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Christelle Langley

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Abstract

The original contribution of this thesis is providing evidence that the basal ganglia may mitigate the effect of fatiguability on cognition in multiple sclerosis (MS). Fatigue is one of the most commonly reported symptoms of MS, but studies are inconsistent in establishing its impact on cognition. Moreover, the underlying mechanisms remain poorly understood. This may be due to the subjective nature of self-report measures and difficulty in assessing fatigue objectively. This thesis set out to use an alertness-motor paradigm and MRI to establish how mental fatigue affects cognition in MS. To determine whether this alertness-motor paradigm provides an objective measure of fatiguability and the underlying neural substrates. 40 MS participants and 40 age-matched healthy controls (HC) performed baseline and post-scan neuropsychological attention tests. Participants completed an alertness-motor paradigm, designed to enhance fatiguability, whilst undergoing magnetic resonance imaging (MRI) in a 3 Tesla scanner. Performance on the neuropsychological tests determined whether induced fatigue impacted cognitive performance. Voxel-based morphometry evaluated structural atrophy and neuroanatomical correlates of fatigue and fatiguability. The alertness-motor paradigm assessed the influence of fatiguability on behavioural performance over time. fMRI established the neuronal substrates that underlie this process. A neural connectivity approach investigated whether whole-brain network topology and basal ganglia functional connectivity were altered by fatiguability. The principle findings showed significant atrophy in the basal ganglia and prefrontal cortex of MS group compared to HC. Grey and white matter volume was not significantly correlated with fatigue or fatiguability scores, but the functional activations demonstrated that the basal ganglia (associative loop) may mitigate the impact fatiguability has on cognition. This was supported by consistent increased functional connectivity of the basal ganglia in groups demonstrating no fatiguability. MS was traditionally viewed as a white matter disease, however, the grey matter function may provide a better understanding of disease symptomology.

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Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: DATE:

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List of Abbreviations

AAL – Automated anatomical labelling

ACC – Anterior cingulate cortex

ANOVA – Analysis of variance

ANT – Attention network test

BA – Brodmanns area

BOLD – Blood oxygenation level dependent

Blipped-CAIPI – blipped- controlled aliasing in parallel imaging

CAIPRINHA – Controlled aliasing in parallel imaging results in higher acceleration

CNS – Central nervous system

CPT – Continuous performance test

CRIC – Clinical research and imaging centre

CSF – Cerebral spinal fluid

DCM – Dynamic causal modelling

DHEA - Hormone dehydroepiandrosterone

DOT – Digital ordering test

EEG - Electroencephalography

EMG – Electromyography

EPI – Echo planar imaging

FACETS – Fatigue: applying cognitive behavioural and energy effectiveness techniques to lifestyle

fMRI – Functional magnetic resonance imaging

FDA – US Food and drug administration

FDR – False discovery rate

FOV – Field of view

FSS – Fatigue severity scale

FWE – Family wise error

GABA – Gamma-aminobutyric acid

GLM – General linear model

HADS – Hospital anxiety and depression scale

HC – Healthy control

HPA – Hypothalamic-pituitary-adrenal

LFP – Local field potential

MB – Multiband

MPRAGE – Magnetisation prepared rapid acceleration gradient echo

MNI – Montreal neurological institute

mSDMT – modified symbol digit modalities task

MRI - Magnetic resonance imaging

MS – Multiple sclerosis

ms – Millisecond

MUA – Multi-unit activity

NMR – Nuclear magnetic resonance

PASAT – Paced auditory serial addition test

PET - Positron emission tomography

pFC – Prefrontal cortex

rCBF – Regional cerebral blood flow

rCMR – Regional cerebral metabolic rate

RF – Radio frequency

RVIP – Rapid visual information processing

ROI – Region of interest

SART – Sustained attention to response task

SE -Spin-echo

SMS – Simultaneous multi-slice

SPM – Statistical parametric mapping

SWP – Small world propensity

TR – Repetition time

TE – Time to echo

VBM – Voxel-based morphometry

Glossary

Fatigue – Refers to the symptom and refers to the self-reported measures of perceived fatigue using questionnaires.

Central fatigue – Refers to reduced performance on cognitive tasks, changes in motivation or the effect of fatigue on the central nervous system.

Peripheral fatigue – Refers to fatigue originating in the muscle. This is typically failure to maintain physical activity and may lead to muscle weakness.

Fatiguability – Refers to quantitative measures of performance fatiguability over time. In this thesis reaction time over time is used as the performance measure of fatiguability.

Chapter 1 Introduction

1.1 Short introduction

Multiple Sclerosis (MS) is a highly prevalent disorder that is characterised as an inflammatory and demyelinating disease of the central nervous system. This results in widespread plaques in the brain and spinal cord. Fatigue is one of the most commonly reported symptoms of MS. Over 90% of individuals with MS reported fatigue as one of their most debilitating symptoms (Branas *et al.*, 2000; Flachenecker *et al.*, 2002). Fatigue leads to impaired quality of life (Janardhan and Bakshi, 2002; Krupp *et al.*, 1988; Mitchell *et al.*, 2005), mental health (Fisk *et al.*, 1994; Schwartz *et al.*, 1996), physical health (Fisk *et al.*, 1994) and increased pain (Schwartz *et al.*, 1996). Some individuals with MS reported that fatigue is even more debilitating than the physical disability they have (Janardhan and Bakshi, 2002). Similarly, fatigue is one of the leading causes for individuals either, decreasing their hours at work or causing complete loss of employment (Smith and Arnett, 2005; van der Hielie *et al.*, 2015). Despite this, fatigue in MS and its underlying mechanisms remain very poorly understood. This may be due to the subjective nature of fatigue, which makes it very difficult to measure. Although several questionnaires measuring fatigue are available, these can only rate the level of perceived fatigue, they do not provide objective measures of fatigue (Braley and Chervin, 2010). Moreover, studies are inconsistent in explaining the relationship between subjective reports of fatigue and cognition which is impaired in approximately 45-65% of individuals with MS (DeSousa *et al.*, 2002). Research examining the relationship between fatigue and cognition often reveal inconsistent results (Bailey *et al.*, 2007; Bryant *et al.*, 2004; Claros-Salinas *et al.*, 2010; Jennekens-Schinkel *et al.*, 1988; Johnson *et al.*, 1997; Krupp and Elkins, 2000; Kujala *et al.*, 1995; Paul *et al.*, 1998; Schwid *et al.*, 2003). Studies using objective measures of fatigue demonstrate a much stronger association between fatigue and cognitive difficulties (DeLuca, 2005; Krupp and Elkins, 2000). As a result, the development of a reliable and objective measurement of fatigue is extremely important.

Several studies suggest that mental fatigue is associated with prolonged task performance, even in healthy individuals (Boksem *et al.*, 2005; Faber *et al.*, 2012; Oken *et al.*, 2006). Studies have often employed psychomotor tests to assess mental effort (fatiguability) and performance (Belenky *et al.*, 2003; Dinges *et al.*, 1997; Philip *et al.*, 2003; van Dongen *et al.*, 2003; Veksler and Gunzelman, 2018). Cognitive fatigue has also been measured with reaction time on cognitive tasks across time (Boksem *et al.*, 2005; Helton and Russell, 2011).

In MS specifically, there seems to be a larger deficit of reaction time (Chiaravalloti *et al.*, 2013; Denney *et al.*, 2005; Denney and Lynch, 2009; Lengenfelder *et al.*, 2006) than measures of simple attention (Benedict *et al.*, 2006; Rao *et al.*, 1991a; 1991b). Furthermore, through its dopaminergic influences, motivation plays a role in prolonged alertness. Individuals who are less motivated will be less alert compared to individuals with high motivation (Oken *et al.*, 2006). A specific brain structure involved in motivation is the basal ganglia, due to its strong dopaminergic inputs. Chaudhuri and Behan (2000; 2004) have proposed that the non-motor function of the basal ganglia may be implicated in central fatigue. Therefore, the alertness mechanism may be uniquely suited to providing an objective measure of performance related fatigue.

1.2 Thesis aims

The relationship between fatigue and cognition remains inconclusive. Furthermore, studies have proposed that the basal ganglia may be implicated in mental fatigue associated with various neurological disorders (Chaudhuri and Behan 2000; 2004), but the exact nature of this relationship is unknown. To this end, an alertness-motor paradigm was employed to induce fatiguability and provide an objective measure of fatigue. There were three main aims for the thesis:

1) to use an alertness-motor paradigm to establish how fatiguability affects cognition in MS;
2) to determine whether the alertness-motor paradigm provides an objective measure of fatigue in MS;
3) to investigate whether the basal ganglia is involved in fatiguability in an MS population.

1.3 Multiple Sclerosis

1.3.1 Clinical presentation and disease phenotypes

In the early to middle 19th century, both Carswell (1838) and Cruveilhier in 1841 (Compston, 1988) illustrated macroscopic plaques in the central nervous system of some individuals. Rindfleish (1868) showed similar lesions in the central nervous system but noted that the lesions were accompanied by inflammation. Charcot (1868) provided the first coherent description of what was described as '*sclerose en plaques disseminees*', or disseminated sclerosis, which then became known as Charcot's disease. The disease was known by many names including Charcot's disease, disseminated sclerosis, polysclerosis and insular sclerosis. It wasn't until McAlpine and colleagues' work (1955) that the term multiple sclerosis (MS) became widely used. As the lesions in MS can manifest throughout the

whole central nervous system, there is often a widespread impact on neurological function and symptomology. Symptoms such as optic neuritis, spinal cord and brain stem syndromes that affect sensorimotor functions, are well recognised characteristics of the disease. Conversely, despite fatigue and cognitive impairments being the most commonly reported symptoms experienced by individuals with MS they are less well characterised and treatment is limited (see section 1.4.4 page 18 and 1.4.5 page 19).

Equally variable is the clinical course of MS. Some individuals have a progressive decline from disease onset, whereas others may have a relatively mild form with limited disability even after years of the disease. Lublin and Reingold (1996) originally defined four MS disease phenotypes based on 1844 participants: Relapsing-remitting (1066, 58%), primary progressive (496, 27%), secondary progressive (109, 6%) and relapsing progressive (173, 9%). The relapsing-remitting phenotype is characterised by acute disruption to neurological functioning (relapse), followed by some degree of recovery and a stable course in between relapses. The primary progressive phenotype involves a gradual decline in functioning from disease onset, with no clear relapses. The rate of this decline may differ between individuals and within the disease course in an individual. The secondary progressive phenotype follows from the initial relapsing-remitting phase, then the disease course transitions into a gradual decline with no more distinct relapses. Finally, the relapsing progressive phenotype has very little consensus on the presentation, other than a combination of relapses and disease progression. More recently Lublin *et al*. (2014) re-examined the original phenotypes and proposed that the relapsing progressive phenotype is removed and added clinically isolated syndrome, which is characterised as a single clinical episode that shows inflammatory demyelination, suggestive of MS, but without further progression or relapses over time. Figure 1.1 illustrates the disease progression of each phenotype with an increased disability over time.

The relapsing-remitting phenotype is the most common presentation of MS and has been estimated to occur between 58%-85% of cases (Confavreux *et al*., 2000; Lublin and Reingold, 1996). Confavreux *et al*. (2000) indicate that after 11 years, 68% of individuals classified as relapsing-remitting remain in this category and 32% had progressed to secondary progressive MS. It is estimated that relapses occur approximately once a year (Compston and Coles, 2002). The relationship between relapses and progression is relatively poorly understood, some studies show a degree of dissociation (Confavreux *et al*., 2000; 2003), whereas others do show an association (Brex *et al*., 2002). Furthermore, there is some debate surrounding the difference between primary and secondary

progressive MS (Thompson *et al.*, 1991). This is largely due to the ambiguity when diagnosing MS phenotypes, as some individuals have one relapse and then develop a progressive disease course, whereas others may initially present progressively and later develop some relapses.

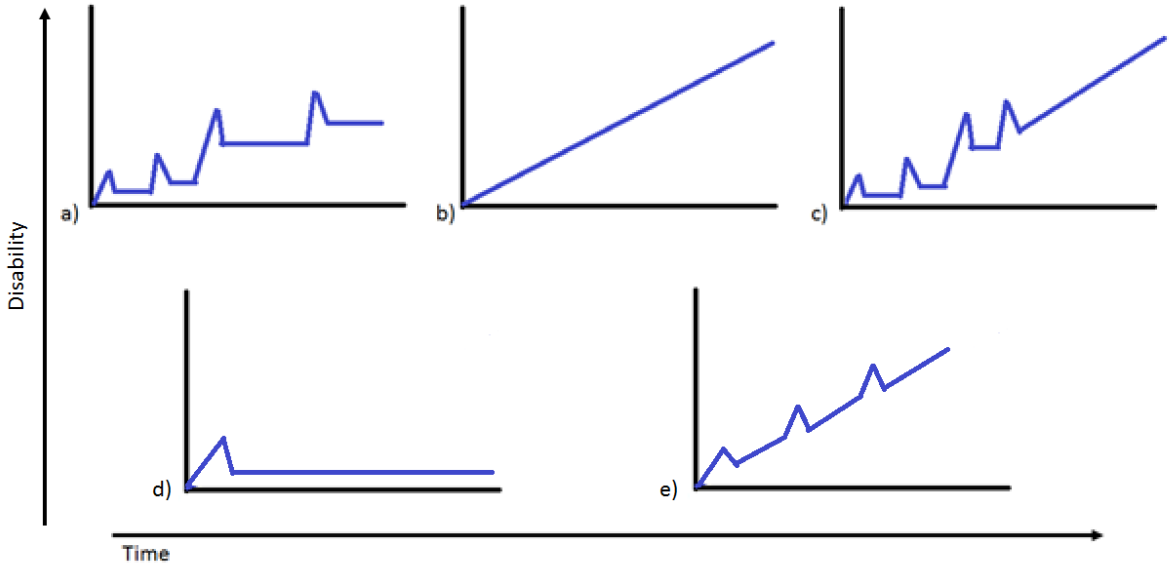


Figure 1.1 Clinical disease course as a function of disease duration. *a) relapsing-remitting. b) primary progressive. c) secondary progressive. d) clinically isolated syndrome. e) relapsing progressive.*

1.3.2 Diagnostic criteria

The first attempt at concrete diagnostic criteria for MS was made by Poser *et al.* (1983). Prior to this, there were only very subjective criteria to determine which patients to include in therapeutic trials (Schumacher *et al.*, 1965). Poser *et al.* (1983) used several criteria to aid diagnosis, including symptom description by patients, clinical and paraclinical evidence of lesions and cerebral spinal fluid (CSF) levels of oligoclonal bands and increased production of immunoglobulin. They established four likely cases for an MS diagnosis; clinically definite MS, laboratory supported definite MS, clinically probable MS, and laboratory supported probable MS. The clinical diagnosis is based on relapses, clinical and paraclinical evidence of lesions, whereas laboratory supported diagnosis is based on CSF findings. Relapses are defined as lasting for longer than 24 hours and must be at least one month after a previous relapse. Similarly, remission is defined as the improvement of symptoms for more than 24 hours. Clinical evidence of lesions can only be established by an experienced clinician. Visual evoked potentials are considered paraclinical evidence of lesions. Laboratory support for the diagnosis occurs where there is increased central nervous system synthesis of immunoglobulin or the presence of immunoglobulin oligoclonal bands in CSF. These criteria were established prior to the consistent use of

magnetic resonance imaging (MRI) and therefore did not include MRI in its diagnostic criteria. MRI became a crucial tool for diagnosis of neurological diseases as it was able to identify lesions non-invasively and *in vivo*. This led to multiple studies attempting to define criteria for MRI diagnosis of MS. Paty *et al*. (1988) proposed that three lesions, where one was periventricular, or simply four or more lesions were indicative of an MS diagnosis. The criteria had a high sensitivity in clinically isolated syndrome but a low specificity for development to clinically definite MS (Lee *et al*., 1991). Fazekas *et al*. (1988) stated that three or more lesions, where at least two were infratentorial, paraventricular, or larger than 6mm, indicated MS. These criteria had both high sensitivity and specificity for MS (Offenbacher *et al*., 1993) but were poor at predicting clinically definite MS following clinically isolated syndrome (Tas *et al*., 1995). Barkhof *et al*. (1997) proposed four parameters which were considered superior to those suggested before. The criteria required at least one gadolinium enhancing, one juxtacortical, one infratentorial and at least three paraventricular lesions to be present for an MS diagnosis. The criteria were later refined where the gadolinium enhancing lesion could be replaced by nine T2 lesions (Tintore *et al*., 2000). Both the Barkhof *et al*. (1997) and Tintore *et al*. (2000) criteria were incorporated into the now widely used McDonald criteria (McDonald *et al*., 2001), but here spinal cord lesions were equivalent to brain lesions. In this criteria lesions disseminated in space was evidenced by a minimum of two MRI lesions, accompanied by CSF changes (Poser *et al*., 1983). Lesions disseminated in time was evidence by new separate lesions developing at least three months after the first relapse. These criteria were found to have a high specificity for MS (Dalton *et al*., 2002; Tintore *et al*., 2003) but low sensitivity (Korteweg *et al*., 2006). The McDonald criteria have been further refined on multiple occasions but remain the most widely used diagnostic criteria for MS (Filippi *et al*., 2016; Montalban *et al*., 2010; Polman *et al*., 2005; 2011).

1.3.3 Epidemiology

In 2008 the World Health Organisation created the MS Atlas, a large collation of epidemiology data across the world in MS. Despite many limitations such as different diagnostic criteria, lack of data in certain countries, and use of evidence from past epidemiological studies, this MS Atlas is the largest collection of MS data across the globe. The results showed a worldwide prevalence of 30 cases per 100 000, but the prevalence varied vastly across regions. The greatest prevalence was noted in Europe (80 per 100 000), followed by Eastern Mediterranean (14.9 per 100 000), the Americas (8.3 per 100 000), Western Pacific (5 per 100 000), South-East Asia (2.8 per 100 000) and Africa (.3 per 100

000). The seemingly low prevalence in the Americas is dominated by the findings of South America, as the prevalence in the USA and Canada is high (135 and 132.5 per 100 000, respectively). Interestingly, the prevalence was higher in high income countries (89 per 100 000) compared to upper middle income countries (32 per 100 000), lower middle income countries (10 per 100 000) and low income countries (.5 per 100 000). There was a similar pattern for the incidence of MS, the worldwide incidence was estimated at 2.5 per 100 000. Regionally the incidence was again highest in Europe (3.8 per 100 000), followed by Eastern Mediterranean (2 per 100 000), the Americas (1.5 per 100 000), the Western Pacific (.9 per 100 000) and Africa (.1 per 100 000). No countries from South-East Asia provided any data on incidence. Again, the incidence was highest in high income countries (3.6 per 100 000), followed by upper middle income countries (2.2 per 100 000), lower middle income countries (1.1 per 100 000) and low income countries (.1 per 100 000).

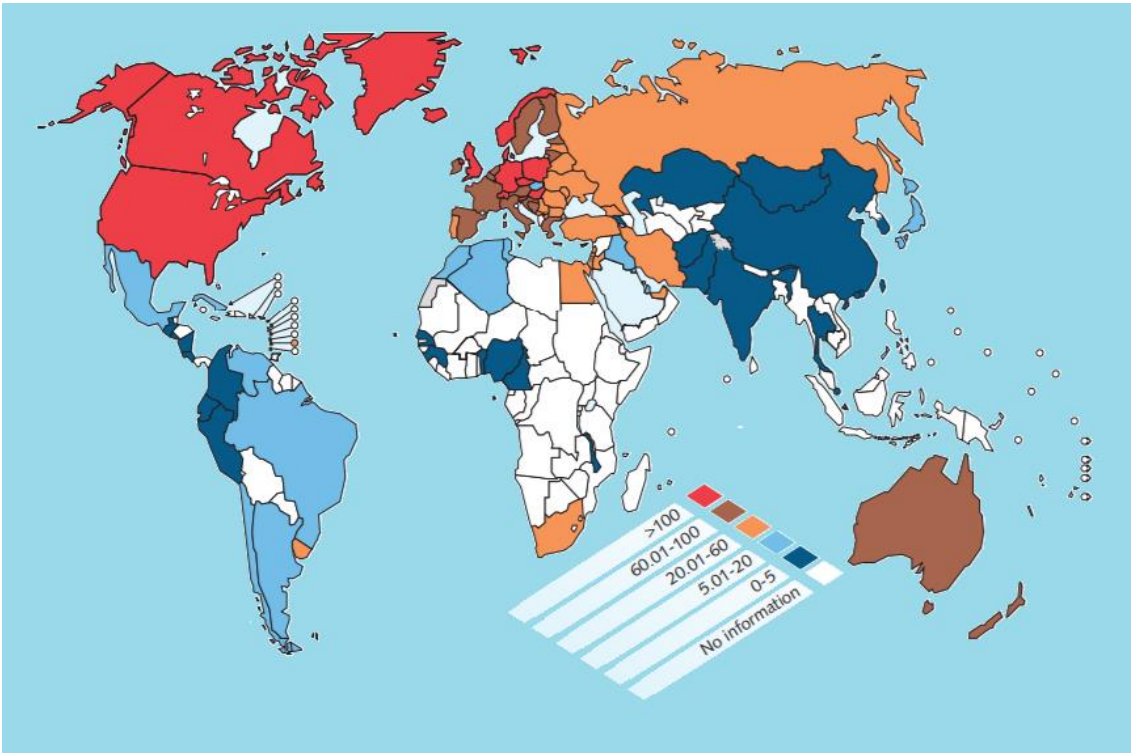


Figure 1.2 Geographical distribution of prevalence of MS. *Note 93 countries took part and values are individuals per 100 000. Image from World Health Organisation, (2008), with permission.*

In terms of age of onset and male/female ratio, there was much more consensus globally. The average age of onset was 29.2 years. Regionally the lowest onset was in Eastern Mediterranean (26.9 years) but closely followed by Europe (29.2 years), Africa (29.3 years), the Americas (29.4 years), South East Asia (29.5 years) and Western Pacific (33.3 years). Furthermore, the age of onset varied minimally for income groups, for low and upper middle income countries the onset was 28.9 years, for lower middle income countries average onset was 29.3 years and 29.5 years for high income countries. The global

male/female was .5 (2 women for every man). Regionally the ratio is lowest in Europe (.6), the Eastern Mediterranean (.55) and the Americas (.5), and highest in South East Asia (.4), Africa (.33) and the Western Pacific (.31). The male/female ratio is identical across all income groups (.5).

Specifically in the UK, epidemiology studies show a relatively high prevalence and incidence of MS (Alonso *et al*., 2007; MacKenzie *et al*., 2013). In 2010, the prevalence of MS was 258.8 per 100 000 in women and 113.1 per 100 000 in men. In the same year, the incidence was 11.52 per 100 000 in women and 4.84 per 100 000 for men (MacKenzie *et al*., 2013). However, Alonso *et al*. (2007) demonstrated that the sex difference is only noticeable in relapsing-remitting MS. The incidence ratio for relapsing-remitting MS is 2.5 more prevalent in women than men, but a ratio of only 1:1 for primary progressive MS. MacKenzie *et al*. (2013) showed that the prevalence of MS is increasing by approximately 2.4% per year. The same authors estimated that 126 669 people were living with MS in the UK in 2010 (203.4 per 100 000 population) and that 6003 new cases were diagnosed that year (9.64 per 100 000/year).

The prevalence of MS carries a very large cost implication. Kobelt *et al*. (2006) reported that the cost in the USA per patient per year was $47 215 on average. Gustavsson *et al*. (2011) estimated that more than half a million individuals in Europe suffered from MS in 2010 and that the cost for this in a single year was more than €14 billion. The authors estimated an annual cost of €29 828 per individual suffering from MS in the UK. Given the estimates of MacKenzie *et al*. (2013), the cost of MS in the UK in 2010 alone was approximately €3.8 billion. MS represents a significant health burden both financially and socially.

1.3.4 Aetiology

The exact aetiology of MS remains unknown, although there have been many possible explanations provided. Due to the geographic distribution of MS, some authors have suggested a possible genetic factor. Studies have shown that races such as Caucasians are susceptible to MS (Ebers and Bulman, 1986), whereas MS is rare in races such as Chinese, Japanese, American Indians (Rosati, 2001), black Africans (Morariu and Linden, 1980) and Aboriginal (Miller *et al*., 1990). Further support for a genetic explanation has come from twin studies. Hawkes and MacGregor (2009) found a concordance rate of approximately 25-30% and a heritability ranging between .25 and .76 in monozygotic twins. Similarly, Compston and Coles (2008) showed the rate of MS increases by 30% in monozygotic

twins, and Hassan-Smith and Duncan (2011) demonstrated a 5% increase in dizygotic twins. Furthermore, the risk for developing MS is greater when a first-degree relative suffers from the disease (Carton *et al.*, 1997; Compston and Coles, 2002; Dyment *et al.*, 1997; Sawcer *et al.*, 1997). When both parents have MS the risk was 10 times higher (Milo and Kahana, 2010). Despite the possibility of a genetic cause, the exact gene remains elusive. In more recent years there has been a shift to large genome-wide association studies (Aulchenko *et al.*, 2008; Australia and New Zealand Multiple Sclerosis Genetics Consortium, 2009; Baranzini *et al.*, 2009; Comabella *et al.*, 2008; DeJager *et al.*, 2009; International Multiple Sclerosis Genetics, 2007; 2011; Jakkula *et al.*, 2010; Martinelli-Boneschi *et al.*, 2012; Matesanz *et al.*, 2012; Nischwitz *et al.*, 2010; Patsopoulos *et al.*, 2011; Sanna *et al.*, 2010; Wellcome Trust Case Control, 2007). The success of these studies is related to the number of samples that they screen. Although these studies have still not identified a specific gene, they suggest that the human leucocyte antigens lead to a susceptibility to MS. The genetic susceptibility alone does not explain changes in the incidence of MS in immigrant populations. Elian and Dean (1987) showed that despite MS being uncommon in West Africa, West African immigrants that were born in the UK had an incidence and prevalence rate more like the UK than that of West Africa. These findings imply more of an environmental factor. Gale and Martyn (1995) observed that individuals who immigrate from a country where MS is common, to a country where it is uncommon, show decreased incidence of MS. Whereas, when individuals immigrate from a country where MS is uncommon to a country where it is common, they retain the lower risk of MS. These authors suggested that the environmental factors in early life established the risk of MS. This was further evidenced by Hammond *et al.* (2000), that showed the age of immigration influenced the risk of developing MS. Immigration studies indicated environmental factors may be more influential than genetics in developing MS, but do not identify a single environmental factor.

Due to the geographical pattern of MS prevalence, studies have examined the influence of sunlight and vitamin D. Ascherio and Munger (2007b) found that higher exposure to sunlight when aged 6-15 years was related to a decreased risk of developing MS. Similarly, Goldacre *et al.* (2004) showed that individuals suffering from MS were less likely to develop skin cancers, suggesting a decreased exposure to sunlight. Although, studies of Israeli individuals do not support sunlight as a possible explanation (Alter *et al.*, 2006; Kahana *et al.*, 2008). Studies showed that vitamin D both in its blood levels and the genetic differences in the vitamin D receptor are related to MS susceptibility (Ascherio and

Munger 2007b; Smolders *et al*., 2009). Exposure to sunlight is a major source of vitamin D therefore, sunlight may influence vitamin D rather than having a direct impact in itself. However, Europeans/Americans (with a high prevalence of MS), who increase their vitamin D intake or sunlight exposure do not change their risk of MS (Milo and Kahana, 2010). This further indicates that the vitamin D hypothesis does not fully explain the development of MS.

Research also suggests that infections or other diseases may result in MS. There are two main hypotheses for this explanation. The hygiene hypothesis suggests that encountering the infection/disease during childhood will be protective against MS. Encountering the same infection/disease during adulthood will lead to MS (Compston and Coles, 2008). Another theory is the prevalence hypothesis which proposes that MS is caused by some pathogen that is more common in regions with a high rate of MS (Kurtzke, 1993). In MS, findings support the hygiene hypothesis over the prevalence hypothesis (Ascherio and Munger, 2007a; 2007b). Multiple different bacterial and viral infections have been implicated in a potential cause for MS; mycobacterium avium paratuberculosis (Galiero *et al*., 2016), canine distemper (Milo and Kahanh, 2010), Epstein Barr (Ascherio and Munger, 2007a), human herpes simplex (Ascherio and Munger, 2007a), chyamydia pneumoniae (Marrie, 2004), acinetobacter species and pseudomonas aeruginosa (Hughes *et al*., 2003). Finally, exposure to solvents (Marrie, 2004), contaminated diet, hormonal intake (Ascherio and Munger, 2007a; 2007b), lifestyle such as smoking, and stress have been associated with MS (Ascherio and Munger, 2007a; 2007b; Marrie, 2004). Despite many different hypotheses, the exact aetiology of MS remains unknown. This may be due to the heterogeneous nature of MS, which becomes evident in the pathology (see section 1.3.5 page 9).

1.3.5 Pathology

MS is characterised by the widespread plaques in the brain and spinal cord. These plaques are the end result of a complex process of demyelination, involving multiple cells and processes in the central nervous system. Due to the MRI nature of this thesis, the main processes to be discussed here are inflammation, neurodegeneration and, to some extent, remyelination. Understanding these processes are necessary as they may impact data measures explored in this thesis.

Inflammation plays a major role in MS pathology and the process is dominated by T cells, macrophages and activated microglia. The inflammatory process in active lesions

coincides with disturbances in the blood brain barrier (Hochmeister *et al.*, 2006; Kirk *et al.*, 2003), proinflammatory cytokines and chemokines with their associated receptors (Cannella and Raine, 1995; Huang *et al.*, 2000). This is a very simplified explanation and there are very specific actions involved in the inflammatory process. In active tissue damage, inflammation is related to macrophages and activated microglia, whereas perivascular inflammation is associated with lymphocyte, mainly T cells with some B cell and plasma cell infiltrates (Booss *et al.*, 1983; Esiri *et al.*, 1980; Franciotta *et al.*, 2008; Hayashi *et al.*, 1988). Furthermore, the expression of proinflammatory and anti-inflammatory cytokines (Cannella and Raine 1995; Mycko *et al.*, 2003) or chemokines and their receptors (Balashov *et al.*, 1999; Holman *et al.*, 2011) which recruit lymphocytes, are related to the inflammatory process. In perivascular inflammation the CD8+ T cells are more plentiful than CD4+ cells, CD20+ cells, B cells or plasma cells (Frischer *et al.*, 2009; Hayashi *et al.*, 1988). Despite the severe white matter disruptions, MS is also characterised by severe damage to the grey matter (Audoin *et al.*, 2006; Battaglini *et al.*, 2009; Bendfeldt *et al.*, 2012; Bisecco *et al.*, 2018; Grothe *et al.*, 2016; Lansley *et al.*, 2013; Onu *et al.*, 2015; Parisi *et al.*, 2014; Prinster *et al.*, 2005; Riccitelli *et al.*, 2012; Sepulcre *et al.*, 2006; van de Pavert *et al.*, 2014; Zhang *et al.*, 2017). A new subtype of MS has recently been identified, where no white matter demyelination is present, only grey matter damage has been reported (Trapp *et al.,* 2018). The authors have named this subtype myelocortical MS, however, it is not presented as a phenotype in this thesis due to its recent identification. Furthermore, it only evident at autopsy, but does provide strong evidence that MS is not exclusively a disease of brain white matter.

Cortical demyelination is characterised by inflammatory infiltrates of the leptomeninges, which have a different inflammatory process than the perivascular inflammatory process, influenced by a variable amount of CD4+ T cells, CD20+ B cells, and plasma cells (Babbe *et al.*, 2000; Frischer *et al.*, 2009). In the meninges, there is a separation of T cells, B cells and plasma cells which form large aggregates. The process is related to B cell proliferation and cells expressing characteristics of follicular dendritic cells (Serafini *et al.*, 2004), which are associated with demyelination of the grey matter (Magliozzi *et al.*, 2007). Furthermore, the inflammatory process differs in the phenotype of MS. In the progressive phenotypes of MS demyelination is still present, but there is a lack of active demyelinating lesions. Instead, it is thought that the existing lesions have a more gradual increase in size (Prineas *et al.*, 2001), which is associated with moderate inflammatory infiltrates, with some T cell but predominantly activated microglia. This results in a slower rate of continual

demyelination (Allen and McKeown, 1979; Allen *et al.*, 2001). There is still some debate whether inflammation is the driving factor for demyelination (Henderson *et al.*, 2009; Marik *et al.*, 2007), or whether the demyelination occurs because of neurodegeneration which is influenced by an inflammatory response (Barnett and Prineas 2004; Stys *et al.*, 2012).

For neurodegeneration, it is essential to understand the basic cells involved in the central nervous system. Oligodendrocytes are completely lost in MS, a process known as apoptosis. But there are some inconsistent reports of the role of apoptosis in MS (Barnett *et al.*, 2009; Boneti and Raine, 1997; Ozawa *et al.*, 1994; Wolswijk, 2000). Ozawa *et al.* (1994) indicated that demyelination was strongly associated with oligodendrocyte apoptosis and that this would also result in a lack of remyelination. In contrast, Boneti and Raine (1997) concluded the opposite and stated that oligodendrocytes showed no evidence of apoptosis in MS. This research hypothesised that oligodendrocyte apoptosis may occur in MS, but that it may not necessarily be a characteristic feature. Barnett *et al.* (2009) showed that oligodendrocyte apoptosis did occur, but was restricted to specific lesions types, providing some explanation for the heterogeneous findings. In astrocytes, hypertrophy, where the cell cytoplasm increases are often observed in MS (van der Valk and DeGroot, 2000). Furthermore, astrocytes react to central nervous system damage and result in glial scars (Fawcett and Asher, 1999). Specifically in MS, astrocyte proliferation is widespread (Allen and McKeown, 1979), but the outcome of this is yet to be established. It has been proposed the astrocytes promote oligodendrocyte survival (Corley *et al.*, 2001), thereby decreasing the likelihood of oligodendrocyte apoptosis. However, the role of astrocytes in MS pathology remains unclear. Microglia act as macrophages in the central nervous system and play a role in both immune and inflammatory responses (Ponomarev *et al.*, 2005). Furthermore, they increase the expression of major histocompatibility complex class I and II antigens and release proinflammatory and anti-inflammatory cytokines (Luo *et al.*, 2017). This makes determining their function difficult as they promote both repair and damage. More recent research suggested that there are several subgroups of microglia and that they may each have a different function (Luo *et al.*, 2017). The exact role of each cell in the process of demyelination remains unclear. Recent research has demonstrated that an oxidative injury may provide a better understanding of neurodegeneration (Lassman, 2018). Studies have shown that degeneration of neurons, myelin and oligodendrocytes are associated with lipid oxidation (Fischer *et al.*, 2013) and this is related to mitochondrial damage (Campbell *et al.*, 2011; Dutta *et al.*, 2006; Mahad *et*

al., 2008). Despite the clear resulting demyelination in MS, the factors that may cause this process are far less clear. Both neurodegeneration and inflammation play a major role in MS pathology, but more work is required to understand the relationship between the two and the driving factor(s).

Regardless of the driving factor, MS pathology is characterised by demyelination. Despite this, it is possible for remyelination to occur (Prineas *et al*., 1979; 1993). If complete remyelination has occurred 'shadow plaques' are observed and are characterised by thin myelin sheath or with low myelin density. Remyelination is specifically associated with the relapsing-remitting phase of MS, this may be because active lesions are more predominant in this phenotype. Authors have posited that remyelination may occur because of oligodendrocyte progenitor cells being recruited to demyelinated regions (Lucchinetti *et al*., 1999; Prineas *et al*., 1989; Raine *et al*., 1981). This is further supported by findings of increased oligodendrocytes in both active and chronic lesions in peri-plaque areas (Chang *et al*., 2002; Solanky *et al*., 2011), suggesting the potential for remyelination. The process of remyelination is extremely heterogeneous, not only between individuals but also between the lesions within an individual. Barkhof *et al*. (2003) showed that approximately 40% of MS lesions showed remyelination demonstrating that some lesions do not undergo remyelination. Lesion location may play a role in this process as lesions in the deep white matter had a higher potential of remyelination than lesions in the periventricular white matter (Patrikios *et al*., 2006). Patrikios *et al*. (2006) also showed extensive remyelination in approximately 20% of MS individuals sampled, to the extent where almost all plaques were shadow plaques. Further research is needed to understand why some individuals undergo remyelination while others do not. The heterogeneity in remyelination may provide an explanation for the heterogeneity in symptomology in MS, specifically related to cognitive impairment and fatigue (see section 1.4.6 page 21). Furthermore, currently MRI is not able to distinguish between remyelinated and demyelinated lesions (Lassman *et al*., 2007) and the development of such techniques is essential both for understanding the process of remyelination, but also for selecting individuals for therapies.

1.4 Fatigue in MS

1.4.1 Definition

Fatigue is one of the most commonly reported symptoms of MS and over 90% report fatigue as one of their most debilitating symptoms (Branas *et al.*, 2000; Flachenecker *et al.*, 2002). Fatigue leads to impaired quality of life (Janardhan and Bakshi, 2002; Krupp *et al.*, 1988; Mitchell *et al.*, 2005), mental health (Fisk *et al.*, 1994; Schwartz *et al.*, 1996), physical health (Fisk *et al.*, 1994) and increased pain (Schwartz *et al.*, 1996). Some individuals with MS reported that fatigue is even more debilitating than the physical disability they have (Janardhan and Bakshi, 2002). Similarly, fatigue is one of the leading causes for individuals either decreasing their hours at work or for the complete loss of employment (Smith and Arnett, 2005; van der Hielie *et al.*, 2015). Despite the widespread impact of fatigue in MS, its underlying mechanisms remain very poorly understood and similarly to cognitive impairments are often underestimated. This may be largely due to the ambiguity and lack of a clear definition. Some definitions include a sense of exhaustion, lack of energy, or tiredness (Krupp *et al.*, 1988). Another definition states 'subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual or desired activity' (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998, page 2). This definition highlights an important distinction between physical (peripheral) fatigue and mental (central) fatigue. Peripheral fatigue is mostly seen in neuromuscular disorders and is more easily defined and measured than central fatigue. The common definition is the reduction of the ability of a muscle to generate force accompanied by an increase in the perceived effort by the individual (Bigland – Ritchie *et al.*, 1978; Liu *et al.*, 2005). Peripheral fatigue can result from increased exertion, or a reduction or complete failure in the neuromuscular system resulting in the inability to generate force (Enoka, 1992). Bigland-Ritchie *et al.* (1984) suggested three potential failure sites, those which lie within the central nervous system (CNS), those concerned with neural transmission between the CNS and the muscle and those within the individual muscle fibres. Failure at one of these sites may lead to increased activity at another site to maintain output, which is known as compensation (Liu *et al.*, 2003). Peripheral fatigue can be measured indirectly through electromyography (EMG) and directly from force measurements. On the other hand, central fatigue has been defined as the enhanced perception of mental effort and limited endurance for sustained physical and mental activities (Chaudhuri and Behan, 2004). It is important to note that the two types of fatigue are not mutually exclusive. The failure of an individual muscle fibre may

cause disruption to the motor output, whereas central fatigue may lead to disrupted transmission to the muscle fibres to begin with. Liu *et al*. (2003) used healthy participants to examine the experience of fatigue on cortical activation and muscle activity using a task which comprised of repeated submaximal maximal voluntary contractions. The authors demonstrated that the EMG signals increased in the working muscle as the experiment progressed, indicating that the muscle was indeed fatigued. In addition to this, the sensorimotor cortex, supplementary motor area, cerebellum, and dorsolateral prefrontal cortex (pFC) showed greater activity as the muscles fatigued. The increased activations of these regions are likely due to a greater central command required to sustain the force output (Liu *et al*., 2003; 2005). Similarly, Post *et al*. (2009) conducted fMRI to examine the cortical activity during a maximal voluntary contraction maintained for 22 seconds. They found that submaximal isometric contractions of 10, 30, and 50% of maximum voluntary force with the left and right index finger activated the precentral gyrus, postcentral gyrus, cingulate cortex, the insular cortex, rolandic opercular and cerebellum. Interestingly Post *et al.* (2009) reported that despite an increase in the overall activity of the brain, voluntary force activation decreased. Significantly more brain regions were activated in response to higher sustained submaximal voluntary contractions compared to lower voluntary contractions (50%, 30%, and 10%). Despite the increase in cortical activity, both maximal voluntary contractions and EMG decreased as the muscles fatigued, indicating that the increase in brain activity was unable to compensate for the decrease in voluntary drive. This was proposed to be a sign of central fatigue. Due to limited scope, this thesis will focus only on central fatigue.

1.4.2 Mechanisms of fatigue

There is very limited information on what may be causing the fatigue associated with MS, but the mechanisms are split into primary mechanisms and secondary mechanisms. Primary mechanisms involve the immune system and occur because of central nervous system damage. Secondary mechanisms involve associated conditions such as sleep disorders or depression. The specific factors thought to be involved in the primary mechanisms of fatigue are proinflammatory cytokines, endocrine influences, axonal loss and different patterns of functional brain activation. The inflammatory process (see section 1.3.5 page 9) demonstrated that cytokines are involved in MS pathology and these are implicated in fatigue (Braley and Chervin, 2010; Flachenecker *et al*., 2004; Heesen *et al*., 2006). The most commonly studied cytokines in the inflammatory process are interferon-γ and tumour necrosis factor (TNF)-α (Hirsch *et al*., 1985; Sharief and Hentges,

1991). Studies have shown that both interferon-γ and TNF-α are elevated in MS patients with fatigue, compared to MS patients without fatigue (Flachenecker *et al*., 2004; Heesen *et al*., 2006). This may be due to the differences in the inflammatory pathology across individuals with MS, rather than the fatigue, as MS is a very heterogeneous condition. Furthermore, the exact nature of the elevated cytokines is poorly understood, therapies using TNF-α antagonists showed that they were effective in reducing sleepiness in obstructive sleep apnoea (Vgontzas *et al*., 2004) and rheumatoid arthritis, without controlling for disease confounds (van Hoogmoed *et al*., 2013). Whereas in MS trials of TNF-α blockers showed detrimental effects (Arnason *et al*., 1999; van Oosten *et al*., 1996). Further work is required to understand the role of cytokines in MS and whether these are related to MS fatigue.

A further proposed primary mechanism for fatigue in MS relates to the endocrine system. The endocrine system consists of several glands, including the pituitary gland, and is involved in hormone production and secretion. The hypothalamic-pituitary-adrenal (HPA) axis and the hormone dehydroepiandrosterone (DHEA) have both been implicated in chronic fatigue syndrome (Cleare, 2003; Maes *et al*., 2005) and in individuals with lupus and rheumatoid arthritis (Chen and Parker, 2004) where fatigue is a common symptom. In MS the results are conflicting. Heesen *et al*. (2006) indicated that the HPA axis dysfunction was not related to MS fatigue, but showed some association to cognitive impairment. Whereas Gottschalk *et al*. (2005) demonstrated that MS individuals with fatigue had higher levels of adrenocorticotropin than MS individuals without fatigue. Similarly, Tellez *et al*. (2006) showed decreased DHEA in MS individuals with fatigue compared to those without fatigue. These results provide some support for a possible endocrine influence in MS fatigue, however, they are very inconsistent. This may be due to the heterogeneity of MS or alternatively that the function of the endocrine system is not fully understood in MS pathology.

Of particular interest to the current thesis, due to the MRI nature, is the proposed primary mechanism where altered cerebral activation may represent a primary mechanism of fatigue in MS (discussed further in section 1.4.7 page 22). In chronic fatigue syndrome, several studies have noticed widespread increased neuronal activation compared to healthy individuals (Caseras *et al*., 2006; Cook *et al*., 2007; Lange *et al*., 2005). Lange *et al*. (2005) proposed that this increased neuronal activation leads to increased use of neural resources, such as glucose and oxygen, and that this may provide an explanation for the increased fatigue. Multiple studies have shown a similar widespread increased activation

in MS compared to healthy participants in a variety of cognitive tasks including memory and attention (Bonnet *et al*., 2010; Lee *et al*., 2000; Lopez-Gongora *et al*., 2015; Mainero *et al*., 2006; Pantano *et al*., 2002a; 2002b; Parry *et al*., 2003; Reddy *et al*., 2000; Rocca *et al*., 2002; Staffen *et al*., 2002; Wishart *et al*., 2004). Tartaglia *et al*. (2008) compared fatigued and non-fatigued MS participants and showed an increased neuronal activation in the cingulate gyri and sensory cortex of the fatigued MS participants compared to the non-fatigued MS participants. These findings indicate that the increased neuronal activation may indeed lead to increased neuronal metabolism. Thereby leading to the increased perception of fatigue. Another explanation may be that the increased neuronal activation represents a form of functional brain reorganisation (Mainero *et al*., 2006) and that this reorganisation process may be related to MS fatigue. Penner *et al*. (2003) showed the opposite. They noted increased neuronal activation, but it was not related to the severity of fatigue. Similarly to the cytokine and endocrine mechanisms, there is variability in the findings across studies. This may be in part due to the heterogeneity of MS or due to the subjective nature of fatigue and the lack of definition and objective measurement for fatigue.

There are several secondary mechanisms that have been proposed for MS fatigue. MS is a disease that has several comorbidities and some of these may play an important role in MS related fatigue. There are several sleep disorders that accompany MS such as restless leg syndrome and chronic insomnia. Restless leg syndrome is approximately 3-5 times more prevalent in MS than in the general population (Auger *et al*., 2005; Italian REMS Study Group *et al*., 2008). Similarly, chronic insomnia is increased in MS individuals compared to healthy individuals and may be increased through pain, disability, depression, and anxiety among others (Chesson *et al*., 2000; Merlino *et al*., 2009; Rae-Grant *et al*., 1999). Attarian *et al*. (2004) showed that fatigued MS participants were more likely to have disrupted sleep compared to both non-fatigued MS and healthy individuals. On the other hand, Kaynak *et al*. (2006) showed that although there were differences on subjective ratings of sleep, there were no objective sleep differences between fatigued MS participants and both non-fatigued MS and healthy individuals. These studies suggest the sleep disturbances may in part contribute to MS related fatigue, but again the findings are inconsistent across studies. Furthermore, a recent systematic review showed that although sleepiness, measured by the Epworth Sleepiness Scale (Johns, 1991), was present in a large proportion of MS individuals, fatigue was more severe than sleepiness.

Moreover, participants reported significant fatigue without increased sleepiness (Popp *et al.*, 2017).

Depression is another common comorbidity of MS and the symptoms can often be mistaken for fatigue. Several studies have shown strong correlations between measures of fatigue and depression in MS (Flachenecker *et al.*, 2002; Kroencke *et al.*, 2000; Patrick *et al.*, 2009; Schwartz *et al.*, 1996). To avoid the possibility of confounds all participants with depression were excluded from the present thesis. Furthermore, due to the widespread symptomology of MS, individuals are often taking a variety of different medications. Studies have shown that immunosuppressive therapies, specifically interferon-β may enhance fatigue (Hadjimichael *et al.*, 2008; Iriarte *et al.*, 2000). Similarly, fatigue is a common side effect of anxiolytics and anti-depressant drugs which are often prescribed to MS individuals. A recent study by Thelen *et al.* (2014) indicated that polypharmacy in MS was associated with increased fatigue and subjective complaints of cognitive impairment. Medication in MS may have an impact on fatigue perception in MS. This is particularly noticeable at the onset of treatment but can reduce over time (Comi and Leocani, 2002) indicating that medication may exacerbate fatigue but is not the primary cause for MS related fatigue.

1.4.3 Measurement of fatigue

There are multiple fatigue rating scales available, which rely on self-reported ratings. There is currently no objective measure of fatigue. Many of the available questionnaires are not MS specific and are used across disorders such as chronic fatigue syndrome, lupus, and rheumatoid arthritis. Only MS specific scales are mentioned here, other fatigue scales used in a wider variety of disorders, are reviewed in Dittner *et al.* (2004). One of the most commonly used scales is the Chalder Fatigue Scale (Chalder *et al.*, 1993), which consists of 14 Likert scale items that measure both the mental and physical fatigue domains. Higher scores indicate enhanced fatigue. The Chalder scale has proven reliable and valid for fatigue measurement in chronic fatigue syndrome, but the findings of its validity in an MS sample is inconsistent (Braley and Chervin, 2010; Chilcot *et al.,* 2016). The Modified Fatigue Impact Scale (MFIS) (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998) contains 21 items for a multidimensional assessment including physical, cognitive and psychosocial functioning items. The MFIS provides good reproducibility and correlates well with other fatigue scales (Tellez *et al.*, 2005). The Fatigue Severity Scale (FSS) (Krupp *et al.*, 1989) contains nine items on a 7-point Likert scale from strongly

disagree to strongly agree. A score of 36 or above is categorised as significant fatigue. The FSS scale is validated in multiple, international MS populations (Armutlu *et al.*, 2007; Rosti-Otajarvi *et al.*, 2017; Valko *et al.*, 2008) and correlates well with various other fatigue scales. Furthermore, the FSS can distinguish between subgroups of individuals with MS, chronic fatigue syndrome and depression (Pepper *et al.*, 1993). Despite there being multiple validated fatigue scales for the use in MS they are all self-reported subjective measures of fatigue and none provide an objective measure of MS fatigue.

1.4.4 Treatments for fatigue

There have been both pharmacological and non-pharmacological therapies proposed for the treatment of fatigue in MS. Amantadine has been shown to involve cholinergic and dopaminergic transmission (Cohen and Fisher, 1989) and has, therefore, been FDA approved for Parkinson's disease. Despite some positive outcomes for MS related fatigue (Canadian MS Research Group, 1987; Cohen and Fisher, 1989; Krupp *et al.*, 1995; Murray, 1985; Rosenblum and Saffir, 1998), the studies used small samples and were not methodologically robust. As such amantadine has not been FDA approved for use in MS (Taus *et al.*, 2003). Pemoline, through dopaminergic effects, stimulates the central nervous system. Studies examining the influence on fatigue do not show positive results (Krupp *et al.*, 1995; Weinshenker *et al.*, 1992) and pemoline has been associated with liver toxicity, as such it is not a good option for treating MS fatigue. Modafinil is FDA approved for sleep disorders such as narcolepsy and obstructive sleep apnoea. For MS, the results remain unconvincing (Rammohan *et al.*, 2002; Stankoff *et al.*, 2005), although it may have a beneficial outcome (Niepel *et al.*, 2013). Studies have shown some benefit of glatiramer acetate for reducing fatigue in MS participants (Galetta *et al.*, 2002; Ziemssen *et al.*, 2008). Furthermore, aspirin has also been proposed as a treatment for MS with some positive outcomes, but only limited studies have examined its effect on MS fatigue (Wingerchuck *et al.*, 2005).

The results for non-pharmacological therapies are also rather inconsistent. Studies examining the use of cognitive behavioural therapy, relaxation therapy, exercise, energy conservation and cooling therapies (Butler *et al.*, 1991; Deale *et al.*, 1997; Di Fabio *et al.*, 1998; Mostert and Kesselring, 2002; Petajan *et al.*, 1996; Roehrs and Karst, 2004; Smith *et al.*, 2009; van Kessel *et al.*, 2008) show some positive effects, but the studies are limited due to the small samples. Thomas *et al.* (2013) developed a fatigue management programme (FACETS) and evaluated it in 164 individuals with MS across 3 centres. Results

demonstrated a moderate reduction of fatigue, that remained at 4-month follow-up and improved at 1-year follow-up (Thomas *et al.*, 2014). However, it did not show significant differences from the improvements noted in participants who received the standard treatment in current local practice. Larger more robust methodological studies are required to examine the effects of non-pharmacological therapies for MS fatigue. There are no reliably effective pharmacological or non-pharmacological treatments for MS fatigue, this may be due to the heterogeneity in MS, but can also be due to the lack of adequate definition and measurement of MS fatigue.

