Functional Magnetic Resonance Imaging in Pediatric-Onset Multiple Sclerosis: Relation to Structural Damage and Cognition

by

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Abstract

Pediatric Multiple Sclerosis (MS) is increasingly being recognized, with special concern being placed on how the demyelinating disease process affects normal brain development and the acquisition of cognitive abilities required for vocational success in adulthood. While pediatric MS patients are at risk for cognitive impairment early in their disease, many youth with pediatric MS appear to function at the same level as their age-matched peers despite the accrual of disease-related structural brain damage. This thesis aims to investigate whether functional magnetic resonance imaging (fMRI) can help understand mechanisms for cognitive preservation as well as the discrepancy between performance and accrual of structural disease insult, using both resting-state and a task-based processing speed paradigm. It was observed overall that pediatric MS patients demonstrate higher resting-state functional connectivity, particularly of the default-mode network, compared to healthy controls. Furthermore, higher functional connectivity of the precuneus within the default-mode network was associated with reduced white matter structural integrity as measured using diffusion tensor imaging (DTI). There is, however, a breakdown specifically of thalamo-cortical networks with increasing lesion accrual and reductions in thalamic volume. It was also observed that
lower cognitive performance was associated with greater functional connectivity of the frontal medial cortex with the anterior cingulate and precuneus during resting-state, suggesting less neural efficiency in patients who demonstrate some cognitive challenges though are not yet impaired. During the performance of an actual processing speed fMRI task, faster performance was associated with activation of a greater number of brain regions suggesting that a compensatory mechanism is present during actual task performance. Overall, this thesis demonstrates that pediatric MS is associated with brain activation abnormalities during resting-state that relates to the extent of neuronal injury. This work also sheds light on the factors which may be accounting for preservation of cognitive abilities, namely greater overall group activation during resting-state and greater activation associating with faster performance during a task that probes processing speed.
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Contributions

Nadine Akbar (author) solely prepared this thesis. All of the following aspects were executed solely by the author: planning, execution (recruitment and data collection) analysis, writing.

Dr. Brenda Banwell (Primary Supervisor): teaching and mentorship, resources, guidance and assistance in manuscript and thesis preparation, editing of thesis.

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Magdalena Lysenko: Recruitment and data collection.

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Dr. Berengere Aubert-Broche: Performed the brain volume segmentation analysis (described in Section 3.2) and provided Figure 7.

Dr. David Araujo: Performed the lesion volume analysis/ measurements(described in Section 3.2) and provided Figure 6.
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List of Abbreviations

AD- Axial Diffusivity
ADEM- Acute Disseminated Encephalomyelitis Syndrome
ADS- Acquired Demyelinating Syndrome
BOLD- Blood Oxygenation Level Dependent
BNBC- Brief Neuropsychological Battery for Children
CIS- Clinically Isolated Syndrome
DIS- Dissemination in Space
DIT- Dissemination in Time
DTI- Diffusion Tensor Imaging
EDSS- Expanded Disability Status Scale
EPI- Echo-Planar Imaging
FA- Fractional Anisotropy
FLAIR- Fluid-attenuated Inversion Recovery
fMRI- Functional MRI
fMRI-SDMT- fMRI version of the Symbol Digit Modalities Test
FWHM- Full-Width at Half Maximum
GLM- General Linear Model
HRF- Hemodynamic Response Function
ICA- Independent Components Analysis
MD- Mean Diffusivity
MS- Multiple Sclerosis
MRI- Magnetic Resonance Imaging
PD- Proton Density
RAVLT- Rey Auditory Verbal Learning Test
RRMS- Relapsing-Remitting MS
RD- Radial Diffusivity
ROI- Region-of-Interest
SDMT- Symbol Digit Modalities Test
TMT-A- Trail Making Test Part A
TMT-B- Trail Making Test Part B
TOMAL-2- Test of Memory and Learning, Second Edition
WASI- Wechsler Abbreviated Scale of Intelligence
WJ-III- Woodcock-Johnson-III Battery
Chapter 1: Literature Review
1.1 Introductory Statement

The study of what the brain does and how it can influence behavior has within the past two decades been informed by the use of functional magnetic resonance imaging (fMRI). fMRI has allowed for the mapping of brain functions to specific brain regions. It has become increasingly recognized, however, that many cognitive functions rely not just on the functioning of one area, but on the integration of function of a number of brain regions. This shift in focus to integrated patterns of activation has largely influenced how brain fMRI data is analyzed, for example using more network-based functional connectivity approaches.

Resting-state fMRI involves observing the brain activation patterns elicited when an individual is simply asked to lie still and not engage in any task. Even in this state of “rest”, certain brain regions consistently demonstrate synchronous activation with other regions in so-called “networks”. The networks seen at rest strike high resemblance to those elicited during task performance, suggesting resting-state as a simpler and easier way to interrogate brain function (i.e. by not requiring a task-based paradigm). For example, commonly observed resting-state networks are demonstrated for visual processing and sensorimotor function which are almost identical to the areas that show heightened activation during performance of tasks eliciting these functions. The most robustly-observed resting-state network is the “default-mode network”, which shows increased activation while at rest, and “de-activation” during cognitive task performance. The default-mode network is associated with its own unique set of cognitive processes such as basic mind-wandering or daydreaming, self-referential processing, overall sensory awareness, thinking about the future, and memory consolidation, all of which are important aspects of everyday experience.

Resting-state fMRI has also resulted in a shift of thinking of the brain as a reflexive organ that only responds to external demands, to one that has a baseline intrinsic organization or state that gets modified in response to internal mental or external environmental demands. For example, when cognitive demands are placed (e.g.
through asking participants to perform an activity) certain networks enhance their activation whereas others “deactivate”. For some individuals it may be more of less difficult to modify this intrinsic activity. For example, individuals may have more difficulty getting into “resting-state” due to anxiety of being in a MRI scanner or continuing ruminating about a previously completed cognitive activity. It is also of interest to consider how resting-state networks might be altered in disease populations and how this may relate to cognition and/or behavior. For example, young adults with autism spectrum disorder (ASD) show an absence of default-mode network activation during rest (Kennedy, Redcay, & Courchesne, 2006). Furthermore, greater levels of abnormal activation are associated with greater social impairment. A possible explanation for these findings is that ASD is associated with different cognitive mentation at rest, such as a lack of self-referential processing, which is associated with failure to modulate default-mode network activity.

fMRI is a valuable tool to study diseases that directly impact neural networks. One such disease, Multiple sclerosis (MS), is a chronic, inflammatory and degenerative disease of the central nervous system associated with widespread white matter as well as gray matter damage. As MS disrupts the transmission of electrical impulses throughout the brain across axons, the question then becomes how this affects neuronal and overall brain function. If we think of MS as damaging to the brain’s “highways” we would speculate that this would result in disruption to the brain’s functional networks. We have seen, however, that many MS patients are able to function at normal or above normal levels on cognitive tasks. We postulate that this functional preservation is likely due to some type of functional reorganization. Using fMRI, functional re-organization has been shown to be present in MS patients but it still remains poorly understood what underlies this reorganization. It could be due to increased recruitment of parallel pathways or unmasking of latent connections (Tomassini et al., 2012).

My thesis will explore resting- and task-specific fMRI in pediatric MS patients. My study cohort is comprised of pediatric MS patients who are largely cognitively intact permitting evaluation of the impact of MS on neural networks prior to impairment, and
potentially at time when compensatory mechanisms might be operative. My thesis also
aims to determine how resting-state fMRI network connectivity relates to the extent of
focal (lesional) and more global MS-related damage in the brain. The final aim of my
thesis relates to how resting-state fMRI network connectivity relates to cognitive
performance on a series of neuropsychological tests, as well as how brain activation
patterns elicited by performance on a speeded task relate to actual performance on this
task. Related to this latter point, cognitive processing speed may be impacted earlier
than global cognitive impairment given the clear insult of MS on myelinated pathways.

My thesis will start with a literature review of key facets of pediatric MS with emphasis
on the cognitive features. The following chapters will follow a traditional format
presenting hypotheses and aims, methods, results, final discussion and future
directions.

1.2 Multiple Sclerosis (MS)

Multiple sclerosis is a chronic and inflammatory disease of the central nervous system.
It is the most common cause of non-traumatic neurological disability in young adults
(Multiple Sclerosis International Federation, www.atlasofms.org). Canada currently has
the highest prevalence of MS worldwide, with approximately 100,000 Canadians
currently living with MS (Evans et al., 2013). The worldwide prevalence of MS
approaches 2.5 million persons (Multiple Sclerosis International Federation,
www.atlasofms.org).

The biological underpinnings of MS seem to involve two primary components: the
inflammatory component and the neurodegenerative component. The inflammatory
component involves the activation of immune cells which pass through blood-brain
barrier to attack myelin. This lends MS to the classification as an auto-immune disease.
Focal demyelination leads to disturbances in the transmission of electrical impulses
along myelinated axons. The neurodegenerative component refers to the damage of the
axons themselves because of loss of integrity due to loss of the protective myelin
sheath. Neuro-degeneration likely involves multiple mechanisms including oxidative
stress, mitochondrial dysfunction and energy deficits, ion channel dysfunction, and/or apoptosis and Wallerian degeneration (Friese, Schattling, & Fugger, 2014).

1.2.1 Etiology

MS etiology is thought to relate to an interplay between genetic and environmental factors. Support for a genetic component is evidenced by an MS concordance rate in monozygotic twins of 38%, which compared to a rate of only 4% in dizygotic twins (Ebers, 2008). The percentage of pediatric MS patients with any family history of MS is 16% (Banwell et al., 2011) with 3%, 5%, and 10% being first, second, or third-degree relatives respectively.

MS prevalence varies around the world (Evans et al., 2013; Kingwell et al., 2013; Makhani et al., 2014). Environmental factors such as vitamin D, Epstein-Barr virus, and smoking have all been implicated in MS risk. There is a global latitude gradient in the prevalence of MS, with lower prevalence reported in populations living close to the equator (Simpson et al., 2011). The highest reported worldwide prevalence rates are noted in Canada, Scandinavian countries, and in Scotland which could be influenced by low ultraviolet radiation during colder months, and subsequent lack of vitamin D. The amount of winter sunlight directly correlates with MS prevalence rates (Ebers, 2008). Diet may also play a role as low MS prevalence is also found in populations that consume large amounts of oily fish, a rich source of vitamin D (Swank & Dugan, 1990). Viral exposures, and the inherent immune responses to such exposures, have also been implicated in MS risk. Nearly 100% of adults with MS test positive for Epstein-Barr virus (EBV) versus 90% of the healthy population (Haahr et al., 1995). The difference in seroprevalence is even more pronounced in pediatric MS (Yea et al., 2013; Banwell, Krupp, Kennedy, et al., 2007; Alotaibi et al., 2004), where 85% of pediatric MS patients but only 40-45% of regional age-matched healthy children have remote EBV exposure. Exposure to environmental pollutants, particularly cigarette smoke has also been implicated in both MS risk. For example, Riise and colleagues (2003) observed higher risk of MS among smokers compared to those who have never smoked.
In the Canadian prospective national pediatric cohort study, of which participants of this thesis were recruited, presence of HLA-DRB1*15 alleles, remote Epstein-Barr virus infection, and low 25-hydroxyvitamin D concentrations increased risk of MS (Banwell et al., 2011), indicative of a similar genetic and environmental contribution to both adult- and pediatric-onset MS.

1.2.2 Clinical features

The most common symptoms of MS include fatigue, cognitive dysfunction, gait difficulties, numbness or tingling, muscle spasticity or weakness, vision problems, dizziness and vertigo, pain, bladder problems, and sexual dysfunction. MS is categorized into four subtypes based on the presence of relapses and disease progression. In the relapsing-remitting MS (RRMS) phenotypes, a relapse (also at times referred to as “exacerbation” or “attack”) of symptoms is defined as an episode of neurological disturbance lasting for longer than 24 hours without fever or infection. Discrete relapses are separated by more than 30 days. These are followed by periods of remission which may or may not present with residual symptoms. Progressive disability may occur following a period of relapsing-remitting disease, or progressive disability can occur from disease onset.

1.2.3 Disease subtypes

The subtypes of MS are as follows and depicted in Figure 1:

a) Relapsing-remitting MS (RRMS): Approximately 85% of adult MS patients are initially diagnosed with this subtype. It is characterized by clearly defined relapses followed by periods of recovery (Lublin & Reingold, 1996). The time between relapses is characterized by lack of disease progression. RRMS is diagnosed in almost all pediatric MS patients.

b) Secondary-progressive MS: This subtype occurs after initial RRMS and is characterized by progression with or without occasional relapses, minor remissions, and plateaus (Lublin & Reingold, 1996). Determining the transition
point to secondary progressive MS is difficult given that there are no clear clinical, imaging, or immunological criteria to define the transition and that transition is usually gradual. In adult MS, the median time to conversion to secondary-progressive MS has been reported as between 10-20 years from disease-onset (Rovaris et al., 2006; Vukusic & Confavreaux, 2003; Weinshenker et al., 1989). Approximately 50% and 90% of adult RRMS patients will transition to secondary-progressive MS within 10 years and 25 years respectively (Eriksson, Andersen & Runmarker, 2003). The factors associated with faster conversion are older age at onset, male sex, spinal-cord related symptoms, and incomplete recovery from relapses (Rovaris et al., 2006). Once disease progression begins, progressive disability accrues independent of discrete relapses.

c) Primary progressive MS: Approximately 10-15% of adult MS patients present with primary progressive MS. This subtype is mostly diagnosed in older patients with the mean age of disease onset being 40 compared to 30 years of age in RRMS (Miller & Leary, 2007; Tremlett, Paty, & Devonshire, 2005; Confavreux & Vukusic, 2006). This subtype carries with it the worst prognosis clinically as disability accrues in downward linear slope from time of symptom onset. This subtype might present with minor fluctuations but no distinct relapses. Primary progressive MS does not appear to really exist in children.
Figure 1. Three subtypes of MS (adapted from Lublin & Reingold, 1996; Lublin et al., 2014)
1.2.4 Diagnostic criteria

The pediatric MS cohort studied in this thesis were diagnosed according to the 2010 revised McDonald criteria (criteria detailed in Polman et al., 2011). Diagnosis of RRMS rests on meeting the criteria of both dissemination in space (DIS) and dissemination in time (DIT) using clinical and magnetic resonance imaging (MRI) findings. RRMS can be confirmed clinically by discrete relapses (as defined above in Section 1.2.2) involving different areas of the CNS (clinical DIS), separated by time (clinical relapse evidence of DIT). MRI findings can also be used to define DIS based on one or more T2 lesion(s) in at least two of four following areas of the CNS: periventricular, juxtacortical, infratentorial, and spinal cord. The 2010 revised McDonald MRI criteria for demonstration of DIT includes: (1) a new T2 and/or T1 gadolinium-enhancing lesion(s) on a follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI, or (2) simultaneous presence of asymptomatic T1 gadolinium-enhancing and nonenhancing lesions at any time. According to the 2010 criteria, MS can be diagnosed at the time of first clinical presentation if the MRI features confirm both DIS and DIT, or can be diagnosed over time based on clinical or MRI evidence of new lesions. MRI features of pediatric MS are described in more detail in Section 1.3.8.

Diagnosis of primary progressive MS rests on the presentation of one year of disability progression (retrospectively or prospectively determined) and two of the following criteria: (a) positive brain MRI, as indicated by nine or more T2 lesions, or four or more T2 lesions with abnormal visual evoked potential, (b) two or more focal T2 lesions in the spinal cord, (c) positive cerebrospinal fluid (Brieva, Rio, & Montalban, 1997).

1.3 Pediatric MS

There has been in increased international awareness of pediatric MS over the last 15 years. Extensive research into this unique patient group was initiated by the Canadian Pediatric Demyelinating Disease network, a program which began in 2004 and is led by my supervisor Dr. Brenda Banwell. The Canadian Pediatric Demyelinating disease study enrols children during the first incident of demyelinating attack, and prospectively
evaluates patients at 3-month, 6-month, 1-year and subsequent annual intervals. The study focuses on clinical, environmental, genetic, and MRI features predictive of subsequent confirmation of MS. The study of putative causal factors (e.g. genes, environment) is particularly informative in children considering that the time window between exposure to the causative agent and actual disease expression is shorter (i.e. closer to biological onset).

1.3.1 Diagnostic considerations

Diagnosis of pediatric MS is based on the same criteria described in the previous Section 1.2.4. For the pediatric MS cohort studied as part of my PhD, patients with MS clinical onset (i.e. first relapse) occurring prior to age 18 were enrolled.

There are a few considerations for the diagnosis of pediatric MS. One of the key issues is to distinguish the first attack of MS from a monophasic demyelinating illness, and in particular to distinguish children manifesting with acute disseminated encephalomyelitis (ADEM). ADEM differs from MS as it mostly occurs only once (i.e. is a monophasic illness), whereas MS is a chronic disease with further, subsequent relapses. The diagnosis of ADEM requires the presence of “encephalopathy” in addition to polyfocal neurological deficits. Encephalopathy refers to alteration in consciousness (e.g. stupor, lethargy) or behaviour change unexplained by fever, systemic illness, or postictal symptoms and is demonstrated as excessive irritability, somnolence, or coma (Krupp et al., 2013).

Criteria for the diagnosis of pediatric MS were published during this work (Krupp et al., 2013). All patients enrolled in this study fulfill requirements of these criteria. According to these criteria, pediatric MS can be diagnosed in the following scenarios:

1. Two or more nonencephalopathic (encephalopathy described above) clinical CNS events (DIT) involving more than one area of the CNS (DIS).
2. One nonencephalopathic episode and MRI findings meeting criteria for DIS. DIT criteria can be demonstrated by at least one new enhancing or nonenhancing lesion at follow-up MRI.

3. One encephalopathic (ADEM) attack followed by a nonencephalopathic clinical event at least three or more months after symptom onset (DIT) and associated with new MRI lesions to meet DIS criteria.

4. For children age 12 or older, a first relapse that does not meet ADEM criteria, and whose MRI findings are consistent DIS and DIT according to the 2010 revised McDonald criteria.

1.3.2 Prevalence

Pediatric MS is increasingly being recognized, with approximately 5% of total MS populations diagnosed with pediatric MS (Ghezzi et al., 1997; Chitnis et al., 2009; Harding et al., 2012). The annual incidence of pediatric MS per 100,000 children is estimated to be 0.18 in Canada (Banwell et al., 2009), with approximately 1000 Canadian children currently living with the disease. A first attack of demyelination can be referred to as acquired demyelinating syndrome (ADS). This may occur as a transient illness or may represent the first attack of MS.

Different ADS presentations include:

1. Optic neuritis: inflammation of the optic nerve associated with vision loss.

2. Transverse myelitis: inflammation of the spinal cord associated with weakness of the limbs, spinal-level sensory, bladder, or bowel dysfunction.

3. ADEM: defined by polyleisional neurologic deficits, accompanied by encephalopathy (defined in 1.3.1)

4. Monofocal demyelination (mono-ADS): defined by neurological deficits localized to a single CNS site but not the optic nerve or spinal cord.
5. Polyfocal demyelination (poly-ADS): defined by multiple neurologic deficits localized to more than one site of the CNS in the absence of encephalopathy.

The annual incidence of ADS in children and adolescents in Canada is reported as 0.9 per 100,000 (Banwell et al., 2009). The percentage of these children who will be diagnosed with MS within 5 years is 21% (Banwell et al., 2011). In children with ADS, female sex and older age of onset are associated with higher likelihood of being diagnosed with MS.

1.3.3 Clinical features

The major features of pediatric MS, as well as a comparison to adult MS are given in Table 1. This table mentions the Expanded Disability Status Scale (EDSS, Kurtze, 1983) which is a commonly employed measure of physical disability in MS with scores ranging from 0 (no disability) – 10 (death due to MS).

The most common presenting symptoms in pediatric MS are as follows: optic neuritis (10-22%), motor dysfunction (30%), sensory symptoms (15-30%), ataxia (5-15%), and brainstem symptoms (25%) (Banwell, Ghezzi, Bar-Or et al., 2007). The clinical profile of pediatric MS versus adult MS is argued to be more “inflammatory” as children with MS have more frequent relapses (Gorman et al., 2009), show greater lesion burden (Ghassemi et al., 2014; Waubant et al., 2009), and primary progressive MS is extremely rare (Banwell, Krupp, Kennedy, et al., 2007). Compared to adults, pediatric MS patients also tend to recover better from relapses than adults, suggesting better remyelination in the pediatric MS context (Harding et al., 2012; Cossburn et al., 2011).

The overall life expectancy for adult-onset MS patients is 6 years lower than the rest of the population (Kaufman et al., 2014; Sadovnick et al., 1992). No life expectancy studies have been conducted in pediatric MS. Much higher rates of suicide are reported in the adult MS population (Pompili et al., 2012), although no such data is available for pediatric MS.
Table 1. Comparison of features of pediatric and adult MS

<table>
<thead>
<tr>
<th></th>
<th>Pediatric MS</th>
<th>Adult MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age of disease onset (years)</td>
<td>• 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>• 30</td>
</tr>
<tr>
<td>Disease subtype at onset</td>
<td>• Relapsing-remitting- 99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>• Relapsing-remitting- 85%, Primary-progressive - 15%</td>
</tr>
<tr>
<td>Initial presentation</td>
<td>• More cerebellar and brainstem symptoms&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>• Variable</td>
</tr>
<tr>
<td></td>
<td>• Pre-pubertal patients more likely to have polyfocal presentation&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Recovery from initial event</td>
<td>• 100&lt;sup&gt;f&lt;/sup&gt;</td>
<td>• Lower and decreases with older age of onset&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Annualized Relapse rate within</td>
<td>• Higher (1.13)&lt;sup&gt;h&lt;/sup&gt;</td>
<td>• Lower (0.40)</td>
</tr>
<tr>
<td>approximately the first 4 years of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disease MRI features</td>
<td>• Multiple white matter lesions</td>
<td>• Multiple white matter lesions</td>
</tr>
<tr>
<td></td>
<td>• Frequent periventricular lesions</td>
<td>• Frequent periventricular lesions</td>
</tr>
<tr>
<td></td>
<td>• Frequent juxtacortical lesions</td>
<td>• Frequent juxtacortical lesions</td>
</tr>
<tr>
<td></td>
<td>• Greater number of infratentorial lesions&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Female to male ratio</td>
<td>• Pre-puberty: 1:1&lt;sup&gt;d,e,k&lt;/sup&gt;</td>
<td>• 3:1</td>
</tr>
<tr>
<td></td>
<td>• Post-puberty: 3:1</td>
<td></td>
</tr>
<tr>
<td>Time to reach irreversibility</td>
<td>• Median time from disease onset to conversion to secondary progressive MS: 20 years</td>
<td>• Median time from disease onset to conversion to secondary progressive MS: 10 years</td>
</tr>
<tr>
<td>disability&lt;sup&gt;c,k,l,m&lt;/sup&gt;</td>
<td>• Median time from disease onset to reach disability landmarks:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EDSS 4 (relatively severe disability): 20 years</td>
<td>EDSS 4 (relatively severe disability): 10 years</td>
</tr>
<tr>
<td></td>
<td>EDSS 6 (assistance required to walk): 30 years</td>
<td>EDSS 6 (assistance required to walk): 20 years</td>
</tr>
<tr>
<td></td>
<td>EDSS 8 (restricted to bed or wheelchair): 40 years</td>
<td>EDSS 8 (restricted to bed or wheelchair): 30 years</td>
</tr>
<tr>
<td>Time point at which irreversible</td>
<td>• 10 years younger than adults (in untreated cohorts)</td>
<td></td>
</tr>
<tr>
<td>disability occurs&lt;sup&gt;c,k,l,m&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EDSS- Expanded Disability Status Scale, (Kurtzke, 1983)
<sup>a</sup>- Boesen et al., 2014; <sup>b</sup>- Banwell, Krupp, Kennedy et al., 2007; <sup>c</sup>- Simone et al., 2002; <sup>d</sup>- Ghezzi et al., 1997; <sup>e</sup>- Huppke et al., 2014; <sup>f</sup>- O’Mahony et al., 2015; <sup>g</sup>- Cossburn et al., 2011; <sup>h</sup>- Gorman et al., 2009; <sup>i</sup>- Waubant et al., 2009; <sup>j</sup>- Ghassemi et al., 2014; <sup>k</sup>- Renoux et al., 2007; <sup>l</sup>- Boiko et al., 2002; <sup>m</sup>- Harding et al., 2012
As reported in Table 1, pediatric MS patients take longer to accrue irreversibly physical disability when compared to adult-onset MS. Despite the longer time from onset, the age at which disability occurs is younger in pediatric-onset MS patients.

As our knowledge about pediatric MS has evolved, so has the recognition that while physical disability rarely occurs during the first few years of disease, the cognitive impact can occur early and have a major impact. Cognitive dysfunction can also negatively affect the acquisition of abilities required for adequate functioning in adulthood. Cognitive deficits in pediatric MS will be discussed in detail in Section 1.3.9.

1.3.4 Treatment

The first-line treatment regimen for pediatric MS is the same as that for adult-onset MS, with demonstrated safety and tolerability of interferons and glatiramer acetate (reviewed in Yeh et al., 2011). Acute relapses are treated with corticosteroids, the most recommended being intravenous methylprednisolone (Waldman et al., 2011; Chitnis et al., 2012). Separate from management of acute attacks, chronic disease suppression requires immunomodulatory disease-modifying therapies. In adults with MS, disease-modifying therapies have been shown to prevent future relapses, slow disability progression, and reduce lesion accrual (Oh & O’Connor, 2015). First-line disease-modifying therapies for MS include glatiramer acetate (Copaxone), interferon beta-1a (Avonex, Plegridy, and Rebif), interferon beta-1b (Betaseron and Extavia). All of these therapies are delivered via injection subcutaneously, with the exception of Avonex, which is injected intramuscularly. Second-line therapies for those patients with inadequate response to first-line therapies or for patients with clinically severe and rapidly evolving MS include natalizumab (Tysabri), fingolimod (Gilenya), teriflunomide (Aubagio), and dimethyl fumarate (Tecfidera), and alemtuzumab (Lemtrada). There is consensus that pediatric MS should be treated with disease-modifying agents soon after diagnosis is confirmed (Waldman et al., 2011; Chitnis et al., 2012). Medications for specific symptoms (e.g. fatigue, pain, depression, spasticity) are also commonly prescribed in pediatric MS.
In the context of this thesis, consideration should be given on how the use of disease-modifying treatment can affect MRI features and cognitive performance in MS. Criteria for the efficacy of MS disease-modifying agents includes the reduction of MRI measures of disease activity (new/enlarging T2 lesions and gadolinium-enhancing T1 lesions), with one agent (Fingolimod) reporting a reduction in brain volume loss (Kappos et al., 2010). Therefore, these agents help reduce the structural MRI disease burden but no studies have been conducted investigating how they might influence fMRI patterns of activation.

Many of the earlier, pivotal, clinical trials in MS did not include cognitive endpoints. From the limited cognitive data that has been collected from these trials, there is indication that disease-modifying agents have a beneficial effect on cognitive performance (i.e. either stabilization or improvement) across numerous cognitive domains such as information processing speed, memory, visuospatial ability, problem solving, and attention (Comi, 2010; Patti, 2012). However, practice effects need to be taken into account, with studies therefore evaluating between-group differences in the slope of cognitive change curves with respect to treatment (Comi, 2010; Patti, 2012).

