The Impact of Mesenchymal Stem Cell Therapy on Cognitive Performance
in People Living with Multiple Sclerosis

by

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Abstract

In Multiple Sclerosis (MS), cognition is directly affected by neural integrity and secondary disease characteristics (anxiety, depression and fatigue). Mesenchymal stem cell therapy (MSCT) has been recently studied due to its potential for neural repair in MS. It is hypothesized that cognitive improvement, a marker of neural repair, will be seen after MSCT. The change in cognition is expected to be correlated with changes in secondary disease characteristics and neural conduction time. Ten participants received MSCT. Cognition, secondary disease characteristics, and conduction time were evaluated pre- and post- (12 month) therapy. Eight participants demonstrated cognitive improvement on at least one test. Secondary disease characteristics and conduction time were not associated with cognition in sample. In conclusion, MSCT appears to be safe with regards to cognition and does not worsen secondary disease characteristics. Future efficacy studies have the potential to show greater cognitive improvement than the current preliminary study.

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1 Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system (CNS) characterized by inflammation leading to demyelination and neural degeneration (Calabrese, Favaretto, Martini, & Gallo, 2013; Lublin et al., 2014). MS causes neural damage in both the white matter and the gray matter (Mallucci, Peruzzotti-Jametti, Bernstock, & Pluchino, 2015). Neural damage is characterized by axonal loss, neural injury, demyelination, glial scarring, oligodendrocyte damage and inflammatory infiltrate (Calabrese et al., 2015; Trapp et al., 1998). Neural damage in MS leads to neural signal conduction deficits, cognitive deficits and physical disabilities. This thesis examines cognition among a sample of People with MS (PMS) undergoing mesenchymal stem cell therapy.

There are three subtypes of the disease: relapsing-remitting MS, secondary progressive MS and primary progressive MS (Lublin et al., 2014). Relapsing remitting MS is characterized by inflammation whereas secondary and primary progressive MS are characterized by neural degeneration (Newsome et al., 2016). The nature of pathology (i.e., inflammation versus neural degeneration) associated with the different disease types, affect cognition and neural conduction differently (Humm, Magistris, Truffert, Hess, & Rösler, 2003; Rocca et al., 2015). The progressive types of MS are associated with more severe cognitive and neural deficits (Humm et al., 2003; Rocca et al., 2015).

The available therapies target the immune responses, which cause the inflammation, and fail to treat the neurodegenerative nature of the disease. Stem cell therapies, such as mesenchymal stem cell (MSC) therapy, are being investigated for their ability to promote neural repair. MSCs are adults stem cells that can differentiate into different types of cells and play a key role in tissue repair after injury (Ding, Shyu, & Lin, 2011; Xie et al., 2016). MSCs were shown to

promote neural repair in vitro and in animal models of MS. This thesis is part of the Mesenchymal Stem Cell Therapy for Canadians with MS (MESCAMS), which is a phase II clinical trial. The MESCAMS study investigates the safety of the MSC therapy. This thesis addresses primarily how MSC therapy can affect cognitive performance. The second goal of this thesis was to examine the association between mood, fatigue and cognition. Finally, the third goal of this thesis was to examine the relationship between two markers of neural repair: neural conduction time and information processing speed, to determine whether MSC therapy affects both markers in a similar way. The three goals are explained in more detail below.

The first goal of this therapy was to examine the impact of MSC therapy on cognition. Cognitive impairment is common in MS and is correlated with measures of brain atrophy. MSC therapy is expected to slow down brain atrophy via neural repair. Thus, it is anticipated that cognition will improve after MSC therapy. As such, the first goal of this thesis was to determine whether cognitive improvement is observed after MSC therapy and if so, to determine the cognitive domain with the highest levels of improvement. Information processing speed, working memory, executive function, as well as visual and verbal learning and memory are the cognitive areas commonly impacted in MS (Chiaravalloti & DeLuca, 2008). Information processing speed is most often affected, possibly due to demyelination which hinders the transmission of the neural signal (Costa, Genova, DeLuca, & Chiaravalloti, 2017). It has been reported that MSC therapy promotes remyelination (Uccelli, Laroni, & Freedman, 2011). Thus, it is expected that information processing speed will demonstrate the highest levels of improvement among the cognitive areas impacted in MS.

The second goal of this thesis was related to the effects of secondary disease characteristics on cognition. Secondary disease characteristics (depression, anxiety and fatigue) are highly

prevalent in the MS population and can cause cognitive deficits (Marrie et al., 2015). Their impact on cognition was investigated in the current sample to determine whether the change in cognition is due to the therapy itself or the secondary disease characteristics.

The third goal of this thesis was to examine the impact of MSC therapy on information processing speed and neural conduction time, which are markers of neural repair. Neural conduction is negatively affected in MS, possibly due to demyelination, and can be assessed using transcranial magnetic stimulation (TMS) (Kobayashi & Pascual-Leone, 2003). TMS measures the conduction time of the neural signal from the cortex to the target muscle. TMS is sensitive to damaged neural pathways (Kobayashi & Pascual-Leone, 2003). Thus, TMS was used in the MESCAMS study to examine if neural repair is observed after the MSC therapy as anticipated.

It was expected that speed of neural conduction would be correlated with speed of information processing. As such it would be expected that MSC therapy would affect both, neural conduction and information processing, in a similar manner (i.e., both would improve, decline, or remain unchanged after the therapy). Previous studies showed that there is an association between neural conduction time and information processing speed in MS (Llufriu et al., 2012). This thesis examined the relationship between information processing and signal conduction before and after MCS therapy.

To summarize, this thesis examined the effects of MSC therapy on cognition. This thesis also considered whether secondary disease characteristics affect cognition in this sample. Finally, the relationship between neural conduction time and information processing speed was explored to determine whether MSC therapy affected cognition and neural conduction in the same manner.

2 Literature Review

2.1 Defining Multiple Sclerosis

The course of MS is defined by two unique phenotypes: relapsing and progressive (Lublin et al., 2014). In the relapsing course of MS, transient inflammation is accompanied by remyelination (Compston & Coles, 2008). As such, neurological repair occurs but does not persist due to the short-lasting remyelination (Compston & Coles, 2008). In contrast, in the progressive course of the disease, microglial activation is widespread leading to progressive neural damage (Compston & Coles, 2008). That is, decreased inflammation accompanied by lack of remyelination leads to neural degeneration and progressive, accumulative disability (Lublin et al., 2014). To summarize, the relapsing course of MS is dominated by inflammatory periods, whereas the progressive course of MS is dominated by neural degeneration and axonal loss (Ontaneda & Fox, 2015).

In general, inflammation protects the host organism from an invading body (Gharagozloo et al., 2017). In MS, the immune system inappropriately attacks the myelin as the invading body. The attack is associated with inflammation, which increases the expression of cytotoxins, leading to further injury (Gharagozloo et al., 2017). In the relapsing-remitting phase, an inflammatory attack is followed by recovery (Compston & Coles, 2008). Interestingly, molecules that were associated with the inflammation-caused cell injury are also associated with recovery and myelin repair (Al-Badri & Castorina, 2018), suggesting that inflammation may also have some positive effects. In the progressive phase of the disease, inflammation is no longer the characterizing feature of the disease (Lublin et al., 2014) and thus no longer plays a role in terms of the disease pathology at these later stages. Thus, the particular role of inflammation varies depending on the stage of the disease.

Magnetic resonance imaging (MRI) is crucial for the diagnosis and prognosis of MS (Zhou, Shiroishi, Gong, & Zee, 2010). After an inflammatory relapse, lesions form in the myelin and axons containing white matter (Giorgio et al., 2014). These lesions are referred to as hyperintense lesions or T2 lesions detected using T2-weighted MRI images. When a relapse is over, the swelling disappears and remyelination takes place decreasing the volume of T2 lesions (Giorgio et al., 2014). However, T2 lesions usually persist after a relapse making them important for understanding the accumulation of MS lesions over time (Giorgio et al., 2014).

As the disease progresses, a relapse could occur in the previously lesioned site leading to permanent neuroaxonal damage known as hypointense lesions on neuroimaging (Giorgio et al., 2014). Hypointense lesions or T1 lesions appear in the grey matter and are detected using T1 weighted MRI scans. T1 lesions are associated with axonal damage, brain atrophy and the accumulation of permanent disease pathologies (Giorgio et al., 2014; Zhou et al., 2010). T1 lesions are used as one of the defining criteria of the progressive phase of MS (Lublin et al., 2014).

The diagnosis of MS requires MRI evidence of dissemination of central nervous system (CNS) lesions in time and space. *Dissemination in space* refers to the occurrence of one or more T2 lesions in at least 2 out of 4 areas of the CNS (Polman et al., 2011). These areas are periventricular (i.e., white matter containing axons and myelin), juxtacortical (i.e., gray matter containing neuronal cell bodies), infratentorial (i.e., cerebellum), and spinal cord (Polman et al., 2011). *Dissemination in time* refers to the occurrence of additional T2 lesions or gadolinium (a contrast agent) enhancing lesions on the follow-up MRI or the presence of both gadolinium enhancing and non-enhancing lesions at any time (Polman et al., 2011). New modifications to the diagnostic criteria were added in the McDonald diagnostic criteria in 2017 to allow for early

diagnosis and treatment of MS. According to the new criteria a person can be diagnosed with MS even if the MRI lesions are not accompanied with physical symptoms, which was a requirement in the previous diagnostic criteria (Thompson et al., 2018; Polman et al., 2011).

2.2 Etiology of Multiple Sclerosis

There is no single known cause of MS, however, there are many factors that increase the risk of MS. Vitamin D deficiency is a risk factor for MS and can impact the disease activity and progression (Ascherio et al., 2014; Prietl, Treiber, Pieber, & Amrein, 2013). Genetic susceptibility is shown to be a risk factor for MS by familial aggregation (i.e., the clustering of a given trait or disorder within a family) and high disease rates within ethnicities (i.e., higher rates of MS are found in Northern European populations compared to African and Asian populations) (Ramagopalan et al., 2011). Specific genes such as the HLA gene and a rare gene variant of vitamin D deficiency have been linked to MS across all populations (Hauser & Oksenberg, 2006; Ramagopalan et al., 2011). The risk of MS increases by 15- fold with first degree relatives (Ramagopalan et al., 2011). Environmental factors such as Epstein Barr virus infection heighten the risk of MS by many times (Pender & Burrows, 2014). By contrast a healthy diet enhances gut microbiota which lowers inflammatory responses associated with MS (Riccio & Rossano, 2015). Diets high in calories and salt negatively impact gut microbiota and increase one's risk of developing MS (Riccio & Rossano, 2015). Thus, although there is no clear single etiology, the disease appears to occur in those with either genetic and/or environmental susceptibility and may be initiated by a viral trigger.

2.3 Multiple Sclerosis Phenotypes

MS can be classified into subtypes, including: clinically isolated syndrome, relapsing remitting, secondary progressive and primary progressive. Clinically isolated syndrome is when

MRI lesions are accompanied by demyelination (Newsome et al., 2016) and is characterized by one relapse suggestive of MS but without the spread of CNS lesions in time and space required for MS diagnosis (Mallucci et al., 2015). The attack lasts at least 24 hours and usually targets the optic nerve, brain stem or the spinal cord (Polman et al., 2011). Thirty to seventy percent of people with clinically isolated syndrome develop MS (Mallucci et al., 2015). Once the clinically isolated syndrome is active and fulfills MS diagnostic criteria of CNS lesions disseminated in time and space, then the condition transitions into relapsing-remitting MS (Mallucci et al., 2015).

Relapsing-remitting MS, as shown in Figure 1, is characterized by relapses lasting from weeks to months followed by recovery lasting from months to years (Mallucci et al., 2015). Relapsing-remitting MS is the onset phenotype in approximately 85 % of people with MS (PMS) (McKay, Kwan, Duggan, & Tremlett, 2015). After 5-15 years of relapsing-remitting MS onset, the disease typically transitions into secondary progressive MS (Mallucci et al., 2015). Secondary progressive MS is characterized by lack of recovery following exacerbations and gradual worsening of symptoms. The exact point of transition of relapsing-remitting MS to secondary progressive MS has not yet been clearly defined (Lublin et al., 2014).

The last phenotype of MS is the primary progressive MS (Figure 1). In the primary progressive MS, there is no relapsing-remitting period. Instead the disease develops into the progressive phase of neural degeneration from disease onset, with its associated lack of inflammation and recovery (Lublin et al., 2014). Primary progressive MS is the onset phenotype in about 15 % of PMS (McKay et al., 2015). The rate of worsening is similar to that seen in the secondary progressive MS (Lublin et al., 2014). In summary, it is important to understand the differences between the different MS types because each of these types affect cognition and neural conduction differently.

Adapted from Lublin et al., 2014

Figure 1. Disease progression in each MS subtype.

2.4 Multiple Sclerosis and Gender

Multiple sclerosis affects males and females differently. The disease affects more females than males; however, males typically experience a more severe course (Golden & Voskuhl, 2017). The transition from disease onset to relapsing-remitting MS and from the relapsing-remitting MS to secondary progressive MS is typically more rapid in males. Males also accumulate more disease-related disability than females (Golden & Voskuhl, 2017).

Clinically isolated syndrome most often develops between 20 and 45 years of age and affects females 2-5 times more than males (Mallucci et al., 2015). Relapsing-remitting multiple sclerosis usually affects young adults between the ages of 20 – 30 and affects females 2-3 times more than males (Mallucci et al., 2015; Pender & Burrows, 2014). Primary progressive MS however, equally affects males and females and typically develops approximately 10 years later than relapsing-remitting MS (Hauser & Oksenberg, 2006; Mallucci et al., 2015; Pender & Burrows, 2014).

2.5 Brain Structure and Cognition

MS lesions occur in the white matter and the grey matter causing physical disability and cognitive dysfunction. There are many factors affecting cognitive function in MS. Some of these

factors are associated with cognitive impairment while other factors play a protective role with regard to cognition. Cognitive dysfunction is directly linked to brain atrophy including grey matter and thalamic volume loss and thinning of the cerebral cortex (Benedict & Zivadinov, 2011). MS lesions including T1 and T2 lesions in the white matter are associated with and, in some cases, predicted future cognitive impairment (Rocca et al., 2015).

In clinically isolated syndrome, T1 lesions observed 3 months after clinical onset predicted cognitive deficit 7 years later (Summers, Swanton, et al., 2008). Similarly, T2 lesions at 3 months follow-up predicted slowing in information processing speed after 7 years (Summers, Swanton, et al., 2008). In people with relapsing remitting MS, T1 lesions predicted poor attention after 5 years (Summers, Fisniku, et al., 2008). In people with primary progressive MS, T2 lesions predicted cognitive dysfunction after 5 years (Penny, Khaleeli, Cipolotti, Thompson, & Ron, 2010). Deficits in white matter are associated with worsening in processing speed and working memory (Rocca et al., 2015). Overall, T1 and T2 lesions are associated with, and can predict future cognitive deficits, in PMS.

The number and volume of cortical lesions are associated with cognitive dysfunction. Lesions in specific areas of the grey matter are related to deficits in the function of the area affected. For example, lesions in the hippocampus are associated with deficits in memory (Rocca et al., 2015). Sanfilipo, Benedict, Weinstock-Guttman and Bakshi (2008) reported that lesions in the thalamus and the putamen early in the disease course are associated with cognitive dysfunction (as cited in Rocca et al., 2015). Deficits in verbal memory and executive function are associated with grey matter lesions (Rocca et al., 2015).

Lesions in the white matter could affect important brain networks leading to disconnection syndrome referred to as damage to “strategic” white matter tracts (Rocca et al.,

2015). Recent studies showed that cortical pathologies trigger damage to connective networks (Louapre et al., 2014; Steenwijk et al., 2015). In one study, the damage to grey matter was associated with pathology of connective tracts of the white matter in people with relapsing-remitting MS (Steenwijk et al., 2015). Functional MRI has revealed that damage to cognitive networks such as the default mode network, frontoparietal and dorsal attentional networks is associated with severe cognitive impairment early in MS (Louapre et al., 2014).

Studies suggest that increased activation as well as changes to the cerebral activity observed in cross-sectional fMRI studies during the early stages of the disease play a protective role attributed to brain plasticity (Loitfelder et al., 2014; Rocca et al., 2015). In other words, plastic changes in functional connectivity are possible early in the disease course and allow affected individuals to maintain cognitive performance for a period of time. However, increased activation causes exhaustion of brain networks over time leading to increased cognitive deficit during the later stages of MS (i.e., efficacy of compensation eventually diminishes given the inability to tolerate increasing disease burden).

There are other risk factors for cognitive impairment in MS. Older age, male gender, earlier disease onset, lower intelligence or education, and grey matter atrophy increase the patient susceptibility to cognitive deficit (Benedict & Zivadinov, 2011). As in the general population, cognitive decline is associated with aging. In addition to this association, people with early disease onset exhibited more cognitive impairment than people with later disease onset (Amato, Ponziani, Siracusa, & Sorbi, 2001). Males with MS are also at a higher risk of cognitive decline (Golden & Voskuhl, 2017). Furthermore, higher rates of cognitive dysfunction are observed when PMS transition from relapsing-remitting MS to secondary progressive MS due to increased grey matter atrophy associated with the progressive course of the disease (Benedict &

Zivadinov, 2011).

2.6 Areas of cognitive deficit

Cognitive dysfunction is observed in 43-70% of PMS during both early and late stages of the disease (Chiaravalloti & DeLuca, 2008). Due to the diverse nature of the disease PMS exhibit different physical symptoms. Likewise, different cognitive functions are impaired due to MS and the severity of impairment varies among PMS. The most commonly affected cognitive areas are complex attention, information processing efficiency (i.e., information processing speed and working memory), executive function, visual and verbal learning, and memory (Chiaravalloti & DeLuca, 2008). Although simple attention (such as a digit-repeating task) is typically intact in MS, sustained attention and divided attention (attending to multiple tasks simultaneously) are often impaired in PMS (Chiaravalloti & DeLuca, 2008). Furthermore, fatigue is a common symptom in MS and can substantially affect tasks with sustained and divided attention (Chiaravalloti & DeLuca, 2008). It has been difficult to characterize the cognitive basis of the attention deficit in MS because most standard neuropsychological assessment tools, that assess attention, involve other cognitive processes as well such as speed of information processing and executive function (Chiaravalloti & DeLuca, 2008). Given that MS is a clinical entity and research has focused on how changes in cognition impact functional performance, the cognitive science literature is scant and thus base cognitive processes have not been evaluated in this area.