1.4.5 Cognitive impairment in MS

Cognitive impairment is a major symptom and is reported in 40-70% of individuals with MS (Benedict *et al.*, 2006; DeSousa *et al.*, 2002; Rao *et al.*, 1991a; 1991b). The deficits affect various aspects of cognitive functioning, including visual processing, verbal memory, visuospatial memory, long-term memory, working memory, attention, executive function and speed of information processing (Amato *et al.*, 2008; 2010; Arnett *et al.*, 1994; Bagert *et al.*, 2002; Beatty *et al.*, 1996; Benedict *et al.*, 2006; Bergendal *et al.*, 2007; Bobholz and Rao, 2003; Bruce *et al.*, 2010a; 2010b; Caine *et al.*, 1986; Calabrese *et al.*, 2006; Chiaravalloti and DeLuca, 2008; DeLuca *et al.*, 1994; 1998; 2004; 2013; 2015; Denney *et al.*, 2005; Drew *et al.*, 2008; Engle *et al.*, 2007; Litvan *et al.*, 1988; Mainero *et al.*, 2006; McCarthy *et al.*, 2005; Pardini *et al.*, 2014; Rao *et al.*, 1986; 1989; 1991a; 1991b; Thornton *et al.*, 2002). Whereas, simple attention, verbal skills, and general intelligence typically remain intact (Benedict *et al.*, 2006; Rao *et al.*, 1991a; 1991b). Furthermore, these cognitive deficits can vary greatly across individuals and are often unrelated to perceived cognition. MS participants often perceive more cognitive difficulties than suggested by objective measures (Middleton *et al.*, 2006; Roberg *et al.*, 2012). Studies have demonstrated that the greatest deficit in MS is speed of information processing and that this impairment drives the disruptions noted in other cognitive domains (Chiaravalloti *et al.*, 2013; Denney *et al.*, 2004; Denney and Lynch, 2009; Lengenfelder *et al.*, 2006). For example, Kalmar *et al.* (2008) showed that speed of information processing can predict performance on the everyday tasks component of the executive function and performance test. Similarly, the paced auditory serial addition test (PASAT), a test of working memory, is significantly impacted by the speed of information processing. As such studies examining cognitive impairment in MS must be sensitive to the contribution of speed of information processing.

There have been some attempts to develop a treatment for the cognitive impairment in MS. These treatments have involved either cognitive rehabilitation programmes or pharmacological approaches. The few studies that have examined cognitive rehabilitation programmes have very inconsistent results. Studies have shown some benefit of treatment for executive functions (Lincoln *et al*., 2002), attention efficiency (Plohmann *et al*., 1998; Pusswald *et al*., 2014) and memory (Chiaravalloti *et al*., 2005a), whereas others have shown no benefit for the same cognitive domains (Allen *et al*., 1995; 1998; Birnboim and Miller, 2004; Lincoln *et al*., 2002; Solari *et al*., 2004). In terms of pharmacological treatments studies have examined the effectiveness of disease modifying therapies, as well as treatments specifically for cognitive impairment. Studies of interferon beta-1b have shown some benefit for cognitive function (Barak and Achiron, 2002; Fischer *et al*., 2000; Pliskin *et al*., 1996), whereas glatiramer acetate has shown no benefit for cognitive functioning (Weinstein *et al*., 1999). Acetylcholinesterase inhibitors is not a recent development and were originally studied in an MS population in 1988 (Leo and Rao, 1988). Some improvement in verbal memory has been noted with physostigmine (Leo and Rao, 1988), but this study had methodological concerns. More recently donepezil has shown some enhanced cognitive function in MS (Christodoulou *et al*., 2006; 2008; Krupp *et al*., 2004). Stimulant drugs such as amantadine hydrochloride have had no impact on cognitive function in MS (Lovera *et al*., 2007). Also, rivastigmine has shown to improve processing speed in MS (Huolman *et al*., 2011). Future studies using rigid methodology with adequate power are needed to reliably assess treatments for cognitive impairment.

Cognitive impairment is also affected by several factors associated with MS. Depression is a major symptom of MS, although the relationship with cognitive impairment remains unclear (Arnett *et al*., 2008a). Some studies have shown that depression is associated with cognitive impairment (Arnett, 2005; Landro *et al*., 2004), whereas others have shown no relationship (DeLuca *et al*., 1994; Fischer, 1988; Good *et al*., 1992). Interestingly a review of the literature determined that when studies had adequate power and included a representative sample of individuals with MS, there was a consistent adverse effect of depression on cognition (Arnett *et al*., 2008a). Furthermore, performance on cognitive tasks may be affected by oral motor (Arnett *et al*., 2008b; Smith and Arnett, 2007) and visual disturbances associated with MS (Bruce *et al*., 2007; Feaster and Bruce, 2011). A major contributor to cognitive function is fatigue in MS. Neuroimaging studies of cognitive impairment have shown that individuals with MS consistently demonstrated activation in regions not activated in healthy individuals. Moreover, they showed

increased activation in regions that are activated in healthy individuals, although the exact regions do differ (Audoin *et al.*, 2003; 2005; Chiaravalloti *et al.*, 2005b; Mainero *et al.*, 2004; Penner *et al.*, 2003). Studies have proposed that this may be due to functional reorganisation (Filippi and Rocca, 2004; Mainero *et al.*, 2004; 2006) and that some of this recruitment may be compensatory to overcome the neuronal loss associated with MS (Mainero *et al.*, 2006; Filippi and Rocca, 2004; Wishart *et al.*, 2004). Furthermore, this increased widespread activation has been proposed as a primary mechanism in fatigue (see section 1.4.2 page 14). In chronic fatigue syndrome, several studies have noticed similar widespread increased neuronal activation compared to healthy individuals (Caseras *et al.*, 2006; Cook *et al.*, 2007; Lange *et al.*, 2005). Lange *et al.* (2005) proposed that this increased neuronal activation leads to increased use of neural resources, such as glucose and oxygen, and that this may provide an explanation for the increased fatigue. Similarly, the functional reorganisation (Filippi and Rocca, 2004; Mainero *et al.*, 2004; 2006) may play a role in MS related fatigue. This relationship is the major interest of this thesis and as such will focus on how fatigue affects a specific aspect of cognition and not cognitive impairment in MS more generally.

1.4.6 Performance measures of fatigue

Behavioural studies employing neuropsychological tests show inconsistent results on whether fatigue in MS affects cognition. One of the earliest studies examined reaction times before and after a four-hour neuropsychological testing period (Jennekens-Schinkel *et al.*, 1988). Results showed that both reaction time and subjective fatigue increased for both the HC and MS groups. There was no difference in the magnitude of the fatiguing effect between the groups. Kujala *et al.* (1995) split their MS participants into groups of cognitively impaired and cognitively preserved. The results show that the cognitively impaired group had slow performance throughout the task, whereas the cognitively preserved group only showed slower performance towards the end of the task, suggesting a fatiguing effect. Johnson *et al.* (1997) administered the PASAT four times over a three-hour testing period in individuals with chronic fatigue syndrome, depression, and MS and compared to healthy individuals. The results indicated that all three patient groups performed significantly worse than healthy individuals at baseline. Over time for the chronic fatigue and depressed groups, the performance increased from session one to two and two to three and then remained unchanged in session four. For the MS group there was a significant increase in performance in session two and then remained stable over sessions three and four. This was the same pattern observed in healthy controls. The

results from this study found that there was no association between fatigue and cognitive performance over time. Paul *et al.* (1998) measured grip strength, learning over time of PASAT performance and vigilance tests, but showed no differences in terms of a fatiguing effect between HC and MS groups. Krupp and Elkins (2000) administered a neuropsychological test battery twice during a four-hour testing period. Results showed that healthy group increased in their performance the second time, whereas the MS group showed decreased performance over time. Schwid *et al.* (2003) administered the PASAT and the digit ordering test (DOT) four times over three testing sessions and performance from the fourth time was analysed. There were no differences on the DOT, but the MS group showed a fatigue effect on PASAT performance. This was not observed in the healthy group. Furthermore, subjective fatigue ratings and PASAT performance were correlated in the MS group. Bryant *et al.* (2004) also subdivided their MS participants into groups with cognitive impairment and those without. All groups, including the healthy individuals, showed a significant fatigue effect. To establish why MS individuals were not showing a greater fatigue effect than healthy individuals, Bryant *et al.* (2004) examined cognitive load. This demonstrated that when the cognitive load was low, the MS groups had a significant fatiguing effect not seen in the HC groups. When cognitive load was high, both groups showed a fatiguing effect. Bailey *et al.* (2007) conducted an n-back task in MS and healthy individuals. The results showed limited evidence of objective cognitive fatigue in the MS group, but they reported significantly increased subjective fatigue. Furthermore, the subjective and objective ratings of fatigue were not correlated. Claros-Salinas *et al.* (2010) administered reaction time tests of alertness, inhibition and divided attention three times a day over two days for individuals with MS, individuals who had suffered from a stroke and healthy individuals. Performance on the tasks decreased for both patient groups compared to controls, this also corresponded to increased subjective ratings of fatigue. These results indicate that there is some association between fatigue and cognition. But this is somewhat inconsistent when subjective measures of fatigue are used and better established when measuring fatigue more objectively. This highlights the importance of a reliable standardised objective measure of fatigue.

1.4.7 Neuroimaging of fatigue and cognition

With the recent advent of MRI, there have been many studies that have evaluated MS fatigue using neuroimaging both structurally and functionally. In terms of structural imaging multiple studies have established that brain atrophy affects both the white matter (Bendfeldt *et al.*, 2010; Parisi *et al.*, 2014; Prinster *et al.*, 2010; Riccitelli *et al.*, 2012; Zhang *et*

al., 2017) and grey matter in MS (Audoin *et al*., 2006; Battaglini *et al*., 2009; Bendfeldt *et al*., 2012; Bisecco *et al*., 2018; Grothe *et al*., 2016; Lansley *et al*., 2013; Onu *et al*., 2015; Parisi *et al*., 2014; Prinster *et al*., 2005; Riccitelli *et al*., 2012; Sepulcre *et al*., 2006; van de Pavert *et al*., 2014; Zhang *et al*., 2017). Only a few studies have examined the relationship between fatigue and brain atrophy (Calabrese *et al*., 2010; Cruz-Gomez *et al*., 2013; Derache *et al*., 2013; Finke *et al*., 2015; Gobbi *et al*., 2013; Riccitelli *et al*., 2011; Sepulcre *et al*., 2009). Sepulcre *et al*. (2009) showed that fatigue scores were correlated with lesion load, specifically with grey matter lesions in the parietal, frontal and anterior cingulate, and white matter lesions in superior and middle frontal regions. Calabrese *et al*. (2010) examined the cortical thickness of the basal ganglia and thalamus in fatigued and non-fatigued MS participants. Results showed atrophy in the striatum, thalamus, superior frontal gyrus and inferior parietal lobe for fatigued MS participants compared to non-fatigued MS individuals. Riccitelli *et al*. (2011) compared MS participants who suffered from fatigue and those who did not. They demonstrated no regional grey matter differences between these two groups but did show that fatigue severity was correlated with atrophy in the precentral gyrus. Similarly, Cruz-Gomez *et al*. (2013) showed high fatigue scores were associated with the supplementary motor area. On the other hand, Derache *et al*. (2013) showed that fatigued MS participants had significant grey matter reduction in the frontal, temporal and parietal cortex compared to non-fatigued MS participants. Furthermore, they demonstrated that fatigue scores were related to atrophy in the basal ganglia. In direct contrast, Finke *et al*. (2015) did demonstrate both grey and white matter atrophy of the basal ganglia in the MS group but observed that this atrophy was unrelated to fatigue. Recent studies have examined fatigue in MS using diffusion tensor imaging. Genova *et al*. (2013) showed that fractional anisotropy in the internal capsule (basal ganglia) was associated with increased subjective ratings of fatigue. Hanken *et al*. (2015) showed that MS participants had increased axial and radial diffusivity in the corpus callosum compared to healthy participants. Furthermore, fatigued MS participants demonstrated increased axial and radial diffusivity in the fibres between the hypothalamus and mesencephalon compared to non-fatigued MS participants. Finke *et al*. (2015) showed reduced white matter integrity in fatigued MS participants compared the healthy individuals. There is little consensus on the exact regions that may be associated with fatigue in MS. This may be because MS is a very heterogeneous population and leads to great difficulties with diagnosis and treatment. However, basal ganglia

atrophy is noted in multiple studies and may lead to a problem with the cortical-subcortical connections. Functional MRI studies can further elucidate this explanation.

Functional imaging is a relatively new area of research but has advanced the understanding of fatigue in MS. An early study using positron emission tomography (PET), compared glucose metabolism in fatigued and non-fatigued MS individuals (Roelcke *et al.*, 1997). The fatigued group showed hypermetabolism in the putamen, pFC, premotor cortex and the supplementary motor area in comparison to the non-fatigued MS group. Subjective fatigue scores correlated negatively with glucose uptake in the pFC. The authors concluded that basal ganglia and prefrontal regions were responsible for fatigue in MS. Fillipi *et al.* (2002) similarly, split their MS participants into fatigued and non-fatigued groups but used fMRI instead of PET. Their results showed reduced activation in the precuneus, cerebellum, pFC and thalamus in the fatigued group compared to the non-fatigued group during performance of a motor task. Furthermore, there was a negative correlation between subjective fatigue scores and signal intensity in the thalamus. Authors also suggested that this may be evidence for a disruption of the cortical-subcortical projections between the thalamus, basal ganglia and motor and prefrontal regions. Tartaglia *et al.* (2008) attempted to induce fatigue using the PASAT test. Participants completed a finger-thumb apposition task in the MRI scanner, then completed the PASAT, followed by a second run of the finger-thumb apposition task. After completing the PASAT task there was increased activation in the cingulate gyri and primary sensory cortex and decreased activation in the premotor and supplementary motor areas in the MS groups. Whereas the healthy group only showed decreased activation. This demonstrates that fatigue can alter cerebral activation. DeLuca *et al.* (2008) used a form of sustained attention, by administering four trials of the modified symbol digit modalities task (mSDMT) during fMRI acquisition. The results showed that MS participants were significantly slower than healthy individuals and this behavioural difference was associated with increased activation in the basal ganglia, pFC, parietal cortex, thalamus and occipital lobes in the MS group. Genova *et al.* (2013) used a task switching paradigm designed to induce fatigue in the MRI scanner. Results showed that increased activity in the caudate was specifically related to MS fatigue compared to the healthy group. Finke *et al.* (2015) observed increased resting-state functional connectivity of the basal ganglia in fatigued MS participants. Pravata *et al.* (2016) showed that fatigued MS participants had stronger resting-state functional connectivity between the superior frontal gyrus and the occipital, frontal and temporal regions. Furthermore, the superior

frontal gyrus was hyperconnected to the caudate and thalamus during PASAT performance, which also correlated with subjective fatigue ratings. Rocca *et al*. (2016) showed that fatigued MS participants had decreased activation in the middle temporal gyrus, supplementary motor area, superior frontal gyrus, postcentral gyrus and basal ganglia compared to both non-fatigued MS and healthy participants. Bonzano *et al*. (2017) used a finger tapping task during three fMRI sessions; baseline, after a finger motor task and after a brief rest period, in an MS group. Increased activation was observed in the basal ganglia, thalamus, and amygdala after the fatiguing task but returned to baseline after rest. There was no group with which to compare. Many of these studies provide evidence that the basal ganglia network is implicated in MS fatigue. The mechanism by which the basal ganglia may be implicated in MS fatigue will be reviewed in section 1.5.6 page 30.

1.5 Basal ganglia

The basal ganglia (Figure 1.3) refer to a group of subcortical nuclei that consist of the striatum (caudate and putamen), globus pallidus (internal and external), subthalamic nucleus and the substantia nigra (pars reticularis and pars compacta). The basal ganglia structures can be simplified by splitting them into input, output and intrinsic nuclei.

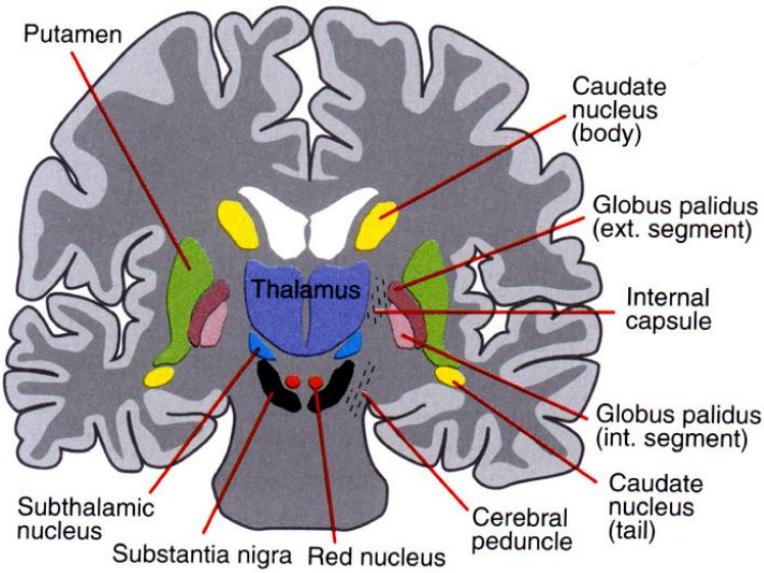


Figure 1.3 Illustration of basal ganglia nuclei. *Note basal ganglia nuclei are striatum (caudate and putamen), globus pallidus (internal and external), subthalamic nucleus and the substantia nigra (pars reticularis and pars compacta). Image from Leisman et al. (2013), with permission.*

1.5.1 Input nuclei

The striatum, which consists of the caudate and the putamen, are the major input nuclei of the basal ganglia. The striatum is considered the largest subcortical structure in the

brain at approximately 10cm^3 (Schröder *et al.*, 1975). It is comprised of GABAergic spiny projection neurons, also known as medium spiny neurons. These medium spiny neurons receive input from the cortex, thalamus, and dopaminergic neurons. The multiple cortical regions that project to the striatum can be grouped into regions underlying cognitive, sensorimotor and affective functions (Redgrave *et al.*, 2010). Similarly, the input from the thalamus can be grouped into motor, cognitive and limbic functions (Erro *et al.*, 2002; Ragsdale and Graybiel, 1991; Smith and Parent, 1986). Finally, the striatum receives input from the dopaminergic neurons (see section 1.5.4 page 28). The projections from the medium spiny neuron can be divided into a direct and indirect pathway (Figure 1.4). The direct pathway consists of the medium spiny neurons that project directly to the basal ganglia pathway (output nuclei section 1.5.3 page 27), whereas the indirect pathway consists of the medium spiny neurons that project, first, to the intrinsic nuclei (see section 1.5.2 page 26). There are also interneurons contained within the striatum, which can be divided into four distinct groups based on their neurochemical and morphological characteristics (Kawaguchi *et al.*, 1995). These are cholinergic interneurons, paravalbumin-containing GABAergic interneurons, calretinin containing GABAergic interneurons and somatostatin-containing interneurons. It is thought that these interneurons create an intra-striatal microcircuit that modulates the activity of the striatal medium spiny neurons (Lanciego *et al.*, 2012). Although the exact function of these interneurons remains unclear (Nelson and Kreitzer, 2014) they may play a role in dopamine modulation (see section 1.5.4 page 28).

1.5.2 Intrinsic nuclei

The globus pallidus can be subdivided into the external globus pallidus and the internal globus pallidus. Similarly, the substantia nigra is subdivided into the substantia nigra pars reticularis and the substantia nigra pars compacta (see section 1.5.4 page 28). The internal globus pallidus and substantia nigra pars reticularis form the major output nuclei (see section 1.5.3 page 27). Whereas the external global pallidus and subthalamic nucleus form intrinsic nuclei in the basal ganglia. The external globus pallidus have connections throughout the basal ganglia and receives GABAergic inputs from the striatum as well as excitatory inputs from the subthalamic nucleus. But can also project signals to the subthalamic nucleus (indirect pathway), output nuclei and back to the striatum (Nelson and Kreitzer, 2014). These findings demonstrate that the external globus pallidus may serve as a relay station between the subthalamic nucleus and the striatum.

The subthalamic nucleus consists mostly of glutamatergic neurons. Originally it was proposed as simply receiving GABAergic input from the external globus pallidus and projecting to basal ganglia output neurons (Albin *et al.*, 1989; DeLong *et al.*, 1990). There has been growing research on a hyperdirect pathway that projects from the cortex to the subthalamic nucleus, bypassing the striatum, and then projecting onwards in the basal ganglia pathway, resulting in faster conduction times (Nambu *et al.*, 2000; 2002; 2004; 2005). This hyperdirect pathway predominantly subserves motor related functions (Nambu *et al.*, 2002). More recently it has been proposed that the hyperdirect pathway has two primary functions. It inhibits motor responses to inhibit unnecessary movement and optimizes goal-directed motor response (Takada *et al.*, 2013). The addition of this pathway suggests that the subthalamic nucleus is perhaps a stronger input nucleus than originally proposed. Furthermore, studies showed that the subthalamic nucleus receives inputs from intralaminar nuclei in the thalamus and caudate (Castle *et al.*, 2005). Along with the feedforward projection from the subthalamic nucleus to the output nuclei, there are also projections back to both other intrinsic nuclei and the striatum (Carpenter *et al.*, 1981; Sato *et al.*, 2000; Smith *et al.*, 1990; Wall *et al.*, 2013).

1.5.3 Output nuclei

The internal globus pallidus and substantia nigra pars reticularis form the major output nuclei. These nuclei share several cyto- and chemoarchitectural characteristics, both consist of inhibitory GABAergic neurons. The output nuclei mainly receive two forms of input from the direct and indirect pathway. The inputs through the direct pathway originate from the medium spiny neurons in the striatum and are a major inhibitory input for the output nuclei. The inputs from the indirect pathway are relayed through the subthalamic nucleus and provide an excitatory input for the output nuclei (Lanciego *et al.*, 2012). The main role of the globus pallidus is to then send signals to the thalamus and brainstem. These outputs are also split into pallido-thalamic and nigro-thalamic projections, although both innervate the ventral anterior motor thalamus, the projections differ on a cellular level. The pallido-thalamic projections are received by the densicelluar and parvicellular areas, whereas the nigro-thalamic projections are received by the magnocellular regions (Lanciego *et al.*, 2012).

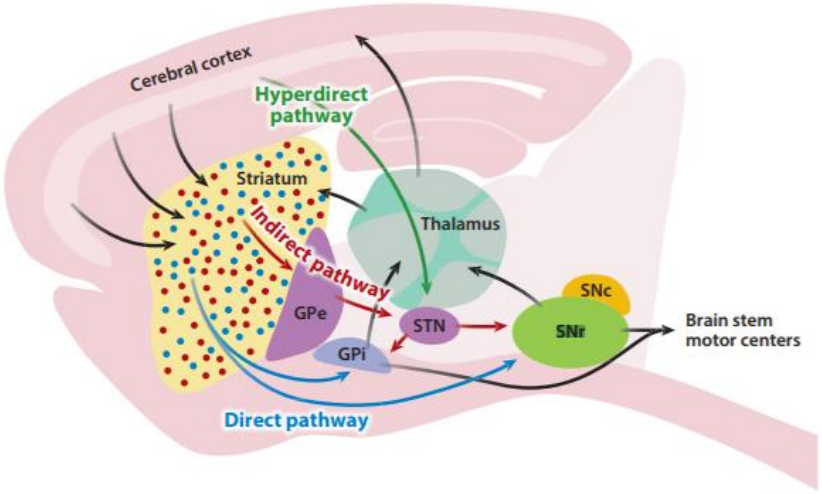


Figure 1.4 Illustration of basal ganglia nuclei and pathways in rodent brain. *Image from Nelson and Kreitzer (2014), with permission.*

1.5.4 The role of dopamine

The substantia nigra pars compacta consists of tyrosine-positive neurons and is a major region supplying dopamine to the basal ganglia. Dopamine plays a major role in basal ganglia excitability, mainly through strong connections from the whole basal ganglia (input, output, and intrinsic nuclei) to dopaminergic substantia nigra regions. Dopamine acts as both a neurotransmitter and neuromodulator in the striatum (Abercrombie *et al.*, 1997; Smith *et al.*, 1994). As a neurotransmitter dopamine is released by action potentials in the peri synaptic region and is fast and short-lasting (Abercrombie *et al.*, 1997). As a neuromodulator, dopamine is released in the extracellular space which produces a much longer lasting action in the striatum (Descarries *et al.*, 1996; Gonon, 1997; Kawagoe *et al.*, 1992). The levels of dopamine are modulated by substantia nigra pars compacta firing rate and dopamine reuptake and metabolism (Hefti *et al.*, 1985; Rodriguez *et al.*, 2003a; 2003b; 2007). Dopamine regulates the excitability of the medium spiny neurons in the striatum through both pre- and postsynaptic mechanisms. Presynaptically, dopamine reduces the glutamatergic release in the striatum, which modulates the large input from the cortex and thalamus (Bamford *et al.*, 2004). Postsynaptically, dopamine increases the excitability of medium spiny neurons and thereby increases the expression of glutamate receptors (Hernandez-Lopez *et al.*, 2000). It seems that the interneurons in the striatum may play a role in dopamine modulation, specifically the cholinergic interneurons. Studies have shown that the cholinergic interneurons reduce presynaptic glutamate release (Hernandez-Echeagaray *et al.*, 1998) and excite the medium spiny neurons (Surmeier *et al.*, 2007). Also, the fast firing paravalbumin-containing GABAergic interneurons mediate

a feed-forward inhibition of medium spiny neurons (Mallet *et al.*, 2005). Research shows that the dopaminergic system is responsible for both the inhibition and excitation of medium spiny neurons in the striatum, which through its strong connection to the rest of the basal ganglia (direct and indirect pathway) has a major impact of basal ganglia function.

1.5.5 Cortical loops

The projections between the cortex and the basal ganglia are functionally divided into five distinct cortico-basal ganglia loops. These are the motor, occulo-motor, associative, limbic and orbital-frontal loops (Alexander *et al.*, 1986). The motor loop projects from the motor and somatosensory cortex to the putamen, which projects on to the ventrolateral globus pallidus (both internal and external). From the internal globus pallidus, the loop continues to the ventrolateral thalamus and then back to the somatosensory cortex. The projections from the external globus pallidus have both feed-forward and feedback projections to the subthalamic nucleus. The occulo-motor loop originates in frontal eye fields and project to the caudate body, from where the loop projects to the caudal and dorsomedial internal globus pallidus and the ventrolateral substantia nigra. From the substantia nigra, the loop is projected back to the frontal eye fields via the ventral anterior and dorsal medial thalamus. The associative loop originally known as the dorsolateral pFC loop originates in the dorsolateral pFC and projects to both the caudate and the putamen. The projections from the putamen follow the indirect pathway, first to the external globus pallidus and then to the internal globus pallidus. The projections from the caudate run directly to both the internal and external globus pallidus. From the external globus pallidus, there are both feed-forward and feedback projections to the subthalamic nucleus, which also projects to the internal globus pallidus. From the internal globus pallidus the loop projects to the ventral anterior thalamus and back to the dorsolateral pFC. The limbic loop, originally known as the anterior cingulate loop, originates in the limbic regions but is mediated by inputs from the anterior cingulate cortex (ACC) and projects to both the caudate and the putamen. The loop stops in the caudate, but from the putamen continues to the internal globus pallidus, from where it projects back to the ACC via the thalamus. At the same time, there are feed-forward and feedback projections between the subthalamic nucleus and external globus pallidus, but they do not form part of the rest of the loop. The orbital-frontal loop projects from the lateral orbital frontal cortex to the ventromedial caudate nucleus, which then extends towards the caudate tail. From the ventromedial caudate it projects to the dorsomedial internal globus pallidus,

then to the anterior ventral and medial dorsal thalamus and finally back to the lateral orbital frontal cortex. The motor, associative and limbic loops are considered the main functional loops and are illustrated in Figure 1.5.

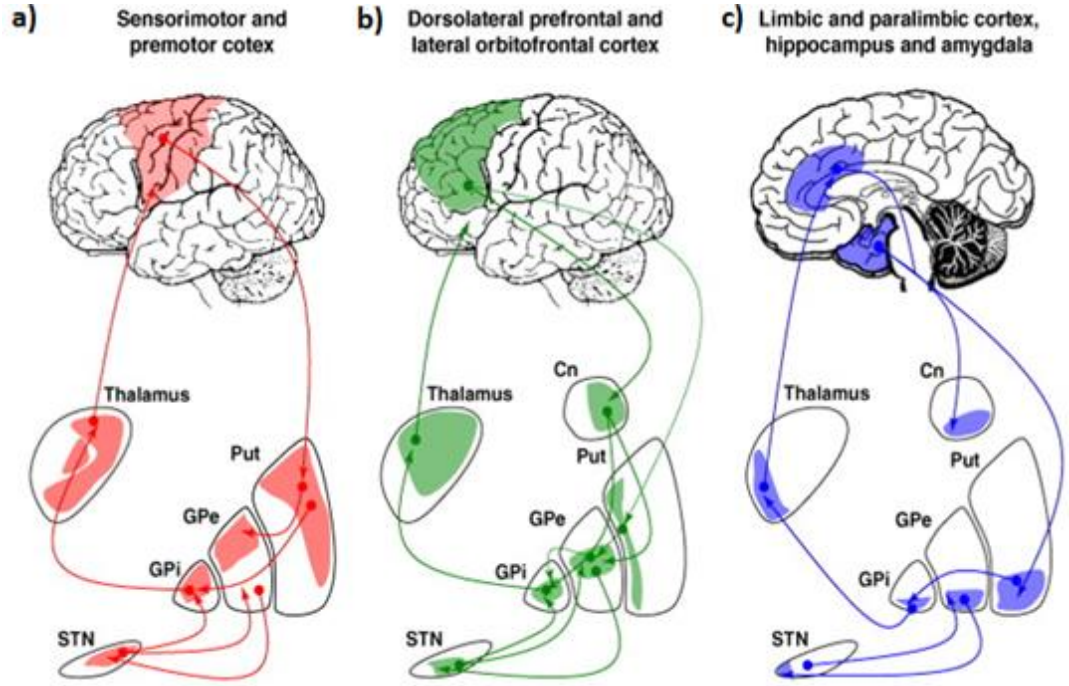


Figure 1.5 Main functional divisions of the cortical-basal ganglia connections. *a) motor loop. b) associative loop. c) limbic loop. Image from Obeso et al. (2008), with permission.*

1.5.6 Basal ganglia and MS fatigue

Chaudhuri and Behan (2000; 2004) proposed that the non-motor function of the basal ganglia may be implicated in central fatigue. Multiple studies examining both brain atrophy (Bisecco *et al.*, 2018; Derache *et al.*, 2013; Finke *et al.*, 2015; Lansley *et al.*, 2013; Prinster *et al.*, 2005; Riccitelli *et al.*, 2012; Sepulcre *et al.*, 2006; van de Pavert *et al.*, 2014; Zhang *et al.*, 2017) and brain function (Bonzano *et al.*, 2017; DeLuca *et al.*, 2008; 2009; Filippi *et al.*, 2002; Finke *et al.*, 2015; Genova *et al.*, 2013; Pravata *et al.*, 2016; Roelcke *et al.*, 1997; Rocca *et al.*, 2016a) have implicated the basal ganglia and its cortical connection loops (Engstrom *et al.*, 2013) in MS fatigue (see section 1.4.7 page 22). Chaudhuri and Behan (2000) proposed that the loss of motivational influence from the striato-thalamic system to the frontal lobes may play an integral role in fatigue. To understand this mechanism in more detail it is important to understand how the basal ganglia network is involved in motivation and reward, through its dopaminergic influences. The basal ganglia network is modulated by reward and more specifically the expectation of reward (Bowman *et al.*, 1996; Hikosaka *et al.*, 1989; Kawagoe *et al.*, 1998; Lauwereyns *et al.*, 2002; Sato and Hikosaka, 2002; Schultz *et al.*, 1992; Takikawa *et al.*, 2002). Studies have suggested that

dopamine differentially innervates the direct and indirect pathways of the basal ganglia based on the dopamine receptors (Gerfen *et al.*, 1990). D1 receptors are excitatory and innervate the direct pathway, and D2 receptors, which innervate the indirect pathway, are inhibitory (Mallet *et al.*, 2006; Nakamura and Hikosaka, 2006; Shen *et al.*, 2008). As such, the direct pathway is active when reward is expected and is therefore involved in reward-oriented movements, whereas the indirect pathway inhibits movements that lack reward by processing non-reward predicting signals (Frank, 2005; Hikida *et al.*, 2010; Hikosaka, 2007; Hong and Hikosaka, 2011; Nakamura and Hikosaka, 2006). When performing any task energy is expended. But a choice based on expected reward and expected energy expenditure must be made before the task is executed. If the expected reward outweighs the expected energy expenditure the action is worth completing. Both expected reward and predicted energy expenditure can be determined using previous knowledge and information of reward and expenditure in long-term memory. For example, the memory of running a marathon but not winning, may inhibit the motivation to run a marathon again as it resulted in extreme energy expenditure but little reward. It may be that the limbic loop is of interest when examining motivation. But similarly, the associative loop projects to the frontal cortex, which is more related to decision making. Chaudhuri and Behan (2000) hypothesised that the failure to integrate the limbic system and the motor functions within the basal ganglia may lead to increased central fatigue. In MS fatigue where perceived exertion is high, motivation may be decreased due to the imbalance between reward and expenditure. This may be even more noticeable in actions that require prolonged effort. Despite the significant, widespread disruption to cognition and everyday functioning from fatigue and the significant contribution of neuroimaging in understanding mental fatigue, it is still a frequently overlooked symptom. Chaudhuri and Behan (2004) strongly stated that it would be incorrect and inappropriate for medical professionals to suggest that fatigue is a medically unexplained and non-organic symptom.

1.6 Alertness

The alertness mechanism is uniquely suited to providing an objective measure of fatigue in MS. Several studies have suggested that mental fatigue is associated with prolonged task performance, even in healthy individuals (Boksem *et al.*, 2005; Faber *et al.*, 2012; Oken *et al.*, 2006). As such this section will elaborate on the definition, neural mechanisms and relationship between fatigue and alertness.

1.6.1 Definition

In 1890, William James stated that everybody knew what attention was, however, this has not been the case at least in terms of cognitive psychology. A definition by Shiffrin (1988) highlights that attention is a very broad term. Here, attention is defined as "all those aspects of human cognition that the subject can control and to all aspects of cognition having to do with limited resources or capacity, and methods of dealing with such constraints" (Shiffrin, 1988, p. 739). Attention can be influenced by both top-down and bottom-up processing. Top-down processing involves information from higher to lower centres of attention, as such, it is dominated by previous knowledge, expectations, and goals, whereas bottom-up processing involves sensory information processing that originates from lower centres and project to higher cognitive centres (Corbetta and Shulman, 2002). These processes have some neural overlap (Katsuki and Constantindis, 2013) but remain distinct process (Pinto *et al.*, 2013). Posner and Petersen (1990) propose that attention can be broadly sub-divided into three distinct domains; orienting to sensory events, detecting signals for processing, and maintaining an alert state. Orienting is related to the prioritisation of sensory input through the selection of targets, often via eye or motor movements. Originally orienting was proposed to involve the pulvinar, superior colliculus and parietal cortex (Posner and Petersen, 1990), more recently frontal areas, such as the frontal eye fields have also been implicated in orienting (Garg *et al.*, 2008; Thompson *et al.*, 2005). Target detection is related to focused attention and has more recently been proposed as an executive component of attention and is proposed to be the main capacity limited process of attention (Petersen and Posner, 2012). The main regions underlying target detection are the ACC and prefrontal regions (Posner and Petersen, 1990). With the wealth of neuroimaging data over the last 20 years these regions have recently been re-examined, however, due to scope will not be discussed here (for brief review see Petersen and Posner, 2012). Particularly interesting to this thesis is the alertness domain. Alertness is the act of preparing a response to an imminent stimulus and is the ability to prepare and maintain response readiness. An increased alertness is associated with a quicker behavioural response (Raz and Buhle, 2006). Alertness can be further subdivided into intrinsic (tonic) and extrinsic (phasic) alertness. Intrinsic alertness is defined as the internal control of attention and occurs without a warning signal. It represents a self-motivated state of awareness (Plohmann *et al.*, 1998; Sturm *et al.*, 1999). Whereas extrinsic alertness represents the ability to increase the response readiness following the presence of a warning stimulus (Plohmann *et al.*, 1998; Posner, 1975). The

presence of a warning signal produces a decrease of reaction time (Perin *et al.*, 2010; Petersen and Posner, 2012).

There is great difficulty when examining alertness as there is a large amount of ambiguity in the terms used. The terms vigilance, sustained attention, arousal and alertness are often being used synonymously and interchangeably in the literature despite the significant differences. The terms arousal and alertness are often synonymously used when referring to states of consciousness (Tassi and Muzet, 2001; Zhou *et al.*, 2015) or in the sleep-wake cycle (Aston-Jones, 2005; Waterhouse *et al.*, 2012). But arousal can also be physiological which is more closely related to the autonomic nervous system, with differences in heart rate and respiratory patterns depending on the state of physiological arousal (Appelhans and Luecken, 2006). Vigilance, for example, can be used synonymously with alertness, but can also refer to attention to potential threats (being hypervigilant) (Oken *et al.*, 2006). As a result, it is often arduous to determine which studies are evaluating the same concept as it requires evaluating the study design, task or aims in more detail. Vigilance is the most similar to alertness in the sense of a response readiness. Mackworth (1948) defined vigilance as the psychological readiness to respond. Whereas sustained attention can be seen as the duration of maintaining response readiness and is defined as the ability to direct and then focus attention on specific stimuli (DeGangi and Porges, 1990). An important distinction between vigilance, sustained attention and alertness relates to the measurement. Sustained attention and vigilance are most concerned with accuracy such as the number of hits and misses (Sturm and Willmes, 2001), whereas alertness focuses on the speed of response. In this thesis, alertness is used in the traditional sense of response readiness, where either intrinsic alertness (no warning stimulus) or extrinsic alertness (presence of warning stimulus) can be measured by reaction time.

1.6.2 Neuroimaging of alertness

fMRI studies have identified a similar frontal-parietal-thalamic-brainstem network activated in both intrinsic and extrinsic alertness. Previous neuroimaging studies have proposed a noradrenergic alertness network comprised of the locus coeruleus, frontal regions and the parietal cortex activation (Posner and Peterson, 1990). This suggests a frontal-parietal pathway sub-serving alertness, originating in the brainstem (Lewin *et al.*, 1996; Pardo *et al.*, 1991), and projecting to frontal and parietal regions such as the ACC, dorsolateral pFC and inferior parietal lobule (Mottaghy *et al.*, 2006; Sturm *et al.*, 1999; 2004). Specifically, structures in the right hemisphere of the brain are thought to be vital

for the normal function of alertness (Pardo *et al.*, 1991; Strum *et al.*, 2004). Individuals with lesions in the right hemisphere often report attentional deficits which provide support for the importance of the right hemisphere in attention (Chica *et al.*, 2012; Howes and Boller, 1975). Research has proposed a voluntary top-down network and an automatic bottom-up network of specific brain regions that are involved in alertness (Michiels *et al.*, 1999; Mottaghy *et al.*, 2006). The automatic bottom-up network is thought to arise from noradrenergic neurons arising from the brainstem that relay through the thalamus and project to frontal and parietal regions in the right hemisphere (Michiels *et al.*, 1999). In the voluntary top-down system, the noradrenergic activity is controlled by the dorsolateral pFC and the ACC, which allows the individual to selectively focus their attention on specific stimuli (Mottaghy *et al.*, 2006). Mottaghy *et al.* (2006) found that the bottom-up network is modulated through the thalamus by the top-down network. Together these findings suggest that the ACC, dorsolateral pFC, thalamus, and brainstem are important structures involved in alertness. The involvement of these regions is further supported by Perin *et al.* (2010), who used an alertness task examining both intrinsic and extrinsic alertness to show increased activation of the dorsolateral pFC, ACC, inferior parietal lobule and thalamus in both tasks. These findings indicate that a network involving these four regions may be crucial to alertness. Although the literature indicates that both intrinsic and extrinsic alertness activate a similar frontal-parietal-thalamic-brainstem network, the right hemisphere is more dominant in intrinsic alertness, whereas the left hemisphere is more dominant in extrinsic alertness (Sturm *et al.*, 2004).

1.6.3 Relationship with fatigue

Several studies suggest that mental fatigue is associated with prolonged task performance, even in healthy individuals (Boksem *et al.*, 2005; Faber *et al.*, 2012; Oken *et al.*, 2006). Boksem *et al.* (2005) measured visual attention for a 3-hour period. The authors showed that increased fatiguability reduced goal-directed attention and resulted in more stimulus-driven processing. Faber *et al.* (2012) conducted an Eriksen flanker task and showed that increased fatiguability resulted in a decreased ability to suppress irrelevant information. The decreased performance is thought to occur as a result of decreased top-down control (Boksem *et al.*, 2005; Helton and Russel, 2010; Warm *et al.*, 2008) and increased boredom and distraction (Pattyn *et al.*, 2008). Studies have often employed psychomotor tests to assess fatiguability and performance. The psychomotor vigilance task (PVT) has been shown to be sensitive to sleep disruptions and is a good objective indicator of cognitive impairment in various conditions such as partial sleep loss (Veksler

and Gunzelman, 2018), chronic sleep restriction (Belenky *et al.*, 2003; Dinges *et al.*, 1997; van Dongen *et al.*, 2003) and sleepiness (Philip *et al.*, 2004). The PVT is a simple reaction time task which requires participants to press a button in response to a digital stimulus. Despite being a reaction time task, often accuracy is the main outcome measure. Studies have also measured cognitive fatigue as reaction time on cognitive tasks across time (Boksem *et al.*, 2005; Helton and Russell, 2011; Howes and Boller, 1975; Sturm and Willmes, 2001). Multiple studies have used reaction time tasks to measure fatigue in MS (see section 1.4.6 page 21). In MS specifically, there seems to be a larger deficit of reaction time (Chiaravalloti *et al.*, 2013; Denney *et al.*, 2004; Denney and Lynch, 2009; Lengenfelder *et al.*, 2006) than measures of simple attention (Benedict *et al.*, 2006; Rao *et al.*, 1991a; 1991b). As such alertness tasks, measuring reaction time, are uniquely suited to providing an objective measure of fatigue in MS. In section 1.5.6 (page 30) the relationship between the basal ganglia and fatigue was reviewed in terms of motivation. Through its dopaminergic influences, motivation plays a role in prolonged alertness. Individuals who are less motivated will be less alert compared to individuals with high motivation (Oken *et al.*, 2006). This further suggests that alertness tasks could potentially provide an objective measure of fatigue and potential basal ganglia dysfunction in MS.

1.7 Thesis chapters

Chapter 2 will provide a brief review of each of the methodological approaches used in this thesis with some explanation of the common methodology conducted throughout the thesis. Chapter 3 provides a systematic review of the fMRI literature in alertness in healthy individuals. This is to determine which paradigms are used to assess alertness and to establish whether there is a consensus neural network that underlies healthy alertness. Chapter 4 conducts a detailed analysis of the neuropsychological test prior to and post completing the alertness-motor paradigm to examine how mental fatigue affects cognition. This is to elucidate the impact of fatiguability on cognitive performance. Chapter 5 is a voxel-based morphometry analysis to determine any structural morphometrical differences between the HC and MS groups, with fatigue correlates of any differences explored. Chapter 6 examines the behavioural performance on the alertness-motor paradigm to determine if there is evidence that the alertness mechanism can provide an objective performance measure of fatigue. Chapter 7 presents the neural correlates of the performance of the alertness-motor paradigm described in chapter 6. Chapter 8 uses a complementary functional connectivity analysis to further elucidate the role of the basal ganglia in fatigue. In Chapter 9 the experimental findings are discussed

in terms of the overall thesis aims, including consideration of the limitations of the current thesis and recommendations for future studies.

Chapter 2 General Methods

2.1 Functional magnetic resonance imaging

2.1.1 Physics of magnetic resonance imaging

Magnetic resonance imaging (MRI) allows for *in vivo* imaging of the body. MRI was founded on the basic principle of nuclear magnetic resonance (NMR). The physics behind NMR was independently discovered by Bloch (1946) and Purcell *et al.* (1946). Nuclei that contain an odd number of neutrons or protons exhibit a property named spin, which is the rotation of the nucleus around an axis (Rahman, 1986). As the number of protons in the nucleus increases the time required for image acquisition increases. Due to its abundance in the human body and the speed of image acquisition, the hydrogen molecule (single proton) is most commonly used in MRI. The combination of spin and the charge of the proton produces a magnetic property. This, in turn, produces angular momentum, where the nucleus will continue to rotate unless disrupted (precess). In the absence of an external magnetic field, the magnetic property is cancelled out. Whereas, in an applied magnetic field (B_0), the protons align with B_0 and create a net magnetization. By applying a rotating magnetic field (B_1), known as a radiofrequency (RF) pulse, the net magnetization is tipped into the transverse plane, perpendicular to B_0. The atoms precess in the direction of B_1 at the Lamour frequency. The Lamour frequency is set by the gyromagnetic ratio (g), which is constant for a given nucleus, and the strength of B_0. The precession slows gradually as the transverse relaxation (xy plane) decreases and the longitudinal relaxation (z plane) increases. The transverse relaxation produces a voltage signal which is measured in NMR (Plewes and Kucharczyk, 2012).

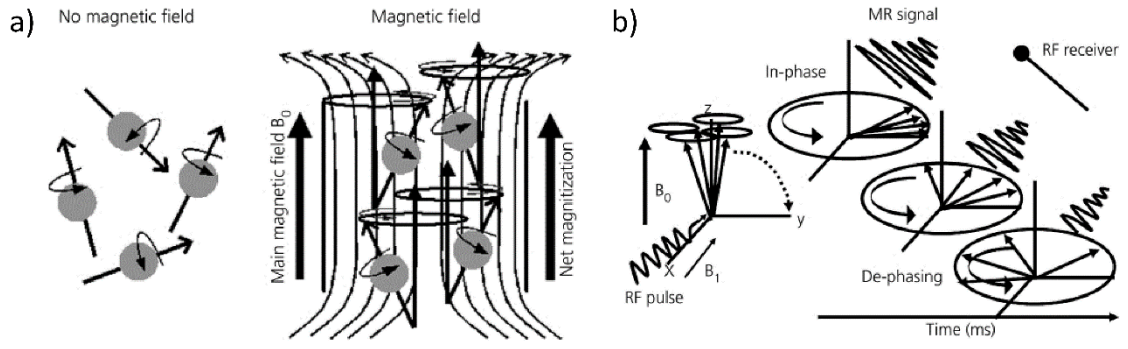


Figure 2.1 Composite image of behaviour of hydrogen atoms during MRI sequence. *a) shows behaviour of hydrogen atoms in non-magnetic and magnetic environments. b) shows hydrogen atoms flipped into the transverse plane (x-y) following an RF pulse. Images from Ferris et al. (2006) with permission.*

Pairs of receiver coils, tuned to the Lamour frequency, detect the direction of rotation and the transverse magnetization of the precession. Because tissue types have unique

longitudinal and transverse relaxation times, MRI can detect the different tissues by altering the pulse sequence (Plewes and Kucharczyk, 2012). The data is collected in a k-space matrix, where each data point is derived from the MR signal detected by the receiver coils. The axes in the matrix correspond to the horizontal (x) and vertical (y) axes of the image. The relationship between the k-space data matrix and the image data is a simple Fourier transformation using frequency and phase encoding methods (Mitchell, 1999).

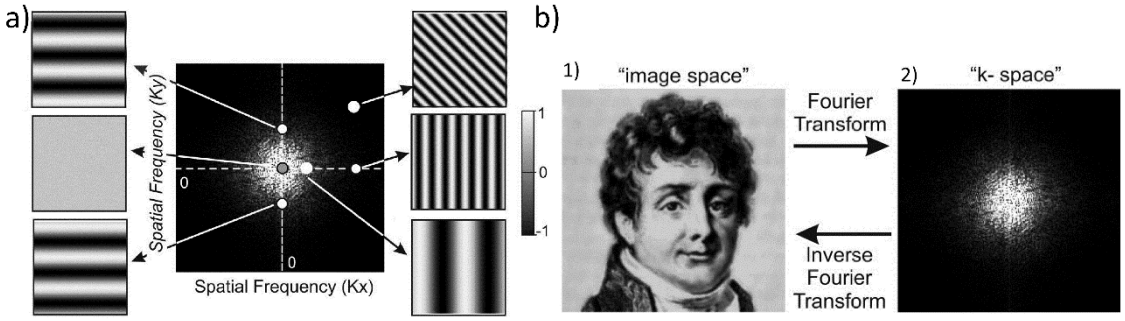


Figure 2.2 Composite image of k-space. *a) represents different locations in k-space and their corresponding stripe patterns. b) represents the difference between image space and k-space where 1) is Jean Baptiste Joseph Fourier in image space and 2) is his Fourier transform in k-space. Images from Plewes and Kucharczyk (2012) with permission.*

2.1.2 Spin and gradient echoes

A significant issue in MRI is signal decay following the initial RF pulse (free induction decay). This decay occurs for two reasons, due to spin relaxation and local magnetic field inhomogeneities which cause spins to precess at different rates. The transverse relaxation cannot be rephased and results in an inevitable loss of MR signal. Hahn (1950) originally offered a solution. By applying a second 90° RF pulse, the dephasing nuclei in the magnetic field are rephased. This is known as spin echo (SE). Carr and Purcell (1954) established the now widely used SE sequence where a 180° RF pulse follows the initial 90° RF pulse. The second 180° RF pulse is applied at echo time/2. This results in an increased MR signal, as the spins continue to precess at the same speed and direction, resulting in a rephasing of the transverse relaxation.

An alternative to SE, gradient echo, instead uses magnetic field gradients to rephase the relaxation. Gradient echo alters the resonance frequency, by applying an external dephasing gradient field. This speeds up the dephasing of free induction decay. A second rephasing gradient of the same strength but opposite polarity is applied, creating a gradient echo. Spin-echo and gradient echo sequences differ in two key respects, no 180° RF rephasing pulse is applied, and the flip angle used is lower than 90°. Using lower flip angles leads to faster longitudinal relaxation and results in shorter scanning times.

Therefore, gradient echo sequences are most routinely used in rapid MR imaging (Tang *et al*, 2014).