1.3.5 Impact on academic and social functioning

One of the most concerning aspects of pediatric MS is how the disease affects school performance. Pediatric MS patients often require reduced class load due to fatigue, and require special assistance within the regular school system (Banwell & Anderson, 2005). The study of MacAllister and colleagues (2005) reported that 35% of pediatric MS patients required academic assistance in school. Examples of impact on school performance included increased time required to finish examinations, difficulty maintaining focus, and frequently missing class due to hospitalizations. The study of Amato and colleagues (2010) reported that school activities and achievements were negatively affected in 28% of pediatric MS patients as determined by requiring extra support teacher(s), repeating a school year, and high number (>30) of school days missed due to medical appointments, relapses, and therapy side effects. These authors
also reported disrupted participation in hobbies and sports (40%) and negative impact of
the disease on family and social relationships (28%).

Long-term follow-up studies on the influence of pediatric MS on long-term/ adult
outcomes have yet to be published. Questions remain as to how diagnosis of MS during
childhood/ adolescence affects outcomes such as how far these youth go in school,
their vocational outcomes (e.g. able to work part-time, full-time), family outcomes such
as getting married and having children, and ability to engage in social and recreational
activities. As studies of pediatric MS only began in the last 15 years, these data are not
yet available.

1.3.6 Psychiatric features

Psychiatric disorders are also commonly reported in pediatric MS. In the study of
MacAllister and colleagues (2005), six (46%) of 13 patients who underwent psychiatric
evaluation (patients not preselected) were diagnosed with an affective disorder
including major depressive disorder and anxiety disorder. In an Italian sample of 39
pediatric MS patients (Amato et al., 2010), psychiatric interview revealed the following:
major depression in 15%, depression and anxiety in 5%, panic disorder in 5%, and
bipolar disorder in 5%. Importantly, these authors noted that the prevalence of
depression (measured using the Children Depression Inventory) increased from 6% at
baseline to 17% at 2-year follow-up. In a study of 45 pediatric MS patients by Weisbrot
and colleagues (2014), 56% of patients met criteria for at least one psychiatric
diagnosis, with 68% of these patients receiving at least two or more diagnoses. The
most common categories of psychiatric diagnoses were anxiety disorders (33%),
attention deficit hyperactivity disorder (27%), and mood disorders (24%), assessed
using semi-structured psychiatric diagnostic interviews. In summary, psychiatric
disturbances are highly prevalent in the pediatric MS population, especially depression
and anxiety, supporting appropriate screening and management.
1.3.7 Fatigue

Fatigue is evident in 70-90% of patients with MS (Freal et al., 1984; Bakshi et al., 2003; Kos et al., 2008) and considered to be one of the worst and most disabling symptoms (Vercoulen et al., 1996; Fisk et al., 1994). MS fatigue can be classified as either primary or secondary. Primary fatigue is thought to be due to disease-related aspects including inflammation and demyelination. Secondary fatigue results from factors such as poor sleep, bladder disturbance, depression, lack of exercise, medication side effects and/or cognitive/mental exertion.

Using the PedsQL Multidimensional Fatigue Scale severe fatigue (two or more SD below published healthy control data) was reported by 32% and 51% of pediatric MS patients and their parents respectively (MacAllister et al., 2009). These authors also found that fatigue was significantly associated with more sleep difficulties, cognitive problems, emotional and academic difficulties. In an Italian sample of 57 pediatric MS patients (Goretti et al., 2012), also using the PedsQL, the percentage of patients who reported fatigue ranged from 9-14% according to self-reports, and 23-39% according to parent reports. This seemingly low prevalence could be due to differences in sample characteristics. Greater self- and parent-reported fatigue were also associated with greater reported depression. Finally, the study of Till and colleagues (Till, Udler, Ghassemi et al., 2012) reported a fatigue prevalence of 44% among 31 pediatric MS patients (simply defined qualitatively as yes/no). In addition, endorsement of fatigue was associated with greater parent-reported behavioural symptoms (e.g. attention problems, withdrawal), and poorer adaptive skills (e.g. functional communication, activities of daily living, social skills).

1.3.8 MRI features

An example of a pediatric MS patient MRI scan is shown in Figure 2. Generally, T2 bright lesions represent inflammation (e.g. influx of inflammatory cytokines), edema (high water content), gliosis (tissue scarring), and demyelination. T1 hypointense lesions are purported to represent focal tissue loss.
Figure 2. Example of MRI sequences depicting lesions in a pediatric MS patient enrolled in the present PhD study. Lesions appear bright (hyperintense) on the fluid-attenuated inversion recovery (FLAIR) image, T2-weighted, and Proton Density (PD) images. T1 hypointense lesions (black holes) appear dark in the T1-weighted image, with examples indicated by red arrows.

In children with ADS, the presence of either (a) one or more T1-weighted hypointense lesions, or (b) one or more periventricular lesions, is associated with an increased likelihood of MS diagnosis (Verhey et al., 2011). Risk is highest when both periventricular and T1 hypointense lesions are present. The classic MS white matter lesions seen in adult MS are also present in pediatric MS. There have been some studies demonstrating, however, differences in lesion characteristics in very young MS patients. For example, MS patients with onset of MS earlier than 11 years of age more often have confluent lesions and have fewer well-defined ovoid T2 bright lesions compared to pediatric MS patients with later onset (Chabas et al., 2008). The same study by Chabas and colleagues (2008) also found that the youngest patients had a much higher reduction in the number of T2 lesions on a second scan (92% patients had reduction) compared to older childhood-onset patients (29%).

Two research groups have demonstrated that pediatric MS is associated with more infratentorial lesions, relative to adult-onset MS. In a study comparing 41 pediatric-onset MS patients with 35 patients with adult-onset MS, pediatric-onset MS patients were
found to have a higher number of T2 bright lesions than adult-onset MS patients, especially in the posterior fossa (Waubant et al., 2009). Pediatric MS patients also had a greater number of gadolinium-enhancing lesions suggesting greater inflammatory disease activity and were more likely to have new T2 bright and T1 gadolinium-enhancing lesions on serial imaging, again implicating a more active course of disease activity in pediatric MS. A limitation of this study, however, was that the groups were not matched for disease duration. A recent study did match for disease duration, and confirmed a greater infratentorial lesion preponderance in pediatric MS patients compared to adult MS patients (Ghassemi et al., 2014). Pontine lesions were particularly notable in pediatric MS patients. The authors hypothesized that, given that myelination proceeds along a caudorostral gradient, there may be preferentially targeting of more mature white matter in children. The number of cortical lesions is reported to be less in pediatric MS compared to adult MS patients (8% versus 66% respectively; Absinta et al., 2011), although some imaging techniques and magnet strengths may underestimate cortical pathology.

In addition to the presence of lesions, patients with pediatric MS have smaller head size compared to age-and-sex matched healthy controls, suggesting that onset of MS during childhood affects primary brain and skull growth (Kerbrat et al., 2012). Pediatric MS patients also have smaller reported total brain volume (Kerbrat et al., 2012) and thalamic volumes (Kerbrat et al., 2012; Mesaros et al., 2008) compared to healthy controls. In addition to lower thalamic volume, reduced volume of the splenium of the corpus callosum, and the globus pallidus was reported in the study of Aubert-Broche and colleagues (2011). These authors also found that reduced volume of the splenium of the corpus callosum was associated with higher total (not co-localized) T2 lesion volume, and that reduced volume of the globus pallidus and optic tract was associated with longer disease duration. The significant correlation with lesion volume suggests that volume loss is a result of Wallerian degeneration of tracts transected by white matter lesions. A recent study by the same group (Aubert-Broche et al., 2014) provided evidence that the reduction in thalamic volume in pediatric MS is also associated with
failure of age-expected growth. Thalamic volume loss may also be due to tissue loss within thalamic lesions themselves. Thalamic lesions in MS have been reported to be demyelinating (Vercellino et al., 2009) and involve both gray and white matter.

Multiple sclerosis is characterized by prominent white matter involvement, as well as by the previously mentioned involvement of gray matter. A method for investigating white matter abnormalities is Diffusion Tensor Imaging (DTI). This section will start with a description of DTI, followed by discussion of DTI abnormalities in pediatric MS.

1.3.8.1 Description of Diffusion Tensor Imaging (DTI)

DTI is a technique used to infer the diffusion of water within tissue. By sampling diffusivity along multiple directions (e.g. 64 directions was used in the DTI protocol of this thesis) evenly spaced on a sphere, a 3D representation of the diffusion can be computed. The diffusion is modeled as an ellipsoid (e.g. Figure 3b) at each voxel. The principal axes of the ellipsoid are given by the three eigenvectors (ε₁, ε₂, and ε₃), with the length of these axes within the ellipsoid being indicative of diffusion distance (r) in a given time (t). The diffusion distance is proportional to the square root of the diffusivity (D) according to “Einstein’s equation” of:

\[ r^2 = 6Dt \]

Three diffusivity values are computed (eigenvalues λ₁, λ₂, λ₃) along the three principal axes/ three eigenvectors (ε₁, ε₂, and ε₃) of the diffusion tensor ellipsoid. If diffusion were unrestricted (e.g. placing a drop of ink within water), then diffusion would occur equally in all directions such as shown in Figure 3a. If diffusion were restricted in two directions (e.g. within a white matter tract) then diffusion would occur preferentially in one direction, such as in the ellipsoid shown in Figure 3b. In the case of water within white matter tracts, diffusion would occur preferentially parallel to the long axis of the axon, rather than perpendicular to it due to restriction from cell membranes. A problem inherent in the ellipsoid model, however, is that it assumes diffusion propagates in time and space in a Gaussian manner. If we considering diffusion where water molecules
encounter different viscosities and obstacles (e.g. cell membranes) it thus may be problematic to consider this diffusion as Gaussian. Techniques that could be used to alleviate this problem are diffusional kurtosis imaging (De Santis et al., 2011; Jensen & Helpern, 2010) or high angular resolution diffusion imaging (HARDI) techniques (Tuch et al., 2002; Frank, 2002).

Figure 3. Depiction of water diffusion in (a) unrestricted tissue and (b) in restricted tissue such as white matter tracts. Diffusion is approximated using a diffusion ellipsoid with the principal axes given by the three eigenvectors ($\varepsilon_1$, $\varepsilon_2$, and $\varepsilon_3$) and the diffusivity along these axes given by the eigenvalues ($\lambda_1$, $\lambda_2$, $\lambda_3$). (Adapted from Winston, 2012 reproduced with permission from the journal *Quantitative Imaging in Medicine and Surgery*)
The diffusion ellipsoid allows for the calculation of axial diffusivity (AD), radial diffusivity (RD), and fractional anisotropy (FA). AD is represented by the value of $\lambda_1$ of the diffusion ellipsoid and is in the units $10^{-3}$ mm$^2$/sec. AD indicates diffusivity parallel to the main axis of white matter tracts and is indicative of axonal loss (Aung, Mar, & Benzinger, 2013; Song et al., 2002). RD is calculated as $(\lambda_2 + \lambda_3) / 2$ of the diffusion ellipsoid and represents diffusivity perpendicular to the main axis. It is also measured in the units $10^{-3}$ mm$^2$/sec and is related to demyelination (Klawiter et al., 2011; Song et al., 2005). FA is calculated by the formula below:

\[
FA = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}
\]

FA incorporates both AD and RD and indicates what fraction of the diffusion tensor displays anisotropic (directionally dependent) diffusion. FA is a dimensionless unit with values ranging from 0-1 with higher values representing increased tissue integrity (Le Bihan et al., 2001; Mori & Zhang, 2006).

Examples of the three different types of DTI images, obtained from a MRI scan of one of the participants in the present PhD study, are shown in Figure 4.
Figure 4: Examples of the three different types of DTI images acquired in this study. The same slice is shown across the three different DTI images. (a) Fractional anisotropy (FA) image in which areas of high FA are brighter than surrounding tissue and occur along white matter tracts. Periventricular lesions are present in this image which have lower FA and appear darker. (b) Axial diffusivity (AD) and (c) Radial diffusivity (RD) images in which areas of higher diffusivity appear bright, such as in cerebrospinal fluid and periventricular lesions.

1.3.8.2 DTI features of pediatric MS

DTI offers a method to evaluate whether “normal-appearing” (i.e. non-lesional) brain tissue is indeed normal. As such, there is a primary focus on normal-appearing brain tissue in many DTI studies conducted in MS, as damage to lesional tissue is already assumed. The first study investigating DTI characteristics in pediatric MS patients was conducted in 2004 by Mezzapesa and colleagues, consisting of a sample of 13 pediatric MS patients (mean age=14.1) and 10 healthy volunteers. Higher average mean diffusivity (MD, represents average diffusion across all three axes/ eigenvectors) of the normal-appearing brain tissue (based on masking out T2-weighted lesions) was found in the pediatric MS group. The same research group conducted a subsequent study (Tortorella et al., 2006) in 23 patients with pediatric MS (mean age=14) and 16 healthy
volunteers. Here, they demonstrated higher average MD and decreased average FA of normal appearing white matter in the pediatric MS group.

In a study which included 38 patients with pediatric RRMS (mean age=14.7, mean disease duration=4.6 years), lower FA of normal-appearing white matter was found in the pediatric MS patients compared to healthy controls (Absinta et al., 2010). In addition, adult RRMS patients (n=27, mean age=41, mean disease duration 5.9 years) had higher average MD within lesion and higher gray matter average MD compared to the pediatric MS patients. Lower MD in the pediatric MS group suggests greater or more efficient tissue repair compared to adult MS patients.

Blaschek and colleagues (2013) studied 14 pediatric patients with MS (mean age=15) and 14 healthy subjects. These authors found that mean FA, MD, and RD values of the entire white matter skeleton (lesions not removed) differed between groups. The most pronounced decrease in FA was found in the splenium of the corpus callosum as well as within the right temporal and right and left parietal regions. They also observed that lower mean white matter skeletal FA was correlated with longer disease duration, suggesting loss of white matter tract integrity with longstanding disease.

Vishwas and colleagues (2010) used region-of-interest (ROI) and tract based analyses to investigate diffusion abnormalities in interhemispheric, projection, and intrahemispheric pathways in 10 patients with pediatric MS (mean age=16.6 years, mean disease duration 1.23 years) and 10 healthy controls. All tracts demonstrated higher mean diffusivity and lower mean FA (with or without lesions removed) in the pediatric MS compared to healthy control group. These authors conducted a later study (Vishwas et al., 2013) comparing DTI measures between 20 children with MS, 27 children with ADS, and 47 healthy controls. Once again diffusion abnormalities were present in callosal fibres, projection fibres, and association fibres in pediatric MS patients compared to healthy controls. Interestingly, the ADS children (imaged at first attack), did not differ from healthy controls suggesting that DTI features may require a longer time from onset to be detected.
The study of Tillema and colleagues (2012) studied 18 pediatric MS patients, 15 patients with CIS, and healthy controls. The pediatric MS group had lower FA, increased RD, and decreased AD compared to healthy controls in the two regions-of-interest studied which were the corpus callosum and internal capsule. The group of Rocca and colleagues (Rocca, Absinta, Amato et al., 2014) found that pediatric-onset MS patients (n=45) had distributed decreased FA in the white matter, as well as increased RD of the splenium of the corpus callosum and posterior parieto-occipital white matter compared to healthy controls. The same authors (Rocca, Absinta, Ghezzi et al., 2014) published findings from 44 RRMS pediatric MS patients and 27 healthy controls and also reported lower mean normal-appearing white matter FA and mean gray matter MD in the pediatric MS group. In both studies, cognitive impairment was associated with a greater extent of DTI abnormalities.

In summary, pediatric MS is characterized by both focal and widespread white matter damage as well as by loss of whole brain, deep gray and cortical tissue. The underlying biological substrates of these metrics are not completely understood but include a mixture of inflammatory and degenerative related processes. These structural features will be examined in this thesis in order to examine the function-structure relationship.

1.3.9 Cognitive features

The cognitive burden of pediatric-onset MS is especially significant given that it can affect the acquisition of abilities that develop over the course of childhood and adolescence. Prevalence rates for cognitive dysfunction in children and adolescents with MS range from 29-53% depending on assessment and sample characteristics (Till, Ghassemi, Aubert-Broche et al., 2011; Amato et al., 2008; Julian et al., 2013). Deficits have been reported across a broad range of domains, including memory, attention, information processing speed, visuomotor integration, language and IQ. For clarity, I have grouped the literature review in this section according to geographic cohorts studied by specific research groups. Higher prevalence rates for cognitive impairment have been reported in Italian cohorts. This could be due to the inclusion of patients with
more severe disease. Though not a formal part of this thesis, part of my PhD time included a Multiple Sclerosis International Federation Scholarship which funded a 3-month project in Italy. My time in Italy was focused on examination of predictors of change in cognitive performance over time using 5-year longitudinal data available from the Canadian and Italian pediatric MS cohorts. A few observations emerged from this work. Firstly, there are differences with respect to recruitment methods. In Canadian studies, participation in research studies is voluntary and considered to be a wholly research exploration, whereas in Italy there is much less distinction between clinical and research-related cognitive evaluation. In the Canadian Pediatric Demyelinating Disease Research program, all patients are seen once every three months. In Italy, visits are less frequent, and those patients who enrol in research studies are likely those who make more frequent hospital visits primarily due to more active disease. In Italy, there thus exists a bias towards enrolling patients who make more clinical visits whereas in Canada there is an equal probability of recruiting patients with and without more active disease as all patients are seen frequently (i.e. once every three months). Secondly, there is likely earlier diagnosis in Canadian MS patients based on easier healthcare access, with Italian samples likely comprising of patients diagnosed later and presenting for cognitive evaluation after longer disease duration. Thirdly, the cognitive tests administered in Canada and Italy are not exactly the same. Finally, there are socioeconomic status differences between the two regions. The Canadian pediatric health-care facility where patients are recruited in Toronto is based in an ethnically diverse region of relatively high socioeconomic status with high levels of parental education. In contrast, many parents of the Italian MS cohort had not completed high school nor entered post-secondary education.

1.3.9.1 Canadian studies

The first reported study of cognition in pediatric MS was published by Banwell and Anderson in 2005. This was a descriptive study evaluating the cognitive profile of 10 pediatric MS patients aged 6-16. The group was divided into patients with short (n=3, first MS attack within 12 months of testing), and long (n=7, mean time since first attack=
5 years) disease duration. Performance on each cognitive test was compared to age-based normative data. Deficits were almost exclusively found in the patients with longer disease duration, who were also younger at MS diagnosis. Deficits were found primarily in the areas of: (a) executive function, particularly those tests requiring self-generated organizational strategies, (b) processing speed, and (c) working memory.

A larger and more comprehensive cognitive study by the same group was published in 2011 (Till, Ghassemi, Aubert-Broche et al., 2011). This study was based on neuropsychological test data and MRI scans of 31 RRMS patients with onset prior to age 18 (mean age of onset=12, mean disease duration=4 years). Thirty-three age and sex-matched volunteers were also enrolled. Cognition was assessed in the following domains: (1) IQ via the WASI (Wechsler Abbreviated Scale of Intelligence), (2) attention and processing speed via Trail Making Test A (TMT-A), Symbol Digit Modalities Test (SDMT), visual matching and rapid picture naming from the Woodcock-Johnson-III Battery (WJ-III), and Conners’ Continuous Performance Test, (3) Attention shifting from Trail Making Test B (TMT-B), (4) Verbal memory from Word Selective Reminding from TOMAL-2 (Test of Memory and Learning, Second Edition), (5) Visuospatial skills from Beery-Buktenica Developmental test of Visual Motor Integration, (6) Language via phonemic verbal fluency from the DKEFS (Delis-Kaplan Executive Function System), vocabulary and similarities from the WASI, Picture Vocabulary from the WJ-III Test of Academic Achievement, and (7) executive functioning from the WCST (Wisconsin Card Sorting Task). Impairment on any test was defined as performance 1.5 SD below the normative age-based mean. Global impairment was defined as being impaired on 3 or more tests. According to this criterion, impairment was detected in 29% of patients. When comparing performance between the two groups, MS patients performed worse on measures of attention and processing speed, visuospatial ability, expressive language, and IQ. The tests which had the greatest frequency of impairment in the pediatric MS group were the Beery-Buktenica Developmental test of Visual Motor Integration, WJ-III visual matching, SDMT, and WASI Block Design. Patients with cognitive impairment were most likely to be male, have longer disease duration, and
younger age of onset. Using a regression model, age of disease onset was a significant predictor of cognitive performance.

Comparison of executive function between 30 pediatric MS patients and 31 healthy controls in another study (Till, Ho, Dudani et al., 2012) revealed differences between groups on the following measures: SDMT, TMT-A, and TMT-B, and verbal fluency. Functional executive skills were also evaluated using the parent reports of the Behavioral Rating Inventory of Executive Function (BRIEF). Parents of pediatric MS patients reported more deficits on the Working Memory and Plan/Organize subscales of the BRIEF, as well as the Metacognition Index (derived from scores on the Initiate, Working Memory, Plan/Organize, Organization of Materials, and Monitor subscales), and the Global Executive Composite which incorporates all eight-scale scores. Based on the Global Executive Composite, a generalized pattern of executive deficits was suggested in 19% of patients. Another finding was that younger age of disease onset was associated with worse SDMT performance and predicted more deficits the Metacognition Index, Working Memory, and Organization of Materials subscales. These correlations were no longer significant after controlling for disease duration.

Verbal and nonverbal memory was evaluated using the TOMAL-2 in 32 pediatric MS patients and 26 age-and sex-matched healthy controls in the paper of Fuentes and colleagues (2012). The TOMAL-2 subtests administered were the following: Word Selective Reminding, Memory for Stories, Abstract Verbal Memory, and Facial Memory. The only subtest that showed significant performance differences between groups was Memory for Stories. Furthermore, there was no evidence of memory impairment in 80%. This suggests that memory abilities are relatively spared relative to other cognitive functions in pediatric MS, a finding that warrants further investigation based on other reports that do note a deficit in this domain.

Slower visuo-perceptual speed has also been observed in pediatric MS, as demonstrated by poorer performance on the visual matching subtest from the WJ-III Test of Cognitive Abilities (Bethune et al., 2011) compared to healthy controls. Todorow
and colleagues (2014) further investigated visuospatial abilities, as well as attention performance, in 15 cognitively-preserved pediatric-onset MS patients compared to 15 age-matched controls. The task utilized in this study asked participants to indicate whether or not a target letter E appeared at either a global or local attended level of the stimulus. In the pediatric-MS group, greater response conflict was present indicating that these patients have more difficulty inhibiting task-irrelevant global information when the task requires the individual to selectively focus on local features. Finally, math performance has been shown to be lower in pediatric MS patients. The study by Till and colleagues (Till, Deotto, Tipu, et al. 2011) revealed that difficulties in written arithmetic ability were present in 26% of patients as demonstrated by at least 1 SD below the normative mean on the Calculation subtest of the WJ-III Tests of Achievement. Pediatric MS patients also performed worse than healthy controls on the Calculation, Letter-word Identification (measuring the ability to read words), and spelling subtests of the WJ-III Tests of Achievement. In addition, younger age of disease onset was associated with lower performance on the Calculation subtest.

1.3.9.2 Italian studies

The first study investigating cognitive performance in an Italian pediatric MS sample was conducted by Amato and colleagues in 2008. This same cohort was evaluated at 2-year and 5-year follow-up. Those results will be discussed in Section 1.3.9.4. In the current section, the results for the initial baseline evaluation will be discussed. In the baseline 2008 study, cognitive performance of 63 MS patients (mean age of onset=12, mean disease duration=3 years) and 57 healthy controls was compared using an extensive battery of neuropsychological tests assessing IQ, memory, attention/concentration, executive functions, and language. Cognitive impairment was evident in 31% of patients if defined as failure (performance under the 5th percentile of healthy controls) on three or more tests, or 53% of patients if defined as failure on two or more tests. Low IQ was particularly evident in this sample (8% with IQ<70 corresponding to mental insufficiency), alongside deficits in memory, complex attention, and executive functions. Linguistic deficits were also prominent in this sample. Using
logistic regression analysis, the only significant predictor of overall cognitive impairment was IQ score lower than 90. The only significant demographic or disease-related variable that was a significant predictor of IQ score less than 70 was younger age of onset.

A further study by this research group (Portaccio et al., 2009) aimed to develop a brief neuropsychological battery to be used in pediatric MS patients. This study enrolled 61 pediatric MS patients and 58 demographically matched healthy controls. A detailed neuropsychological test battery was administered including measures of: (a) IQ using the Weschler Intelligence Scale for Children (WISC-R), (b) memory using the Selective Reminding Test and the Spatial Recall Test, (c) sustained attention and concentration using the SDMT and TMT-A and TMT-B, (d) abstract reasoning using the Modified Card Sorting Task, (e) expressive language using semantic and phonemic verbal fluency and oral denomination test, and (f) receptive language using the token test, and phrase completion test from the battery for the analysis of aphasic deficits. Failure on any test was defined as performance below the 5th percentile of healthy controls. Using a cut-off of at least 4 tests failed, 41% of patients were classified as cognitively impaired. The most frequently affected cognitive domains were IQ (30% of patients impaired), verbal and spatial memory (23% impaired on each of immediate recall measures of Selective Reminding Test and Spatial Recall Test), information processing speed (SDMT, TMT) and language (semantic verbal fluency 18% and token test 18%). Using a discriminant functional analysis, the tests with the highest discriminating ability were WISC-R Vocabulary, SDMT, Trails B, and Selective Reminding Test (Continuous Long-term Retrieval variable) and were selected for inclusion in the Brief Neuropsychological Battery for Children (BNBC). Using criteria as failure on at least one test of the BNBC, this yielded a sensitivity of 76-96% and specificity of 76-81% for detecting impairment as defined on the full battery. This battery also only takes 30 minutes to administer, advocating its use in pediatric MS populations.

A recent study of Rocca and colleagues (Rocca, Absinta, Amato et al., 2014) aimed to relate cognitive performance to structural and fMRI features in a sample of 35 pediatric
MS patients and 16 sex- and age-matched controls. Cognitive performance was evaluated using the same detailed battery as described in the previous paragraph (Portaccio et al., 2009). Cognitive impairment was defined as performance below the 5th percentile of healthy controls on two or more tests. According to these criteria 45% of pediatric MS patients were classified as cognitively impaired. The most frequently impaired cognitive domains were spatial and verbal memory (18%), language (12%), and attention (8%).

### 1.3.9.3 United States studies

The study by MacAllister and colleagues (2005) evaluated cognitive function in 37 children and adolescents with clinically definite MS. The mean disease duration was only 20 months for patients in this study. The cognitive battery included the following measures: (a) TMT A and B, (b) Controlled Oral Word Association Test, measuring verbal fluency, (c) Boston Naming Test, (d) Listening to Paragraphs subtest of the Clinical Evaluation of Language Fundamentals–III, assessing receptive language, (e) Verbal and Visual Learning of the Wide Range Assessment of Memory and Learning, and (f) Beery Test of Visual Motor Integration. Cognitive impairment was defined as performance 1.5 or more SD below the mean of published norms on at least two cognitive tasks. Thirty-five percent of the sample demonstrated significant cognitive impairment, with 59% demonstrating impairment on at least one neuropsychological test. The most common impairments were in complex attention (29% of patients) and language (19% with deficits in naming, and 14% with poor receptive language). With respect to memory function, immediate recall was impaired in 3% and 8% of patients, whereas delayed recall was impaired in 19% and 11% across the verbal and visual domains respectively. Therefore, both encoding and retrieval deficits were present. Visuospatial deficits were evident in 5% of patients.