Long-term memory impairment is observed in 40-65% of PMS (Langdon, 2011). Both verbal and visual-spatial long-term memory are affected (Langdon, 2011). Although it was previously believed that impairment in long term memory was due to deficits in retrieval of information in MS, later studies have shown that the encoding of new information is the primary cause of the deficit in the majority of cases (Chiaravalloti & DeLuca, 2008). Poor learning skills

in PMS are associated with slow processing speed and vulnerability to interference (inability to ignore distractors) (Chiaravalloti & DeLuca, 2008).

People with MS may also exhibit deficits in tasks involving executive functions including planning, organization, reasoning and fluency (Chiaravalloti & DeLuca, 2008). Approximately 19% of PMS exhibit impairment in executive functions (Guimarães & Sá, 2012). One study demonstrated that PMS exhibit deficits in executive function tasks such as disinhibition and poor fluency (Drew, Tippet, Starkey, & Isler, 2008). Fluency tasks involve testing the ability of spontaneously generating words beginning with one letter (i.e., phonemic fluency) or belonging to one category (i.e., semantic fluency) (Langdon, 2011). In these tasks perseveration errors (the repetition of a prior response) may indicate a deficit in inhibition which underlies executive function impairment (Chiaravalloti & DeLuca, 2008). PMS were reported to make more perseverative errors compared to healthy controls (Griffiths et al., 2005). Thus, executive function is also impacted by MS.

Efficiency of processing information involves both working memory (encoding and manipulating information for a short period of time) and speed of information processing (the speed at which this information can be manipulated). Approximately, 20-30% of PMS have impaired speed of information processing (Guimarães & Sá, 2012). It has been hypothesized that information processing speed is the primary deficit causing cognitive impairment in MS (Costa et al., 2017). Deficits in processing speed are correlated with deficits in working and long-term memory. When PMS are given more time to process information their performance on tasks examining executive function (e.g., Tower of London) is as accurate as healthy controls (Denney, Lynch, & Parmenter, 2008). Leavitt and co-workers showed that increasing the time for information processing improved the performance of PMS in tasks requiring high cognitive

load (Leavitt, Lengenfelder, Moore, Chiaravalloti, & DeLuca, 2011). Thus, information processing is impacted in MS but more importantly it might be the underlying cause of cognitive impairment in the higher level cognitive functions.

The MS subtypes differ in terms of their cognitive profile. One study compared groups of the three subtypes of MS with matched healthy controls (Huijbregts et al., 2004). All three subtypes were cognitively impaired compared to healthy controls. Working memory was impacted in relapsing remitting MS and in primary progressive MS. People with secondary progressive MS showed impairment in information processing (Huijbregts et al., 2004). Thus, different areas of cognition are impacted in each type of the disease.

Both information processing speed and working memory are commonly impacted in MS and the literature is inconsistent as to which one is the underlying cause of cognitive impairment in MS. In a study where people with relapsing-remitting MS and secondary progressive MS participated, researchers compared working memory and speed of processing in PMS to determine which of the two is responsible for the information processing deficit (DeLuca, Chelune, Tulsky, Lengenfelder, & Chiaravalloti, 2004). Their data showed that speed of processing was the cause of the information processing deficits in PMS (DeLuca et al., 2004). Others have found that in early-phase relapsing-remitting MS in particular, working memory may be the primary deficit (Berrigan et al., 2013). Thus, studies should aim to evaluate both information processing and working memory along with other cognitive domains commonly impacted by MS to have a comprehensive understanding of cognitive deficit in PMS.

Beatty and Goodkin (1990) reported that The Mini-mental State Exam (a standard screening tool in assessing cognition) is inadequate at detecting the cognitive deficits specific to MS (as cited in Amato, Zipoli, & Portaccio, 2008). Thus, a few different neuropsychological

assessment batteries were developed to assess the cognitive areas commonly compromised in MS. One brief battery designed specifically for MS is the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) (Langdon et al., 2012). It was developed for quick administration by MS clinic staff in the absence of a neuropsychologist (Amato, Zipoli, & Portaccio, 2008; Langdon et al., 2012). A more detailed battery is the Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS), which includes measures of spatial processing, verbal and visual-spatial learning and memory, processing speed, working memory and executive functions (Benedict et al., 2002). This comprehensive tool was developed by a panel of expert neuropsychologists and psychologists from the United States, Canada, the United Kingdom and Australia (Walker et al., 2017). The MACFIMS comprehensively assesses the cognitive functions commonly impacted by MS (Benedict et al., 2002). Other batteries have also been developed but the BICAMS and MACFIMS are most frequently utilized in clinic settings. Cognitive assessment depends on the battery administered. Thus, a reliable validated battery for cognitive functions associated with MS should be used to understand cognitive performance in PMS.

2.7 Secondary Disease Characteristics

Secondary disease characteristics of MS include anxiety, depression and fatigue. It is important to understand and evaluate secondary disease characteristics given that they are related to cognitive deficits. Depression and anxiety are the most common comorbidities in MS with a prevalence of 23.7% (95% CI 17.4 – 30%) and 21.9% (95% CI 8.76 – 35%) respectively (Marrie et al., 2015).

Depression is characterized by low mood and inability to enjoy life as they normally would. It is accompanied with physical symptoms such as fatigue, altered sleep and appetite and

depressive thoughts like worthlessness, feelings of guilt and the feeling that life is not worth living. Up to 50% of PMS are diagnosed with depression, which is 3 times the rate of the healthy population. Major depression, severity of the depression, and social isolation are the leading causes of suicide in the MS population (Feinstein, 2011). Twenty five percent of PMS contemplate suicide (Feinstein, 2011). The rate of suicide in the Canadian MS population is seven-fold higher than the healthy population (Feinstein, 2011). Those numbers highlight the alarming nature of the deadly combination of depression and MS. Both males and female are prone to depression equally (Feinstein, 2011). However, young males are the highest risk of suicide within the first three years of the disease (Feinstein, 2011). Physical symptoms (e.g., fatigue and insomnia) of depression can confound the symptoms of MS (Feinstein, 2011). To avoid false diagnosis of depression, self-report questionnaires (e.g., the Hospital Anxiety and Depression Scale) emphasize mood symptoms rather than physical symptoms when diagnosing depression in persons with the disease (Feinstein, 2011).

In 2009, an fMRI study examined the mechanisms underlying depression in people with MS (Passamonti et al., 2009). The study compared activation patterns between healthy controls and PMS who were not depressed or anxious when they were shown an emotional vs. a neutral stimulus (Passamonti et al., 2009). PMS have different cerebral activation patterns compared to healthy controls (Passamonti et al., 2009) given that they displayed a consistent hyper-activation of the cerebral cortex, referred to by the authors as a compensatory mechanism to prevent showing emotional symptoms and maintain a euthymic state (Passamonti et al., 2009). As such, in the neither depressed nor anxious PMS, the brain is already busy compensating for continuous emotional stimuli in daily life to maintain a euthymic state. As such, when new MS symptoms appear little resources are left to maintain the euthymic state, presumably leaving PMS prone to

depression (Feinstein, 2011). This hyper-activation of the cerebral cortex may thus help explain why PMS are more prone to depression than healthy individuals.

There were many proposed causes for depression among people with MS. First, there are altered brain structures in MS such as the abnormal connection between the amygdala and the prefrontal regions. Brain atrophy quantified using MRI accounted for 43% of variance in depression (Feinstein, Magalhaes, Richard, Audet, & Moore, 2014). MRI shows that depression is associated with chronically brain damaging lesions rather than transient lesions (Feinstein, 2011). Other factors are also associated with depression in MS such as pain, fatigue, anxiety, cognitive impairment, and sleep disturbances (Feinstein et al., 2014; Mendis, Rickards, & Cavanna, 2010).

There are a number of treatment options for depressed PMS. Antidepressants are modestly effective. Supportive emotional therapy, cognitive behavioral therapy and mindfulness based interventions are used to treat depression. The latter two are limited to cognitively intact individuals (Feinstein, 2011). Studies have shown that cognitive behavioral therapy is as effective as antidepressants followed by supportive emotional therapy (Mohr, Boudewyn, Goodkin, Bostrom, & Epstein, 2001). Electroconvulsive therapy is reserved as a last treatment option when persons do not respond to other therapies (Feinstein, 2011).

Anxiety is another secondary disease characteristic that impacts MS. Anxiety has been less studied than depression. Studies show that 65% of the cases with anxiety are not detected due to the method used in evaluating anxiety (Marrie et al., 2018). Interventions to treat anxiety are even less common than those to treat depression. Some factors that affect anxiety are young age at disease onset, the EDSS score, pain and fatigue (Beiske 2008). In addition, people with MS are reported to have an increase in the level of anxiety after injections with the disease

modifying drug (Théaudin et al., 2015). However, the extent of this transient anxiety's effect on general anxiety is not clearly understood (Théaudin et al., 2015).

Fatigue is the most commonly reported symptom by PMS and is considered the main debilitating chronic symptom of MS (Induruwa, Constantinescu, & Gran, 2012). Fatigue is shown to precede MS by years (Induruwa, Constantinescu, & Gran, 2012). PMS struggle with many aspects of life in relation to fatigue (Induruwa, Constantinescu, & Gran, 2012). First, it is difficult to identify tiredness as MS-related fatigue and be aware of it (Induruwa, Constantinescu, & Gran, 2012). Second, they struggle with accepting the fact that they have to change their lifestyles to accommodate their fatigue (Induruwa, Constantinescu, & Gran, 2012). Third, explaining to others the new life style that accommodates fatigue is also challenging (Induruwa, Constantinescu, & Gran, 2012).

Depression, anxiety and fatigue tend to co-occur and predict future occurrence of one another (Brown et al., 2009; Wood et al., 2013). Both mood and fatigue influence other factors such as the quality of life, work productivity, activity impairment, and substantial pain (i.e., pain severe enough to impair function) (Glanz et al., 2012; Lobentanz et al., 2004; Marck et al., 2017).

Mood and fatigue also affect cognitive function. Depression and fatigue are common predictors of self-reported cognitive function (Beier, Amtmann, & Ehde, 2015). Gilchrist and Creed found associations between depression and cognitive dysfunction rather than neurological symptoms and disability (Gilchrist & Creed, 1994). In addition, depression and social stress were shown to cause cognitive dysfunction in PMS (Feinstein, Kartsounis, Miller, Youl, & Ron, 1992; Gilchrist & Creed, 1994). In order to treat cognitive dysfunction comorbidities such as fatigue and depression must be treated first (Bagert, Camplair, & Bourdette, 2002).

2.8 MS Treatments

There is no known cure for MS, however, there are many disease-modifying therapies that act as immunomodulatory and/or immunosuppressant therapies (i.e., can modulate and suppress responses of the immune system). Ultimately, disease-modifying therapies aim to achieve “no evident disease activity” by improving quality of life, lowering relapse rate and severity, lowering the number of new MRI lesions, and protecting PMS against future disease related disability (Giovannoni et al., 2015). There are four categories of disease modifying therapies for relapsing-remitting MS: platform therapies, oral therapies, infusion therapies and emerging therapies. Each of these therapies is discussed in more detail in Appendix A. Thus, despite therapeutic benefits such as reduction in the number of relapses, they do not treat the neurodegenerative aspect of the disease.

A major limitation of the available MS therapies is that they lack any neural repair. The available therapies target the immune responses but not the continuous accumulation of neural degeneration associated with the disease. Thus, research has moved in a new direction to investigate treatment options that better target the CNS. For example, cell-based therapies such as bone marrow transplantation (hematopoietic stem cell transplantation), widely used for cancer treatment, has gained more attention in the field of MS therapies.

2.8.1 Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation paved the way to introduce new stem cell therapies such as the mesenchymal stem cell therapy. This section introduces hematopoietic stem cell therapy and its benefits and limitations.

The rationale behind bone marrow transplantation, particularly, hematopoietic stem cell transplantation is that MS is an autoimmune disease caused by the abnormal reaction of the

immune system. Thus, the theory is that the destruction of the immune system and replacing it with a normally functioning one will suppress future relapses (Freedman, 2007). It is widely established that using autologous cells (derived from the patient's own cells) is better than using allogeneic cells (obtained from healthy tissue-matched individuals) for the transplantation procedure (Freedman, 2007; Uccelli, Laroni, & Freedman, 2013). This is due to the fact that allogeneic cells can be rejected by the patient's immune system, thus making them less effective (Uccelli et al., 2013). Allogeneic cell transplantation is the only option for people with leukemia because the entire bone marrow has to be replaced (Freedman, 2007). As for PMS, autologous cells can be obtained, multiplied in number, and administered to the patient.

Although hematopoietic stem cell transplantation has been an effective therapy for some diseases such as leukemia, the procedure is rather aggressive and has higher risk levels in PMS. The reason is that in PMS the immune system has to be suppressed (or completely ablated) before the transplantation of bone marrow takes place (Atkins & Freedman, 2013). Suppression of the immune system leaves PMS more vulnerable to other diseases.

The success of bone marrow transplantation in treating diseases such as leukemia inspired the use of hematopoietic stem cell transplantation in MS. Hematopoietic stem cells seem not to be affected by the abnormal function of the immune system, thus when transplanted back to the patients they do not attack the CNS (Walker et al., 2014). Then PMS undergo suppression of the immune system using cyclophosphamide (chemotherapy medication) and the hematopoietic stem cell transplantation takes place (Walker et al., 2014). The therapeutic effects of immunoablative hematopoietic stem cell transplantation are rather unique. Participants enrolled in a study at the Ottawa Hospital, have been followed up to 13 years with no relapses, no T2 lesions, alleviation of brain atrophy, and disability improvement (Atkins et al., 2016;

Walker et al., 2014). This type of positive response is unheard of in other MS therapies. However, this therapy is limited to people with active MS (i.e., new inflammatory activity) because its main target is to replace the malfunctioning immune system. Thus, people living with progressive MS are excluded from this therapy because their disease is no longer characterized by inflammation associated with immune system dysfunction but is rather associated with neurodegeneration (Saccardi et al., 2012).

2.8.2 Hematopoietic Stem Cell Transplantation and Cognition

Only a few studies have explored the effect of hematopoietic stem cell transplantation paired with immunoablation in a population of PMS (Walker et al., 2014; Walker et al., 2016). Walker and colleagues (2014) found that PMS (n = 7) treated with immunoablation showed declines in cognitive performance soon after the treatment, however, cognitive functioning returned to baseline levels two years after the treatment. They argued that the early cognitive decline was caused by chemotoxicity (Walker et al., 2014). After one year, Walker et al. reported an improvement in Paced Auditory Serial Addition Test (PASAT) scores. The PASAT assess information processing speed and working memory by requiring individuals to add a number to the previous number stated throughout 60 continuous number presentations. Participants continued to improve for up to 36 months after the transplantation (Walker et al., 2014). However, this improvement was not statistically significant, possibly due to the small sample size. This study suggests that there may be a potential for improvement in cognitive performance following the immunoablative hematopoietic stem cell transplantation and serves as an impetus for future investigation in this field. The continuous improvement of cognitive function over three years could indicate that neural repair maybe associated with hematopoietic stem cell therapy. In conclusion, hematopoietic stem cell therapy served an important role in leading the

field of stem cell therapy and leading the way for investigating new stem cell therapies that perhaps do not require immune ablation.

2.8.3 Mesenchymal Stem Cell Transplantation

Recent research has focused on mesenchymal stem cell (MSC) transplantation to treat MS (Bai et al., 2009; Connick et al., 2012; Liang et al., 2009; Uccelli, Laroni, & Freedman, 2011). Mesenchymal stem cells are self-renewing stromal cells (i.e., originate from the connective tissue of an organ) and have the ability to differentiate into different cells such as bone, cartilage, fat and muscle cells (Ding et al., 2011). MSCs can be derived from different cell types such as bone marrow, umbilical cord, menses blood, endometrial polypus (the inner lining of the uterus), as well as adipose tissue (fatty tissue) (Ding et al., 2011). MSC have the unique ability to inhibit T cells (i.e., immune cells) growth both in vivo and in vitro (Ahn et al., 2014).

MSCs were first tested in animal models of MS (experimental autoimmune encephalomyelitis - EAE). Animals with EAE treated with MSC had less demyelination and fewer attacks of the immune system on the CNS (Zappia et al., 2005). Another study showed the neuro-protective effects of MSC as well as their ability to enhance oligodendrocyte generation when injected into EAE (Zappia et al., 2005). Desirable outcomes of animal research in using MSC as treatment for MS includes the suppression of T-cells and other immune cells responsible for the malfunction in MS (Anderson et al., 2017).

A few studies have examined the safety, the required dosage, and the route of administration of MSC in human subjects with small sample sizes per study. Yamout and colleagues administered autologous MSC intrathecally (injected directly into CNS) to 10 PMS with a range of dosage between 32×10^6 and 100×10^6 cells per patient. Six participants showed improvement in terms of disease course. The patient who was treated with 100×10^6 MSCs

developed seizures. In addition, three PMS were not given a large enough dose because their cells did not multiply to adequate numbers ($< 2 \times 10^6$) (Yamout et al., 2010).

In another study, people with optic lesions treated with MSC (n=10), showed improvement in their vision, specifically higher visual acuity and lower contrast sensitivity. They also had higher optic nerve cross sectional area (Connick et al., 2012). Llufríu and colleagues administered lower amounts of MSC ($1-2 \times 10^6$ MSCs/ Kg of body weight) than Yamout et al. (2010) and showed that MSC are safe at this dosage. There were no safety issues in this study except for one participant who had facial flushing when MCSs were administered (Llufríu et al., 2014). Although treatment did not result in a significant decrease of gadolinium-enhancing lesions (a marker for active MS lesions), there were lower means of gadolinium-enhancing lesions 6 months and 12 months after treatment in the MSC group compared to the placebo group (Llufríu et al., 2014).

A recent pilot trial for autologous MSC for MS reported that the transplantation is a safe and well tolerated therapy for PMS (Cohen et al., 2017). The study reported no severe or serious adverse events caused by the MSC therapy (Cohen et al., 2017).

Many studies have been conducted to understand the mechanism of action of MSC. Since MS is initiated by a maladaptive attack of the immune system on the myelin of the nervous system it would make sense to study the therapeutic effects of MSC affecting the immune system. This has been supported by the finding that MSC exhibit immunosuppressive effects and inhibit T cell and B cell proliferation (Trounson & McDonald, 2015). However, more importantly there was direct influence of MSC on the CNS. MSCs modulate the differentiation of progenitor cells (undifferentiated cells) leading more to differentiate into oligodendrocytes (myelin producing cells) and cells expressing the myelin protein and fewer astrocytes (Uccelli et

al., 2011). In summary, MSC are showing promising effects on both the immune system and the CNS. MSC therapy is the first therapy for MS that taps into neural protection and repair.