2.1.3 Blood oxygenation level dependent signal

Functional magnetic resonance imaging (fMRI) allows for the detection of activity patterns in the brain. fMRI is a relatively new technique and has only been implemented for the last 28 years (Ogawa *et al*., 1990), however, it has greatly advanced our understanding of the brain in this time. The basic principle that underlies fMRI is the blood oxygenation level dependent (BOLD) signal. This technology exploits the basic physics of NMR and the intrinsic magnetisation of deoxyhaemoglobin, present in the venous system. The oxygenated haemoglobin molecule is diamagnetic as it has no unpaired electron, whereas deoxyhaemoglobin is paramagnetic due to the unpaired electron (Pauling and Coryell, 1936; Thulborn *et al*., 1982). The presence of deoxyhaemoglobin creates local magnetic field distortions which result in a decreased MR signal, whereas reduced deoxyhaemoglobin would result in an increased MR signal. As fMRI measures the venous system, which contains deoxyhaemoglobin, it seems paradoxical to have an increase in MR signal. This is because both regional cerebral blood flow (rCBF) and regional cerebral metabolic rate (rCMR) increase during neuronal activation. However rCBF increases by approximately 29% whereas the rCMR for oxygen only increases by about 5% (Fox and Raichle, 1986). This results in an increase in oxygenated blood in the venous system during neuronal activation. Therefore, the ratio between oxygenated and deoxygenated blood increases and as a result the MR signal also increases. The change in the ratio between oxygenated and deoxygenated blood is known as the haemodynamic response function, which is used in fMRI to infer activation in the brain (Gore, 2003; Kim and Bandettini, 2011).

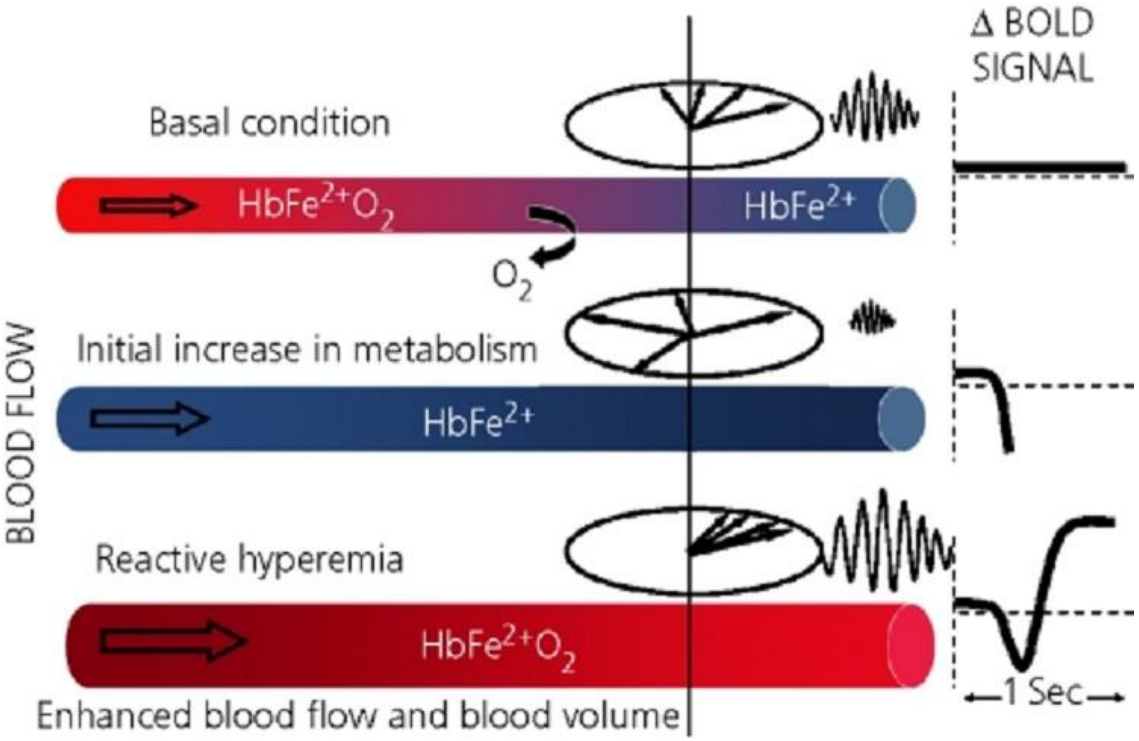


Figure 2.3 Schematic of BOLD signal. *In the basal condition, there is an increased concentration of deoxygenated haemoglobin, during the initial increase in metabolism there is a loss of oxygen, which produces a decreased signal, this is followed by a sudden increase of cerebral blood flow and volume and therefore a decrease in deoxygenated haemoglobin leading to an increased MRI signal. Image from Ferris et al., (2006) with permission.*

There is not a direct relationship between the BOLD signal and neuronal activity. The action potentials that reflect immediate neuronal firing generate electrical impulses, which are detected in electroencephalography (EEG) and magnetoencephalography (MEG) but not in fMRI. The BOLD signal measures the haemodynamic response to an action potential which increases approximately 2 seconds after an action potential. Several studies have suggested that the BOLD signal correlates better with local field potential (LFP). The LFP is thought to consist of both excitatory and inhibitory post synaptic potentials (Logothetis, 2003). The fact the LFP is a combination of both post-synaptic and pre-synaptic activity across several neurons, it is referred to as peri-synaptic activity. Logothetis *et al*. (2001) created a device that allowed for the measurement of LFP, fMRI and the spiking of neurons in monkey visual cortex, thereby examining the correlates of the BOLD signal. The electrophysiology data was filtered into both lower frequency LFPs and high frequency multi-unit activity (MUA). There was a strong correlation between the BOLD signal and LFPs, and a weaker correlation between BOLD and MUA. This finding suggests that the BOLD signal is better associated with LFPs than the spike rate. The correlation between the BOLD signal and MUA was determined to occur as a result of the correlation between spike rate and LFP, rather than a direct correlation between spike

rate and BOLD signal (Logothetis and Wandell, 2004). This correlation is further supported by studies that have suppressed the spiking in the visual cortex, but still showed a robust relationship between BOLD and LFPs (Rauch *et al.*, 2008; Viswanathan and Freeman, 2007). These findings also suggested a dissociation between spike rate and BOLD signal but did suggest that spiking occurred as a result of the LFPs. Shmuel *et al.* (2006) showed that decreases in LFPs and MUA correlated with decreases in BOLD signal. Similarly, decreases in LFPs, but not spiking, was correlated with decreased blood oxygenation (Devor *et al.*, 2007). These findings strongly support that the BOLD signal is related to LFPs and that the BOLD signal, therefore, represents peri-synaptic activity. However, as mentioned previously LFPs relate to both excitatory and inhibitory potentials, both of which lead to an increased BOLD signal, thereby making it difficult to interpret whether activation in a brain region has an excitatory or inhibitory function.

2.1.4 Simultaneous multi-slice fMRI

Generally, MR imaging is temporally slow. Echo planar imaging (EPI) was introduced to increase the temporal resolution (Mansfield, 1977; Ordidge *et al.*, 1981; 1982). In a conventional SE sequence, one line in k-space (or one phase-encoding step) is collected during each repetition time (TR) period. Whereas in an EPI pulse sequence, multiple lines of k-space are acquired during a single TR period. This is possible as instead of measuring only a single echo following an RF pulse, EPI imaging acquires multiple echoes. Each echo is phase encoded differently on the phase-encoding axis (in k-space) using phase-encoding blips. Each line in the k-space matrix is then represented by the frequency encoding gradient and each phase-encoding blip corresponds to a new line in the k-space (Poustchi-Amin *et al.*, 2001).

More recently a method called simultaneous multi-slice (SMS) or multiband (MB) imaging has been developed (Moeller *et al.*, 2010). SMS uses a combination of MB RF excitations and parallel imaging, by under sampling k-space in one phase encoding dimension. This results in highly accelerated, high-resolution whole brain coverage, as multiple slices are excited and acquired simultaneously. With this technique, each channel of the receiver coil receives a linear combination of signals and is weighted by coil sensitivity profiles, from each of the slices. Together with matrix inversion, these are used to reconstruct the signal for individual slices. Unlike parallel imaging alone, there is no loss of the signal-to-noise ratio due to reduced data collection because the slices are excited and sampled identically. However, there is a reduction of signal-to-noise because

of g-factor, due to slice aliasing. The g-factor is dependent upon the number of aliased replicates for every point and the differences in coil sensitivities. To overcome this slice aliasing, techniques that manipulate the aliasing have been proposed. The first method proposed was 'controlled aliasing in parallel imaging results in higher acceleration' (CAIPRINHA) (Breuer *et al.*, 2006), which controlled aliasing by alternating the MB RF pulses and thereby providing individual slices with different phase cycles. However, Setsompop (2012) improved on CAIPRINHA and suggested a method that has an even lower g-factor penalty called 'blipped-controlled aliasing in parallel imaging' (blipped-CAIPI). This technique increases the distance between aliasing pixels by creating inter-slice image shifts in the phase encoding direction. The shifts between the slices are created by simultaneously using EPI phase encoding blips and sign and amplitude modulated slice-gradient blips.

A further concern in SMS imaging is that of signal leakage (L-factor). The traditional reconstruction used for SMS imaging is the slice-GRAPPA technique (Moeller *et al.*, 2012) and was based upon a linear convolution model for in-plane acceleration. An improvement of this technique, split-slice GRAPPA, was proposed by Cauley *et al.* (2014) and was found to reduce the signal leakage artefacts further. However, signal leakage is specifically related to the in-plane acceleration with SMS imaging. The total SMS factor used is calculated by multiplying the MB factor and in-plane acceleration factor. Several studies have evaluated the SMS imaging technique, and all recommended the use of moderate acceleration factors (Boyacioğlu *et al.*, 2015; Preibisch *et al.*, 2015; Todd *et al.*, 2016; Xu *et al.*, 2013). Boyacioğlu *et al.* (2015) suggested that SMS imaging provides both increased sensitivity and increased spatial specificity compared to standard sequences at high field strength. Similarly, Preibsch *et al.* (2015) and Todd *et al.* (2016) indicated that SMS imaging techniques allow for increased sensitivity and acquisition time without compromising image quality. However, they suggest using moderate acceleration factors, such as 8. Todd *et al.* (2016) further suggested that experiments must be optimised cautiously to minimise the false positive rate.

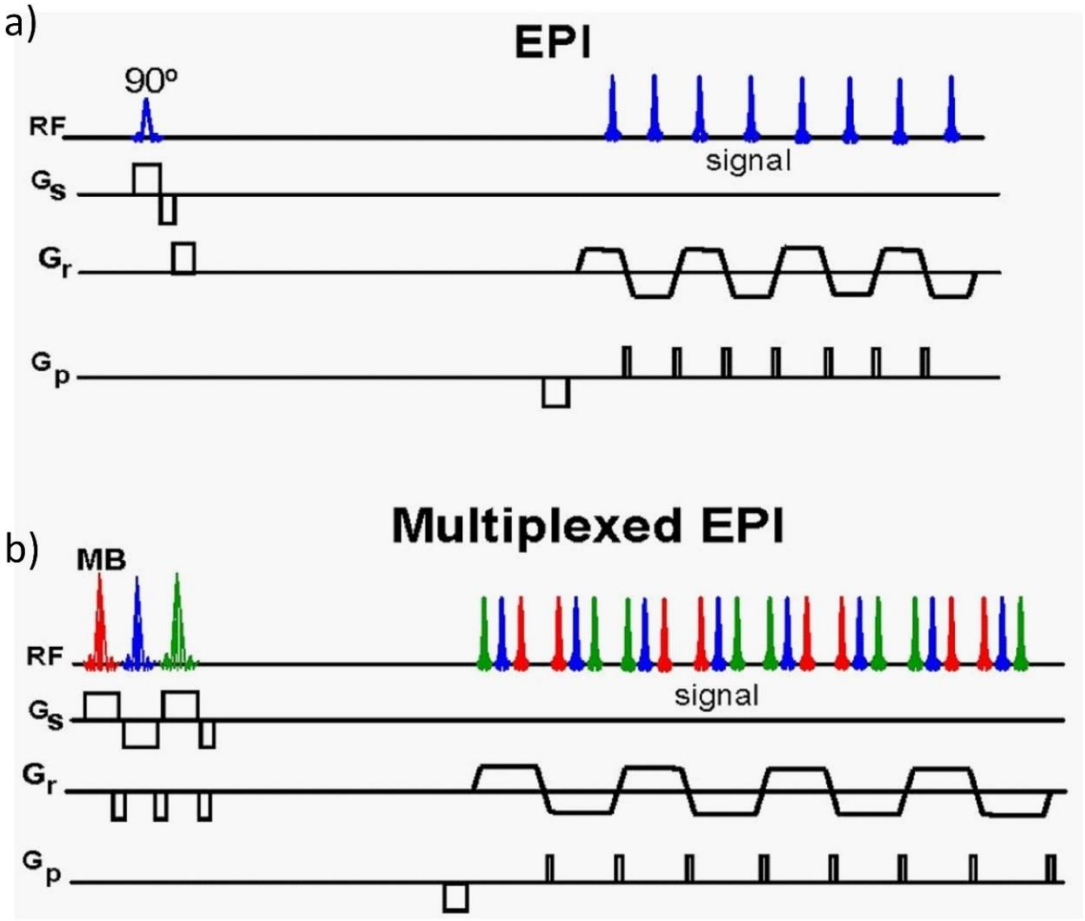


Figure 2.4 Composite image of EPI and SMS imaging pulses. *a) displays a conventional single slice gradient-echo EPI. b) displays an SMS modified sequences, the composite pulse used for acquisition of 3 simultaneous slices (MB=3). Image adapted from Feinberg et al. (2010) with permission.*

2.2 Analysis approaches

2.2.1 Voxel-based morphometry

The brain changes as it develops into adulthood. It decays with age and may also undergo changes due to disease. Morphometry evaluates the basic anatomical structure of the brain by evaluating the size and the shape of the brain and its structures. Voxel-based morphometry (VBM) is a technique that uses the anatomical scan acquired from MRI to evaluate the structure of the brain. In VBM the brain is segmented broadly into grey matter, white matter and cerebrospinal fluid (CSF) and enables the examination of volumetric differences between groups, for example, healthy control (HC) and multiple sclerosis (MS) groups. Furthermore, both the global, whole brain and regional, specific region of interest (ROI), volumes can be evaluated using this technique (Ashburner and Friston, 2000).

The first step of VBM requires the T1-weighted anatomical image of an individual to be normalised to a group template, this is to allow for voxel by voxel agreement across

participants. This is achieved by using a non-linear registration that stretches or compresses regions with respect to the template. A deformation field is created, which holds information on the extent to which the input image must be altered to match the template image. The deformed image is then segmented into the different tissue groups (grey matter, white matter, CSF) based upon the likelihood of finding a tissue class at a given location, using tissue priors and the intensity of the image. This creates a segmented image which contains values of tissue concentration at each voxel. These segmented images are then spatially smoothed and the tissue concentration from different participants are combined using a voxel wise statistical analysis (Ashburner and Friston, 2000). The volumes of brain structure can be correlated with various behavioural measures. For example, Maguire *et al.* (1999), found that taxi drivers had increased regional brain volumes in regions responsible for spatial navigation compared to non-taxi drivers. Similarly, Scholtz *et al.* (2009) showed that learning a new skill, such as juggling, increases white matter volume in associated regions. Disease progression can also impact brain volume, multiple studies have used VBM to examine atrophy in MS in both the white matter (Bendfeldt *et al.*, 2010; Parisi *et al.*, 2014; Prinster *et al.*, 2010; Riccitelli *et al.*, 2012; Zhang *et al.*, 2017) and grey matter (Audoin *et al.*, 2006; Battaglini *et al.*, 2009; Bendfeldt *et al.*, 2012; Bisecco *et al.*, 2018; Grothe *et al.*, 2016; Lansley *et al.*, 2013; Onu *et al.*, 2015; Parisi *et al.*, 2014; Prinster *et al.*, 2005; Riccitelli *et al.*, 2012; Sepulcre *et al.*, 2006; van de Pavert *et al.*, 2014; Zhang *et al.*, 2017). These studies highlight the usefulness of the VBM technique in examining brain structure.

2.2.2 Graph theory

fMRI is an exceptional non-invasive technique for understanding activation in the human brain, however, the general linear model (GLM) analysis employs a system level approach. This provides quality information about the system required for task performance and the differences between tasks and groups, but connectivity approaches allow for further definition of the integration of the system. Moreover, they can provide some information about how the system integrates and communicates. There are various approaches that allow for connectivity analysis. Dynamic Causal Modelling (DCM) (Friston *et al.*, 2003) is a Bayesian generative model that attempts to explore the mechanistic of brain activation at a neuronal level. DCM allows for inferences to be made about the coupling among brain regions, as well as how this coupling is influenced by different experimental conditions. However, DCM generally deals with a relatively small number of ROIs. This is largely because DCM was designed to specifically test hypotheses of neuronal mechanisms that

underlie brain activations in a particular experiment, it is not an exploratory technique (Stephan *et al.*, 2010). Due to the heterogeneous nature of MS, there is a lack of clear ROIs in this field and as such DCM is perhaps not the most useful connectivity approach.

A, perhaps, more meaningful approach for this thesis, which allows for more exploratory analysis, is based on a mathematical technique called graph theory. In mathematics, graph theory is the study of graphs, which are mathematical structures used to model pairwise relationships between objects. A graph consists of a set of nodes, and a set of connections between the nodes, generally called edges. In an unweighted graph, the edges are either present or absent, whereas in a weighted graph the edge carries information on the strength of the connection between two nodes. Nodes can also be either directed or undirected, for example, a directed edge represents that one node exerts some influence on another, but not the other way around. Undirected edges on the other hand only suggest that two nodes are connected, without referring to the directionality of the connection. It is possible to represent a graph as an adjacency matrix, where the number of rows and columns are equal to the number of nodes. Pearson's correlations are often used for creating the adjacency matrix. In an undirected graph, the matrix is symmetric unlike in a directed graph matrix. In a weighted graph, the values in the matrix represent the weight of each edge, which is determined by the strength of the correlation. In an unweighted graph, the values in the matrix are either 0, if the edge is absent, or 1, if the edge is present (Kaiser, 2011). However, a threshold on the correlation must be set to determine whether the edge is present or not. There is little consensus in the literature as to how this threshold should be determined. Authors argue that the setting of thresholds is completely arbitrary (De Vico Fallani *et al.*, 2014; Rubinov and Sporns, 2010; 2011), but the threshold used directly impacts the results of the analysis (Garrison *et al.*, 2015; Jalili, 2016; Zalesky *et al.*, 2012).

Graph theory is becoming increasingly used in the field of neurocognitive science to understand both the structural and functional organisation of the brain. In the context of neuroscience, a graph is synonymous with a brain network and as such will be referred to as a brain network from this point. Neuroanatomically a node can be an ROI in the brain and the edges are the functional or structural connections between the ROIs. Complex functional brain networks are represented statistically by network measures, which provide quantification of global and local properties of the brain network (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010), key elements of which are displayed in Figure 2.5. For example, the characteristic path length is the average shortest path length

between all pairs of nodes in a network, and the clustering coefficient is the fraction of triangles around an individual node (Watts and Strogatz, 1998). Network measures can be used, among others, to characterise the functional integration, segregation, and centrality of a brain network or to quantify the importance of a single brain region to the whole network. The network measures can be used to evaluate connectivity differences between groups, such as MS and HC. The specific network measures used for this thesis are explained in more detail in section 8.2.4 (page 161).

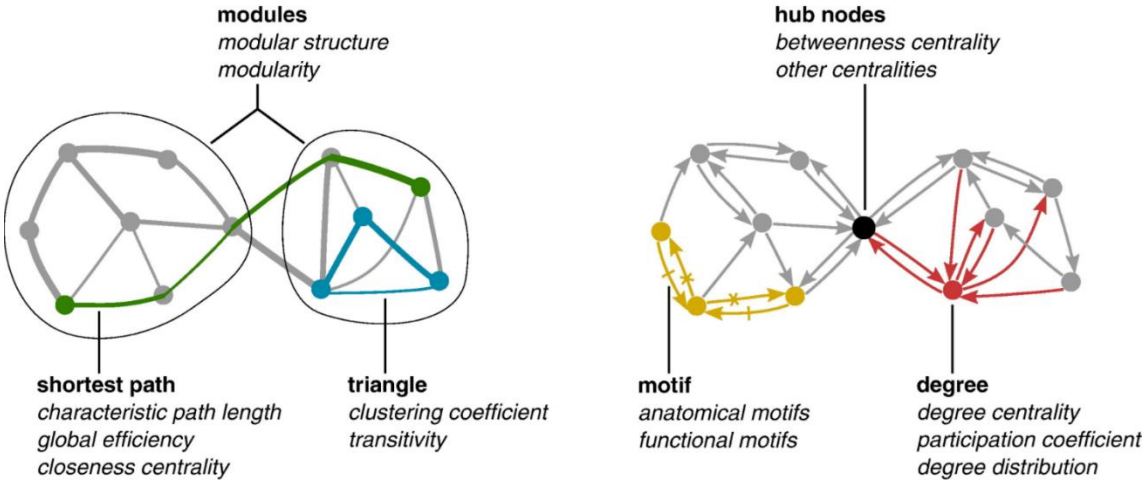


Figure 2.5 Network measures. *An illustration of key complex network measures (in italics) described in Rubinov and Sporns (2010). Measures of integration are based on shortest path lengths (green), while measures of segregation are often based on triangle counts (blue) but also include more sophisticated decomposition into modules (ovals). Measures of centrality may be based on node degree (red) or on the length and number of shortest paths between nodes. Hub nodes (black) often lie on a high number of shortest paths and consequently often have high betweenness centrality. Patterns of local connectivity are quantified by network motifs (yellow). An example three-node and four-link anatomical motif contains six possible functional motifs, of which two are shown—one motif containing dashed links, and one motif containing crossed links. Image from Rubinov and Sporns (2010) with permission.*

A key aspect of a brain network is the definition of ROIs. A recent review (Stanley *et al.*, 2013) suggests that these can be determined using various techniques. The voxel-wise approach is data driven and uses each voxel in the brain as an ROI. However, this may mean as many as 140,000 nodes in the brain network. Extensions to the voxel-wise approach have attempted to group voxels into functional units in fMRI. There are three main approaches to this; detecting sharp transitions in resting state functional connectivity MRI patterns (Barnes *et al.*, 2010; 2011; Cohen *et al.*, 2008; Nelson *et al.*, 2010), identifying functionally similar clusters (Craddock *et al.*, 2012; Mumford *et al.*, 2010; Thirion *et al.*, 2006; Vejmelka and Palus, 2010) and region growing methods (Blumensath *et al.*, 2013). ROIs can also be defined by the anatomy of the brain using structural anatomical atlases. One of the most commonly used atlases in the literature is the Automated Anatomical Labelling (AAL) atlas (Tzourio-Mazoyer *et al.*, 2002). When using

the anatomical atlas, it is possible to do whole brain analysis using all the ROIs specified in the atlas or to use only a partial brain network defined *a priori* based on pre-existing literature (Ciftci, 2011). Finally, it is possible to determine ROIs using only pre-existing functional activation. In this technique co-ordinates of activation are chosen, either directly from previous literature, or established after meta-analytic approaches (Cole *et al.*, 2013; Power *et al.*, 2011). This involves creating spheres, typically between 3-6mm radii, fixed on either the peak activity (Power *et al.*, 2011; Stevens *et al.*, 2012) or the centre of coordinates of a presumed functional area (Dosenbach *et al.*, 2007). However, this technique means that any voxel not contained in the sphere is not incorporated into the analysis. Which means that poorly defined spheres may miss crucial information.

Several studies have evaluated the technique of graph theory for determining the organisation of the human brain. These have suggested that it is a promising technique that may specifically shed new light on connectivity disorders that previous methods have not been able to explore (Bullmore and Sporns, 2009; He and Evans, 2010; van den Heuvel and Hulshoff Pol, 2010).

2.3 Experimental design

2.3.1 Screening questionnaires

Questionnaires are an efficient way of collecting data from the participant's perspective. Questionnaires can be designed in multiple ways by using structured questions, unstructured questions, or a combination of both. One major limitation of using questionnaires is that they are self-reported. This can lead to participants interpreting questions differently and may lead to issues of social desirability. As such there are several considerations that need to be made when choosing the questionnaires to administer, such as the validity and reliability of the questionnaire. Furthermore, there are some practical considerations as to the best way to administer the questionnaires (for review see Testa and Simonson, 2017). Despite this, they can provide valuable information from the participant that objective testing cannot.

In the current thesis, two questionnaires were administered (appendix A page 243 and appendix B page 244). The Fatigue Severity Scale (FSS) (Krupp *et al.*, 1989) was used to ensure that the HC participants did not have significant fatigue and that the MS participants did have significant fatigue. The scores from this questionnaire were also used for analysis purposes, as a measure of fatigue. The FSS required the participants to rate whether they strongly disagree (1) or strongly agree (7) with nine different statements

relating to fatigue. A score of 36 or above was categorised as high in fatigue. The FSS was specifically chosen as it has widely been used in MS research and validated in multiple, international MS populations (Armutlu *et al.*, 2007; Rosti-Otajarvi *et al.*, 2017; Valko *et al.*, 2008). This allows for easier comparison of other studies examining fatigue in MS. A further questionnaire administered was the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983). This was used only as a screening tool to exclude any participants with comorbidities as these can have a significant impact on fatigue and cognition (see section 1.4.2 page 14 and 1.4.5 page 19). In the HADS participants are asked to rate how frequently/definitely they feel with regards to a given statement relating to depression or anxiety. A higher score indicates increased depression or anxiety, there were a total of 7 statements related to depression and 7 statements related to anxiety. Participants with a score above 12 in either section were excluded. The scale was specifically designed for use with physically ill patients (Zigmond and Snaith, 1983), and has been validated in an MS population (Honarmand and Feinstein, 2009).

2.3.2 Neuropsychological testing

Neuropsychological tests are a very useful technique to determine brain function, by exploring the behavioural and cognitive performance. There are large numbers of tests designed to measure multiple domains, such as memory, attention, and intelligence among others. These tests can be used in the diagnosis of disorders, such as the Mini Mental State Exam (Folstein *et al.*, 1983), but can also be used to test for cognitive deficits in clinical groups. In MS specifically, studies using neuropsychological tests, have established impairments in verbal memory (Mainero *et al.*, 2006), attention (Beatty *et al.*, 1996; Mainero *et al.*, 2006), executive function (Denney *et al.*, 2005) and speed of information processing (DeLuca *et al.*, 1994; 1998; 2004; 2013; 2015; Litvan *et al.*, 1988; Mainero *et al.*, 2006). Furthermore, neuropsychological tests can be used to measure how cognitive performance is affected by specific manipulations. For example, studies have used neuropsychological testing to examine the effectiveness of rehabilitation programmes (Doniger *et al.*, 2018), to examine the effect of sleep loss on cognition (Kendall *et al.*, 2006; Taillard *et al.*, 2006) and of most interest to the current thesis, to examine how fatigue affects cognitive performance (Bailey *et al.*, 2007; Bryant *et al.*, 2004; Claros-Salinas *et al.*, 2010; Jennekens-Schinkel *et al.*, 1988; Johnson *et al.*, 1997; Krupp and Elkins., 2000; Kujala *et al.*, 1995; Paul *et al.*, 1998; Schwid *et al.*, 2003). Given the number of potential uses of neuropsychological tests, there needs to be some consideration of the choice of test based on the aim of the study in this thesis.

2.3.3 fMRI paradigm

Functional MRI is uniquely able to provide patterns of brain activity related to specific tasks being conducted. The task used to elicit these neuronal patterns requires much consideration. Even with a specific focus on alertness, there are multiple existing paradigms that can be used. The Attention Network Test (ANT) was designed to simultaneously study three different attentional networks, namely alerting, orienting and cueing. There are also revised versions of the original ANT that are used to test the three attentional networks. Both the Continuous Performance Test (CPT) and the Sustained Attention to Response Task (SART) can be designed in multiple different ways but requires the participant to respond to a target stimulus as fast as possible, among multiple distracting stimuli. Similarly, the Rapid Visual Information Processing (RVIP) requires participants to maintain prolonged attention whilst choosing target stimuli during a rapidly appearing array of stimuli. Many of these tests are used for sustained attention, where the accuracy is the primary outcome. In alertness, the primary outcome is reaction time, and as such simple reaction time tasks that only require a response to a target stimulus, without distractors are frequently used. The paradigms used during past fMRI studies to evaluate alertness, as well as the strengths and weakness of these paradigms will be examined in Chapter 3 (page 54).

2.4 Multiple comparisons and family wise error

There are two main forms of statistical error, Type I and Type II. Type I errors are known as false positives, where the null hypothesis is mistakenly rejected and is controlled by the significance level (α). On the other hand, type II errors are false negatives, where the null hypothesis is false but is accepted, the probability of which is specified by the power (β) of a study. This becomes even more concerning when testing multiple comparisons, as more than one hypothesis test is performed, which increases the likelihood of Type I errors. This is the case in neuroimaging research, as the brain consists of approximately 100,000 voxels. This means that with an α of .05, there would be about 5000 false positives, and even with a more stringent α= .001, 100 false positives may still occur. In fMRI research, there are multiple ways of controlling for multiple comparisons. Family wise error (FWE) tests any false positives and assumes all activation is zero, this can be implemented using Bonferroni or Random Field methods. The false discovery rate (FDR) looks at the proportion of false positives among the rejected results. There are also some Bayesian statistical approaches (for review see Nichols and Hayasaka, 2003). The Bonferroni correction uses basic probability and takes the probability threshold (α) for

each of the probability values (*n*). Therefore, if the FWE rate is .05, .05/100 000= .0000005. However, this correction is often too stringent as it assumes no correlation between voxels, whereas voxels are generally spatially correlated. Random field theory considers the spatial smoothness of the data. The calculation progresses in three stages, firstly the smoothness of the data is estimated, this is then used to calculate an expected Euler characteristic, which is finally used to calculate the threshold needed (see Brett *et al*., 2003). This is the default method used in SPM and is particularly useful for multi subject fMRI (Hayasaka and Nichols, 2003). The advantages of these parametric methods are briefly discussed in Flandin and Friston (2017). However, selecting stringent thresholds require large sample sizes, which has several of its own implications.

2.5 Sample size calculations

The fMRIpower software package (fmripower.org) which employs novel methods developed by Mumford and Nichols (2008) was used to calculate the sample size needed for the present study. This method estimates power for detecting significant activation within specific regions of interest. Power calculations were based on four regions of interest identified by Perin *et al*. (2010) for the alertness mechanism. No masks were created; the coordinates of interest were simply used to identify the required sample size. Pilot data using a two-sample t-test with a p-threshold of .05 was used to determine sample size. The effect sizes are expressed in standard deviation (s.d.) units which are analogous to Cohens d. The power calculation (Figure 2.6, page 51) revealed that 40 participants, per group, would allow for at least 80% power to detect an effect of size .95 s.d. in the anterior cingulate cortex (a), .98 s.d. in the inferior parietal lobe (b), .64 in the prefrontal cortex (c) and .65 in the thalamus (d).

This does not account for whole brain activation. For example, in the inferior frontal operculum, the power analysis shows that even with 100 participants per group there would be less than 6% power to detect an effect size of .01 s.d. This represents a large problem when conducting fMRI studies. A recent article by Cremers *et al*. (2017) highlights that fMRI research has particularly low statistical power, specifically when dealing with between subject effects and brain behaviour correlations. The authors suggest that increasing the sample size, using less stringent thresholds or focusing on a particular region of interest will allow for increased power. However, each of these have some major disadvantages. Increasing the sample size is often too costly and time-consuming. Furthermore, using less stringent thresholds leads to a large amount of Type I and Type II errors. Similarly focusing on a particular region of interest is difficult given the complex

organisation of the brain, specifically when examining patient populations. Of concern in the current thesis, is also that MS represents a very heterogeneous population. This leads to even greater concerns of consistency across individuals and focusing on a particular region of interest would allow for very little understanding of the widespread pathology. Given the limited time and funding of this thesis, it was determined that 40 participants, based on sample size calculations from previous regions of activation, would be used and the significance thresholds would be lowered to less stringent levels where required, based on the recommendations by Cremers *et al.* (2017).

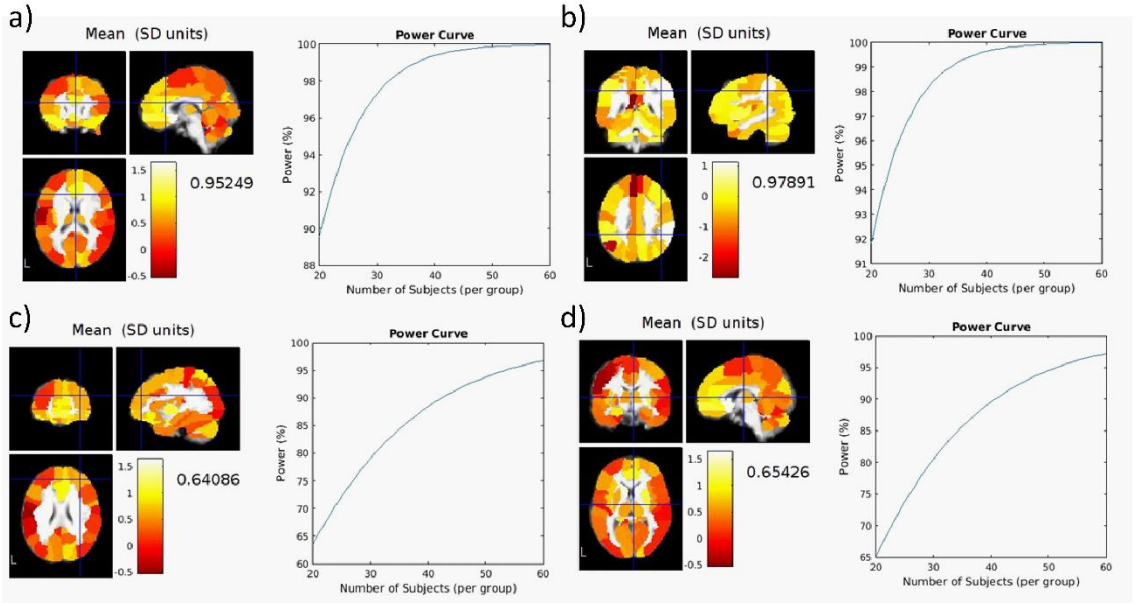


Figure 2.6 Sample size calculations. *a) anterior cingulate cortex, b) inferior parietal lobule, c) prefrontal cortex, d) thalamus. The colour maps on the left display the mean effect size and the power curve on the right displays the percentage of power based on the number of participants per group.*

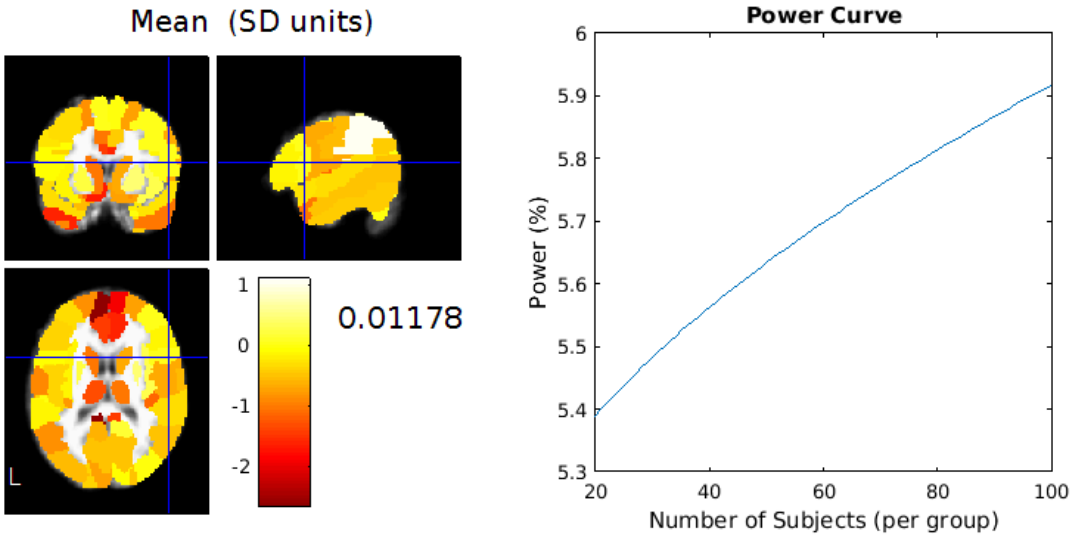


Figure 2.7 Sample size calculation for inferior frontal operculum. *The colour map on the left displays the mean effect size and the power curve on the right displays the percentage of power based on the number of participants per group.*

2.6 Participant recruitment

A total of 40 female MS participants (aged 32-67, mean 49.8) were recruited from The Brain Centre at Southmead Hospital, North Bristol Trust with the help of the Clinical Specialist Research Physiotherapist. MS participants were screened and only included if they scored 36 or above on the FSS (Krupp *et al.*, 1989) and had no alternative diagnosis for their fatigue. MS participants were excluded if they were aged below 18 and above 70, male, showed any contraindications for MRI, had excessive upper limb tremor, did not have normal or corrected to normal vision, scored above 12 for either subsection, on the HADS (Zigmond and Snaith, 1983), or wore any contracture that disrupted positioning in the MRI scanner. Participants were excluded if they had reaction times (refer to 6.2.3 page 107) two standard deviations above or below the mean. This resulted in 3 MS participants being excluded. The remaining 37 MS participants (aged 35-67, mean 50.11 years) were used in the analyses.

Forty age matched HC (aged 31-68, mean 49.5) were recruited in Bristol and the surrounding areas using posters, websites, and emails. Healthy volunteers were recruited if they were aged +/- 3 years from the age of an MS participant and were female. Exclusion criteria for HC group included any contraindications for MRI, those who did not have normal or corrected to normal vision, males, scored above 12 for either subsection, on the HADS, and any individual who scored above 36 on the FSS. Ethical approval was granted by the NHS REC Frenchay Committee (see appendix C page 245). Participants were excluded if they had reaction times (refer to 6.2.3 page 107) two standard deviations above or below the mean. This resulted in 4 HC participants being excluded. The remaining 36 MS participants (aged 31-68, mean 49.69 years) were used in the analyses.

2.7 Image acquisition

MRI scans were performed in a 3 Tesla Siemens Skyra scanner with the use of a 32 channel radiofrequency head coil. Two fMRI scans, for each condition of the paradigm, were acquired using T2*-weighted multiband gradient echo planar imaging (refer to 2.1.4, page 41). Each scan lasted approximately 15 minutes. 39 slices orientated parallel to the anterior-posterior commissure plane, covering the whole brain were acquired. The parameters for the functional scans were: time to repetition (TR): 906 ms; time to echo (TE): 30 ms; field of view (FoV): 192 mm; voxel size: 3x3x3 mm and a multiband acceleration factor of 3. The functional scans were acquired in the axial plane and no in plane acceleration was used. The blipped-CAIPI method was used to reduce slice aliasing and no slice-GRAPPA

technique was applied as no in-plane acceleration was used. A further T1-weighted inversion recovery 'magnetisation prepared rapid acquisition gradient echo' (MPRAGE) was acquired in the sagittal plane, comprising of 192 slices; TR: 1800 ms; TE: 2.25 ms; .9mm isotropic voxel; and a FoV of 240 mm for co-registration with functional scans. Furthermore, this MPRAGE image was used for the segmentation of grey matter, white matter and cerebral-spinal fluid for the VBM and brain connectivity analysis.

2.8 Procedure

On the day of attendance, the participants were fully explained the procedure of the entire experiment and the initial screening form (appendix D page 250) was checked again to ensure no contraindications to the MRI. The participants completed all 5 neuropsychological tests (appendix E-G page 252-257) in one of the clinic rooms at CRIC Bristol. The participants were explained the alertness-motor paradigm prior to scanning to ensure that they fully understood the requirements of the task. A member of the research team then cleaned a small region on the arm of the participants, to aid conduction. Five EMG electrodes were then placed on to their left arm. Two electrodes were placed onto each of the two arm muscles responsible for handgrip, flexor, and extensor. The fifth electrode was placed behind the elbow as a reference electrode. The participants completed the CRICBristol standard second MR screening form (appendix H page 258), to ensure that they were no longer carrying any metal, before entering the scan room. The participants were then asked to lie on the MRI bed with their head positioned in the head coil with a small mirror attachment (which was required for participants to see the screen and to receive instructions during the functional MRI scans). Small earplugs were provided to reduce the noise inside the MRI scanner. Furthermore, the participants were given an alarm button to squeeze if they needed to alert the MR operator during the scan. The EMG cables were attached to the electrodes on the participants left arm, and they were provided with the MR safe hand dynamometer to squeeze as instructed by the paradigm. The MR operator would ensure that the participant was comfortable before moving them into the bore of the magnet. Before any scanning, the MR operator would also ensure that they were able to communicate with the participant through the intercom system.

Chapter 3 Understanding the neural substrates of the alertness mechanism: A systematic review of functional magnetic resonance imaging.

3.1 Introduction

Early studies of attention proposed that attention can be broadly sub-divided into three distinct domains, where alertness represents only one of these sub domains (Posner and Petersen, 1990). Alertness can be defined as the preparation of attention to respond to a target stimulus and the ability to maintain response readiness over time. Early studies have proposed a noradrenergic alertness network comprising of the locus coeruleus, frontal regions and involving parietal cortex activation (Posner and Peterson, 1990). This suggests a frontal-parietal pathway sub-serving alertness which is further supported by neuroimaging studies (Lewin *et al*., 1996; Pardo *et al*, 1991), but also extended by more recent studies to include the anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (pFC), inferior parietal lobule as well as showing activation of the thalamus and brainstem (Mottaghy *et al*., 2006; Sturm *et al*., 1999; 2004). There have been significant advancements with the emergence of functional magnetic resonance imaging (fMRI). The synthesis of the fMRI literature is necessary to establish whether there is a consensus among the studies over the neural correlates of the healthy alertness mechanism.

There is great difficulty when examining the literature on alertness mechanisms as several terms such as vigilance, sustained attention, arousal and alertness are often being used synonymously and interchangeably in the literature despite the significant differences (Oken *et al*., 2006). The ambiguity of terms makes searching the literature more laborious and may result in relevant articles being missed. As such, very concise criteria must be set when attempting to synthesise the literature examining the alertness mechanism.

The alertness mechanism is uniquely suited to providing an insight into the effect of fatiguability or fatigue. DeLuca (2005) noted that tasks of alertness may be most sensitive to the effect of fatigue and fatiguability. Several studies suggest the mental fatigue is associated with prolonged task performance, even in healthy individuals (Boksem *et al*., 2005; Faber *et al*., 2012; Oken *et al*., 2006). There are many behavioural tasks that have been used to test alertness, the Continuous Performance Task (CPT), the Attention Network Test (ANT) and the Sustained Attention Response Task (SART) to name only a few. Establishing the tasks that have been used in the fMRI literature and examining the

strengths and weaknesses of these will allow for the most appropriate paradigm for the current thesis to be selected.

The primary aim of this systematic review was to synthesise the existing literature on the healthy alertness mechanism, to determine whether there is a consensus on the neural mechanism that underlies alertness. A secondary aim was to establish and evaluate the existing paradigms assessing alertness, to determine a good paradigm to use for assessing fatigue in an MS group. To specifically overcome the problem of ambiguous definition of alertness terminology used in the literature, very precise inclusion and exclusion criteria, specifically with regards to the experimental paradigm were set.

3.2 Methods

The systematic review searched PubMed, Scopus, Psychinfo and EMBASE from 1991, the first successful conception of blood oxygen level dependent (BOLD) signal in MRI for functional brain mapping, to 20th January 2017, the commencement of the systematic review. Keywords searches in the databases included the medical subject heading (MeSH) terms 'alertness', 'sustained attention' and 'functional magnetic resonance imaging' along with variations such as 'fMRI', 'functional MRI', 'functional MR imaging', 'BOLD', 'blood oxygen level dependent (signal)', 'alerting', 'alert*', 'phasic', 'tonic', 'intrinsic', 'extrinsic', 'arousal', 'wakefulness', 'readiness' and 'vigilance'. The reference lists of eligible studies were also hand searched for any missed publications. All studies examining the alertness mechanisms in healthy adults were included. All identified studies were input into the covidence software (www.covidence.org). Titles and study abstracts of studies identified by the initial search were reviewed and duplicates were excluded. Two reviewers (CL, SD) independently reviewed potential articles, and any disagreements were resolved by consensus. The systematic review assessed the quality of studies using the best practise criteria set by Nichols *et al*. (2015). The quality studies were examined by assessing whether the eligible studies had clearly stated their; research objectives, recruitment procedure, inclusion and exclusion criteria, population demographics, imaging protocol, image pre-processing protocol, the paradigm used and study limitations. A subjective rating of perceived study limitations and overall quality was made. No studies were excluded based on the quality assessment, due to the low number of eligible studies. For full protocol see appendix I (page 259).

3.3 Results

The searches found 1406 articles, 1372 were excluded at the title screening and abstract phase. The number of papers being excluded at this stage was largely due to the ambiguous use of the search terms by various authors. A total of 34 studies were reviewed at full text, one was excluded as it was a review, eight studies did not report adequate results and study outcomes for inclusion, another eight studies were excluded for using the incorrect paradigm, thereby not truly assessing alertness, one study was excluded due to using a child population and three studies were duplicates. Therefore, a total of thirteen studies met the inclusion criteria Bartes - Serrallonga *et al.*, (2014); Clemens *et al.*, (2011); Clemens *et al.*, (2013); Fan *et al.*, (2005); Grahn and Manly, (2012); Hilti *et al.*, (2013); Kellerman *et al.*, (2011); Lawrence *et al.*, (2003); Perin *et al.*, (2010); Sturm *et al.*, (2006); Tana *et al.*, (2010); Thiel *et al.*, (2004) and Xuan *et al.*, (2016). The search flow is represented in Figure 3.1 (page 56). The characteristics of included studies are outlined in Table 3.1. Upon quality assessment, the mean number of criteria fulfilled by the included studies (n=13) was six (range 5-8). Only Hilti *et al.* (2013) fulfilled all the criteria (Table 3.2).

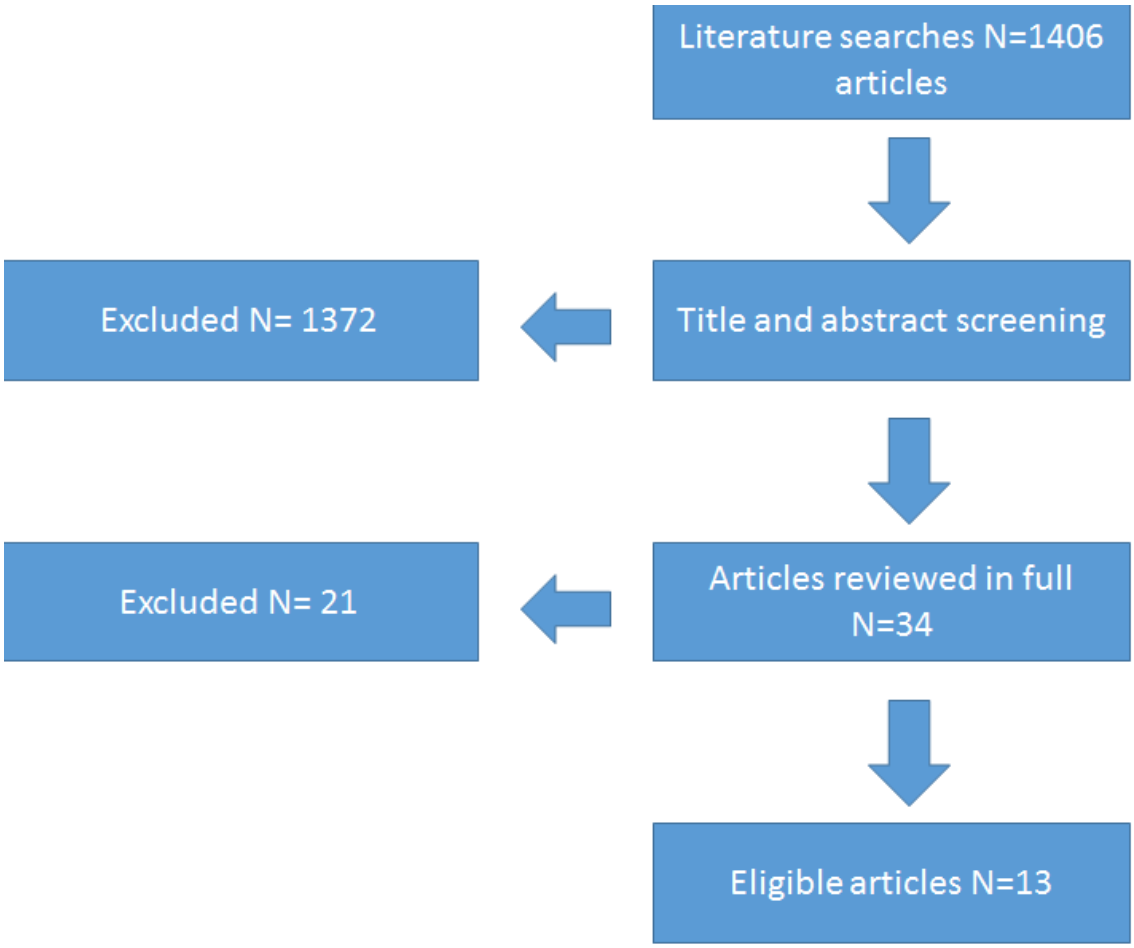


Figure 3.1 Flow diagram of the review procedure.