Smerbeck and colleagues (2011) compared performance on a neuropsychological test battery between 43 pediatric MS patients to 45 healthy controls in order to determine which tests were the most sensitive in distinguishing between groups. Statistically
significant differences between patients and controls were found on a measure of spatial memory (the Brief Visuospatial Memory Test- Revised, Total Learning and Delayed Recall) and processing speed (SDMT). There was also a trend for significance on the WASI vocabulary (p=.046). No differences were evident on measures of confrontational naming or general intelligence, suggesting that tests probing visual processing are the most sensitive in pediatric MS.

Finally, the most recent and largest study from the United States Pediatric MS network compiled cognitive data from six US Pediatric MS Centres of Excellence for a total sample size of 187 pediatric MS (mean disease duration 2.2 years and mean age of symptom onset=12 years) and 44 patients with CIS (mean time since symptom onset 0.8 years) (Julian et al., 2013). Neuropsychological evaluation spanned a wide array of abilities including (a) general intelligence using the WASI, (b) reading and language using the Wechsler Individual Achievement Test-II Pseudoword Decoding, and the Expressive One Word Picture Vocabulary Test, (c) attention, working memory, and processing speed using Digit Span and Digit Symbol Coding from the Wechsler Adult Intelligence Scale, (d) executive functioning using the Contingency Naming Test, and Delis Kaplan Executive Function System versions of the TMT, (e) verbal episodic learning and recall using the California Verbal Learning Test, (f) visuospatial functioning using the Beery-Buktenica Developmental test of Visual Motor Integration, and (g) fine motor speed and coordination using the Grooved pegboard test. Impairment on any measure was defined using a liberal threshold of performance at least 1 SD below the normative mean as per published, age-stratified normative data. Cognitive impairment was defined based on impairment on more than one third of the test scores obtained. Using this criteria, impairment was detected in 35% of the pediatric MS group, with the most frequent deficits in fine motor coordination (54%), visuomotor integration (50%), and information processing speed (35%).
1.3.9.4 Longitudinal progression of cognitive deficits

In the first longitudinal cognitive study in pediatric MS, MacAllister and colleagues (2007) evaluated longitudinal neuropsychological performance in a cohort of 12 pediatric MS patients (mean age of onset=12.5 years) evaluated at baseline and after a mean of 22 months. Using criteria of more tests being impaired at follow-up, five patients (42%) demonstrated cognitive decline. At follow-up evaluation, there were several tests which showed increases in impairment frequency. This was the most pronounced for TMT-B (5 patients at baseline, 9 at follow-up) and Beery Test of Visual Motor Integration (1 patient at baseline, 4 patients at follow-up). This study also found that greater decline was present in patients with younger age of disease onset.

Two-year and 5-year follow-up from the 2008 cohort of Amato and colleagues (described in 1.6b) have been published. At two-year follow-up, data from 56 of the 61 patients evaluated at baseline and a new group of 50 healthy controls were collected (Amato et al., 2010). At two-year follow-up, the percentage of patients classified as cognitive impaired, defined as failure on 3 or more tests, increased from 31% to 70%. Seventy-five percent of patients were classified as cognitively deteriorating. Older age was found to be a significant predictor of deteriorating cognitive performance. Five-year follow up of the same group (n=48) revealed cognitive improvement in many (67%) based on comparison to performance at 2 years (Amato, Goretti, Ghezzi et al., 2014). Comparison to performance at baseline, however, showed that cognitive deterioration was present in 56% of patients, with cognitive stability and improvement present in 19% and 25% respectively. The overall pattern appears that after two years overall cognitive performance deteriorates, and then by five years improves. It is important to note, however, that different healthy control groups (used for defining cognitive impairment) were used at each time point. Also, the test version administered at 2-year follow-up was different from the version used at baseline and 5-years. Thus, the use of perhaps a more difficult version at time 2 might have had a small effect on explaining the marked deterioration in cognitive performance from baseline to 2 years.
Within a short follow-up of one year, cognitive deterioration was present in 25% of pediatric MS patients (Till et al., 2013). In contrast, the study by Charvet and colleagues (2014a) found that the rate of cognitive impairment was 37% at baseline and actually dropped to 33% after mean follow-up of 1.64 years. Considering the results in the aforementioned paragraph, it is clear that variability exists with respect to change in cognitive performance (i.e. decline, improvement, stability) across time in pediatric MS. This may be related to baseline sample characteristics and length of follow-up. For example, Hosseini and colleagues (2014) demonstrated that older age of disease onset was associated with greater increases in TMT-B and SDMT scores across time (Hosseini et al., 2014). This suggests that older age of onset may help facilitate development age-expected increases in processing speed and prevent decline seen in patients with younger age of disease onset.

1.3.9.5 Relationship of cognitive performance to structural MRI

Reduced thalamic volume has been shown to be the brain metric most strongly correlated with cognitive dysfunction in pediatric MS (Till, Ghassemi, Aubert-Broche et al., 2011). The relationship was stronger than that of T2 lesion volume. Notably, these authors also found that thalamic volume could explain 42% of the variance in full scale IQ. Similarly in adults, thalamic volume (quantified by third ventricle width, absolute and relative volume) has also been demonstrated to be the strongest structural correlate of cognitive impairment (Houtchens et al., 2007). The important role of the thalamus is not unexpected given the importance of the thalamus as a relay centre for cognitive processing. How thalamic volume reduction relates to measures of fMRI functional connectivity is not known and will be investigated in this thesis.

The study examining executive functioning by Till and colleagues (Till, Ho, Dudani et al., 2012) found that lower task performance on the TMT-B and SDMT were associated with lower frontal lobe and thalamic volumes. These two structural measures, as well as normalized brain volume, were also correlated with the Initiate, Working Memory, and Plan/Organize subscales of the parent-reported Behavioral Rating Inventory of
Executive Function (BRIEF). The study examining memory performance by Fuentes and colleagues (2012) found that the total number of words learned (immediate recall) on the TOMAL-2 Word Selective Reminding subtest was positively correlated with total hippocampal volume and total brain volume. This was despite hippocampal volume not differing between groups, whereas differences between-groups in amygdala, thalamic, and total brain volumes were present. Why hippocampal volume is spared in pediatric MS should be an area of further inquiry. These authors also observed that visual recognition memory performance was positively correlated with thalamic volume and that overall full scale IQ, as measured by the WASI, was positively correlated with hippocampal volume and thalamic volume in the MS group.

In healthy developing children and adolescents, the developmental increase of white matter integrity has been shown the parallel the development of IQ (Schmithorst et al., 2005; Tamnes et al., 2010), information processing speed (Mabbott et al., 2006; Ferrer et al., 2013), and working memory (Nagy, Westerberg, & Klingberg, 2004; Vestergaard et al., 2011). Studies investigating the relationship between white matter integrity and cognitive performance have also been conducted in pediatric MS. In the study of Bethune and colleagues (2011), lower performance on two tasks of processing speed (visual matching from the WJ- III Test of Cognitive Abilities and SDMT) was correlated with lower FA values in the corpus callosum in pediatric MS patients. This relationship is fitting given that intact information transfer across regions is likely determined by the integrity of interconnecting white matter tracts. The correlation between lesion volume and cognitive processing speed was not, however, statistically significant. Todorow and colleagues (2014) observed that greater response conflict, indicating the ability to inhibit task-irrelevant global information, was correlated with lower FA in the anterior body of the corpus callosum in a sample of 13 pediatric MS patients and 15 healthy controls. Finally, the study of Till and colleagues (Till, Deotto, Tipu et al., 2011) showed that arithmetic ability positively correlated with FA values across all segments of the corpus callosum and in right frontal and parietal regions. In summary, all of these results
suggest that white matter integrity reduction in pediatric MS is associated with lower performance on tasks requiring speeded information transfer.

Studies investigating the structure-cognition relationship have also been conducted in Italian pediatric MS cohorts. Rocca and colleagues (Rocca, Absinta, Amato et al., 2014) compared lesion volumes, gray and white matter volumes, and DTI characteristics between pediatric MS patients with (n=16) and without (n=19) cognitive impairment. These authors observed the following in patients with cognitive impairment compared to patients who were cognitively intact: (a) increased probability of lesions in the right thalamus, middle and posterior (close to the precuneus) corpus callosum, and bilateral parieto-occipital white matter, (b) reduced gray matter volume of the right precuneus and left middle temporal gyrus, (c) reduced white matter volume of the splenium of the corpus callosum, posterior cingulum, left parahippocampus, white matter close to the precuneus bilaterally, and bilateral superior longitudinal fasciculus, (d) decreased FA and increased RD of the posterior corpus callosum and cingulum, and decreased FA of the bilateral parieto-occipital white matter. Therefore, structural abnormalities (more lesions, reduced brain volumes, reduced white matter integrity) are present in pediatric MS patients with cognitive impairment, particularly in patients with lesions and loss of brain volume in posterior brain regions. In another publication, the same group reported lower normal-appearing white matter average FA, and higher gray matter MD in cognitive impaired patients compared to cognitively preserved patients (Rocca, Valsasina, Absinta, et al., 2014). Finally, this research group recently published that cognitive impairment is also not related to cortical lesions in pediatric MS (Rocca, De Meo, Amato et al., 2014) implicating primarily white matter damage as a substrate for cognitive dysfunction in pediatric MS.

### 1.3.9.6 Other factors that influence cognitive performance

As described throughout Sections 1.3.9.1-4, age of disease onset has a strong influence on cognitive outcomes, with younger age of disease onset being associated with worse cognitive performance. This suggests a key vulnerability of the developing
neural networks to MS-related insult. A consistent relationship between physical
disability and cognitive function has not been demonstrated in both adult and pediatric
MS. In pediatric MS, this lack of relationship is likely due to the limited amount of
physical disability early in the disease of these patients.

With respect to the relationship between mood and cognitive function, higher anxiety
and depression (as reported by the caregiver of pediatric MS patients) has been shown
to correlate with worse executive function as measured by performance on the TMT
Part A and B and Digit Span task (Holland et al., 2012). Weisbrot and colleagues (2014)
observed that pediatric MS patients with either a mood or anxiety disorder were more
likely to be cognitively impaired compared to those with other psychiatric diagnosis (e.g.
attention deficit hyperactivity disorder, oppositional defiant disorder), or no psychiatric
diagnosis.

With respect to the relationship between fatigue and cognitive function, greater fatigue
(report both by the pediatric MS patient and his/her parent) is associated with worse
performance on the TMT Parts A and B (Holland et al., 2012). Goretti and colleagues
(2012) observed that higher patient self-reported cognitive fatigue was associated with
impaired performance on a test of problem-solving (Tower of London). Higher parent-
reported cognitive fatigue was associated with impaired performance on tests of verbal
memory (Selective Reminding Test), processing speed and attention (TMT-B), and
language (Token Test). Greater parent-reported difficulties compared to patient self-
reported difficulties, across the spectrum of symptoms in pediatric MS, is a common
observance that could be related to poorer insight in pediatric MS patients themselves.

In summary, given the demonstrated association between cognitive performance and
fatigue and depression, it is important that these factors are accounted for in any study
evaluating cognition in MS as they may exert an influence.
1.3.9.7 Management of Cognitive Dysfunction

No cognitive rehabilitation studies have been published in pediatric MS. With the demonstrated efficacy of cognitive rehabilitation interventions in adult MS patients, future studies in pediatric MS are warranted. This could include the use of computer-assisted rehabilitation (e.g. Amato, Goretti, Viterbo et al., 2014), teaching of specific memory and behavioural strategies (e.g. Chiaravalloti et al., 2013; Hanssen et al., 2014), or completion of home-based written and computer-based materials (e.g. Gich et al., 2015). Currently, cognitive deficits in pediatric MS are generally managed on a case-by-case basis, primarily involving academic accommodations according to areas of deficit. For example, children with attention deficits may be placed in a distraction-free area during class, children with reduced processing speed may get more time to finish exams, or children with memory deficits may be taught compensatory strategies such as using a day planner. Further research into cognitive rehabilitation interventions in pediatric MS is clearly needed.

1.4 Functional MRI

Earlier in my thesis a description was provided of the structural MRI features in MS given that this is used for diagnosis as well as to characterize the damage that occurs in this disease. For example, with respect to the latter, it was noted that reduced thalamic volume was an important feature of pediatric MS due to degenerative-related processes and failure of age-expected growth. Focus will now be shifted to function and how MS can affect neural patterns of activation. Measuring the impact of MS on activation of neural networks can be investigated in two ways either using (a) resting-state and (b) task-based fMRI paradigms. Details on the exact analytical methods used in this thesis are provided in Chapter 4. A brief overview of some basic principles, however, is provided here.
1.4.1 Resting-state fMRI

Resting-state fMRI refers to brain activation patterns observed when not engaged in a specific task. Basic instructions typically include asking the participant to lie still with eyes either closed, open, or fixated on a specific visual target. The duration of resting-state is suggested as a minimum of 5 minutes (van Dijk et al., 2010) with increased reliability with longer durations (Birn et al., 2013). Resting-state fMRI is used to identify “networks”, or groups of brain regions that show functional connectivity, or temporal synchronization of activation, with one another. Commonly observed resting-state networks (described later on in this section) are evident during sleep (Horovitz et al., 2009), anaesthesia (Kiviniemi et al., 2003), as well as during task performance (Smith, Fox, Miller et al., 2009). Such findings suggest that resting-state measures intrinsic properties of the brain. The validity of resting state networks is further supported by the fact that they correspond to specific behavioural or cognitive functions (e.g. visual processing, sensorimotor function, working memory, Damoiseaux et al., 2006) and are also strongly correlated with actual neuronal patterns of synchronization (Shmuel & Leopold, 2008). Finally, these networks are highly replicable across individuals (Beckmann et al., 2005) and show high test-retest reliability, even in children (Thomason et al., 2011).

In resting-state analysis, the measure of interest is functional connectivity. Resting-state functional connectivity is inferred based on “the temporal synchronization of low-frequency fluctuations (0.01-0.1 Hz) arising from spontaneous neuronal activities in distant brain regions” (Fox et al., 2005). Functional connectivity has also been defined as the “synchronization of neural activity between anatomically separate brain regions” (Lowe et al., 2000). Functional connectivity between disparate brain regions could be due to (a) two regions being structurally connected to one another, i.e. one region providing direct input to another region, (b) one region receiving input indirectly through mediation by another region, (c) two regions both receiving shared input from another third region. Regions can also appear functionally connected to one another due to
non-neuronal physiological parameters (e.g. breathing, cardiac pulsation) or motion. Ways to potentially control for this are described in Chapter 3.

The two most common techniques for identifying resting-state networks are (a) seed-based, and (b) Independent Components Analysis (ICA) approaches. Both techniques are described in detail in Chapter 3. Based on utilizing either a seed-based or ICA approach, the most reproducible or commonly reported resting-state networks are described below.

**Default-mode network**

The default-mode network has been the most reproducible resting-network with its constituent regions showing heightened activation while at rest (Raichle et al., 2001). This network shows suppression of activation during cognitively demanding tasks and is often called a “task-negative” network. The most commonly used seed region for the default-mode network is the precuneus (Utevsky, Smith, & Huettel, 2014). Other regions that are part of this network include the posterior cingulate, medial prefrontal, inferior parietal, lateral temporal cortices and hippocampal formation (Buckner, Andrews-Hanna, & Schacter, 2008). The functions related to the default mode network are basic mind-wandering or daydreaming, self-referential processing, overall sensory awareness, thinking about the future, and memory consolidation. These associated functions are appropriate if we consider the mental processes at work when asking participants to simply lie still for about 5 minutes in an MRI scanner.

**Executive control networks**

Executive controls networks are lateralized and also commonly referred to as “task-positive” networks (Seeley et al., 2007). The main seed region is the middle frontal gyrus of whichever hemisphere is being interrogated. The regions constituting this network include the middle frontal gyrus and parietal lobe which is why this network is also often commonly referred to as the “left/ right frontoparietal” networks. The areas of this network show increased activation during cognitive task performance. This is
especially true for tasks probing higher-order cognitive, working memory, and attentional control processes.

**Salience network**

The salience network determines where internal bodily information or information from the external world should be channeled, much acting as a controller or network switcher (Seeley et al., 2007; Goulden et al., 2014). Is it speculated that information identified as important requiring immediate action or cognitive processing gets operated on by the executive control network; otherwise it is directed to the default-mode network. This network also becomes activated in order to mediate response to pain or emotional information. The seed region of the salience network is most often the insula. Other regions of this network include the anterior cingulate and thalamus. The salience network is built around paralimbic structures and other regions related to processing and response to stimuli produced within the body. With respect to cognitive processing, the salience network is related to attention, and processing errors and conflicts.

Other resting state networks (based on Damoiseaux et al., 2006; Smith, Fox, Miller et al., 2009; van den Heuvel et al., 2010) include the following:

(a) Primary (occipital pole) visual and secondary (extrastriate) visual networks.

(b) Auditory network including the superior temporal gyrus, Heschl’s gyrus, and posterior insula regions.

(c) Cerebellar network covering the entire cerebellum.

(d) Sensorimotor network consisting of the pre- and postcentral gyri as well as supplementary motor and secondary somatosensory cortices.

(e) A dorsal attention network has also been described which includes the intraparietal and superior frontal cortices (Spreng et al., 2013; Corbetta & Shulman, 2002; Fox et al., 2005). This network is associated with voluntary goal-driven “top-down” control of
attention. For example, this network is engaged when attention is cued by the presence of an arrow. This is contrast to the ventral attention network which is more automatic/reflexive and associated with attention to unexpected stimuli.

1.4.2 Development of resting-state fMRI networks throughout childhood and adolescence

It is important to understand the normal trajectory of resting-state network development in healthy children before considering how they might be affected in pediatric MS. The default-mode network is evident by 2 years of age, and is even similar to adults at that age (Fransson et al., 2011). The overall location of hubs of resting-state networks is also the same from 10 to 20 years of age, at which point the developmental peak has likely been reached (Hwang, Hallquist, & Luna, 2013). What changes, however, is the strength of connections between areas of the same network and how functionally segregated resting-state networks actually become.

The seminal work on the development of resting-state networks in childhood and adolescence has been conducted by the group of Fair and colleagues (2009). These authors describe a pattern whereby young children (under 8 years of age) demonstrate greater functional connectivity between regions that are close to each other in space. With development, regions close in space become “functionally” segregated into separate networks, i.e. considering the default-mode, executive control, and salience networks. Greater functional segregation between the executive control and default-mode networks has been shown to associate with greater cognitive control in adolescents (Dwyer et al., 2014). The developmental increase in long-range functional connections (i.e. of regions of the same network) appears to parallel myelination of interconnecting network pathways. The increased long-range connections are also purportedly due to experience. According to this explanation, regions that are independently co-activated with one another become more functionally connected (i.e. part of the same network) through principles of Hebbian-guided plasticity (Bi & Po, 1999).
1.4.3 fMRI findings in MS

MS is a clinical population where many fMRI studies have been conducted. This is primarily in adults, with different disease types, and varying levels of clinical disability and structural brain pathology. A review of studies conducted in adult MS patients will be provided in this section, categorized according to resting-state or task-based fMRI. The few studies conducted in pediatric MS will also be described.

1.4.3.1 Resting-state fMRI studies in adult MS

A consistent pattern observed in the MS literature is heightened resting-state connectivity in RRMS patients compared to healthy controls. This has been demonstrated for numerous resting-state networks including the default-mode (Basile et al., 2013; Faivre et al., 2012; Hawellek et al., 2011; Zhou et al., 2014), frontoparietal (Faivre et al., 2012), salience (Faivre et al., 2012), visual processing (Faivre et al., 2012), and sensorimotor (Basile et al., 2013) networks. However, in RRMS patients with cognitive impairment, lower functional connectivity of the default-mode (Cruz-Gomez et al., 2014; Louapre et al., 2014), fronto-parietal (Cruz-Gomez et al., 2014; Louapre et al., 2014), salience (Cruz-Gomez et al., 2014) and dorsal attention (Louapre et al., 2014) networks has been observed compared to cognitively intact MS patients. Investigating anterior cingulate connectivity specifically, Loitfelder and colleagues (2012) found higher functional connectivity of the anterior cingulate with the right postcentral gyrus, left angular gyrus, and left posterior cingulate cortex in 31 MS patients compared to 31 healthy controls using a seed-based approach. In addition, better cognitive performance [as demonstrated by performance the SDMT and Paced Auditory Serial Addition Test (PASAT)] was associated with increased anterior cingulate functional connectivity to the cerebellum, middle temporal gyrus, occipital pole, and the angular gyrus in the MS group. Wojtowicz and colleagues (2014) observed that reduced performance variability (i.e. greater stability) on a complex processing speed task was associated with greater resting-state functional connectivity between the ventral medial prefrontal cortex and frontal pole of the default-mode network. All of these combined
results point to overall heightened functional connectivity of RRMS patients across the spectrum of resting-state networks. This heightened connectivity is even more pronounced in patients with better and intact overall cognitive performance.

Interesting work by Leavitt and colleagues (2014) has pointed to the specificity of memory function, and not cognitive status overall, to the heightened functional connectivity of the default-mode network observed in MS patients. These authors studied 43 MS patients with (n=20) and without (n=23) memory impairment. Functional connectivity among default-mode hubs was not clearly present in the memory impaired group. Better memory performance was associated with greater default-mode network functional connectivity in both between-group and correlational analyses, even after controlling for overall cognitive efficiency (as defined by performance on the SDMT). These authors therefore suggest considering the default-mode network more specifically as a memory network, rather than an overall cognitive network, at least in the context of MS.

In contrast to patients with RRMS, patients with progressive forms of MS demonstrate lower resting-state connectivity compared to healthy controls. For example, Rocca and colleagues (Rocca, Valsasina, Absinta et al., 2010) observed lower functional connectivity of the default-mode network in 57 patients with progressive forms of MS (n=33 secondary-progressive MS, n=24 primary-progressive MS). They further divided their sample into those with and without cognitive impairment. Cognitively impaired patients showed lower resting-state activity of the anterior cingulate cortex, suggesting this region as important in underlying cognitive impairment in progressive MS.

1.4.3.2 Resting-state fMRI studies in pediatric MS

To date, only two studies have utilized resting-state fMRI in pediatric MS patients (Rocca, Absinta, Amato et al., 2014; Rocca, Valsasina, Absinta et al., 2014). Both reports have been conducted by the same group with presumably large overlap of patients. The first study reported lower overall functional connectivity of posterior regions (right precuneus, right angular gyrus) of the default-mode network (delineated
using an ICA approach) in the pediatric MS group compared to healthy control group (Rocca, Absinta, Amato et al., 2014). The sample comprised of 35 pediatric MS patients (mean age = 15 years, mean disease duration= 2.1 years) with a high rate of cognitive impairment (46%) and 16 healthy controls (cognitive findings described in Section 1.3.9.2). Within the default-mode network, cognitively preserved patients (n=19) demonstrated higher functional connectivity of the right anterior cingulate whereas the cognitively impaired patients (n=16) demonstrated reduced functional connectivity of the right precuneus. The association of cognitive preservation with higher functional connectivity is the same pattern observed in adult MS (described in previous Section 1.4.3.1).

The second study by these authors evaluated functional connectivity across 10 resting-state networks in addition to the default-mode network (Rocca, Valsasina, Absinta et al., 2014). The sample was slightly larger than the previously published report (Rocca, Absinta, Amato et al., 2014) consisting of 44 patients and 27 healthy controls. Lower functional connectivity of regions within the following networks were demonstrated in the pediatric MS group compared to healthy control group: (a) right postcentral gyrus and left cerebellum of the sensorimotor network, (b) left cerebellum of the secondary visual network, (c) left angular gyrus of the default-mode network, (d) left middle temporal gyrus of the executive control network, and (e) bilateral precuneus of the bilateral working memory network. The opposite pattern of higher functional connectivity was found for the right medial frontal gyrus of the attention network in the pediatric MS group compared to the healthy control group. Thus, overall lower functional connectivity was demonstrated in resting-state networks including the default-mode network in line with the findings described in the previous study/paragraph, with one exception (the attention network). Post-hoc analyses were also conducted comparing pediatric MS patients with (n=19) and without (n=25) cognitive impairment. This revealed that cognitively preserved patients had higher functional connectivity of the right precuneus of the left working memory (i.e. frontoparietal) network. Higher functional connectivity in cognitively preserved patients was demonstrated in the previous study/paragraph albeit
in a different region and network (i.e. anterior cingulate cortex of the default-mode network). This study also found that higher T2 lesion volume was correlated with lower functional connectivity of the right medial frontal gyrus of the fronto-parietal attention network. This suggests a breakdown of the attention network with accumulation of structural disease burden.

1.4.3.3 Task-based fMRI studies in adult MS

Task-based fMRI identifies patterns of increased activation during tasks of working memory and attention (Audoin et al., 2003; 2005; Mainero et al., 2004; Penner et al., 2003; Staffen et al., 2002) in patients with MS compared to healthy controls. This is evident very early on in the disease, including in patients studied within the first few years post onset (Audoin et al., 2003; 2005; Mainero et al., 2004; Penner et al., 2003; Staffen et al. 2002). Increased activation is also more evident in patients who perform at the same (versus worse) level as healthy controls behaviourally on the fMRI tasks utilized in these studies (Audoin et al., 2005; Mainero et al., 2004; Penner et al., 2003).

Task-based fMRI studies also allow for the opportunity to investigate how activation patterns change with increasing task difficulty. A widely used task in the fMRI literature is the n-back working memory task. In this task, a series of pseudo-randomized digits (1-9) are presented at a rate of one every two seconds. Participants then have to indicate “yes” or “no” whether the current digit presented is the same as the one presented one (1-back task), two (2-back task), or three (3-back task) digits previously. The activation patterns during high (i.e. 2-back or 3-back) and low (i.e. 1-back) working memory load conditions can then be contrasted. In RRMS patients, easiest task load is associated with higher activation compared to healthy controls. With increasing task difficulty, these increases become less pronounced (Cader et al., 2006; Amann et al., 2011). A multi-centre study by Rocca and colleagues (Rocca, Valsasina, Hulst et al., 2014) studied brain activation patterns during performance of the n-back task in 42 adult RRMS patients and 52 healthy controls. They also divided the RRMS group into patients with and without cognitive impairment. In patients who were cognitively
preserved, increased recruitment of the right dorsolateral prefrontal cortex was evident during performance of the n-back compared to patients with cognitive impairment and healthy controls. A more interesting aspect of this study was the change in activation patterns with increasing n-back task difficulty. Decreased task-related activation of the bilateral frontoparietal (i.e. task-positive network), and decreased task-related deactivation of the default-mode network (i.e. task-negative network) with increasing task difficulty was present in RRMS patients with cognitive impairment. Stated differently, increased task-related activation of frontoparietal networks, and increased task-related deactivation of the default-mode with increasing task difficulty was present in RRMS patients who were cognitively preserved. This suggests that RRMS patients who are cognitively preserved are better able to modulate the activation levels of these opposing networks as task difficulty rises.

