reliance on posterior regions may be adequate for visuospatial processing, it is likely inefficient for more complex cognitive operations, such as language comprehension [19] and WM [10], which would have implications for the social and executive deficits typically observed in ASD.
The finding of reduced activation within the frontal cortex may be most significant for ASD due to its crucial role in WM and executive function [10,41-42,59-61]. Consistent with previous WM studies of adults [10,12] and adolescents [14] with ASD, we found that children with ASD demonstrated reduced activation of the DLPFC (BA 9). In this area, TD children showed a graded increase in activity from D4 to D6, whereas activity seemed to plateau in children with ASD. The DLPFC is believed to play a critical role in holding information “online” [26] and mediating strategic organization and data compression processes [62-63], hence its sensitivity to increasing cognitive demand in ours and other studies [62-65]. Reduced linear activation across difficulty in this area suggests that children with ASD may fail to use appropriate organizational strategies, such as “chunking” methods, which facilitate WM by simplifying cognitive load/demand. Furthermore, normative studies have demonstrated that with increasing age, individuals rely more on DLPFC in WM processes [41,60,66], suggesting that specialization of this region for WM coincides with structural maturation across development. It is possible that evidence of early abnormal growth patterns in ASD in the frontal cortex, the DLPFC in particular [15], adversely impacts its functional integrity; these speculations require further research.

Reduced linear activation of the precuneus across difficulty in ASD subjects is also of interest, given evidence supporting parietal involvement in addition to the prefrontal cortex during spatial WM processing [42,60,66,67]. The precuneus forms part of the proposed occipito-parietal network, or alternatively the visual ‘dorsal pathway’, responsible for spatial visual processing (i.e., object location) [68]. Conversely, the occipito-temporal, or visual ‘ventral pathway’, includes the fusiform gyri and is critical to object identification (i.e., colour and shape) [68]. Given that CMT was a visuospatial task with gradual increases in WM that needed to be processed, areas responsive to both spatial search (occipito-parietal regions) and categorization (occipito-temporal regions) should show increased recruitment across cognitive load. Although group differences in fusiform activity were absent, we observed a significant difference in the precuneus; TD children showed increasing activity from D3 to D6, whereas the ASD group showed an overall decreasing pattern of activation. Impaired parietal activity is consistent with prior studies of WM in ASD [10,14], and lends support to the proposed dorsal stream deficits in ASD, as well as in other developmental disorders [69-72], while ventral processing is relatively intact. Furthermore, normative developmental studies suggest that the dorsal pathway has a
more protracted maturational trajectory than the ventral stream [60], and WM fMRI studies demonstrate greater parietal [4,66] but less fusiform recruitment [60] with age. Thus, spared fusiform/occipital activity and abnormal parietal functioning in our ASD group may reflect immature WM processing typically seen in young children. However, it is not clear whether the recruitment of mature neural substrates underlying WM processes is delayed, persistently weak or arrested. Future longitudinal work will help clarify the developmental path of WM neural circuitry in ASD.

Overall our results are consistent with previous neuroimaging studies of WM indicating prefrontal and parietal system abnormalities in ASD, as well as the tendency to rely on posterior brain regions associated with lower-level cognitive processing. The present study also converges on the growing body of literature proposing dorsal stream deficits in visuospatial processing in this population. We are contributing new information on WM differences early in school-aged children with and without ASD, demonstrating that even in pre-teens, there are significant differences in the brain activation patterns with increasing cognitive load that differentiate the groups. This research also provides insight into default mode and WM networks, in which TD children demonstrate balancing activation patterns between these two mental processes that are both modulated by cognitive load, whereas children with ASD do not.

It is important to consider the limitations of the current study when interpreting results. With comparable CMT and WMTB-C behavioural performance between ASD and TD groups, we eliminated performance as a confounding factor. Consequently, our sample was less representative of low-functioning individuals with ASD, and thus, results are generalizable to higher-functioning individuals only. Future fMRI research is required to understand WM function across various levels of functioning and a range of symptoms. In addition, given our choice of control subjects (TD children), findings can only provide information about differences from the norm. Comparisons to other atypical populations who share similar cognitive but different clinical profiles (i.e. ADHD) [5] will further our understanding about the neural patterns that are unique to ASD, potentially explaining characteristic behaviour in this complex group.

Despite these limitations, findings from the current study have a number of important implications. Several researchers have stressed the importance of executive cognitive skills for
social function [5,73], a core deficit of ASD. However, there remains a gap in the literature regarding the link between neuropsychopathology and clinical symptoms of autism. With respect to our findings of atypical neural activity underlying WM processes in ASD, this may impair the ability to hold information “online” that may effect one’s ability to evaluate and select appropriate responses during peer interactions, translating into socially inappropriate behaviour. Future neuroimaging studies should work towards understanding the relation between ASD symptomology and neural activation patterns associated with WM processing. Overall, our findings will help guide future longitudinal work by localizing areas of vulnerability to developmental disturbances. Developmental information will allow us to identify the nature and timing of atypical development, which is critical in establishing age-appropriate cognitive or pharmacological remediation for WM function and behavioural deficits in ASD.
References


Appendices

**Appendix A.** fMRI data for medicated and non-medicated children with ASD

Percent signal change as a function of difficulty between task difficulty and baseline conditions in children with ASD who were on medication versus those who were not. Areas of the brain shown are from regions where children with ASD significantly differed from TD children in the linear trend analyses.

Notes. Potential differences between medicated and non-medicated children with ASD were also examined statistically using the FSL FEAT, and no significant differences were found between children with ASD who were and were not on medication. However, due to low N (only 6 subjects on medication), this statistical test may not be reliable. Therefore scatter plots were created to visually examine the data for significant group differences, and this reaffirmed that medication does not appear to affect the findings.