Table 3.1 Characteristics of included studies

Author (Year)	Type of Paradigm	Study Population	Main Study Focus	Imaging	Brain Region	Hemisphere	Coordinate System
Bartes-Serrallonga (2014)	Continuous Performance Test	40 Right-handed 20 Females Age Range 18-25 Mean Age 19.6 +/- 1.7	To evaluate neural correlates of sustained attention.	3T SPM8 Realignment Normalisation Smoothing	Inferior Parietal Lobule Middle Frontal Gyrus Superior Frontal Gyrus Anterior Cingulate Precuneus Brainstem Middle Occipital Gyrus Inferior Occipital Gyrus Precentral Gyrus Fusiform Gyrus Angular Gyrus Inferior Frontal Gyrus	Bilateral Bilateral Bilateral Right Right Right Left Left Left Left Left Left	MNI
Clemens (2011)	Wahrnehmungs und Aufmerksamkeits funktionen.	16 Right-handed 4 Females Age Range 22-36 Mean Age 26	To determine the specific neural correlates of intrinsic alertness.	3T Brain Voyager 3D motion correction Slice Timing Co-registration Smoothing Linear Trend Removal High Pass Filtering	Inferior Occipital Gyrus Insula Medial Dorsal Nucleus Inferior Parietal Lobule Middle Frontal Gyrus Inferior Frontal Gyrus Anterior Cingulate Supplementary Motor Ponto Mesencephalic Cerebellum	Bilateral Right Right Right Left Left Left Left Left Left Left	MNI
Clemens (2013)	Wahrnehmungs und Aufmerksamkeits funktionen.	32 Right-handed 0 Females Age Range None Mean Age 27 SD 3	To reveal the neural correlates of attentional processing across varied attention functions and tasks (test	3T Brain Voyager QX2.3 Linear Trend Removal Slice Timing High Pass	Middle Frontal Gyrus Precentral Gyrus Medial Dorsal Nucleus Brainstem Posterior Cingulate Inferior Occipital Gyrus Insula	Bilateral Right Right Right Right Right Left	MNI

			and training) assessing the same functions.	filtering 3D motion correction Co-registration	Medial Frontal Gyrus Lateral Globus Pallidus Superior Parietal Lobule Cerebellum	Left Left Left Left	
Fan (2005)	Attention Networks Test	16 Right-handed 8 Females Age Range 18-36 Mean Age 27.2 SD 5.7	To isolate the neural networks responsible for performance on the three indices of the ANT.	3T SPM99 Slice Timing Realignment Co-registration Normalisation Smoothing	Thalamus Superior Temporal Gyrus Superior Colliculus Inferior Parietal Lobe Fusiform Gyrus Inferior Frontal Gyrus Cerebellar Vermis Superior Parietal Lobe	Bilateral Right Right Left Left Left Left Left	MNI
Grahn (2012)	Sustained Attention to Response Task	18 Right-handed 10 Females Age Range 19-29 Mean Age 22.9 SD 3.5	To identify regions of common recruitment involved in increased sustained attention.	3T SPM5 Realignment Slice Timing Segmented Normalisation Smoothing	Supplementary Motor Inferior Frontal Operculum Precentral Gyrus Midbrain	Bilateral Bilateral Bilateral Right	MNI
Hilti (2013)	Rapid Visual Information Processing Task	20 Right-handed 10 Females Age Range 20-28 Mean Age 23.5	To test: (1) whether reaction time (RT) increased over time. (2) whether there were correlations between RT and BOLD responses (3) whether brain regions were differentially activated in good versus poor performers	3T Brain Voyager QX 1.9.9 rotated into AC-PC Normalisation Slice Timing 3D motion correction Linear Trend Removal High Pass filtering Co-registration Smoothing	Middle Frontal Gyrus Precuneus Inferior Parietal Lobule Insula Postcentral Gyrus Superior Frontal Gyrus Dorsal Cingulate Cortex Precentral Gyrus Claustrum Superior Parietal Lobe	Bilateral Bilateral Bilateral Bilateral Right Left Left Left Left Left	MNI

Kellerman (2011)	Attention Networks Test	16 Right-handed 10 Females Age Range 24-40 Mean Age 30.4 SD 4.9	To test: (1) Latencies in visual processing (2) Latencies reflecting alerting (3) Latencies reflecting orienting (4) Latencies in executive control.	1.5T SPM2 Realignment Slice Timing Normalisation Smoothing	Precentral Gyrus Middle Frontal Gyrus Inferior Temporal Gyrus Superior Parietal Lobule Cingulate Gyrus Thalamus Inferior Occipital Gyrus Superior Frontal Gyrus Superior Parietal Lobule Lingual Gyrus Insula Precuneus	Bilateral Right Right Right Right Right Left Left Left Left Left Left	MNI
Lawrence (2003)	Rapid Visual Information Processing Task	25 Right-handed 15 Females Age Range 18-36 Mean Age 24.3 SD 5.5	Not Reported	3T AFNI v.2.4 3D motion correction	Insula Middle Frontal Gyrus Medial Frontal Gyrus Middle Occipital Gyrus Parietal Lobule Culmen Superior Frontal Gyrus Supplementary Motor Anterior Cingulate Fusiform Gyrus Posterior Cingulate Thalamus Middle Temporal Gyrus Parahippocampal Gyrus Angular Gyrus Inferior Occipital Gyrus Declive	Bilateral Bilateral Bilateral Bilateral Bilateral Bilateral Right Right Right Right Left Left Left Left Left Left Left	MNI
Perin (2010)	Simple Reaction Time Task	16 8 Females Age Range None Mean Age 28	To determine brain regions involved in phasic and intrinsic alertness.	1.5T SPM5 Realignment Slice Timing Normalisation Smoothing	Prefrontal Cortex Orbitofrontal Cortex Anterior Cingulate Supplementary Motor Inferior Parietal Cortex Superior Parietal Lobule Thalamus	Right Right Right Right Right Right Right	MNI

					Putamen Brainstem	Right Right	
Sturm (2006)	Novel task based on the 'Neglect' component of the Test Battery for Attentional Performance	10 Right-handed 5 Females Age Range 21-28 Mean Age 29	To analyse the role of intrinsic alerting in spatial attention.	1.5T SPM99 Realignment Co-registration Normalisation Smoothing	Inferior Occipital Gyrus Superior Frontal Gyrus Superior Temporal Gyrus Thalamus Superior Parietal Lobule Inferior Parietal Lobule Prefrontal Cortex Inferior Frontal Gyrus Precuneus Cuneus Precentral Gyrus Cingulate Gyrus Cerebellum	Bilateral Bilateral Bilateral Bilateral Right Right Right Right Left Left Left Left Left	Talairach
Tana (2010)	Continuous Performance Test	8 Right-handed 1 Female Age Range None Mean Age 18.8 SD 2.9	To study the neural networks of brain regions involved during the Continuous Performance Test.	1.5T SPM8b Normalisation	Insula Cingulate Gyrus Supramarginal Gyrus Cerebellum Lateral Occipital Cortex Lingual Gyrus Precentral Gyrus	Bilateral Right Right Left Left Left Left	MNI
Thiel (2004)	Cued-Detection Task	15 Right-handed 8 Females Age Range 20-34 Mean Age 26.6	To study neural correlates of spatial and non-spatial attentional subcomponents, alerting, orienting and reorienting of attention.	1.5T SPM99d	Fusiform Gyrus Inferior Occipital Gyrus Precuneus	Right Left Left	MNI
Xuan (2016)	Revised Attention Networks Test	24 Right-handed 11 Females Age Range 16-49 Mean Age 26.3	To use the revised attention network test to examine the neural	3T SPM8 Realignment Slice Timing Co-registration	Precentral Gyrus Middle Occipital Gyrus Postcentral Gyrus Cerebellum Thalamus Anterior Insula	Bilateral Bilateral Bilateral Bilateral Bilateral Bilateral	MNI

			substrates underlying the attentional functions and the interactions among them.	Normalisation Smoothing	Precuneus Putamen Superior Occipital Gyrus Supramarginal Gyrus Middle Temporal Gyrus Fusiform Gyrus Superior Parietal Lobule Calcarine Cortex Pons Rolandic Operculum Middle Frontal Gyrus Locus Coeruleus Anterior Cingulate Cortex Inferior Occipital Gyrus Superior Frontal Gyrus Inferior Parietal Lobule	Bilateral Bilateral Bilateral Bilateral Right Right Right Right Right Right Right Right Left Left Left Left	

Table 3.2 Quality assessment of included studies

Author (Year)	Objectives	Demographics	Recruitment procedure	Inclusion/ Exclusion	Imaging Protocol	Pre-processing Protocol	Paradigm	Study Limitations Reported	Perceived Study Limitations	BIAS Rating
Bartes-Serrallonga (2014)	YES	YES	YES	YES	YES	YES	YES	NO	LOW	LOW
Clemens (2011)	YES	YES	YES	NO	YES	YES	YES	NO	MEDIUM	MEDIUM
Clemens (2013)	YES	YES	YES	YES	YES	YES	YES	NO	HIGH	HIGH
Fan (2005)	YES	YES	NO	NO	YES	YES	YES	NO	MEDIUM	MEDIUM
Grahn (2012)	YES	YES	NO	NO	YES	YES	YES	NO	MEDIUM	MEDIUM
Hilti (2013)	YES	YES	YES	YES	YES	YES	YES	YES	LOW	LOW
Kellerman (2011)	YES	YES	NO	NO	YES	YES	YES	YES	MEDIUM	LOW
Lawrence (2003)	NO	YES	YES	YES	YES	YES	YES	NO	LOW	MEDIUM
Perin (2010)	YES	YES	NO	YES	YES	YES	YES	NO	MEDIUM	MEDIUM
Sturm (2006)	YES	YES	YES	YES	YES	YES	YES	NO	HIGH	HIGH
Tana (2010)	YES	YES	YES	YES	YES	YES	YES	NO	HIGH	HIGH
Thiel (2004)	YES	YES	NO	YES	YES	NO	YES	NO	MEDIUM	HIGH
Xuan (2016)	YES	YES	NO	YES	YES	YES	YES	NO	LOW	LOW

3.3.1 Experimental paradigm

Two of the studies used the ANT (Fan *et al.*, 2005; Kellerman *et al.*, 2011). In the ANT participants had to indicate by a button press if a target arrow pointed to the left or right. The target arrow was surrounded by four flankers (vertically, Kellerman *et al.*, 2012; horizontally, Fan *et al.*, 2005), which had either the same orientation as the middle target arrow (congruent condition) or pointed to the opposite direction (incongruent condition). Three different cueing conditions were used. Items probing alertness were preceded by a central cue (replacing the fixation cross). Whereas, cues probing orientation were preceded by a spatial cue, which was located at the position where the upcoming stimulus was going to be presented. Control trials were not cued at all (fixation cross present). One study used the revised ANT (Xuan *et al.*, 2016). This test was designed to magnify the interactions among the three attentional functions. It is based upon the original ANT task and manipulates the validity of spatial cues to measure the orienting operations of disengaging and moving + engaging. When a cue was displayed to the participant they were informed whether this was valid or invalid.

The CPT was used by two studies (Bartes – Serrallonga *et al.*, 2014; Tana *et al.*, 2010). In Tana *et al.* (2010) participants were presented with the 26 different letters of the English alphabet and required to respond by pressing a button as fast as possible when any letter other than 'X' appeared. In Bartes-Serrallonga *et al.* (2014) participants were presented with a series of 27 four-digit numbers (digits from 1 to 9, without repetition). Participants are required to respond by pressing a button as fast as possible when the same number occurred twice sequentially. In each CPT block, only 4 numbers were repeated in relation to the previous number. The control task consisted of the digits '1 2 3 4' presented at the same rate and intervals as the CPT. The CPT and control tasks were displayed in alternating blocks. Two studies used the Wahrnehmungs- und Aufmerksamkeits Funktionen (WAF, in English, translates to perception and attention functions) (Clemens *et al.*, 2011; 2013), which involved participants responding as fast as possible to a target stimulus which was a black circle when no target stimulus was visible participants viewed a fixation cross in the centre of the screen. No cueing was used.

One study used the SART (Grahn and Manly, 2012), in which participants were displayed digits, (1-9) and were asked to respond as soon as the digit turned grey. Participants were instructed to not press at all when the number '3' appeared. The task was then split into an easy and a hard condition. In the easy condition participants were correctly told that

no 3's would be displayed. In the hard condition, the number 3 was presented at unpredictable intervals. Two used the Rapid Visual Information Processing Task (RVIP) (Hilti *et al.*, 2013; Lawrence *et al.*, 2003). In Lawrence *et al.* (2003) the task consisted of 90-sec blocks of continuous stimuli presentation, consisting of a stream of single digits presented in the centre of the screen. Participants were asked to press a button as soon as they saw a target sequence of three different odd or three different even numbers appearing consecutively. The target sequences were not composed of specific ascending or descending sequences, but of any three odd or even digits, e.g. 1-5-9. In the study by Hiliti *et al.* (2013) participants were presented with 10 digits, 0-9 and required to respond by pressing a button as fast as possible when the number '0' appeared. One study used a cued-detection task (Thiel *et al.*, 2004), where the cue stimulus consisted of the central diamond brightening for 100 ms. The target stimulus was either a filled diamond or a filled circle which appeared in one of the peripheral boxes. Five different cueing conditions were used. A spatial cue, the left or right side of the central diamond brightened for 100 ms, indicating the side for subsequent target appearance. In 80% of these spatial trials, the target appeared on the side indicated by the cue (validly cued trials), in 20% of the cases the target appeared on the opposite side (invalidly cued trials). In neutral cue trials, the diamond brightened as a whole, *i.e.* no spatial information on where the target would appear. In no cue trials, the central diamond remained unchanged, giving no indication that a target would appear subsequently. Six percent of all trials were catch trials in which a spatial cue, but no target appeared.

One study used a simple reaction time task (Perin *et al.*, 2010). Participants completed a sensorimotor control, intrinsic alertness and extrinsic alertness condition. In both the intrinsic and extrinsic conditions participants were asked to respond as soon as they detected a white square. A white cross at the centre of the screen was used as a fixation point. In the intrinsic condition, the fixation point remained permanently, whereas in the extrinsic alertness the central fixation cross was displayed for 400 ms and preceded the stimulus with randomized, varying stimulus onset asynchronies acting as a warning stimulus. In the control sensorimotor condition, the participants had to perform an automatic and slow self-paced finger tapping with a frequency around 1 Hz while watching passively a fast (10 Hz) flickering white square. The high frequency of the flickering avoided the synchronization with the tapping. One study used a novel task based on the 'Neglect' component of the Test Battery for Attentional Performance (Sturm *et al.*, 2006). Participants were requested to monitor a fixation box and respond as soon as they

registered a flickering stimulus. This was presented either peripherally in the distributed spatial attention condition or centrally in the focused spatial attention condition.

3.3.2 Reporting of image acquisition methods

All thirteen studies reported the imaging protocol and parameters used for the functional scans (Bartes-Serrallonga *et al.*, 2014; Clemens *et al.*, 2011; Clemens *et al.*, 2013; Fan *et al.*, 2005; Grahn and Manly, 2012; Hilti *et al.*, 2013; Kellerman *et al.*, 2011; Lawrence *et al.*, 2003; Perin *et al.*, 2010; Sturm *et al.*, 2006; Tana *et al.*, 2010; Thiel *et al.*, 2004; Xuan *et al.*, 2016). Seven studies also reported the anatomical scan protocol and parameters used (Bartes-Serrallonga *et al.*, 2014; Clemens *et al.*, 2011; Clemens *et al.*, 2013; Fan *et al.*, 2005; Hilti *et al.*, 2013; Perin *et al.*, 2010; Sturm *et al.*, 2006). One study reported the anatomical scan protocol but did not report the parameters used (Lawrence *et al.*, 2003). Five studies reported no anatomical scan protocol or acquisition parameters (Grahn and Manly, 2012; Kellerman *et al.*, 2011; Tana *et al.*, 2010; Thiel *et al.*, 2004; Xuan *et al.*, 2016). Eight of the thirteen studies used 3 Tesla scanners (Bartes-Serrallonga *et al.*, 2014; Clemens *et al.*, 2011; Clemens *et al.*, 2013; Fan *et al.*, 2005; Grahn and Manly, 2012; Hilti *et al.*, 2013; Lawrence *et al.*, 2003; Xuan *et al.*, 2016) and the other five studies used 1.5 Tesla scanners (Kellerman *et al.*, 2011; Perin *et al.*, 2010; Sturm *et al.*, 2006; Tana *et al.*, 2010; Thiel *et al.*, 2004).

3.3.3 Brain regions reported to be activated

A total of 25 active brain regions were found among the 13 eligible studies. All regions and the percentage of studies that showed significant activation in each region is represented in Figure 3.2. The brain regions consistently reported (at least 50% of studies) were the precentral gyrus, inferior occipital gyrus, middle frontal gyrus, superior and inferior parietal lobule, thalamus, insula, cerebellum and brainstem regions. Due to the consistency of the activations in these regions, they are considered crucial to the alertness mechanism. Although, all these regions were simultaneously activated in only a single study (Xuan *et al.*, 2016). There are some studies that show activation of the cingulate gyrus and temporal regions, but these were not consistently reported. There are also some regions that show spurious activation as these are only reported to be activated during a single study, as such, they are not considered essential to the alertness mechanism.

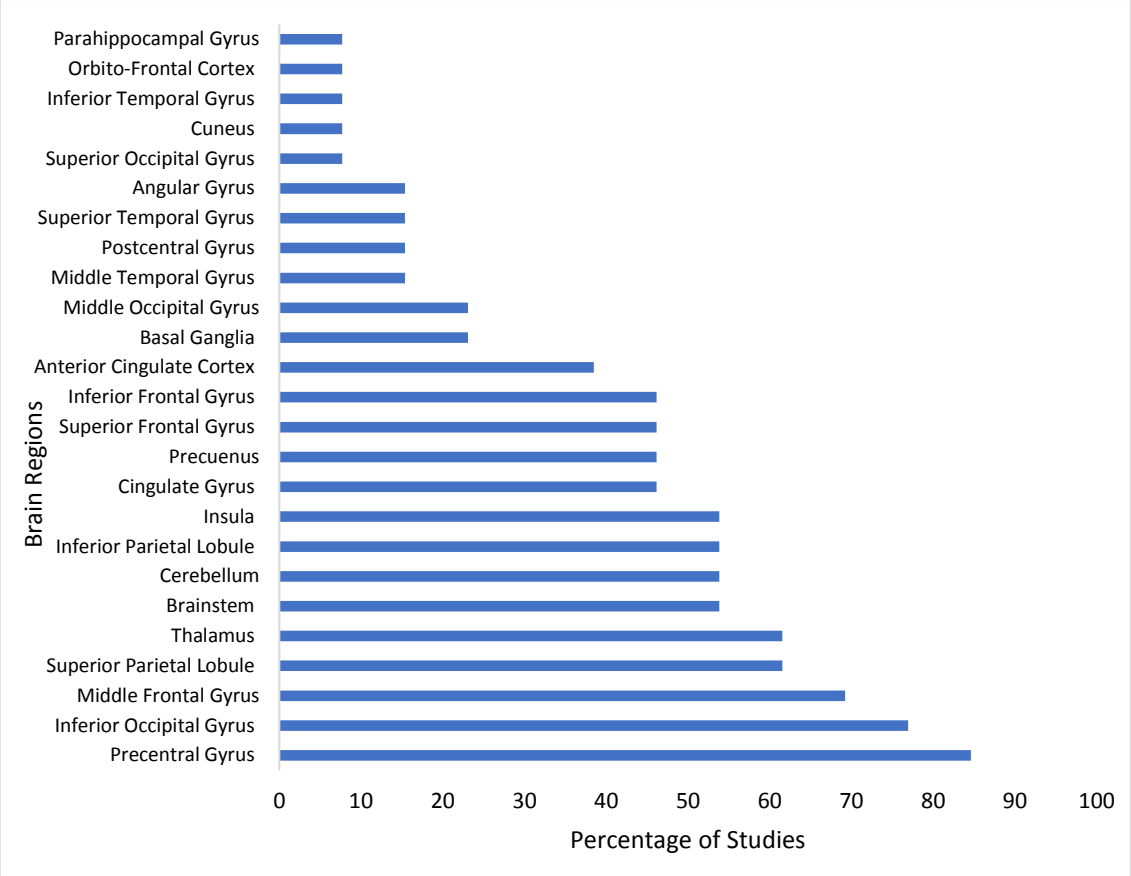


Figure 3.2 Brain regions and the percentage of studies reporting significant activation in each region.

3.4 Discussion

The primary aim of this chapter was to establish whether there is a consensus on the network that underlies healthy alertness. To this end, a systematic review, with very concise inclusion and exclusion criteria to avoid the ambiguity of the alertness term, was conducted and identified thirteen studies to be included. The synthesis of these studies showed that, even with varying paradigms, there are some regions of consensus across studies. The majority of studies included in this review showed activation in the precentral gyrus, middle frontal gyrus, brainstem, inferior occipital gyrus, inferior parietal lobule, superior parietal lobule, thalamus and the insula. Previous literature has suggested a frontal-parietal-brainstem-thalamic network involved in alertness (Mottaghy *et al.*, 2006; Sturm *et al.*, 1999; 2004). These studies suggest that the alertness mechanism is controlled by an automatic bottom-up and voluntary top-down network (Mottaghy *et al.*, 2006; Sturm and Wilmes, 2001). The bottom-up network consisting of the noradrenergic system which originates in the locus coeruleus in the brainstem, and relays through the thalamus which projects to frontal and parietal regions (Posner and Petersen, 1990; Robbins, 1984). This noradrenergic activity is governed by a top-down network consisting of the ACC and

the dorsolateral pFC, enabling the individual to selectively focus attention to the target stimulus (Mottagy *et al*., 2006; Sturm *et al*., 1999; 2004). The results of the systematic review are partially consistent with the previously hypothesised frontal-parietal-thalamic-brainstem network (Lewin *et al*., 1996; Pardo *et al*., 1991; Mottaghy *et al*., 2006; Sturm *et al*., 1999; 2004). The results are consistent with the bottom-up network, originating in the brainstem, then relaying through the thalamus and projecting to frontal and parietal regions (Posner and Petersen, 1990; Robbins, 1984). Whereas the top-down system seems to be more complex than initially suggested. Although the dorsolateral pFC is consistently reported in the studies included in this review, the ACC is not. Several included studies (seven) do show activation of the insula. Studies have shown that the insula and ACC together form a saliency network where the ACC is responsible for determining behaviourally relevant stimuli and guiding attention and the insula is responsible for the switching of attention (Menon *et al*., 2001; Menon & Uddin, 2010; Ridderinkhof *et al*., 2004). Despite the ACC not being activated in most of the studies, it is still activated in five studies, all using a different paradigm. This suggests that there may be some involvement of the ACC, but perhaps not as strongly as originally proposed. As a result, it may be that the noradrenergic system is modulated by the dorsolateral pFC and the salience network, where both the ACC and insula are involved.

The consistent reporting of the precentral gyrus and inferior occipital gyrus could be argued to be specific to the tasks using visual stimuli and button responses rather than being responsible for the alertness component. However, studies have shown that the premotor cortex and precentral regions are involved in the control, coordination and preparation of motor movements (Halsband *et al*., 1993; Lu and Ashe, 2005). Multiple studies have suggested that the precentral gyrus, may be associated not only with motor execution but as a preparatory mechanism (Pedersen *et al*., 1998; Porro *et al*., 2000; Toma *et al*., 2002). The cerebellum, which is also reported active in the majority of studies, is also an important region involved in motor responses. More specifically studies have suggested that it is involved in predictive, optimisation of movement (Manto, 2010; Morton and Bastian, 2006). Berardelli *et al*. (1996) showed that patients with cerebellar lesions have problems with controlling the timing element required for rapid movement. Specifically, for gripped force Manto (2010) suggested that the cerebellum plays a role in predictive force grip by tuning muscles for readiness to execute a movement. It is important to note that optimization of movement can come in very different forms, *i.e.* rapid movement would increase speed, but sometimes accurate movement would require

a slower response. As such there is variability in the exact cerebellar regions involved. The joint consistent reporting of the precentral gyrus and the cerebellum suggest that alertness requires an element of readiness for action.

Similarly, the occipital gyrus is predominantly activated during visual tasks and contains regions in the visual cortex that are active during the processing of objects (Tootell *et al.*, 1998). Several studies have shown that the inferior occipital cortex is essential to the representation and discriminations of objects, such as colour, shape, *etc.* (Brefczynski and DeYoe, 1999; Gitelman *et al.*, 1999). In the paradigms mentioned throughout this systematic review, the activation of inferior occipital cortex may be due to processing differences between a fixation point (*i.e.* cross) and the target stimulus (*i.e.* square). However, studies have shown that the inferior occipital cortex is not only activated in visual alertness tasks but also in auditory alertness tasks (Daumann *et al.*, 2010; Thiel and Fink, 2007), suggesting an element of attentional demand. Brefczynski and DeYoe (1999) indicated that the inferior occipital cortex was related to the attentional shifts in processing and not just the processing of visual stimuli itself. Similarly, Somers *et al.* (1999) suggest that the visual cortex is influenced by attentional demand, specifically by increasing the responses to attended stimuli and inhibiting responses to distractor stimuli. The consistent reporting of the inferior occipital gyrus suggests that alertness requires an attentional readiness.

The current thesis is particularly focused on the effect of fatigue and fatiguability on cognition. Previous studies have reported that the alertness mechanism may provide a good understanding of fatiguability (Boksem *et al.*, 2005; DeLuca, 2005; Faber *et al.*, 2012; Oken *et al.*, 2006). As such, the secondary aim was to establish and evaluate the paradigms used to assess alertness in the fMRI literature. Paradigms are evaluated for their suitability to address the thesis aims. The systematic review indicated that a wide variety of paradigms are used, despite only thirteen studies being included, there were a total of nine different paradigms. The ANT was devised to test three attention domains simultaneously, namely alerting, orienting and executive. Similarly, the revised-ANT measures these three sub-domains. A significant limitation of this task is that it simply uses different subtractions to assess each of these domains and does not consider the possible interactions between the networks. As such a more specific task assessing only alertness would be more advantageous. The CPT, SART, and RVIP all require the participant to respond to a target stimulus among several distractors. In some conditions, the target stimulus may be cued and in others, no cue is presented. These tests can be designed in

multiple different ways, which allows them to be tailored to the specific study. Furthermore, given the number of ways in which cues can be presented (congruent, incongruent, absent) allows task difficulty to be flexible. These tasks involve an accuracy element, where target stimuli must first be identified among distractors before a response is made. Although this is not a direct limitation of the behavioural tasks, this was not ideal for the current question. Previous studies have often suggested that alertness is measured by simple reaction time tasks (Kinomura *et al*., 1996; Sturm *et al*., 1999; Sturm and Wilmes, 2001), despite this only one of the studies included in this review used this type of task (Perin *et al*., 2010). In MS specifically, there seems to be a larger deficit of reaction time (Chiaravalloti *et al*., 2013; Denney *et al*., 2005; Denney and Lynch, 2009; Lengenfelder *et al*., 2006) than measures of simple attention (Rao *et al*., 1991a; 1991b; Benedict *et al*., 2006). Therefore, a reaction time task is more appropriate in an MS population. Furthermore, the Perin *et al*. (2010) task contained no distractor stimuli and allowed for alertness to be split into its two sub-components intrinsic (no cue) and extrinsic (with warning cue) alertness. Without the distractors, the task is slightly less complex and more appropriate for an MS population. Moreover, as it measures both intrinsic and extrinsic alertness, this task may elucidate subtle differences between the two forms of alertness. As a result, this simple reaction time task was chosen as the most appropriate task for the current thesis research aims.

Further to the aims of the systematic review, the lack of studies in this field was surprising, only thirteen studies have been conducted in this field over the past 25 years, suggesting that it is very understudied. It must be noted that only four of the thirteen studies had a sample size above 20 (Bartes-Serrallonga *et al*., 2014; Clemens *et al*., 2013; Lawrence *et al*., 2003; Xuan *et al*., 2016), and of these only one reported p-values that were family-wise error (FWE) corrected. This calls into question the reliability of the results from these studies and a study with a large sample size is required to determine more reliable and valid results. Moreover, due to the small number of studies, it is not possible to comment on whether these mechanisms may have a hemispheric dominance or whether there is any difference in the pattern of activation across different paradigms. Given the large number of varying paradigms used it makes it difficult to determine whether the different paradigms produce different patterns of activations. Although the activations do appear consistent across the paradigms, with more studies it would be possible to detect specific patterns of activation in each of the paradigms used. This may also allow for the subtle difference in the alertness mechanism to be teased apart.

There is clearly a lack fMRI studies evaluating the alertness mechanism, even in healthy individuals. The inconsistent and poorly defined terminology also poses a significant problem for this field, this systematic review proposes that researchers should be more selective about the terms used based on the research topic. Using the terms intrinsic and extrinsic alertness would allow for the difference between cued and non-cued alertness to be easily identified. Furthermore, the terms are more concise and therefore do not have the same overlapping use, across fields, that the terms vigilance and arousal have.

3.5 Conclusions

The systematic review has revealed that there is some consensus as to the network that underlies healthy alertness. The majority of studies reported activations in the precentral gyrus, inferior occipital gyrus, middle frontal gyrus, superior and inferior parietal lobule, thalamus, insula, cerebellum and brainstem regions. It must be noted that all these regions are simultaneously activated in only one study. This result provides a good understanding of the alertness mechanism in healthy individuals, which will allow for a better interpretation of the results in later chapters in both HC and MS groups. Moreover, it has established the tasks that are used in the fMRI literature to elicit the alertness mechanism. Evaluating the strengths and weaknesses have demonstrated that using a simple reaction time task, such as in Perin *et al.*, (2010) would be the most appropriate for the aims of this thesis. Further to our originals aims, the results of the systematic review suggest that there is a large gap in this field of research. In the past 25 years, only 13 fMRI studies have been conducted on alertness with only one of these studies having both a sample size of above 20 and reporting corrected p-values. This may be due to the ambiguity of the current terms used in the literature. As such a more concise, standardised term and definition must be used in conjunction with a larger powered study.

Chapter 4 The influence of induced fatiguability on attention performance in multiple sclerosis.

4.1 Introduction

Behavioural studies employing neuropsychological tests show inconsistent results on whether fatigue in multiple sclerosis (MS) affects cognition (Bailey *et al.*, 2007; Bryant *et al.*, 2004; Claros-Salinas *et al.*, 2010; Jennekens-Schinkel *et al.*, 1988; Johnson *et al.*, 1997; Krupp and Elkins, 2000; Kujala *et al.*, 1995; Paul *et al.*, 1998; Schwid *et al.*, 2003). Some studies show that cognitive performance is adversely affected by fatigue, whereas others find no association. The results are mostly inconsistent when subjective measures of fatigue are used and better established when measuring fatigue more objectively. This highlights the importance of a reliable standardised objective measure of fatigue. Studies examining the effect of fatigue on cognitive performance will conduct baseline measures of cognitive performance, this would be followed by the fatiguing event, and then the same neuropsychological test will be applied after the fatiguing event. In healthy groups, multiple studies have indicated that repeated performance of a cognitive test can aid performance on the later tests, known as the learning or testing effect (Agarwal *et al.*, 2007; Carrier and Pashler, 1992; McLeod *et al.*, 2004; Roediger and Butler, 2011; Roediger and Karpicke, 2006a; 2006b; Wheeler and Roediger, 1992; Wrisley, 2005). Sumovski *et al.* (2010) showed a similar effect in an MS population. In direct contrast, Krupp and Elkins (2000) demonstrated that MS participant had a significant decline in performance following a continuous performance task, whereas healthy controls still exhibited the learning effect and performed better. These findings demonstrated that the learning effect may be disrupted by increased fatiguability in an MS group.

Specifically in MS, both the phenotype with which the participant has been diagnosed and disease duration may affect cognitive performance (Dulau *et al.*, 2017; Rosti-Otajarvi *et al.*, 2014). However, this tends to be a weak relationship and is not consistent across tasks. This is likely due to the large heterogeneity in MS. For example, Leavitt *et al.* (2018) explored cognition in only the relapsing-remitting phenotype, and yet established four distinct cognitive phenotypes within their cohort: not impaired (56%), processing speed impaired (8%), memory impaired (19%) and both processing speed and memory impaired (17%). Furthermore, these cognitive impairments are associated with disability, depression, quality of life, employment status, adherence to therapy and fatigue (Arnett *et al.*, 2008a; Beatty *et al.*, 1990; Benedict *et al.*, 2005; Bruce *et al.*, 2010a; 2010b; Hojsgaard

et al., 2018; Larocca *et al.*, 1985; Marrie *et al.*, 2005; Piras *et al.*, 2003; Rao *et al.*, 1991a; 1991b; Roberg *et al.*, 2012). These findings highlight the importance of understanding the specific population in the sample before being able to make complex interpretations.

The primary aim of the current chapter was to examine the impact of induced fatiguability on cognitive performance in both an MS group, already suffering from persistent fatigue and a healthy control (HC) group. The secondary aims were to examine whether MS phenotype or disease duration affected cognitive performance, and to examine any cognitive impairment in the MS group compared to the HC group. Fatiguability was induced using an alertness-motor paradigm (see section 5.2.3-5.2.5 page 90-92), that was conducted during an MRI scan, between testing sessions. It is expected that the HC group demonstrate a learning effect in post-scan scores, but that this would be attenuated in the MS group if the paradigm induced fatiguability. Furthermore, it is expected that the MS groups would show significant disruptions to cognitive performance, specifically related to speed of information processing. In terms of differences between MS phenotypes, no meaningful differences are expected.

4.2 Methods

4.2.1 Participants

A total of 40 MS participants and 40 healthy individuals were recruited according to the procedure described in the general methods section. 3 MS and 4 HC outliers were removed (refer to section 2.6 page 52). In the MS group, there were 4 participants diagnosed with primary progressive MS, 10 with secondary progressive MS, and 26 with relapsing-remitting MS. Disease duration ranged between 1 and 36 years with a mean of 12.6 years.

Phenotype	Number of Participants	Age	Disease Duration	FSS Score
Relapsing Remitting	24	47.88 (32-67)	9.42 (1-29)	51.63 (43-62)
Secondary Progressive	10	55.8 (40-65)	23.7 (12-36)	55 (46-63)
Primary Progressive	3	58.67 (52-59)	8.67 (6-14)	48.67 (48-54)

Table 4.1 Demographics of MS group. *Mean (Range).*

4.2.2 Neuropsychological questionnaires

All participants, in both the MS and HC groups, were screened for fatigue, using the Fatigue Severity Scale (FSS) (Krupp *et al.*, 1989), to ensure that the MS did have significant

fatigue and the HC did not. Furthermore, the scores from the FSS was used as a measure of fatigue and used in the analysis.

4.2.3 Neuropsychological tests

The participants completed a total of five paper pencil attention tasks, which were grouped into three categories of tasks. All five tasks were completed prior to and post MRI scan, lasting approximately 60 minutes (see section 5.2.3 page 90). The first category was the Stroop tasks. This category consisted of three different tests, colour naming, colour words and the interference task (Figure 4.1). During all the tasks in this group reaction time was measured and the number of errors, both corrected and uncorrected by the participant, were noted. Each error added one second to the total reaction time. In the colour naming task, the participants were presented with coloured blocks which were either printed in red, green or blue. The participants were required to read aloud the colours in which the blocks were printed. The page consisted of 11 rows with 10 blocks in each row, where the first row was a practice row.

During the colour words task, the participants were asked to read aloud the words printed on the page. All the words were printed in black ink and consisted of RED, BLUE or GREEN. The page contained 11 rows with 10 words in each row, again the first row constituted a practice row.

The last task in this category was the interference task. The page consisted of 10 words per row printed either in red, blue or green ink and were incongruent with the meaning of the word *e.g.* the word RED was printed in blue ink. There was a total of 11 rows, with the first row being for practice. Participants were required to read aloud the colour in which the word was printed (*e.g.* green ink) and not what the word itself read (*e.g.* BLUE). The incongruity between the word and the colour in which it is printed causes severe disruption and is known as the Stroop Effect. Stroop (1935) suggested that this disruption occurs due to the interference created by the incongruity and the inhibition required to not simply read the word. Studies have suggested that performance on the Stroop task is related to frontal lobe regions and therefore related to executive functions (Stuss *et al.*, 2001; Tsuchida and Fellows, 2013; Vendrell *et al.*, 1995). As a result, the Stroop test is often used as a test of executive function. Despite this, there is not a direct relationship between the frontal lobes and executive function (Alvarez and Emory, 2006; Stuss, 2011). As such one must be careful when interpreting the Stroop task in terms of executive function. As a result, this thesis cautiously used the Stroop tasks as a test of selective attention.

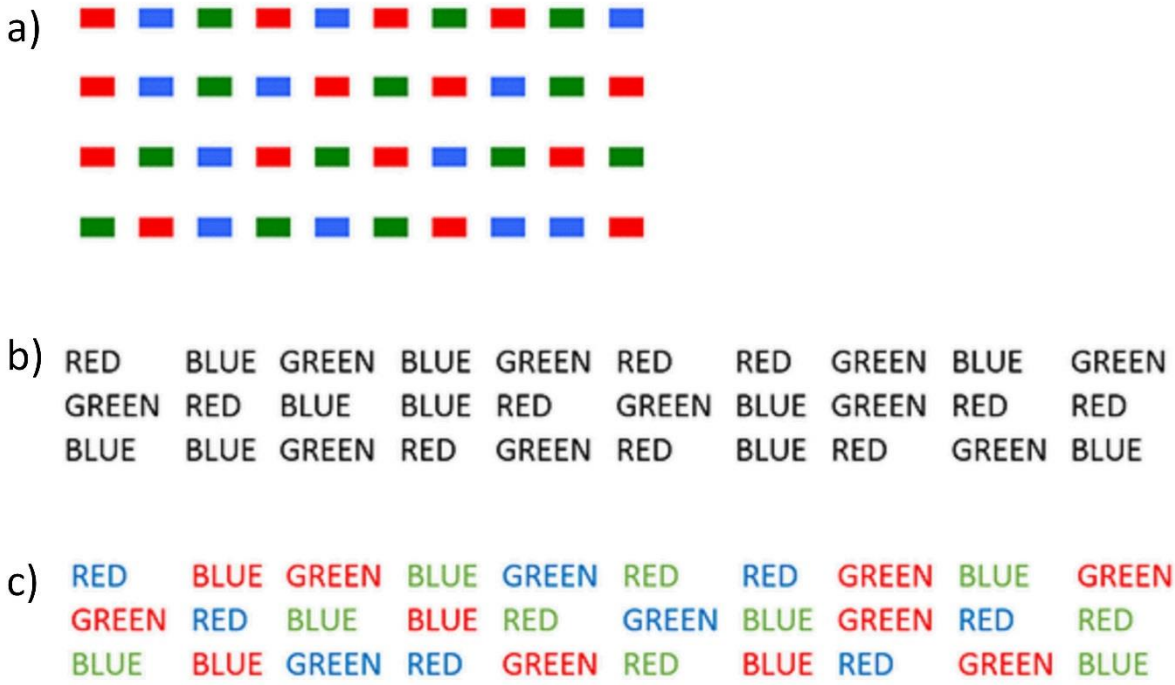


Figure 4.1 Composite image of shortened Stroop tasks. *a) colour naming. b) colour words. c) interference task. See appendix E (page 252-254) for the full task.*

The second category of tests was the trail making tasks (Figure 4.2, page 75), there were two tests in this category. Both tests consisted of 25 circles distributed over a sheet of paper. In the first test (trail making part A), the circles were numbered 1-25, the participants were asked to connect the numbers in ascending order. In the second test (trail making part B) the circles included both numbers (1 – 13) and letters (A – L), similarly to part A, the participants were asked to connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (*e.g.* 1-A-2-B-3-C, etc.). The participants were instructed to connect the circles as quickly as possible, without lifting the pen or pencil from the paper. If the participants made an error, it was pointed out immediately and they were given the opportunity to correct the error. Errors were not noted separately but add to the overall reaction time of the task. The trail making tasks were used as a simple test of visual attention.

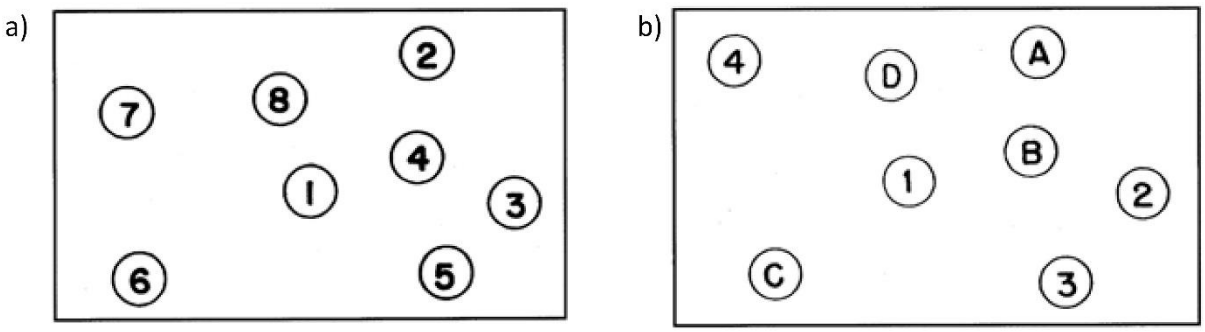


Figure 4.2 Composite image showing an example of trail making tasks. *a) Part A, numbers only. b) Part B numbers and letters. See appendix F (page 255-256) for the full task.*

The final category was the d2 (Figure 4.3). This category only consisted of one d2 task, but three outcome measures. In the d2 test, participants were presented with 14 rows each containing 47 characters. Each character consisted of either the letter, 'd' or 'p' marked with one, two, three or four small dashes. The participants were required to scan the lines and circle all occurrences of the letter 'd' that had a total of two dashes. They were instructed to ignore all the letters 'p' regardless of the number of dashes and the letters 'd' that contain one, three or four dashes. The d2 tasks were used to measure focused attention.

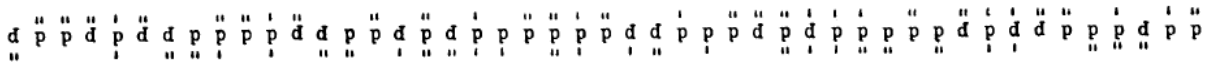


Figure 4.3 Single row of D2 task. *See appendix G (page 257) for the full task.*

4.2.4 Behavioural data analysis

Independent sample t-tests, for each of the five tasks, was used to compare baseline performance between the HC and MS groups. 2x2 ANOVA, for each of the five tasks, were conducted to compare performance pre- and post-scan performance between HC and MS groups. Within group performance between pre-scan and post-scan performance was analysed using paired t-tests, for both HC and MS groups. In the MS group only, a partial correlation, controlling for age, using Pearson's correlation coefficient, was conducted between test scores and disease duration. A one-way ANOVA was conducted to examine whether test scores differed across MS phenotypes. A partial correlation, controlling for age, between neuropsychological test scores and fatigue scores was also conducted. Due to outliers, a Spearman's rank correlation was also conducted (Appendix L), however this did not alter the results. As such Pearson's correlation coefficient is reported.

4.3 Results

4.3.1 Neuropsychological tests in the HC group

Paired t-tests between pre-scan and post-scan performance on the neuropsychological tests showed that HC performed significantly better post-scan on the colour naming task ($t(35)= 3.62$, $p< .01$), colour word task ($t(35)= 3.84$, $p< .01$), interference task ($t(35)= 2.12$, $p= .04$) , trail-making A task ($t(35)= 4.26$, $p< .01$), trail-making B task ($t(35)= 2.51$, $p= .01$), and D2 speed ($t(35)= -6.86$, $p< .01$) and no significant differences on, D2 errors ($t(35)= 1.39$, $p= .17$), D2 ratio ($t(35)= 1.75$, $p= .09$). Reaction times on tasks are displayed in Figure 4.4 and performance on D2 is displayed in Figure 4.5.

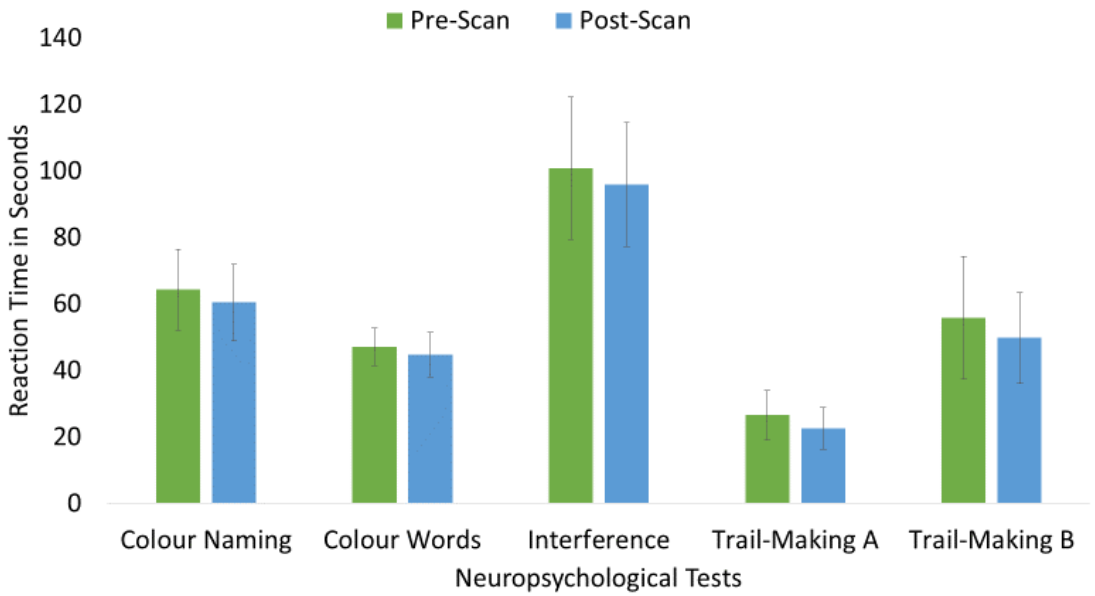


Figure 4.4 Reaction time on neuropsychological tests, excluding D2 task. *Pre-Scan scores are displayed in green, Post-Scan scores are displayed in blue. The error bars represent one standard deviation from the mean.*

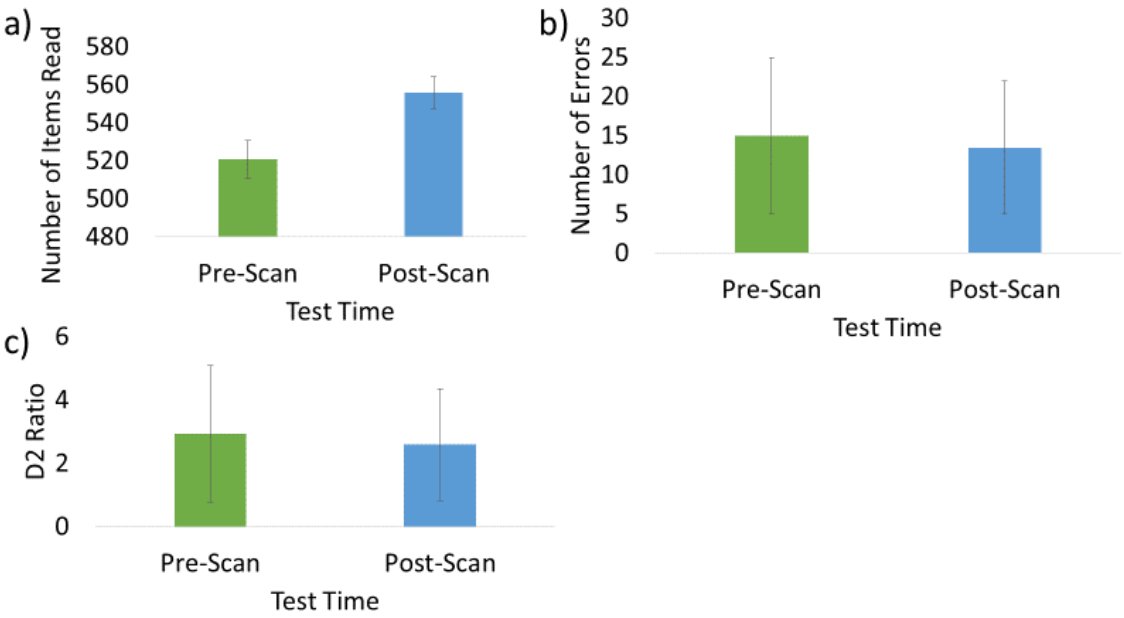


Figure 4.5 Composite image for D2 task. *a) D2 speed (maximum 658). b) D2 error. c) D2 ratio (errors/speed).*

4.3.2 Neuropsychological tests in the MS group

For the MS group, the paired t-tests between pre-scan and post-scan performance on the neuropsychological tests revealed significantly better post-scan performance on the trail-making A task ($t(36)= 2.99$, $p= .01$) and significantly decreased post-scan performance on the colour word task ($t(36)= -2.47$, $p= .02$). There were no performance differences between pre-scan and post-scan on the colour naming task ($t(36)= -1.06$, $p= .29$), interference task ($t(36)= -1.14$, $p= .26$), trail-making B task ($t(36)= .67$, $p= .51$), D2 speed ($t(36)= -.86$, $p= .40$), D2 errors ($t(36)= .24$, $p= .81$), or D2 ratio ($t(36)= .45$, $p= .66$). Reaction times on tasks are displayed in Figure 4.6 and performance on D2 is displayed in Figure 4.7.

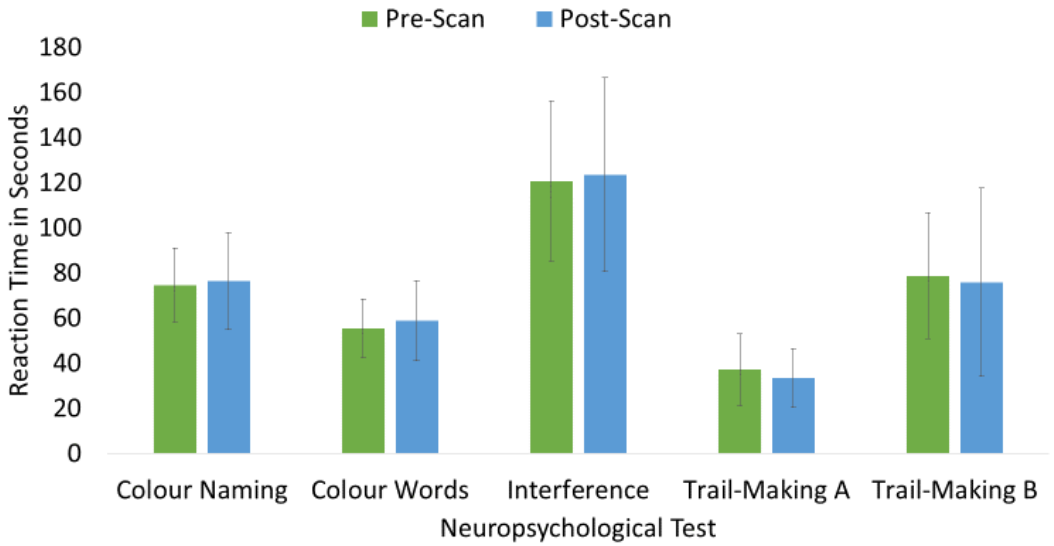


Figure 4.6 Reaction time on neuropsychological tests, excluding D2 task. *Pre-Scan scores are displayed in green, Post-Scan scores are displayed in blue. The error bars represent one standard deviation from the mean.*

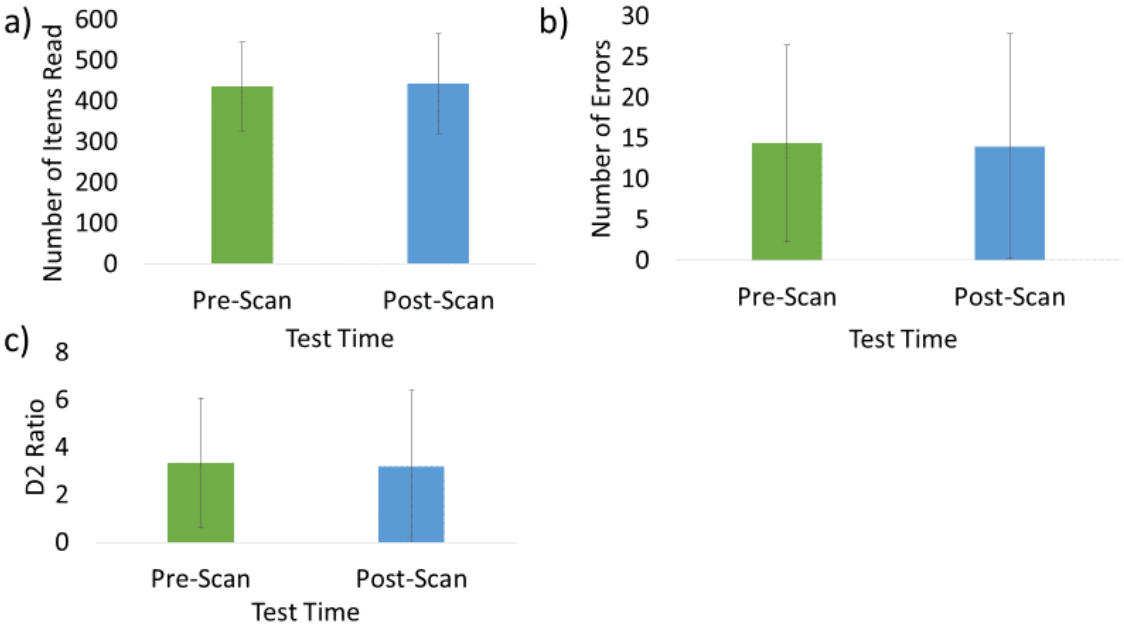


Figure 4.7 Composite image for D2 task. *a) D2 speed (maximum 658). b) D2 error. c) D2 ratio (errors/speed).*

When examining whether phenotype affected the cognitive performance, the MS group were split into their phenotypes and a one-way ANOVA was conducted. There were no significant main effects for any of the neuropsychological tests colour naming task ($F(2,34)= .31$, $p= .74$, $\eta^2= .02$), colour word task ($F(2,34)= 1.26$, $p= .30$, $\eta^2= .08$), interference task ($F(2,34)= 314$, $p= .74$, $\eta^2< .01$), trail-making A task ($F(2,34)= .11$, $p= .89$, $\eta^2= .015$), trail-making B task ($F(2,34)= 359$, $p= .70$, $\eta^2= .01$), D2 speed ($F(2,34)= .61$, $p= .55$, $\eta^2= .04$), D2 errors ($F(2,34)= 2.06$, $p= .14$, $\eta^2= .01$) and D2 ratio ($F(2,34)= 3.05$, $p= .06$, $\eta^2= .14$).