1.4.3.4 Task-based fMRI studies in pediatric MS

Two task-based fMRI studies have been published in pediatric MS (Rocca, Absinta, Ghezzi et al., 2009; Rocca, Absinta, Moiola et al., 2010). These studies investigated activation during basic motor performance. The first study in 2009 (Rocca, Absinta, Ghezzi et al., 2009) obtained fMRI during repetitive flexion-extension of last four fingers of the right hand in 17 pediatric MS patients and 9 healthy controls. The pediatric MS group showed increased recruitment of the left primary sensorimotor cortex during task performance compared to healthy controls. Greater activation of the left primary sensorimotor cortex was correlated with higher lesion volume. These authors also demonstrated reduced functional connectivity across the following regions: left primary sensorimotor cortex, left thalamus, left insula, left somatosensory motor cortex, and supplementary motor area. The evidenced increasingly lateralized (i.e. left-sided during this right-handed task) activation and reduction of functional connectivity suggest a potential mechanism preserving motor functional capacity in pediatric MS.

The later 2010 study by the same group (Rocca, Absinta, Moiola et al., 2010) compared activation patterns during simple finger tapping between five groups: pediatric healthy,
adult healthy, pediatric RRMS, adult clinically isolated syndrome (CIS), and adult RRMS patients. It was found that the pediatric RRMS group showed the same functional connectivity pattern as the pediatric healthy group. The adult CIS and adult RRMS groups showed greater functional connectivity both intra- and inter-hemispherically between regions of the sensorimotor network compared to the pediatric RRMS group. This was more so for the adult RRMS group. The authors suggest that because the pediatric MS group failed to show a disrupted motor network, acquiring MS in childhood may confer an added benefit at least with respect to motor disability. Whether the same result will hold true as pertains to still developing cognitive functions has yet to be determined.

1.4.3.5 Summary of fMRI findings in MS

There is apparent variability within the MS literature with respect to fMRI patterns of activation as elicited by both resting-state and task-based fMRI paradigms. A broad conceptualization was presented in the editorial of Schoonheim and colleagues in 2010 who postulated that RRMS patients experience heightened activation early-on until structural damage (lesion volume, brain atrophy) reaches a level where activation can no longer compensate. At this point, activation reaches a peak and subsequently declines, followed by concomitant cognitive disability. The reason for the inconsistency within the MS fMRI literature can likely be attributed to the variability in terms of time since diagnosis, extent of MS-related structural damage, and baseline cognitive performance of the MS patients studied. Newly diagnosed patients will show heightened activation whereas those patients with later stages of the disease will show reduced activation accompanied by cognitive impairment. Longitudinal studies are required in order to truly be able to model the relationship between functional activation, disease duration, structural damage accrual, and cognitive performance. In pediatric MS, the relationship is even harder to define based on the limited fMRI literature that currently exists, and given the added element of childhood/adolescent development of functional networks (described in Section 1.4.2).
The fMRI version of the Symbol Digit Modalities Test (fMRI-SDMT) was used in this thesis to investigate brain activation patterns elicited by cognitive task performance. An overview of the traditional paper-and-pencil version of this task, followed by a description of the fMRI version and studies conducted (in both healthy and adult MS populations) with the fMRI version will now be described.

1.5 The Symbol Digit Modalities Test (SDMT) as a measure of processing speed

Information processing speed is one of the most commonly affected cognitive functions in pediatric MS. One of the most commonly employed measures of processing speed in MS is the Symbol Digit Modalities Test (SDMT). The SDMT measures the speed of matching numbers to symbols. In the traditional paper-and-pencil version, participants have to respond orally the number that goes with each symbol according to a key of nine such digit-symbol pairings shown at the top of the page. The number of correct responses in 90 seconds is recorded.

The definition by Vernon (1983) states that information processing speed is assessed by relatively simple tasks that measure the time required to perform elementary cognitive operations. These tasks involve more than basic motor and/or sensory processes, however, are not complex enough to require much semantic knowledge, memory, and strategy. Information processing speed can also be conceptualized as a limiting capacity system that is challenged (e.g. via a time limit). The question then arises whether the SDMT is truly a measure of information processing speed or are other abilities involved? The answer I would put forth is that the SDMT measures primarily processing speed, and that additional functions are involved which include visual scanning and perception, decision making, error processing, and working memory.

The validity of the traditional paper-and-pencil SDMT as a screening instrument in pediatric MS was recently demonstrated based on comparison to performance on a neuropsychological test battery (Charvet et al., 2014b). Here, 31 pediatric MS patients
completed a neuropsychological evaluation within one year of completing the SDMT. Thirty-seven percent of patients demonstrated impairment on the SDMT. Impairment, however, was defined using liberal criteria of 1 or more SD below the normative mean values. Sensitivity was 77% and specificity was 81% for the SDMT in detecting cognitive impairment on the neuropsychological test battery. This supports the SDMT as an effective screening instrument for cognitive impairment in pediatric MS. In addition, older age at testing and increased disability were predictors of poorer SDMT performance. Another study (Portaccio et al., 2009) found that the SDMT was one of the most frequently impaired measures, with high discriminating ability between pediatric MS patients and healthy controls. The SDMT, in addition to three other measures, was thus selected for inclusion in a screening battery for cognitive impairment in pediatric MS (the Brief Neuropsychological Battery for Children with MS, BNBC; Portaccio et al., 2009).

Performance on the paper-and-pencil SDMT has also been shown to correlate strongly with the white matter integrity of the corpus callosum in pediatric MS (Bethune et al., 2011). This supports the SDMT’s role as a measure of processing speed reliant on intact information transfer across white matter tracts. Good test-retest reliability of the SDMT has been demonstrated in adult MS patients (Benedict et al., 2008). It is undetermined, however, what the test-retest reliability is in pediatric MS patients who are undergoing age-related increases in processing speed and developmental myelination of white matter pathways. Despite the widespread use of the SDMT in the pediatric MS population, there have been no studies to date on the neural underpinnings of performance on this task or any other information processing speed measure. As such, this PhD thesis will investigate this using the fMRI-version of the SDMT (fMRI-SDMT).

1.5.1 fMRI-version of the SDMT: Findings from healthy and MS adult populations

The fMRI-version of the SDMT (fMRI-SDMT) used in this thesis differs from the paper-and-pencil SDMT. The fMRI-SDMT, however, has been shown to correlate highly (.51-
.74) with the full-scale Wechsler Adult Intelligence Scale-Revised (which includes a similar digit-coding task), helping support its validity (Rypma & Prabhakaran, 2009). In the fMRI-SDMT, participants indicate “yes” or “no” whether or not a probe symbol-digit pairing matches any of those shown in an array (diagram and description given in Section 3.1). Response time and accuracy are then measured. The commonly reported measures are mean response time per trial (across only correct trials), and percent accuracy across all 52 trials. By measuring response time the fMRI-SDMT is likely more sensitive than the traditional paper-and-pencil SDMT in detecting subtle information processing speed deficits.

Studies using the fMRI-SDMT in healthy young (18-30) adults have noted very high accuracy rates (e.g. 97%, Rypma et al., 2006 and 94%, Rao et al., 2014), with inter-individual variability in response times (mean=1.33 sec, SD=0.177 sec, Rypma et al., 2006 and mean=1.7 sec, SD=0.27 sec. Rao et al., 2014). There are two published reports using this task in adult MS patients (Genova et al., 2009, and Leavitt et al., 2012), both using the same MS sample data but employing different techniques for the analysis. Within the reported sample, the MS group (n=16) had significantly slower response times (mean=2.03 sec, SD=0.371) compared to the healthy control group (mean=1.67 sec, SD=0.264). The MS group also performed significantly worse than the healthy control group on three out of four neuropsychological processing speed tasks that were also administered, adding further validity to the fMRI-SDMT as a sensitive measure of processing speed.

Performance of the fMRI-SDMT in healthy young adults has been shown to elicit activation of bilateral occipital, bilateral parietal, precuneus, anterior cingulate, posterior cingulate, bilateral temporal, right cerebellum, and right cuneus regions (Rao et al., 2014). The fMRI-SDMT has also been shown to produce significant white matter activation. In a study of 17 healthy young adults (Gawryluk et al., 2014), white matter activation was present in 88% of participants in either the corpus callosum or internal capsule. Comparing fMRI-SDMT activation patterns between adult MS patients and healthy controls, differences have been observed. The study of Genova and colleagues...
(2009) found that both the MS and healthy controls groups showed activation of several brain regions related to visual and motor function (i.e. occipital areas and precentral gyrus). Compared to the healthy control group, the MS group showed more negative activation (less activation relative to baseline activation levels) in response to stimulus presentation in the anterior cingulate cortex, inferior and middle frontal gyri, and hypothalamus. In addition, more negative activation of these regions was associated with higher lesion load, therefore suggesting structural damage as a pathological substrate for the lower activation observed that perhaps led to the poorer performance in the MS group overall.

Significant relationships have also been demonstrated between activation patterns and actual behavioural performance on the fMRI-SDMT task in both healthy adults and MS patients. All of these studies have provided support for the “neural efficiency” hypothesis. The neural efficiency hypothesis states that subjects performing a complex task will use a limited number of brain circuits and/or fewer neurons while poor performers use more circuits and/or neurons, some of which are inessential or detrimental to task performance (Grabner et al., 2003). Supporting this hypothesis in healthy young adults, faster performers of the fMRI-SDMT show less neural activity than slower performers in regions including the bilateral occipital, bilateral parietal, and precuneus (Rao et al., 2014). In addition, faster performers require less prefrontal cortex control in order to perform the task. This was determined based on a fewer number of functional connections present between the prefrontal cortex and other frontal and parietal regions during performance of the fMRI-SDMT. This suggests that faster individuals have greater efficiency of interactions between brain regions. The study of Leavitt and colleagues (2012) aimed to investigate whether this would be true in MS, re-analyzing the same data from the study mentioned in the previous paragraph (Genova et al., 2009) using a different approach. Compared to healthy controls, the MS group relied on a greater number of functional connections from multiple regions to the frontal cortices bilaterally in order to perform the task. The MS group was also significantly slower on this task, supporting the idea of less neural efficiency in MS. Adding further
support to this idea, more functional connections originating and terminating in the right
dorsal prefrontal cortex were associated with worse performance accuracy in the MS
group. The first report by Genova and colleagues (2009) also provided support for the
neural efficiency hypothesis in that increased response time was associated with
increased activation. Areas which demonstrated this relationship were the anterior
cingulate cortex and thalamus across both groups, and additionally in the inferior frontal
gyrus, left cuneus, precuneus, and bilateral cerebellum in the healthy controls (i.e.
healthy controls showed more areas in which activation co-varied with response time
compared to MS patients).
Chapter 2:
Aims and Hypothesis
2.1 Rationale and Overall Hypothesis

As summarized in Section 1.4.3.1 adult RRMS patients demonstrate heightened functional connectivity of the default-mode network. It is hypothesized that this same pattern will be observed in pediatric MS, all of which have RRMS (i.e. no progressive forms of MS). The hypothesis also needs to consider how the normal trajectory of resting-state network development in childhood/adolescence might be negatively impacted by pediatric MS. Since MS affects developmental myelination, this will affect the transmission of signals across white matter tracts connecting regions within the same network. As mentioned in Section 1.4.2, the hubs of the canonical resting-state networks are established at a young age, likely before MS disease onset. Therefore, the location and presence of the hubs of the default-mode network is hypothesized to be intact. What is likely to differ, however, is how directly these hubs are functionally connected to one another, as this may occur through more indirect pathways. This overall might lead to less constrained and more widespread network activation, for example, as evidenced by a spatially larger default-mode network.

Studies in pediatric MS (Rocca, Absinta, Amato et al., 2014) and adult MS samples (Rocca et al., 2012; Cruz-Gomez et al., 2014) have reported significant correlations between higher lesion volume and reduced functional connectivity. Studies in adult MS have also demonstrated that white matter disruptions (as measured by DTI) corresponds to reduced functional connectivity of corresponding resting-state networks (Lowe et al., 2008; Louapre et al., 2014). This suggests a direct relationship between anatomical and functional connectivity of neural networks. It is thus hypothesized that pediatric MS patients with extensive structural damage will show a breakdown (i.e. reduced functional connectivity) of resting-state networks, purportedly when the extent of injury exceeds the brain’s threshold for neuroplastic adaptation.

Prior to conducting the data analysis of my thesis, it was hypothesized that pediatric MS patients would perform worse than healthy controls on the neuropsychological battery administered, as well as on the fMRI-SDMT. Given the common deficits observed in
memory, attention, processing speed, and executive function in pediatric MS (described in Section 1.3.9) it was hypothesized that neuropsychological test measures probing these functions would demonstrate significant between-group differences. We, however, did not observe any cognitive differences between groups and as such the prevalence of cognitive impairment was too low (3 out of 23) to allow for between-groups comparisons of those patients with and without impairment. The first analysis thus involved the correlation between resting-state functional connectivity and cognitive performance as quantified by mean composite cognitive z-score. I hypothesize that greater functional connectivity with frontal regions will be associated with worse overall cognitive performance, supporting the idea of reduced neural efficiency in pediatric MS.

With respect to the fMRI-SDMT, slower response times on the fMRI-SDMT in the pediatric MS group compared to healthy controls was also hypothesized, especially considering that this task involves a precise and perhaps more sensitive measure of behavioural response time via button press. Using the fMRI-SDMT we investigated which brain areas are activated during the performance of this task and whether these activation patterns differ between groups. We also investigated the correlation between BOLD activation and actual behavioral performance on this task. As described in Section 1.5.1, adult MS studies have also shown a pattern whereby MS patients overall, and especially those with poorer accuracy on the fMRI-SDMT, demonstrate greater frontal recruitment during the performance of this task. I hypothesize this to be also present in pediatric MS patients as this would imply that this compensatory frontal activation is required in order to maintain task performance. Also, given that pediatric MS affects the developmental myelination of frontal white matter pathways, this compensatory activation might be even more necessary to overcome structural abnormalities.

The overall hypothesis of this thesis can be summarized as follows: In this group of pediatric MS patients with preserved cognitive performance, reduced neural efficiency will be present as evidenced by greater fMRI activation corresponding to reduced cognitive performance. In patients with extensive structural damage, however, a
breakdown of resting-state networks will be present as evidenced by lower functional connectivity.

2.2 Specific Aims and Hypotheses

**Aim 1:** To determine differences between pediatric MS patients and healthy controls in resting-state fMRI functional connectivity.

Hypothesis: The pediatric MS group will show heightened and less spatially constrained (i.e. involving more surrounding regions) functional connectivity of the default-mode network compared to the healthy control group.

**Aim 2:** To determine the relationship between resting-state network connectivity and structural damage in pediatric MS.

Hypothesis: Higher lesion volume, reduced thalamic volume and lower white matter microstructural integrity (as determined using DTI-derived measures) will be correlated with reduced resting-state functional connectivity in pediatric MS.

**Aim 3:** To determine the relation between fMRI patterns of activation and cognitive performance in pediatric MS.

Hypothesis: Within the pediatric MS group, greater functional connectivity of frontal regions during resting state will be associated with worse neuropsychological performance. In addition, during the performance of the fMRI-SDMT, greater frontal recruitment will be present and greater frontal activation will correlate with worse behavioural performance (longer response time and reduced accuracy) in the pediatric MS group.
Chapter 3:
Methods
3.1 Study Protocol

Participants

This study enrolled a total of 23 pediatric-onset MS patients and 20 healthy controls. Different portions of this thesis have slightly different sample sizes as participants were excluded for a variety of reasons (e.g. scanner or motion artifact, cognitive impairment), all of which is outlined in Figure 8.

Recruitment

The pediatric-onset MS group was recruited primarily by approaching patients at the MS clinic at the Hospital for Sick Children. In addition, all eligible patients in the database of the Canadian Pediatric Demyelinating Disease Study currently, or previously cared for at the Hospital for Sick Children, were contacted via telephone or email (approximately 115 patients, all previously agreeing to be contacted for future studies). This database included patients that have transitioned to adult care (note that study age range = 13-25). Additional means of recruitment included online advertisement on the MS Society of Canada Research Portal and the Sickkids research4kids website. Recruitment was also attempted at other adult MS clinics in Southern Ontario (London, Hamilton, Kingston) however there were unfortunately no patients meeting eligibility criteria at these other sites. Of note, York University is approximately a 45 minute drive from downtown Toronto (i.e. The Hospital for Sick Children, St. Michael’s Hospital) and is only easily accessed by car, which may have influenced recruitment. An additional influence on recruitment could be that patients had to endorse their capacity to undergo a 90-minute MRI and thus claustrophobic patients could not be included.

Healthy controls were first recruited via advertisement (approximately the first 10 controls), followed by one-by-one matching of each patient with a corresponding gender and age (within 6-months) matched control. Advertisements for the study were posted on the York University research portal and Hospital for Sick Children research4kids website. Recruitment was also done by word-of-mouth by the study investigators.
**Ethics**

The research protocol was reviewed and approved by the Ethics Boards at the Hospital for Sick Children, as well as the site of MRI scanning (York University). Written informed consent was obtained from all participants and/or parent or legal guardian.

**Inclusion and Exclusion Criteria**

Participants were required to be between the ages of 13-25. Pediatric-onset MS patients had to be younger than age 18 at time of first MS attack, and meet revised McDonald 2010 MS diagnostic criteria (Polman et al., 2011, described in Section 1.2.4). All patients were required to have relapsing-remitting MS. MS participants were required to be at least four weeks from clinical relapse or corticosteroid treatment. Cognitive testing or MRI during clinical relapse is not implemented in MS studies because of visual/ motor disturbances and MRI abnormalities (Benedict et al., 2014). Furthermore, cognitive declines, especially of processing speed, have been demonstrated in patients currently experiencing a relapse (Benedict et al., 2014). MS patients with visual or motor difficulties that would preclude testing were excluded.

Participants were not recruited into the study if they endorsed a history of head trauma, alcohol abuse, illicit drug use, had visual or motor difficulties that would preclude testing, or any other major medical illness potentially affecting cognitive function. Controls were not recruited if they reported any current or past neurological illness, psychiatric diagnosis or known learning disability. Participants were also screened for safety to undergo an MRI. All participants were required to be proficient in English. All participants were also required to be more than 6 months from any previous neuropsychological evaluation to control for potential practice effects.

**Demographic, disease-related information and questionnaires**

For each participant, the study took place in one 4-hour session at York University consisting of questionnaires, neuropsychological assessment, and the MRI scan. Demographic and disease-related information were obtained from clinical records. The
Expanded Disability Status Scale (EDSS) score (Kurtzke, 1983) reported within the last 6 months, relapse history, disease duration, and medications were recorded.

All participants completed the following questionnaires: (i) Dutch Handedness Questionnaire, (Van Strien, 2002) (ii) Centre for Epidemiological Studies Depression Scale for Children (CES-DC) (Weissman, Orvaschel, & Padian, 1980), (iii) Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (PedsQL), (Varni et al., 2002) and (iv) Barratt Simplified Measure of Social Status (BSMSS) (Barratt, 2006). For the BSMSS scale scores range between 8 and 66 with higher scores indicating higher socioeconomic status. Scores are derived by the level of education and occupation of each parent.

**Neuropsychological Assessment**

All participants underwent a 45-minute neuropsychological assessment emphasizing the functions that are most commonly affected in pediatric MS including attention, processing speed, and memory. Measures included the following:

(a) General intelligence: Wechsler Abbreviated Scales of Intelligence (WASI; Wechsler, 1999) subtests including (i) Matrix Reasoning, a measure of nonverbal reasoning with items asking participants to fill in the missing piece of a puzzle, and (ii) Vocabulary, a measure of verbal expression and word knowledge asking the participant to describe the meanings of words. These calculated scores from these two subtests were used to calculate full IQ.

(b) Information processing speed and attention: (i) Symbol Digit Modalities Test (SDMT) Oral version (Smith, 2002), which involves speeded verbal transcription of symbol-number pairings (described in Section 1.5). The number of correct responses in 90 seconds is recorded. (ii) Decision Speed from the Woodcock-Johnson III (WJ-III) Test of Cognitive Abilities (Woodcock, McGrew, & Mather, 2001) which includes items requiring the participant to locate quickly two pictures that are most similar conceptually. The number of correct responses within three minutes is recorded. (iii) Trail Making Test
(TMT)-Parts A and B (Reitan, 1958) which requires speeded number (Part A) and both
number-letter (Part B) sequencing within the format of a speeded visual-motor task. The
time to completion is recorded for both subtests.

(c) Memory: (i) Rey Auditory Verbal Learning Test (RAVLT) (Schmidt, 1996) which
consists of a list of 15 unrelated words presented during five learning trials. Total correct
responses across all learning trials (immediate recall) and number of words recalled
spontaneously after a 20 minute delay (delayed recall) were measured. (ii) Auditory
Working Memory from the Woodcock-Johnson III (WJ-III) Test of Cognitive Abilities
(Woodcock, McGrew, & Mather, 2001) in which a series that contains digits and words
are presented (e.g. snake-soup-7-2-glove-9) and the participant is then asked to
attempt to reorder the information, repeating first the objects in sequential order and
then the digits in sequential order (e.g. snake-soup-glove-7-2-9). The number of items in
each series increases in difficulty starting from two and going up to eight. The number of
correctly repeated series is recorded.

Cognitive impairment was defined as standardized performance falling below the 5th
percentile (i.e. z-score 1.64 SD or more below the normative mean) on two or more of
the nine cognitive subtests on at least two separate measures. This is standard criterion
adopted in both adult and pediatric MS studies (e.g. Portaccio et al., 2009; Rao et al.,
1991). For each test, z-scores were calculated based on individual test age and sex-
based normative data. A mean composite z-score was calculated for each participant
using z-scores on all nine subtests.

**Functional MRI version of the SDMT**

Participants completed an fMRI-adapted SDMT (review of studies utilizing this task
described in Section 1.5.1). A screenshot of the fMRI-SDMT stimuli is given in Figure 5.
Using a keypad controlled by the dominant index and middle fingers, participants
indicated “yes” or “no” whether or not a probe symbol-digit pairing matches any of those
shown in an array above. The array changes with each trial in order to avoid
memorization of the pairings. This task is an event-related design whereby stimuli are
presented for 4 seconds followed by a variable inter-stimulus interval of 0, 4, 8, or 12 seconds. During the inter-stimulus interval a fixation cross is presented in the middle of the screen. There are a total of 52 trials for an entire duration of 5 minutes. Prior to completing the task in the scanner, practice was given to ensure task familiarity and capacity (i.e. to ensure that MS patients did not have significant visual or motor impairment to preclude performance). Stimuli were presented on a back projection screen and viewed through a mirror mounted onto the 32-channel head coil. E-prime software (Psychology Software Tools, Inc.) was used to present the stimuli and record responses. The behavioural variables recorded by E-prime are response time and correct (yes/no) for each trial. For each participant mean response time (per trial, only correct responses) and overall percent accuracy (across all trials) were calculated.

![Example of fMRI SDMT stimuli](Image from Rao, Motes, & Rypma, 2014; reproduced with permission from Frontiers)

**Figure 5.** Example of fMRI SDMT stimuli. Participant indicates “yes” or “no” whether single pairing matches any of those seen in the key above. Response time and accuracy are measured. (Image from Rao, Motes, & Rypma, 2014; reproduced with permission from Frontiers)

**MRI Protocol**

All data were acquired on a Siemens MAGNETOM 3T Tim Trio MRI scanner at York University with a 32-channel head coil. The entire scan lasted approximately 90 minutes and included the following sequences, in the following order:

1. Localizer, which is a single-slice, 3-axis scan that gives a view of the subjects head in order to isolate the brain and orient the slices.
2. B₀ field map, to identify inhomogeneities in the magnetic field which can later be used to control for artifact (not used in this thesis).

3. fMRI-alphaspan task (20 minutes), which is a working memory task not analyzed as part of this thesis.

4. fMRI-SDMT task (5 minutes, 150 volumes) using a gradient-echo T₂*-weighted echo-planar imaging sequence (repetition time (TR)=2000ms, echo time (TE)=30ms, voxel size =3.0x3.0x4.0mm³, field of view (FOV)=256x191x136mm, 34 axial slices, flip angle=90°).

5. fMRI-go-no-go task (6 minutes), which is a response inhibition task not analyzed as part of this thesis.

6. Sagittal high-resolution three-dimensional (3D) magnetization prepared rapid acquisition gradient echo (MPRAGE) T₁-weighted image for the purposes of anatomical localization (TR=2300ms, TE=2.96ms, inversion time (TI)=900ms, voxel size=1.0x1.0x1.0mm, field of view (FOV)=256x240x192mm, number of slices =192, flip angle=9°).

7. fMRI resting-state (6 minutes) in which participants were instructed to lie still with their eyes closed, without falling asleep. They were also instructed not to think about anything in particular and let their mind wander. The duration was six minutes (180 volumes). Images were recorded using a gradient-echo T₂*-weighted echo-planar imaging (EPI) sequence (TR=2000ms, TE=30ms, voxel size =3.0x3.0x4.0mm³, matrix size=63x85x34, 34 axial slices, flip angle=90°).

8. DTI, which was recorded using a diffusion encoded spin echo EPI sequence (ep2d_diff sequence from Siemens), with diffusion weighting in 64 directions and b-value of 1000s/mm² (TR=4600 ms, TE=93ms, FOV=256x256x108mm, number of slices=36, voxel size=2.0x2.0x3.0mm).
9. Proton-density and $T_2$-weighted images acquired using dual-echo, turbo spin-echo sequence (TR/TE1/TE2=4500/10/83, voxel size=1.0x1.0x3.0mm, 256x256 matrix, 250mm FOV, flip angle=90°).

10. Fluid attenuated inversion recovery (FLAIR) sequence (TE/TI/TR=88/2407.5/9000 ms, voxel size=1.0x1.0x1.0mm, 256x256 matrix, 250mm FOV, flip angle=120°).

3.2 Structural MRI analysis

Lesion volumes

A depiction of how lesion volumes were obtained is shown in Figure 6. $T_2$ lesions were segmented using an automated Bayesian classifier (Ghassemi et al., 2014) based on the $T_2$, PD, FLAIR images. This was followed by manual review and correction of the results by a trained specialist (David Araujo). $T_1$ lesions were identified within $T_2$ lesions using an automated threshold technique, as previously described (Ghassemi et al., 2014). Total $T_1$ and $T_2$ lesion volumes are reported in cubic centimeters ($cm^3$).
Figure 6. Determination of lesion volumes. T₂ lesion volumes are obtained according to the following steps: (a) T₂-weighted, PD-weighted, FLAIR (not shown in this figure), and T₁-weighted images are pre-processed in order to correct for intensity non-uniformity (Sled et al., 1998). Skull and scalp are removed. This is followed by a mixture of linear and non-linear registration to MNI152 common stereotaxic space to allow for voxel-wise anatomical alignment across modalities (Collins et al., 1998). The intensity range is also normalized within each image using a linear transformation (Nyul & Udupa, 1999). Lesion segmentation is done using the T₂, PD, and FLAIR images using a Bayesian classifier incorporating anatomically determined prior probabilities for each tissue class. Outputs get manually reviewed and corrected. Only supratentorial lesion volumes are reported in this thesis. (b) T₁ lesion volumes are obtained by first transforming the finalized T₂-weighted lesion labels into T₁ space. T₁ hypointense lesions were identified as any voxels within T₂ lesions having an intensity less than 85% if surrounding normal-appearing white matter.
Total brain and thalamic volumes

Brain and thalamic volumes were computed based on the T₁-weighted image. This method incorporates the following steps: (a) mixture of linear and non-linear transformation (Collins et al., 1994) of the raw T₁-weighted image to ICBM152 population template space (Fonov et al., 2011), (b) brain extraction using a library of tissue priors that are available in the ICBM152 population template (Eskildsen et al., 2012), (c) warping of the thalamus onto the raw T₁-weighted image using the inverse of the transformation generated in step (a). An example of how thalamus segmentation appears is shown in Figure 7. Absolute volumes were reported in cubic centimeters (cm³). Thalamic volumes were normalized for head size by dividing by total intracranial volume. This correction allows indication of whether volume loss in the thalamus is disproportionately larger than that of global brain volume loss.