2.8.3.1 MSC and cognition

The effect of MSC therapy on cognition has been evaluated in only one MS clinical trial since the primary emphasis of these trials has been safety rather than cognitive outcomes. Even in this one study only one test of cognition was administered (the Paced Auditory Serial Addition Test (PASAT)) rather than a comprehensive assessment of cognition (Cohen et al., 2017).

PASAT is a measure of information processing speed and working memory. In 2017, Cohen and colleagues reported performance results on the PASAT at 7 time points before and after the treatment. Even though performance scores were higher at 6 months after the treatment (50.8 ± 8.8) than scores on the date of initial screening (45.3 ± 11.4) it was not a statistically significant improvement (Cohen et al., 2017). This increase is likely due to practice effects associated with the PASAT especially given that there was also an increase in scores from the prescreening session to the baseline session (both occurred before the therapy). The PASAT is criticized for being prone to practice effects (Tombaugh, 2006).

Despite the scarcity of studies investigating the role of MSC on cognitive performance in the MS population there are many studies conducted on other neurological conditions that investigate this matter. Cognitive improvement was observed in animal models as well as human subjects of other neurological conditions such as Alzheimer's disease (AD) and traumatic brain injury (TBI) after being treated with MSC therapy (Cui et al., 2017; Kim et al., 2016; Zhao et al., 2016; Zheng et al., 2017). Zheng and colleagues treated Alzheimer's disease in a mouse model with human amniotic mesenchymal stem cell transplantation. They reported improvement in spatial memory and learning in the treated mice based on their performance in the Morris water

maze and the novel object recognition test (Zheng et al., 2017). The mechanism by which MSC could improve cognition in AD could be due to their role in alleviating oxidative stress and promoting neurogenesis (Cui et al., 2017). Cui and colleagues also reported memory improvement in AD mice models treated with human umbilical cord MSC as seen in the Morris water maze.

In the TBI mice model, mice that were treated with bone-marrow derived MSC required less learning time than the non-treated mice in a fear discrimination task (Zhao et al., 2016). Treated TBI mice spent more time exploring novel objects compared to non-treated mice. Mice intravenously injected with MSC briefly after the induction of TBI showed less impairment in cognitive tasks such as pattern separation (the ability to distinguish between two similar patterns) and spatial memory than non-treated mice. MSC treatment was reported to suppress neuro-inflammation at the site of the TBI (Kim et al., 2016).

Other studies, reported the effects of treating people, with neurological conditions, with MSC therapy on cognition. In 2016, Kota and colleagues treated people with TBI with propranolol followed by MSC. The study showed that MSC increased neurogenesis 7 days after TBI and decreased cognitive and memory impairment (Kota et al., 2016). In another study, 22 people with hypoxic ischemic encephalopathy (a condition caused by oxygen deprivation due to the lack of blood flow into the brain) were treated with human umbilical cord MSC (Xie et al., 2016). There was significant cognitive improvement measured by the Mini-mental State Examination reported in the MSC treated group (Xie et al., 2016). Both anxiety and depression were significantly lowered 2 weeks after MSC therapy although there was no significant difference between the treatment and non-treatment groups after 30 weeks (Xie et al., 2016). Wang and colleagues conducted a human trial to evaluate MSC's ability to improve neurological

function in people with TBI (Wang et al., 2013). People with TBI treated with MSC showed significant improvement in motor, communication and social cognition scores on the Functional Independence Measure six months after the treatment compared to the control group (Wang et al., 2013). Thus, previous studies, examining neurological conditions other than MS, reported therapeutic benefits of MSC therapy on cognition.

Although the previously mentioned studies strongly suggest positive effects of MSC therapy on cognition, studies that include comprehensive neuropsychological testing are scarce. In addition, to our knowledge, neuropsychological evaluation was not conducted in studies investigating MSC therapy for MS. Despite the fact that cognitive enhancement was observed among people with TBI after MSC therapy the same results might not necessarily be achieved in MS (Wang et al., 2013) given the different pathological mechanisms of the two conditions. Thus, cognition should also be measured in PMS treated with MSC therapy because people with an inflammatory disease like MS might not experience cognitive enhancement as has been demonstrated in people with TBI (Cohen et al., 2017).

2.9 Transcranial magnetic stimulation for MS

Transcranial magnetic stimulation (TMS) is a non-invasive technique that can evaluate the integrity of neural motor pathways (Robert Chen et al., 2008). TMS allows for the extraction of a number of measures that estimate the neural pathway although only two will be used in this thesis. The first measure is central motor conduction time, which estimates the conduction time from the primary motor cortex to the spinal motor neurons (Kobayashi & Pascual-Leone, 2003). Central motor conduction is measured by subtracting the time the signal takes to travel from the spinal cord to the target muscle from the time the signal takes to travel from the motor cortex to the same muscle (Kobayashi & Pascual-Leone, 2003). The second measure of conduction time is

the latency of transcallosal inhibition which follows the signal from the cortex to the target muscle. To measure transcallosal inhibition, a single TMS pulse is administered to the head which causes a period of inhibition, or a silent period (Codecà et al., 2010). This inhibition is evident in both the contralateral and the ipsilateral muscles. In order to achieve the ipsilateral inhibition, the signal has to travel through the corpus callosum. The delay until the signal is received in the ipsilateral muscle is called the latency of transcallosal inhibition (LTI). PMS have longer LTI than healthy individuals (Chen, Yung, & Li, 2002).

Pathologies caused by MS such as demyelination and axonal loss can be detected using measures derived from TMS (Kale, Agaoglu, Onder, & Tanik, 2009). In fact, central motor conduction time can be used as a diagnostic and prognostic measure of MS (Humm et al., 2003). In MS, central motor conduction is altered in two different ways. First, prolongation of conduction time (i.e., an increase in the time required for a signal to travel from the cortex to the muscle) as a result of the search for adaptive pathways (alternative signaling routes) (Lubetzki & Stankoff, 2014). Second, conduction deficit, where a signal is not transmitted due to the loss of conducting axons or due to conduction block (Humm et al., 2003).

To summarize there are neural signaling deficits in MS. TMS offers measures of signaling conduction time that allows for the quantification of the neural deficits caused by MS. Information processing speed is also impacted in MS. Information processing depends primarily on the transmission of the signal between neurons which could be similar to the conduction of neural signals measured in TMS.

This thesis will explore whether there is a relationship between information processing speed and signaling conduction time as measured in TMS. Khedr, Ahmed, Darwish, and Ali (2011) showed that cognitive impairment, measured using the Mini-Mental State Examination

(MMSE), in patient with Alzheimer's disease was correlated with transcallosal inhibition (Khedr et al., 2011). This thesis will specifically test information processing speed rather than general cognitive performance. The reason for choosing information processing speed is that TMS measures the speed of information conduction by measuring the time the neural signal takes to travel from the cortex to the muscle.

3 Rationale

PMS experience cognitive impairment that can hinder their daily life activities such as running a household, participating in the community and maintaining employment (Chiaravalloti & DeLuca, 2008). Research has shown that more than one cognitive area can be compromised due to MS. The most severely impacted areas of cognition are executive function, visual learning and memory, information processing speed, working memory and attention (Chiaravalloti & DeLuca, 2008). Deficits in these areas, if severe enough, could potentially hinder one's ability to make decisions and live an independent life.

Cognitive impairment is associated with the underlying pathologies of the disease. Brain atrophy including grey matter lesions, thalamic volume loss and thinning of the cerebral cortex are associated with cognitive impairment (Benedict & Zivadinov, 2011). Associations are also found between white matter T1 and T2 lesions and cognitive deficits (Rocca et al., 2015). Furthermore, loss of brain connective tracts and networks such as the default brain network and attentional networks are linked to cognitive deficits during the early stages of MS (Louapre et al., 2014).

Secondary disease characteristics such as mood dysfunction and fatigue are prevalent in PMS and impact quality of life, work productivity, pain and cognition (Feinstein et al., 1992), (Glanz et al., 2012; Lobentanz et al., 2004; Marck et al., 2017). Depression and social distress

impair cognition more so than neurological symptoms (Feinstein, Kartsounis, Miller, Youl, & Ron, 1992; Gilchrist & Creed, 1994).

Many treatments have been approved and prescribed to PMS. Most of these disease-modifying therapies target the relapsing phase of the disease leaving those in the progressive phase with very few treatment options. As the phenotype of the disease changes from the relapsing to the progressive phase, less inflammation and more neural damage takes place. The current therapies are effective in lowering the annual relapse rate as well as slowing the progression of disability caused by the disease through their anti-inflammatory and immune-modulating mechanisms. However, these mechanisms are not effective in the progressive stage of the disease since neural damage, rather than inflammation, becomes more prevalent.

New emerging therapies are being tested aiming for neural repair as one of the potential outcomes. The Mesenchymal Stem Cell Therapy for Canadian MS Patients (MESCAMS) study is part of the International Mesenchymal Stem Cell Transplantation Study Group (IMSTSG) a phase II study to evaluate the safety, efficacy and the potential benefits of MSC therapy. There is research literature supporting the role of MSC therapy in improving cognitive performance in neurological conditions such as Alzheimer's disease and traumatic brain injury. The underlying pathology of these neurological conditions is different from MS; hence the critical importance of assessing the impact of MSC therapy on cognition in those with MS. If cognitive improvement can be documented in this population then it could potentially serve as a proxy measure of neural repair.

In this study, people with MS were treated with MSC therapy. In contrast to immunoablative hematopoietic stem cell transplantation, MSC therapy does not involve chemotherapy which is the presumed cause for cognitive deficits soon after the immunoablative

hematopoietic stem cell transplantation. In contrast, neural repair is one of the predicted outcomes of MSC therapy. If neural repair occurs, then cognitive improvement may serve as a marker of this underlying repair. Therefore, neuropsychological assessments, based on the previously validated MACFIMS battery (Benedict et al., 2006), was administered to PMS before and after the treatment in order to measure any change in cognition resulting from MSC therapy. Based on findings in other neurological conditions, it is hypothesized that cognitive improvement will be demonstrated between baseline and follow-up. Levels of cognitive performance will be determined at baseline and follow-up to monitor change and to determine the level of impairment present at both time points. If, as predicted, MSC therapy helps neural repair, then participants with more impairment at baseline are likely to show more improvement at follow-up. Given that this is the first study of its kind to investigate cognition after MSC therapy in PMS, the study should be considered exploratory. As such, all cognitive domains will be evaluated. It is expected that attention/information processing speed would be the domain most likely to benefit from the therapy. In MS, lesions are dispersed in the CNS causing deficits in the signal conduction. If MSC therapy results in neural repair, as expected, then signal transmission between neurons will be enhanced causing the signal to travel faster. Signaling speed should be detected using tests of information processing speed given that it is the domain that is most affected by signal transmission.

In patients with MS, the latency of transcallosal inhibition is longer than healthy controls possibly due to loss of myelination. Thus, if the latency of transcallosal inhibition is decreased after MSC therapy this may be a marker of remyelination and neural repair. Similarly, neural repair may also be reflected in better cognitive performance. Therefore, it is expected that if

cognitive improvement occurs it should be correlated with shorter latencies of transcallosal inhibition.

3.1 Hypotheses

3.1.1 Primary Hypothesis

Treatment with MSCs will enhance cognition in one or more cognitive domains in PMS. As discussed above, information processing speed and transcallosal speed are expected to improve.

3.1.2 Secondary Hypothesis

A negative relationship is expected between secondary disease characteristics (i.e., anxiety, depression, fatigue) and cognition. Greater mood dysfunction and fatigue is expected to be related to lower performance on cognitive tasks.

3.1.3 Exploratory Hypotheses

A positive correlation between the latency of measures of conduction time (latency of transcallosal inhibition and central motor conduction time) and information processing speed will be detected (i.e., shorter conduction time will be associated with faster processing speed). If speed of information processing is enhanced by MSC therapy then a similar positive correlation will be observed between both latency of transcallosal inhibition and central motor conduction time and processing speed as well. This relationship will be examined at baseline and follow-up.

4 Methods

Studies with large sample sizes are required to draw conclusions on the safety and the efficacy of MSC therapy in PMS. However, only a few participants can be enrolled per center because of the labour-intensive burden of such a study and because the safety of the procedure has yet to be established. Therefore, an international consensus on appropriate procedures has

been reached by the International Mesenchymal Stem Cells Transplantation Study Group (IMSCTSG) (Freedman et al., 2010). Eight countries around the world are participating in the same study and are following a consensus-approved protocol outlined in the Mesenchymal Stem Cells for Multiple Sclerosis for Clinical Trials. Having multiple sites will expedite the testing phase since proliferating the mesenchymal stem cells takes 1 month/participant. These clinical trials are double-blind, randomized, with a cross-over for a period of 12 months utilizing an “early” vs. “delayed” treatment paradigm (Figure 2).

The Canadian branch of this international initiative is called the MEsenchymal Stem cell therapy for CANadian MS patients (MESCAMS). In Canada, there are two centers where the study is taking place. One is in Winnipeg led by Dr. James Marriott and the other is at the Ottawa Hospital MS clinic led by Dr. Mark Freedman. The current thesis utilized data from the Ottawa site. The portion of this study associated with the current thesis project has been approved by both the Ottawa Health Science Network Research Ethics Board and the Carleton University Research Ethics Board. The larger study protocol has been approved by the appropriate research ethics boards at each site.

4.1 Participants

Twenty PMS with ages between 18-50 years who are capable of and willing to sign the consent form were enrolled in MESCAMS. Participants were recruited through patient visits to the MS clinic at The Ottawa Hospital. The MESCAMS study runs for a longer time than the time anticipated for the completion of this thesis. Therefore, only participants that completed week 48 assessment by March 2018 were included in this thesis. On this basis, this thesis included 10 participants. PMS with disease duration of 2-10 years and an Expanded Disability Scale Score (EDSS) score between 3.0 and 6.5 were included in MESCAMS. This is to ensure that

participants are still in the active phase of the disease which is more responsive to treatments compared to the latter phases of the disease (after 10 years of the disease onset). The study enrolled those living with relapsing-remitting MS, primary progressive MS, and secondary progressive MS. In order to be included, participants' medical history must show that they did not respond to any clinically proven therapies for the past year. There are inclusion criteria specific to each subtype of the disease Appendix B.

Exclusion criteria were based on unmet conditions of the inclusion criteria or any risk of increasing a co-morbid disorder or increasing mortality. Exclusion criteria included a history of a co-morbid disorder such as active or chronic infection of HIV1-2 or chronic hepatitis B or hepatitis C. PMS who received a treatment with immunosuppressive drug within the past 3 months were excluded (if they are willing to stop taking the drug then they are allowed to enroll 3 months after the drug termination date). PMS who received treatment with cladribine or alemtuzumab were also excluded. Those treated with disease modifying therapies (such as; interferon-beta, glatiramer acetate, teriflunomide, dimethyl fumarate) or corticosteroids within the past 30 days or had a relapse within the past 60 days were excluded. Excluding those treated with disease modifying therapies allows for interpreting the anticipated improvements to MSC therapy rather than the disease modifying therapy.

Those who are unable to provide a written informed consent were excluded. If a participant was taking part in another clinical trial he/she was excluded from this trial if both trials are simultaneous. In addition, according to the neurologist's medical decision, if a participant was unable to follow the instructions of the trial protocol he/she was also excluded. In other words, the neurologist can exclude a participant if he/she shows a history of not following doctor's orders and not adhering to medical treatments (e.g., taking their drugs on time).

4.2 Study design

In this double-blind phase II clinical trial participants were randomly assigned into two arms of the study. In arm 1, participants received the treatment by infusion at week 0 where in arm 2 participants receive sham infusion at week 0. At week 24, a cross-over in the treatment took place where those who received the sham originally (arm 2) received the treatment and arm 1 (received treatment originally) received the sham (Figure 2). All participants returned for a follow up visit at week 48. This design allows for all participants to receive the treatment while maintaining a control group. It allowed for comparing between those that received a treatment at week 0 and those that received the sham to explore the effects of the treatment. This study design also entailed an extended follow up condition where patients that received the treatment at week 0 will be monitored for 48 weeks rather than 24 weeks.

Figure 2. Study design and the flow of patients in each arm.

4.3 Treatments

Given the cross-over design, participants received one treatment and one placebo within the course of the trial. The packaging of both the treatment and the placebo was identical and included the participant's randomization number. The investigational product guidelines provided by the Therapeutics Products Directorate of Health Canada were followed.

- Mesenchymal Stem Cells: in Plasma-Lyte A (Baxter) suspension media, containing 5% Human Albumin and 10% dimethylsulfoxide (total volume of 5mL dimethylsulfoxide in final cell product) and autologous MSCs at a dose of 1 to 2×10^6 MSC/Kg participant's body weight at randomization
- Matching placebo: Plasma-Lyte A (Baxter) suspension media, containing 5% Human Albumin and 10% dimethylsulfoxide (total volume of 5mL dimethylsulfoxide in final cell product)

Participants were randomly assigned into an early treatment arm or a delayed treatment arm. In the early treatment arm, participants received a single dose of autologous MSCs at a concentration of 1 to 2×10^6 MSC/Kg through intravenous (IV) infusion at week 0 and a sham IV infusion after 24 weeks. In the delayed treatment arm, participants received sham IV infusion at week 0 and a single dose of autologous MSCs at a concentration of 1 to 2×10^6 MSC/Kg IV infusion after 24 weeks.

The MESCAMS study was not completed before the submission of this thesis. Thus, the blind was not broken and those who received early vs. those who received late treatment were not identified in this thesis.

4.4 Procedures

Signed informed consent were obtained from all participants before any assessment or test was conducted. All participants enrolled met all the inclusion criteria and did not meet any of the exclusion criteria. Randomization took place after a signed consent form was obtained. An on-line tool was made available to the authorized researcher by the contract research organization in charge in order to randomly assign participants to the early vs. delayed treatment

arms. This was a double-blinded trial where all of the participants, staff, as well as the treating neurologist are blinded to study arm until after data analysis is complete.

Participants' recruitment and the cell harvest and infusion procedures were carried out by the MS clinic at The Ottawa Hospital and the lab facilities associated with the study respectively. Neuropsychological assessment administration, scoring and analysis were carried out by Maha Abu-AlHawa.

4.4.1 Cell harvest, expansion and infusion

Eight weeks prior to the first infusion, the bone marrow was harvested in accordance with the clinical practice guidelines. About 20 ml of bone marrow was harvested from the posterior–superior iliac spine.