The partial correlation demonstrated a significant positive correlation between disease duration and D2 error (R= .55, p< .01) and D2 ratio (R= .58, p< .01). There were no significant correlations between disease duration and colour naming (R= -.14, p= .41), colour words (R= -.05, p= .79), interference task (R= -.09, p= .61), trail-making A task (R= -.08, p= .64), trail-making B task (R= .12, p= .50) and D2 speed (R= .03, p= .88).

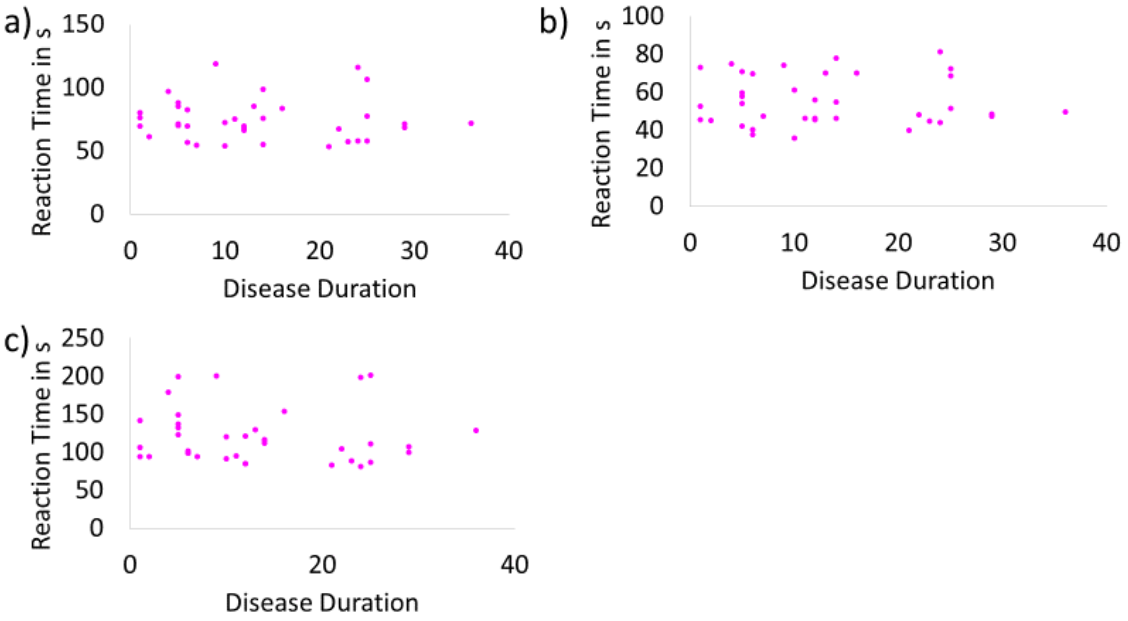


Figure 4.8 Composite image of scatterplot between FSS and performance on the Stroop group tasks. *a) Colour naming and disease duration. b) Colour words and disease duration. c) Interference and disease duration. Each circle represents one participant.*

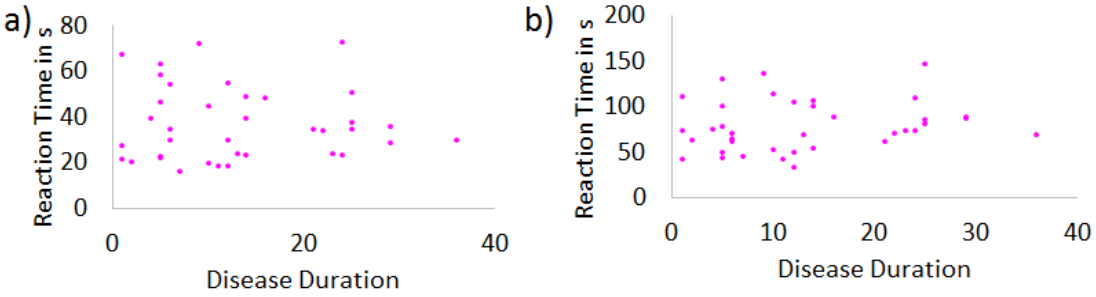


Figure 4.9 Composite image of scatterplot between FSS and performance on the trail making group tasks. *a) Trail making part A and disease duration. b) Trail making part B and disease duration. Each circle represents one participant.*

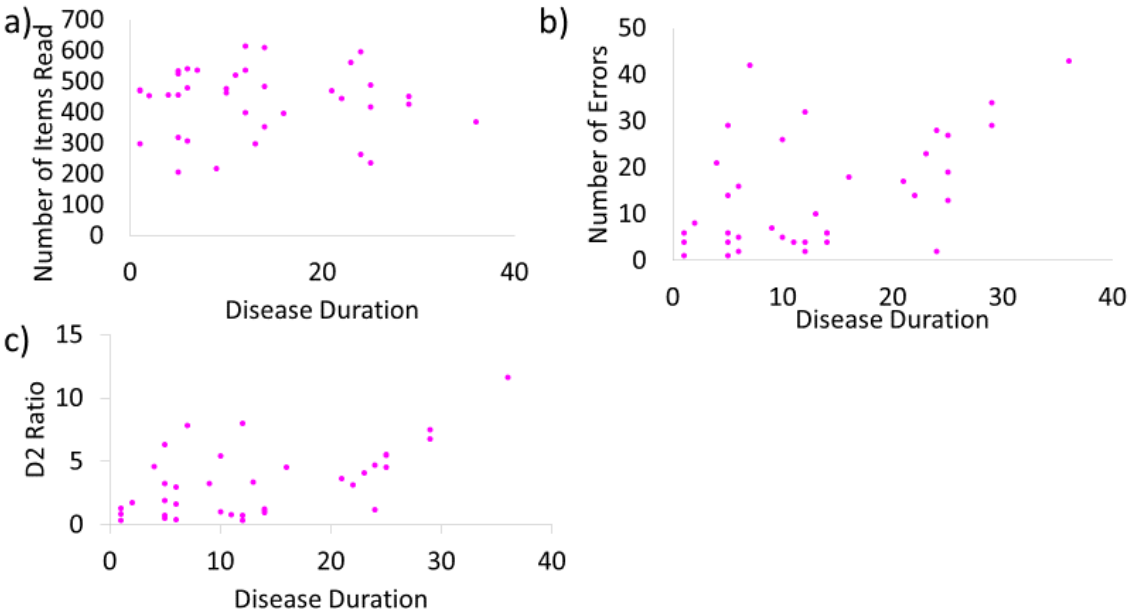


Figure 4.10 Composite image of scatterplot between FSS and performance on the D2 task. *a) D2 speed and disease duration. b) D2 errors and disease duration. c) D2 ratio and disease duration. Each circle represents one participant.*

4.3.3 Group comparison of neuropsychological tests

The comparison between baseline performance in the HC and MS groups on the neuropsychological tests revealed significant differences between groups on the colour naming task ($t(71)= -3.04$, $p< .01$, $d= .72$), colour word task ($t(49.68)= -3.58$, $p< .01$, $d= .89$), interference task ($t(59.37)= -2.92$, $p< .01$, $d= .65$), trail-making A task ($t(51.03)= -3.64$, $p< .01$, $d= .73$), trail-making B task ($t(62.65)= -4.17$, $p< .01$, $d= .79$), and D2 speed ($t(71)= 3.70$, $p< .01$, $d= .89$). There were no significant differences on D2 errors ($t(71)= .23$, $p= .82$, $d= .01$), and D2 ratio ($t(71)= -.69$, $p= .49$, $d= .12$).

The 2x2 ANOVA revealed a significant main effect over time for the trail making A ($F(1,71)= 24.42$, $p< .01$, $\eta^2= .26$) and D2 speed ($F(1,71)= 21.16$, $p< .01$, $\eta^2= .23$). There were no main effects over time for colour naming ($F(1,71)= .73$, $p= .40$, $\eta^2= .01$), colour words ($F(1,71)= .41$, $p= .52$, $\eta^2= .01$), interference ($F(1,71)= .30$, $p= .59$, $\eta^2= .01$), trail making B ($F(1,71)= 3.18$, $p= .08$, $\eta^2= .04$), D2 error ($F(1,71)= .97$, $p= .33$, $\eta^2= .01$) or D2 ratio ($F(1,71)= 1.67$, $p= .20$, $\eta^2= .02$). There were significant time x group interactions for colour naming ($F(1,71)= 7.23$, $p= .01$, $\eta^2= .09$), colour words ($F(1,71)= 14.54$, $p< .01$, $\eta^2= .17$), interference ($F(1,71)= 5.07$, $p= .03$, $\eta^2= .07$), and D2 speed ($F(1,71)= 10.21$, $p< .01$, $\eta^2= .13$) and no significant interactions for trail making A ($F(1,71)= .04$, $p= 84$, $\eta^2= .01$), trail making B ($F(1,71)= .39$, $p= .53$, $\eta^2= .01$), D2 error ($F(1,71)= .35$, $p= .56$, $\eta^2= .01$) or D2 ratio ($F(1,71)= .29$, $p= .59$, $\eta^2= .01$). Performance on the Stroop tasks are displayed in Figure 4.11, trail making tasks are displayed in Figure 4.12 and performance on D2 is displayed in Figure 4.13.

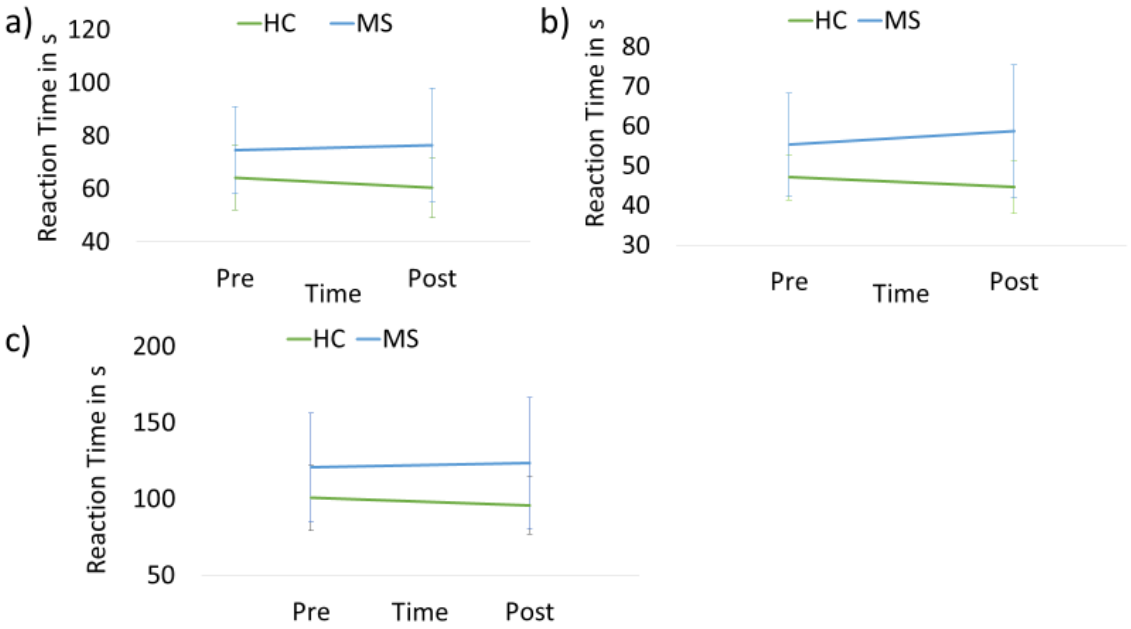


Figure 4.11 Reaction time on Stroop group tasks. *HC group are displayed in green, MS group are displayed in blue. The error bars represent one standard deviation from the mean.*

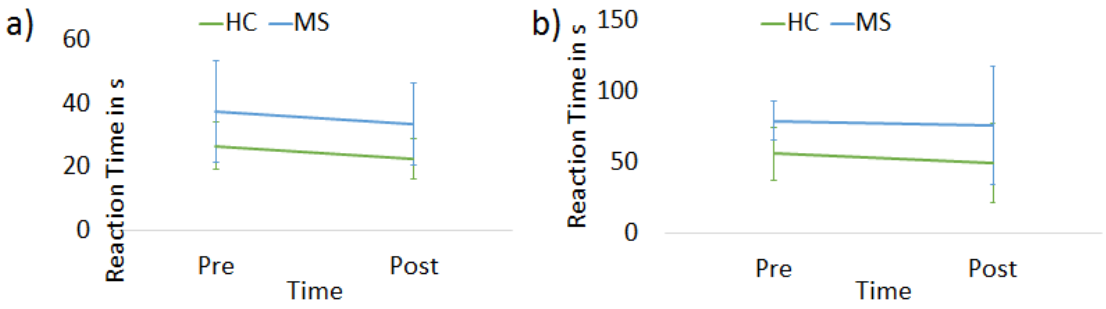


Figure 4.12 Reaction time on trail making tasks. *HC group are displayed in green, MS group are displayed in blue. The error bars represent one standard deviation from the mean.*

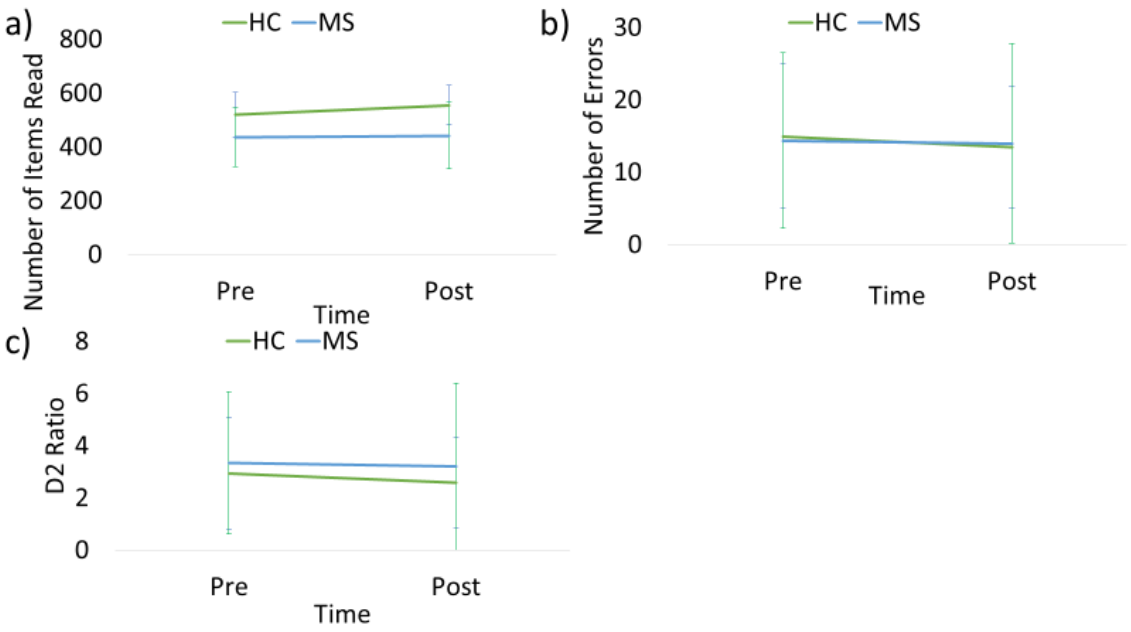


Figure 4.13 Composite image for D2 task performance. *a) D2 speed (maximum 658). b) D2 error. c) D2 ratio (errors/speed). HC group are displayed in green, MS group are displayed in blue.*

4.3.4 Correlates between fatigue scores and neuropsychological tests

In the HC group, the partial correlation indicated a significant positive correlation between fatigue scores and the interference task (R= .35, p= .04), D2 error (R= .36, p= .03) and D2 ratio (R=.35, p= .04). There were no significant correlations between fatigue or colour naming (R= .25, p= .14), colour words (R= .25, p= .15), trail-making part A (R= .21, p= .22), trail-making part B (R= .07, p= .71) and D2 speed (R= -.13, p= .46).

In the MS group there were no significant partial correlations with fatigue scores colour naming (R= .08, p= .65), colour words (R= .08, p= .66), interference task (R= .05, p= .79), trail-making part A (R= .08, p= .66), trail-making part B (R= .28, p= .10) and D2 speed (R= -.04, p= .80), D2 error (R= .31, p= .06) and D2 ratio (R=.29, p= .08). Scatterplots are displayed for the Stroop group task in Figure 4.14, for the trail making group in Figure 4.15 and for the D2 test in Figure 4.16.

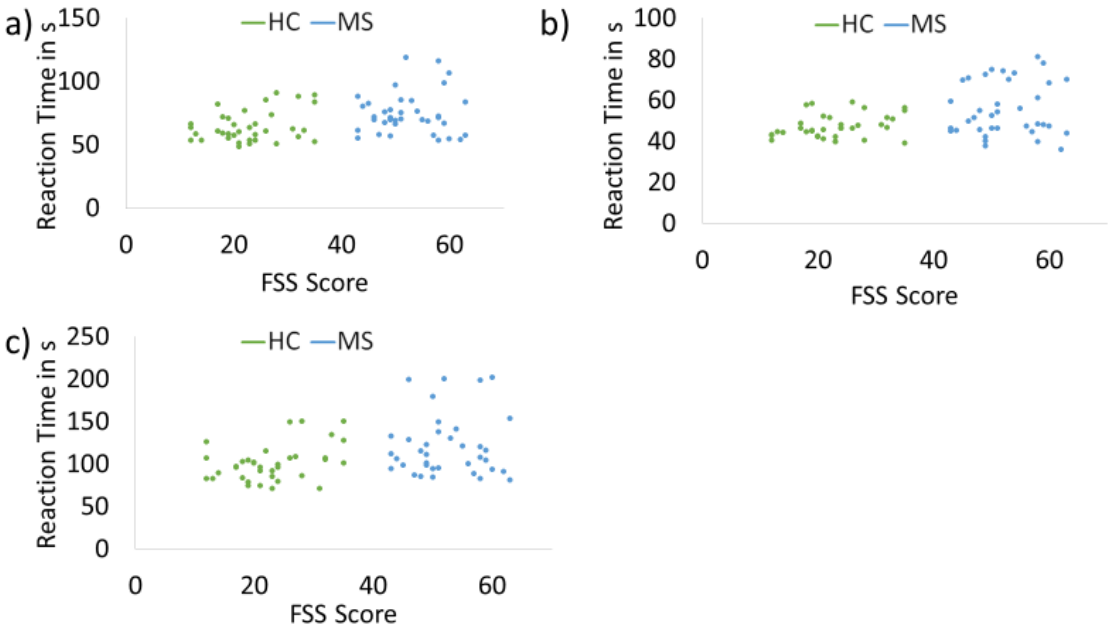


Figure 4.14 Composite image of scatterplot between FSS and performance on the Stroop group tasks. *a) Colour naming and FSS. b) Colour words and FSS. c) Interference and FSS. Each circle represents one participant. HC group are displayed in green, MS group are displayed in blue.*

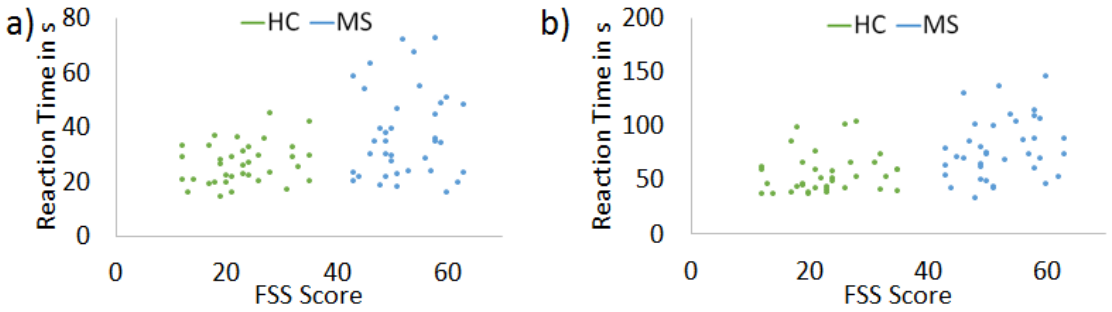


Figure 4.15 Composite image of scatterplot between FSS and performance on the trail making group tasks. *a) Trail making part A and FSS. b) Trail making part B and FSS. Each circle represents one participant. HC group are displayed in green, MS group are displayed in blue.*

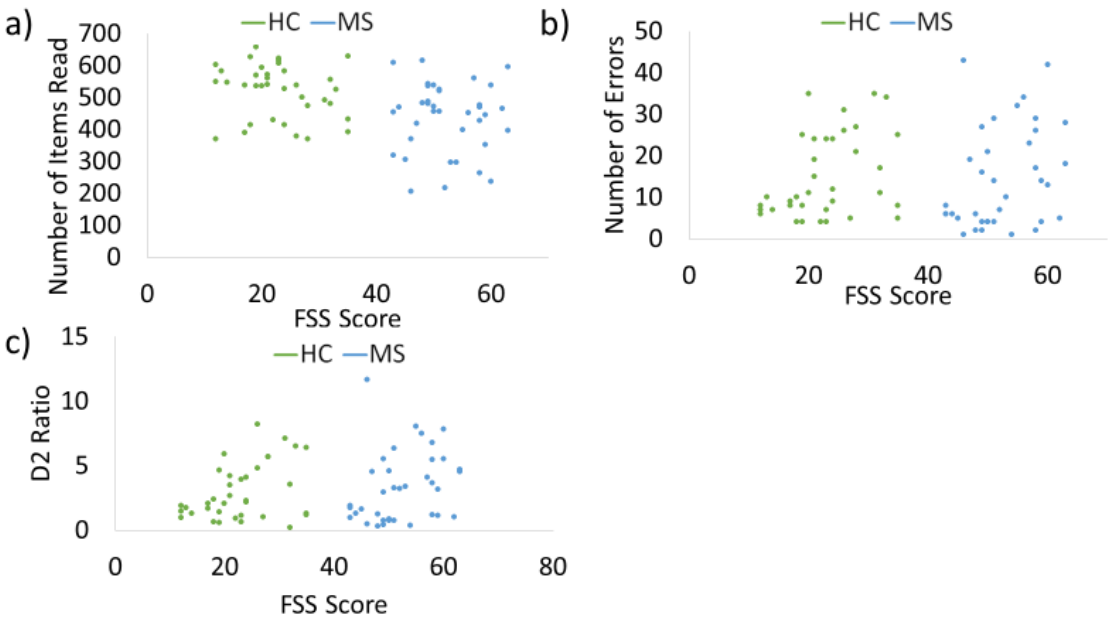


Figure 4.16 Composite image of scatterplot between FSS and performance on the D2 task. *a) D2 speed and FSS. b) D2 errors and FSS. c) D2 ratio and FSS. Each circle represents one participant. HC group are displayed in green, MS group are displayed in blue.*

4.4 Discussion

The primary aim of the current chapter was to examine the impact of induced fatiguability on cognitive performance in both an MS group, already suffering from persistent fatigue and an HC group. The results revealed that in the HC group performance significantly improved post scan in all reaction time tasks, but not on the tasks of accuracy. Overall the group showed no decreased performance when completing the neuropsychological tests a second time. The findings suggest that HC perform significantly better in speed of response when completing the tasks a second time, but measures of accuracy remain similar. This is in line with previous findings that demonstrated a learning effect in HC (Agarwal *et al.*, 2007; Carrier and Pashler, 1992; McLeod *et al.*, 2004; Roediger and Butler, 2011; Roediger and Karpicke, 2006a; 2006b; Wheeler and Roediger, 1992; Wrisley, 2005). However, the present study was not only interested in a simple learning effect but how induced fatiguability would influence this effect. The consistent learning effect observed in the HC group may suggest that the alertness-motor paradigm failed to induce fatiguability entirely. However, the more likely explanation is that the alertness-motor paradigm does induce fatiguability and the HC group are able to compensate for the increase in fatiguability and still show improved performance. This is consistent with previous findings that show a learning effect in healthy individuals following prolonged task performance (Claros-Salinas *et al.*, 2010; Johnson *et al.*, 1997; Krupp and Elkins, 2000; Schwid *et al.*, 2003).

The results revealed a very different pattern in the MS group. There was only improved performance in the trail-making part A test, a simple test of visual attention. The MS group showed decreased performance in the colour words task, but consistently showed no difference on the colour naming, interference, trail-making part B tasks, and no difference for D2 speed, D2 error, and D2 ratio. This suggests that the MS group may have an absence of the learning effect, rather than a detrimental, fatiguing effect. This is inconsistent with previous findings that have shown both a learning effect (Sumovski *et al.*, 2010) and fatiguing effect (Krupp and Elkins, 2000) in MS. Again, the lack of fatiguing effect in the MS group may be due to the alertness-motor paradigm not inducing fatiguability. This is unlikely as if the paradigm did not induce fatiguability a learning effect would be expected, as in Sumovski *et al.* (2010). The MS group demonstrated a consistent attenuation of the learning effect, rather than a fatiguing effect. Therefore, it is possible that the MS group are able to overcome the fatiguing effect with some effectiveness, but not to the same extent as seen in the HC group. This is is further evidenced by the 2 x 2 ANOVAs that showed the HC improve significantly more than the MS group on tests of reaction time. As seen in the current results using the neuropsychological tests alone, this may lead to some ambiguous results and therefore make interpretations difficult. The analysis employed in the following chapters may further elucidate exactly which explanation is most appropriate. Furthermore, Hanken *et al.* (2015) demonstrated that memory performance, selective attention, language comprehension and visuospatial processing remain largely unaffected by fatigue in MS, whereas fatigue significantly impacts tasks of alertness or vigilance. Which may suggest that the performance during the alertness-motor paradigm may be more indicative of fatigue.

To establish whether MS phenotype or disease duration affected cognitive performance on attention tasks, the MS group was split into its three phenotypes. The results from this analysis revealed that there were no performance differences on any of the neuropsychological tests between the phenotypes. This is inconsistent with previous findings (Dulau *et al.*, 2017; Rosti-Otajarvi *et al.*, 2014), which did show some differences across the phenotypes. However, these studies did suggest that there was only a weak relationship. Given the heterogeneity in MS, it is difficult to generalise these findings. Furthermore, the group sizes per phenotype were very different in the current sample. The findings, in the current sample, do support that the MS group, despite different phenotypes, are homogenous in cognitive performance and can be assessed together as one group. Disease duration correlated positively with D2 errors and D2 ratio but did not

correlate with any other neuropsychological test. This finding suggests that disease duration is correlated more strongly with measures of accuracy, but seemingly unrelated to measures of response speed, in the current study sample.

A further secondary aim was to examine group differences between the MS and HC groups. The comparison between the two groups on baseline performance showed that the MS group had significantly increased reaction time for the colour naming, colour word, interference task, trail-making part A and trail-making part B tests. Furthermore, they read a decreased number of items on the D2 test. Interestingly, there were no differences between groups on D2 error or D2 ratio. The D2 ratio is heavily impacted by the number of errors, the similar number of errors between groups, but decreased number of items read suggest that the MS group are able to complete the D2 task to a similar level of accuracy as the HC group, but have a significant disruption to the speed of information processing. This is further evidenced by the increased reaction time on all the other tasks. Strongly suggesting that the MS group have a greater impairment to speed of information processing but can complete tasks as accurately as HC. These findings are consistent with previous studies showing cognitive deficits in MS (Amato *et al.*, 2008; 2010; Arnett *et al.*, 1994; Bagert *et al.*, 2002; Beatty *et al.*, 1996; Benedict *et al.*, 2006; Bergendal *et al.*, 2007; Bobholz and Rao, 2003; Bruce *et al.*, 2010a; 2010b; Caine *et al.*, 1986; Calabrese *et al.*, 2006; Chiaravalloti and DeLuca, 2008; DeLuca *et al.*, 1994; 1998; 2004; 2013; 2015; Denney *et al.*, 2005; Drew *et al.*, 2008; Engle *et al.*, 2007; Litvan *et al.*, 1988; Mainero *et al.*, 2006; McCarthy *et al.*, 2005; Pardini *et al.*, 2014; Rao *et al.*, 1986; 1989; 1991a; 1991b; Thornton *et al.*, 2002), with some previous studies similarly suggesting that the cognitive impairments seen in MS are largely a results of the deficit in speed of information processing (Chiaravalloti *et al.*, 2013; Denney *et al.*, 2004; Denney and Lynch, 2009; Lengenfelder *et al.*, 2006). Moreover, the results indicated a significant difference between the HC group and the MS group in terms of improvement between baseline and subsequent testing periods. As such the HC showed greater improvement than the MS group, further evidencing the attenuation of the learning effect in the MS group. Studies have shown that there is a strong association between speed of information processing and fatigue in MS (Andreasan *et al.*, 2010). Interestingly the performance on these neuropsychological tests were unrelated to fatigue scores in the MS group. Whereas in the HC measures of accuracy were related to fatigue scores. The neuropsychological tests are measures of cognition rather than fatiguability. As such this result suggests a dissociation between patient perception

of the impact of fatigue and objective cognitive performance (Middleton *et al.,* 2006; Roberg *et al.,* 2012).

4.4.1 Conclusions

In conclusion, the results in this chapter demonstrate that the HC group show a learning effect when completing a set of attentional tests a second time, this effect is absent in the MS group. This suggests the HC group can overcome the influence of fatiguability on cognitive performance to a greater extent than the MS group. However, it is possible that the alertness-motor paradigm is unable to induce fatiguability. The results from neuropsychological tests alone do not elucidate which explanation is more appropriate. The MS group have widespread cognitive disruption in speed of information processing across a variety of attentional tests but show similar accuracy levels compared to HC. This indicates that speed of information processing may be disrupting cognitive performance on a variety of tasks in MS. However, this performance in the MS group is unrelated to fatigue scores, suggesting a dissociation between patients' perception of the impact of fatigue and objective measures of performance fatigue in MS. The alertness-motor paradigm examined in Chapter 6 (page 105) and Chapter 7 (page 125) provides a more direct objective measure of fatigue and could better elucidate the relationship between fatigue and cognition.

Chapter 5 A voxel-based morphometric magnetic resonance imaging study in a multiple sclerosis group with fatigue.

5.1 Introduction

Widespread demyelination in the brain and spinal cord is a core characteristic of multiple sclerosis (MS). Early studies of MS suggested that it was a disease related to white matter atrophy (Poser *et al.*, 1983; Prins *et al.*, 2015) and that grey matter lesions were rarely seen (Poser *et al.*, 1983). However, more recent studies have established that brain atrophy affects both the white matter (Bendfeldt *et al.*, 2010; Parisi *et al.*, 2014; Prinster *et al.*, 2010; Riccitelli *et al.*, 2012; Zhang *et al.*, 2017) and grey matter in MS (Audoin *et al.*, 2006; Battaglini *et al.*, 2009; Bendfeldt *et al.*, 2012; Bisecco *et al.*, 2018; Grothe *et al.*, 2016; Lansley *et al.*, 2013; Onu *et al.*, 2015; Parisi *et al.*, 2014; Prinster *et al.*, 2005; Riccitelli *et al.*, 2012; Sepulcre *et al.*, 2006; van de Pavert *et al.*, 2014; Zhang *et al.*, 2017). A review conducted by Bö *et al.* (2006) demonstrated that the atrophy in grey matter involves both cortical and sub-cortical structures. Furthermore, they demonstrated that MS is characterised by both white and grey matter atrophy, but the lesions can be inflammatory, which can make detecting volumetric tissue loss difficult. Moreover, recent post-mortem evidence has revealed a sub-type of MS where only grey matter damage is present and no white matter damage is observed (Trapp *et al.,* 2018).

Perhaps of more interest is how the brain atrophy relates to clinical and behavioural measures such as disability and cognition. Several studies have examined correlates of brain atrophy, predominantly in grey matter. Some find only a weak relationship (Bodini *et al.*, 2013; Prinster *et al.*, 2005; 2010;), whereas some do show a stronger association (Grothe *et al.*, 2016; 2017; Lansley *et al.*, 2013; Mackenzie-Graham *et al.*, 2016; Morgen *et al.*, 2005; Nocentini *et al.*, 2014; Onu *et al.*, 2015; Prinster *et al.*, 2005; 2010; Riccitelli *et al.*, 2012; Sastre-Garriga *et al.*, 2009). Despite consensus on the association between brain atrophy and clinical or cognitive measures, the exact regions involved are inconsistent. This pattern of results may be due to the heterogeneity of MS. The inconsistency makes it very difficult to compare across studies and come to a consensus on the specific mechanisms that may be damaged in MS.

Of particular interest to the current thesis, is how fatigue relates to atrophy in MS. Only a few studies have examined the relationship between fatigue and brain atrophy (Cruz-Gomez *et al.*, 2013; Derache *et al.*, 2013; Finke *et al.*, 2015; Gobbi *et al.*, 2013; Riccitelli *et al.*, 2011; Sepulcre *et al.*, 2009). These studies show very inconsistent results. Although all

studies show atrophy in the MS group, the exact regions vary vastly. Moreover, some show an association between atrophy and fatigue (Cruz-Gomez *et al.*, 2013; Derache *et al.*, 2013; Riccitelli *et al.*, 2011; Sepulcre *et al.*, 2009), whereas others observed no association (Finke *et al.*, 2015). Studies examining the relationship between fatigue and cognition have demonstrated that inconsistent results are predominantly noted when using subjective measures of fatigue, whereas using objective measures of fatigue yield more consistent results (Bailey *et al.*, 2007; Bryant *et al.*, 2004; Claros-Salinas *et al.*, 2010; Jennekens-Schinkel *et al.*, 1988; Johnson *et al.*, 1997; Krupp and Elkins, 2000; Kujala *et al.*, 1995; Paul *et al.*, 1998; Schwid *et al.*, 2003). This may be a similar concern with the relationship between fatigue and atrophy, as such an objective measure of fatigue is necessary. The alertness mechanism is uniquely suited to being able to provide a more objective performance measure of fatigue (Boksem *et al.*, 2005; Oken *et al.*, 2006; Faber *et al.*, 2012).

The primary aim of this chapter was to investigate whether fatigue is associated with brain atrophy in MS. Here fatigue is measured with a subjective self-report measure, fatigue severity scale, and objective performance measure, alertness-motor paradigm. The secondary aim is to establish the regions showing volumetric differences between MS and HC groups. To this end, voxel-based morphometry (VBM) to compute the global tissue volume and establish regional differences between the groups was used. If the atrophy is associated with fatigue scores, the neurodegeneration in MS directly impacts the fatigue in MS. If the atrophy is unrelated to fatigue scores, the neurodegeneration in MS does not directly impact fatigue but may contribute in another manner. Furthermore, it is expected that the MS group will have widespread regions of atrophy in both grey and white matter compared to the HC group.

5.2 Methods

5.2.1 Participants

A total of 40 MS participants and 40 healthy individuals were recruited according to the procedure described in the general methods section. 3 MS and 4 HC outliers were removed (refer to section 2.6 page 52).

5.2.2 Questionnaires

As fatigue is the main focus of this thesis, only the FSS score was used in the analysis. The FSS scores were correlated with both global and regional brain volume.

5.2.3 Alertness-motor paradigm

The paradigm consisted of interleaved periods of three different tasks, sensorimotor control, intrinsic alertness and extrinsic alertness, requiring the participant to exert a certain force (low, medium, high). Each task was repeated four times, resulting in four blocks containing each of the three tasks at each of the three force levels. The order of tasks was pseudorandomised (Figure 5.1, page 90). Furthermore, there were two conditions to the paradigm; physical handgrip, and mental imagery. Every participant completed both conditions. The group of participants that completed the handgrip condition before the mental imagery condition will be referred to as the handgrip first group throughout the thesis. The group of participants that completed the mental imagery condition before the handgrip condition will be referred to as the imagery first group throughout the thesis. In the physical condition, participants performed the full paradigm whilst squeezing a hand dynamometer at the required force. In the mental imagery task, participants performed the full paradigm but were asked to only imagine squeezing the hand dynamometer at the required force.

Block 1	T3			T1			T2		
	Low	Medium	High	High	Medium	Low	Low	High	Medium
Block 2	T2			T1			T3		
	High	Low	Medium	High	Medium	Low	High	Medium	Low
Block 3	T3			T1			T2		
	Medium	High	Low	Low	Medium	High	Medium	Low	High
Block 4	T2			T1			T3		
	Low	Medium	High	Low	Medium	High	Low	High	Medium

Figure 5.1 Task design. *Note: T1= sensorimotor task, T2= intrinsic alertness task, T3= extrinsic alertness task.*

5.2.4 Physical handgrip condition

During the physical handgrip condition, for each of the three tasks, the participants were asked to squeeze the hand dynamometer quickly, for approximately 1 second, and then release. They were instructed to squeeze at the force indicated by the task: low force, medium force or high force. A schematic of the complete paradigm is represented in Figure 5.2 (page 92). Electromyography (EMG), force and reaction time were measured across the paradigm.

In the sensorimotor task (T1), the instructions were displayed on the screen followed by the level of force which was to be exerted by the participant. The task consisted of a white

square and a black screen alternating every 100ms. The high frequency of the flickering avoided the synchronisation with the squeezing. The stimuli were presented 105 times each and lasted for a total of approximately 21 seconds. This was visualised as a white square flashing on the screen with a black background. Participants were instructed to attend to the appearance of the white square, and simultaneously squeeze the hand dynamometer at the required force. The participants were instructed to squeeze the handgrip at their own pace and were not required to squeeze at the appearance of each white square.

During the intrinsic alertness task (T2), the first stimulus to be displayed on the screen was the task instructions and the level of force required. The task commenced with a white cross, which was displayed in the centre of the screen for varying durations (1750, 1780 or 1810 ms) as a fixation point. The presentation of the white square stimulus followed the cross. Participants were instructed to squeeze the hand dynamometer at the required force as soon as the white square appeared on the screen. The blocks consisted of 21 square occurrences, 7 for each of the three forces. Each block lasted approximately 19 seconds in total.

In the extrinsic alertness task (T3) the instructions and level of force required were the first stimuli to be displayed. As in the intrinsic condition, the task instruction was followed by the appearance of a white cross, the cross was displayed for a fixed duration (1500 ms). The white cross was followed by the presentation of a black screen, which was displayed for varying durations (250, 280 or 310 ms). Following the black screen presentation, the white square stimulus was displayed. Participants were instructed to squeeze the hand dynamometer at the required force as soon as the white square appeared on the screen. The black screen represented an external warning cue, which indicated that the white square would appear next. The blocks consisted of 21 square occurrences, 7 for each of the three forces. Each block lasted approximately 19 seconds in total.

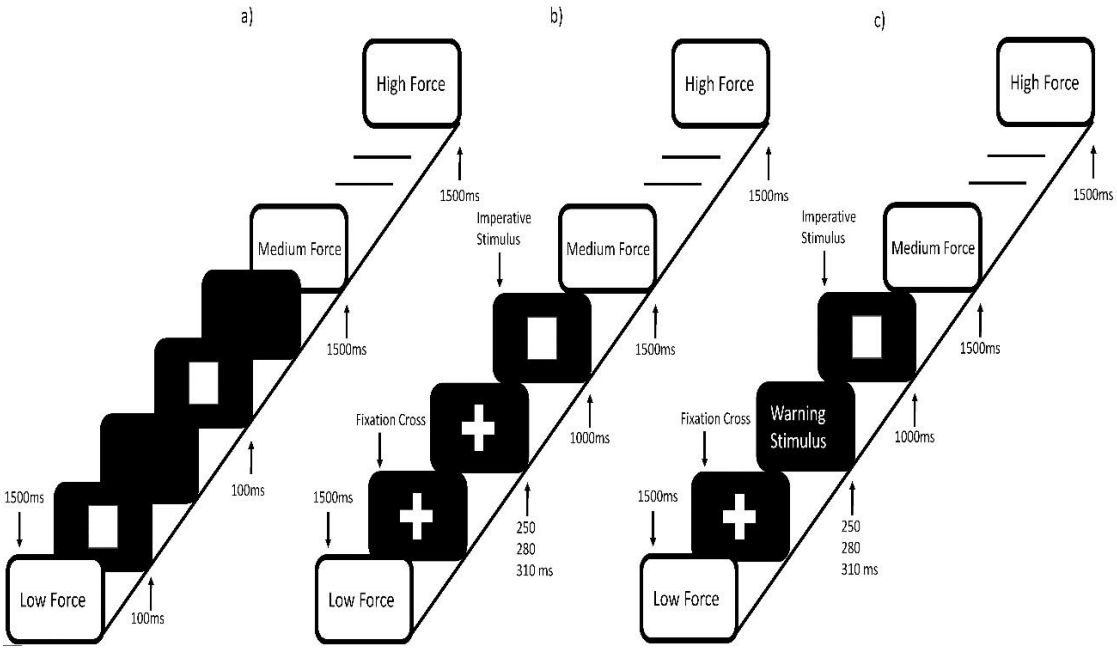


Figure 5.2 Schematic of alertness-motor paradigm. *a) sensorimotor control. b) intrinsic alertness. c) extrinsic alertness.*

5.2.5 Mental imagery condition

For the mental imagery condition, the experimental paradigm was identical to the physical handgrip condition and the tasks were presented in the same pseudorandomised order. The only difference between the conditions was that, in the mental imagery condition, the participants were required to only imagine squeezing the hand dynamometer and not to execute the physical action. Force data was not recorded, because there was no motor output. EMG data was still recorded for the mental imagery condition of the experiment. The mental imagery data was not analysed but simply used as a further manipulation to induce fatiguability.

5.2.6 Image acquisition and VBM analysis

A T1-weighted inversion recovery 'magnetisation prepared rapid acquisition gradient echo' (MPRAGE) was acquired according to the parameters stated in the general methods (refer to section 2.7 page 52). All image pre-processing and analysis were conducted in MATLAB (Mathworks Inc., Natick, MA, USA) and statistical parametric mapping package SPM12 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm). Firstly, structural data was visually inspected and manually repositioned to correspond with templates, if required. Images were then segmented into grey matter, white matter and cerebrospinal fluid (CSF) using the Computational Anatomy Toolbox (CAT12), with default settings. Next, the images were modulated to compensate for the possible volume changes that can occur from spatial normalisation. The images were then spatially

normalised to MNI space to allow for group level statistics and between group comparison. For quality assurance, the images were reviewed for sample homogeneity. All images had high correlation values and as a result, none of the images were discarded. Finally, both grey matter and white matter images were smoothed with a Gaussian kernel with a full width at half maximum (FWHM) of 8mm, to increase the signal-to-noise ratio. Global tissue volumes for grey matter, white matter and CSF, as well as total intracranial volumes, were extracted from the segmentation report.

An independent sample t-test was conducted in SPSS 24 to compare global tissue volumes between HC and MS groups. Furthermore, partial correlations were conducted between global tissue volumes and fatigue scores measured subjectively and objectively, controlling for age and total intracranial volume. Due to outliers, Spearman's rank correlations (Appendix L) were also conducted, however this did not alter the results, therefore Pearson's correlation coefficients are reported. Significance threshold was set at $p<.05$. For regional volume differences, statistical analyses were carried out using random effects models in SPM12. A two-sample t-test using the general linear framework was conducted to compare regional grey matter and white matter differences between MS and healthy participants on a voxel-by-voxel basis. Additionally, the total intracranial volume and age were added as covariates of no interest, to correct for the influence of total brain size and age. To restrict each analysis to the correct tissue class an absolute threshold of .1 was used. To identify any regions that correlated with FSS scores a multiple regression was conducted. Reaction time was calculated for the intrinsic and extrinsic alertness tasks, as the time between the onset of the imperative stimulus and the onset of the participant squeezing the handgrip. The reaction times were entered into a multiple regression to identify any regions that were related to a performance measure of fatigue.

5.3 Results

5.3.1 Global tissue volume

The two-sample t-test to detect differences in global tissue volume between MS and healthy participants showed a significant difference in CSF and grey matter tissue volume. MS group had greater CSF volume (t(71)= 2.79, p= .01, d= .62), and reduced grey matter volume (t(71)= -2.51, p= .01, d= .59) and no difference in white matter volume (t(71)= -1.48, p= .15, d= .32) compared to the HC group. The volume of tissue for both groups is displayed in Figure 5.3.

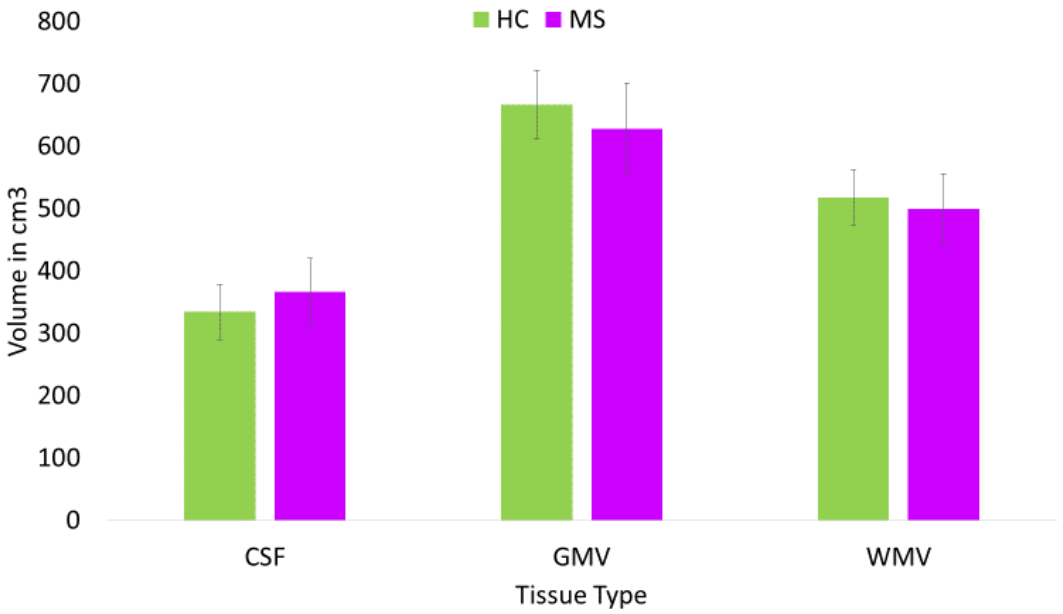


Figure 5.3 Volume of tissue types for the HC and MS groups. *HC values displayed in lime, MS values displayed in violet. The error bars represent one standard deviation from the mean. CSF cerebrospinal fluid, GMV grey matter volume, WMV white matter volume.*

In the HC group the partial correlation of FSS score revealed no significant correlations between FSS scores and tissue volume (grey matter R= -.32, p= .06; white matter R= .16, p= .36, CSF R= -.08, p= .66), intrinsic alertness reaction time and tissue volume (grey matter R= -.18, p= .29; white matter R= -.01, p= .97, CSF R= .01, p= .96), or extrinsic alertness reaction time and tissue volume (grey matter R= -.19, p= .27; white matter R= -.06, p= .74, CSF R= -.04, p= .84). The partial correlations in the MS group showed a significant positive correlation between FSS scores and CSF fluid (R= .45, p= .01) but no significant differences with other tissue types (grey matter R= -.07, p= .69; white matter R= -.30, p= .08). There were no significant differences between intrinsic alertness reaction time and tissue volume (grey matter R= .03, p= .88; white matter R= -.18, p= .30, CSF R= .16, p= .36) or extrinsic alertness reaction time and tissue volume(grey matter R= .09, p=

.59; white matter R= -.25, p= .14, CSF R= .15, p= .39). Scatterplots are displayed in Figure 5.4, Figure 5.5 and Figure 5.6.