Figure 7. Example of thalamus segmentation.
Diffusion Tensor Imaging analysis

DTI processing was conducted using FMRIB Software Library (FSL) software library tools (www.fmrib.ox.ac.uk/fsl/) (Smith et al. 2004). First, DTI data were corrected for MRI eddy currents and head motion using affine registration to a reference volume, i.e., the one without diffusion weighting (b=0). Then, images were brain-extracted using Brain Extraction Tool (BET)(Smith, 2002) and entered into the program DTIFIT which fits a diffusion tensor model at each voxel. Images were created for each subject representing fractional anisotropy (FA), axial (longitudinal) diffusivity (AD, λ₁), radial (perpendicular) diffusivity [RD, (λ₂ + λ₃)/2] on a voxel-wise level. Tract-Based Spatial Statistics (TBSS) analysis (Smith et al. 2007) created a nonlinear registration of all FA images to 1x1x1mm³ standard space, followed by creation of a mean FA image which was further refined to create a mean FA white matter skeleton thresholded at a FA value of 0.2. As all subject’s FA images were in a standard space, the white matter skeleton was applied yielding FA skeletonized images for each subject. The same white matter skeleton was applied to the AD and RD images. For voxelwise analyses of DTI measures (FA, AD, RD), differences between groups were tested in a general linear model (GLM) framework using permutation testing [number of permutations=5000, FSL’s Randomise (Winkler et al. 2014), described in Section 3.3.4]. Threshold-free cluster enhancement was used in which voxelwise p-values incorporate the amount of cluster-like local spatial support (Smith & Nichols, 2009). The generated p-value maps were thresholded at p=.05.
3.3 Functional MRI analysis

In order to accomplish the three research aims of this thesis, different fMRI analytic software were used, each with specific methods for analysis. The software used included AFNI (Analysis of Functional NeuroImages) (Cox, 1996), FSL version 6.00 (FMRIB’s Software Library) (Smith et al., 2004), and CONN functional connectivity toolbox version 14b (Whitfield-Gabrieli & Nieto-Castanon, 2012). Figure 8 provides a schematic of the analyses performed and their corresponding research aims (described in Section 2.2).
Figure 8. Schematic of thesis methods. Resting-state and fMRI-SDMT data were first pre-processed with scans discarded due to artifacts or manuscript specifics. For the latter, this corresponded to a journal reviewer request to remove subjects with cognitive impairment. All resting-state data were analyzed using both seed-based region-of-interest and ICA approaches. For both of these analyses, between-group differences in functional connectivity were tested using voxel-wise and ROI-based functional connectivity measures as part of Aim 1 (green). Functional connectivity was correlated with structural volumes (lesion and thalamic volumes, DTI data) as part of Aim 2 (pink). Aim 3 (purple), examining the relationship between functional activation and cognitive performance, involved both resting-state and task-based fMRI-SDMT data. With respect to resting-state data, functional connectivity was correlated with neuropsychological test data. With respect to fMRI-SDMT data, t-tests were conducted examining differences between groups in BOLD activation patterns, and correlations were sought between BOLD activation patterns and cognitive performance. For each analysis, the method, software program, and control for multiple comparisons is indicated.
3.3.1 fMRI data pre-processing

The influence of noise on the integrity of fMRI-data can be large. The main purpose of fMRI pre-processing is to perform a series of transformations on the raw fMRI data in order to reduce the influence of noise. This helps ensure that the residual data is meaningful signal ideally maximally related to neuronal activation as opposed to noise or artifact such as due to scanner hardware, motion, or physiological parameters (e.g. breathing, heart rate). fMRI data pre-processing also helps improve the sensitivity of subsequent analyses.

This section provides a description of standard fMRI pre-processing and additional required steps that were used in my analysis, as well as the order in which they were done.

1. **Conversion to NIfTI (Neuroimaging Informatics Technology Initiative) file format.** The images acquired directly from the scanner are of the DICOM (Digital Imaging and Communications in Medicine) file format and must be converted to NIfTI file format. This is a universal file format that is commonly used by neuroimaging software such as FSL and AFNI. The conversion process is done using the dcm2nii command.

2. **Removal of first four volumes.** The first four volumes (of the total 180 acquired) are removed to ensure that the effects of $T_1$ recovery have reached a steady state as we are only interested in visualizing $T_2^*$ decay with BOLD fMRI. This results in a functional time series with 176 volumes.

3. **Conversion to Standard “Neuroscience” Orientation.** FMRI data were acquired in a specific orientation framework whereby the x-axis is right-to-left, y-axis is posterior-to-anterior and z-axis is inferior-to-superior. For the $T_1$ sagittal MPRAGE, the orientation acquired is: (a) x-axis: anterior-to-posterior, (b) y-axis: inferior-to-superior, and (c) z-axis: left-to-right. When the fMRI data gets aligned with the anatomical data during co-registration (step 7), both datasets have to be in the same orientation. Thus, we convert
both the functional and anatomical data to the following standard “neuroscience” orientation which is x-axis: left-to-right, y-axis: posterior-to-anterior, and z-axis: inferior-to-superior. This is done using the 3daxialize command in AFNI.

4. Motion correction. Motion correction is considered one of the most important steps of pre-processing. This is because movement can produce greater signal change than the actual BOLD signal change in response to greater neuronal activation (i.e. which is only 3%). In addition, where there is excessive motion, the correspondence between a particular voxel and anatomical localization (e.g. to a specific atlas region) is lost and will affect interpretation of results. With motion correction (using AFNI’s 3dvolreg for resting-state data, and FSL’s mcflirt for fMRI-SDMT), all volumes are aligned to a specific reference volume (e.g. first or middle volume) using a rigid-body (6 parameter, translation and rotation along each of the 3 axes) spatial transformation. The motion correction step creates output files giving the displacement (in mm) across the six dimensions (x,y,z, and rotation in x,y,z) for each volume. It also outputs the maximum displacement across any dimension. Maximum displacement greater than a voxel size (i.e. 3mm) was the criteria adopted for discarding scans.

5. Brain extraction. The removal of non-brain tissue (e.g. skull, scalp, eyeballs) for the fMRI images is done using the Brain Extraction Tool (BET) in the FSL software library (Smith, 2002). The technique has been demonstrated to be quick and comparable to manual hand-based segmentations and other popular automated methods. This technique involves the following steps: (a) a histogram of intensity values is plotted, and thresholds (e.g. at the 2% ends of the distribution) of low and high intensity are estimated to provide a rough binarized image separating brain from non-brain (i.e. especially background/ air), (b) the centre of gravity is found and a rough estimate of the size of the head from the binarized image is created, (c) starting from the centre of the brain a small sphere is formed which moves outwards. A triangular tessellation is performed whereby the sphere surface is allowed to deform as it moves toward the edge of the brain while trying to maintain smoothness. If a clean outline is not formed then the process is repeated with a higher smoothness restraint.
A modified version of the BET algorithm, entitled 3dSkullStrip which is part of AFNI, was used on the T₁ Sagittal MPRAGE anatomical image. This method is very similar to BET with a few modifications. These include using information from outside the brain to create the outline, and additional operations to reduce clipping certain brain areas and reduce leakage into the skull.

6. **Spatial smoothing.** Spatial smoothing is done primarily to improve the signal-to-noise ratio. Spatial smoothing involves replacing each voxel's intensity value by a weighted average of neighboring voxel intensities. The highest weighting is applied to the voxel being smoothed itself and decreases with distance away from this voxel. The extent of smoothing is defined by the value of full-width at half maximum (FWHM), which is the distance between two points at which the weighting is 0.5. For my analysis, the FWHM was specified to be 5mm. With spatial smoothing, intensity peaks get smoothed and valleys get filled in making the intensity values more normally distributed. Gaussian distribution of intensity values is an important assumption in fMRI statistical models. Spatial smoothing also allows for better overlap of atlas brain regions across subjects, but at the same time reduces spatial resolution. When smoothing is performed the spatial filter (defined by the FWHM) must be smaller than the spatial extent of the signal expected or significant effects might not be detected. This means that the underlying signal that aims to be detected must be larger than the effect of the smoothing. According to this criterion, a smoothing filter of 5mm is sufficiently small for investigation of default-mode network functional connectivity given the large spatial extent of the default-mode network regions.

7. **Spatial normalization.** This step involved spatial transformation of fMRI images to ICBM152 template (Collins et al., 1994) space, followed by resampling to 3mm³ cubic voxels. The transformation was done using a combination of linear (FSL’s FLIRT, Jenkinson & Smith, 2001; Jenkinson et al., 2002) and nonlinear (FSL’s FNIRT, Andersson, Jenkinson, & Smith, 2010, Smith et al., 2004) registration tools and incorporating the T₁-weighted anatomical image. In the first step, the brain-extracted T₁ image gets linearly transformed (using FLIRT) to the ICBM152 template to create an
affine matrix. This matrix is used to create a non-linear transformation matrix using FNIRT. These two matrices are then applied to create an anatomical image now in ICBM152 space. The fMRI EPI data gets linearly registered to the T₁ anatomical image using FLIRT and an affine matrix is created. This matrix gets combined with the non-linear transformation matrix created earlier from the T₁-weighted anatomical image to create an fMRI image now in ICBM152 template space. The voxel size of the fMRI images were resampled to 3mm³ cubic voxels.

8. Regression of white matter, CSF, and motion-related signal. Given the interest in determining activation of gray matter with fMRI, signal from white matter and CSF are essentially noise. Signals from white matter and CSF are non-neuronal (e.g. cardiac-induced CSF pulsations) and minimally influenced by BOLD changes. Once fMRI data are spatially normalized and in a common (e.g. MNI152) template space, anatomical atlases can be overlaid onto each subjects scan in order to define specific regions. Using anatomical overlays available in FSL, four white matter (right and left superior longitudinal fasciculus, right and left anterior corona radiata) and four CSF (left and right lateral ventricle, right and left third ventricle) voxels were selected as part of the fMRI analysis I conducted in my thesis. The timeseries of these voxels (i.e. of their specific MNI x,y,z coordinates) were used to generate four white matter and four CSF timeseries regressors. Timeseries for translation and rotation within each of the three dimensions (x,y,z) can also be regressed out. For my analysis, these six motion timeseries and the eight white matter and CSF timeseries were regressed out of each voxel’s timeseries using AFNI’s 3dDetrend.

9. Temporal filtering. Temporal filtering of each voxel timeseries is done in order to study signal fluctuations within a narrow range of frequencies associated with resting-state networks. These frequencies are within the bandwidth of 0.01 to 0.1 Hz (Damoiseaux et al., 2006). Frequencies lower than 0.1 Hz are usually due to low frequency drift for example due to scanner artifact or motion. Frequencies higher than 0.01 are usually due to physiological artifact such as breathing (approximately 0.2 Hz),
heart rate (1 Hz) or scanner artifact. As such, temporal filtering is another step done in order to reduce noise in fMRI data.

As a note, slice timing correction was not done in this thesis as there is recent consensus that slice timing correction creates more problems than it actually solves (Poldrack, Mumford, & Nichols, 2011). Reasons for this include that it may propagate artifacts throughout the entire image, it interacts in unpredictable ways with motion correction, and that spatial smoothing is done across proximal slices eliminating the need for slice timing correction.

3.3.2 Methods of resting-state fMRI data analysis

Once resting-state fMRI data have been pre-processed, the two most common methods of analysis include seed-based and ICA approaches. Both techniques were used in my thesis and will now be described.

3.3.2.1 Seed-based approach

The seed-based approach relies on a priori selection of a seed-region. In doing so, focus can be directed onto a specific a-priori region of interest or network (e.g. based on previous research) herein considered an advantage of this approach. The seed region selected for this thesis was the precuneus. The rationale for the precuneus was that this region is considered the major hub of the default-mode network, which is the major focus of investigation into resting-state neural activity, and has been shown to play a core role in cognition more broadly (Utevsky et al., 2014). In addition, the precuneus has shown structural and functional abnormalities in pediatric MS (Rocca, Absinta, Amato et al., 2014; Rocca, Valsasina, Absinta et al., 2014). As all pre-processed data are in common ICBM152 template space, ROI masks can be overlaid onto each subject’s resting-state scan. The Harvard-Oxford atlas (one of the anatomical atlases part of FSL, Desikan et al., 2006) was used to create ROI masks of precuneus and for other regions that showed high functional connectivity with the precuneus. The validity of the Harvard-Oxford anatomical atlas has been previously demonstrated by
comparing automated and manual tracing schemes to one another yielding an average intraclass correlation coefficient of 0.835 across all ROIs (Desikan et al., 2006). In addition, a mean distance error or mismatch of less than 1mm between the manual and automated set of ROIs was demonstrated.

After the creation of a precuneus mask (includes both right and left) based on the Harvard-Oxford cortical-subcortical atlas, the average timeseries of voxels within the precuneus can be extracted for each subject. Using the AFNI program 3dfim+, the cross-correlation of this seed precuneus timeseries with the measured timeseries for each voxel is calculated. The computed r-values are transformed to z-scores for the subsequent t-test analysis. The outputted correlation maps can be thought of as functional connectivity maps, with areas of high correlation representing brain voxels/regions that show a shared temporal pattern of activation with the precuneus seed, thereby approximating the default-mode network.

**Seed-based between-groups comparisons**

Based on the voxel-wise correlation maps, mean differences between groups were tested using the program 3dttest++ in AFNI. This 2-sample t-test assumed unequal variance between groups. The output is a voxel-wise brain map of t-values which can be thresholded at a particular p-value and cluster size based on the program AlphaSim (described in more detail in Section 3.3.4.2).

The analysis for my thesis also involved the creation of additional ROI masks based on areas of high functional connectivity with the precuneus seed region. This resulted in a total of 18 ROIs. Focus on these specific ROIs reduces the need for multiple comparisons correction in contrast to voxel-wise approaches, allows for focus of regions which have underlying functional substrates, and allowed for study of the default-mode network in particular. Mean timeseries were extracted for each of these 18 ROIs and Pearson correlation coefficients computed between every possible pair of ROIs to create a connectivity matrix (all done using Matlab). R-to-z transformations were performed in order to normalize distributions prior to statistical analysis. A two-sample t-
test, assuming un-pooled variance, was performed to assess for group differences in ROI-based functional connectivity.

An issue relevant to seed-based functional connectivity analysis is that noise is not distributed equally across the brain. For example, voxels close to the edge of the brain where there may be motion artifact, or close to CSF and thus highly modulated by both cardiac and respiratory cycles, may produce signals that are much more variable. Higher variability of signal in these voxels will then require greater covariance to be present with other voxels in order for these correlations to be statistically significant. Therefore, areas of significant correlation could be those that have the least amount of noise/time-series variability. Thinking about between-group comparisons, a group in which there may be greater overall noise/variability in signal may show lower correlations/functional connectivity values. Therefore a non-significant t-test may be due to greater noise rather than there being no difference between groups. With respect to t-test analysis, for areas with a large amount of noise or variability in functional connectivity for one or both groups, the group differences would have to be larger in order to reach statistical significance. Ideally, the influence of noise is eliminated based on the pre-processing fMRI steps I have described in the previous Section 3.3.1, though this may not always be the case.

Another way of removing noise from resting-state fMRI data involves the use of Independent Components Analysis (ICA) which will be described in Section 3.3.2.2.

**Seed-based Correlations with Structural Volumes and Cognitive performance**

Testing for the association between functional connectivity and brain volumes (lesion volume, thalamic volume), and cognitive composite z-score (calculation of this score described in Section 3.1), was done using the CONN functional connectivity toolbox version 14b (Whitfield-Gabrieli & Nieto-Castanon, 2012) as it allows for ROI-based correlations to be computed. Using this toolbox, the GLM (described in Section 3.3.3.2) is used to determine the correlation between functional connectivity and the variable of interest, and converted to t-values for assessing significance. The functional
connectivity values (one for each pair of 18 ROI’s result in a total of 162 values per subject) entered into this analysis were the z-transformed r-values.

3.3.2.2 Independent Components Analysis (ICA) approach

A problem inherent to fMRI data is that contains a mixture of meaningful and un-meaningful signal. In fMRI data, sources of signal variability that are not related to neural activity, and as such un-meaningful, could include physiological pulsation, scanner artifact, or motion. ICA attempts to solve this problem by decomposing data into linear combinations of statistically independent latent component variables (Beckmann et al., 2004) which can be discarded from subsequent analyses if deemed un-meaningful. Another advantage of ICA is that it is a data-driven and model-free approach in comparison to a priori hypothesis-driven methods (e.g. seed-based, GLM approaches).

Figure 9 provides a visual representation of the ICA process for a single subject. Each independent spatial pattern, herein referred to as “component”, identifies activation that is common to a group of voxels. These groups of voxels show shared variance with respect to their time series of activation. Components, however, are maximally independent to each other with respect to space. Each component has its own time course paired with a spatial map. The sum of the products of these timecourses and spatial maps will return the original data. Based on visual inspection of components via their spatial pattern and time-course, components are categorized as meaningful (e.g. network of interest) or not (e.g. noise or artifact related). For example, components which show a ring-link pattern around the edges of the brain and are of low temporal frequency are likely due to motion. The categorization process, however, can introduce some bias when trying to match certain components to certain networks and when a component includes a mixture of meaningful network signal with noise. In addition, one network may map onto more than one component.
Figure 9. Depiction of spatial ICA of a single subject’s fMRI data (image from Ylipaavalniemi & Vigário 2008, reproduced with permission from Elsevier). The pre-processed fMRI signal is contained in a time (t) by space (v) data matrix X. t represents the number of time points (e.g. 176 2-sec intervals for resting-state) and v is the number of voxels in the volume. The ICA output of S contains rows of independent spatial patterns, sources of variability or components, and the output of A contains columns of the activation time-series of these components.

The program used to conduct ICA analysis in this thesis was FSL’s MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components, Smith et al., 2004; Beckmann & Smith, 2004). ICA can be conducted on individual subject’s data, as shown above, or across subjects. For ICA across subjects, data are temporally concatenated across all subjects to create a single dataset (such as adding another dimension of “subjects” to the matrix X in Figure 9, as described in http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC), which is then decomposed into entire-group components.

Using MELODIC, the number of components that data are decomposed to is determined based on use of a Bayesian model, which compares the statistical evidence of each level of dimensionality (i.e. number of components) in the data (Beckmann & Smith, 2004).
ICA Between-Groups Comparisons

For my thesis work, all subject’s (i.e. combined pediatric MS and healthy control) data were entered into ICA and the outputted components classified as being meaningful (i.e. corresponding to canonical resting-state networks) and retained or artifact related and discarded. Subject-specific versions of the retained ICA components were generated using a dual regression technique (Filippini et al., 2009; Beckmann et al., 2009, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/DualRegression). For each ICA component (at the group or individual-level), the voxel-wise intensity values on these spatial maps represent parameter estimates within the general linear model (GLM, described in Section 3.3.3.2). Parameter estimate values indicate the amount of covariance of the particular voxel timeseries with the particular component timeseries (i.e. level of co-activation/synchronization). Differences between groups on parameter estimates form the basis of determining functional connectivity differences between groups. For my thesis work, group differences in parameter estimates were tested within a GLM framework (t-statistics reported), with control for multiple comparisons via permutation testing [number of permutations=5000, FSL’s Randomise (Winkler et al. 2014, described in Section 3.3.4.2). For clusters that showed significant differences between groups, mean parameter estimate values were calculated for each subject and used to correlate with DTI measures.

ICA Correlations with DTI measures

Testing for the association between ICA-derived measures of functional connectivity (mean parameter estimate values) and DTI measures was done using SPSS version 22.0, using Pearson correlation coefficients and assessed for significance using a p-value of <.05.
3.3.3 Methods of task-based fMRI data analysis

3.3.3.1 Approximating the BOLD response

Analysis of the fMRI-SDMT data from my thesis involved use of the GLM. Use of the GLM involves the prediction and modelling of the Blood Oxygenation Level Dependent (BOLD) response elicited by stimulus presentation. The BOLD response is driven primarily by increased synaptic activity and reflects a post-synaptic response to local neural activity (Logothetis et al, 2001; Buxton, 2013; Lindquist et al., 2009). The BOLD response is due to a combination of changes to cerebral blood flow, cerebral metabolic rate of oxygen, and cerebral blood volume in relation to greater energy demands of more active neurons (Buxton, 2009). Greater neural activity leads to greater oxygen demands leading to increased cerebral blood flow. Despite the influx of oxygen, only a small proportion is actually extracted by brain tissue. As a result, the ratio of deoxyhemoglobin to oxyhemoglobin within the blood surrounding active tissue then decreases. Given that deoxyhemoglobin is paramagnetic, it demonstrates a difference in magnetic susceptibility creating magnetic field distortions in blood and surrounding extracellular area. With a lower deoxyhemoglobin/oxyhemoglobin ratio, there is less magnetic field distortion and less $T_2^*$ signal decay which is seen as brighter signal on the $T_2^*$-weighted image that is used in fMRI. Thus, voxels with higher intensity on the $T_2^*$-weighted image are thought to contain neurons that are more active.

The “hemodynamic response function” (HRF) is the hypothetical BOLD response to an idealized impulse of neural activation (Ashby, 2011). The HRF is hypothetical in the sense that it is an estimation based on research findings. This estimation has been primarily based on the presentation of stimuli widely spaced in time (e.g. 30 seconds) and averaging the evoked response at each time point. For my thesis, the HRF that the stimulus was convolved to in order to create the modelled response was the double-gamma response function implemented in the FSL software package. This HRF has the following characteristics: (a) peak height of 0.5-3% BOLD amplitude signal change, (b) time to peak of 6 seconds after stimulus onset, (c) a rise that begins within 1-2 seconds and returns to baseline by 12-20 seconds after stimulus onset, and (d) post-stimulus
undershoot that can persist up to 20 seconds or more after the stimulus. A response which includes the post-stimulus undershoot is called double-gamma in that the first gamma models the shape of the initial stimulus response (i.e. the standard positive function) and the second models the post-stimulus undershoot (small, inverted gamma). It is unclear what the post-stimulus response actually represents, but has been demonstrated to be present in the work of Friston and colleagues (1998) and Glover (1999).

An issue in the modelling of BOLD response is the variability of the HRF across individuals (e.g. shape, time to peak), and across different brain regions. One way to control for timing shifts in the HRF (e.g. offsets in time to peak) is to add the temporal derivative as a basis function (Friston et al., 1998; Lindquist et al., 2009). This means that the modelled signal can be decomposed into the sum of the stimulus and temporal derivative waveform. Incorporation of the temporal derivative was done in my thesis.

3.3.3.2 General Linear Model (GLM)

The GLM is linear modelling applied simultaneously across all voxels of the brain, leading to the name “generalized”. In the context of my thesis, the GLM was used to test whether each voxel’s time-series was a good fit to the modelled BOLD response to presentation of fMRI-SDMT stimuli. A description of the fMRI-SDMT was given in Section 3.1. Based on the timing of stimulus presentation, and convolution of the BOLD response, a modelled response is created as shown in Figure 10. Only stimulus events/trials which were answered correctly were entered into this model. As can be seen in Figure 10, the modelled response depends on convolution of the spikes of stimulus presentation with the HRF. As described above in Section 3.3.3.1, the HRF was modelled as a double-gamma, and incorporated the temporal derivative in order to capture small offsets in the timing of the BOLD response.
With just one type of stimulus, as in the fMRI-SDMT, the linear model would be indicated by the formula 1 below:

\[ y = a1 \times x1 + b + e \]

In this formula y is the observed time-series data, which is a 1D vector of intensity values. x1 is the model waveform, which is also a 1D vector with one value for each time point. b is a constant or baseline (rest) intensity value in the data and e is the residual error in the model fitting. a1 is the value of the parameter estimate, with higher values representing good fit to the modelled response. Parameter estimates can also be considered a measure of effect size indicating the influence of stimulus on measured BOLD response. Each parameter estimate value is divided by the variance of this estimate to obtain a t-value which can be used to assess statistical significance. A z (Gaussianised t) statistic image is generated for each subject and cluster thresholding applied at \( z = 2.3 \) (described in Section 3.3.4.3). Additional confound regressors can be added to this model. For the fMRI-SDMT analysis, the six motion parameter time-series generated during pre-processing were added as regressors. The model-based voxel-wise GLM for modelling single-session data in FSL is done through the program FILM (FMRIB’s Improved Linear Model) (Smith et al., 2004; Woolrich et al., 2001) which
includes correction for temporal autocorrelation in each voxel’s time series without requiring low-pass temporal filtering.

Inherent in the use of the GLM is the problem of presence of un-modelled artifactual signal in the data. Structured noise that is temporally non-orthogonal to the modelled response will bias the parameter estimates to higher values (e.g. stimulus-correlated motion) (Beckmann & Smith, 2004). On the contrary, noise that is orthogonal to the modelled response will increase the residual error, lowering the parameter value estimates. Related to this, areas with more noise may have a larger amount of residual error, resulting in lower parameter value estimates in these regions. Given the effects that non-modelled artifact can have on the analysis, trying as best to remove the influence of noise during fMRI pre-processing and GLM analysis is crucial.

**Between groups GLM Analysis**

For the fMRI-SDMT data, higher-level (between-subject) analyses were carried out using FLAME (FMRIB’s Local Analysis of Mixed Effects) (Woolrich et al., 2004). By being a mixed-effects analysis, FLAME models the between-subject variability therefore allowing for inferences to be made about the wider pediatric MS population in general. In FLAME, the lower-level/ within-subjects summary statistics (e.g. parameter estimates, variance of parameter estimates) are used to infer the higher-level/ group statistics using Bayesian modelling. Separate modelling of the variance was done for each group. t-statistics were computed indicating whether there were differences between groups on the mean parameter estimates for each voxel. A significant t-value would indicate significant differences between groups in BOLD response to stimulus presentation.

**Correlational analysis within the GLM**

FLAME also allows for covariates to be entered into the GLM in order to determine whether they can explain significant variability in BOLD response. Mean response time per trial (e.g. 1100ms) was used as a covariate in order to determine the correlation
between response time/ behavioural performance and BOLD activation. These covariate values must be demeaned (by the group mean) for interpretation purposes. Mainly, we want the model to be able to predict the BOLD response when the value of the covariate is the average value rather than when it’s a value of 0. t-statistics are computed indicating the voxels in which there was a significant correlation between parameter estimates and response time for each voxel. A significantly positive t-value would indicate that greater BOLD response to stimulus is associated with longer/ slower response times, whereas a negative t-value would indicate that greater BOLD response is associated with shorter/ faster response times.

3.3.4 Correction for multiple comparisons

If we consider than statistics are run for every voxel of the brain, this would result in approximately 50,000 comparisons being done at once. At an alpha level of $p<.05$, this would result in 2,500 false positives. As such, the problem of multiple comparisons is a large one in the context of neuroimaging analysis. One way to reduce the number of comparisons is to perform statistics on ROI’s rather than voxels. As described in the seed-based ROI analysis (Section 3.3.2.1), functional connectivity between pairs of ROIs were computed and entered into between-group and correlational analyses. Therefore, in contrast to voxel-wise analyses, less correction for multiple comparisons is required. Similarly, the correlations computed between ICA and DTI measures involved computing mean values (i.e. functional connectivity or DTI measures) for clusters that were significantly different between groups in the voxel-wise analysis. Since the number of significant clusters was small, the number of correlations calculated was small, reducing the number of multiple comparisons.