MSCs were isolated from the bone marrow in accordance with the international approved protocol (i.e., the Mesenchymal Stem Cells for Multiple Sclerosis - clinical trials). The isolated MSCs were then expanded ex vivo for a period of eight weeks; required for the optimal dose and quality control. The cell therapy facility prepared two identical looking suspensions, that is, a treatment suspension and a placebo suspension. The appropriate suspension was sent according to the randomization schedule.

At the scheduled visits participants were assessed for vital signs and adverse events. The planned assessments took place according to the schedule of the trial.

4.4.2 Cognitive Assessments

In this study, neuropsychological assessments were administered to participants at week 0, week 24 and week 48. For the purposes of this Master's thesis project, only data from weeks 0 and week 48 were analyzed in order to ensure that all participants received the active treatment. A pre-treatment assessment was administered to all participants at week 0 before infusion took

place. At week 24, all participants were assessed using alternate forms of the neuropsychological assessments where applicable. At week 48, a third assessment was administered as a follow up for all participants (alternate forms when available). For participants in arm 1, this assessment was a 12-month follow-up post-treatment. As for participants in arm 2 of the study, the week 48 assessment was a 6-month follow-up post-treatment.

The neuropsychological assessment administered was a comprehensive battery based on the MACFIMS battery (Benedict et al., 2006). The assessment included tests that assess cognitive areas commonly affected by MS. The tested areas of cognition were: estimated pre-morbid ability (a potential proxy measure of cognitive reserve), information processing speed, working memory, visual perceptual abilities, learning and memory, and executive functioning. The neuropsychological assessment also included self-report questionnaires (demographics questionnaire, Modified Fatigue Impact Scale and Hospital Anxiety and Depression Scale) (Table 1).

Table 1

Neuropsychological assessment battery

Cognitive function	Neuropsychological assessment	Time (min)	Source
Pre-morbid ability/ cognitive reserve	Wechsler Test of Adult Reading (WTAR)	10	(Wechsler, 2001)
Information processing speed	Symbol Digit Modalities Test (SDMT)	5	(Rao, 1990)
	Paced Auditory Serial Addition Test (PASAT)	10	(Gronwall, 1977)
Working memory	Auditory Constant Trigrams (Based on the Brown-Peterson task)	10	(Brown, 1958) & (Peterson & Peterson, 1959)
Visual perceptual abilities	Judgment of Line Orientation	5	(Benton, 1994)
Learning and memory	California Verbal Learning Test-II	15	(Delis D. C., Kramer J. H., Kaplan E., 1987)
	Brief Visuospatial Memory Test-Revised	15	(Benedict, 1997; Benedict, Schretlen, Groninger, Dobraski, & Shpritz, 1996; Benedict & Groninger, 1995)
Executive Functioning	Delis-Kaplan Executive Function System (D-KEFS)		(Delis, Kaplan, & Kramer, 2001)
	• Sorting Test	10	
	• Color-Word Test	10	
	• Verbal Fluency Test	10	
	Trail Making Test	5	(Reitan, 1955)
Questionnaires	Demographic Questionnaire	10	
	Modified Fatigue Impact Scale	5	(Multiple Sclerosis Council for Clinical Practice Guidelines, 1998)
	Hospital Anxiety and Depression Scale Multiple Sclerosis	5	(Zigmond & Snaith, 1983)
Total time		125	

4.4.2.1 Pre-morbid ability

Pre-morbid ability estimates cognition prior to any acquired brain dysfunction. Neuropsychological assessments compare between pre-morbid ability (prior to a neurological condition) and the current cognitive performance to estimate the relative cognitive change post a neurological condition (Berg, Durant, Banks, & Miller, 2016). Pre-morbid ability was tested using the Wechsler Test of Adult Reading. This test requires correct pronunciation of 50 phonetically irregular words (e.g., cough and subtle). Performance on this measure correlates highly with intelligence.

4.4.2.2 Information processing speed

Two tests were used to assess information processing speed: the Symbol Digit Modalities Test (SDMT) and the Paced Auditory Serial Addition Test (PASAT). For the SDMT, participants were given a sheet of paper with a coding key at the top of the page where symbols are paired with numbers. The rest of the page contained symbols only and the participant was asked to say out loud the number that should be paired with each symbol. Since this was a speeded task the participant were required to say the numbers as fast as they can. The examiner wrote down the participant's responses and terminated the test after 90 seconds. The oral version of the test was used rather than the written in order to ensure MS-related mobility impairments did not impede performance. The SDMT is assumed to measure information processing speed. The total correct score is the outcome used to evaluate performance on this task.

For the PASAT, the participant listened to an audio recording of numbers presented at 3 second intervals for the first 60 numbers followed by a brief break. The next 60 numbers were presented at 2 second intervals. After each number, the participant was asked to orally add the last number they heard to the one before it and the examiner wrote the responses. For example, if

the participant heard 3 - 4 they would respond 7. If the next number was 5 they would respond 9. This task requires information processing speed, working memory and basic math skills. The total correct score is the outcome used to evaluate performance on this task and was used in the data analysis.

4.4.2.3 Working memory

Working memory in this battery was assessed using the Auditory Consonant Trigrams test (ACT). ACT is a modified version of the Brown-Peterson Task which assesses working memory under conditions of interference. In this task, the participant heard three consonant letters followed by a number (e.g., S G N 95). The participant then counted backwards in increments of three from that number (e.g., 92, 89, 86...). After a delay of 3 seconds, 9 seconds, or 18 seconds the participant responded with the three letters mentioned before the number. The task was scored based on the number of correct consonants relayed. The mathematical task was only the distractor task and was not scored. The total correct score is the outcome used to evaluate performance on this task and was used in the data analysis.

4.4.2.4 Visual perceptual abilities

Judgement of Line Orientation was used to assess visual perceptual abilities. Participants were presented with a vertically-oriented spiral-bound booklet with a stimulus on the top page and a key figure on the bottom page. The key figure shows 11 numbered lines separated by 18 degree angles and the stimulus page shows two lines that were not numbered. The participant's task was to relay the corresponding number of these two lines based on the numbered lines on the adjacent page. The total correct score is the outcome used to evaluate performance on this task and was used in the data analysis.

Figure 3. A sample of the Benton Judgement of Line Orientation Test.

4.4.2.5 Learning and memory

Both verbal and visual learning and memory were assessed by the California Verbal Learning Test second version (CVLT-II) and the Brief Visuospatial Memory Test revised version respectively (BVMT-R). In the CVLT-II, the examiner read out loud 16 words from 4 different categories (e.g., animals, transportations, vegetables and clothing items) mixed in order. Participants were asked to repeat as many of the words as they can remember regardless of the order. After a short delay, during which participants were asked to repeat a second list of words different from the first one, participants were required to recall as many words from the first list as they can without mentioning any from the second list. Then participants were told to recall all the words that fall within each category (i.e., cued recall). A long delay with both free and cued recall were administered approximately 20 minutes later. Lastly, a recognition task was administered where the examiner read a fixed list of 40 words that included the 16 words from the first list as well as distractor words and the participant had to identify the target words. The total correct score across all five learning trials is the outcome used to evaluate learning on the

CVLT-II and the total delayed recall score was used to evaluation memory. Both scores were used in the data analysis.

In the BVMT-R, participants were presented with a one-page display with 6 simple geometric figures on it for 10 seconds (Figure 4). The display was then removed and participants were asked to draw the figures accurately and in their correct location. The task was repeated 3 times. After a delay of 20 minutes participants were asked to draw the figures from memory. Finally, a recognition task was performed where participants were presented with 12 figures, one at a time, including the 6 from the display and 6 similar figures. The participant's task was to identify the figures that were on the original display. The total correct score across the three learning trials is the outcome used to evaluate learning on this task and the total delayed recall scores was used to evaluate memory. Both scores were used in the data analysis.

Figure 4. A sample of the Brief Visuospatial Memory Test – Revised

4.4.2.6 Executive function

Three subtests from the Delis-Kaplan Executive Function System (D-KEFS) were administered to evaluate executive functions. In the Verbal Fluency subtest, for 60 seconds participants were asked to say as many words as they can that begin with a specific letter (i.e., phonemic fluency). This was repeated for 3 different letters. The next part of the task required participants to mention as many words as they can that belong to one category (e.g., boys' names) (i.e., semantic fluency). The third part of the task entailed alternating between two

semantic categories (e.g., fruits, furniture). The total correct scores for phonemic fluency, semantic fluency and category switching were used to evaluate performance on these tasks and were used in data analysis.

The second D-KEFS test was the Sorting task. In this task participants were presented with a set of 6 cards. Each set can be sorted in many different ways (e.g., shape, color, etc.). Participants were asked to sort the cards into two groups with three cards in each group. After each sort, they were encouraged to find another way to sort the cards. The task continued until the participant cannot think of any further sorting strategies. The task was then completed with a second set of cards. The total confirmed correct sorts and the total correct descriptions were the outcome scores used to evaluate performance on this task and were used for the data analysis.

The last D-KEFS task was the Color-Word interference task (a modified Stroop task). This test entailed four displays. In the color condition various color patches (red, blue, green) were printed in pseudorandom order in horizontal lines on the card and the participant must name the colors as quickly as they can. In the word condition, the color words (red, blue, green) were written in black ink and the participant must simply read the words. In the interference condition the words were written in contrasting ink colors (e.g., the word red was written in green ink) and the participant must name the ink color rather than read the word. The last display was the same as the third where the ink color was typically relayed except when the participant encountered a word inside a square. In that circumstance, the word is read rather than naming the ink color (i.e., switching condition). The time required to complete each of the conditions are the outcomes used to evaluate performance on this task and were used in the data analysis.

The Trail Making test was another executive measure (but also measures attention and processing speed), in which the participant was presented with a sheet with numbered circles placed randomly. The task was to connect the circles according to their number from 1-23 (Trails A). In the second part of the task the sheet contained both numbers and letters (Trails B). Participants are asked to connect the circles by alternating between numbers and letters (e.g., 1-A, 2-B, 3-C). The time required to complete Trails A and Trails B were used to evaluate performance on these tasks and were used in data analysis.

4.5 Secondary disease characteristics

The Modified Fatigue Impact Scale (Fisk & Doble, 2002) and the Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983) were administered at each neuropsychological assessment at baseline and follow-up. The Modified Fatigue Impact Scale (total score) was used to assess fatigue and the Hospital Anxiety and Depression Scale (total scores for each subscale) was used to assess anxiety and depression in this sample.

4.6 Exploratory studies

Ethics permission to use TMS data (i.e., transcallosal connectivity) was obtained. Values of transcallosal connectivity measured by transcallosal magnetic stimulation were obtained from Dr. Francois Tremblay (Bruyere Continuing Care). Dr. Tremblay and his team recorded the TMS measures at baseline, week 24 and week 48 in order to evaluate the neural pathways and detect if any improvement occurred after MSC therapy.

Ipsilateral silent period (iSP) is a measure of TMS used to evaluate interhemispheric connectivity (Davidson & Tremblay, 2016). Latency of transcallosal inhibition (LTI) is an index of the speed of communication between the two hemispheres which can be derived from the iSP.

Central motor conduction time measures the time required for a neural signal to travel within the central nervous.

5 Analysis

5.1 Descriptive analyses of cognitive performance at baseline and follow up

The sample of MS participants was examined to characterize the demographics of the sample. Frequencies were calculated with respect to: age, sex, education, employment, disease duration, EDSS score, and disease subtype.

The sample was examined to determine if cognitive impairment (at least 1.5 standard deviations below the normal control mean) exists at both baseline (week 0) and follow up (week 48). Cognitive performance was examined on all the tests of the neuropsychological battery administered. Participants were considered to be cognitively impaired if they demonstrated impairment on at least two neuropsychological measures. Since the MESCAMS study is double-blind and the completion date will be later than the submission date of this thesis, participant's week 24 data were not analyzed to ensure that all participants have received the active treatment. The study design entails that participants are randomly assigned to a treatment group (early treatment group vs. late treatment group) the early treatment group received the treatment at week 0 and the late treatment group received the treatment at week 24. Participants and researchers were blinded to the group assignment. As such, it is unknown to the researchers which participants had received the treatment at week 24 and which had not. Therefore, cognitive performance at week 0 was compared to cognitive performance at week 48 to ensure that the data analysis examined cognition prior to and after the treatment.

5.2 Primary analysis

In order to evaluate cognitive performance between baseline and follow up reliable change indices that account for practice were applied (Walker et al., 2014). Individual change would potentially be masked and practice effects (inherent to serial neuropsychological testing) could lead to misinterpretation of the results if pre-post group within-group analyses were applied.

$$\text{Reliable change index} = \frac{(\text{time 2} - \text{time 1}) - \text{practice}}{SE_{\text{diff}}}$$

$$\text{Practice} = \text{mean of time 2} - \text{mean of time 1}$$

$$SE_{\text{diff}} = \sqrt{(2 * SE_m^2)}$$

$$SE_m = SD1(\sqrt{[1 - r_{xx}]})$$

Time 1 is the variable raw score at baseline, time 2 is the raw score at follow up. The practice for each variable is the mean difference between follow-up and baseline scores of the normative sample (Shilling, Jenkins, Morris, Deutsch, & Bloomfield, 2005). SE_{diff} is the standard error of difference which represents the spread of the distribution of change scores expected had no change occurred. The standard error of the measurement (SE_m) of the particular tool (e.g., SDMT) was obtained to calculate the standard error of the difference (Shilling et al., 2005). In order to calculate the standard error of the measure, the reliability coefficient (r_{xx}) of the measure and the standard deviation (SD) of the variable (e.g., SDMT raw score) at time 1 are required (Shilling et al., 2005).

Reliable change values are considered significant at 90% confidence intervals if the reliable change index value lies outside of +/- 1.64 (Shilling et al., 2005; Walker et al., 2014). Cognitive improvement can be inferred if the reliable change index score was greater than 1.64

while a reliable change index score of less than -1.64 implies the occurrence of cognitive decline. In other words, if the reliable change index value lies outside of ± 1.64 then the variance in the measurement alone does not explain the change of scores observed (i.e., change can be attributed to the treatment). Reliable change coefficients and control means were obtained from the sources listed in Table 2.

Table 2

Reliable change indexes, sources of test-retest correlation coefficient values

Test	Sources
Trail Making test	(Levine, Miller, Becker, Selnes, & Cohen, 2004)
BVMT	(Benedict, 1997)
CVLT-II	(Delis, Kramer, Kaplan, & Ober, 2000)
PASAT	(Berard, Smith & Walker, 2018)
D-KEFS Sorting	(Delis, Kaplan, & Kramer, 2001)
D-KEFS Verbal	(Delis, Kaplan, & Kramer, 2001)
D-KEFS color-word	(Delis, Kaplan, & Kramer, 2001)
JOLO	(Berard, Smith & Walker, 2018)
SDMT	(Smith, 2002)
ACT	(Abu-AlHawa, Berard, Osman, Gresham, Walker, 2016)
Depression and anxiety	(Zigmond & Snaith, 1983)

5.3 Secondary analyses

5.3.1 Depression and anxiety

Characterization of depression and anxiety at baseline was conducted using cut-off scores. Cut-off values for anxiety and depression were obtained to identify participants with clinically meaningful levels of depression and anxiety (Watson, Ford, Worthington, & Lincoln, 2014). Participants scoring 11 or more on each of the anxiety and depression sections of the

Hospital Anxiety and Depression Scale were considered anxious and depressed. Change in levels of depression and anxiety was examined at both the group level using pair wise t-test analysis and the individual level using reliable change analysis.

The results of the cognitive RCI analyses and the mood RCI analyses were examined to determine whether those who demonstrated cognitive decline also demonstrated mood decline, and those who demonstrated cognitive improvement also demonstrated mood improvement after the therapy. Also, Fisher's Exact Test was used to investigate whether participants with cognitive impairment also showed impairment in mood. In addition, Pearson's correlation was used to examine if the change over time in mood is related to the change over time in cognitive performance in each cognitive domain. Furthermore, Pearson's correlation was conducted to determine if there is a relationship between mood and each of the cognitive tests at both baseline and follow-up.

5.3.2 Fatigue

The change in fatigue from baseline to follow-up was examined using a paired sample t-test. The relationship between changes in fatigue and cognition from baseline and follow-up was examined using Pearson correlation analysis. The analysis compared between fatigue difference scores and the difference scores in cognitive performance per domain. The relationship between fatigue and the individual cognitive tests was also examined using Pearson's correlation.

5.4 Exploratory analyses

The change of latency of transcallosal inhibition and central motor conduction time from baseline to follow-up was examined using paired sample t-tests. Impairment in central motor conduction time (CMCT) was identified at baseline and follow-up. The normal range of CMCT is 7.4+/- 1.5, therefore, scores higher than 8.5 indicated impairment in CMCT. The results of the

RCI analysis in cognition and the levels of impairment in CMCT were examined to determine whether CMCT and cognition were similarly impacted by the therapy. In other words, to examine whether both cognition and CMCT improved after the therapy or both CMCT and cognition declined after the therapy.

To test the relationship between neural-motor variables and information processing speed, Pearson's correlation analyses were used. The neural-motor measures used were: latency of transcallosal inhibition and central motor conduction time. Information processing speed was measured using the following tests: Trail Making Test, D-KEFS Color-Word Interference test, and Symbol Digit Modalities Test at baseline and follow-up (week 48). In addition, Pearson correlation of the difference scores were conducted to examine whether information processing speed and conduction time change in a similar manner after the therapy.

6 Results

6.1 Demographics

Within the sample of 10 PMS there were 4 participants with relapsing remitting MS, 4 with secondary progressive MS and 2 with primary progressive MS. The sample consisted of 3 males and 7 females with the mean age of 36.30 ($SD = 5.68$) years at week 0. The ages ranged between 28 and 43 years. Mean education was 13.9 ($SD = 2.33$) years ranging from 9 to 16 years. The average standard score of the premorbid ability test (Wechsler's test for Adult Reading) was 100.9 at baseline. There were three employed participants. Two of them were working full-time and one was working part-time. Seven participants were unemployed. Six out of the 7 were unemployed because of an MS-related disability and one participant was unemployed by choice. The mean disease duration was 5.60 ($SD = 1.71$) years with a minimum of 2 and a maximum of 8 years. The mean Expanded Disability Status Scale score was 4.75

($SD= 1.23$) with a minimum of 3.00 and a maximum of 6.50. An EDSS score of 4.75 lies between EDSS scores of 4.5 and 5.0. A score of 4.5 indicates significant disability but the person maintains the ability to work a full day with minimal assistance and the ability to walk without aid or rest for 300m. An EDSS of 5.0 indicates that the disability is severe enough to impair full daily activities and the person is only able to walk without aid or rest for 200m (Kurtzke, 1983).