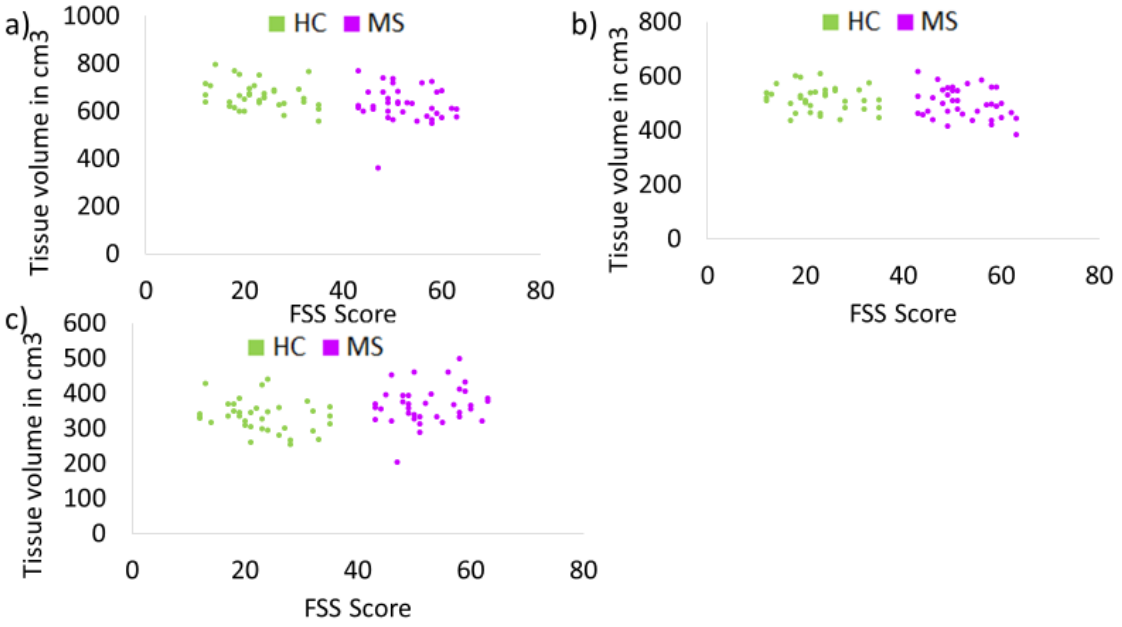


Figure 5.4 Scatterplots for correlations between fatigue scores and tissue volume. *a) grey matter and FSS. b) white matter and FSS. c) CSF and FSS. Each circle represents a participant.*

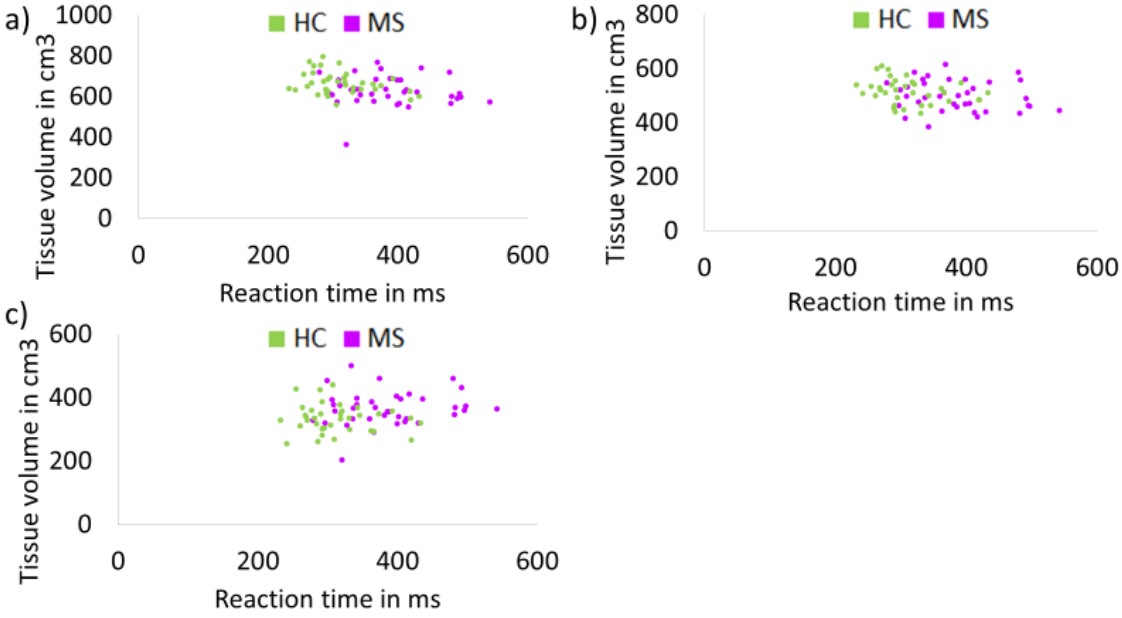


Figure 5.5 Scatterplots for correlations between intrinsic task reaction time and tissue volume*. a) grey matter and reaction time. b) white matter and reaction time. c) CSF and reaction time. Each circle represents a participant.*

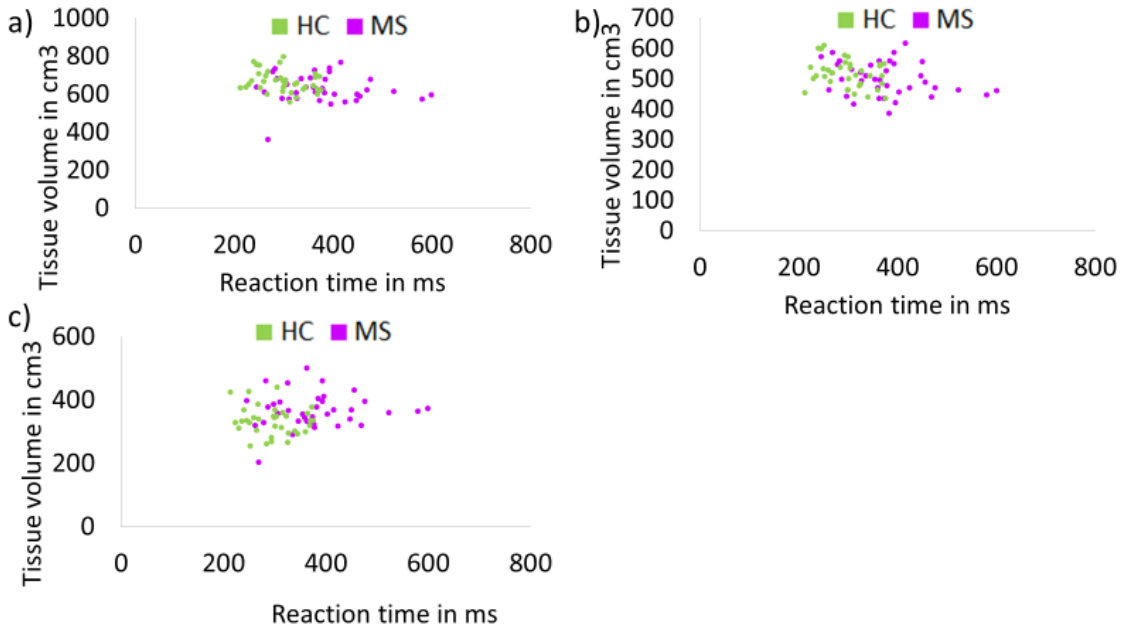


Figure 5.6 Scatterplots for correlations between extrinsic task reaction time and tissue volume*. a) grey matter and reaction time. b) white matter and reaction time. c) CSF and reaction time. Each circle represents a participant.*

5.3.2 Regional tissue volume

To examine the regional grey matter differences between the MS and HC groups, a two-sample t-test was conducted. The results, displayed in Table 5.1, revealed that the MS group had significant grey matter volume reductions in the bilateral thalamus, pallidum and right prefrontal cortex (pFC), superior occipital gyrus and left precentral gyrus and middle temporal gyrus. Whereas the HC group had no reduced grey matter volume compared to the MS group.

Table 5.1 Regions of reduced grey matter in the MS group compared to the HC group.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Thalamus	N/A	20	-30	4	6.36
Right	Pallidum	N/A	20	-3	-9	5.85
Right	Superior Occipital Gyrus	18	27	-82	36	5.28
Right	Prefrontal Cortex (pFC)	6	34	22	44	4.94
Left	Precentral Gyrus	6	-46	-5	32	6.41
Left	Thalamus	N/A	-20	-32	3	6.25
Left	Middle Temporal Gyrus	22	-56	-46	0	5.7
Left	Pallidum	N/A	-22	-6	-6	5.08

Note BA= Brodmanns Area

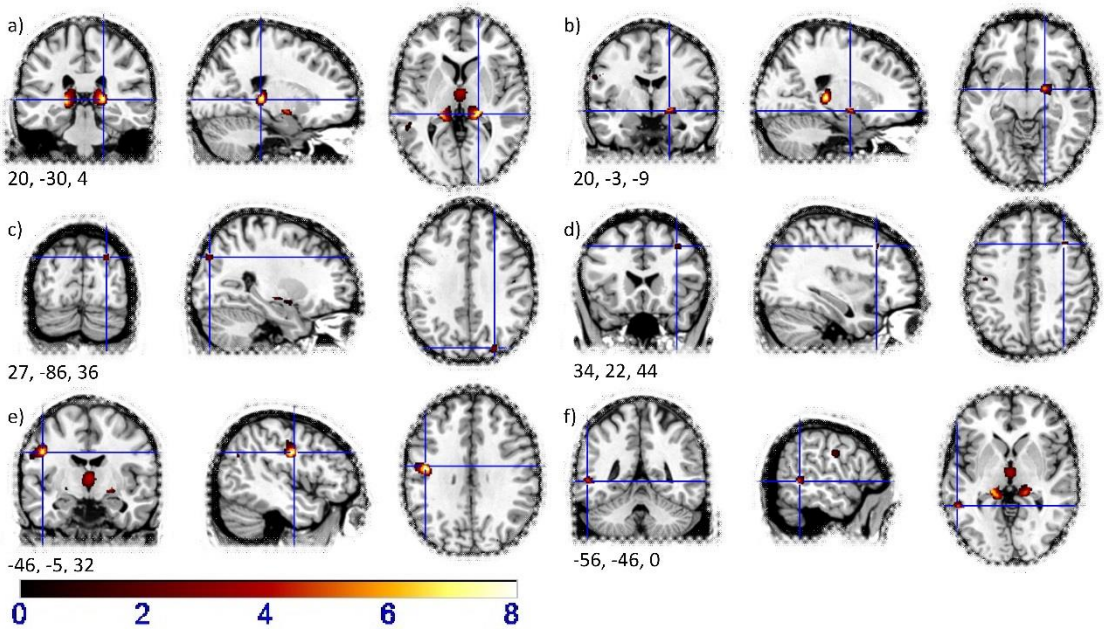


Figure 5.7 Reduced grey matter regions in the MS group compared to the HC group. *a) Thalamus, b) Pallidum, c) Superior Occipital Gyrus, d) Prefrontal Cortex (pFC), e) Precentral Gyrus, f) Middle Temporal Gyrus. MNI Coordinates (x,y,z). p<0.05 family wise error (FEW) corrected.*

To examine the regional white matter differences between the MS and HC groups, a two-sample t-test was conducted. The results, displayed in Table 5.2, showed that the MS group displayed significant white matter volume reductions in the right superior longitudinal fasciculus and left posterior corona radiata and anterior thalamic radiation. No regions of reduced white matter volume were observed in the HC group compared to the MS group.

Table 5.2 Regions of reduced white matter in the MS group compared to the HC group.

Hemisphere	Brain Region	MNI Coordinate (x,y,z)			T Value
Right	Superior Longitudinal Fasciculus	46	-36	-4	6.22
Right	Fornix	27	-26	-6	5.59
Right	Corpus Callosum	3	-10	21	5.57
Left	Superior Longitudinal Fasciculus	-26	-51	24	5.81
Left	Anterior Thalamic Radiation	-24	-42	27	5.68
Left	Superior Corona Radiata	-20	-10	36	5.17

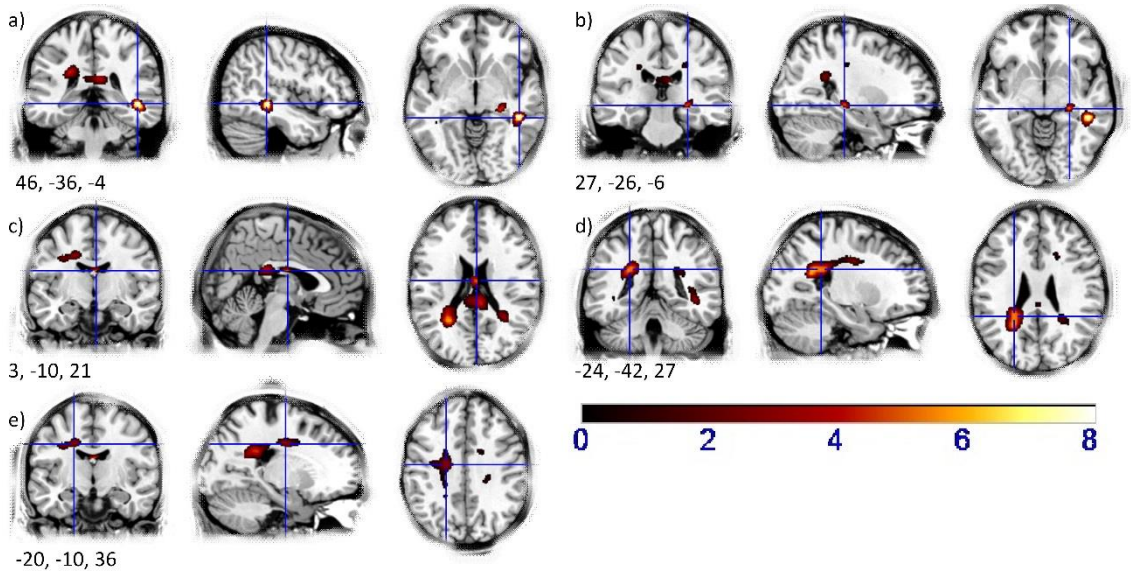


Figure 5.8 Reduced white matter regions in the MS group compared to the HC group. *a) Superior Longitudinal Fasciculus. b) Fornix. c) Corpus Callosum. d) Anterior Thalamic Radiation. e) Superior Corona Radiata. MNI Coordinates (x,y,z). p<0.05 FWE corrected.*

5.3.3 Multiple regression

To examine brain regions that correlate with fatigue, multiple regressions between grey matter, white matter and FSS scores and reaction time was conducted. There were no correlates of subjective fatigue that survived the FWE p< .05 threshold. Similarly, the multiple regressions between grey matter, white matter and reaction time, as a performance measure of fatigue, also revealed no correlates that survived the FWE p< .05 threshold.

5.4 Discussion

The primary aim of this chapter was to investigate whether fatigue is associated with brain atrophy in MS. Here fatigue is measured with a subjective self-report measure, and objective task performance measure. The current results indicate that tissue volume, both globally and regionally, in both HC and MS groups is unrelated to fatigue and fatigability scores. This suggests that structure alone does not directly impact functional outcomes. Interestingly, in the MS group CSF volume correlated positively with fatigue measures on both subjective and performance measures. CSF has been used in MS to examine the level of several different proteins that indicate neuronal loss or damage as well as a marker of neuroinflammation (Dujmovic, 2010; Matejčíková *et al.*, 2015). As such neurodegeneration may play a role in fatigue perception in MS. These results suggests that fatigue in MS is more complex than a simple relationship to brain atrophy. The current results strongly indicate that the structural atrophy of specific regions in the MS brain are not implicated

in fatigue. However, it is possible that the functional component of these atrophied regions relate to MS fatigue. This explanation will be explored in the following chapters.

The secondary aim of this chapter was to determine whether there are morphometrical differences in the brain between the MS and HC groups and whether these differences are related to fatigue. This was investigated by global tissue volume and regional volume differences in both white and grey matter. The results from the global volume analysis showed that the MS group had significantly increased CSF volume compared to the HC group. This result indicates higher levels of neurodegeneration in the MS group than in the HC group (Dujmovic, 2010; Matejcikova *et al*., 2015).

The grey matter analysis revealed significant widespread cortical and subcortical grey matter reductions in the MS group compared to the HC group. The widespread reductions are further evidenced by the global reduction in grey matter volume in the MS group compared to the HC group. The specific grey matter reductions in the MS group, revealed by the present analysis, are observed in the thalamus, basal ganglia, pFC, precentral gyrus, temporal gyrus and the occipital lobe. There are regions that overlap with the findings of previous studies. The thalamus (Ceccarelli *et al*., 2008; Derache *et al*., 2013; Hyncicova *et al*., 2017; Lansley *et al*., 2013; Riccitelli *et al*., 2011; van de Pavert *et al*., 2014) has multiple projections throughout the brain (Bisecco *et al*., 2018; Bolkan *et al*., 2017; Buschman and Miller, 2014; Kuramoto *et al*., 2009; McKenna and Vertes, 2004; Mogenson *et al*., 1987; Vertes *et al*., 2012). Through these widespread projections the thalamus acts as a relay centre for a multitude of functions including motor (Mattay *et al*., 2002; Sillito *et al*., 2006; Wurtz and Albano, 1980), sensory (Sillito *et al*., 2006; Wurtz and Albano, 1980), and higher order cognitive function such as memory (Vertes *et al*., 2012) and attention (Mottaghy *et al*., 2006; Perin *et al*., 2010; Sturm *et al*., 1999; 2004). Furthermore, the thalamus is involved in other functions such as the regulation of arousal in sleep and wakefulness (Coull *et al*., 2004; Schiff *et al*., 2008), where damage to the thalamus may result in a coma (Bernat, 2006). This makes it difficult to determine whether the thalamus is directly involved in the pathological process, or whether it is susceptible to pathology from the areas to which it is connected (Minagar *et al*., 2013). The basal ganglia (Bisecco *et al*., 2018; Calabrese *et al*., 2010; Derache *et al*., 2013; Finke *et al*., 2015; Lansley *et al*., 2013; Prinster *et al*., 2005; Riccitelli *et al*., 2012; Sepulcre *et al*., 2006; van de Pavert *et al*., 2014; Zhang *et al*., 2017) consists of multiple densely connected nuclei, including but not limited to the caudate, pallidum and putamen. It has important connections to the cerebral cortex and the thalamus by cortical-subcortical circuits, which have both motor and non-motor

functions. Given the multiple cortical loops associated with the basal ganglia, it can serve multiple functions including motor, emotions and behaviour. Furthermore, the basal ganglia network is associated with motivation. It has strong connections to the striatum and limbic system, through the thalamus (Haber and Calzavara, 2009), and projects to frontal cortices (Chaudhuri and Behan, 2000). The widespread connections indicate that the basal ganglia network incorporates information from the limbic system with the dopaminergic, striatal, and then projects to the frontal cortex for decision making in goal-directed behaviour. Chaudhuri and Behan (2004) indicate that both structural and metabolic lesions can interrupt the function of the connections between the basal ganglia, thalamus, limbic system and higher cortical areas, which may result in central fatigue. Studies have shown that damage to the basal ganglia is present in disorders characterised by increased fatigue such as Parkinson's disease (Friedman and Friedman, 1993; van Hilten *et al.*, 1993), post-polio fatigue (Bruno *et al.*, 1998) and MS (Roelcke *et al.*, 1997). Similarly to the thalamus, it is difficult to determine whether the basal ganglia is affected directly by the pathology of MS, or whether it is disrupted by pathology from the areas to which it is connected. However, the basal ganglia is implicated in fatigue (Chaudhuri and Behan, 2000; 2004) and may suggest that the atrophy in this region plays a significant role in the enhanced perception of fatigue in MS.

The pFC (Audoin *et al.*, 2006; Battaglini *et al.*, 2009; Bendfeldt *et al.*, 2012; Cerasa *et al.*, 2013; Derache *et al.*, 2013; Finke *et al.*, 2015; Gobbi *et al.*, 2013; Hyncicova *et al.*, 2017; Morgen *et al.*, 2005; Mesaros *et al.*, 2008; Nocentini *et al.*, 2014; Parisi *et al.*, 2014; Riccitelli *et al.*, 2011; 2012; Sepulcre *et al.*, 2009) is strongly associated with executive function. Studies using multiple modalities have shown that the pFC underlies executive function including lesion studies (Fuster, 1997; Goldman- Rakic, 1996; Stuss and Benson, 1986), structural studies (Morgen *et al.*, 2005; Yuan and Raz 2014), functional imaging studies (Engle *et al.*, 1999; Knight *et al.*, 1995; Rossi *et al.*, 2009) and meta-analyses (Buchsbaum *et al.*, 2005; Laird *et al.*, 2005). The reduced volume found in the current study may indicate that the MS group may experience disruption to executive functioning.

Further reduced volume in the MS group was noted in the precentral gyrus (Lansley *et al.*, 2013; MacKenzie-Graham *et al.*, 2013; Morgen *et al.*, 2005; Riccitelli *et al.*, 2011; 2012), which is involved in both the preparation and execution of movement (Pedersen *et al.*, 1998; Porro *et al.*, 2000; Toma *et al.*, 2002). Individuals with MS may suffer from significant motor disturbances. This is usually associated with increased disease duration and progression (Lansley *et al.*, 2013). The reduced volume in the precentral gyrus of the MS

group, compared to HC, may explain the motor disruptions associated with MS. The temporal cortex has shown atrophy in MS in multiple studies (Battaglini *et al.*, 2009; Bendfeldt *et al.*, 2012; Cerasa *et al.*, 2013; Derache *et al.*, 2013; Hyncicova *et al.*, 2017; Morgen *et al.*, 2005; Parisi *et al.*, 2014; Prinster *et al.*, 2005; 2010; Zhang *et al.*, 2017). The middle temporal gyrus has been associated with both verbal and non-verbal semantic processing (Chertkow *et al.*, 1997; Hoffman *et al.*, 2007; Visser *et al.*, 2012), multi-modal sensory integration (Binder *et al.*, 2009; Mesulam, 1998) and lexical retrieval (Baldo *et al.*, 2013; DeLeon *et al.*, 2007; Grossman *et al.*, 2004). The decreased volume of tissue in these temporal regions may, therefore, result in a multitude of functional disruptions. The occipital lobe (Bisecco *et al.*, 2018; Ceccarelli *et al.*, 2008; Gobbi *et al.*, 2013) contains the primary visual cortex, and as such is often associated with the processing of visual information. However, some studies have demonstrated that it can also be influenced by attention and can increase attentional resources towards a specific stimulus (Brefczynski and DeYoe, 1999; Daumann *et al.*, 2010; Somers *et al.*, 1999; Thiel and Fink, 2007). The decreased volume in MS may lead to disruptions in the attentional demand in the visual cortex. As evidenced by the current findings, individuals with MS show widespread grey matter abnormalities. This may provide some explanation for the functional disruptions associated with MS. However, morphometry is a simple measure of volume, and it is not possible to definitively state that reduced volume may lead to functional disruptions. Furthermore, due to the widespread nature of the atrophy in MS, it is extremely difficult to determine the specific pathology underlying MS.

The HC group showed no global or regional reduced grey matter compared to the MS group. This result is expected as the MS group shows neurodegeneration. However, given that grey matter lesions can be inflammatory, specifically through meningeal inflammation (Bruck, 2017; Calabrese *et al.*, 2015; Haider *et al.*, 2014; Junker and Bruck, 2012; Magliozzi *et al.* 2007; Serafini *et al.* 2004), some regions showing inflammation may be detected as having decreased volume in the HC group. This is not the case in the current sample. It is possible that this result is observed because the majority of the sample had relapsing-remitting MS, characterised by acute flares, associated with inflammation, and then periods of remission. It is more than likely that in the current sample individuals are in a period of remission as participation in research studies during relapse would be highly taxing. This implication is discussed in further Chapter 9 (section 9.6 page 187).

There were no global white matter differences between the HC and MS groups. Given that MS is considered as a demyelinating disease, this finding is interesting as it would be

expected that MS individuals would show decreased white matter volume. However, Trapp *et al.* (2018) identified a subtype of MS where no white matter demyelination is present, thereby suggesting that the white matter atrophy may be overemphasised. Moreover, MS white matter lesions can also result in inflammation (Bö *et al.*, 2006; Prins *et al.*, 2015) and undergo a process of remyelination, known as shadow plaques. Studies have shown that in approximately 20% of MS participants almost all lesions were shadow plaques (Patrikios *et al.*, 2006). On an individual level, Barkhof *et al.* (2003) demonstrated that about 40% of lesions showed remyelination. The process of acute inflammation and neurodegeneration followed by remyelination may provide an explanation for the relapsing-remitting phenotype of MS, which made up the majority of the current sample. Furthermore, there is a large amount of heterogeneity in the remyelination process. Given that participants are likely in a remission phase upon taking part, it is possible to suggest that the lack of global white matter differences are a result of this process.

Despite there being no global differences between the MS and HC group for white matter volume, there are some regional differences between the groups. The MS group had reduced white matter in the superior longitudinal fasciculus, fornix, corpus callosum, posterior corona radiata and anterior thalamic radiation. This shows some consistency with previous findings of reduced volume in the superior longitudinal fasciculus (Parisi *et al.*, 2014; Zhang *et al.*, 2017), corona radiata (Bendfeldt *et al.*, 2010; Zhang *et al.*, 2017) and thalamic radiations (Sbardella *et al.*, 2013), although this study showed reduction in anterior, not posterior thalamic radiations. The superior longitudinal fasciculus is the main association fibre tracts that connect the parietal and frontal lobes (Wang *et al.*, 2016). The reduced volume in the MS group indicates that there may be a disruption with the projections from sensory input regions to the frontal lobe, thereby representing a connectivity deficit in MS. This is further evidenced by the reduced volume of the corona radiata and thalamic radiation as similarly, these are both involved with projections to frontal regions. The corona radiata specifically has projections to the motor cortex (Cho *et al.*, 2007), suggesting that it is involved in motor function by projecting motor inputs to the motor cortex. The anterior thalamic radiation has projections to the frontal cortex, cerebellar and brainstem regions (Wakana *et al.*, 2004), indicating that it may project information to the frontal cortex. Similarly, the corpus callosum is the main white matter fibre bundle connecting the left and right hemisphere of the brain. As such atrophy to this part of the brain may suggest a connectivity deficit between the hemispheres. The fornix, on the other hand, provides a more localised connection. It connects the limbic system,

originating in the hypothalamus, to the thalamus and cingulum (Wakana *et al.*, 2004). This indicates a potential connectivity disruption of the limbic system in individuals with MS. Together these findings may demonstrate some form of connectivity deficit is MS in long range inter and intra-hemispheric connections, as well as more local limbic connectivity. However, the current study only measures volumetric differences and did not conduct tractography analyses. Studies using diffusion tensor imaging of normal appearing white matter in MS have shown widespread abnormalities including both increased mean diffusivity and decreased fractional anisotropy (Bammer *et al.*, 2000; Cercignani *et al.*, 2000; Ciccarelli *et al.*, 2001; Droogan *et al.*, 1999; Filippi, 2001; Filippi *et al.*, 2000; Guo *et al.*, 2001; Rocca *et al.*, 2000). These results do support the possibility of a connectivity deficit in MS. These results imply that combining studies examining the white matter tracts with diffusion tensor imaging and morphometry in MS may help to further elucidate the pathology of MS.

When examining the grey and white matter atrophy together, there is a clear disruption to the motor regions of the brain in MS, as this group showed both decreased grey matter in the precentral gyrus and decreased white matter in the corona radiata that project from the brainstem to the motor cortex. Furthermore, the results strongly indicate some disruption to the associative and limbic cortical-subcortical loops (see section 1.5.5 page 29). The associative loop functionally connects the basal ganglia, thalamus and pFC, whereas the limbic loop functionally connects the basal ganglia, thalamus, limbic system and anterior cingulate cortex (ACC). The results of the VBM analysis showed grey matter atrophy in the MS group in the thalamus, basal ganglia and pFC, as well as white matter atrophy in connections between these such as the fornix and the thalamic radiations. This provides evidence for a cortical-subcortical disruption in MS.

In neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, there are localised pathological processes that result in localised structural changes related to disease progression. In Alzheimer's disease the hippocampus is most affected (Huang and Mucke, 2012; Putcha *et al.*, 2011) and in Parkinson's disease the basal ganglia (Blandini *et al.*, 2000; Hughes *et al.*, 1992). In MS there is much less consensus and it remains difficult to determine whether the atrophy noted in the current study are as a result of pathological processes in the same way. Due to the widespread nature of the plaques in MS, a combination of techniques are required to elucidate the pathology and how this impacts behavioural outcomes such as fatigue and cognition.

5.4.1 Conclusion

In grey matter, the MS group demonstrate widespread regional volume reductions compared to the HC group. The grey matter in the MS group was also significantly reduced globally compared to the HC group. These reductions are in regions associated with motor, sensory, relay and higher cognitive functions, suggesting that the atrophy may provide an explanation for the widespread functional disruptions in MS. However, morphometry is not able to explicitly state functional associations, it provides a structural measure of tissue volume. In the white matter, there are no global volume differences between the HC and MS groups. There were some regional differences. The MS group showed reduced white matter in long range inter and intra-hemispheric connections, as well as more local limbic connections. Taking both grey and white matter atrophy into account provides evidence for a cortical-subcortical disruption in MS, specifically to the limbic and associative loops. MS was traditionally seen as a white matter disease, but more recent evidence suggests that grey matter atrophy is also pervasive in MS. No specific regions showed correlations with fatigue scores, measured both subjectively and through performance on an alertness-motor paradigm. This may suggest that neuroinflammation, evidenced by high CSF in MS, may be associated with MS fatigue, but the structural atrophy of specific regions in the MS brain are not implicated in fatigue. However, it is possible that the functional component of these atrophied regions, relate to MS fatigue. This explanation will be explored in the following chapters.

Chapter 6 Towards an objective measure of fatigue: the effect of prolonged performance on an alertness-motor task on behavioural performance in multiple sclerosis.

6.1 Introduction

The literature regarding the impact of fatigue on cognition in multiple sclerosis (MS) is very inconsistent (Bailey *et al.*, 2007; Bryant *et al.*, 2004; Claros-Salinas *et al.*, 2010; Jennekens-Schinkel *et al.*, 1988; Johnson *et al.*, 1997; Krupp and Elkins, 2000; Kujala *et al.*, 1995; Paul *et al.*, 1998; Schwid *et al.*, 2003). The inconsistency is most apparent when subjective measures of fatigue are used but are better established when measuring fatigue more objectively. In Chapter 4 (page 71) the results demonstrated a learning effect on a set of attentional tests in the healthy control (HC) group following performance of an alertness-motor paradigm. Whereas the MS group did not show a consistent difference between baseline and subsequent testing periods. The results did not elucidate whether the alertness-motor paradigm was unable to induce fatiguability, or whether the groups were able to overcome the fatiguing effect. Where the HC group were more effective at overcoming induced fatiguability than the MS group.

The alertness mechanism is uniquely suited to being able to provide an insight into the effect of fatiguability or fatigue. DeLuca (2005) noted that tasks of alertness may be most sensitive to the effect of fatigue and fatiguability. Several studies report that mental fatigue is associated with prolonged task performance, even in healthy individuals (Boksem *et al.*, 2005; Faber *et al.*, 2012; Oken *et al.*, 2006). Furthermore, in MS, Hanken *et al.* (2015) demonstrated that memory performance, selective attention, language comprehension and visuospatial processing remain largely unaffected by fatigue, whereas fatigue significantly impacts tasks of alertness or vigilance. This suggests that the alertness mechanism may be a useful tool for MS fatigue measurement.

There are multiple tasks available for measuring alertness. Studies have often employed psychomotor tests to assess fatiguability and performance (Belenky *et al.*, 2003; Dinges *et al.*, 1997; Philip *et al.*, 2003; Van Dongen *et al.*, 2003). Despite being a reaction time task, often accuracy is the main outcome measure. Studies have also measured fatiguability as reaction time on cognitive tasks across time (Boksem *et al.*, 2005; Helton and Russell, 2011). Following the systematic synthesis of thirteen available studies examining neural substrates of alertness (refer to Chapter 3 page 54), a simple reaction time task was deemed

to be the most appropriate task for the current thesis. As such an adaptation of the alertness paradigm designed by Perin *et al.* (2010) was used.

The primary aim of the current chapter was to use an alertness-motor paradigm to establish how fatiguability affects cognition, in both HC and in an MS population suffering from persistent fatigue. The secondary aims were to establish behavioural differences between the MS and HC groups, examine whether in the MS group phenotype or disease duration affected behavioural performance and to evaluate whether this alertness-motor paradigm could provide a good performance measure of fatigue. It is expected that the MS group would have increased reaction times compared to the HC group, due to the increased persistent fatigue. If the alertness-motor paradigm induces fatiguability, there would be increased reaction times towards the end of the task. Furthermore, due to the increased task demand, it is expected that fatiguability would be higher in the groups that completed the mental imagery condition first.

6.2 Methods

6.2.1 Participants

A total of 40 MS participants and 40 healthy individuals were recruited according to the procedure described in the general methods section. 3 MS and 4 HC outliers were removed (refer to section 2.6 page 52).

6.2.2 Neuropsychological questionnaires

All participants, in both the MS and HC groups, were screened for fatigue, using the Fatigue Severity Scale (FSS) (Krupp *et al*., 1989), to ensure that the MS did have significant fatigue and the HC did not. Furthermore, the scores from the FSS was used as a measure of fatigue and used in the analysis. The Borg scale of perceived effort (Borg, 1982) (see appendix J page 268) was used to assess and compare the perception of fatigue following each condition of the paradigm between MS and HC groups. The Borg scale requires participants to rate their perceived effort between 6 and 20, with a higher value representing higher effort. For example, a value of 9 is very light, a value of 13 is somewhat hard and a value of 17 is very hard. As fatigue is the main interest of the thesis, only the FSS score was used in the correlation analysis. The Borg scale of perceived effort was added after the start of data collection and as such was collected in a subset of 35 HC and 25 MS participants.

6.2.3 Biopac recordings

The participants were asked to squeeze the hand dynamometer at either low, medium or high force during the scan. Before the scan, participants were told that there was no threshold at which to squeeze, but that the force exerted was what they perceived as a low, medium or high squeeze. The force exerted was detected and transduced by the hand dynamometer. This apparatus was attached to a radiofrequency filter, which enabled the data to be transferred from the scan room to the DA100C transducer amplifier. The transducer amplifier was connected to an MP150 device. This allowed the force exerted by the participant to be transformed into an optical signal and visualised on the software, AcqKnowledge, on a PC in the MRI control room. Force data was sampled at a frequency of 50Hz. The electromyography (EMG) data was recorded in the same way. Participants had 5 electrodes in total placed on their left arm. Two were placed at the bottom of the arm measuring activity in the flexor, two electrodes were placed on the top of the arm measuring activity in the extensor and one electrode was placed just above the elbow as a reference electrode. EMG data was not analysed, because a measure of actual force was available. However, it was used to ensure that the participants were imagining during the mental imagery condition. Both scanner triggers and triggers created by the paradigm, which were displayed via Eprime, were also recorded on this software. The triggers created by the paradigm were related to when the white square appeared on the screen. This allowed for the analysis of participant reaction times, the difference between the onset of force grip squeeze and the onset of the white square. These recordings are taken in both the mental imagery and the motor task, but reaction times and force are only computed for the physical run where an actual force grip was produced.

6.2.4 Alertness-motor paradigm

The paradigm consisted of interleaved periods of three different tasks, sensorimotor control, intrinsic alertness and extrinsic alertness, requiring the participant to exert a certain force (low, medium, high). Each task was repeated four times, resulting in four blocks containing each of the three tasks at each of the three force levels. The order of tasks was pseudorandomised (Figure 5.1, page 90). Furthermore, there were two conditions to the paradigm, physical handgrip, and mental imagery, every participant completed both conditions. The group of participants that completed the handgrip condition before the mental imagery condition is referred to as the handgrip first group throughout the thesis. The group of participants that completed the mental imagery

condition before the handgrip condition is referred to as the imagery first group throughout the thesis. In the physical condition, participants performed the full paradigm whilst squeezing a hand dynamometer at the required force. In the mental imagery, task participants performed the full paradigm but were asked to only imagine squeezing the hand dynamometer at the required force. Following each condition, participants were asked to rate their mental effort on the Borg perceived effort scale (see appendix J page 268). This scale rated the perceived effort between 6 and 20. This was conducted on a subset of the participants.

Block 1	T3			T1			T2		
	Low	Medium	High	High	Medium	Low	Low	High	Medium
Block 2	T2			T1			T3		
	High	Low	Medium	High	Medium	Low	High	Medium	Low
Block 3	T3			T1			T2		
	Medium	High	Low	Low	Medium	High	Medium	Low	High
Block 4	T2			T1			T3		
	Low	Medium	High	Low	Medium	High	Low	High	Medium

Figure 6.1 Task design. *Note: T1= sensorimotor task. T2= intrinsic alertness task. T3= extrinsic alertness task.*

6.2.5 Physical handgrip condition

The sensorimotor control task was a self-paced task and as such, no reaction time or force data was extracted from this task. Furthermore, it was a control task and as such is not analysed in this chapter. During the physical handgrip condition for each of the tasks, participants were asked to squeeze the hand dynamometer quickly, for approximately 1 second, and then release. They were instructed to squeeze at the force indicated by the task: low force; medium force or high force. A schematic of the complete paradigm is represented in Figure 5.2 (page 92).

During the intrinsic alertness task (T2), participants would view a white fixation cross at the centre of the screen for varying durations (1750, 1780 or 1810 ms). When the white square, target stimulus, appeared, participants were instructed to squeeze the handgrip at the required force. In the extrinsic alertness task (T3) the instructions and level of required force were the first stimuli to be displayed. The white fixation cross was displayed for a fixed duration (1500 ms). The white cross was followed by the presentation of a black screen, warning stimulus, which was displayed for varying durations (250, 280 or 310 ms). After the warning cue the target stimulus, requiring participants to squeeze the handgrip, was displayed.

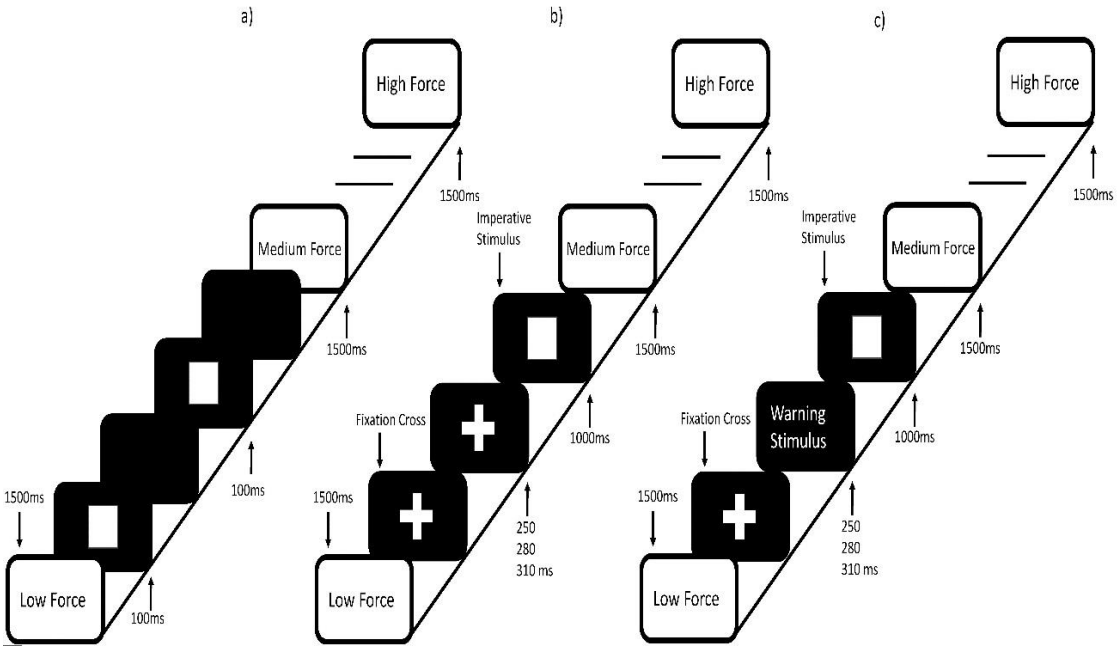


Figure 6.2 Schematic of alertness-motor paradigm. *a) sensorimotor control. b) intrinsic alertness. c) extrinsic alertness.*

6.2.6 Mental imagery condition

For the mental imagery condition, the experimental paradigm was identical to the physical handgrip condition and the tasks were presented in the same pseudorandomised order. The only difference between the conditions was that, in the mental imagery condition, the participants were required to only imagine squeezing the hand dynamometer and not to execute the physical action. Force data was not recorded, because there was no motor output. EMG data was still recorded for the mental imagery condition of the experiment. The mental imagery data was not analysed but simply used as a further manipulation to induce fatiguability.

6.2.7 Behavioural analysis

The force data was extracted from the Biopac recordings and analysed between the HC and MS groups using independent sample t-test, for each of the three force levels (low, medium, high). A five-way 2 x 2 x 3 x 2 x 2 mixed model analysis of variance (ANOVA), with block (1, 4), task type (T2, T3) and force grip (low, medium, high) as within group measures and task order (imagery first or handgrip first) and group (MS or HC) as between group measure, was conducted. This was to explore whether task order or block (time) affected the force grip. For the reaction time analysis, any outliers above or below 2 standard deviations of the mean were excluded. A four-way 2 x 2 x 2 x 2 mixed model ANOVA, with block (1, 4) and task type (T2, T3) as within group measures and task order (imagery first or handgrip first) and group (MS or HC) as between group measures, was

conducted. This was to explore whether task order or block (time) affected the reaction time. For mixed model ANOVA, where the sphericity assumption, as assessed by Mauchly's test of sphericity (Mauchly, 1940), was violated, the Greenhouse-Geisser correction was used (Greenhouse and Geisser, 1959). The sensorimotor task was not analysed. *Post-hoc* Tukey HSD were conducted to further explore the means that differ significantly. For all independent sample t-tests, Levene's test for equality of variances (Levene, 1960) was used to assess violations of homogeneity of variance. If this assumption was violated the associated values were used. When comparing between the HC and MS groups, the participants were not split according to condition order, as task order did not affect global performance (see appendix K page 269). When examining within group effects, such as the effect of fatigue across time, the analysis was split by condition order.

To determine if disease duration affected reaction time and force performance, two partial correlation, controlling for age, using Pearson's correlation coefficient, was conducted between reaction time and disease duration, and between force and disease duration. Furthermore, to examine whether MS phenotype affected reaction time or force, a one-way ANOVA between each behavioural measure and MS phenotype was conducted separately. The MS groups were split into handgrip first and imagery first groups, to determine if performance was impacted by condition order. FSS scores were correlated with reaction time and force measures, using partial correlations, controlling for age, with Pearson's correlation coefficient. For all correlations Spearman's rank correlations (Appendix L) were also conducted, due to outliers, however this did not alter the results. As such Pearson's correlation coefficients are reported.

For the Borg ratings of perceived effort, independent t-tests were used to compare between the HC and MS group. Paired t-tests were conducted to examine ratings of perceived effort between each condition. To explore whether the order in which participants completed the conditions affected perceived effort paired t-tests were conducted separately for the handgrip first and imagery first groups. All analyses were conducted using the software SPSS 24 and the significance threshold was set at $p<.05$.

6.3 Results

6.3.1 Reaction time performance

A mixed model four-way 2 x 2 x 2 x 2 ANOVA showed a significant main effect of task ($F(1,69)= 6.26$, $p= .02$, $\eta p^2= .08$); block ($F(1,69)= 15.09$, $p< .01$, $\eta p^2= .18$); task order ($F(1,69)= 5.09$, $p= .03$, $\eta p^2= .07$); and group ($F(1,69)= 34.85$, $p< .01$, $\eta p^2= .34$). There were significant interaction effects between block and group ($F(1,69)= 3.8$, $p= .05$, $\eta p^2= .05$); block, task order and group ($F(1,69)= 3.80$, $p= .05$, $\eta p^2= .05$); and task, block and task order ($F(1,69)= 4.92$, $p= .03$, $\eta p^2= .07$) and no significant interaction effects between task and task order ($F(1,69)= .02$, $p= .88$, $\eta p^2= .01$); task and group ($F(1,69)= .35$, $p= .55$, $\eta p^2= .01$); task, task order and group ($F(1,69)= .32$, $p= .58$, $\eta p^2= .01$); block and task order ($F(1,69)= .11$, $p= .74$, $\eta p^2= .01$); task and block ($F(1,69)= .15$, $p= .71$, $\eta p^2= .01$); task, block and group ($F(1,69)= 2.08$, $p= .15$, $\eta p^2= .03$); and task, block, task order and group ($F(1,69)= .09$, $p= .76$, $\eta p^2= .01$).

To explore the main and interaction effects *post-hoc* Tukey HSD was conducted. For the significant main effect of task the *post-hoc* analysis showed that reaction time was reduced in the extrinsic alertness task compared to the intrinsic alertness task ($M_{T_2}= 349.26$ $M_{T_3}= 337.47$, $p< .05$). However, the interaction between task, task order and group revealed that this was only true for the HC groups ($M_{T_2H}= 318.75$, $M_{T_3H}= 300.15$, $p< .05$, $M_{T_2I}= 296.16$, $M_{T_3I}= 281.92$, $p< .05$), but not for the MS groups ($M_{T_2H}= 410.34$, $M_{T_3H}= 405.02$, $p> .05$, $M_{T_2I}= 365.19$, $M_{T_3I}= 358.29$, $p> .05$). For the main effect of block there was increased reaction time in block 4 compared to block one of the task ($M_1= 331.13$, $M_4= 355.17$, $p< .05$). In terms of group the MS group had significantly increased reaction time compared to the HC group ($M_{MS}= 376.30$, $M_{HC}= 299.81$, $p< .01$). For task order the handgrip first group had increased reaction time compared to the imagery first group ($M_H= 358.31$, $M_I= 326.69$, $p< .05$). The significant interaction effect between block and group revealed that the MS group demonstrated increased fatiguability compared to the HC group ($M_{MS_1}= 367.70$ $M_{MS_4}= 402.93$; $M_{HC_1}= 293.54$ $M_{HC_4}= 306.08$, $p< .05$). The interaction between block, task order and group showed that the HC handgrip first group had increased fatiguability ($M_1= 299.02$, $M_4= 338.48$, $p< .01$) whereas this was not evident in the HC imagery first group ($M_1= 282.22$, $M_4= 284.01$, $p> .05$). For the MS group both the handgrip first ($M_1= 394.34$, $M_4= 420.02$, $p> .05$) and imagery first ($M_1= 339.59$, $M_4= 384.89$, $p> .01$) had increased fatiguability, but for the handgrip first group this was during intrinsic alertness ($M_{T_{2I}}= 395.88$, $M_{T_{24}}= 424.81$, $p> .05$; $M_{T_{3I}}= 392.81$, $M_{T_{34}}= 415.23$, $p> .05$) and for the imagery first group it was during extrinsic alertness ($M_{T_{2I}}= 356.62$, $M_{T_{24}}= 381.75$, $p> .05$; $M_{T_{3I}}= 322.55$, $M_{T_{34}}= 388.03$, $p< .05$).

In summary, the results indicated that the HC group respond to the warning effect and the MS group do not, however there is no significant difference between the two groups. This is likely due to the variability in the MS group. There was a significant overall increase in reaction time over the paradigm. Specifically, the handgrip first groups in both the MS and HC groups showed increased fatiguability compared to the imagery first groups. The MS handgrip first group showed increased fatiguability compared to the MS imagery first group during intrinsic alertness, whereas the MS imagery first group demonstrated increased fatiguability compared to the MS handgrip first group during extrinsic alertness. Moreover, the MS group showed increased fatiguability compared to the HC group. The reaction times are displayed in Figure 6.3.

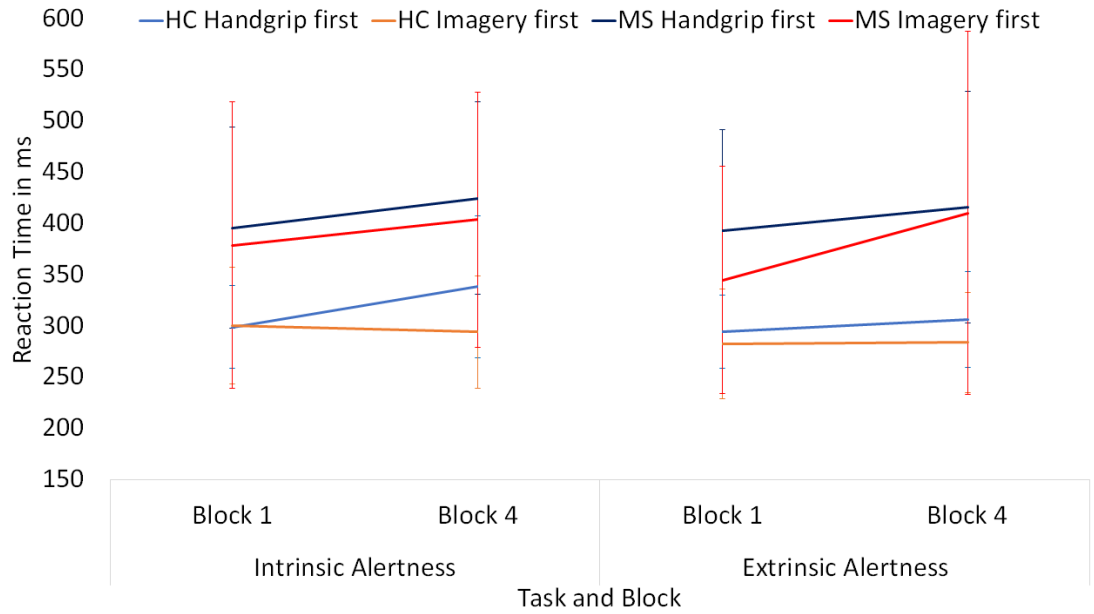


Figure 6.3 Line graph of reaction time in ms. *The HC handgrip first group are displayed in blue, the HC imagery first group are displayed in orange, the MS handgrip first group are displayed in navy and the MS imagery first group are displayed in red. The error bars represent one standard deviation.*

6.3.2 Impact of phenotype and disease during on reaction time in the MS group

The one-way ANOVA revealed no significant main effect for intrinsic alertness ($F(2,37)= .49$, $p= .61$, $\eta^2= .03$) or extrinsic alertness $F(2,37)= .49$, $p= .61$, $\eta^2= .02$) reaction time. The partial correlations demonstrated similar results. There were no significant correlations between disease duration and intrinsic alertness ($R= .07$, $p= .68$) or extrinsic alertness ($R= 0.02$, $p= .89$) reaction time.

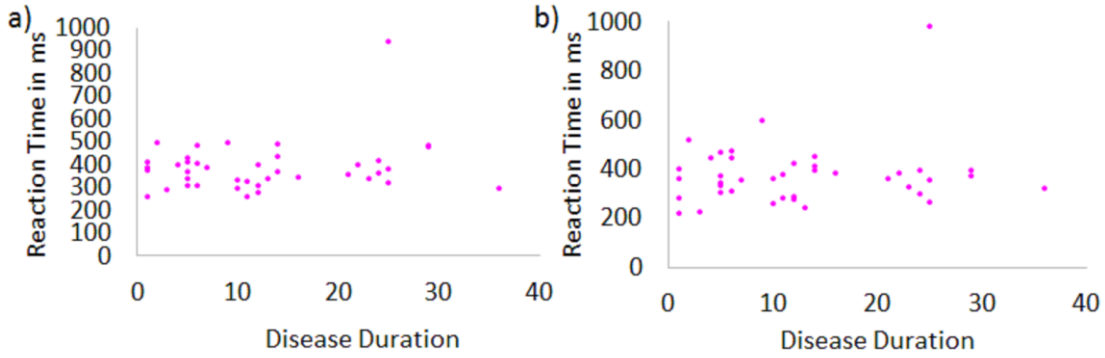


Figure 6.4 Composite image of scatterplot between disease duration and reaction time on the paradigm. *a) intrinsic alertness and disease duration. b) extrinsic alertness and disease duration.*

This result was replicated when the MS group was split into groups based on which condition of the paradigm they completed first. The MS handgrip first group showed no main effect of phenotype, or correlation with disease duration on reaction time for the intrinsic alertness task ($F(2,37)= .39$, $p= .69$, $\eta^2= .05$; $R= .19$, $p= .46$) or extrinsic alertness task ($F(2,37)= 2.49$, $p= .11$, $\eta^2= .29$; $R= -.12$, $p= .63$). For the MS imagery first group there was no main effect of phenotype on reaction time for intrinsic ($F(2,37)= 1.89$, $p= .19$, $\eta^2= .11$) or extrinsic ($F(2,37)= 2.83$, $p= .11$, $\eta^2= .16$) alertness task. There were also no correlations between disease duration and reaction time for intrinsic alertness ($R= .32$, $p= .22$) and extrinsic alertness ($R= .35$, $p= .17$).

6.3.3 FSS correlates with reaction time

There were significant positive partial correlations in the HC group between FSS scores and reaction time for both intrinsic alertness ($R= .43$, $p= .01$), and extrinsic alertness ($R= .49$, $p< .01$). In the MS group there were no significant partial correlations between FSS scores and intrinsic alertness reaction time ($R= .20$, $p= .25$) or extrinsic alertness reaction time ($R= .07$, $p= .67$). Scatterplots are shown in Figure 6.5.