The various methods used for multiple comparison correction implemented as part of AFNI, FSL, and CONN will now be described.
3.3.4.1 Control of the false discovery rate

Control of the false discovery rate first involves determining the alpha level at which each voxel rejects the null hypothesis. Setting the false discovery rate threshold at 0.05 means that among all voxels in which the null hypothesis was rejected, 5% would be false positives. In my thesis, correction based on false-discovery rate was not used for any voxel-wise analyses, but rather for the functional connectivity ROI-based correlations with other variables (i.e. structural volumes and cognitive performance) as implemented by the CONN functional connectivity toolbox (described in Section 3.3.2.1). Therefore, for all correlations that were run between functional connectivity and the structural and cognitive variables, control was set so that the probability that any significant correlation was a false positive was 5%, and p-values were reported according to false-discovery rate correction.

3.3.4.2 Resampling techniques

The program Alphasim (Ward, 2000) uses Monte Carlo simulations to select appropriate cluster thresholds for voxel-wise analyses. For my thesis, AlphaSim was used to correct for multiple comparisons as part of the voxel-wise t-test of seed-based functional connectivity differences (Section 3.3.4.2). Using AlphaSim, the user specifies the number of iterations of which random noise images are generated. For my thesis, this value was 1000 (Ward, 2000). Alphasim takes into account the fact that the underlying signal/activation of interest spans more than one voxel and so voxels that are next to one another will be correlated (i.e. voxels are not independent). By specifying the smoothing filter (i.e. 5mm FWHM described in Section 3.3.1), the correlation of signal between adjacent voxels is taken into account. With Alphasim, Gaussian data are simulated and smoothed based on the smoothing threshold, with surrogate statistical images created under the null hypothesis based on the inputted alpha threshold ($\alpha=.01$ for my analysis). The output is an ascending list of cluster sizes, each with a corresponding probability that random noise alone would generate a significant cluster of that size under the null hypothesis. For example, based on running AlphaSim on the data of my thesis, a cluster size of 38 voxels would be obtained 1% of the time and was
considered a suitable cluster size threshold for the t-test analysis conducted in my thesis (AlphaSim results presented in Figure 11). Voxels were considered part of the same cluster if their centers were equal to or less than 3mm apart.

Figure 11. AlphaSim results indicating that the probability of obtaining a significant result under the null hypothesis decreases with increasing cluster size. A cluster size of 38 (corresponding to an alpha probability of p=.01) was chosen as the significance threshold for this thesis.

Another resampling method used in my thesis was permutation testing implemented by the “randomise” algorithm as part of FSL (Winkler et al., 2014). Randomise was used for voxel-wise between-group comparisons of ICA-derived functional connectivity and
DTI measures. Randomise involves simulating the distribution of the maximum t-statistic (across the whole brain) based on re-labeling of group assignment. For my thesis, the number of permutations/ re-labellings was 5000 (Nichols & Holmes, 2002). The maximum t-value obtained from the actual data is then compared to the distribution of the maximum t-value derived from the permutations/ random assignment of labels. The adjusted p-value is then the probability that a t-statistic of that value or higher could occur simply by chance. The threshold for statistical significance in this thesis was an adjusted p value of .05.

3.3.4.3 Cluster thresholding

Cluster thresholding, implemented as part of FSL software, was applied as part of analysis of the fMRI-SDMT data. This involved two steps (a) the raw statistical image is thresholded at a specific z-value (for this thesis z=2.3, Friston et al., 1994; Worsley et al., 1992) and clusters of contiguous supra-threshold voxels are identified, (b) the p-value for each cluster is calculated on the basis of its size according to Gaussian Random Field theory. Using this Gaussian Random Field theory approach, random noise images are generated and the probability distribution of clusters of different sizes is determined, incorporating the smoothing threshold (e.g. 5mm FWHM). The corrected p-value of a particular cluster is then the probability that a cluster of that size could occur simply by chance.
Parts of this chapter are included in the following manuscripts:


4.1 Sample characteristics

Demographic and disease-related characteristics of the sample are reported in Table 2. As the two approaches (seed-based versus ICA) had slightly different sample sizes (i.e. seed-based approach only included those with cognitive preservation, outlined in Figure 8), they are given separate columns in the table. These two pediatric MS samples did not differ on any demographic or disease-related variables. Within the ICA sample of 19 pediatric MS patients, seven (37%) versus four (25%) healthy controls reported elevated scores on the depression scale (CES-DC score > 15, Weismann et al., 1980). Nine (47%) versus two (13%) healthy controls endorsed moderate or greater levels of fatigue (PedsQL score ≥36, Parrish et al., 2013). There were, however, no significant differences between groups on these measures. No differences were observed between groups in handedness (Dutch Handedness Questionnaire score) or socioeconomic status (BSMSS score).
<table>
<thead>
<tr>
<th>Table 2. Demographic and disease-related data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Seed-based ROI sample Pediatric-onset MS (n=16)</strong></td>
</tr>
<tr>
<td>Mean (range) age at scan</td>
</tr>
<tr>
<td>Sex (F/M)</td>
</tr>
<tr>
<td>Mean (SD) disease duration b, months</td>
</tr>
<tr>
<td>Mean (SD) age at MS onset c, years</td>
</tr>
<tr>
<td>Median (range) EDSS score</td>
</tr>
<tr>
<td>Median (range) total number of relapses since MS onset</td>
</tr>
<tr>
<td>Current use of disease-modifying therapies (Y/N)</td>
</tr>
<tr>
<td>Mean (SD) Socioeconomic status-BSMSS score</td>
</tr>
<tr>
<td>Mean (SD) Depression- CES-DC score</td>
</tr>
<tr>
<td>Mean (SD) Fatigue- PedQL Multidimensional Fatigue score</td>
</tr>
<tr>
<td>Handedness (Right/Left)d</td>
</tr>
</tbody>
</table>

EDSS = Expanded Disability Status Scale Score; BSMSS = Barratt Simplified Measure of Social Status (BSMSS); CES-DC = Centre for Epidemiological Studies Depression Scale for Children; PedQL = Pediatric Quality of Life Inventory Multidimensional Fatigue Scale; a- Based on comparison of ICA (n=19) pediatric MS sample, b- Months since first attack; c- Age at first attack; d- Based on Dutch Handedness Questionnaire.
4.2 Cognitive performance

4.2.1 Neuropsychological test performance

The cognitive data presented in this section are for participants included in the fMRI-SDMT analysis (n=20 MS, n=16 healthy controls, outlined in Figure 8). As shown in Table 3, MS patients and healthy controls did not differ on any cognitive measure. A profile of the cognitive scores is depicted in Figure 12. As can be seen from Figure 12 impairment (z-score less than -1.64) was not evident in the pediatric MS group on any measure. The two tests that the pediatric MS group performed the least well compared to healthy controls was the TMT and Decision speed. These are tests that both tap into attentional set-shifting and processing speed. Based on the assessment battery, only two (10%) of the 20 pediatric-onset MS patients demonstrated evidence of cognitive impairment (defined in Section 3.1) versus one (6%) of the 16 healthy controls. For the fMRI-SDMT analysis, patients with cognitive impairment were not excluded from the analysis.

In the pediatric MS group, no cognitive variables were significantly correlated with age of disease onset or disease duration. Higher EDSS score (greater physical disability) was correlated with worse performance on the TMT A (r=-.550, p=.012) and WJ-III Decision Speed (r=-.530, p=.016).
### Table 3. Mean (SD) cognitive performance on the fMRI-SDMT and neuropsychological test battery

<table>
<thead>
<tr>
<th>fMRI-SDMT Behavioural Measures</th>
<th>Pediatric-onset MS (n=20)</th>
<th>Healthy Controls (n=16)</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) Response time (sec)</td>
<td>1.61 (.275)</td>
<td>1.60 (.267)</td>
<td>-.115</td>
<td>.909</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>.208 (.033)</td>
<td>.210 (.033)</td>
<td>.137</td>
<td>.892</td>
</tr>
<tr>
<td>Percent accuracy</td>
<td>98.9 (1.26)</td>
<td>98.8 (1.41)</td>
<td>138</td>
<td>.851</td>
</tr>
</tbody>
</table>

Neuropsychological test z-scores

- **RAVLT- Total Immediate Recall**: -.101 (1.17) vs -.400 (1.44), t-test = -.687, p-value = .497
- **RAVLT- Delayed Recall**: -.302 (1.09) vs -.140 (1.21), t-test = .424, p-value = .674
- **WASI Vocabulary**: .422 (.744) vs .522 (.714), t-test = .407, p-value = .686
- **WASI Matrix Reasoning**: .277 (.773) vs .416 (.554), t-test = .561, p-value = .579
- **WASI Full IQ**: 106.4 (10.2) vs 108.1 (11.8), t-test = .427, p-value = .673
- **SDMT**: .704 (1.26) vs .225 (1.23), t-test = -1.141, p-value = .262
- **TMT-A**: .137 (1.23) vs .555 (.793), t-test = 1.179, p-value = .247
- **TMT-B**: -.686 (2.37) vs .035 (1.29), t-test = 1.091, p-value = .283
- **WJ-III Decision Speed**: -.157 (.859) vs .062 (1.34), t-test = .594, p-value = .557
- **WJ-III Auditory Working Memory**: .343 (.638) vs .352 (.616), t-test = .044, p-value = .965
- **Composite z-score**
  - **RAVLT = Rey Auditory Verbal Learning Test; WASI = Wechsler Abbreviated Scales of Intelligence; SDMT = Symbol Digit Modalities Test; TMT-A and TMT-B = Trail Making Test Parts A and B; WJ-III = Woodcock-Johnson III Test of Cognitive Abilities- 3rd edition; a- Only correct trials; b- Mean z-score of the 9 test variables**

The table above provides a summary of cognitive performance on various tests. The data includes mean (SD) values for different measures and compares the Pediatric-onset MS group (n=20) with the Healthy Controls group (n=16). The t-test and p-value values indicate the statistical significance of the differences between the groups for each measure.
4.2.2 fMRI-SDMT performance

No differences were found between groups on behavioural performance of the fMRI-SDMT with respect to mean response time and coefficient of variation in response time, both computed using only correct trials (Table 3). The frequency distribution of mean and median response times is shown in Figure 13 and shows the similarity between groups on performance. Percent accuracy also did not differ between groups and were near ceiling (average of 99% for both groups).

Faster response time on the fMRI-SDMT was correlated with a greater number of correct responses on the paper-and-pencil version of the SDMT when calculated across the entire sample ($r=-.335$, $p=.05$), suggesting that this paradigm was successful in
assessing processing speed. No significant correlation was found between age and fMRI-SDMT mean response time in either the MS or healthy control group ($r=-.121$, $p=.632$ and $r=-.100$, $p=.712$ respectively). In the pediatric MS group, higher EDSS (greater physical disability) was correlated with longer mean fMRI-SDMT response time ($r=.548$, $p=.019$). Age ($r=-.112$, $p=.658$), disease duration ($r=.069$, $p=.785$), and age of disease onset ($r=-.234$, $p=.349$) did not demonstrate significant correlations with fMRI-SDMT response time.
Figure 13. Frequency distribution of (a) mean and (b) median response times across correct trials (maximum number of correct trials= 52) of the fMRI-SDMT task.
4.3 Structural MRI Results

4.3.1 Lesion, brain and thalamic volumes

Lesion volumes are reported in Table 4. As shown in Table 4, the MS group had lower thalamic volumes (both absolute and normalized by brain volume) than healthy controls, but there was no difference between groups in total brain volume.

**Table 4.** Mean (SD) structural MRI volumes

<table>
<thead>
<tr>
<th></th>
<th>Pediatric-onset MS (n=19)</th>
<th>Healthy Controls (n=16)</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₂-Lesion Volume (cm³)</td>
<td>14.1 (26.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₁-Lesion Volume (cm³)</td>
<td>8.94 (18.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain Volume (cm³)</td>
<td>1328 (127.9)</td>
<td>1296 (133.8)</td>
<td>-.722</td>
<td>.476</td>
</tr>
<tr>
<td>Thalamic Volume (cm³)</td>
<td>11.64 (1.39)</td>
<td>12.65 (1.078)</td>
<td>2.38</td>
<td>.023*</td>
</tr>
<tr>
<td>Normalized thalamic volumeᵃ</td>
<td>.00880 (.00090)</td>
<td>.00978 (.00035)</td>
<td>4.10</td>
<td>&lt;.001**</td>
</tr>
</tbody>
</table>

ᵃ- Normalized thalamic volume = thalamic volume/brain volume

Thalamic volume was negatively correlated with T₂ and T₁ lesion volume ($r = -.804$, $p < .001$ and $r = -.764$, $p = .001$ respectively). Within the MS group, longer disease duration was correlated with greater T₁ lesion volume ($r = .52$, $p = .04$) and T₂ lesion volume ($r = .586$, $p = .017$), and younger age of onset was associated with lower absolute (cm³) thalamic volume ($r = .619$, $p = .011$). There were no significant associations present between structural MRI variables and physical disability (EDSS score), or age in the MS group.
4.3.2 DTI measures

Pediatric-onset MS patients demonstrated lower white matter FA compared to healthy controls of the entire white matter skeleton (t=2.84, p=.008, reported in Table 5). Areas demonstrating the largest differences between groups were the corpus callosum, posterior thalamic radiation, sagittal striatum (including the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus), and corona radiata (Figure 14, Table 5). The pediatric-onset MS group also demonstrated higher white matter RD, but no differences in AD compared to the healthy control group. There were no brain regions in which the healthy controls showed lower FA or higher diffusivity relative to the MS patients. As can

Within the MS group, none of the DTI metrics showed a significant association with age, sex, age of disease onset, or EDSS. Longer disease duration was correlated with lower FA and higher RD of the splenium of the corpus callosum (FA: r=-.462, p=.46; RD: r=.485, p=.035). All FA, AD, and RD measures correlated significantly with T2- and T1-lesion volumes (FA: r=-.782, p<.001; AD: r=.684, p=.001, and RD: r=.788, p<.001 for correlations with the whole white matter skeleton) as well as thalamic volumes (FA: r=.707, p=.001; AD: r=-.782, p<.001; RD: r=-.806, p<.001 for correlations with the whole white matter skeleton).
Figure 14. WM skeleton (green) depicting areas in which the pediatric-onset MS group demonstrated lower FA (red) compared to the healthy control group (p<.01, corrected). Mean lesion map overlay of the pediatric MS group is also shown (blue) with darker areas representing voxels with higher probability of harboring lesions. High correspondence is demonstrated between areas with lower FA and high lesional probability.
**Table 5. Mean (SD) DTI data**

<table>
<thead>
<tr>
<th>Fractional Anisotropy</th>
<th>Pediatric MS (n=19)</th>
<th>Healthy Control (n=16)</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Entire White Matter Skeleton</strong></td>
<td>.437 (.025)</td>
<td>.458 (.015)</td>
<td>2.84</td>
<td>.008**</td>
</tr>
<tr>
<td><strong>Within Significant FA voxels</strong></td>
<td>.551 (.041)</td>
<td>.616 (.020)</td>
<td>5.84</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Corpus Callosum- Body</strong></td>
<td>.637 (.058)</td>
<td>.714 (.032)</td>
<td>5.02</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Corpus Callosum- Genu</strong></td>
<td>.644 (.065)</td>
<td>.701 (.040)</td>
<td>3.05</td>
<td>.005**</td>
</tr>
<tr>
<td><strong>Corpus Callosum- Splenium</strong></td>
<td>.748 (.049)</td>
<td>.796 (.016)</td>
<td>4.63</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Posterior Thalamic Radiation- Left</strong></td>
<td>.596 (.053)</td>
<td>.669 (.027)</td>
<td>5.26</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Posterior Thalamic Radiation- Right</strong></td>
<td>.601 (.043)</td>
<td>.680 (.024)</td>
<td>6.50</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Corona Radiata- Left Superior</strong></td>
<td>.445 (.047)</td>
<td>.497 (.029)</td>
<td>4.03</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Corona Radiata- Right Superior</strong></td>
<td>.443 (.046)</td>
<td>.493 (.026)</td>
<td>4.02</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Corona Radiata- Left Posterior</strong></td>
<td>.485 (.050)</td>
<td>.542 (.029)</td>
<td>4.15</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Corona Radiata- Right Posterior</strong></td>
<td>.463 (.047)</td>
<td>.522 (.021)</td>
<td>4.91</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Sagittal Stratum- Left</strong></td>
<td>.536 (.041)</td>
<td>.594 (.023)</td>
<td>4.75</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td><strong>Sagittal Stratum- Right</strong></td>
<td>.536 (.045)</td>
<td>.607 (.028)</td>
<td>5.44</td>
<td>&lt;.001**</td>
</tr>
</tbody>
</table>

**Axial Diffusivity (10⁻³mm²/sec)**

| White Matter Skeleton                        | 1.26 (.042)        | 1.24 (.023)        | -1.43  | .163    |
| Within Significant FA voxels                | 1.53 (.057)        | 1.51 (.029)        | -1.07  | .294    |

**Radial Diffusivity (10⁻³mm²/sec)**

| White Matter Skeleton                        | .622 (.052)        | .585 (.027)        | -2.51  | .017*   |
| Within Significant FA voxels                | .534 (.013)        | .433 (.049)        | -3.22  | .004**  |

*a- mean values reported are based only on those voxels that demonstrated significantly different FA values between groups in t-test analysis (shown in Figure 14); b- mean values reported are based only on those voxels that demonstrated significantly different FA values between groups in t-test analysis, restricted to certain white matter structures as defined by FSL’s JHU white-matter tractography atlas (Mori et al., 2008)
4.3.3 Correlation between structural MRI and cognitive variables

No significant correlations were present between cognitive and structural MRI variables in the pediatric MS group (Table 6).

**Table 6.** Correlation between cognitive and structural MRI variables in the pediatric MS group (n=19)

<table>
<thead>
<tr>
<th>Variable</th>
<th>FA of White Matter Skeleton</th>
<th>( T_2 ) Lesion Volume (cm(^3))</th>
<th>( T_1 ) Lesion Volume (cm(^3))</th>
<th>Normalized Thalamic Volume (fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cognitive z-score</td>
<td>.240 (.309)</td>
<td>-.062 (.731)</td>
<td>-.064 (.789)</td>
<td>.117 (.622)</td>
</tr>
<tr>
<td>RAVLT Total Recall z-score</td>
<td>.139 (.558)</td>
<td>.132 (.580)</td>
<td>.152 (.522)</td>
<td>-.037 (.877)</td>
</tr>
<tr>
<td>RAVLT Delay z-score</td>
<td>.228 (.333)</td>
<td>.063 (.791)</td>
<td>.093 (.695)</td>
<td>.007 (.977)</td>
</tr>
<tr>
<td>WASI Vocabulary z-score</td>
<td>.094 (.694)</td>
<td>-.049 (.837)</td>
<td>-.032 (.894)</td>
<td>.274 (.242)</td>
</tr>
<tr>
<td>SDMT z-score</td>
<td>.285 (.222)</td>
<td>-.345 (.137)</td>
<td>-.339 (.144)</td>
<td>.277 (.237)</td>
</tr>
<tr>
<td>Trail Making Test A z-score</td>
<td>.060 (.801)</td>
<td>-.042 (.860)</td>
<td>-.050 (.834)</td>
<td>-.055 (817)</td>
</tr>
<tr>
<td>Trail Making Test B z-score</td>
<td>.179 (.451)</td>
<td>-.085 (.721)</td>
<td>-.063 (.793)</td>
<td>.084 (.723)</td>
</tr>
<tr>
<td>Woodcock-Johnson III Decision Speed z-score</td>
<td>.311 (.182)</td>
<td>-.314 (.177)</td>
<td>-.322 (.166)</td>
<td>.307 (.187)</td>
</tr>
<tr>
<td>Woodcock-Johnson III Auditory Working Memory z-score</td>
<td>.118 (.621)</td>
<td>-.163 (.491)</td>
<td>-.171 (.472)</td>
<td>-.014 (.953)</td>
</tr>
<tr>
<td>fMRI-SDMT mean response time</td>
<td>-250 (.316)</td>
<td>-.133 (.600)</td>
<td>-.150 (.551)</td>
<td>.050 (.845)</td>
</tr>
<tr>
<td>fMRI-SDMT Coefficient of variation</td>
<td>-.284 (.254)</td>
<td>-.071 (.778)</td>
<td>-.110 (.664)</td>
<td>-.216 (.390)</td>
</tr>
</tbody>
</table>

Pearson correlation coefficients and p-values reported
4.4 Resting-state functional connectivity abnormalities in pediatric MS

4.4.1 Voxel-wise correlations with precuneus seed

The mean precuneus functional connectivity maps and t-test differences between groups are shown in Figure 15. Relative to the healthy control group, the pediatric-onset MS group demonstrated higher functional connectivity between the precuneus and: (a) bilateral anterior cingulate (z=4.21, p<.001, cluster size=372 voxels), (b) bilateral frontal medial cortex (z=3.48, p<.001, cluster size=78 voxels), (c) cerebellum (z=3.72, p<.001, cluster size=115 voxels), (d) left middle frontal gyrus (z=3.62, p<.001, cluster size=52 voxels), and (e) left frontal pole (z=4.62, p<.001, cluster size=248 voxels) (value at peak of cluster mass reported).
Figure 15. Mean resting-state functional connectivity maps in (a) pediatric-onset MS group (n=16) and (b) healthy control group (n=15). Mean Pearson correlation coefficients of individual voxel timeseries with precuneus seed region timeseries are displayed thereby approximating the default-mode network of brain function. These images were thresholded at an r-value > 0.5 and cluster threshold of 30 voxels, (c) Between-groups t-test results showing significant clusters in which the pediatric-onset MS group showed higher functional connectivity with the precuneus than healthy controls (p < .01, and minimum cluster threshold of 38 voxels based on Alphasim Monte Carlo simulations). These areas included the (1) bilateral anterior cingulate cortex (z=4.21, p < .001), (2) bilateral frontal medial cortex (z=3.48, p < .001), (3) cerebellum (z=3.72, p < .001), (4) left middle frontal gyrus (z=3.62, p < .001), and (5) left frontal pole (z=4.62, p < .001) (value at peak of cluster mass reported). Sagittal, axial, and coronal slice location indicated by crosshairs. Images are displayed in radiological convention (Left=Right, Right=Left).
4.4.2 Seed-based ROI analysis

Sixteen ROIs were selected based on having cluster peaks of highest functional connectivity with the precuneus seed (based on z-transformed r-values) in the pediatric MS group (Table 7): (a) left and (b) right posterior cingulate cortex; (c) left and (d) right paracingulate gyrus; (e) left and (f) right frontal medial cortex; (g) left and (h) right superior occipital cortex; (i) left and (j) right anterior cingulate cortex; (k) thalamus; (l) left and (m) right insular cortex; (n) left and (o) right middle frontal gyrus and (p) cerebellum. The regions were defined using an overlay of the Harvard-oxford cortical and subcortical structural atlas and were used in subsequent analyses presented in Sections 4.5 and 4.6.
Table 7. Regions of highest functional connectivity with the precuneus

<table>
<thead>
<tr>
<th>Cluster size (voxels)</th>
<th>Primary Region(s)</th>
<th>MNI co-ordinates (mm)</th>
<th>Value at cluster peak (z-score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2166</td>
<td>Bilateral precuneus, bilateral Posterior Cingulate</td>
<td>3 -60 42</td>
<td>7.96</td>
</tr>
<tr>
<td>1267</td>
<td>Bilateral Frontal Medial Cortex</td>
<td>3 45 -15</td>
<td>7.62</td>
</tr>
<tr>
<td>395</td>
<td>Right Superior Occipital</td>
<td>54 -63 -27</td>
<td>6.94</td>
</tr>
<tr>
<td>356</td>
<td>Right Middle Frontal Gyrus</td>
<td>24 33 27</td>
<td>6.62</td>
</tr>
<tr>
<td>231</td>
<td>Left Superior Occipital</td>
<td>-39 -78 33</td>
<td>6.47</td>
</tr>
<tr>
<td>201</td>
<td>Cerebellum</td>
<td>21 -90 -30</td>
<td>6.23</td>
</tr>
<tr>
<td>79</td>
<td>Left Middle Frontal Gyrus</td>
<td>-27.5 6.5 54.5</td>
<td>5.57</td>
</tr>
<tr>
<td>71</td>
<td>Bilateral Paracingulate and Anterior Cingulate</td>
<td>2.5 18.5 51.5</td>
<td>5.34</td>
</tr>
<tr>
<td>55</td>
<td>Left Insula</td>
<td>-54.5 -17.5 -14.5</td>
<td>5.28</td>
</tr>
<tr>
<td>49</td>
<td>Right Insula</td>
<td>35.5 -20.5 -2.5</td>
<td>5.14</td>
</tr>
<tr>
<td>25</td>
<td>Thalamus</td>
<td>20.5 -26.5 9.5</td>
<td>5.95</td>
</tr>
</tbody>
</table>

Clusters of highest functional connectivity with the precuneus seed region in the pediatric-onset MS group (n=16). These clusters were used as a guide for the selection of Regions-of-Interest for further analyses. The r-to-z transformed functional connectivity values and Montreal Neurological Institute (MNI) co-ordinates (x,y,z in mm) at the peak of each cluster are reported. All reported clusters had a p-value <.01.
Functional connectivity correlation matrices for both groups utilizing the specified ROIs (described above) are shown in Figure 16. The functional connections showing higher connectivity in patients relative to controls were that of the right anterior cingulate with the right precuneus (t=2.29, p=.03) and of the left anterior cingulate with the left superior occipital cortex (t=2.22, p=.04), right precuneus (t=2.55, p=.02), left precuneus (t=2.60, p=.01), and cerebellum (t=2.14, p=.04). Lower functional connectivity of the thalamus with the right anterior cingulate (t=-2.56, p=.02) was also observed in the pediatric-onset MS group.

Functional connectivity was not correlated with disease duration, age of disease onset, EDSS scores, depression, fatigue, or socioeconomic status (Barratt SES) for any ROI.
Figure 16. Average r-to-z transformed functional connectivity matrices for the (a) pediatric-onset MS group, (b) healthy control group. (c) t-test results indicating which functional connections were significantly different between groups at p<.05. t-tests indicated that the pediatric-onset MS group had higher functional connectivity of the right anterior cingulate cortex with the right precuneus (t=2.29, p=.03) and of the left anterior cingulate cortex with the left superior occipital cortex (t=2.22, p=.04), right precuneus (t=2.55, p=.02), left precuneus (t=2.60, p=.01), and cerebellum, (t=2.14, p=.04) compared to the healthy control group. Lower functional connectivity of the thalamus with the right anterior cingulate cortex (t=-2.56, p=.02) was also observed in the pediatric-onset MS group.
4.4.3 Independent Components Analysis

Group ICA of all 35 subjects revealed eight canonical resting-state networks (Figure 17) out of the 19 components that were outputted from ICA (n=11 were discarded as noise/artifact related or mis-registration). These networks are a replication of commonly reported resting-state networks, and were labelled accordingly (Smith, Fox, Miller et al., 2009; Damoiseaux et al., 2006; van den Heuvel, Mandl, & Pol, 2008). These networks included the default-mode network (3 components, including precuneus in Figure 17j), primary and secondary visual networks, salience network, frontoparietal network (separate right and left components, and one bilateral component, Figure 17i), sensorimotor network (2 components), and dorsal attention network.