6.2 Cognitive Impairment

Nine out of ten patients were impaired on at least two tests at baseline while one patient (MS05) was only impaired on one test at baseline (Table 3). Thus, patient MS05 does not meet criteria for cognitive impairment (z-score less than -1.5 in at least two tests) and is considered cognitively intact at baseline. Seven out of ten patients were impaired at follow-up. Patients MS02 and MS08 were impaired at baseline but were not impaired on any test at follow-up.

Attention/information processing speed and memory are the two domains with the highest rates of impairment (9 of 10 participants) and (8 of 10 participants) respectively in our sample followed by learning (5 of 10 participants) executive functions (4 of 10 participants), language (3 of 10 participants) and visual-spatial abilities (2 of 10 participants).

Table 3

Standard scores (z scores) on neuropsychological measures.

Domain	Patient					
	MS01		MS02		MS03	
	T1	T3	T1	T3	T1	T3
Attention/Information Processing Speed						
Trails A	-4.80	-10.25	0.15	0.75	0.39	0.62
Trails B	-6.55	-13.74	0.76	0.94	-0.35	-1.00
PASAT 3 sec	-1.90	-0.87	0.58	0.99	0.47	0.78
PASAT 2 sec	-2.29	-2.10	1.14	1.04	-0.43	0.74
SDMT	-3.53	-4.80	-1.26	-1.23	-1.68	0.61
Color naming	-2.65	-3.00	-1.00	-0.35	-0.35	0.00
Word reading	-3.00	-3.00	-1.00	-0.65	0.00	0.35
ACT 9 sec	-2.38	-2.14	0.00	0.32	-2.78	-1.09
ACT 18 sec	-1.45	-3.47	0.16	0.77	-0.80	-1.54
Visual-Spatial Abilities						
JOLO	-0.55	-2.01	-3.08	-1.13	-0.64	0.74
Learning						
BVMT-R total score	-0.62	-1.43	1.62	-0.91	-0.98	-0.34
CVLT-II total score	0.30	-0.60	0.50	1.00	-1.40	-1.50
Memory						
BVMT-R delayed recall	-0.32	-0.80	1.25	-1.14	-0.01	-1.23
CVLT-II short delay free recall	-0.50	-1.00	-1.50	0.50	-3.50	-2.00
CVLT-II long delay free recall	0.00	-1.00	-2.50	-0.50	-3.50	-1.50
Executive Function						
Trails difference between A and B (B-A)	-3.20	-6.63	0.61	0.44	-0.21	-0.75
D-KEFS verbal fluency: Letter fluency	-2.35	-2.65	0.65	1.65	-1.35	0.65
D-KEFS verbal fluency: Category switching total	-1.35	-1.35	-0.65	1.35	0.65	-0.65
D-KEFS verbal fluency: Switching accuracy	-0.35	-1.00	-0.35	1.35	1.35	-1.00
D-KEFS Sorting: confirmed correct sorts	0.35	-0.65	1.35	0.65	0.00	1.35
D-KEFS Sorting: free sorting description scores	0.35	-0.65	1.35	1.00	-0.65	1.35
D-KEFS Color-Word: Inhibition	-2.65	-3.00	0.35	0.00	-0.65	-0.65
D-KEFS Color-Word: Inhibition/switching	-2.35	-3.00	-0.65	-0.35	-1.00	-1.35
Language						
D-KEFS verbal fluency: category fluency	-1.35	-1.65	0.35	0.65	0.00	0.35

Domain	Patient					
	MS04		MS05		MS06	
	T1	T3	T1	T3	T1	T3
Attention/Information Processing Speed						
Trails A	-5.30	-5.40	-0.14	-0.04	-5.58	-5.00
Trails B	-3.75	-5.27	0.33	0.15	-9.73	-9.17
PASAT 3 sec	-0.76	-0.45	0.89	0.99	-2.05	-1.62
PASAT 2 sec	-2.39	-1.22	0.35	1.53	-2.09	-1.16
SDMT	-2.61	-3.07	-0.20	-0.77	-3.63	-3.94
Color naming	-2.65	-3.00	-1.35	-1.65	-1.65	NA
Word reading	-2.65	-3.00	-2.65	-3.00	-2.65	NA
ACT 9 sec	-1.59	-1.79	0.00	-0.39	-0.91	NA
ACT 18 sec	-1.45	-1.54	-0.48	-0.77	-2.61	NA
Visual-Spatial Abilities						
JOLO	0.33	0.04	0.62	-1.43	-0.65	-0.64
Learning						
BVMT-R total score	-0.82	-0.22	-0.97	-0.49	-4.08	-3.42
CVLT-II total score	-0.60	-1.40	0.30	-0.80	-1.50	-2.65
Memory						
BVMT-R delayed recall	-0.80	-0.26	-0.39	0.23	-3.21	-3.21
CVLT-II short delay free recall	-2.00	-1.00	0.50	-1.00	-1.50	-3.50
CVLT-II long delay free recall	-2.50	-2.00	0.50	-0.50	-2.00	-3.50
Executive Function						
Trails difference between A and B (B-A)	-0.42	-1.73	0.39	0.17	-3.69	-3.58
D-KEFS verbal fluency: Letter fluency	-2.00	-0.65	-1.35	-1.00	-2.35	-3.00
D-KEFS verbal fluency: Category switching total	-0.65	0.00	1.35	2.00	-1.35	-2.00
D-KEFS verbal fluency: Switching accuracy	-0.35	0.35	1.35	2.00	-0.35	-1.65
D-KEFS Sorting: confirmed correct sorts	0.00	0.35	-0.35	-0.65	-1.00	-1.65
D-KEFS Sorting: free sorting description scores	0.00	0.35	-0.35	-0.65	-1.00	-1.65
D-KEFS Color-Word: Inhibition	-2.00	-3.00	0.00	0.35	-0.35	NA
D-KEFS Color-Word: Inhibition/switching	-1.00	-2.35	0.35	0.65	-2.35	NA
Language						
D-KEFS verbal fluency: category fluency	-1.35	-1.00	-0.35	0.35	-2.65	-3.00

* Bolded numbers represent cognitive impairment

Domain	Patient					
	MS07		MS08		MS09	
	T1	T3	T1	T3	T1	T3
Attention/Information Processing Speed						
Trails A	-1.14	-0.14	-2.32	-0.14	0.16	0.50
Trails B	-1.13	-4.66	-1.37	-0.03	-0.02	0.22
PASAT 3 sec	-0.85	-0.85	0.68	0.37	-0.97	-0.14
PASAT 2 sec	-0.95	0.20	0.06	1.24	-1.22	0.45
SDMT	-1.81	-1.12	-1.58	-1.23	-2.50	-0.54
Color naming	-1.00	-0.65	-1.00	-0.35	0.00	0.00
Word reading	-1.00	-0.65	0.65	0.35	0.65	0.65
ACT 9 sec	-3.14	-4.24	0.79	1.02	-1.35	-1.44
ACT 18 sec	-2.26	-4.63	0.80	0.00	1.29	0.00
Visual-Spatial Abilities						
JOLO	-1.43	-1.13	1.50	0.91	0.40	1.09
Learning						
BVMT-R total score	-2.61	-2.00	-2.93	-1.10	-3.32	-2.01
CVLT-II total score	-1.00	-3.00	0.50	0.60	0.90	1.00
Memory						
BVMT-R delayed recall	-0.97	-3.82	-3.68	-0.88	-0.79	-2.08
CVLT-II short delay free recall	-3.00	-4.50	-1.00	0.50	0.50	0.00
CVLT-II long delay free recall	-3.00	-4.50	-0.50	0.50	1.50	0.00
Executive Function						
Trails difference between A and B (B-A)	-0.37	-4.07	0.06	0.06	0.12	0.12
D-KEFS verbal fluency: Letter fluency	-1.35	-1.35	0.65	2.00	1.35	1.00
D-KEFS verbal fluency: Category switching total	-0.65	-1.35	1.35	0.00	0.65	0.65
D-KEFS verbal fluency: Switching accuracy	0.35	-1.65	1.35	-0.35	0.65	1.00
D-KEFS Sorting: confirmed correct sorts	-1.00	-1.35	0.65	1.00	-0.65	-0.35
D-KEFS Sorting: free sorting description scores	-1.35	-1.35	1.00	1.35	-0.65	-0.35
D-KEFS Color-Word: Inhibition	-0.65	0.65	0.35	1.00	1.00	0.35
D-KEFS Color-Word: Inhibition/switching	-1.35	-1.35	0.35	0.65	0.35	1.35
Language						
D-KEFS verbal fluency: category fluency	-1.00	-2.00	1.00	0.65	0.00	0.35

* Bolded numbers represent cognitive impairment

Domain	Patient	
	MS10	
	T1	T3
Attention/Information Processing Speed		
Trails A	-0.30	-0.64
Trails B	-0.67	-0.51
PASAT 3 sec	0.36	0.69
PASAT 2 sec	0.09	0.51
SDMT	-0.75	-0.54
Color naming	0.65	-0.35
Word reading	0.00	0.65
ACT 9 sec	-1.80	-3.24
ACT 18 sec	-1.19	-1.36
Visual-Spatial Abilities		
JOLO	0.74	0.74
Learning		
BVMT-R total score	3.41	1.26
CVLT-II total score	0.10	-0.20
Memory		
BVMT-R delayed recall	1.09	1.28
CVLT-II short delay free recall	-1.50	-0.50
CVLT-II long delay free recall	-1.00	-1.00
Executive Function		
Trails difference between A and B (B-A)	-0.10	0.17
D-KEFS verbal fluency: Letter fluency	0.35	-0.35
D-KEFS verbal fluency: Category switching total	0.35	0.35
D-KEFS verbal fluency: Switching accuracy	0.35	0.35
D-KEFS Sorting: confirmed correct sorts	-0.35	0.00
D-KEFS Sorting: free sorting description scores	-0.35	0.00
D-KEFS Color-Word: Inhibition	0.35	0.35
D-KEFS Color-Word: Inhibition/switching	0.35	1.00
Language		
D-KEFS verbal fluency: category fluency	0.35	-0.35

* Bolded numbers represent cognitive impairment

As noted in table 4, participants MS06 and MS07 demonstrated the most widespread impairment. Although participants MS05, MS09, and MS10 were impaired as measured by performance on two or more tests (Table 3), these individuals were not impaired in any domain when collapsing individual test scores into a summary score per domain (Table 4).

Table 4

Mean standard scores (z scores) on summary scores in each cognitive domain

Domain	Patient					
	MS01		MS02		MS03	
	T1	T3	T1	T3	T1	T3
Attention/Information Processing Speed	-3.17	-4.82	-0.05	0.29	-0.61	-0.06
Visual-spatial Abilities	-0.55	-2.01	-3.08	-1.13	-0.64	0.74
Learning	-0.16	-1.02	1.06	0.05	-1.19	-0.92
Memory	-0.27	-0.93	-0.92	-0.38	-2.34	-1.58
Executive Function	-1.44	-2.37	0.33	0.76	-0.23	-0.13
Language	-1.35	-1.65	0.35	0.65	0.00	0.35

Domain	Patient					
	MS04		MS05		MS06	
	T1	T3	T1	T3	T1	T3
Attention/Information Processing Speed	-2.57	-2.75	-0.36	-0.44	-3.43	-4.18
Visual-spatial Abilities	0.33	0.04	0.62	-1.43	-0.65	-0.64
Learning	-0.71	-0.81	-0.34	-0.65	-2.79	-3.04
Memory	-1.77	-1.09	0.20	-0.42	-2.24	-3.40
Executive Function	-0.80	-0.84	0.17	0.36	-1.56	-2.26
Language	-1.35	-1.00	-0.35	0.35	-2.65	-3.00

Domain	Patient					
	MS07		MS08		MS09	
	T1	T3	T1	T3	T1	T3
Attention/Information Processing Speed	-1.48	-1.86	-0.37	0.14	-0.44	-0.03
Visual-spatial Abilities	-1.43	-1.13	1.50	0.91	0.40	1.09
Learning	-1.81	-2.50	-1.22	-0.25	-1.21	-0.51
Memory	-2.32	-4.27	-1.73	0.04	0.40	-0.69
Executive Function	-0.80	-1.48	0.72	0.71	0.35	0.47
Language	-1.00	-2.00	1.00	0.65	0.00	0.35

Domain	Patient	
	MS10	
	T1	T3
Attention/Information Processing Speed	-0.40	-0.53
Visual-spatial Abilities	0.74	0.74
Learning	1.76	0.53
Memory	-0.47	-0.07
Executive Function	0.12	0.23
Language	0.35	-0.35

* Bolded numbers represent cognitive impairment

6.3 Reliable Change Analysis

Nine of 10 participants demonstrated declines on at least one measure, although 8 of 10 demonstrated improvements on at least one measure. Nonetheless, there was more decline overall. With the exception of visuospatial abilities and executive function that demonstrated equivocal results, all other domains assessed demonstrated more decline than improvement (Table 5). Half of the sample showed more improvement (green total higher than red total) while the other half showed more decline (red total higher than green total).

Table 5

Reliable change indices in each task

	MS01	MS02	MS03	MS04	MS05
Attention/Information Processing Speed					
Trails A	7.98	-0.41	0.14	0.55	0.27
Trails B	7.68	0.12	0.81	1.87	0.49
PASAT 3 sec	0.70	-0.08	-0.21	-0.21	-0.46
PASAT 2 sec					
SDMT	-1.84	-1.11	1.31	-0.99	-1.11
Color naming	10.22	-2.38	-2.38	7.22	0.62
Word reading	6.89	-0.54	-0.54	4.53	0.47
ACT 9 sec	-0.21	0.14	1.18	-0.56	-0.56
ACT 18 sec	-1.15	0.75	0.43	0.43	0.11
Visual-Spatial abilities					
JOLO	-2.53	2.02	2.59	-0.26	-3.67
Learning					
BVMT-R total score	-2.20	-4.69	-0.03	-0.03	-0.34
CVLT-II total score	-1.09	0.48	-0.17	-0.83	-1.35
Memory					
BVMT-R delayed recall	-1.87	-5.18	-2.98	0.33	0.33
CVLT-II short delay free recall	-0.94	1.80	1.11	0.77	-1.28
CVLT-II long delay free recall	-1.33	1.68	1.31	0.55	-0.95
Executive Function					
D-KEFS verbal fluency: Letter fluency	-1.64	4.40	9.06	4.87	1.15
D-KEFS verbal fluency: Category switching total correct responses	0.31	1.68	0.78	0.86	0.86
D-KEFS verbal fluency: Category switching total switching accuracy	-0.25	1.64	-1.61	0.83	0.83
D-KEFS Sorting: confirmed correct sorts	-1.45	-0.83	0.42	-0.20	-0.83
D-KEFS Sorting: free sorting description scores	-2.93	-0.55	5.70	0.34	-1.44
D-KEFS Color-Word: Inhibition	0.91	0.91	0.41	6.40	-2.09
D-KEFS Color-Word: Inhibition/switching	5.15	-1.77	1.69	9.48	-3.07
Language					
D-KEFS verbal fluency: category fluency	-2.19	0.76	1.25	0.76	2.23

	MS06	MS07	MS08	MS09	MS10
Attention/Information Processing Speed					
Trails A	-0.28	-0.96	-2.61	0.00	0.82
Trails B	-0.13	3.93	-1.07	0.12	0.18
PASAT 3 sec	-0.08	-0.59	-0.98	0.44	-0.21
PASAT 2 sec					
SDMT	-0.87	0.22	-0.14	1.80	-0.26
Color naming	NA	-1.18	-2.38	-0.58	2.42
Word reading	NA	-0.88	0.47	-0.54	-1.55
ACT 9 sec	NA	-1.95	0.14	-0.56	-0.91
ACT 18 sec	NA	-1.79	-0.52	-1.15	0.11
Visual-Spatial abilities					
JOLO	0.31	0.88	-0.82	1.45	0.31
Learning					
BVMT-R total score	-0.03	-0.03	1.83	0.90	-4.07
CVLT-II total score	-1.48	-3.04	0.09	0.09	-0.43
Memory					
BVMT-R delayed recall	-0.77	-6.26	4.72	-2.97	-0.77
CVLT-II short delay free recall	-2.31	-1.62	1.11	-0.60	0.43
CVLT-II long delay free recall	-2.08	-1.71	0.55	-1.33	-0.20
Executive Function					
D-KEFS verbal fluency: Letter fluency	-2.10	-0.24	6.73	0.22	-3.50
D-KEFS verbal fluency: Category switching total correct responses	-0.23	0.04	-0.50	0.59	0.31
D-KEFS verbal fluency: Category switching total switching accuracy	-0.79	-1.34	-1.07	0.29	0.29
D-KEFS Sorting: confirmed correct sorts	-1.14	-1.14	-0.20	-0.20	-0.20
D-KEFS Sorting: free sorting description scores	-2.93	-1.74	0.34	0.34	0.94
D-KEFS Color-Word: Inhibition	NA	-6.58	-4.58	4.40	-1.09
D-KEFS Color-Word: Inhibition/switching	NA	3.42	-3.50	-4.36	-3.93
Language					
D-KEFS verbal fluency: category fluency	-1.70	-3.67	-1.21	1.74	-2.68

*numbers in red represent cognitive decline

*numbers in green represent cognitive improvement

NA: Not available

6.4 Secondary disease characteristics

6.4.1 Measures of Mood

Only one participant had clinically meaningful levels of depression at baseline and one participant had a clinically meaningful level of depression at follow-up (Figure 5). A score ≥ 11 indicates a clinically meaningful level of depression (Watson et al., 2014).

Figure 5. Levels of depression at baseline and follow-up measured by the Hospital Anxiety and Depression Scale

Five of the ten participants had a clinically meaningful level of anxiety at baseline and three participants had a clinically meaningful level of anxiety at follow-up. An anxiety score ≥ 11 indicate a clinically meaningful level of anxiety (Watson et al., 2014). Both participants who showed a clinically meaningful level of depression also showed a clinically meaningful level of anxiety at the respective time point (baseline/ follow-up) (Figures 5 & 6).

Figure 6. Levels of anxiety at baseline and follow-up measured by the Hospital Anxiety and Depression Scale.

In order to assess if the change in anxiety and depression levels from baseline to follow-up are significant, reliable change analyses were conducted (Table 6).