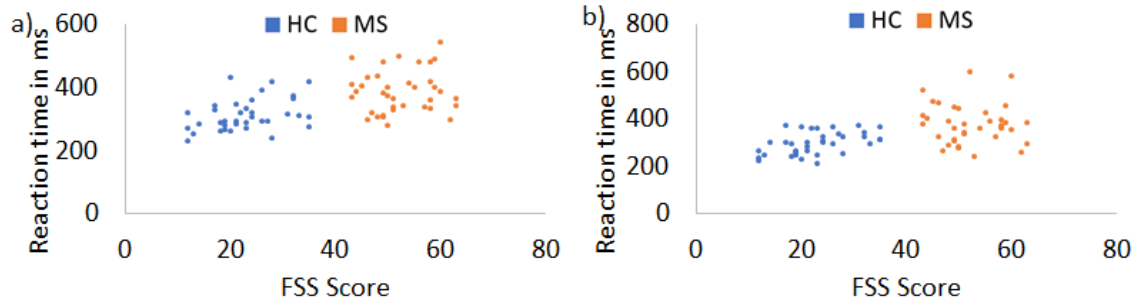


Figure 6.5 Scatterplots for reaction time and fatigue scores. *a) Intrinsic alertness, b) extrinsic alertness values. HC are displayed in blue, MS are displayed in orange. Each circle represents a participant.*

6.3.4 Force grip performance

A mixed model five-way 2 x 2 x 2 x 2 x 3 ANOVA showed a significant main effect of block ($F(1,69)= 13.65$, $p< .01$, $\eta p^2= .17$); force ($F(2,38.92)= 278.51$, $p< .01$, $\eta p^2= .80$); task order ($F(1,69)= 4.40$, $p= .04$, $\eta p^2= .06$); and group ($F(1,69)= 10.07$, $p< .01$, $\eta p^2= .13$) and no significant main effect of task ($F(1,69)= 1.23$, $p= .27$, $\eta p^2= .02$). There were significant interaction effects between task and task order ($F(1,69)= 12.49$, $p< .01$, $\eta p^2= .15$); block and task order ($F(1,69)= 9.93$, $p< .01$, $\eta p^2= .13$); block and group ($F(1,69)= 4.39$, $p= .04$, $\eta p^2= .06$); force and group ($F(2, 38.92)= 13.27$, $p< .01$, $\eta p^2= .16$); task and block ($F(1,69)= 6.34$, $p= .01$, $\eta p^2= .08$); task, block and task order ($F(1,69)= 11.31$, $p< .01$, $\eta p^2= .14$); task, force and task order ($F(2,58.12)= 3.38$, $p= .04$, $\eta p^2= .05$); and block, force and task order ($F(2,41.75)= 3.74$, $p= .03$, $\eta p^2= .05$).There were no significant interaction effects between task and group ($F(1,69)= 1.13$, $p= .29$, $\eta p^2= .02$); task and force ($F(2,38.92)= 3.94$, $p= .20$, $\eta p^2= .02$); block, task order and group ($F(1,69)= .34$, $p= .56$, $\eta p^2= .01$); force and task order ($F(2,68)= .06$, $p= .94$, $\eta p^2= .01$); force, task order and group ($F(2,38.92)= 2.06$, $p= .13$, $\eta p^2= .03$); task, block and group ($F(1,69)= .54$, $p= .46$, $\eta p^2= .01$); task, block, task order and group ($F(1,69)= .18$, $p= .67$, $\eta p^2= .01$); task, force and group ($F(2,68)= .28$, $p= .76$, $\eta p^2= .01$); task, force, task order and group ($F(2,58.12)= .49$, $p= .62$, $\eta p^2= .01$); block and force ($F(2,38.92)= 1.74$, $p= .18$, $\eta p^2= .03$); block, force and group ($F(2,38.92)= .09$, $p= .92$, $\eta p^2= .01$); block, force, task order and group ($F(2,58.12)= 2.37$, $p= .10$, $\eta p^2= .03$); task, block and force ($F(2,38.92)= .46$, $p= .63$, $\eta p^2= .01$); task, block, force and task order ($F(2,58.12)= .22$, $p= .79$, $\eta p^2= .01$); task, block, force and group ($F(2,38.92)= .86$, $p= .43$, $\eta p^2= .01$); and task, block, force, task order and group ($F(2,58.12)= 1.20$, $p= .31$, $\eta p^2= .02$).

The main effect of force was expected, as force grip should increase between low, medium and high forces. *Post-hoc* Tukey HSD between each of the levels of force confirmed that this was indeed the case ($M_L= 3.43$, $M_M= 5.55$, $p< .01$, $M_M= 5.55$, $M_H= 9.58$, $p< .01$). Although the MS group did increase force between the levels, the HC group increased the force grip significantly more between each level ($M_{MSL}= 3.13$, $M_{MSM}= 4.63$, $p< .05$, $M_{MSM}= 4.63$, $M_{MSH}= 7.95$, $p< .05$, $M_{HCL}= 3.74$, $M_{HCM}= 6.50$, $p< .01$, $M_{HCM}= 6.50$, $M_{HCH}= 11.25$, $p< .01$). The significant main effect of block revealed that force was decreased in block 4 compared to block 1 ($M_1= 6.58$, $M_4= 5.97$, $p< .05$). In terms of group the MS group exerted significantly decreased force compared to the HC group ($M_{HC}= 7.17$, $M_{MS}= 5.24$, $p< .01$). The interaction between task and task order showed that the handgrip first groups had increased force grip during the extrinsic alertness task than during the intrinsic alertness task ($M_{T_2}= 6.53$, $M_{T_3}= 7.10$, $p< .05$), whereas the imagery

first groups did not exert different forces between the two tasks (M_{T_2}= 5.65 , M_{T_3}= 5.36, p< .05). The MS group exhibited increased fatiguability compared to the MS group (M_{MS_1}= 5.84, M_{MS_4}= 4.64, p< .01, M_{HC_1}= 7.34, M_{HC_4}= 6.99, p> .05). The interaction between task and block revealed that forced decreased between block 1 and block 4 to a greater extent during the extrinsic alertness task compared to the intrinsic alertness task ($M_{T_{21}}$= 6.37, $M_{T_{24}}$= 5.86, p> .05, $M_{T_{31}}$= 6.79, $M_{T_{34}}$= 5.73, p> .05). The handgrip first groups demonstrated increased fatiguability compared to the imagery first groups (M_{H_1}= 7.52, M_{H_4}= 6.12, p< .01, M_{I_1}= 5.56, M_{I_4}= 5.44, p> .05). This fatiguability is more noticeable during the extrinsic alertness task than the intrinsic alertness task ($M_{T_2H_1}$= 6.94, $M_{T_2H_4}$= 6.14, p< .05, $M_{T_3H_1}$= 8.10, $M_{T_3H_4}$= 6.10, p< .01). Where the handgrip first groups showed a stable decreased force during all grip strengths (M_{L_1}= 4.70, M_{L_4}= 3.34, p< .05, M_{M_1}= 7.01, M_{M_4}= 5.46, p< .05, M_{H_1}= 10.86, M_{H_4}= 9.56, p< .05) the imagery first groups show only decreased force in the high grip strength (M_{L_1}= 2.72, M_{L_4}= 2.87, p> .05, M_{M_1}= 4.72, M_{M_4}= 4.91, p> .05, M_{H_1}= 9.24, M_{H_4}= 8.55, p< .05). However, the handgrip first groups exerted greater force than the imagery first groups (M_H= 6.82, M_I= 5.38, p< .05). Again this difference is more noticeable during the extrinsic alertness task compared to the intrinsic alertness task (M_{HT_2L}= 3.68, M_{IT_2L}= 2.75, p< .05, M_{HT_3L}= 4.36, M_{IT_3L}= 2.84, p< .01, M_{HT_2M}= 5.93, M_{IT_2M}= 5.17, p> .05, M_{HT_3M}= 6.54, M_{IT_3L}= 4.45, p< .01, M_{HT_2H}= 10.02, M_{IT_2H}= 9.02, p< .05, M_{HT_3H}= 10.40, M_{IT_3H}= 8.77, p< .01).

In summary, the results demonstrated that both the HC and MS successfully exerted different forces between the low, medium and high grip strengths. The MS group exhibited increased fatiguability compared to the HC group. Similarly the handgrip first groups had increased fatiguability compared to the imagery first groups, however the handgrip first group did consistently exert greater force than the imagery first group which may confound this result. The force grips are displayed in Figure 6.6.

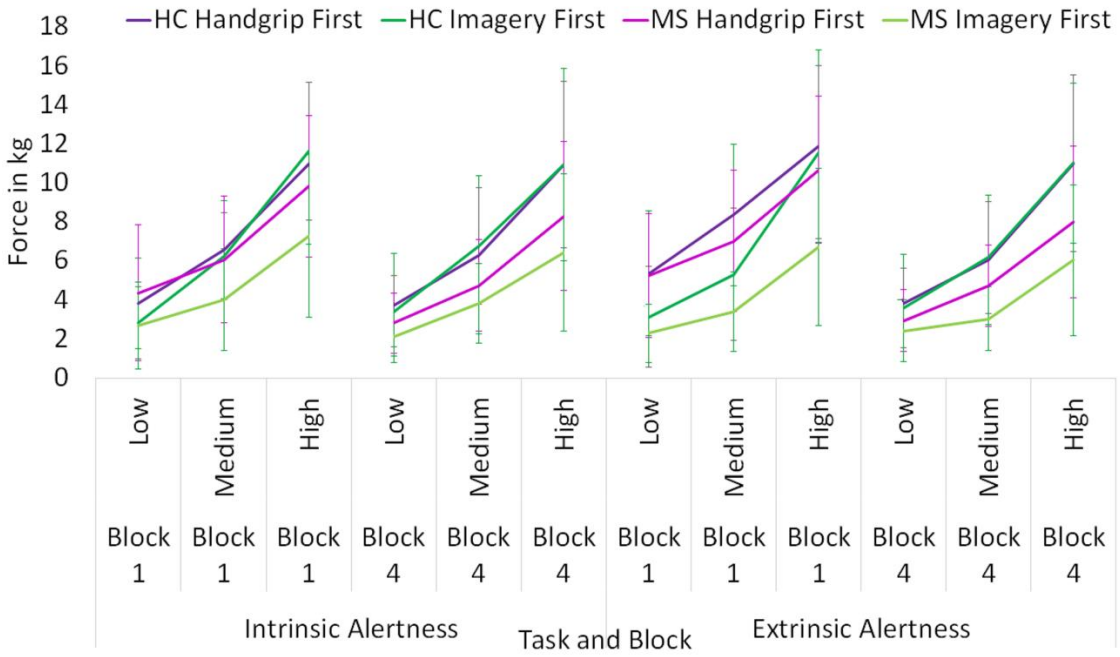


Figure 6.6 Line graph of force grip in kg. *The HC handgrip first group are displayed in purple, the HC imagery first group are displayed in green, the MS handgrip first group are displayed in magenta and the MS imagery first group are displayed in lime. The error bars represent one standard deviation.*

6.3.5 Impact of phenotype and disease duration on force in the MS group

The one-way ANOVA showed no significant main effect for intrinsic alertness force in low ($F(2,37)= .05$, $p= .95$, $\eta^2 < .01$), medium ($F(2,37)= .02$, $p= .98$, $\eta^2 < .01$) and high grip strengths ($F(2,37)= .59$, $p= .56$, $\eta^2= .03$) or for extrinsic alertness in any grip strength (low $F(2,37)= .40$, $p= .67$, $\eta^2= .02$, medium $F(2,37)= .23$, $p= .80$, $\eta^2= .01$, high $F(2,37)= .29$, $p= .75$, $\eta^2= .02$). Furthermore, there were no significant correlations between disease duration and intrinsic alertness (low $R= -.31$, $p= .06$; medium $R= -.21$, $p= .21$; high $R= -.03$, $p= .86$) or extrinsic alertness (low $R= -.25$, $p= .14$; medium $R= -.21$, $p= .23$; high $R= -.02$, $p= .91$) force grip.

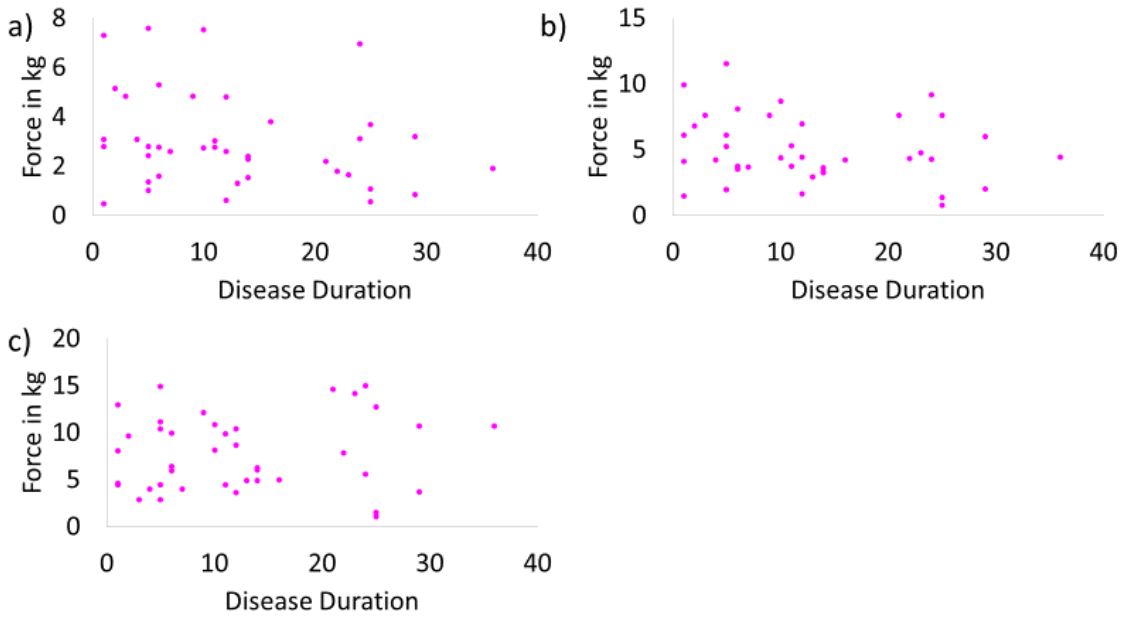


Figure 6.7 Composite image of scatterplot between disease duration force in the intrinsic alertness Task. *a) low force and disease duration. b) medium force and disease duration. c) high force and disease duration.*

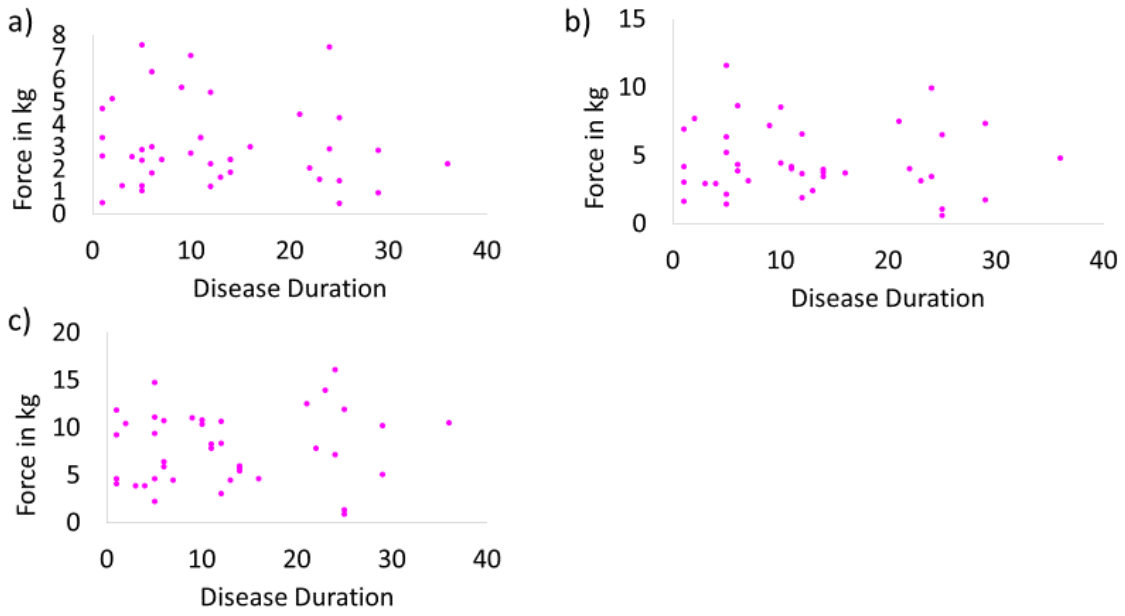


Figure 6.8 Composite image of scatterplot between disease duration force in the extrinsic alertness task. *a) low force and disease duration. b) medium force and disease duration. c) high force and disease duration.*

The one-way ANOVA for the MS handgrip first group revealed there was no main effect of phenotype on force grip for intrinsic alertness (low $F(2,37)= .39$, $p= .69$, $\eta^2= .05$, medium $F(2,37)= 2.49$, $p= .11$, $\eta^2= .29$, high $F(2,37)= .03$, $p= .98$, $\eta^2< .01$) and extrinsic alertness (low $F(2,37)= .04$, $p= .96$, $\eta^2< .01$, medium $F(2,37)= .22$, $p= .80$, $\eta^2= .03$, high $F(2,37)= 1.81$, $p= .19$, $\eta^2= .21$). Furthermore, there were no correlations between disease duration and force grip for intrinsic alertness (low $R= .19$, $p= .46$, medium $R= .12$, $p= .63$, high $R= .37$, $p= .14$) and

extrinsic alertness (low R= .36, p= .14, medium R= .23, p= .35, high R= .42, p= .08). For the MS imagery first group there was no main effect of phenotype on force grip for intrinsic (low $F(2,37)= .36$, p= .56, $\eta^2= .02$; medium $F(2,37)= .58$, p= .46, $\eta^2= .03$; high $F(2,37)= .06$, p= .81, $\eta^2< .01$) and extrinsic (low $F(2,37)= .01$, p= .94, $\eta^2< .01$; medium $F(2,37)= .06$, p= .81, $\eta^2< .01$; high $F(2,37)= .22$, p= .64, $\eta^2= .01$) alertness task. There were also no correlations between disease duration and force grip for intrinsic alertness (low R= .10, p= .71; medium R= .13, p= .63; high R= .26, p= .32) and extrinsic alertness (low R= .20, p= .44; medium R= .24, p= .34; high R= .21, p= .42).

6.3.6 FSS correlates with force grip

Partial correlations in the HC group revealed a significant negative correlation between fatigue scores and high force during intrinsic alertness (R= -.38, p= .03). There were no significant correlations between FSS scores and low (R= -.04, p= .83) or medium force (R= -.21, p= .22) during the intrinsic alertness task. Similarly during the extrinsic alertness task force did not correlate with FSS scores (low R= -.03, p= .86; medium R= - .21, p= .24; high R= -.27, p= .11). For the MS group there were no significant correlations between FSS scores and force during either the intrinsic alertness task (low R= -.01, p= .95; medium R= -.07, p= .69; high R= -.07, p= .69) or during the extrinsic alertness task (low R= .01 p= .95; medium R= -.07, p= .68; high R= -.03, p= .85). Scatterplots are shown in Figure 6.9 and Figure 6.10.

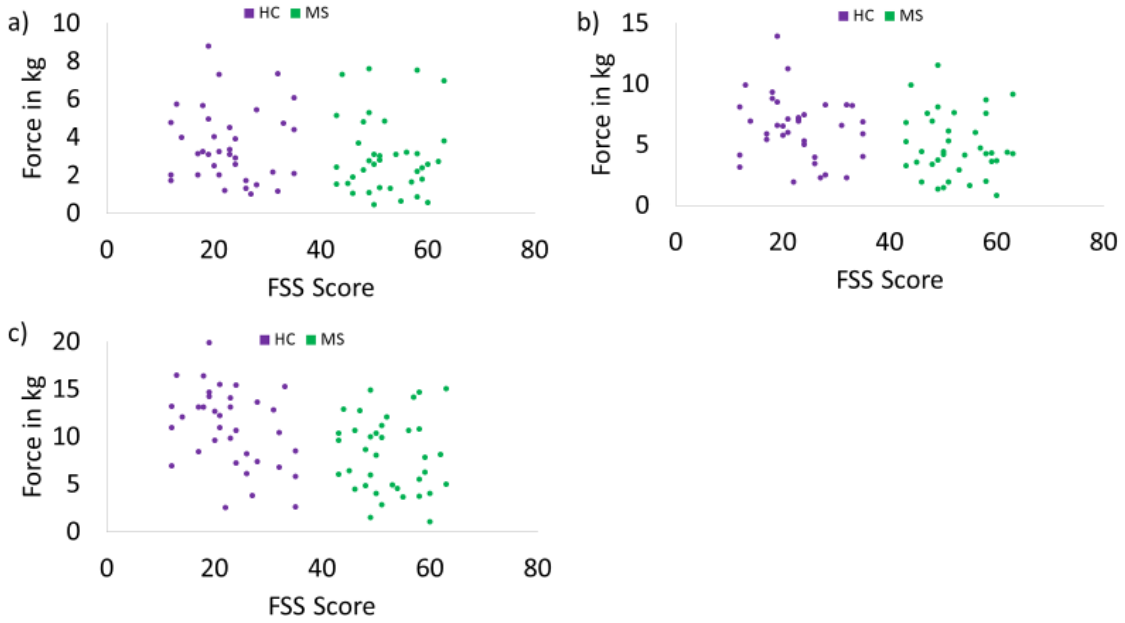


Figure 6.9 Scatterplot for force and fatigue scores during intrinsic alertness. *a) low force. b) medium force. c) high force. HC are displayed in purple, MS are displayed in green. Each circle represents a participant.*

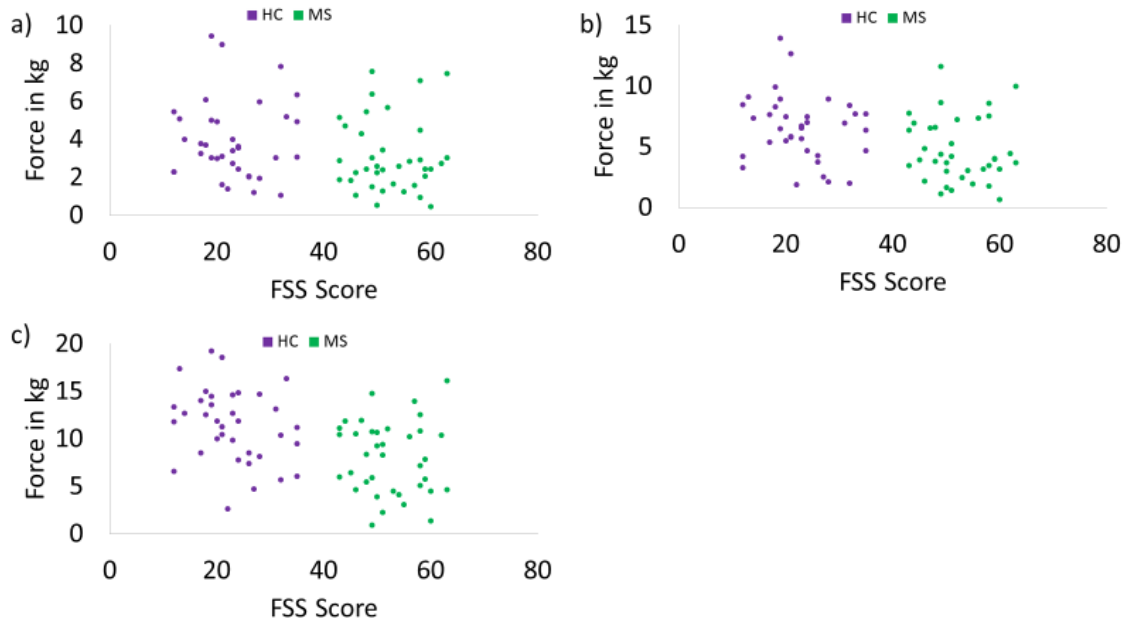


Figure 6.10 Scatterplot for force and fatigue scores during extrinsic alertness. *a) low force. b) medium force. c) high force. HC are displayed in purple, MS are displayed in green. Each circle represents a participant.*

6.3.7 Borg ratings of perceived effort

The independent sample t-tests revealed that the MS group rated both the handgrip ($t(58)= -5.21$, $p< .01$, $d= 1.39$) and mental imagery condition ($t(58)= -3.89$, $p< .01$, $d= 1.04$) as significantly more effortful than the HC group, as assessed by the Borg scale. Furthermore, a paired t-test to compare Borg ratings between the handgrip and mental imagery conditions showed that participants rated the mental imagery condition as significantly more effortful than the handgrip condition, regardless of group ($t(59)= -5.72$, $p<.01$). When splitting the groups based on which condition they completed first, there were no differences in Borg ratings between the handgrip first and imagery first groups for both HC (handgrip $t(33)= .89$, $p= .38$, $d= .31$; mental imagery $t(33)= .28$, $p= .78$, $d= .10$) and MS (handgrip $t(23)= -1.97$, $p= .06$, $d= .79$; mental imagery $t(23)= -.32$, $p= .75$, $d= .13$).

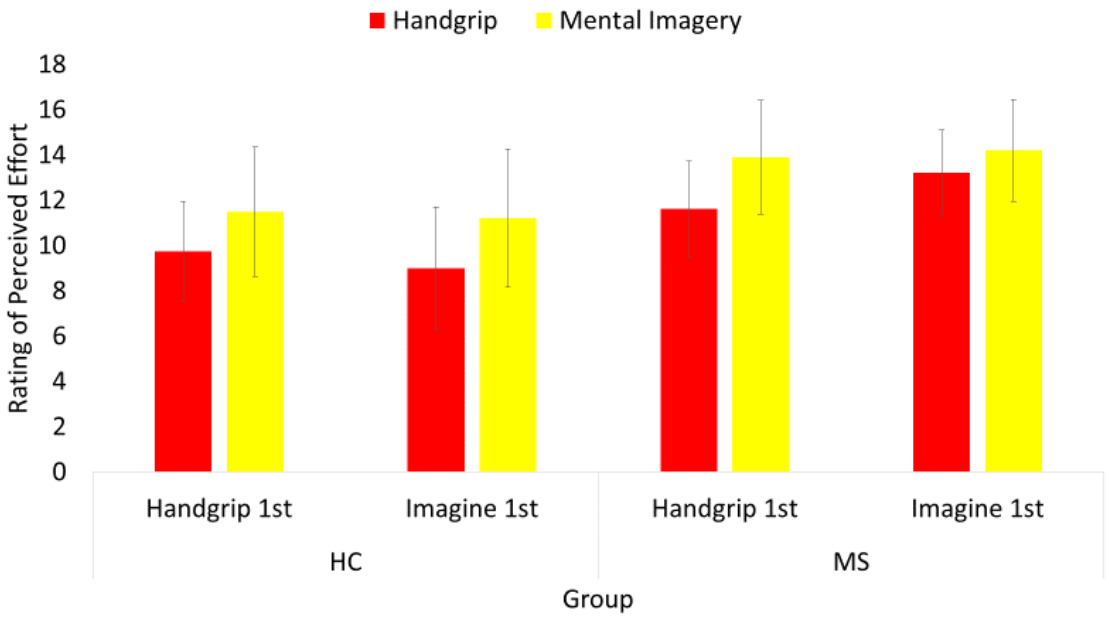


Figure 6.11 Bar chart for the Borg ratings of perceived effort. *The ratings for the physical handgrip condition are displayed in red, the ratings for the mental imagery condition are displayed in yellow. The error bars represent one standard deviation.*

6.4 Discussion

The primary aim of the current chapter was to use an alertness-motor paradigm to establish how fatiguability affects cognition, in both healthy individuals and in an MS population, suffering from persistent fatigue. Both reaction time and force were measured across the alertness-motor paradigm, both of which correlated significantly with fatigue scores from the FSS. This confirmed that they did provide an accurate performance measure of fatigue.

6.4.1 Reaction time performance

Due to the presence of a warning signal the extrinsic alertness task should produce a decrease of reaction time (Perin *et al.*, 2010; Petersen and Posner, 2012), due to increased readiness. The results from the four-way ANOVA indicated that the HC group respond to the warning effect, but the MS group do not, which indicates a deficit in extrinsic alertness in the MS group. However, there was not a significant difference in the change between the two groups. This result indicates that the MS group do reduce their reaction time in extrinsic alertness, but not enough to produce an overall warning effect. This is likely due to the heterogeneity in the MS group creating a large amount of variance. Large variance creates a problem when statistically comparing mean performance. In future perhaps an approach using response variability may be more appropriate but was not conducted here.

There was a significant overall increase in reaction time over the paradigm, suggesting that the paradigm does successfully induce fatiguability even in a healthy population. The MS group showed significantly slower reaction times compared to the HC group, further evidenceing a deficit in repsonse speed. Moreover, the MS group showed increased fatiguability compared to the HC group, which suggests that fatiguability effects cognition to a greater extent in an MS population already suffering from persistent fatigue, compared to a healthy non-fatigued group. Once the participants were further split into groups depending on the condition order (handgrip first or imagery first) the results showed that the handgrip first groups in both the MS and HC groups showed increased fatiguability compared to the imagery first groups. This result suggests that there is some benefit from completing the mental imagery condition first.

It was hypothesised that due to the increased task demand, completing the mental imagery condition first would increase the effect of fatiguability on cognition. These results suggest that completing the mental imagery condition first actually produces a learning effect. This is consistent with previous findings showing a learning effect upon completing a task a second time, even when the task was fatiguing (Claros-Salinas *et al.*, 2010; Johnson *et al.*, 1997; Krupp and Elkins, 2000; Schwid *et al.*, 2003). Moreover, some previous studies have shown that practising mental imagery has some effectiveness at decreasing perceived fatigue (Bovend'Eerdt *et al.*, 2010; Catalan *et al.*, 2011; Seebacher *et al.*, 2017; 2018), however the results of these studies are very limited. Interestingly, this finding is at odds with the perceived effort, as measured by the Borg scale, where both HC and MS groups consistently rate the mental imagery condition as more effortful than the handgrip condition. This result indicates that there may be a discrepancy between perceived fatigue and objective measures of fatigue, even in healthy individuals.

This finding has two important implications, it further highlights the necessity of measuring fatigue in an objective manner and suggests that the perceptions of the individual must be considered when examining such a unique concept as fatigue. However, once split by task and task order the results showed that the benefit of completing the mental imagery condition first was only noted during the intrinsic alertness task. During the extrinsic alertness task both HC groups did not show evidence of fatiguability. This result demonstrates that that intrinsic alertness is more susceptible to the effects of fatiguability. This may be due to the cue involved in extrinsic alertness that supersedes the necessity for prolonged alertness. Whereas during the extrinsic alertness task the MS handgrip first group did not demonstrate fatiguability, but the MS

imagery first group did. This result is more consistent with the original hypothesis that mental imagery would increase fatiguability. To interpret this result, it is important to remember that the MS group have a significant deficit to extrinsic alertness, and do not respond to the warning cue as expected. It is, therefore, possible to suggest that when a significant deficit is present, completing the mental imagery condition first increases the fatiguability, thereby producing a fatiguing effect.

6.4.2 Force grip performance

In terms of force the five-way ANOVA revealed that both the HC and MS successfully exerted different forces between the low, medium and high grip strengths, therefore conducting the task correctly. There was a smaller difference between forces in the MS group than the HC group. Similarly, the MS group exerted lower force grip than the HC group. This was expected due to factors such as muscle weakness in the MS group. As with reaction time, the MS group had significantly increased fatiguability compared to the HC group. This further evidences that fatiguability effects cognition to a greater extent in an MS population already suffering from persistent fatigue, compared to a healthy non-fatigued group.

There was a significant overall increase in reaction time over the paradigm, further supporting that the alertness-motor paradigm does successfully induce fatiguability even in a healthy population. Inline with the reaction time results, once the participants were further split into handgrip first or imagery first groups, the results indicated increased fatiguability in the handgrip first groups compared to the imagery first groups. The results further suggest that there is some benefit to conducting the mental imagery condition first and is consistent with previous studies showing improved movements following a period of mental imagery (Bovend'Eerdt *et al.*, 2010; Seebacher *et al.*, 2017; 2018; Yaguez *et al.*, 1998; Yue and Cole, 1992). However, this was confounded by the fact that the imagery first groups also exerted significantly less force than the handgrip first groups. Due to the lower force grip, the imagery first groups may have been better able to sustain that force throughout the paradigm. This becomes particularly apparent in the split MS groups. In both the intrinsic and extrinsic alertness task the MS handgrip first group exhibited decreased force in all three force levels, low, medium and high across the paradigm. Whereas the MS imagery first group had decreased force in the high force level during the intrinsic alertness task, which is the most associated with fatigue. As such the MS imagery first group do show increased fatiguability, but due to the

consistently low force exerted during the paradigm this is only apparent in the most fatigue inducing scenario. The interaction effects showed that the HC groups decreased force and demonstrated increased fatiguability during low and medium but not high force grips. Given that only the medium, and to a greater extent, the high force levels correlated with fatigue scores, it may be that the force differences are more due to inattentiveness than motor fatigue. The results across HC and MS groups indicate that the behavioural performance during the intrinsic alertness task of the alertness-motor paradigm is more susceptible to the influence of fatiguability than during the extrinsic alertness task. However, the results are slightly confounded due to the mental imagery condition. The implication for the current experimental design is discussed in more detail in section 9.6 (page 187).

The secondary aims were to examine whether in the MS group phenotype or disease duration affected behavioural performance and to evaluate whether this alertness-motor paradigm could provide a performance measure of fatigue. When examining whether MS phenotype or disease duration impact reaction time or force grip, the results demonstrate no significant differences across phenotype and no correlation with disease duration during either the intrinsic or extrinsic alertness task. In line with Chapter 4 (page 71), these results confirm that the current sample is homogenous in behavioural performance. However, this must be distinguished from the general heterogeneity of MS that has been highlighted throughout the thesis. The correlates with fatigue scores demonstrated that reaction time, but not force correlated significantly with fatigue scores in the HC group, suggesting that the alertness-motor paradigm does indeed provide a performance measure of fatigue. However, in the MS group both measures were unrelated to fatigue scores. This further evidences a dissociation between objective and subjective measure of fatigue in an MS population and highlights the difference between perceived fatigue and performance fatigue (Middleton *et al.,* 2006; Roberg *et al.,* 2012).

6.4.3 Conclusion

The results provide further evidence for a speed of information deficit in MS. Moreover, they provide evidence that fatiguability has a detrimental effect on cognition. The alertness-motor paradigm can successfully induce fatiguability, to a greater extent through the intrinsic alertness task. Moreover, the MS group showed increased fatiguability compared to the HC group, which suggests that fatiguability effects cognition to a greater extent in an MS population already suffering from persistent fatigue,

compared to a healthy non-fatigued group. However, counter to the hypotheses the results are slightly confounded due to the mental imagery condition. Examining the neural substrates that underlie this pattern of behavioural results may elucidate subtle neurological differences to clarify these results.

Chapter 7 Neural correlates of fatigue: a functional magnetic resonance imaging study in multiple sclerosis.

7.1 Introduction

Functional neuroimaging studies can detect neuronal processes that underlie behavioural functions. Moreover, it can provide a complementary technique to provide insight into the functional specialization of the brain. In the systematic review (Chapter 3 page 54) examining the neural correlates of alertness, the synthesis of the thirteen available fMRI studies revealed a consensus network involving nine regions. These included the precentral gyrus, inferior occipital gyrus, prefrontal cortex (pFC), superior and inferior parietal lobule, thalamus, insula, cerebellum and brainstem regions, with several studies, but not the majority, reporting activation of the anterior cingulate cortex (ACC). It must be noted that only one study reported all these regions to be active within the same study (Xuan *et al.*, 2016). The result from the systematic review provides a good basis for understanding the neural correlates that may underlie alertness and allows for good comparison to the results of the current study. In line with the aims of this thesis, the neural correlates of fatigue are of most interest in this chapter.

Previous studies have attempted to provide a similar understanding of the neural basis for fatigue. In chronic fatigue syndrome studies observed widespread increased activation compared to controls (Caseras *et al.*, 2006; Cook *et al.*, 2007; Lange *et al.*, 2005), which led to the hypothesis that increased neural activation may increase the metabolic demand required, thereby increasing fatigue (Lange *et al.*, 2005). A similar widespread increased activation has been noted in MS (Bonnet *et al.*, 2010; Lee *et al.*, 2000; Lopez-Gongora *et al.*, 2015; Mainero *et al.*, 2006; Pantano *et al.*, 2002a; 2002b; Parry *et al.*, 2003; Reddy *et al.*, 2000; Rocca *et al.*, 2002; Staffen *et al.*, 2002; Wishart *et al.*, 2004). The direct relationship to fatigue remains unknown (Mainero *et al.*, 2006; Penner *et al.*, 2003; Tartaglia *et al.*, 2008). Studies that directly attempt to examine fatigue in MS showed mixed results. There is some inconsistency across regions involved, but the majority implicate the basal ganglia in MS fatigue (Bonzano *et al.*, 2017; DeLuca *et al.*, 2008; Fillipi *et al.*, 2002; Finke *et al.*, 2015; Genova *et al.*, 2013; Pravata *et al.*, 2016; Roelcke *et al.*, 1997; Rocca *et al.*, 2016a). Chaudhuri and Behan (2000; 2004) proposed that through its association with the dopamine system, dysfunction to the basal ganglia and its connections can lead to disruption of motivation, which may play a role in central fatigue. Despite this, the exact nature of the association between MS fatigue and the basal ganglia also remains unknown.

Chapter 7 Neural correlates of fatigue: a functional magnetic resonance imaging study in multiple sclerosis.

In Chapter 6 (page 105), the impact of induced fatiguability on behavioural performance during an alertness-motor paradigm in both a HC and an MS group with persistent fatigue was explored. The primary aim of this chapter was to examine the neural correlates that underlie the behavioural performance associated with fatiguability observed in Chapter 6 (page 105). The secondary aims were to explore the pattern of neuronal activation that underlies the behavioural difference between the MS and HC groups. If the alertness-motor paradigm induces fatiguability, then basal ganglia activation is expected in the final blocks of the task. Furthermore, increased activation of the basal ganglia is expected in the MS group compared to the HC group.

7.2 Methods

7.2.1 Participants

A total of 40 MS participants and 40 healthy individuals were recruited according to the procedure described in the general methods section. 3 MS and 4 HC outliers were removed (refer to section 2.6 page 52).

7.2.2 Neuropsychological questionnaires

All participants, in both the MS and HC groups, were screened for fatigue, using the Fatigue Severity Scale (FSS) (Krupp *et al*., 1989), to ensure that the MS did have significant fatigue and the HC did not. Furthermore, the scores from the FSS was used as a measure of fatigue in the analysis.

7.2.3 Alertness-motor paradigm

The alertness-motor paradigm consisted of interleaved periods of three different tasks; sensorimotor control, intrinsic alertness and extrinsic alertness, requiring the participant to exert a defined force (low, medium, high). The sensorimotor task was a self-cued motor control condition, where participants were asked to squeeze at the required force when the flashing white square appeared on the screen. For the intrinsic alertness task participants fixated on a white cross and were instructed to squeeze, at the required force, when the white square appeared. In the extrinsic alertness, task participants fixated on a white cross but received a black screen as a warning cue before the white square appeared. Participants were instructed to squeeze, at the required force when the white square appeared and were told that the black screen would proceed the white square. A schematic of the paradigm is displayed in Figure 7.2. Each task was repeated four times, resulting in four blocks containing each of the three tasks at each of the three force levels. The order

of tasks was pseudorandomised (Figure 7.1). Furthermore, there were two conditions to the paradigm, physical handgrip, and mental imagery. Every participant completed both conditions. In the physical condition, participants performed the full paradigm whilst squeezing a hand dynamometer at the required force. In the mental imagery, task participants performed the full paradigm but were asked to only imagine squeezing the hand dynamometer at the required force. The paradigm is explained in detail in section 5.2.3-5.2.5 (page 90-92). The order of the mental imagery and handgrip condition was pseudorandomised, as a result, half the participants completed the handgrip condition before the mental imagery condition and *vice versa*. The group of participants that completed the handgrip condition before the mental imagery condition is referred to as the handgrip first group. The group of participants that completed the mental imagery condition before the handgrip condition is referred to as the imagery first group. When comparing between HC and MS groups, the participants were not split according to condition order, as condition order did not affect global performance (see appendix K page 269). When examining within group effects, such as the effect of fatiguability across time, the analysis was split by condition order.

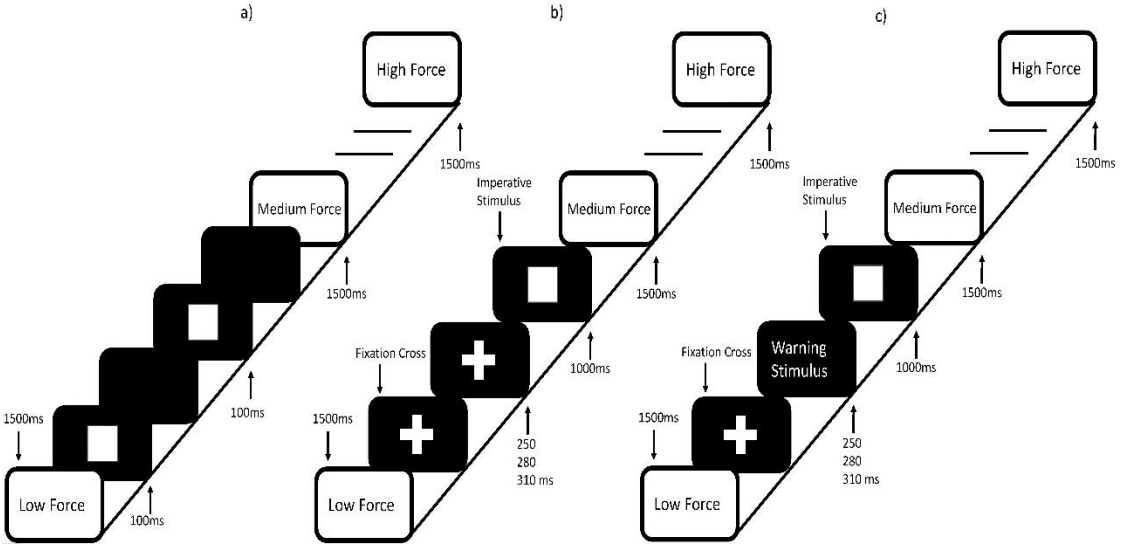


Figure 7.1 Schematic of alertness-motor paradigm. *a) sensorimotor control. b) intrinsic alertness. c) extrinsic alertness.*

Block 1	T3			T1			T2		
	Low	Medium	High	High	Medium	Low	Low	High	Medium
Block 2	T2			T1			T3		
	High	Low	Medium	High	Medium	Low	High	Medium	Low
Block 3	T3			T1			T2		
	Medium	High	Low	Low	Medium	High	Medium	Low	High
Block 4	T2			T1			T3		
	Low	Medium	High	Low	Medium	High	Low	High	Medium

***Figure 7.2 Task design**. Note: T1= sensorimotor task. T2= intrinsic task. T3= extrinsic task.*

7.2.4 Image Pre-processing

Pre-processing and analysis of fMRI data were performed using MATLAB (Mathworks Inc., Natick, MA, USA) and Statistical Parametric Mapping package SPM12 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm). Pre-processing steps consisted of correction for head movements, co-registration, segmentation, image normalisation into standard space based on the MNI template using forward deformations field and smoothing using 8mm full-width half-maximum (FWHM) Gaussian kernel to account for residual inter-individual differences. To correct for signal changes due to head movement, 6 realignment parameters were included in the design matrix. An additional set of harmonic regressors was used to account for any temporal low-pass frequency variance within the data that is typical to fMRI signal with a cut-off of 1/128 Hz. All regressors were convolved with the canonical haemodynamic response function.

7.2.5 fMRI analysis

Individual analyses were run according to a block design. Several planned t-contrasts were created based on the different tasks completed (T1, T2, T3), to determine whether the fitted parameter value at each voxel was significantly greater than 0. To selectively map neural activity related to intrinsic alertness the sensorimotor task (T1) was subtracted from the intrinsic alertness (T2) task. To map neural activity related to extrinsic alertness the intrinsic alertness (T2) contrast was subtracted from the extrinsic alertness (T3) tasks (for a subtraction review see (Friston *et al.*, 1996). First-level individual analyses included a global activation, across the whole task, and block activation, specified for the first and last block of the paradigm. Brain regions during the global activation were identified according to two contrasts intrinsic alertness (T2 – T1) and extrinsic alertness (T3-T2). Activated brain regions during the last block of the paradigm were identified according to

two contrasts: intrinsic alertness block 4 (T2 Block 4- T2 Block 1), extrinsic alertness block 4 (T3 Block 4- T3 Block 1).

The analyses of consistent effects across groups of participants (random-effects, second-level analysis) were tested using the general linear model (GLM) framework. One-sample t-tests were conducted for each of the four contrasts specified during the individual analysis, for both global (T2-T1, T3-T2) and block activations, where participants were split into handgrip first or imagery first in both the HC and MS groups (T2 Block 4- T2 Block 1, T3 Block 4- T3 Block 1). To compare between the MS and HC group planned two-sample t-tests were conducted separately for the intrinsic alertness (T2-T1) and extrinsic alertness (T3-T2) contrasts. Planned two sample t-tests were then conducted to explore the effect of condition order (handgrip first or imagery condition first) on the last block of the intrinsic alertness task (T2 Block 4- T2 Block 1) and extrinsic alertness task (T3 Block 4- T3 Block 1) for both the HC and MS group.

To explore whether specific brain regions were associated with fatigue scores, a multiple regression between FSS scores and the sensorimotor task (T1), intrinsic alertness (T2-T1), extrinsic alertness (T3-T2). Neither masking nor error correction was applied, the significance threshold was set at FWE corrected p<.05 for global activations but for block activation and comparisons the threshold was lowered to uncorrected p <.001 with a T-value of 3.5 and above, based on recommendations in Cremers *et al*. (2017) because significant behavioural differences were observed in Chapter 6 (page 105). Only the results of the intrinsic and extrinsic alertness task are reported here as the sensorimotor task was simply a control condition.

7.3 Results

Here only a brief recap of significant behavioural performance results, essential for interpreting the fMRI analysis, are reported. For full results refer to section 6.3 (page 111).

7.3.1 Reaction time performance

The HC handgrip first group had increased reaction in the last block of intrinsic alertness (p< .05), but no reaction time differences between block 1 and 4 for extrinsic alertness (p> .05). For the HC imagery first group there were no reaction time differences between the beginning and the end of the paradigm for intrinsic alertness (p> .05) or extrinsic alertness (p> .05). The MS handgrip first group had increased reaction time in the last block of intrinsic alertness (p< .01) but no reaction time

differences between block 1 and 4 for extrinsic alertness (p> .05). For the MS imagery first group the opposite was observed, there was an increased reaction time in block 4 for extrinsic alertness (p< .05), and no reaction time differences between the beginning and the end of the paradigm for intrinsic alertness (p> .05). The MS group had increased fatiguability compared to the HC group (p< .05).

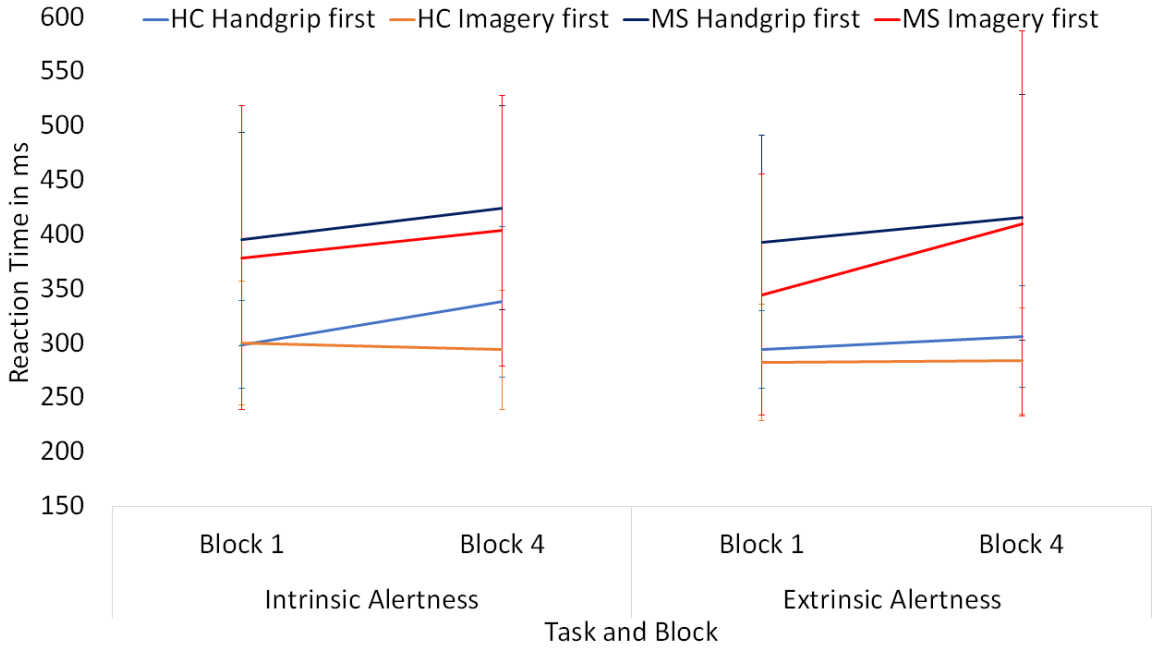


Figure 7.3 Line graph of reaction time in ms. *The HC handgrip first group are displayed in blue, the HC imagery first group are displayed in orange, the MS handgrip first group are displayed in navy and the MS imagery first group are displayed in red. The error bars represent one standard deviation.*

7.3.2 BOLD activation of the HC group during global task performance

Regions of significant activation during intrinsic alertness (T2-T1) for the HC group are displayed in Table 7.1. Activation is predominantly in the bilateral inferior occipital lobe.

Table 7.1 Regions of peak activation for intrinsic alertness in the HC group.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Inferior Occipital Gyrus	19	30	-86	-6	11.67**
Left	Inferior Occipital Gyrus	18	-26	-90	-4	10.80**
Left	Fusiform Gyrus	N/A	-36	-82	-12	10.46**

Note BA= Brodmanns Area. **FWE corrected p<.05

Brain regions that displayed significantly increased BOLD signal during extrinsic alertness (T3-T2) for the HC group are shown in Table 7.2. Activations are in the bilateral prefrontal cortex.

Table 7.2 Regions of peak activation for extrinsic alertness in the HC group.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Prefrontal Cortex (pFC)	6	10	32	52	6.99**
Left	Prefrontal Cortex (pFC)	6	-8	34	54	5.71**
Left	Thalamus	N/A	-18	-14	2	5.62**

Note BA= Brodmanns Area. **FWE corrected p<.05

7.3.3 BOLD activation of the split HC groups during the final block of intrinsic alertness

Brain regions that demonstrated significant activation during the last block of the intrinsic alertness task (T2 Block 4- T2 Block 1) for the HC handgrip first group are displayed in Table 7.3. The analysis indicated increased activation of bilateral caudate and frontal regions, right occipital lobe and left temporal regions.