For the ICA analysis, because no clusters survived correction for multiple comparisons at p<0.05 (using permutation testing as part of FSL’s randomise), an uncorrected p-value threshold of 0.005 was selected and only those clusters>30 voxels spatially within or touching the network were considered significant (Forman et al., 1995). Using this new adopted significance threshold, there were two components in which the pediatric-onset MS group demonstrated higher functional connectivity than the healthy control group (Figure 17k). These two components comprised the frontoparietal network bilaterally, and default-mode network. Within the bilateral frontoparietal network, higher functional connectivity was demonstrated at the level of the anterior cingulate cortex and left middle frontal gyrus in the pediatric MS group (statistics reported in Table 8). Within the default-mode network, higher functional connectivity was demonstrated within the anterior cingulate cortex and right precuneus. Healthy controls did not demonstrate higher connectivity relative to the MS patients in any network.
Figure 17. Group ICA components including (a) default mode network, (b) primary visual network, (c) secondary visual network, (d) salience network, (e) right frontoparietal network, (f) left frontoparietal network, (g) sensorimotor network, and (h) dorsal attention network. The ICA components are shown in FSL red-yellow encoding using a 3< z-score <10 threshold. The (i) bilateral frontoparietal and (j) precuneus (posterior default-mode) networks were the only networks which demonstrated significant differences between groups (p<.005 uncorrected, cluster size> 30 voxels). Areas in blue below these networks (k), with numbered clusters 1-4, indicate those areas within these networks in which the pediatric-onset MS group demonstrated higher connectivity compared to healthy controls. C1 indicates the anterior cingulate cluster and C2 the left middle frontal gyrus cluster of the bilateral frontoparietal network. C3 indicates the right precuneus and C4 the anterior cingulate cluster of the precuneus posterior default-mode network. Statistics for the connectivity values of these clusters are referred to throughout the text. All images are displayed in radiological convention. (Left=Right, Right=Left). The most informative slices are shown.
Table 8. Differences between groups in functional connectivity

<table>
<thead>
<tr>
<th>Clusters</th>
<th>Pediatric (n=19)</th>
<th>MS Control (n=16)</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1: Bilateral frontoparietal network, anterior cingulate cortex</td>
<td>3.20 (2.34)</td>
<td>-2.67 (3.08)</td>
<td>-6.40</td>
<td>&lt;.001 **</td>
</tr>
<tr>
<td>C2: Bilateral frontoparietal network, left middle frontal gyrus</td>
<td>11.92 (5.75)</td>
<td>3.43 (4.64)</td>
<td>-4.74</td>
<td>&lt;.001 **</td>
</tr>
<tr>
<td>C3: Precuneus network, right precuneus</td>
<td>9.51 (5.74)</td>
<td>1.49 (4.42)</td>
<td>-4.56</td>
<td>&lt;.001 **</td>
</tr>
<tr>
<td>C4: Precuneus network, anterior cingulate cortex</td>
<td>3.21 (4.66)</td>
<td>-5.77 (4.83)</td>
<td>-5.59</td>
<td>&lt;.001 **</td>
</tr>
</tbody>
</table>

Mean (SD) functional connectivity values within clusters that differed significantly between groups in the voxel-wise dual regression analysis (clusters depicted in Figure 17k).

4.5 Relationship between structural damage and resting-state functional connectivity

4.5.1 Relationship between structural volumes and functional connectivity

Using seed-based correlations with structural volumes (described in Section 3.3.2.1), higher T2 lesion volumes were correlated with lower functional connectivity between the thalamus and right superior occipital region (t=-2.87, p=.012, Table 9). Normalized thalamic volume was positively correlated with functional connectivity between the thalamus and right superior occipital (t=2.27, p=.04), left middle frontal (t=2.81, p=.014), left precuneus (t=2.16, p=.049), and left paracingulate (t=2.26, p=.04) regions. Total brain volume was not significantly correlated with any functional connectivity measure.
Table 9. Structural MRI correlations with functional connectivity in the pediatric MS group

<table>
<thead>
<tr>
<th>Structural Measure</th>
<th>FC- Region 1</th>
<th>FC- Region 2</th>
<th>T-statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T2 Lesion Volume</strong></td>
<td>Thalamus</td>
<td>R Sup Occ</td>
<td>-2.87</td>
<td>.012</td>
</tr>
<tr>
<td>Normalized thalamic volume*</td>
<td>Thalamus</td>
<td>R Sup Occ</td>
<td>2.27</td>
<td>.040</td>
</tr>
<tr>
<td></td>
<td>Thalamus</td>
<td>L Mid Fron</td>
<td>2.81</td>
<td>.014</td>
</tr>
<tr>
<td></td>
<td>Thalamus</td>
<td>L Precuneus</td>
<td>2.16</td>
<td>.049</td>
</tr>
<tr>
<td></td>
<td>Thalamus</td>
<td>L Paracingulate</td>
<td>2.26</td>
<td>.040</td>
</tr>
</tbody>
</table>

FC= functional connectivity; R SupOcc= right superior occipital cortex; LMidFron= left middle frontal gyrus; a- Normalized thalamic volume= absolute thalamic volume/absolute brain volume

4.5.2 Relationship between DTI measures and functional connectivity

Using ICA correlations (described in Section 3.3.2.2) higher functional connectivity of the right precuneus within the default-mode network (cluster 3, Figure 17k) was associated with lower FA and higher RD in the pediatric-onset MS group (all correlations reported in Table 10). With respect to FA, functional connectivity of the precuneus within the default-mode network was significantly correlated with whole white matter skeleton FA (r=-.525, p=.021), and FA of the following structures: (a) genu of corpus callosum (FA: r=-.553, p=.014; RD: r=.679, p=.001), (b) left sagittal striatum (FA: r=-.467, p=.044; RD: r=.588, p=.008), and (c) right sagittal striatum (FA: r=-.615, p=.005; RD: r=.658, p=.002). Widespread significant associations with RD were also found (all reported in Table 10). No significant correlations between functional connectivity and DTI measures were present in the healthy control group.
Table 10. Correlation between DTI measures and functional connectivity in the pediatric MS group

<table>
<thead>
<tr>
<th></th>
<th>C1- FrontoPar, ACC</th>
<th>C2- FrontoPar, LMidFron</th>
<th>C3- Default-mode, RPrecuneus</th>
<th>C4- Default-mode, ACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional Anisotropy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire White Matter Skeleton</td>
<td>-.329 (.169)</td>
<td>-.153 (.532)</td>
<td>-.525 (.021)*</td>
<td>.042 (.865)</td>
</tr>
<tr>
<td>Within Significant FA voxels a</td>
<td>-.181 (.459)</td>
<td>.022 (.929)</td>
<td>-.351 (.140)</td>
<td>.035 (.886)</td>
</tr>
<tr>
<td>Corpus Callosum- Body b</td>
<td>-.085 (.729)</td>
<td>.104 (.672)</td>
<td>-.252 (.298)</td>
<td>.009 (.972)</td>
</tr>
<tr>
<td>Corpus Callosum- Genu b</td>
<td>-.296 (.218)</td>
<td>-.083 (.735)</td>
<td>-.553 (.014)*</td>
<td>.028 (.909)</td>
</tr>
<tr>
<td>Corpus Callosum- Splenium b</td>
<td>-.288 (.232)</td>
<td>.105 (.669)</td>
<td>-.409 (.082)</td>
<td>.021 (.932)</td>
</tr>
<tr>
<td>Posterior Thalamic Radiation- Left b</td>
<td>-.120 (.623)</td>
<td>.088 (.720)</td>
<td>-.094 (.703)</td>
<td>.137 (.575)</td>
</tr>
<tr>
<td>Posterior Thalamic Radiation- Right b</td>
<td>-.004 (.986)</td>
<td>.078 (.751)</td>
<td>-.147 (.548)</td>
<td>.022 (.930)</td>
</tr>
<tr>
<td>Corona Radiata- Left Superior b</td>
<td>.033 (.895)</td>
<td>-.054 (.827)</td>
<td>-.136 (.580)</td>
<td>-.022 (.930)</td>
</tr>
<tr>
<td>Corona Radiata- Right Superior b</td>
<td>-.172 (.481)</td>
<td>.103 (.675)</td>
<td>-.152 (.534)</td>
<td>.176 (.472)</td>
</tr>
<tr>
<td>Corona Radiata- Left Posterior b</td>
<td>-.088 (.721)</td>
<td>.046 (.850)</td>
<td>-.429 (.067)</td>
<td>.093 (.706)</td>
</tr>
<tr>
<td>Corona Radiata- Right Posterior b</td>
<td>-.112 (.647)</td>
<td>.170 (.486)</td>
<td>-.348 (.144)</td>
<td>-.054 (.826)</td>
</tr>
<tr>
<td>Sagittal Stratum- Left b</td>
<td>-.111 (.651)</td>
<td>-.078 (.751)</td>
<td>-.467 (.044)*</td>
<td>.061 (.804)</td>
</tr>
<tr>
<td>Sagittal Stratum- Right b</td>
<td>-.150 (.535)</td>
<td>.045 (.855)</td>
<td>-.615 (.005)**</td>
<td>.014 (.955)</td>
</tr>
</tbody>
</table>

Radial Diffusivity ($10^{-3}$mm$^2$/sec)

<table>
<thead>
<tr>
<th></th>
<th>C1- FrontoPar, ACC</th>
<th>C2- FrontoPar, LMidFron</th>
<th>C3- Default-mode, RPrecuneus</th>
<th>C4- Default-mode, ACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire White Matter Skeleton</td>
<td>.361 (.129)</td>
<td>.014 (.956)</td>
<td>.661 (.002)**</td>
<td>-.040 (.872)</td>
</tr>
<tr>
<td>Within Significant FA voxels a</td>
<td>.256 (.289)</td>
<td>-.130 (.597)</td>
<td>.486 (.035)*</td>
<td>-.005 (.983)</td>
</tr>
<tr>
<td>Corpus Callosum- Body b</td>
<td>.176 (.472)</td>
<td>-.145 (.553)</td>
<td>.350 (.141)</td>
<td>.092 (.626)</td>
</tr>
<tr>
<td>Corpus Callosum- Genu b</td>
<td>.318 (.185)</td>
<td>.105 (.669)</td>
<td>.679 (.001)**</td>
<td>-.043 (.862)</td>
</tr>
<tr>
<td>Corpus Callosum- Splenium b</td>
<td>.314 (.191)</td>
<td>-.176 (.472)</td>
<td>.501 (.029)*</td>
<td>-.008 (.973)</td>
</tr>
<tr>
<td>Posterior Thalamic Radiation- Left b</td>
<td>.140 (.568)</td>
<td>-.170 (.486)</td>
<td>.217 (.372)</td>
<td>-.109 (.656)</td>
</tr>
<tr>
<td>Posterior Thalamic Radiation- Right b</td>
<td>.084 (.733)</td>
<td>-.201 (.408)</td>
<td>.341 (.153)</td>
<td>.034 (.891)</td>
</tr>
<tr>
<td>Corona Radiata- Left Superior b</td>
<td>.201 (.408)</td>
<td>.037 (.880)</td>
<td>.399 (.091)</td>
<td>.102 (.677)</td>
</tr>
<tr>
<td>Corona Radiata- Right Superior b</td>
<td>.385 (.104)</td>
<td>-.207 (.394)</td>
<td>.273 (.259)</td>
<td>-.132 (.591)</td>
</tr>
<tr>
<td>Corona Radiata- Left Posterior b</td>
<td>.245 (.313)</td>
<td>-.141 (.566)</td>
<td>.582 (.009)**</td>
<td>-.052 (.831)</td>
</tr>
<tr>
<td>Corona Radiata- Right Posterior b</td>
<td>.227 (.351)</td>
<td>-.176 (.471)</td>
<td>.601 (.007)**</td>
<td>.063 (.798)</td>
</tr>
<tr>
<td>Sagittal Stratum- Left b</td>
<td>.204 (.402)</td>
<td>-.032 (.896)</td>
<td>.588 (.008)**</td>
<td>-.028 (.911)</td>
</tr>
<tr>
<td>Sagittal Stratum- Right b</td>
<td>.147 (.548)</td>
<td>-.086 (.727)</td>
<td>.658 (.002)**</td>
<td>.013 (.957)</td>
</tr>
</tbody>
</table>

Functional connectivity measures correspond to those clusters depicted in Figure 17k that differed between groups and include C1 (anterior cingulate cortex) and C2 (left middle frontal gyrus) of the bilateral frontoparietal network, C3 (right precuneus) and C4 (anterior cingulate cortex) of the precuneus default-mode network. Pearson correlation coefficients (r-values) are reported with their corresponding p-values; a- mean values reported are based only on those voxels that demonstrated significantly different FA values between groups in t-test analysis (shown in Figure 14); b- mean values reported are based only on those voxels that demonstrated significantly different FA values between groups in t-test analysis, restricted to certain white matter structures as defined by FSL’s JHU white-matter tractography atlas (Mori et al., 2008)
4.6 Relationship between cognition and functional activation patterns

4.6.1 Relationship between neuropsychological test performance and resting-state functional connectivity

The composite z-score (described in Sections 3.1, 4.2.1) was used to examine the relationship between cognition and functional connectivity as not enough patients met criteria for cognitive impairment. Using seed-based correlations with cognitive performance (described in Section 3.3.2.1), lower cognitive performance (lower composite z-score) was associated with higher functional connectivity of the left frontal medial cortex and the following regions: right precuneus ($t=-4.04, p=.0012$), right anterior cingulate ($t=-5.73, p<.001$), and left anterior cingulate ($t=-4.10, p=.001$) in the pediatric MS group. No significant correlations between cognitive performance and functional connectivity were present in the healthy control group.

4.6.2 fMRI-SDMT activation patterns and differences between groups

Both groups demonstrated widespread bilateral activation of the occipital cortex, precentral gyrus, and thalamus during performance of the fMRI-SDMT (Figure 18a, b). The healthy control group showed greater BOLD activation of the right middle frontal gyrus compared to the pediatric-onset MS group (Figure 18c).
Figure 18. Mean fMRI-SDMT activation maps of (a) pediatric-onset MS group, and (b) healthy control group. In the pediatric-onset MS group, the highest peaks of activation were located in the left occipital pole [z=6.82 at cluster peak, MNI coordinates (x,y,z in mm)= -26,-96,-14, p<.001], left precentral gyrus (z=4.59 at cluster peak, MNI coordinates=28,2.44, p<.001), and thalamus (z=3.39 at cluster peak, MNI coordinates=20,2.8, p=.027). In the healthy control group, the highest peak area of activation was in the left occipital pole (z=6.51 at cluster peak, MNI coordinates= -20,-96,-12, p<.001). (c) t-test results indicating the voxels in which the pediatric-onset MS group demonstrated significantly lower activation compared to the healthy control group thresholded at z>2.3 and family-wise error corrected cluster significance of p=.05. This was the right middle frontal gyrus (z=3.21 at cluster peak, MNI coordinates=42,30,28, p=.0026). Sagittal, axial, and coronal slice location indicated by crosshairs. All images are displayed in radiological convention (subject’s right side shown on the left).
4.6.3 fMRI-SDMT activation correlations with behavioral performance

Within the MS group, faster response time correlated with greater BOLD activation of the bilateral occipital, anterior cingulate, right superior parietal and thalamus regions (Figure 19, clusters of significant correlation reported in Table 11). No significant correlations between regional activation and response time were detected in the healthy control group. Coefficient of variation of response times and BOLD activation were also not correlated in either group.

Figure 19. Regions in which BOLD activation significantly negatively correlated with fMRI-SDMT mean response time in the pediatric MS group. Images are thresholded at z>2.3 and family-wise error corrected cluster significance of p=.05. Statistics are reported in Table 11. All images are displayed in radiological convention.
Table 11. Regions in which BOLD activation was significantly negatively correlated with response time in the pediatric MS group

<table>
<thead>
<tr>
<th>Cluster size</th>
<th>Brain Region (local maxima)</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>z-score at peak</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1951</td>
<td>Right Inferior Occipital</td>
<td>38</td>
<td>-86</td>
<td>-14</td>
<td>-3.81</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>901</td>
<td>Anterior Cingulate</td>
<td>-4</td>
<td>6</td>
<td>32</td>
<td>-3.69</td>
<td>.0003</td>
</tr>
<tr>
<td>862</td>
<td>Right Superior Parietal</td>
<td>12</td>
<td>-56</td>
<td>74</td>
<td>-3.54</td>
<td>.0005</td>
</tr>
<tr>
<td>487</td>
<td>Thalamus</td>
<td>16</td>
<td>16</td>
<td>8</td>
<td>-3.33</td>
<td>.0234</td>
</tr>
<tr>
<td>459</td>
<td>Left Superior Occipital</td>
<td>-10</td>
<td>-86</td>
<td>46</td>
<td>-4.09</td>
<td>.0321</td>
</tr>
</tbody>
</table>

MNI co-ordinates (in mm) are reported for the peak of each cluster.
Chapter 5:
Discussion and Future Directions
5.1 Heightened resting-state fMRI functional connectivity in pediatric MS

The main finding related to aim 1 (results Section 4.4) was that pediatric MS patients show heightened long-range resting-state functional connectivity of the precuneus, the major hub of the default-mode network, with anterior regions (anterior cingulate, frontal medial, left frontal), as well as the cerebellum, using a seed-based approach. These observations are in line with my a priori hypothesis that heightened, and less spatially constrained, functional connectivity of the default-mode network would be present in pediatric MS patients compared to healthy controls. Using the ICA approach, higher functional connectivity was also demonstrated within the frontoparietal network. As demonstrated in the adult RRMS literature (described in Section 1.4.3.1), greater functional connectivity is associated with intact cognitive performance. Intact cognitive performance was observed in our pediatric MS group. Therefore, I speculate that preserved cognitive performance is mediated by compensatory mechanisms, herein evidenced by heightened resting-state connectivity.

Only two studies have evaluated resting state functional connectivity in pediatric MS (Rocca, Absinta, Amato et al., 2014; Rocca, Valsasina, Absinta et al., 2014). Contrary to our findings, results from these studies show that pediatric MS patients have decreased functional connectivity as compared to healthy controls. However, these differences may be accounted for by the large proportion of patients in their study with cognitive impairment (45%). On the contrary, our seed-based ROI findings specifically included cognitively intact pediatric MS patients allowing us to evaluate the potential for early compensatory mechanisms. It is possible that functional disruptions of the default-mode network arise with increasing disease-related pathology and the consequent manifestation of cognitive impairment. Of interest, Rocca and colleagues (Rocca, Absinta, Amato et al., 2014) showed that functional connectivity was higher in cognitively preserved versus impaired patients, particularly in the anterior cingulate, as found in our sample.
The hubs of the default-mode network in the pediatric MS group, as indicated by areas of high correlation in Figure 15, are similar to those reported in adults, suggesting that pediatric-onset MS did not disrupt normal development of the default-mode network. However, groups differed with respect to the level of constraint of connectivity of these hubs, as well as their connectivity with regions that are likely parts of different networks. With respect to the first point, the amount of functional connectivity immediately surrounding the precuneus and frontal medial cortex in Figure 15 appears larger. Further supporting this using the ICA approach, greater functional connectivity was also observed immediately surrounding the hubs of the default mode network (i.e. the precuneus) and frontoparietal (i.e. left middle frontal gyrus) networks. This less constrained activation surrounding the hubs of these networks could be due to a less direct and constrained flow of electrical impulses to/from these hubs based on damage to interconnecting white matter pathways.

The higher connectivity between the precuneus and regions that are typically not thought of as being major hubs of the default-mode network, or primarily part of different networks (i.e. anterior cingulate and cerebellum), suggests a greater inter-network relationship in pediatric MS patients compared to controls. Of note, all subjects completed 30 minutes of cognitive fMRI tasks prior to the resting-state acquisition. These tasks included the fMRI-SDMT analysed as part of this thesis, in addition to two tasks probing working memory and response inhibition. It is possible that the pediatric MS group had greater difficulty “turning off” activation of previously engaged regions/networks. Higher functional connectivity was also observed within the fronto-parietal network during resting state, typically considered a “task-positive” network. It has been shown that increased functional coupling between areas previously engaged during an fMRI cognitive task is present during an immediately following resting state scan (Stevens, Buckner, & Schacter, 2010; Omer & Grady, 2010). A recent study has also shown that mental exhaustion induces resting-state enhancement of the anterior cingulate (Esposito et al., 2014) in healthy adults. The anterior cingulate is also considered one of the major hubs of the salience network which includes the fronto-
insular circuit (Seeley al., 2007). The salience network possesses robust connections to subcortical and limbic structures and is related to emotional and attentional control of sensory information via integration with the autonomic nervous system. It guides the activation of the executive control networks in order to direct the individual to cognitive processing of relevant stimuli, and is largely responsible for switching between the default-mode and executive control/ task-positive networks. The increased connectivity between the default-mode and anterior cingulate/ salience network could thus be indicative of patient group’s greater deployment of neuronal resources and greater alertness/ attentiveness immediately following the processing of cognitively demanding stimuli in the fMRI tasks completed prior to resting state.

We also observed higher functional connectivity between the precuneus and cerebellum. There have been studies that have reported lower functional connectivity of the cerebellum in both pediatric (Rocca, Valsasina, Absinta et al., 2014) and adult (Dogonowski et al., 2013) MS patients. These studies however included a larger proportion of patients with physical and/or cognitive disability. The study by Loitfelder and colleagues (2012) reported that higher connectivity between the anterior cingulate and cerebellum was associated with better cognitive performance on the Paced Auditory Serial Addition Test (PASAT), a test of working memory. These same two regions also showed heightened functional connectivity in our sample of cognitively preserved patients. These combined results suggest that heightened connectivity with the cerebellum could then be a mechanism for preservation of cognitive abilities.

Another finding of interest was lower functional connectivity between the thalamus and anterior cingulate cortex. Given the proclivity of the thalamus to structural damage in pediatric MS, this suggests that there is disruption of thalamo-cortical networks in pediatric MS. This will be discussed in more detail in the following section.
5.2 Structure-function relationship in pediatric MS is dependent on measure of structural brain pathology used

Volumetric analyses and DTI evaluation of tissue integrity revealed key differences between pediatric MS patients and healthy controls. As expected thalamic volumes in our pediatric MS patients were lower relative to healthy controls. Thalamic volume was significantly negatively correlated with lesion volume. This supports the idea that reduced thalamic volume could be due to Wallerian degeneration as a result of white matter tracts transected by lesions. DTI results confirm a widespread loss of white matter integrity in our pediatric-onset MS patients, consistent with findings reported in other pediatric MS studies (Absinta et al., 2010; Blaschek et al., 2013; Vishwas et al., 2010, 2013; Tillema et al., 2012; Rocca, Absinta, Amato et al., 2014; Rocca, Valsasina, Absinta, et al., 2014; Bethune et al., 2011). The corpus callosum, posterior thalamic radiations, sagittal striatum, and corona radiata were the white matter regions most impacted. These structures may be more vulnerable in pediatric MS given that they are in close proximity to regions with high lesional proclivity (e.g. periventricular areas). The corpus callosum is the major right to left white matter trajectory and has been shown to be particularly impacted by MS (Blaschek et al. 2013; Vishwas et al., 2013; Tillema et al., 2012; Rocca, Absinta, Amato et al, 2014). We also found that DTI measures, including lower FA of the corpus callosum, were correlated with longer disease duration as well as greater $T_2$ and $T_1$ lesion volume, implicating increasing loss of tissue integrity with increasing disease activity.

Our MS patients demonstrated lower functional connectivity between the thalamus and anterior cingulate cortex (Results described in Section 4.4.2). Furthermore, lower thalamo-cortical functional connectivity in patients was associated with greater brain pathology, as measured by reduced thalamic volume and higher $T_2$ lesion volume. A greater number of significant correlations were present between functional connectivity and thalamic volume than between functional connectivity and lesion volume highlighting the thalamus as a critical structure involved in network connectivity. Patients with lower thalamic volume showed reduced functional connectivity between the
thalamus and right superior occipital region as well as with the left middle frontal, left precuneus and left paracingulate regions. This result suggests that with reductions in thalamic volume, there is an overall disruption or breakdown of thalamo-cortical functional networks.

Within our pediatric MS group, T₂ lesion volume correlated with reduced functional connectivity between the thalamus and right superior occipital region, while in the study of Rocca and colleagues (Rocca, Valsasina, Absinta, et al., 2014), T₂ lesion volume correlated with reduced connectivity of the right medial frontal gyrus of the attention network. In both studies, the very limited relationship between T₂ lesion volume and different functional connectivity relationships suggests that T₂ lesion volume is not markedly influencing on functional networks broadly.

Using DTI as a measure of brain tissue integrity, we evaluated whether reduced integrity as measured by whole brain or regional FA was associated with difference in functional connectivity. My original hypothesis was that loss of tissue integrity would associate with reduced connectivity. In contrast we observed that reduced white matter microstructural integrity was associated with higher functional connectivity of the precuneus of the default-mode network. In a task-based fMRI study in pediatric MS (Rocca et al., 2010), lower FA in the normal appearing white matter of the corpus callosum was associated with increases (rather than decreases) in connectivity between the right cerebellum and the left primary sensorimotor cortex during the performance of a simple motor task. In adult MS patients, DTI evidence of reduced tissue integrity has also been associated with heightened connectivity of the default-mode network during resting-state (Zhou et al., 2014; Hawellek et al., 2011). Conceptually, loss of tissue microstructure is unlikely to lead to improved connectivity of neural networks, but it is possible that loss of tissue integrity leads to loss of inhibition of specific neural networks which in turn leads to increased connectivity. It is also possible that in the context of reduced tissue microstructure, connectivity is preferentially focused to fewer networks leading to increased connectivity within these preferred pathways. Therefore, with reduced DTI white matter microstructural integrity increased functional connectivity
could then be present in the more prominent resting-state networks i.e. the default-mode and frontoparietal networks.

Overall, both support and non-support for my a priori hypothesis (i.e. greater structural damage would be associated with reduced functional connectivity) was demonstrated. Support was given by the finding that lower thalamic volume was associated with lower functional connectivity of the thalamus. Lack of support was given by the finding that lower white matter microstructural integrity was associated with heightened functional connectivity of the default-mode network. This suggests that the interplay between structural damage and functional connectivity is complex and influenced by the specificity of the network being interrogated, as well as the metric of structural damage that is used, each metric of which visualizes different underlying aspects of brain pathology.

5.3 fMRI activation patterns vary according to cognitive performance

The regional activation pattern in pediatric MS patients during performance of the fMRI-SDMT, a task that interrogates processing speed, was examined. Both pediatric MS patients and healthy youth demonstrated the expected bilateral activation of the motor and visual cortices. Activation of the right middle frontal gyrus, however, was significantly lower in our pediatric-onset MS patients. This is contrary to my a priori hypothesis of greater frontal recruitment in the pediatric MS group. Our findings have some similarity to that reported by Genova and colleagues (2009), who found more negative activation of bilateral frontal and parietal regions, including the right middle frontal gyrus, during fMRI-SDMT performance in 16 adult MS patients compared to healthy controls. Lower activation correlated with T2 lesion load, a finding that we did not replicate in our pediatric MS patients. The failure to replicate the contribution of lesion volume to activation on the SDMT cannot be ascribed to a lower T2 lesion volume in our pediatric MS patients. The average T2 lesion volume in our patients was 14.1cm³ (reported in Table 4) compared to the average T2 lesion volume in the adult MS patients
study of Genova and colleagues (2009) of 7.8 cm$^3$. A high lesion volume in pediatric MS patients relative to adults has been previously reported (Ghassemi et al., 2014, described in Section 1.3.8). Lesion tissue remains relatively better preserved in pediatric MS patients compared to adults as shown by magnetization-transfer ratio studies (Brown et al. 2014) which may explain why lesions are less contributory to network connectivity early in pediatric MS.