Table 6

Reliable change indices for depression and anxiety measured using the Hospital Anxiety and Depression Scale

	MS01	MS02	MS03	MS04	MS05
HADS depression	-0.49	-0.97	0.00	-1.94	2.91
HADS anxiety	-0.40	0.00	0.00	-3.24	3.64

	MS06	MS07	MS08	MS09	MS10
HADS depression	0.97	-0.97	2.43	-1.46	-0.97
HADS anxiety	2.02	-0.81	-0.40	-1.62	-0.81

Table 6 shows that one participant experienced a reliable decrease in levels of anxiety and depression (i.e., mood improved over time). Two participants had a reliable increase in levels of

depression and two participants had a reliable increase in levels of anxiety (i.e., mood worsened over time).

Normality of anxiety and depression variables was examined in (Appendix D, Table 1) using Shapiro-Wilk normality test. The Shapiro-Wilk test revealed that variables were normally distributed. There are no significant within group differences between baseline and follow-up in levels of anxiety $t(9) = .27, p = .79$ and depression $t(9) = .09, p = .93$, suggesting that although individual participants demonstrated changes in their mood, there were no consistent trends among all participants.

Reliable change analyses examining cognition (table 5) were compared to reliable change analyses examining mood (table 6) to determine whether participants that declined cognitively demonstrated corresponding declines in mood. The comparison showed that those who declined cognitively did not necessarily demonstrate corresponding declines in mood.

Fisher's Exact Test analyses were performed to assess the relationship between mood (i.e., anxiety and depression as measured by HADS) and cognitive impairment (per domain) at baseline and follow-up. Scores of 11 or higher on both the anxiety and depression portions of the HADS are considered to be indicative of a clinically meaningful level of anxiety and depression (Marrie et al., 2018). Mean values lower than -1.5 were considered indicative of impaired cognition in each cognitive domain (Table 4). There was no relationship between cognitive impairment and either anxiety (Table 7) or depression (Table 8) at both baseline and follow-up, meaning that mood had no apparent impact on cognitive performance at either time-point.

Table 7

The relationship between anxiety and cognition.

Domain	Baseline		Follow-up	
	Fisher Exact Test value (p-value)	95% confidence interval (lower – upper)	Fisher Exact Test value (p-value)	95% confidence interval (lower – upper)
Attention/information processing speed	0.75 (1.00)	.324 – 1.74	.86 (1.00)	.31 – 2.39
Visual-spatial abilities	.80 (1.00)	.52 – 1.24	.86 (1.00)	.63 – 1.16
Learning	1.00 (1.00)	.54 – 1.86	1.29 (1.00)	.55 – 3.02
Memory	4.00 (.21)	.66 – 24.37	2.57 (.18)	.50 – 13.11
Executive function	.80 (1.00)	.52 – 1.24	1.29 (1.00)	.55 – 3.02
Language	.80 (1.00)	.52 – 1.24	1.07 (1.00)	.42 – 2.71

Note: ($df = 1$, $N = 10$).

Table 8

The relationship between depression and cognition

Domain	Baseline		Follow-up	
	Fisher Exact Test value (p-value)	95% confidence interval (lower – upper)	Fisher Exact Test value (p-value)	95% confidence interval (lower – upper)
Attention/information processing speed	.22 (.30)	.06 - .75	.56 (1.00)	.31 – 1.00
Visual-spatial abilities	.89 (1.00)	.71 – 1.12	.89 (1.00)	.71 – 1.12
Learning	.78 (1.00)	.55 – 1.10	.78 (1.00)	.55 – 1.10
Memory	.44 (1.00)	.21 - .92	.67 (1.00)	.42 – 1.06
Executive function	.89 (1.00)	.71 – 1.12	.78 (1.00)	.55 – 1.10
Language	.89 (1.00)	.71 – 1.12	.67 (1.00)	.42 – 1.06

Note: ($df = 1$, $N = 10$).

When assessing the relationship between mood and cognition (at the cognitive domain level) no significant results were found (Tables 7 and 8). Using summary scores for domain level can mask a meaningful relationship between mood and performance on the individual cognitive

tests. Thus, the relationship between mood and cognition was assessed for each cognitive test. There was no significant correlation between depression and any of the individual cognitive tests at both baseline and follow-up (Appendix D: Tables 2 & 3). There was a significant correlation between anxiety and some specific tasks of processing speed (color-word interference: color-naming task and word-reading task) at baseline, $r(8) = -.64, p < .05$, and $r(8) = -.67, p < .05$, respectively. There was also a correlation between anxiety and color-naming at follow-up, $r(7) = -.72, p = .03$. Anxiety was correlated with color-word interference: inhibition task, $r(7) = -.74, p < .05$ at follow-up. These correlations indicate that participants with higher levels of anxiety performed better on the a few cognitive tests.

There was no correlation between the change in anxiety and depression and the change in performance in each cognitive domain (Appendix D, Table 4).

6.4.2 Fatigue

Overall our sample demonstrated high levels of fatigue at both baseline ($M = 53.11, SD = 6.86$) and follow-up ($M = 50.11, SD = 8.58$) as measured by the Modified Fatigue Impact Scale (Figure 7). There was one outlier in the sample at baseline (MS05) which was excluded to obtain normality required for the t-test analysis. There was no significant difference between fatigue levels at baseline and follow-up $t(8) = 1.51, p = .17$.

Figure 7. Fatigue at baseline and follow-up.

The relationship between the change in fatigue levels from baseline to follow-up and the change in cognition (per domain) was explored using correlation analysis (Appendix D: Table 4). There was no significant correlation between change in fatigue and change in cognitive performance (i.e., variations in the level of fatigue did not relate to variations in cognitive performance).

When evaluating the relationship between fatigue and cognition at single time points there was a significant correlation found at follow-up between fatigue and measures of executive function (Delis-Kaplan Executive Function System Sorting task: confirmed correct sorts) $r(8) = 0.64, p < .05$ and free sorting description score $r(8) = 0.64, p < .05$. At follow-up, fatigue was also correlated with an attention task: The Auditory Consonant Trigrams 9 seconds and 18 seconds $r(7) = .84, p < .05$ and $r(7) = .69, p < .05$, respectively. These results indicate that participants who were more fatigued performed better on certain cognitive tasks.

Given that this thesis followed a fixed neuropsychological battery to examine cognition, the order of the tests was not changed. Fatigue could have had an influence on cognitive

performance in the latter tasks. Thus, cognitive impairment in the early tasks vs. the latter tasks was examined. It did not seem as there was a specific pattern in cognitive performance in early tasks vs. late. Thus, fatigue did not seem to influence cognitive performance in the latter tasks.

6.5 Neural conduction time and cognition

Figure 8 & 9 show that six participants exhibited longer latency of transcalloal inhibition (i.e., slower conduction) at follow-up in their right hand (Figure 8). And four participants had longer latency of transcalloal inhibition (i.e., slower conduction) at in their left hand at follow-up (Figure 9). It is worth noting that only two participants exhibited worsening in conduction speed in both hands. As for the rest of the participants, the direction of change was not consistent in both hands (e.g., a shorter conduction time was recorded from the left hand but a longer conduction time was recorded from the right hand). In healthy individuals, the latency of transcalloal inhibition does not generally change over time $r(32) = .97$ (Davidson & Tremblay, 2013). Thus, any change in latency of transcalloal inhibition seen in this sample is meaningful.

Figure 8. Latency of transcalloal inhibition in the right hand at baseline and follow-up.

Figure 9. Latency of transcalloal inhibition in the left hand at baseline and follow-up.

As demonstrated in Figures 10 & 11, nine of the participants exhibited increased central motor conduction time in the right hand (i.e., slower conduction) at follow-up (Figure 10), whereas only six participants showed increased conduction time in the left hand at follow-up (Figure 11).

Figure 10. Central motor conduction time in the right hand at baseline and follow-up.

Figure 11. Central motor conduction time in the left hand at baseline and follow-up.

Overall, central motor conduction time did not decrease at follow-up in either the right hand or the left hand, Figures 7 and 8. A normal central motor conduction time is 7.2 ± 1.2 . Thus, conduction time at or higher than 8.5 indicates abnormal central motor conduction time. All participants demonstrate abnormal central motor conduction time in at least one hand at one time point (Table 9). All participants demonstrated increased conduction time in their right hand (i.e., slower responses) at follow-up, indicating worsening.

Table 9

Central motor conduction time in the right and left hands at baseline and follow-up

	Left hand		Right hand	
	Baseline	Follow-up	Baseline	Follow-up
MS01	13	13	16	18
MS02	8	8	8.5	10
MS03	6.5	8.5	9.5	12
MS04	13	12	12	14.5
MS05	14	13.5	16	19
MS06	17.5	20.5	21.5	21.5
MS07	8	8.5	9	11
MS08	7.5	10.5	14	16
MS09	5.5	10.5	12.5	18
MS10	21	22	31.5	39.5

Note: Numbers highlighted in green are within the normal range

Note: Numbers highlighted in red represent conduction times slower than the normal range

No significant relationship was found between the latency of transcallosal inhibition in either hand and performance on cognitive tasks (Table 10). However, there was a positive correlation found between central conduction time measured from the left hand and the Symbol Digit Modalities Test score. Indicating that as the speed of central conduction decreases, counterintuitively, the speed of information processing increases.

Table 10

Pearson's correlation between neural-motor difference scores and information processing speed difference scores from baseline to follow-up

		Symbol Digit Modalities Test <i>df</i> =8	Trail Making Test: Trail A <i>df</i> =8	Trail Making Test: Trail B <i>df</i> =8	D-KEFS Color- Word Interference Test: Color- naming <i>df</i> =7	D-KEFS Color- Word Interference Test: Word- reading <i>df</i> =7
Central motor conduction time: Right hand	<i>r</i>	0.397	0.023	-0.170	0.027	-0.128
	<i>p</i>	0.256	0.949	0.639	0.944	0.743
Central motor conduction time: Left hand	<i>r</i>	.707*	-0.333	-0.441	-0.477	-0.621
	<i>p</i>	0.022	0.347	0.202	0.194	0.074
Latency of transcalloal inhibition: Right hand	<i>r</i>	-0.077	-0.479	-0.352	-0.185	-0.183
	<i>p</i>	0.833	0.161	0.318	0.634	0.638
Latency of transcalloal inhibition: Left hand	<i>r</i>	-0.416	0.523	0.266	0.337	0.549
	<i>p</i>	0.232	0.121	0.457	0.376	0.126

Note: * significant at $p < .05$

7 Discussion

This thesis examined the effect of mesenchymal stem cell therapy on cognitive function in people with MS. To understand the impact of mesenchymal stem cell therapy in this study three hypotheses were proposed. First, it was expected that if mesenchymal stem cell therapy has neuroprotective effects then this would be reflected in an improvement in cognitive performance. Secondly, given that dealing with such a disease on a daily basis takes its toll on people and may also lead to secondary effects of the disease such as anxiety, depression and fatigue, we hypothesized that individuals with higher levels of anxiety, depression and fatigue will also

demonstrate higher levels of cognitive impairment. Thirdly, given that the speed of neural signal transmission can be measured using transcranial magnetic stimulation, it was expected that if there is a positive effect of mesenchymal stem cell therapy on neural regeneration then faster neural signaling should be apparent. Thus, we expected that both cognition and neural signaling will be affected in the same way following the therapy. Findings related to each hypothesis will be discussed in turn.

7.1 Sample demographics

The sample demographics were similar to that of the MS population in Canada. There were 7 females and 3 males similar to the female to male ratio of MS in Canada 2.6:1 (Gilmour, Ramage-Morin, & Wong, 2018). The average years of education was 13.9 years which is essentially equivalent to two years of university. This is similar to Canadian demographics where the majority of working-age adults have a postsecondary degree or higher (Statistics Canada, 2017). The average standard score of the premorbid intelligence test (Wechsler's test for Adult Reading) was 100.9 at baseline. The range of premorbid intelligence ranged from low average to high average (84 – 117). Only one participant did not complete high school. There were 8 patients with relapsing remitting MS as the onset phenotype and 2 with primary progressive MS as the onset phenotype. This is also similar to the published ratio of the onset phenotype of MS of relapsing remitting MS to primary progressive MS of 8.5:1.5. There were 4 participants with relapsing remitting MS, 4 with secondary progressive MS and 2 with primary progressive MS. Thus, there was a greater proportion of progressive MS than the typical for the MS population as a whole. This was expected given the type of study (i.e., those with relapsing remitting MS are more likely than those with progressive MS to respond to other therapies and would not seek out a study such as this).

The two domains that were most frequently impaired were attention/information processing speed and memory. The most common cognitive deficit in MS is information processing speed (Chiaravalloti & DeLuca, 2008). It underlies the overall cognitive deficit as well as deficits in higher order cognition (i.e., executive function) (Chiaravalloti & DeLuca, 2008; DeLuca, Chelune, Tulskey, Lengenfelder, & Chiaravalloti, 2004). In addition, the extent of deficit in information processing speed correlates with the extent of deficit in memory (DeLuca et al., 2004).

Memory impairment was observed 50% of the participants. This is similar to the literature where memory impairment is seen among 40-65% of PMS. Learning new information, rather than retrieving old information, is the primary cause for memory impairment in MS (DeLuca et al., 1994; DeLuca, Leavitt, Chiaravalloti, & Wylie, 2013; Rendell, Jensen, & Henry, 2007; Thornton, Raz, & Tucke, 2002). Poor learning is associated with slow information processing (Chiaravalloti & DeLuca 2008). Thus, findings are similar to the literature in that speed of information processing and memory are the most affected domains in MS.

7.2 Cognition and mesenchymal stem cell therapy

It was hypothesized that treatment would enhance cognition in at least one or more domains. When data was analyzed using the group as a whole, there was no improvement in any of the domains. In contrast, when analyzing change at the level of each individual participant, one participant demonstrated improvement (with no areas of decline) at follow-up, MS08. The absence of widespread cognitive improvement in all other participants suggested that MSC therapy does not appear to be of significant cognitive benefit in most individuals. This lack of improvement was also observed in the central motor conduction time where all participants worsened at follow-up. The small sample size of this study as well as the heterogeneous sample

(i.e., the sample included both relapsing-remitting MS and progressive MS) are possible factors as to why no improvement was detected.

Previous studies, showed that MSCs cross the blood brain barrier, possibly due to their innate ability to cross and possibly due to the disruption in the BBB caused by MS pathology (Hasan et al., 2017; Lee et al., 2012; Zanier et al., 2011). However, there was a lack of the anticipated therapeutic effects of MSC therapy in the current study. Four potential reasons were proposed as to why improvement was not generally observed. First, dosage and frequency of the treatment may not be sufficient for therapeutic gains. Although some studies only administered one dosage for the entire period of the trial (Cohen et al., 2017; Connick et al., 2012; Llufríu et al., 2014) others have administered three doses over the period of three months (Harris et al., 2018) and even 7 doses over the period of seven days (Riordan et al., 2018). The latter studies reported significant therapeutic improvements such as lower Expanded Disability Scaled Score, and improved muscle strength, bowel and bladder function, walk times and quality of life compared to the former studies. In addition, Riordan et al., reported that the initial therapeutic quality of life improvements after 1 month of follow-up subsided at the 1 year follow-up. Harris et al., (2014) suggested that more frequent administration of mesenchymal stem cells would ensure more sustained production of immunomodulatory and neuroprotective factors.

Second, the follow-up period might be too short to detect clinically meaningful change. This is related to a major weakness in the current study. Researchers are, as yet, unable to break the blind as the study is not yet complete. In this study, there was an early and a late treatment groups. Thus, participants in the late treatment group were followed-up for 6 months only while participants in the early treatment group were followed for 12 months. The outcomes for each group might have been different since improvement can continue for longer than 6 months.

Llufriu et al. 2014, showed that gadolinium-enhancing lesion number further decreased in the second 6 months compared to the first 6 months after the therapy. Thus, had all participants had a longer follow-up interval, then improvement may have been more likely to be detected.

Third, route of administration might have an impact on the efficacy of the therapy. Harris et al., (2014) administered the mesenchymal stem cells intrathecally (i.e., directly injected into the central nervous system) and reported more therapeutic benefits than studies that used IV injections as the route of administration (Cohen et al., 2017; Connick et al., 2012; Llufriu et al., 2014). Harris reported that the therapeutic potential of MSC could be increased when the intrathecal route of administration is used because it ensures that MSCs are directly injected in the affected areas (i.e., CNS). More mesenchymal stem cells were able to migrate and survive at the lesion sites and differentiate into neurons when administered intrathecally than intravenously (Yeon Lim et al., 2011). It would be expected that greater cognitive improvement would be detected when greater number of mesenchymal stem cells are found at the lesion site. Therefore, the intrathecal route of administration could enhance cognition more than intravenous route of administration.

Fourth, there was cognitive impairment seen after the therapy. This could be because of new lesions occurring given that patients are not taking any disease modifying drugs during the study period. The people enrolled in this study have very active and aggressive disease (otherwise they would not be participating in the study). As such, their disease likely has a greater chance of advancing over the one year study period when not adequately treated than the general MS population.

Despite the lack of improvement overall, there were some areas of cognition that were more likely to demonstrate improvement than others. Specifically, executive function and in

particular, tasks that had a speed component demonstrated more improvement. Three of the 4 participants who improved in speeded executive function tasks also demonstrated improvement in the information processing speed domain. This was expected because information processing speed is a core task required for adequate executive function. Similar to our hypothesis, tasks showing the greatest improvement were those in the information processing speed domain. Research suggests that impairment in processing speed underlies cognitive impairment in multiple sclerosis (Costa et al., 2017). Genova and colleagues in 2013 were able to show that deficits in executive function as measured by the DKEFS color-word test were due to impairment in processing speed. They reported that brain imaging variables, measuring the integrity of the axons, correlated with information processing speed and not executive function (Genova, DeLuca, Chiaravalloti, & Wylie, 2013). Thus, information processing speed rather than executive function could be the area most impacted by MSC therapy in the current small sample.

It is speculated that improvement in other cognitive domains will follow after this improvement in information processing speed. In other words, if participants were followed for longer periods, those who demonstrated improvement in information processing speed will continue to improve. Eventually, improvement will be demonstrated in higher order cognitive domains such as learning and executive function. Bergendal and colleagues, showed that decline in information processing speed leads to decline in overall cognitive function in MS (Bergendal, Fredrikson, & Almkvist, 2007). Thus, cognitive enhancement might occur following improved information processing speed. These speculations require further investigation as the current study included 10 participants only.

There were two participants who demonstrated cognitive decline (according to the reliable change analysis) without improvement in any cognitive domain. Participant MS01 had

an aggressive primary progressive MS which has lacked any period of recovery from the onset of the disease. This participant exhibited motor disability resulting in difficulty using the pencil to draw on the Trail Making Test and the Brief Visuospatial Memory Test- Revised. This participant also had dysarthria; a speech disorder caused by poor control over the musculature involved in speech production. This affects the participant's oral-motor speed which would further affect her performance on tests measuring the speed of information processing based on an oral response (e.g., D-KEFS Verbal Fluency Test, the Symbol Digit Modalities Test - Oral version and well as the D-KEFS Color-Word Interference Test). These disabilities could ideally be accounted for using different statistical analyses. Future studies may wish to use regression-based techniques (e.g., standardized regression-based formulae) that can account for motor dysfunction when considering change over time in cognition. To that end, such studies should include a measure of pure motor speed and/or speaking speed that could be used to then subtract out any motor-based performance difficulties from the overall cognitive performance scores. One such measure administered in this study that measures cognition and subtracts the motor response component from the score is the Trail Making Test (Trail B-Trail A). However, there are no published data to allow the calculation of reliable change on the difference score. Thus, one cannot conclude whether the impairment in this participant was based on cognition rather than motor response disability.

The second participant who demonstrated cognitive decline only with no improvement in any test was MS06. This participant had achieved the fewest years of education (9 years) in this sample. Lower intelligence and education are factors that increase susceptibility to cognitive deficit in MS (Benedict & Zivadinov, 2011). Years of education are an essential factor affecting cognitive reserve. Cognitive reserve refers to one's ability to cope with brain damage by "using

pre-existing compensatory approaches or enlisting new compensatory approaches” (Stern, 2012). As such, when brain damage occurs, a person with high cognitive reserve would show less cognitive impairment than another person with the same degree of damage but with low cognitive reserve. Similarly, cognitive reserve attenuates the impact of MS related brain atrophy on information processing deficit in PMS. In other words, those with higher cognitive reserve are able to withstand more brain atrophy before impairment in information processing speed becomes evident (Modica et al., 2016; Sumowski, Chiaravalloti, Wylie, & Deluca, 2009). Specifically, higher levels of education are associated with lower levels of cognitive deficit in MS (Da Silva et al., 2015). As such, cognitive decline for this participant might be related to the low cognitive reserve.

7.3 Secondary disease characteristics

7.3.1 Depression and anxiety

To understand mood in this sample, depression and anxiety levels based on the Hospital Anxiety and Depression Scale were examined at baseline and follow-up at the group and individual levels. There was no difference in mood levels before and after the treatment at the group level. This could indicate the safety of the therapy given that previous disease modifying drugs (e.g., interferon- β) have been reported on occasion to negatively impact mood (de Jong et al., 2017) and there was no such finding currently. However, one cannot conclude that mesenchymal stem cell therapy is an alternative for interferon- β , as interferon- β has been approved to lower relapse rate and delay relapses (de Jong et al., 2017). Instead, the fact that depression can be affected by therapies indicates that new therapies should include measures of mood when assessing the safety of new therapies.

To examine mood at the individual level, reliable change analyses were performed. Six out of ten participants did not demonstrate reliable change in mood. However, in the remaining four participants, both anxiety and depression changed in the same manner (i.e., there was either negative change in both anxiety and depression or positive change in both of them). There are no studies in the literature that include mood as an outcome measure following mesenchymal stem cell therapy. Therefore, with the small numbers in this sample one cannot make definitive conclusions on how the therapy affects mood.

In order to understand the relationship between mood and cognition in this sample a number of analyses were performed. First, results from the reliable change analyses examining cognition (Table 5) were compared to results from the reliable change analyses examining mood (Table 6) to determine whether participants that declined cognitively demonstrated corresponding declines in mood. The comparison showed that those who declined cognitively did not necessarily demonstrate corresponding declines in mood. Some participants that declined in mood showed improvement in cognition while another participant that declined in mood also declined in cognition. The participant who showed improvement in mood demonstrated an overall decline in cognition. This could suggest that mood changes might not be related to changes in cognitive performance in the current sample. However, due to the small sample size general conclusions on mood and cognitive performance following MSC therapy cannot be made.

Second, the relationship between mood and each cognitive domain was tested using Fisher Exact test. There was no relationship found between mood and any of the cognitive domains. The small sample size ($N=10$) led to small numbers per cell in the Fisher Exact test and could possibly be the reason why no significant results were found. This analysis looked at

cognitive impairment per domain rather than total cognitive impairment because nine of the ten participants were impaired at baseline when looking at total impairment. Thus, these per domain analyses offered more variability than when results are collapsed to obtain a measure of total impairment. However, subtle variations that might be detected when looking at the individual cognitive tests may still have been masked when collapsing tests within each domain. In order to overcome the limitations of the Fisher Exact test, Pearson's correlational analysis were conducted to examine the relationship between the change in mood and the change in cognition. There was no relationship between the change in depression and anxiety scores and change in performance under each cognitive domain.

The relationship between cognitive function per test (instead of per domain) and mood were examined using Pearson's Correlation. As mentioned above, there was no relationship between mood and cognitive function when looking at cognition per domain. However, when looking at cognitive performance in each task, there was a negative correlation between anxiety and the color-word interference task. In other words, if a participant is more anxious then they are likely to process information faster (perform better in the color-word interference task). Color-word interference is an executive function task that depends heavily on information processing speed. Findings of this study are opposite to Goretti and colleagues (2014) who found that the impairment on a task of information processing speed (SDMT) was mainly caused by anxiety and independent from depression and fatigue. The current finding might be a false positive for two reasons: the first reason is that our finding contradicts the published literature. The second reason is that there was no relationship between anxiety and cognition in any of the other analyses conducted in this study. Thus, the correlation may have simply occurred by chance.

Although the literature indicates that there is a positive link between depression and cognitive impairment in MS, no such relationship was found in this sample (Chiaravalloti & DeLuca, 2008). Overall there was no relationship between mood and cognition in this sample. Thus, cognitive deficits in this sample are more likely to be related to MS pathology rather than mood.

7.3.2 Fatigue

When examining fatigue at the group level no significant difference in fatigue levels were observed before and after the therapy. The therapy did not seem to change fatigue levels neither to worsen nor alleviate. The examination of fatigue at the individual level was not possible due to the lack of test-retest correlation analyses for the Modified Fatigue Impact Scale in the published literature (i.e., there is no clear indicator of how much change over time is typical for fatigue in those with MS). There was also no significant correlation between the change in fatigue and the change in performance in any of the cognitive domains.

Fatigue was correlated with two cognitive tasks at follow-up only. The results show that the higher the fatigue levels the better the performance was on tasks of executive function (D-KEFS Sorting) and working memory (The Auditory Consonant Trigrams). The D-KEFS Sorting, requires participants to sort a group of cards in as many ways as possible without repeating the old sorts, which means one has to remember which sorts were already made to prevent repeating them. The Auditory Consonant Trigrams requires retaining a stimulus in working memory under conditions of interference. Both tasks are dependent on sustaining mental effort over time and holding information in working memory; both of which are greatly impacted by fatigue (Chiaravalloti & DeLuca, 2008). Thus, the findings in the current study were opposite to the published literature. The literature indicates that higher levels of fatigue are associated with

worse cognitive performance and the opposite was found in the current study. In general, it would be expected that tasks measuring the same cognitive domains would have a similar relationship with fatigue. This was not the case in the current study. There were other tasks administered that assess working memory and tasks that require sustained mental effort (e.g., Paced Auditory Serial Addition test); however, these did not correlate with fatigue. In addition, the correlation between fatigue and D-KEFS Sorting and the Auditory Consonant Trigrams was only found at one time point and not the other. Thus, this correlation is probably the result of a Type One error. Larger numbers are required to test this relationship.

Previous research indicates that secondary disease characteristics have a great impact on cognition. However, no relationship was found between cognition and the secondary disease characteristics studied in the current study. In our sample, the cognitive deficits and the mood/fatigue issues seem to be separate issues that are both present, but unrelated to each other.

7.4 Transcranial magnetic stimulation

Transcranial magnetic stimulation measures neural integrity by measuring the conduction time of the neural signal. It was hypothesized that measures of conduction time would correlate with processing speed. The results did not support this hypothesis despite published evidence suggesting that measures of conduction time correlate with cognitive tasks such as information processing speed (Llufriu et al., 2012). This discrepancy could be due to the variation in physical disability and cognitive impairment in our sample. Llufriu et al., (2012) studied participants with relapsing remitting MS who were receiving a disease modifying treatment and were free from relapses for the previous month. Thus, their sample was very homogenous, where a similar level of disease pathology was demonstrated in all participants. In contrast, there was a lot of variability in our sample because participants demonstrated three different subtypes of MS:

relapsing-remitting, secondary progressive and primary progressive. The course of the disease is very different in these three types wherein the relapsing remitting subtype is characterized by inflammation and the progressive types are characterized by neural degeneration. Thus, the neural pathology is different between the three types of the disease which means the conduction time is different from one type of the disease to the other (i.e., shorter conduction time is seen in relapsing-remitting MS compared to progressive MS).

It was hypothesized that measures of conduction time would negatively correlate with information processing speed. In other words, if neural conduction time was shortened after the therapy (presumably due to neural repair) then the speed of information processing would be faster. However, the findings did not support this hypothesis. Unexpectedly, a positive correlation was found between central motor conduction time and the Symbol Digit Modalities Test at follow-up which could be a false positive result. The fact that no such correlation was found on other speed-dependent tasks in the battery (e.g., Trail Making Test) suggests that this finding was likely an anomaly.

It was hypothesized that participants with cognitive impairment would also exhibit abnormal central motor conduction time. However, the results did not support this hypothesis. This was probably due to the variability in conduction time caused by the MS pathology. Measures of conduction time are consistent over time in healthy controls. However, in PMS conduction time varies greatly due to the number of lesions, relapses and physical disability that accumulates (Davidson & Tremblay, 2013). This variability is observed at the individual level (between the right and the left hemispheres) and inter-individually within PMS. A healthy person's conduction time is identical when measured from the right hand and the left hand wherein PMS the conduction time is different between the two hands because the number and

volume of lesions in each hemisphere is different. Inter-individual differences include differences in the location of the lesions, differences in the extent of myelin degradation, and differences in the recovery after an attack (Humm et al., 2003). In addition, people with relapsing-remitting MS have lower conduction time than people with progressive MS. This could be due to the fact that in the relapsing remitting phase of the disease there is recovery after an attack, wherein progressive MS neurodegeneration is dominant with no recovery (Humm et al., 2003).

There was no relationship observed in the current sample between measures of conduction time and cognition. This could be due to the variability with the sample which included all three types of MS (i.e., creating variation in physical disability). This could also be due to the small sample size. Another source of variability is the difference in lesion number between the two hemispheres. In a healthy control both hemispheres have the same latency of transcallosal inhibition but not in an MS patient (Davidson & Tremblay, 2013). Adding to this variation are the differences in cognitive performance in each patient. In other words, when comparing two variables (measure of conduction time and cognition) where great variability exists within each variable in the sample then consistent patterns of a fixed relationship between variables is less likely to be found. Future studies should consider examining each subtype of MS separately and increase their sample size.

7.5 Limitations

There are a number of weaknesses in this study. First, the limitations of the cell production facility and the timeline of the therapy resulted in a small sample size. Treating the mesenchymal stem cells and cultivating them takes one month per participant. The facility (lab)

can handle stem cells from only 1-2 patients at a time. Thus, the slow procedure limits the number that can be enrolled.

Second, our inability to break the treatment arm blind at this point of the study is a large limitation. We are unable to draw conclusions on whether participants treated earlier (baseline) demonstrate changes in cognition compared to those treated later (at 6 months) (i.e., if treatment effects were delayed then the early treatment group may show changes in cognition and the late treatment group may not). Previous studies of stem cell therapy show that therapeutic gains and cognitive improvement are evident at longer periods of follow-up. Once the blind is broken, if we find the early treatment group demonstrated improvement in the second six months then recommendations can be made to employ longer follow-up intervals.

Third, the statistical analyses that can be conducted with 10 participants are very limited. If a larger number of participants are enrolled in the future one can use more advanced statistical techniques to study the effect of mesenchymal stem cell therapy on cognition while having anxiety, depression and fatigue as covariates.

Fourth, practice effect in the RCI analyses are obtained from healthy controls rather than patients with MS. It is proposed that people with MS might not benefit from practice as much as healthy controls. In other words, the RCI analysis when accounting for practice could be an overly stringent method of evaluating reliable change in cognition in an MS population. Thus, in order for people with MS to show the same reliable cognitive improvement as a healthy control, people with MS would have to demonstrate more improvement than the healthy controls given that they might not benefit from practice as much as healthy controls. To resolve this issue, it is proposed that in future studies practice effects be calculated from a sample with MS rather than healthy controls if possible. Unfortunately, there is a lack of studies that report cognitive

performance in an MS population over a period of time. Another possible solution is to calculate reliable change first by accounting for practice and second without accounting for practice and compare the two results. It is possible that some individuals would not show reliable change even after taking out practice from the RCI formula. To summarize, patients with MS might not benefit from practice as much as healthy controls. Therefore, caution must be taken when evaluating reliable change in an MS population.

Another potential limitation of this study was the fixed order of cognitive tests administered (rather than a counterbalanced model of test administration). The current study followed an established battery. Thus, the order of tests administered was not changed throughout the study. This neuropsychological assessment is 90 minutes long and fatigue could possibly impact cognition in the tasks towards the end of the assessment. However, in order to determine if this was indeed a limitation that impacted results, data was examined. When examining cognitive performance in the early vs. late tasks no specific patterns were detected suggesting that fatigue did not appear to negatively impact performance on tasks administered later in the battery. Nonetheless, this should be considered in future trial designs.

7.6 Future directions

In 2016, Walker and colleagues highlighted the importance of long term follow-up intervals after hematopoietic stem cells transplantation. They reported continuous improvement in cognitive performance over a period of three years. It is possible that stem cell therapy requires long intervals for the cells to migrate, multiply and start functioning. Thus, future studies should consider following-up with the participants for longer intervals.

Future studies should also consider factors to improve the efficacy of the MSC therapy. One possibility is to administer MSC more frequently. Such that, instead of administering the

therapy once a year, it might be more beneficial to administer the treatment several times a year. Multiple administrations were performed by two studies and shown clinical improvements in the treated samples (Harrison, Armstrong, Harrison, Lange, & Iverson, 2014; Riordan et al., 2018). Thus, increasing the frequency of MSC therapy administered might be more beneficial.

In order to better understand the effects of MSC on cognition, a larger sample size is needed. Thus, future studies should administer a cognitive assessment tool before and after the treatment. Sites that lack the expertise to assess cognition can administer brief batteries (e.g., The Brief Cognitive Assessment for Multiple Sclerosis) (Langdon et al., 2012). As such, larger data on cognitive performance can be gathered and more conclusive remarks can be made on how MSC therapy impacts cognition.

Future research should also consider comparing those with relapsing MS and those with progressive MS in terms of how they are impacted by MSC therapy. Cognitive function and neural conduction in relapsing MS versus progressive MS should also be examined. In summary, because the disease pathology is very different in relapsing MS versus progressive MS studies should study each separately.

Finally, MSC promotes neural repair by repairing the axons. Newly formed axons will make new synapses with other cells. Offering physical exercise and cognitive rehabilitation in addition to MSC therapy will ensure that the newly formed synapses are active and might show better results (i.e., enhanced cognition and less physical disability) after MSC therapy.

8 Conclusion

Multiple sclerosis is the most frequent neurodegenerative disorder in young adults. Many attempts have been made to find a cure for the disease but as yet, none have been successful. One of the promising therapies is immunoablative hematopoietic stem cell transplantation where

treatment with chemotherapy is necessary. The transplantation therapy is only available for patients with an aggressive disease course while still in the inflammatory phase of the disease. This leaves those with a progressive course, where less inflammation and more neural damage is taking place, without this treatment option. Focus has turned to mesenchymal stem cells as a potentially safer alternative given that immunoablation with chemotherapy is unnecessary. Mesenchymal stem cells have been shown to exhibit anti-inflammatory and neural protective properties. In this thesis, cognitive function and how it is affected by mesenchymal stem cell therapy was studied. It was concluded that mesenchymal stem cell therapy does not appear to negatively impact cognition in any consistent fashion across participants. With the lack of consistent decline, there was also a lack of cognitive improvement. It was also concluded that the therapy does not worsen or alleviate studied secondary disease characteristics (anxiety, depression and fatigue). The changes in anxiety, depression and fatigue from baseline to follow-up were not associated with change in cognition in our sample. Exploratory analysis between measures of neural conduction time and cognitive function were conducted. No relationship between cognition and conduction time was found. To conclude, the outcomes of the therapy are not clear yet. At this point we can only surmise that mesenchymal stem cell therapy does not appear to have a consistent beneficial or deleterious effect on cognition, mood or fatigue.

This thesis examined mesenchymal stem cell therapy which has been proposed as a potential treatment for those in the degenerative stage of MS who have very limited treatment/therapeutic options. It is a potentially safer option than previous stem cell therapies given that chemotherapy-induced immunoablation is not required pre-treatment. This study also used a comprehensive neuropsychological battery to evaluate cognition in all the commonly impacted domains in MS.

To summarize, the main purpose of this trial was to assess the safety rather than the efficacy of the therapy. Based on these preliminary findings, it is concluded that the therapy appears to be safe for both cognition and mood, although there are no clear benefits in the variables assessed to suggest a neural repair signal.

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Appendix A

A.1 Platform therapies

Platform therapies refer to disease modifying therapies that are used as the first option of therapy and are continued for a long period of time (Stuart, Cohan, Richert, & Achiron, 2004). Platform therapies include two categories; glatiramer acetate and interferon beta administered subcutaneously (injected under skin tissue) or intramuscularly (injected into muscle tissue). Glatiramer acetate is an immunomodulatory drug whereas interferon beta agents are generally anti-inflammatory; lowering neural inflammation caused by immune-mediated attacks on myelin (Lalive et al., 2011; Tanaka, 2014). Glatiramer acetate and interferon beta reduce the rate of annual relapses by 34% and 36% respectively. Potential side effects of Interferon beta include: increased risk of stroke, migraine, depression, and abnormal production and growth of blood cells (de Jong et al., 2017). However, both platform therapies are considered safe due to the rare occurrence of side effects (Soelberg Sorensen, 2017).