The lower activation of frontal regions in our pediatric MS patients relative to controls may relate to MS impact on brain regions that are undergoing active maturation. Studies conducted in healthy adolescents have shown greater recruitment of frontal regions with increasing age (Rubia et al., 2000), indicating a role for these pathways at this epoch of the age. Pediatric MS may be less able to recruit these pathways if MS affects their primary formation as the disease affects the development of crucial white matter pathways likely responsible for this age-related increase in frontal activation. We were able to detect activation patterns in pediatric MS even in a group that performed the task well. Future selection of cognitive tasks that utilize frontal networks, and are even more intellectually challenging, may reveal even greater differences in fMRI patterns between pediatric MS patients and controls.

Despite equivalent overall fMRI-SDMT performance between groups, faster performance was associated with greater activation of a number of regions including the bilateral occipital cortices, anterior cingulate, right superior parietal cortex and thalamus in the pediatric MS group. This is opposite to my a priori hypothesis that greater frontal activation would be associated with worse performance. Instead, our results suggest that greater processing efficiency requires heightened activation. This is similar to what has been observed in healthy adults completing a high-speed relative to low speed attention task (Lazeron, et al., 2003). Using the same fMRI-SDMT paradigm as we used, Genova and colleagues (2009) observed that activation of the thalamus and anterior cingulate was correlated with reaction time on the fMRI-SDMT in adult MS, a relationship that we confirmed in our pediatric patients. These regions are also considered part of the salience network (Seeley et al., 2007), which is thought to sub-
serve, among other activities, the ability to process cognitive error processing and attention. The salience network may thus be an important contributor to performance on the SDMT. Genova and colleagues (2009) also found a greater number of areas (including the anterior cingulate and thalamus) correlating with processing speed performance in healthy controls compared to MS patients. On the contrary, we found no relationship between processing speed and regional activation in any of our healthy controls. This suggests that the regions that may mediate processing speed, or at least performance on the SDMT, may differ in pediatric MS patients compared to healthy controls.

In addition to evaluating a task-based paradigm, we also evaluated whether resting-state connectivity differed as a function of global cognitive performance using seed-based correlations (Section 4.6.1). Within our MS group, higher functional connectivity of the frontal medial cortex was associated with lower composite cognitive scores, although the overall cognitive scores were not in the impaired range. This is in support of my a priori hypothesis that heightened frontal functional connectivity would be associated with worse neuropsychological performance, supporting the concept of reduced neural efficiency (described in Section 1.5.1) in pediatric MS.

The pattern observed in this thesis of higher functional connectivity being associated with worse cognitive performance has also been observed in some studies in adult-onset MS. For example, Faivre and colleagues (2012) demonstrated that higher resting-state connectivity within the posterior default-mode network was associated with decreased performance on the word list generation semantic fluency task. In addition, higher frontoparietal network connectivity was associated with lower working memory performance as measured using Paced Auditory Serial Addition Test (PASAT). The study by Hawellek and colleagues (2011) found that a loss of cognitive efficiency (as determined by factor analysis of performance across a series of neuropsychological tests) was associated with a gain in functional connectivity among core parts of the default-mode network and executive control network. Resting-state fMRI studies in solely cognitively preserved adult MS patients have not been conducted, and so we
cannot directly compare our results to those from adult-onset MS. A more relevant comparison will be serial analyses on initially intact pediatric MS patients who demonstrate accrual of cognitive deficits over time to see at what time-point increased connectivity is lost.

5.4 General discussion

The findings from my thesis can be summarized below.

**Resting-state functional connectivity:**

1. Cognitively intact pediatric MS patients differ from healthy controls in resting-state functional connectivity of the default-mode network, also in thalamo-cortical, and frontal medial cortex functional connectivity.

2. Within the MS group, default-mode network connectivity correlates with DTI measures of white matter tissue microstructure, thalamo-cortical connectivity is influenced by thalamic volume, and frontal medial cortex connectivity correlates with cognitive performance.

Figure 20 conceptualizes the directionality of these relationships. In the pre-clinical phase of MS, cognition is intact, little or no structural damage has been incurred, and functional connectivity of the default-mode network, thalamus and frontal medial cortex are normal. As white matter tissue microstructure is compromised in “normal-appearing” brain tissue (evidenced by abnormal DTI), and prior to visible lesion formation or volume loss, an early compensatory activation of default-mode network connectivity occurs while connectivity of the thalamus and frontal medial cortex remains normal. As white matter DTI abnormalities increase, lesions accumulate, and thalamic volume declines, the functional connectivity of the thalamus declines and compensatory default-mode network activation remains high. In addition, the frontal medial cortex has yet to be impacted in the face of still normal cognition. As cognitive performance declines, activation of the frontal medial cortex engages in the same compensatory increase in connectivity as has been evidenced earlier by the default-mode network, while
accumulating lesion burden and progressive loss of thalamic volume lead to further reductions of thalamo-cortical connectivity. Finally, in cognitive impaired patients I hypothesize that the connectivity of the default-mode network, as well as thalamo-cortical, and frontal medial cortex connectivity will all be reduced and that these reductions will be particularly notable in patients with the greatest extent of thalamic volume loss, highest lesion burden, and most extensive loss of white matter tissue micro-structure.
Figure 20. Grid conceptualization of resting-state fMRI characteristics of pediatric-onset MS
Task-based activation patterns:

1. Despite normal performance on the fMRI-SDMT, pediatric MS patients demonstrated lower activation of the right middle frontal gyrus compared to healthy controls.

2. Within the MS group, faster performance was associated with greater activation of the bilateral occipital cortices, anterior cingulate, right superior parietal cortex, and thalamus.

3. Higher activation of the anterior cingulate (implied based on heightened functional connectivity with the default-mode network) was also demonstrated in pediatric MS patients during resting state fMRI.

5.4.1 Relevance of anterior cingulate activation in pediatric MS

Related to point 3 above, the findings from my thesis suggest that the anterior cingulate plays an important role in pediatric MS. With respect to the resting-state findings, heightened functional connectivity of the anterior cingulate may be due to two factors. Firstly, the anterior cingulate may be required as a mediator region for relaying signals from the different nodes of the default-mode network. According to this explanation, in order for the default-mode network as a whole to remain intact, activation of the anterior cingulate may be necessary. Secondly, the anterior cingulate could be active while at rest because of additional cognitive processes active in the pediatric MS group. With respect to the second explanation, the related functions of the anterior cingulate are many and include motor control, attentional/cognitive control, conflict monitoring, social perception and theory of mind, and emotional regulation (Kelly et al., 2009; Patel et al., 2014; Kerns et al., 2004). It has been suggested that the anterior cingulate is one of the most non-specific regions of the brain that shows activation in a multitude of cognitive tasks (Poldrack, 2012). It is a region with high level of “forward inference” meaning that given a particular cognitive process, significant activation of voxels in the anterior cingulate will likely be present (Henson, 2006). The anterior cingulate, however, has low “reverse inference” meaning that activation of a voxel in this area does not easily predict
which cognitive process is being performed (Poldrack, 2006). I think that the non-specificity and heterogeneity of function of the anterior cingulate lends itself well to the fact that this area was more activated in the MS group. As such, if greater activation in the pediatric MS group was present overall it is likely that it would present in this area. However, if we investigate exactly where in the anterior cingulate higher activation occurred we might be able to understand the cognitive processes occurring in pediatric MS that might be associated with this heightened activation. If we first look at anterior cingulate regions of increased functional connectivity in Figure 15, and those showing higher activation in faster performers of the fMRI-SDMT in Figure 19, we can infer which cognitive processes are occurring based on studies describing the functional subdivisions of the anterior cingulate. Both Kelly and colleagues (2009) and Margulies and colleagues (2007) have used the functional subdivisions indicated in Figure 21. The areas of greatest overlap between this figure and Figures 15 and 19 of my thesis suggest that the pediatric MS group is more engaged in cognitive control and conflict monitoring both during rest and while performing the fMRI-SDMT. Cognitive control is an attentional process involved in the management of cognitive processes in order to accomplish goals, and is associated with working memory, response selection, and inhibition (Botvinick et al., 2001). Conflict monitoring involves more evaluative functions such as recognition of conflict or interference, error processing, reasoning and decision making (Botvinick et al., 2001). Considering the results from the fMRI-SDMT (i.e. greater anterior cingulate activation being associated with faster response time), greater deployment of attentional resources (i.e. greater exertion of greater cognitive control and conflict monitoring) is suggested to be present in patients who performed better/faster on the fMRI-SDMT. Considering the results from resting-state (greater anterior cingulate functional connectivity), greater deployment of attentional resources in the pediatric MS group as a whole is also suggested to be present while at “rest”. The pediatric MS group may have been continuing to focus on monitoring their performance, following instructions, or thinking about errors that they may have made on the previous fMRI tasks, alluding to more difficulty actually getting into “resting-state” and abnormal patterns of resting-state functional connectivity.
5.5 Future directions

5.5.1 Generalizability of results

Pediatric MS is a rare disease. As such, my PhD cohort was small with only 23 patients. While the ability to interrogate neural networks in a high-functioning pediatric MS cohort was achieved, the generalizability of the findings to patients with a broader range of cognitive performance will require further work. As shown in Figure 20, I propose a model wherein accumulation of MS mediated pathological insult to the CNS results in both alterations in neural network connectivity and cognitive performance. To explore this hypothesis, a collaborative multi centre model is required, with inclusion of
pediatric-onset MS patients of variable cognitive functioning. It would also be of interest to perform longitudinal studies to illustrate the relationships between accrual of tissue damage and changes in cognitive performance over time. The cross-sectional relationship between breakdown of resting-state networks and accompanying cognitive impairment has been demonstrated in adult patients with progressive MS (Rocca, Valsasina, Absinta et al., 2010). Whether the relationships in pediatric-onset MS patients will mirror the relationships in adult-onset MS patients must account for maturational changes in neuronal development and as such may yield different findings from those reported in adults.

5.5.2 Clinical applicability of findings

Resting-state fMRI can be readily acquired during clinical MRI studies with data acquired in as little as 5 minutes, and without experimental task set-up. However, the role of fMRI in clinical practice is far from determined. For example, is higher or lower activation relative to normal activation beneficial or detrimental? Increased activation can be interpreted as compensatory, or inefficient. Reduced connectivity in the context of disease could be interpreted as failure to activate required networks. Alternatively, lower activation patterns could be reflective of higher efficiency as per the “neural efficiency hypothesis” (described in Section 1.5.1). My thesis showed that higher functional connectivity of the frontal medial cortex is associated with reduced cognitive performance in a group of patients who are overall cognitively intact. As depicted in Figure 20, it is hypothesized that later cognitive impairment will be associated with a breakdown of functional connectivity of the frontal medial cortex, particularly with other regions in the default-mode network. With respect to clinical application, heightened resting-state connectivity of the frontal medial cortex could potentially help identify those patients potentially at risk for future cognitive decline. Longitudinal studies following the functional connectivity profile of patients who are initially cognitively intact then develop cognitive impairment would be very informative in this regard and are required in order to fully evaluate the clinical potential of resting-state fMRI in pediatric MS.
fMRI clearly illustrates that activation of neural networks is a dynamic process in health and disease. As such, use of fMRI as a metric to evaluate therapeutic attempts for cognitive rehabilitation is exciting. A new area of therapeutic research involves real-time fMRI for neurofeedback-based activation of specific neural networks. Specifically, patients are shown their own brain activation pattern during a specific task, and are taught how to modulate this activation pattern, in effect utilizing patient driven neuroplasticity. For example, up-regulation of activation of the dorsolateral prefrontal cortex after two training sessions was associated with improved verbal working memory performance in healthy young adults (Zhang et al., 2013). Based on my findings, I would suggest that modulation of activity of the frontal medial cortex would be a strategy to improve cognitive performance in early pediatric MS. Considerations for neuro-modulation therapy will include sustainability, must translate into patient-centered meaningful improvements in cognitive tasks, and will yield interesting research into whether functional activation ultimately lead to structural change. Preliminary support for such possibilities comes from research in which memory-related enhancement of clinical function is associated with increased hippocampal volume (Fotuhi, Do, & Jack 2012).

5.5.3 Mental processes occurring during resting-state

Resting-state is purported to measure brain activation while the brain is cognitively “at rest”. Therefore, one of the challenges of resting-state fMRI is to try to achieve a uniform capacity for participants to enter this state of cognitive rest. In our own data, we questioned whether recent task-based activities rendered our MS patients less capable of moving from a pattern of task-related activation to rest. Across individuals, what defines a resting state might differ. Some individuals may engage in complex thought or planning while others may become drowsy. This individual variability is likely to influence regional brain activation patterns to a greater or lesser extent. Some individuals find MRI challenging in terms a sense of claustrophobia, some participants may become more readily bored, and others may find the experience relaxing. Future studies may wish to develop tools to more objectively measure the mental processes
occurring during resting state and how this might actually correlate with observed functional connectivity patterns. The work of Diaz and colleagues (2013) has begun to shed light on this issue by their development of the 50-item self-report survey the Amsterdam Resting-State Questionnaire (ARSQ). For their study, the ARSQ was administered immediately following 5-minutes of eyes-closed rest. The rest period occurred either at home (based on a computerized instruction to relax for 5 minutes, n=813 participants), during resting-state EEG (n=68) or resting-state fMRI (n=89). With respect to fMRI, participants completed the ARSQ questionnaire via button response while still in the scanner. Factor analysis revealed seven dimensions of resting-state cognition including the following, with example items given for each (adapted from Diaz et al., 2013):

(a) Discontinuity of Mind: “I felt restless”, “I had difficulty holding onto my thoughts”, “I had busy thoughts”

(b) Theory of Mind: “I thought about other people”, “I placed myself in other people’s shoes”

(c) Self: “I thought about myself”, “I thought about my feelings”, “I thought about my behaviour”

(d) Planning: “I thought about things I need to do”, “I thought about the past”, “I thought about the future”

(e) Sleepiness: “I felt tired”, “I felt sleepy”, “I had difficulty staying awake”

(f) Comfort: “I felt comfortable”, “I felt relaxed”, “I felt happy”

(g) Somatic Awareness: “I was conscious of my body”, “I thought about my heartbeat”, “I thought about my breathing”

Participants were also given a series of questionnaires to evaluate general mental well-being. It was found that lower mental health was associated with lower reported comfort and higher discontinuity of mind. With respect to the resting-state EEG data acquired, sleepiness was associated with different patterns of neural activity. No correlations were performed between resting-state fMRI measures, with the authors of this study (Diaz et al., 2012) noting this would be provided in future publications. In my thesis, I observed
heightened resting-state functional connectivity of the anterior cingulate in the pediatric MS group which I suspect might be related to greater cognitive control and conflict monitoring given the functional localization of this region as discussed earlier in Section 5.4.1. Such processing patterns may lead to greater endorsement of items related to the “self” or “planning” on the ARSQ. Future studies using the ARSQ after resting-state acquisition and observing the relation to activation patterns might be an interesting avenue for further research.

5.5.4 Cognitive reserve

In the MS literature, there are numerous accounts of the clinico-radiological paradox (Barkhof, 2012; Hackmack et al., 2012; Davis, 2014). This refers to the weak correlation of MRI lesion load with clinical disability. With respect to the specific correlation between lesion volume and cognitive performance, this relationship can be described as moderate at best. This concept is visualized in examples of participant data collected from this thesis in Figure 22.
Figure 22. MRI (FLAIR) images of two MS patients who took part in this PhD study. Despite heavy lesion burden and pronounced brain atrophy, the participant in (a) did not demonstrate evidence of cognitive impairment. The participant in (b) was one of the few patients who did demonstrate cognitive impairment, and this was despite low lesion load and minimal atrophy.

The patient in Figure 22a has markedly greater T2 lesion volume and obvious brain volume reduction relative to the patient illustrated in Figure 22b. However, the participant in Figure 22a, has intact cognitive performance whereas the patient in 22b does not. One possible explanation for the capacity of the patient depicted in Figure 22a to remain cognitively intact despite marked disease burden could relate to cognitive reserve. The concept of cognitive reserve has been investigated in the adult MS literature, but not in the pediatric MS literature and warrants some discussion. In adult-onset MS, higher cognitive reserve has been shown to reduce the influence of brain atrophy and lesion volume (local inflammatory injury) on cognition (Amato et al., 2013; Booth et al., 2013; Sumowski, Chiaravalloti, & DeLuca 2009, Sumowski et al., 2013; Pinter et al., 2014). Cognitive reserve can be broadly considered as an aggregate of cognitive potential (loosely defined by premorbid IQ and education), intellectual enrichment, and socioeconomic factors (i.e. nutrition, academic opportunities). In
pediatric populations, cognitive reserve is difficult to determine given that these individuals have yet to reach their educational peak. Determining the baseline IQ of a patient prior to MS disease onset is rarely achievable; unless the child was examined using standardized testing as part of scholastic activities. School-based testing programs, however, are academically focused and do not provide robust information on IQ. Intellectual enrichment can be evaluated by estimation of participation in cognitively challenging tasks (i.e. reading, mathematics, video games, etc.). Socioeconomic factors may also certainly influence cognitive potential if one considers early life nutrition and its effect on brain growth, academic opportunities, and participation in both scholastic and recreational activities. Parental IQ could be formally measured as part of studies in pediatric MS, but is usually estimated using scales such as the Barratt Simplified Measure of Social Status (BSMSS, described in Section 3.1). Though not presented in this thesis, part of my PhD involved a 3-month project in Italy in which I aimed to investigate predictors of change in processing speed/SDMT performance across time based on 5-year longitudinal data available from Canadian and Italian pediatric MS cohorts. An observation gleaned from this work was the highly significant difference in parental education and occupation levels between the Canadian and Italian cohorts, purportedly due to cultural factors. The median parental education level (based on the BSMSS) was partial high school in the Italian cohort, whereas it was partial college in the Canadian cohort. It was found that higher BSMSS score was associated with greater age-expected improvement in SDMT performance in pediatric MS across 5 years. Italian pediatric MS studies consistently report higher levels of cognitive impairment than Canadian pediatric MS studies. Higher rates of cognitive impairment could then possibly be due to the higher cognitive reserve present in Canadian pediatric MS patients. A product of higher reserve could be that it allows the brain to withstand greater amounts of lesions and atrophy before cognitive deficits actually become manifest. This could occur by a process of functional compensation such as the heightened resting-state functional connectivity demonstrated in our sample.
No single study can adequately control for the multiplicity of factors influencing an individual’s cognitive reserve. However, the factors that might influence cognitive reserve should be considered when comparing patients and controls and when considering within group analyses. Assuming that a composite cognitive reserve score can be determined, regression modelling can then be used to determine whether higher reserve influences fMRI findings, brain volume and impact of MS on cognitive performance over time. It would then be interesting to compare resting-state fMRI functional connectivity and/or BOLD activation patterns during the performance of a cognitively demanding task as a function of reserve. One might speculate that patients with greater reserve will preserve normal functional connectivity and activation patterns or perhaps demonstrate even more efficient neural networks as has been demonstrated in adults with high intelligence (van den Heuvel et al., 2009) and well as adult MS patients with higher premorbid intellectual enrichment (Sumowski et al., 2010).

5.5.5 Influence of fatigue on functional activation patterns

While we have proposed that increased activation may represent a compensatory process, it is possible that this compensation has a “price”. Patients with MS consistently report fatigue, although this has been less well studied in pediatric MS. The current fatigue scales for MS are focused on physical fatigue and not only may be ill-suited to capture physical limitations in an active pediatric MS population, but also may not be sensitive to cognitive fatigue. In support of this, we found no relationship between the PedsQL scale for fatigue and resting-state functional connectivity in our patient group. A better way to measure the relationship between fatigue and BOLD activation would be to query participant-perceived fatigue during an fMRI task. In MS, greater perception of fatigue is thought to be reflected by greater allocation of cerebral resources in order to perform at a similar level as healthy controls. In a study by Genova and colleagues (2013), cognitive fatigue during the performance of a demanding mental switching task was measured using a visual analogue scale by asking subjects to report from 0-100 how mentally fatigued they feel “right now, at this moment”. These authors found that higher reported fatigue was associated with higher functional activation,
particularly of the caudate. It would be interesting to adopt a similar paradigm in pediatric MS. In addition to querying fatigue in real-time, it would also be important to select tasks that become progressively more fatiguing. An example of a task that may be suited to explore the role of fatigue is the mentally demanding n-back task which was described in Section 1.4.3.3. Fatigue can also be evaluated with respect to its impact on resting-state fMRI. Evaluation of resting-state fMRI following a cognitive fMRI task can be stratified both as a function of patient-reported task-based fatigue, and by the time between task-based fMRI and recording of resting-state functional connectivity. Resting-state fMRI arguably should be performed both prior to and following any task-based fMRI to permit a better assessment of the impact of fatigue.

5.5.6 Importance of fMRI task selection

A key facet relates to the selection of the task to be performed. Practical issues must be considered. For patients with MS, problems with vision or motor function may render some individuals incapable of participating in a particular task-based fMRI study. Selection of cognitive tasks first requires that the participant is capable of performing the task well enough to be included in the study in order to measure activation related to the task itself. Patients with profound cognitive impairment may not be able to participate, effectively leading to a “floor effect” for a particular fMRI study. A “ceiling effect” in which tasks that are not sufficiently cognitively demanding may fail to fully capture more subtle changes in neural networks. The SDMT in cognitively intact pediatric MS patients may simply not be sensitive enough to early cognitive impairment. Future studies using testing paradigms that are suited to a broad range of cognitive functionality will be required. Based on the cognitive findings in our patient cohort, the tests that differed the most between groups were the TMT-B and WJ-III Decision Speed, though these differences were not statistically significant (Figure 12). The study of Till et al. (Till, Ho, Dudani, et al., 2012) found that the TMT-B also showed the greatest differences groups (here being statistically significant), with almost half of pediatric MS patients demonstrating impairment on this measure. The TMT-B and WJ-III Decision Speed are both tasks that probe attentional set-shifting and processing speed. Therefore, creating
An fMRI paradigm that evaluates the ability to shift set and includes a timed component would be a priority for pediatric MS fMRI research.

My thesis utilized a cross sectional analysis. Pediatric MS is both a relapsing and progressive disease and is occurring in the context of active brain maturation. Prospective serial analyses are required to determine change in fMRI patterns and cognitive performance over time. Serial analyses permit consideration of two key facets that relate to “time”. One is age at onset and the impact of active MS on neural networks that are developing at that time. The second aspect of time relates to disease duration and the cumulative effect of chronic disease on both established and still developing neural networks. Finally, individual factors may lead to differential impact of age of onset and disease duration in a given patient. This variability may explain why, despite disease duration of up to 10 years in a couple of patients in my study, we were unable to find a relationship between cognitive performance and time from disease onset.

5.6 Concluding Summary

fMRI is a tool used to interrogate brain function and help understand the nature of deficit in clinical populations. My thesis has demonstrated that fMRI is a sensitive technique for detecting abnormalities in pediatric MS patients despite no overt functional deficits. If these patients were solely observed clinically, it likely would have been inferred that nothing is actually wrong with their brain function given that they perform at the same level as their sex- and age-matched peers. This thesis has shown, however, that there are robust differences in observed brain function in pediatric MS patients who appear behaviourally intact and that these can be detected using fMRI. I think that the real benefit of fMRI may lie in detecting subtle abnormalities in patients who have yet to demonstrate deficits, such as the patients in my thesis. Ultimately, it is of interest to determine whether fMRI abnormalities early in the disease can predict patient-relevant outcomes such as quality of life, employment, family responsibilities, and engagement in social and recreational activities.
As expected, I observed that structural brain pathology was present in pediatric MS patients in white matter tracts, and in the thalamus. An important part of this thesis was the demonstration that such damage was associated with differences in network connectivity while at rest. In some networks there was a breakdown of function (thalamo-cortical), whereas in others there was enhanced connectivity (default-mode). This suggests that different networks of the brain have different responses and adaptive properties to cope with structural damage and so should be examined separately. The fact that functional re-organization was present in relation to structural damage has been seen consistently in other clinical populations. What remains to be fully understood is how this re-organization occurs (e.g. through recruitment of additional parallel or latent pathways) which cannot be determined by fMRI. Further study on the actual mechanisms underlying functional reorganization is warranted and can largely help inform rehabilitation efforts in clinical populations. Related to this point is the idea that some type of reserve may be very important for facilitating adaptive functional reorganization. Based on highly intact cognitive functioning of patients in my thesis, and based on comparison to pediatric MS patients in Italy, I suspect our Canadian patients have a high level of cognitive reserve. Even at a young age, it appears as though reserve is operating. The factors related to this reserve (e.g. greater intellectual enrichment, more educational opportunity, greater engagement in recreational activities) warrants further study and can also help inform the general, including pediatric, population how to prevent the onset of cognitive difficulties if recently or later diagnosed with neurological disease.

I will end this thesis with a final point that I think that resting-state cannot really be considered “rest” and is different in brain diseases such as pediatric MS. Perhaps the most robust finding of this thesis was greater anterior cingulate functional connectivity present during resting-state in the pediatric MS group, which I suspect to be due to greater cognitive processing (e.g. cognitive control, conflict monitoring) occurring while at “rest”. I think it is very important to consider that the brain activation patterns elicited during resting state could be influenced by what the participant is really thinking about
during the imaging acquisitions. As such, the networks characteristics (e.g. functional connectivity of regions) will vary according to these thinking patterns. In order to allow a better comparison across groups and across studies I think that greater control of resting-state is required and one such way could be acquiring resting state at the beginning of an MRI scan where the influence of previously completed cognitive tasks is non-existent. It is impossible to control a person’s thinking patterns but the administration of questionnaires, such as the previously mentioned ARSQ, post resting-state acquisition to actually ask patients what they were thinking about during resting-state could also be very helpful.

In summary, fMRI is a very sensitive technique to identify abnormalities in clinical populations, likely before clinical deficits are observed. fMRI is also sensitive in the sense that it is largely influenced by mental processes which may operate in different ways in different clinical populations. The field of cognitive neuroscience should then recognize the utility of fMRI in clinical applications, as well as recognize the intricacies involved in interpreting resting-state fMRI data with special consideration of whether the brain can really be considered as being at “rest” during resting-state.
References


89. Gawryluk JR, Mazerolle EL, Beyea SD, D’Arcy RCN. Functional MRI activation in white matter during the Symbol Digit Modalities Test. Front Hum Neurosci. August 8 2014; [Epub ahead of print].


189. Rypma B, Prabhakaran V. When less is more and when more is more: The mediating roles of capacity and speed in brain-behavior efficiency. *Intelligence.* 2009;37(2):207–222.


231. Van Strien JW. The Dutch Handedness Questionnaire. FSW, Department of Psychology, Erasmus University Rotterdam, 2002.

232. Varni J, Burwinkle T, Katz E, et al. The PedsQL in pediatric cancer: reliability and validity of the Pediatric Quality of Life Inventory Generic Core Scales,