A.2 Oral therapies

Oral therapies are easier to administer than platform therapies and include dimethyl fumarate, fingolimod, and teriflunomide. Teriflunomide has an immunomodulatory and anti-inflammatory mechanism of action and can reduce annual relapse rates up to 31% - 36% (Miller, 2015). Side effects of teriflunomide include, headache, gastrointestinal problems (e.g., nausea and diarrhea), skin rashes, hair loss, increased liver activity, damage to peripheral nerves which in turn causes pain, weakness and numbness (Miller, 2015; Soelberg Sorensen, 2017). Dimethyl fumarate (DMF) is an anti-inflammatory agent that reduces annualized relapse rate by 53% when administered twice daily (Fox et al., 2012; Gold et al., 2012). However, 10% of participants discontinued the DMF due to flushing and gastrointestinal symptoms occurring in 38% and 36%

of cases respectively (Fox et al., 2012; Gold et al., 2012; Soelberg Sorensen, 2017). Fingolimod prevents lymphocytes (T cells) from entering the peripheral blood circulation. It reduces annualized relapse rate by 48% (Calabresi et al., 2014). Close observation must be maintained after first dosage due to adverse effects of the drug on the heart (i.e., slow heart-beat, and blockade between atria and ventricle of the heart) (Soelberg Sorensen, 2017).

A.3 Infusion therapies

The third category of therapies used to treat relapsing-remitting MS is *infusion therapies* including; natalizumab and alemtuzumab. Natalizumab prevents lymphocytes from penetrating the blood brain barrier. It is administered intravenously every 28 days and reduces annualized relapse rate by 68% (Polman et al., 2006). However, in rare cases treatment with natalizumab is associated with progressive multifocal leukoencephalopathy (PML) caused by John Cunningham virus (JCV). Risk factors of PML post-natalizumab treatment are; the period of treatment, previous use of immunosuppressant therapies, and JCV positive blood serum (Polman et al., 2006). The drug is tolerated well within the first year, however, the risk of PML increases substantially after the second year of treatment. Alemtuzumab is another infusion therapy administered intravenously once daily for three or five days within the first year. Alemtuzumab therapy should not be repeated. In one study, participants were monitored for 4 years after the 12-month treatment with alemtuzumab; 80% of participants were free from sustained accumulation of disability with low relapse rate (Havrdova et al., 2017). The most common serious side effects of alemtuzumab therapy are autoimmune thyroid disease (34%) and herpes viral infections (16%).

A.4 Emerging therapies

There are three *emerging therapies* for the treatment of relapsing remitting MS; daclizumab, ocrelizumab, and anti-LINGO. Both daclizumab and ocrelizumab lower the annual relapse rate in treatment groups when compared to placebo. Anti-LINGO therapy promotes myelin repair (Tran et al., 2014). In a phase 2 study, anti-LINGO was administered to participants experiencing optic neuritis for the first time (Cadavid et al., 2017). The study showed improvement on tests of the optic nerve which is an outcome of a possible remyelination of the optic nerve.

A.5 Positive outcomes of disease modifying therapies

The effects of disease modifying therapies are not limited to improving physical symptoms; they extend to alleviating fatigue, and may also improve quality of life and cognition. There is preliminary scientific evidence that suggests that treatment with disease modifying drugs, specifically beta interferon, may help reduce fatigue and cognitive deficit in people with relapsing-remitting MS (Melanson et al., 2010). Both beta interferons and glatiramer, 1st line treatments, were given to PMS and yielded improved quality of life (Johnson & Due, 2009) . In a group of PMS treated for six months with natalizumab who were observed for 10 years, improvement was noted in attention, memory, mood and well-being (Lang, Reiss, & Mäurer, 2012).

A.6 Disease modifying therapies for progressive MS

On the other hand, disease modifying therapies aimed for the treatment of progressive MS are scarce. Some of the studied therapies are fingolimod, ocrelizumab, and natalizumab. Both fingolimod and natalizumab did not limit progressive clinical disability (Lublin et al.,

2016). However, PMS treated with ocrelizumab showed 24% improvement in clinical disability progression when compared to placebo group (Amato et al., 2013).

In 2017, Stahnke and Holt showed that Ocrelizumab did indeed have disease modifying effects on primary progressive MS (Stahnke & Holt, 2018). PMS treated with Ocrelizumab showed lower percent rate of disability progression compared to the placebo group at 12 and 24 weeks. They also reported lower percentage change of T2 lesions and overall brain atrophy at week 24 as an effect of Ocrelizumab therapy. This therapy is intravenously administered therefore, side effects include reactions at the injection site. Other side effects include possible infections such as upper-respiratory tract, herpes, and nasopharyngitis. This is considered a breakthrough in the field of progressive MS therapies as this is the first ever therapy to slow the progression of the disease in primary progressive MS.

Appendix B

B.1 Inclusion criteria

Inclusion criteria specific to each type of MS:

Those living with relapsing remitting MS who did not respond to one or more of the approved therapies (such as; beta-interferon, glatiramer acetate, natalizumab, mitoxantrone, fingolimod, dimethyl fumarate, teriflunomide, and alemtuzumab) for the past 1 year with one or more relapses for the past 12 months, 2 or more relapses for the past 24 months, or one or more gadolinium enhancing lesions (GEL) on MRI taken within the past 12 months were enrolled.

Those living with secondary progressive MS and had an increase of one point in the EDSS score within the past 12 months and one or more relapses or one or more GEL on MRI in the past 12 months were enrolled. Those living with primary progressive MS and exhibited: an increase of one or more points on the EDSS score within the last 12 months, one or more GEL on MRI

within the past 12 months, and positive cerebrospinal fluid were enrolled in the study. Overall the inclusion criteria ensure that patients who do not respond to the approved therapies and those with a history of worsening symptoms (i.e., active disease) are included in the study.

These criteria allow PMS with little treatment options to get the first chance at accessing this novel therapy. The inclusion criteria are focused on PMS who cannot benefit from the approved therapies. The study includes people living with progressive MS because there are limited treatment options available for progressive MS. The study also allows the inclusion of people with relapsing remitting MS who did not respond to the approved treatments for the past year.

B.2 Exclusion criteria

People with MS who met any of the following conditions were excluded from the study:

PMS with the following conditions were excluded from the study:

- 1- Previous history of cancer or tumor reported by the patient with the exceptions of basal cell carcinoma and carcinoma that has not been active for more than one year
- 2- A co-morbid disorder endangering the participant's life
- 3- Previous history of a hematologic disease (blood disease) including myelodysplasia or current abnormal white blood cells count
- 4- Pregnancy or risk of pregnancy, to eliminate the risk of pregnancy participants are asked to practice contraceptive measures, those unwilling to do so are excluded
- 5- Kidney failure or an estimated glomerular filtration rate of less than 60 mL/min/1.73m² or being incapable of undergoing MRI screening
- 6- Allergic reaction to gentamicin or related aminoglycosides

Appendix C

The MESCAMS followed a previously published procedure in Davidson and Tremblay 2016 as showing below.

C.1 General procedure for TMS and recording of MEPs

TMS was administered with participants comfortably seated in a recording chair. Magnetic stimulation was delivered with a Magstim 200 stimulator (Magstim Co. Whitland, UK) connected to a figure-eight coil (P/N 3190, Magstim Co.). Before testing, participants were fitted with a Waveguard™ TMS compatible EEG cap (ANT Neuro, Madison, WI, USA), which was carefully positioned according to nasion-inion and interaural lines, to allow for consistent coil placement. In addition, a U-shaped neck cushion was used to restrain head movements and prevent neck fatigue during testing. Motor evoked potentials (MEPs) were recorded using small auto-adhesive surface electrodes (Ag/AgCl, Kendall Medi-Trace™ 130) placed over the first dorsal interosseous (FDI) muscles of the right and left hand. Electromyographic (EMG) signals were amplified and filtered (bandwidth, 5Hz–1kHz) with a polygraph amplifier (RMP-6004, Nihon-Kohden Corp.). Signals were digitized at 5kHz sampling rate using custom software on a PC running LabView™ (National Instrument Corp.) under Microsoft Windows™ XP equipped with a digital/analog acquisition card (PCI-63203, National Instrument Corp. Austin, TX, USA). TMS sessions started with the determination of the hotspot for the FDI. With the coil held ~45° in the mid-sagittal plane, the approximate location of the hand motor area on the left hemisphere was explored in 1-cm steps until reliable MEPs could be evoked in the target muscle. This site was then marked with a sticker to ensure consistent coil positioning during testing. After determination of the “hotspot”, the coil was held in place manually by one of the experimenters (FT). The experimenter frequently reassessed the coil position to ensure that it remained over the

optimal stimulation site throughout the experiment. All testing took place during regular working hours (9:00am–5:00pm) to reduce variability due to diurnal variations in corticospinal excitability (Doeltgen & Ridding, 2010). All TMS testing proceeded from one hemisphere first and, then, to the opposite hemisphere, with a short break (~15min) between, the order of testing being counterbalanced across the two groups of participants. Note that for each hemisphere testing always proceeded first with corticomotor excitability at rest, then afferent-induced modulation, followed by tests for the cSP and finally the iSP, as described below.

C.2 Ipsilateral silent period (iSP) and transcallosal motor inhibition (TCI)

As in our previous work (Davidson & Tremblay, 2013a, 2013b), interactions between hemispheres were probed with the iSP, which reflects transcallosally-mediated inhibition between motor cortices (Meyer, Rörich, von Einsiedel, Kruggel, & Weindl, 1995; Bernd-Ulrich Meyer, Rörich, & Woiciechowsky, 1998). As reported previously, our procedure to elicit the iSP was based on the work of (Giovannelli et al., 2009) and involved bilateral contractions. Briefly, as for the cSP protocol, participants were instructed to synchronize their contractions with the duration of an auditory tone lasting 3s, with the TMS pulse (130% rMT) being delivered at 2s in the course of the trial. To elicit the iSP, participants were instructed to exert a maximal pinching force against the pinch gauge with the hand ipsilateral to the TMS pulse, while maintaining a light pinching force (15–20% maximum) against a second pinch gauge held in the opposite hand. This procedure was repeated until 5–8 trials were recorded for each hemisphere with sufficient resting time between trials (30–60s) to prevent fatigue.

C.3 Central motor conduction time

Procedures used to measure central motor conduction time were obtained from (Chen et al., 2008).

The CMCT provides a sensitive measure of conduction within the central motor pathway (Chen et al., 2008). To determine the CMCT, two measures of latency are required: cortical and peripheral. For the cortical motor latency (CMT), the corticomotor latency derived from the clinical evaluation of MEP characteristics, as described above (Appendix C.1), will be used. The peripheral motor latency (PML) will be determined using foraminal magnetic stimulation directed at specific cervical and lumbar segments. Foraminal magnetic stimulation is performed using the same round coil that is used for cortical stimulation. The coil is centered either over the C7/C8 (cervical segments) or L4/L5 (lumbar segments) with the windings of the coil following the orientation of the target root. The coil is placed flat on the skin and centered over the body midline or 1–2 cm lateral to the spine. Contrary to cortical stimulation, foraminal stimulation uses just a relatively low stimulus intensity which is just suprathreshold for giving rise to a small response in the target muscle (typically <1 mV). For each segment, the CMCT is derived by computing the difference between the CMT and the PMT.

Appendix D

Table 1

Mean, skewness, kurtosis and normality test of anxiety and depression.

Variable	Mean (SD)	Mean SE	Skewness (SE)	Kurtosis (SE)	Shapiro- Wilk	df	P- value
Anxiety T1	9.30 (3.59)	1.14	-1.53 (.69)	2.45 (1.33)	.86	10	.08
Anxiety T3	8.90 (3.38)	1.07	-.28 (.69)	-.46 (1.33)	.97	10	.92
Depression T1	7.80 (2.20)	.70	-.47 (.69)	-.50 (1.33)	.95	10	.64
Depression T3	7.70 (2.36)	.75	1.40 (.69)	1.93 (1.33)	.86	10	.07
Fatigue T1	50.00 (11.78)	3.72	-1.66 (.69)	3.16 (1.33)	.842	10	.05
Fatigue T3	50.40 (8.14)	2.57	.01 (.69)	-.79 (1.33)	.922	10	.37
Fatigue T1 (outlier removed)	53.11 (6.86)	2.29	-.68 (.72)	.25 (1.40)	.940	9	.58
Fatigue T3 (outlier removed)	50.11 (8.58)	2.86	.13 (.72)	-1.02 (1.40)	.923	9	.41

Skewness and Kurtosis parameters were examined. A value greater than 1.96 in these parameters was considered significant at $p < .05$ (Ghasemi & Zahediasl, 2012). Thus, a positive kurtosis was observed in Anxiety at baseline.

Table 2

Correlation between secondary disease characteristics (anxiety, depression, and fatigue) and cognition at baseline

	T1 - HADS - Anxiety		T1 - HADS - Depression		T1 - Modified Fatigue Impact Scale - Total Score	
	Pearson Correlation	Sig. (2-tailed)	Pearson Correlation	Sig. (2-tailed)	Pearson Correlation	Sig. (2-tailed)
T1 - Trails A - Raw Score	-0.007	0.984	0.371	0.291	0.232	0.519
T1 - Trails B - Raw Score	-0.072	0.844	0.179	0.621	0.177	0.624
T1 - Trails - Difference Between B - A	-0.118	0.745	-0.014	0.969	0.105	0.773
T1 - BVMT - Total - Raw Score	-0.205	0.570	0.243	0.499	0.199	0.581
T1 - BVMT - Delayed - Raw Score	-0.271	0.449	0.144	0.691	0.027	0.941
T1 - PASAT - 3" - Total Correct - z-Score	-0.070	0.849	-0.260	0.468	-0.123	0.734
T1 - PASAT - 2" - Total Correct - Raw Score	-0.174	0.630	-0.348	0.324	-0.161	0.657
T1 - D-KEFS Verbal Fluency - FAS Total - Raw Score	0.274	0.444	0.094	0.795	0.166	0.646
T1 - D-KEFS Verbal Fluency - Category Total - Raw Score	0.172	0.634	0.067	0.855	0.054	0.881
T1 - D-KEFS Verbal Fluency - Total Correct Responses - Raw Score	-0.027	0.941	-0.453	0.188	-0.345	0.329
T1 - D-KEFS Verbal Fluency - Shifting Accuracy - Raw Score	0.034	0.926	-0.495	0.145	-0.420	0.227
T1 - CVLT - Total Correct - Raw Score	-0.266	0.457	0.144	0.691	-0.268	0.455
T1 - CVLT - Short Delay Free Recall - Raw Score	-0.547	0.102	-0.195	0.589	-0.568	0.087
T1 - CVLT - Long Delay Free Recall - Raw Score	-0.394	0.260	-0.147	0.686	-0.549	0.101
T1 - SDMT - Total Correct - Raw Score	-0.167	0.644	-0.302	0.396	-0.249	0.488
T1 - D-KEFS - Sorting - Confirmed Correct Sort - Raw Score	0.056	0.878	0.226	0.530	0.483	0.157
T1 - D-KEFS - Sorting - Free Sort Description Score - z-Score	-0.100	0.784	0.258	0.472	0.340	0.337
T1 - JOLO - Total Correct - Raw Score	0.110	0.762	-0.129	0.723	-0.074	0.840
T1 - D-KEFS - Color Word - Color Naming Total Time - Raw Score	-0.339	0.337	0.234	0.516	-0.053	0.884
T1 - D-KEFS - Color Word - Word Reading Total Time - Raw Score	-.643 [*]	0.045	-0.039	0.916	-0.253	0.481
T1 - D-KEFS - Color Word - Inhibition Total Time - Raw Score	-0.167	0.645	0.456	0.185	0.108	0.766
T1 - D-KEFS - Color Word - Inhibition/Switching Total Time - Raw Score	-0.115	0.752	0.183	0.612	0.170	0.638
T1 - CCC - 9 Second Total - Raw Score	-0.246	0.493	-0.344	0.330	-0.097	0.789
T1 - CCC - 18 Second Total - Raw Score	0.077	0.832	-0.006	0.988	-0.036	0.921

Table 3

Correlation between secondary disease characteristics (anxiety, depression, and fatigue) and cognition at follow-up

	HADS - Anxiety - Raw Score		HADS - Depression - Raw Score		Modified Fatigue Impact Scale - Total	
	Pearson Correlati	Sig. (2-tailed)	Pearson Correlati	Sig. (2-tailed)	Pearson Correlati	Sig. (2-tailed)
Trails A - Raw Score	-0.466	0.175	0.162	0.654	-0.324	0.362
Trails B - Raw Score	-0.306	0.390	0.092	0.801	-0.492	0.149
Trails - Difference Between B - A	-0.145	0.690	0.024	0.947	-0.587	0.074
BVMT - Total - Raw Score	-0.315	0.375	-0.040	0.913	0.319	0.369
BVMT- Delayed - Raw Score	-0.346	0.327	0.178	0.623	0.422	0.225
T3 - PASAT - 3" - Total Correct - Raw Score	-0.037	0.919	0.134	0.713	0.578	0.080
T3 - PASAT - 2" - Total Correct - Raw Score	0.353	0.317	0.194	0.592	0.524	0.120
D-KEFS Verbal Fluency - Verbal Total - Raw Score	0.045	0.901	0.060	0.869	0.543	0.105
D-KEFS Verbal Fluency - Category Total - Raw Score	-0.055	0.879	0.166	0.647	0.522	0.122
D-KEFS Verbal Fluency - Total Correct Responses - Raw Score	-0.261	0.466	0.094	0.796	0.336	0.343
D-KEFS Verbal Fluency - Shifting Accuracy - Raw Score	-0.345	0.329	0.039	0.915	0.354	0.315
CVLT - Total Correct - Raw Score	-0.271	0.449	0.164	0.650	0.377	0.283
CVLT - Short Delay Free Recall - Raw Score	-0.292	0.413	0.283	0.428	0.479	0.161
CVLT - Long Delay Free Recall - Raw Score	-0.175	0.628	0.324	0.361	0.368	0.295
D-KEFS - Sorting - Confirmed Correct Sort - Raw Score	-0.079	0.828	0.016	0.965	.639 *	0.047
D-KEFS - Sorting - Free Sort Description Score - Raw Score	-0.036	0.921	0.005	0.989	.641 *	0.046
JOLO - Total Correct - Raw Score	0.224	0.535	-0.197	0.585	0.162	0.655
D-KEFS - Color Word - Color Naming Total Time - Raw Score	-.719 *	0.029	0.229	0.553	-0.266	0.488
D-KEFS - Color Word - Word Reading Total Time - Raw Score	-.675 *	0.046	0.180	0.642	-0.252	0.513
D-KEFS - Color Word - Inhibition Total Time - Raw Score	-.736 *	0.024	-0.067	0.864	-0.259	0.502
D-KEFS - Color Word - Inhibition/Switching Total Time - Raw Score	-0.512	0.159	-0.031	0.936	-0.339	0.373
CCC - 9 Second Total - Raw Score	0.298	0.436	0.440	0.236	.843 **	0.004
CCC - 18 Second Total - Raw Score	0.126	0.748	0.032	0.934	.688 *	0.040