Table 7.3 Regions of peak activation for intrinsic alertness in the last block for the HC handgrip first group.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Middle Occipital Gyrus	18	32	-94	16	4.83*
Right	Prefrontal Cortex (pFC)	6	32	10	40	4.68*
Right	Cuneus	18	22	-90	16	4.03*
Right	Caudate	N/A	4	10	2	4.00*
Right	Orbital Frontal Gyrus	11	26	60	-12	3.90*
Right	Vermis	N/A	0	-84	-28	3.83*
Right	Inferior Temporal Gyrus	38	44	14	-40	3.78*
Right	Pallidum	N/A	8	0	-4	3.75*
Left	Prefrontal Cortex (pFC)	6	-40	10	50	6.38*
Left	Inferior Temporal Gyrus	38	-52	14	-32	5.83*
Left	Claustrum	N/A	-26	26	0	5.10*
Left	Caudate	N/A	-14	10	14	4.84*
Left	Inferior Frontal Gyrus	46	-38	28	4	4.75*
Left	Culmen	N/A	-36	-40	-34	4.39*
Left	Orbital Frontal Gyrus	10	-30	56	0	4.15*
Left	Declive	N/A	-26	-90	-14	4.02*
Left	Pyramis	N/A	-30	-66	-28	3.90*
Left	Pallidum	N/A	-10	2	-2	3.83*
Left	Inferior Parietal Lobule	40	-54	-48	44	3.76*
Left	Superior Parietal Lobule	7	-24	-68	52	3.70*
Left	Fusiform Gyrus	37	-56	-32	-28	3.66*
Left	Thalamus	N/A	-10	-6	2	3.54*

Note BA= Brodmanns Area. *uncorrected p <.001

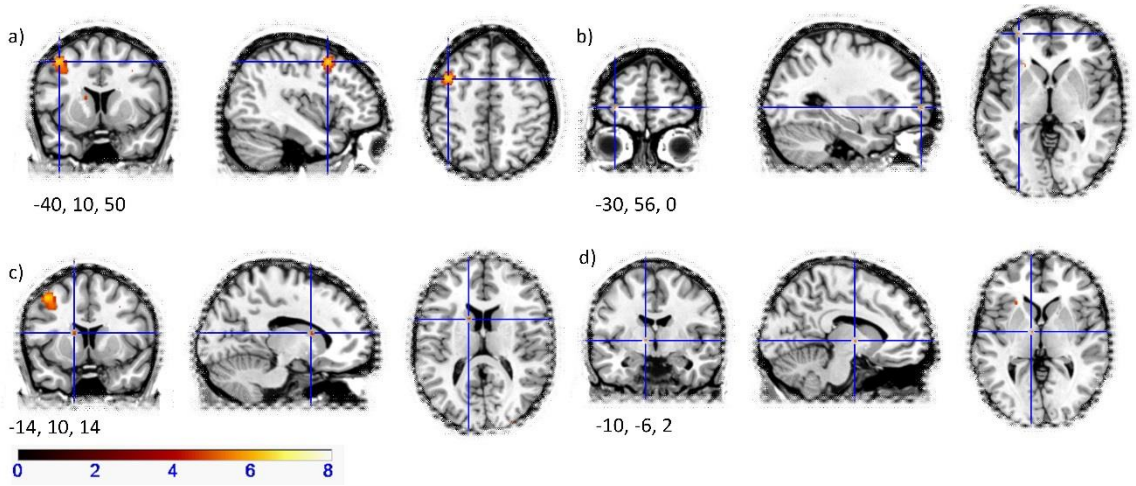


Figure 7.4 Regions of increased activation in the HC handgrip first group during the last block of the intrinsic alertness task. *a) Prefrontal Cortex (pFC). b) Orbital Frontal Gyrus. c) Caudate. d) Thalamus. MNI Coordinates (x,y,z). p<0.001 uncorrected.*

Regions of significant activation during the last block of the intrinsic alertness task (T2 Block 4- T2 Block 1) for the HC imagery first group are shown in Table 7.4.

Table 7.4 Regions of peak activation for intrinsic alertness in the last block for the HC imagery first group.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Claustrum	N/A	32	14	16	3.79*
Right	Middle Temporal Gyrus	38	52	0	-20	3.78*
Right	Parahippocampal Gyrus	28	30	-36	8	3.75*
Right	Paracentral Lobule	5	2	-34	62	3.69*
Right	Inferior Temporal Gyrus	37	48	-48	-4	3.62*
Left	Medial Frontal Gyrus	8	-2	46	36	4.25*
Left	Precuneus	7	-14	-58	72	3.96*
Left	Middle Temporal Gyrus	22	-46	-36	-2	3.96*
Left	Superior Temporal Gyrus	13	-36	-36	22	3.82*
Left	Inferior Frontal Gyrus	45	-56	20	20	3.67*
Left	Paracentral Gyrus	5	-12	-16	74	3.60*
Left	Orbital Frontal Gyrus	47	-42	32	-2	3.54*

Note BA= Brodmanns Area. *uncorrected p <.001

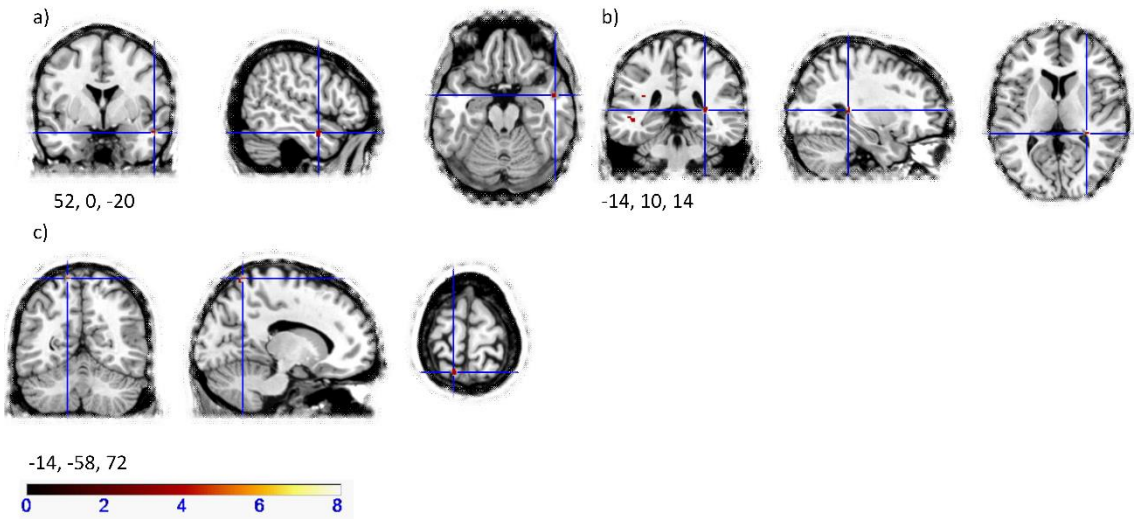


Figure 7.5 Regions of increased activation in the HC imagery first group during the last block of the intrinsic alertness task. *a) Middle Temporal Gyrus. b) Parahippocampal Gyrus. c) Precuneus. MNI Coordinates (x,y,z). p<0.001 uncorrected.*

The direct comparison of the split HC groups during the last block of intrinsic alertness (T2 Block 4- T2 Block 1) showed increased activation in the right orbital frontal gyrus and bilateral cuneus for the HC handgrip first group (Table 7.5). The HC imagery first group had moderately increased BOLD signals in the right parahippocampal gyrus and left precuneus (Table 7.6).

Table 7.5 Regions of greater peak activation for the HC handgrip first group compared to the HC imagery first group that during the final block of intrinsic alertness.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Orbital Frontal Gyrus	10	24	60	-12	3.97*
Right	Cuneus	18	24	-93	24	3.76*
Left	Cuneus	19	-26	-90	36	3.83*
Left	Inferior Temporal Gyrus	20	-54	-38	-14	3.61*

Note BA= Brodmanns Area. *uncorrected p <.001

Table 7.6 Regions of greater peak activation for the HC imagery first group compared to the HC handgrip first group that during the final block of intrinsic alertness.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Parahippocampal Gyrus	34	12	-16	-32	3.53*
Left	Precuneus	7	-12	-56	74	3.53*

Note BA= Brodmanns Area. *uncorrected p <.001

7.3.4 BOLD activation of the split HC groups during the final block of extrinsic alertness

Once the HC group was split into the handgrip first and imagery first groups there were no behavioural differences in within or between group performance, and as such, the contrast was not included. Without a behavioural difference, the neuronal patterns of activation do not provide any interpretable information.

7.3.5 BOLD activation of the MS group during global task performance

Regions of significant activation during intrinsic alertness (T2-T1) for the MS group are shown in Table 7.7. Activation is predominantly in the bilateral inferior occipital gyrus and middle temporal gyrus.

Table 7.7 Regions of peak activation for intrinsic alertness in the MS group.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Inferior Occipital Gyrus	18	24	-90	0	11.93**
Right	Middle Temporal Gyrus	20	40	-66	-10	6.28**
Left	Middle Occipital Gyrus	18	-28	-88	0	9.89**
Left	Inferior Occipital Gyrus	19	-36	-78	-8	8.83**

Note BA= Brodmanns Area. **FWE corrected p<.05

For extrinsic alertness (T3-T2) in the MS group, no activations survived the FWE corrected p< .05 significance level or the less stringent uncorrected p< .001, T-Value> 3.5 threshold.

7.3.6 BOLD activation of the split MS groups during the final block of intrinsic alertness

During the last block of the intrinsic alertness task (T2 Block 4- T2 Block 1) increased activation in the bilateral parahippocampal gyrus and left temporal regions in the MS handgrip first group were observed (Table 7.8).

Table 7.8 Regions of peak activation for the MS handgrip first during the last block of the intrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Parahippocampal Gyrus	28	28	10	-28	4.41*
Right	Orbital Frontal Gyrus	45	6	34	-18	3.82*
Right	Cerebellar Tonsil	N/A	24	-52	-36	3.80*
Right	Middle Temporal Gyrus	21	62	-10	-20	3.79*
Right	Precuneus	31	12	-52	34	3.51*
Left	Inferior Temporal Gyrus	21	-60	-24	-18	4.65*
Left	Middle Temporal Gyrus	21	-58	4	-20	3.70*
Left	Parahippocampal Gyrus	34	-12	-4	-18	3.66*
Left	Pallidum	N/A	-24	-6	-2	3.61*
Left	Prefrontal Cortex (pFC)	6	-42	20	52	3.58*

Note BA= Brodmanns Area. *uncorrected p <.001

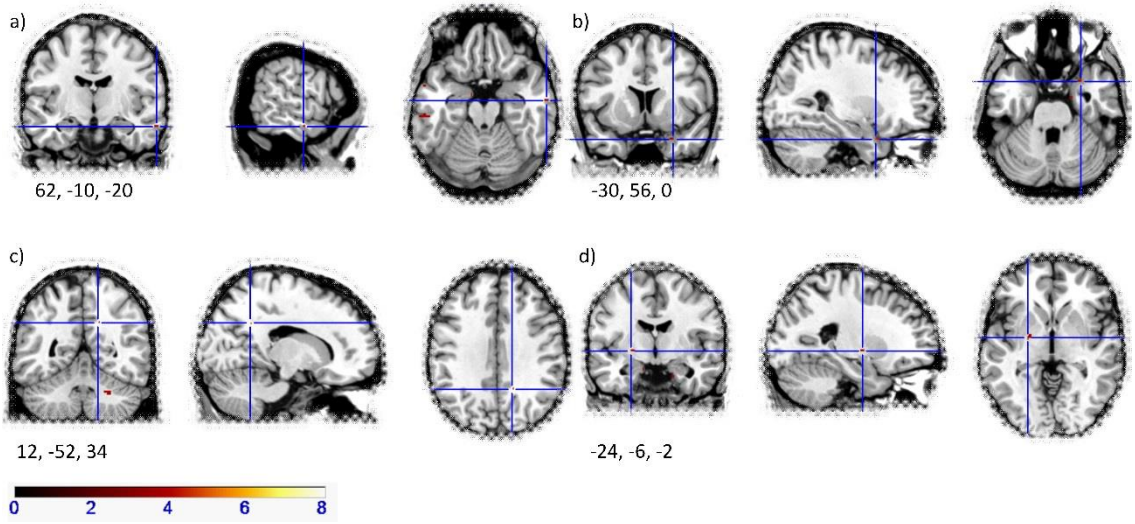


Figure 7.6 Regions of increased activation in the MS handgrip first group during the final block of the intrinsic alertness task. *a) Middle Temporal Gyrus. b) Parahippocampal Gyrus. c) Precuneus. d) Pallidum. MNI Coordinates (x,y,z). p<0.001 uncorrected.*

For the MS imagery first group the brain regions with significantly increased BOLD signals during the last block of the intrinsic alertness task (T2 Block 4- T2 Block 1) are shown in Table 7.9.

Table 7.9 Regions of peak activation for the MS imagery first group that during the final block of the intrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Middle Occipital Gyrus	31	34	-72	24	4.82*
Right	Caudate	N/A	44	-28	-12	4.14*
Right	Culmen	N/A	38	-40	-32	4.10*
Right	Orbital Frontal Gyrus	47	36	20	-18	3.93*
Left	Superior Temporal Gyrus	22	-62	-46	18	4.49*
Left	Tuber	N/A	-42	-78	-24	4.41*
Left	Precentral Gyrus	6	-38	6	38	4.19*
Left	Inferior Parietal Lobule	19	-38	-74	46	4.13*
Left	Putamen	N/A	-24	14	6	4.06*
Left	Inferior Occipital Gyrus	18	-34	-84	-2	4.05*
Left	Inferior Temporal Gyrus	20	-54	-32	-16	3.92*
Left	Caudate	N/A	-4	16	-4	3.88*
Left	Middle Occipital Gyrus	18	-28	-88	2	3.85*
Left	Middle Temporal Gyrus	22	-48	-26	-12	3.81*
Left	Pyramis	N/A	-16	-90	-28	3.70*
Left	Prefrontal Cortex (pFC)	10	-46	50	6	3.63*
Left	Precuneus	7	-10	-68	38	3.58*
Left	Supramarginal Gyrus	40	-48	-48	26	3.57*

Note BA= Brodmanns Area. *uncorrected p <.001

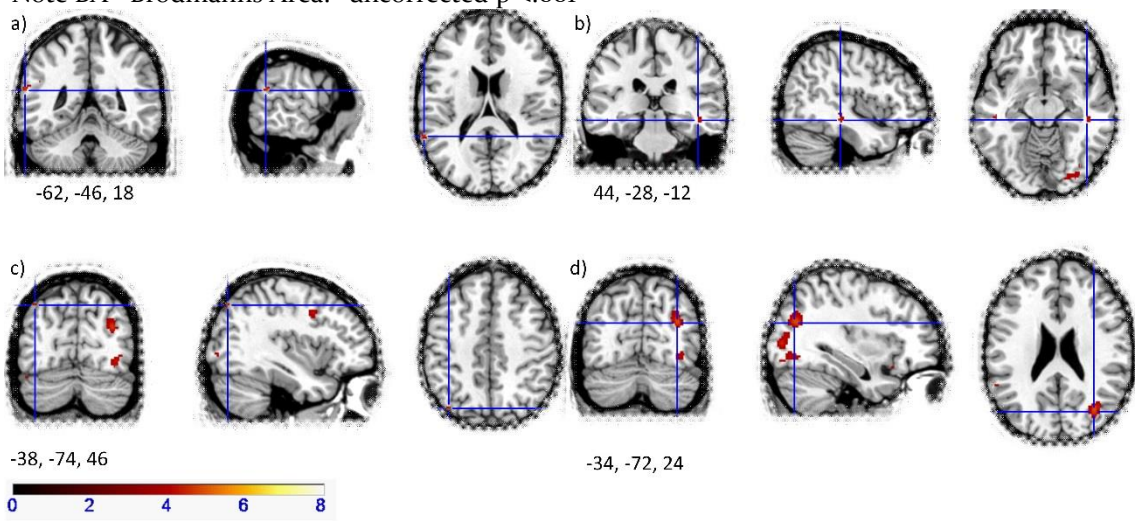


Figure 7.7 Regions of increased activation in the MS imagery first group during the last block of the intrinsic alertness task. *a) Superior Temporal Gyrus. b) Putamen. c) Inferior Parietal Lobule. d) Middle Occipital Gyrus. MNI Coordinates (x,y,z). p<0.001 uncorrected.*

The direct comparison of the split MS groups during the last block of intrinsic alertness (T2 Block 4- T2 Block 1) showed increased activation in the MS imagery first group in the basal ganglia, precuneus and frontal regions.

Table 7.10 Regions of greater peak activation for the MS imagery first group compared to the MS imagery first group in the last block of the intrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Cingulate Gyrus	23	2	-8	26	3.71*
Right	Middle Occipital Gyrus	18	30	-98	10	3.50*
r						
Left	Caudate	N/A	-4	16	-4	4.27*
Left	Precuneus	7	-8	-70	42	4.01*
Left	Insula	13	-40	14	2	3.83*
Left	Declive	N/A	-44	-76	-22	3.79*
Left	Tuber	N/A	-52	-60	-28	3.77*
Left	Superior Frontal Gyrus	10	-22	62	2	3.72*
Left	Prefrontal Cortex (pFC)	10	-42	54	4	3.62*
Left	Inferior Frontal Gyrus	44	-60	10	18	3.57*

Note BA= Brodmanns Area. *uncorrected p <.001

7.3.7 BOLD activation of the split MS groups during the final block of extrinsic alertness

The bilateral parahippocampal gyrus and caudate as well as the right posterior cingulate and left thalamus exhibited increased BOLD signal in the MS handgrip first group during the last block of the extrinsic alertness task (T3 Block 4- T3 Block 1) (Table 7.11).

Table 7.11 Regions of peak activation for the MS handgrip first group during the last block of the extrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Posterior Cingulate	29	12	-40	14	7.94*
Right	Precuneus	7	28	-48	10	6.93*
Right	Parahippocampal Gyrus	28	38	-12	-20	6.47*
Right	Caudate	N/A	10	0	18	6.31*
Right	Superior Frontal Gyrus	8	12	56	38	5.62*
Right	Middle Temporal Gyrus	21	62	2	-26	4.41*
Right	Orbital Frontal Gyrus	46	54	44	-2	4.26*
Right	Lingual Gyrus	19	28	-70	6	4.23*
Right	Paracentral Lobule	4	8	-28	74	4.17*
Right	Superior Temporal Gyrus	38	50	10	-18	4.09*
Right	Inferior Temporal Gyrus	21	60	-20	-20	3.99*
Left	Thalamus	N/A	-18	-28	12	7.15*
Left	Caudate	N/A	-10	0	22	6.43*
Left	Parahippocampal Gyrus	28	-24	-40	4	6.18*
Left	Orbital Frontal Cortex	45	-16	32	-12	6.13*
Left	Claustrum	N/A	-26	28	-10	5.36*
Left	Inferior Frontal Gyrus	45	-56	28	2	5.25*
Left	Superior Temporal Gyrus	38	-42	6	-20	5.06*
Left	Paracentral Lobule	4	-8	-34	72	4.58*
Left	Middle Temporal Gyrus	21	-62	-12	-14	4.13*
Left	Pallidum	N/A	-14	4	-5	3.72*
Left	Superior Parietal Lobule	7	-24	-78	48	3.71*
Left	Prefrontal Cortex (pFC)	6	-28	22	52	3.66*
Left	Superior Frontal Gyrus	6	-10	12	66	3.61*

Note BA= Brodmanns Area. *uncorrected p <.001

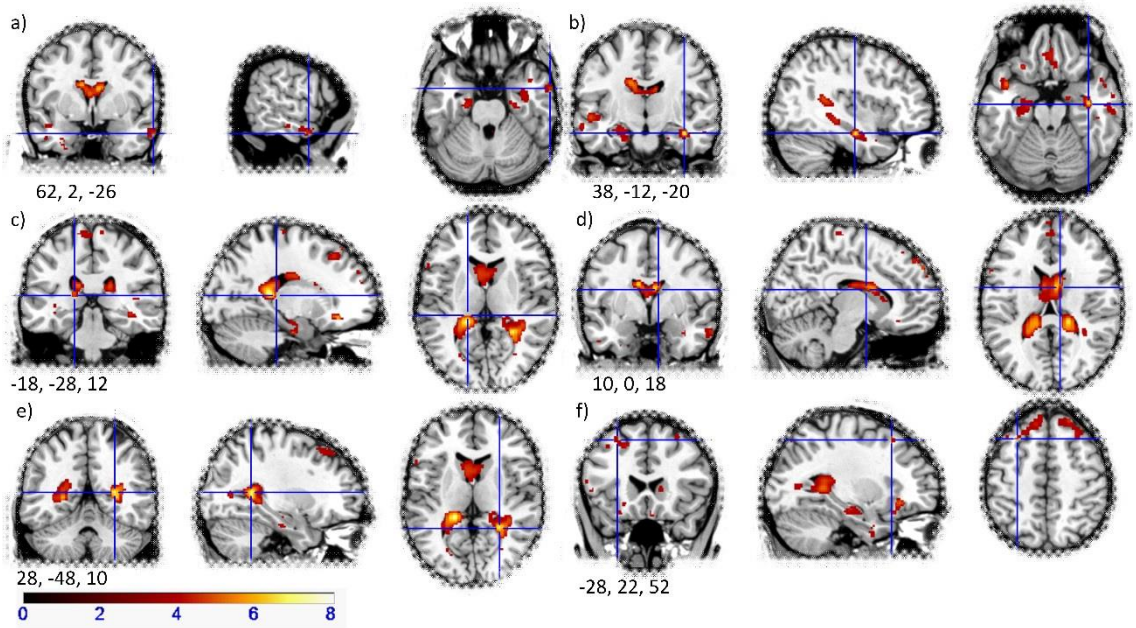


Figure 7.8 Regions of increased activation in the MS handgrip first group during the last block of the extrinsic alertness task. *a) Middle Temporal Gyrus. b) Parahippocampal Gyrus. c) Thalamus. d) Caudate. e) Precuneus. f) Prefrontal Cortex (pFC). MNI Coordinates (x,y,z). p<0.001 uncorrected.*

The brain regions with significant activation during the last block of the extrinsic alertness task (T3 Block 4- T3 Block 1) for the MS imagery first group are shown in Table 7.12. The bilateral superior frontal gyrus, parahippocampal gyrus, specifically left hemisphere, and caudate demonstrated the most marked increased BOLD signal.

Table 7.12 Regions of peak activation for the MS imagery first group during the final block of the extrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Superior Frontal Gyrus	6	14	38	56	4.39*
Right	Middle Temporal Gyrus	21	66	-14	-18	3.89*
Right	Caudate	24	12	10	20	3.65*
Right	Parahippocampal Gyrus	28	30	-42	6	3.54*
Left	Superior Frontal Gyrus	9	-26	60	22	4.76*
Left	Parahippocampal Gyrus	28	-28	-36	6	4.21*
Left	Orbital Frontal Gyrus	46	-48	48	0	4.15*
Left	Inferior Parietal Lobule	40	-56	-54	42	4.01*
Left	Caudate	N/A	-8	6	22	3.78*
Left	Inferior Frontal Gyrus	46	-52	42	4	3.76*

Note BA= Brodmanns Area. *uncorrected p <.001

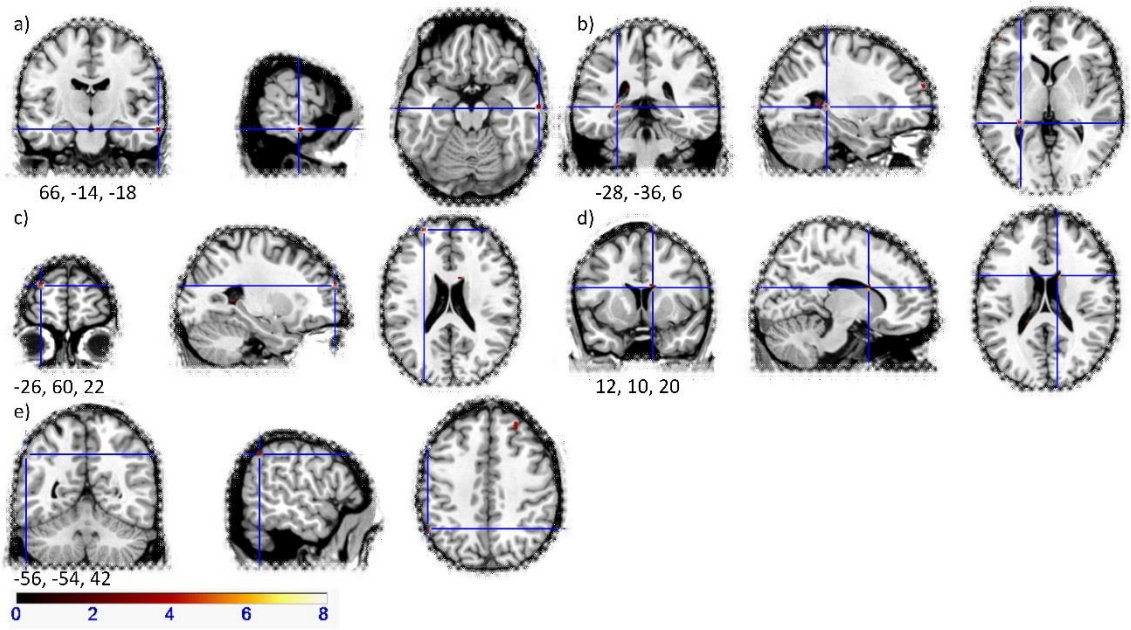


Figure 7.9 Regions of increased activation in the MS imagery first group during the last block of the extrinsic alertness task. *a) Middle Temporal Gyrus. b) Parahippocampal Gyrus. c) Superior Frontal Gyrus. d) Caudate. e) Inferior Parietal Lobule. MNI Coordinates (x,y,z). p<0.001 uncorrected.*

For the direct comparison between MS handgrip first and MS imagery first groups during the last block of the extrinsic alertness task (T3 Block 4- T3 Block 1) showed increased activation in the MS handgrip first group in the basal ganglia, parahippocampal gyrus, and inferior temporal gyrus. The results are summarised in Table 7.13 below.

Table 7.13 Regions of greater peak activation for the MS handgrip first group compared to the MS imagery first group during the last block of the extrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Parahippocampal Gyrus	28	46	-26	-20	4.23*
Right	Fusiform Gyrus	20	42	-36	-22	4.03*
Right	Caudate	N/A	12	13	18	3.95*
Right	Superior Temporal Gyrus	41	44	-30	6	3.76*
Right	Precentral Gyrus	6	42	-10	36	3.76*
Right	Middle Temporal Gyrus	21	58	6	-30	3.61*
Left	Parahippocampal Gyrus	28	-16	-16	-28	4.39*
Left	Prefrontal Cortex (pFC)	10	-52	38	-10	4.35*
Left	Middle Temporal Gyrus	21	-50	-2	-36	4.10*
Left	Inferior Temporal Gyrus	20	-46	-44	-24	3.98*
Left	Caudate	N/A	-12	-2	16	3.83*
Left	Precentral Gyrus	4	-42	-10	38	3.81*
Left	Superior Temporal Gyrus	22	-50	-14	-6	3.80*
Left	Inferior Frontal Gyrus	44	-60	16	14	3.60*

Note BA= Brodmanns Area. *uncorrected p <.001

7.3.8 Group Comparison between the HC and MS Groups

For intrinsic alertness (T2-T1) the HC group had increased activations in the right anterior cerebellum and left Prefrontal Cortex (pFC) compared to the MS group. Whereas the MS group showed greater activation in bilateral precentral gyrus and inferior temporal gyrus compared to the HC group. All regions of greater activations are displayed in Table 7.14 and Table 7.15.

Table 7.14 Regions of greater peak activation for the HC group compared to the MS group during intrinsic alertness.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Cerebellar Tonsil	N/A	48	-52	-48	3.55*
Left	Prefrontal Cortex (pFC)	46	-48	52	2	3.58*

Note BA= Brodmanns Area. *uncorrected p <.001

Table 7.15 Regions of greater peak activation for the MS group compared to the HC group during intrinsic alertness.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Inferior Temporal Gyrus	20	50	-36	-18	4.16*
Right	Precentral Gyrus	4	46	-10	58	3.99*
Right	Declive	N/A	22	-78	-16	3.83*
Right	Substantia Nigra	N/A	12	-24	-10	3.72*
Right	Calcarine Cortex	18	16	-84	2	3.63*
Right	Precuneus	7	14	-56	52	3.61*
Right	Culmen	N/A	0	-88	-20	3.55*
Left	Precentral Gyrus	4	-38	-8	56	3.96*
Left	Declive	N/A	-14	-62	-18	3.85*
Left	Culmen	N/A	-26	-36	-30	3.75*
Left	Lingual Gyrus	18	-22	-75	4	3.73*
Left	Postcentral Gyrus	1	-58	-14	48	3.60*
Left	Inferior Temporal Gyrus	20	-46	-36	-20	3.54*

Note BA= Brodmanns Area. *uncorrected p <.001

For extrinsic alertness (T3-T2), the MS group showed no regions of greater activation than the HC group. The HC group displayed increased activation of the bilateral postcentral gyrus and left Prefrontal Cortex (pFC) compared to the MS group (Table 7.16). No regions survived the original FWE corrected p< .05 significance threshold, but reaction time differences were observed, therefore the threshold was lowered to uncorrected p <.001 with a T-value of 3.5 and above.

Table 7.16 Regions of greater peak activation for the HC group compared to the MS group for extrinsic alertness.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Postcentral Gyrus	4	68	-6	24	3.75*
Left	Postcentral Gyrus	4	-64	-12	36	4.04*
Left	Middle Temporal Gyrus	21	-62	2	-16	3.78*
Left	Precentral Gyrus	6	-62	0	34	3.66*
Left	Prefrontal Cortex (pFC)	8	-28	46	38	3.59*
Left	Superior Temporal Gyrus	38	-48	22	-34	3.54*
Left	Superior Frontal Gyrus	8	-24	48	38	3.53*

Note BA= Brodmanns Area. *uncorrected p <.001

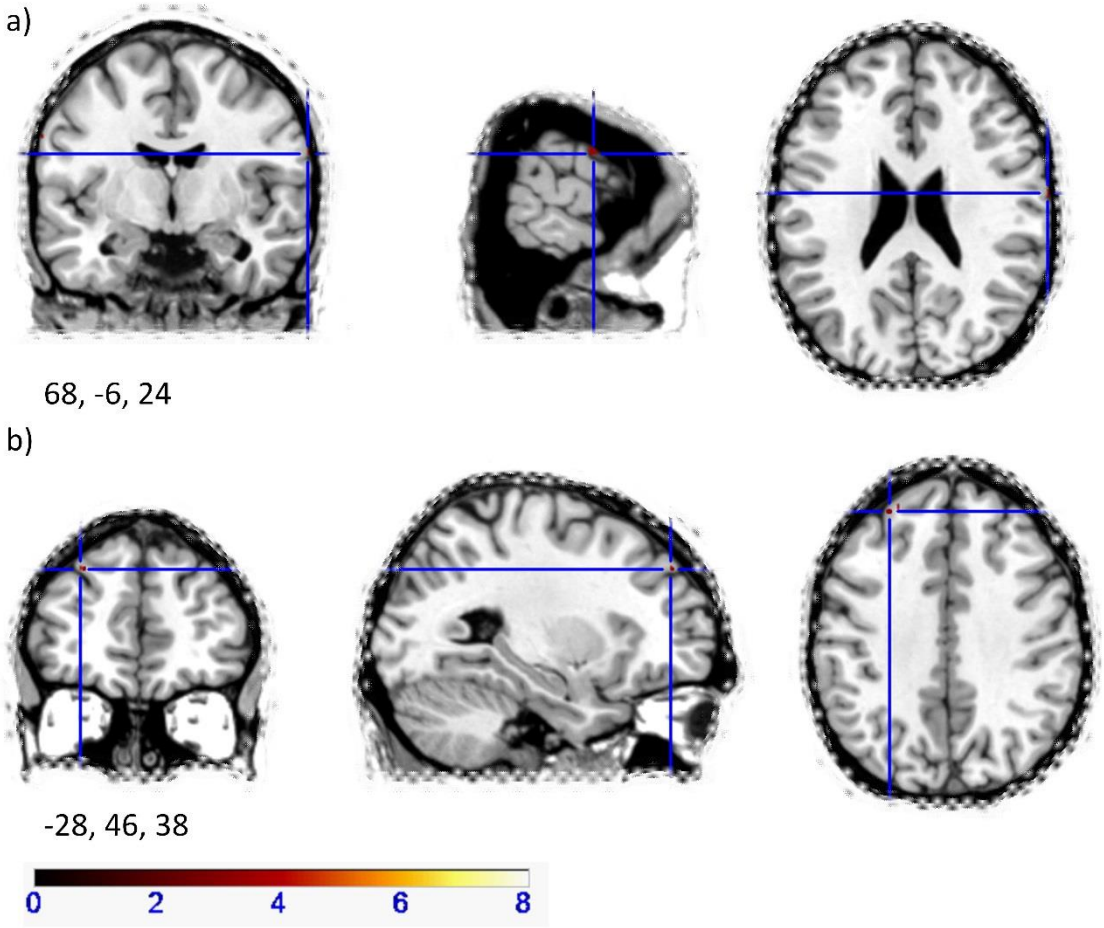


Figure 7.10 Regions of increased activation in the HC group compared to the MS group during extrinsic alertness. *a) Prefrontal Cortex (pFC). b) Postcentral Gyrus. MNI Coordinates (x,y,z). p<0.001 uncorrected.*

7.3.9 Multiple regression with FSS scores

For intrinsic alertness (T2-T1), fatigue was correlated both positively (Table 7.17) and negatively (Table 7.18) only in the left hemisphere in the postcentral gyrus.

Table 7.17 Regions of peak activation positively correlated with fatigue scores in the intrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Left	Postcentral Gyrus	1	-50	-18	56	3.65*

Note BA= Brodmanns Area. *uncorrected p <.001

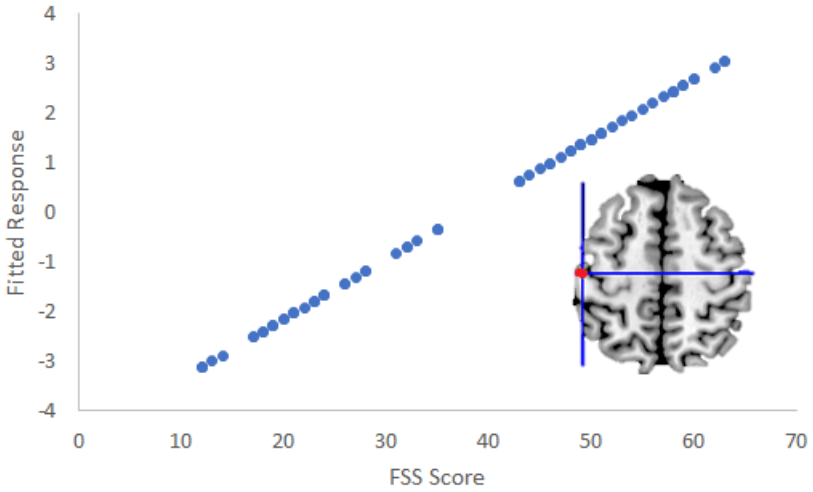


Figure 7.11 Scatterplot of fitted responses at [-50, -18, 56] and FSS scores.

Table 7.18 Regions of peak activation negatively correlated with fatigue scores in the intrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Left	Middle Temporal Gyrus	38	-32	24	-36	4.46*
Left	Cerebellar Tonsil	N/A	-52	-56	-40	3.99*

Note BA= Brodmanns Area. *uncorrected p <.001

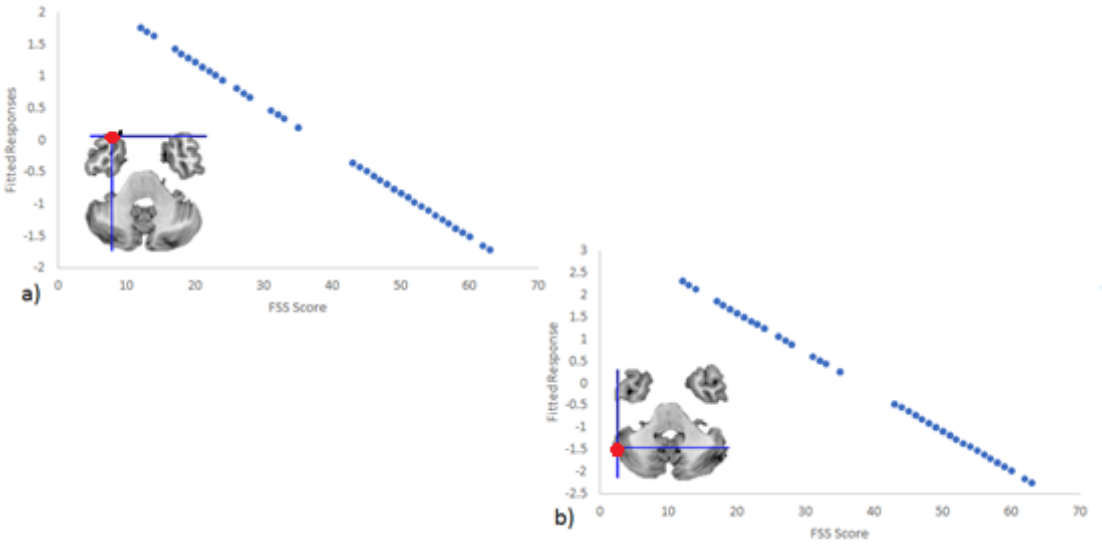


Figure 7.12 Composite image of scatterplots of fitted responses and FSS scores. *a) fitted responses at [-32, 24, -36]. b) fitted responses at [-52, -56, -40].*

During extrinsic alertness (T3-T2) FSS scores were correlated positively with the left inferior temporal gyrus (Table 7.19) and negatively with the right Prefrontal Cortex (pFC) and putamen and left pre- and postcentral gyrus (Table 7.20).

Table 7.19 Regions of peak activation positively correlated with fatigue scores in the extrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Left	Inferior Temporal Gyrus	20	-64	-32	-20	3.78*

Note BA= Brodmanns Area. *uncorrected p <.001

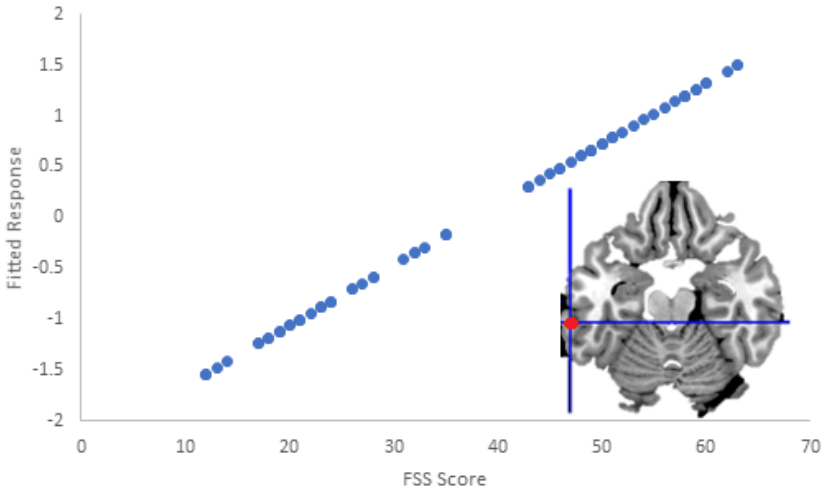


Figure 7.13 Scatterplot of fitted responses at [-64, -32, -20] and FSS scores.

Table 7.20 Regions of peak activation negatively correlated with fatigue scores in the extrinsic alertness task.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Prefrontal Cortex (pFC)	9	60	26	24	4.35*
Right	Putamen	N/A	28	4	16	3.98*
Left	Postcentral Gyrus	6	-62	-2	34	3.89*
Left	Precentral Gyrus	6	-54	2	46	3.71*

Note BA= Brodmanns Area. *uncorrected p <.001

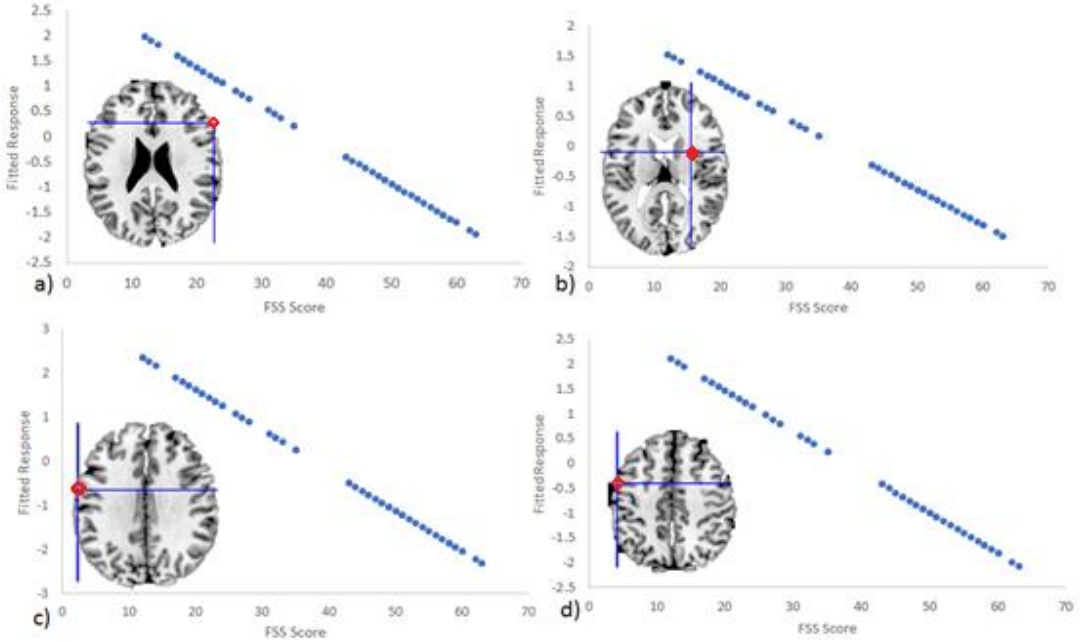


Figure 7.14 Composite image of scatterplots of fitted responses and FSS scores. *a) fitted responses at [52, 26, 35]. b) fitted responses at [28, 4, 16]. c) fitted responses at [-62, -2, 34]. d) fitted responses at [-54, 2, 46].*

7.4 Discussion

The primary aim of this chapter was to examine the neural correlates that underlie the fatiguability noted in the behavioural performance and the secondary aims were to explore whether the pattern of activation between the MS and HC groups differ, in line with the behavioural differences. To understand the impact of fatiguability fully, the basic alertness contrasts and comparison between groups are discussed first.

7.4.1 Alertness mechanism in the HC group

The results from the current study suggest that the intrinsic alertness mechanism involves a basic attentional system that determines relevant stimuli and then increases the readiness to respond to this stimulus. During intrinsic alertness, the HC group activate the bilateral inferior occipital gyrus. This region of activation is not consistent with the frontal-parietal-brainstem-thalamic network reported in some previous studies (Mottaghy *et al.*, 2006; Perin *et al.*, 2010; Sturm *et al.*, 1999; 2004). The inferior occipital gyrus is the main region consistently activated in alertness tasks in the majority of the fMRI studies in the systematic review (refer to Chapter 3 page 54). The inferior occipital gyrus was reported to be active in ten of thirteen available fMRI studies reviewed (Bartes-Serrallonga *et al.*, 2014; Clemens *et al.*, 2011; 2013; Fan *et al.*, 2005; Kellerman *et al.*, 2011; Lawrence *et al.*, 2003; Sturm *et al.*, 2006; Tana *et al.*, 2010; Thiel *et al.*, 2004; Xuan *et al.*, 2016). The occipital lobe is generally accepted to be associated with the processing of visual information. In the current study, the intrinsic alertness contrast has subtracted the control sensorimotor task and thereby indicates that the inferior occipital lobe is associated with intrinsic alertness and not just visual processing. Studies have shown that the inferior occipital cortex is not only activated in visual alertness tasks but also in auditory alertness tasks (Daumann *et al.*, 2010; Thiel and Fink, 2007), suggesting an element of attentional demand. Brefczynski and DeYoe (1999) indicated that the inferior occipital cortex was related to the attentional shifts in processing and not just the processing of visual stimuli itself. Similarly, Somers *et al.* (1999) suggested that the visual cortex is influenced by attentional demand, specifically by increasing the responses to attended stimuli and inhibiting responses to distractor stimuli. The consistent reporting of the inferior occipital gyrus suggests that alertness requires an attentional readiness.

The alertness mechanism is controlled by an automatic bottom-up and voluntary top-down network (Mottaghy *et al.*, 2006; Sturm and Wilmes, 2001). The bottom-up network consists of the noradrenergic system. It originates in the locus coreleus in the brainstem

and relays through the thalamus which projects to frontal and parietal regions (Posner and Petersen, 1990; Robbins, 1984). This noradrenergic activity is governed by a top-down network, consisting of the ACC and the dorsolateral pFC, enabling the individual to selectively focus attention to the target stimulus (Mottagy *et al.*, 2006; Sturm *et al.*, 1999; 2004).

The results of the current study suggest that extrinsic alertness requires higher order attentional control. The extrinsic alertness task involves a cue which warns participants that the target stimulus is about to appear, this should result in a decreased reaction time. In the HC group, the behavioural results confirm this, which demonstrates that the HC respond to the warning effect and are completing the task correctly. In terms of neuroimaging, the HC group exhibited increased activation in the bilateral dorsolateral pFC. The pFC is associated with executive function, this finding has been robust across multiple modalities. Several lesion studies have reported that damage to the pFC produces poor judgment, planning, and decision-making (Fuster, 1997; Goldman-Rakic, 1996; Stuss and Benson, 1986). Structural imaging found that larger volume and thickness of the pFC were associated with better executive performance (Morgen *et al.*, 2005; Yuan and Raz, 2014). Furthermore, functional imaging studies provide even further evidence of an association between pFC activation and executive functions (Engle *et al.*, 1999; Knight *et al.*, 1995; Rossi *et al.*, 2009). Similarly, meta-analyses of neuroimaging studies have shown that the pFC, along with the ACC, was consistently activated during the Wisconsin card sort test (Buchsbaum *et al.*, 2005) and the Stroop task (Laird *et al.*, 2005), both of which are tests of executive function. The activation of the pFC during extrinsic alertness indicates that the warning cue creates a necessity for some higher cognitive function compared to the intrinsic alertness. This may be due to the need to inhibit a response to the warning cue (wait for stimulus), rather than simply maintaining readiness. This means the participants have more information about when the stimulus will appear, allowing for increased preparation. The activations of the extrinsic task are more consistent with previous literature of a noradrenergic system projecting through the thalamus to frontal regions (Posner and Petersen, 1990; Robbins, 1984), which in turn enable the participant to selectively focus attention to the target stimulus (Mottagy *et al.*, 2006; Sturm *et al.*, 1999; 2004).

In summary, the results demonstrate that the intrinsic alertness mechanism is a basic state of readiness where brain regions involved with increased attentional readiness are

activated. The extrinsic alertness mechanism, on the other hand, involves higher order attentional control involving the pFC.

7.4.2 Alertness mechanism in the MS group

The MS group can adequately recruit the basic muscle and attentional readiness mechanism required by the intrinsic alertness task, but they do require some compensatory mechanisms to complete the task. During the intrinsic alertness task, the MS group demonstrated similar activations to the HC group in the occipital gyrus. This provides further support that the intrinsic alertness task requires a more basic state of readiness and indicates that the MS group can adequately recruit an intrinsic alertness mechanism. Behaviourally, the MS group have significantly slower reaction times compared to the HC group. It is possible that the MS group are recruiting the appropriate mechanism, but the speed of information processing is disrupted, evidenced by increased reaction times. This may be due to the persistent fatigue in this group.

The direct comparison between HC and MS group during the intrinsic alertness revealed more widespread activation in the MS group including the cerebellum, precentral gyrus, postcentral gyrus, inferior temporal gyrus, calcarine cortex and lingual gyrus. In the HC group consistent increased activation of the pFC was observed. The inferior temporal gyrus has connections to visual cortices, limbic and frontal regions. It has been implicated as the link between the visual cortices and frontal (executive) and limbic (memory) regions (Miyashita, 1993; Riches *et al.*, 1991). Its increased activation in the MS group may suggest that the MS group adequately process information from visual cortices, but with the lack of frontal activation, they fail to project this information to higher order regions and as such use the inferior temporal gyrus to process the information. Furthermore, the inferior temporal gyrus is positively correlated with fatigue scores in this dataset. As such the increased activation seen in the MS group may be contributing to the increased fatigue.

The precentral gyrus and cerebellum have been found to be involved in the preparation and optimization of motor responses (Berardelli *et al.*, 1996; Manto *et al.*, 2010; Morton and Bastian, 2006; Pedersen *et al.*, 1998; Porro *et al.*, 2000; Toma *et al.*, 2002). The increased activation in the MS group demonstrates that, although they can increase motor readiness required in the intrinsic alertness task, they recruit additional brain regions to perform this task. This is consistent with previous findings of increased activation in the motor cortex of MS patients compared to HC during simple finger movements (Lee *et al.*,

2000) and finger tapping (Reddy *et al.*, 2000). Similarly, the increased activation of the lingual gyrus and calcarine cortex may represent a more widespread compensatory neural network to complete the task. Widespread activation to complete a cognitive task is well established in the MS literature (Bonnet *et al.*, 2010; Lee *et al.*, 2000; Lopez-Gongora *et al.*, 2015; Mainero *et al.*, 2006; Pantano *et al.*, 2002a; 2002b; Parry *et al.*, 2003; Reddy *et al.*, 2000; Rocca *et al.*, 2002; Staffen *et al.*, 2002; Wishart *et al.*, 2004). The need to recruit additional brain regions has been proposed to contribute to cognitive fatigue, due to increased metabolic demand (Lange *et al.*, 2005). The direct relationship to fatigue remains unknown (Mainero *et al.*, 2006; Penner *et al.*, 2003; Tartaglia *et al.*, 2008).

The MS group further demonstrate significant disruptions to the pFC, which may indicate a significant disruption to executive functioning. Despite being able to recruit the appropriate mechanisms to complete the intrinsic alertness task, a direct comparison between the HC and MS groups revealed increased activation of the pFC in the HC group. The pFC is an established region involved in executive function (Buchsbaum *et al.*, 2005; Engle *et al.*, 1999; Fuster, 1997; Goldman-Rakic, 1996; Knight *et al.*, 1995; Laird *et al.*, 2005; Rossi *et al.*, 2009; Stuss and Benson, 1986; Yuan and Raz, 2014). Interestingly, this region is also correlated negatively with fatigue scores in the current data. Which is consistent with the increased activation in the HC group who have lower fatigue scores. The results suggest that the pFC may not be required for intrinsic alertness, but its activation is associated with decreased fatigue scores, and the MS group do not recruit the pFC to the same extent as the HC group. The disruption to pFC becomes more apparent in the extrinsic alertness task. The behavioural reaction time analysis indicated that the MS group do not respond to the warning cue and are therefore unable to perform extrinsic alertness. No neuronal activations that survive even the less stringent threshold are observed in the MS group. The results indicate that the MS group are using a basic intrinsic alertness mechanism to complete the extrinsic alertness task, and as such exhibited a significant behavioural deficit. The direct comparison between the HC and MS group revealed an increased activation of the pFC, precentral, postcentral and temporal regions in the HC group. The increased activation in the HC group is not surprising, given that the MS group do not recruit any brain regions associated with extrinsic alertness. Interestingly, both the precentral and postcentral gyrus, which demonstrated increased activation in the HC group, are negatively correlated with fatigue scores. Together with the increased activation in the inferior temporal gyrus of the MS group, associated with increased fatigue scores, the results provide functional evidence of increased fatiguability

in the MS group during basic alertness. Similarly, the results showed that the MS group are unable to recruit mechanisms they may allow for reduced fatiguability, thus fatigue reduction in the extrinsic alertness task. The increased activation of the pFC in the HC group provides further support that the MS group have a deficit in higher cognitive functions. This deficit becomes more noticeable in the extrinsic alertness task, as the pFC is required for successful task performance.

7.4.3 Fatiguability in the HC group

The results discussed in this section did not survive the multiple comparisons threshold. As such the interpretations are made cautiously. The results are indicative of trends that may be seen when adequately powered, however inferences cannot be drawn from the uncorrected results. In the healthy population, there is consistent increased activation towards the end of the task. Increased activation, in clinical populations, has been proposed to be associated with fatigue (Lange *et al.*, 2005), but the trends noted in the current results in the HC group do not support this. Rather than increased activation being generally associated with fatigue, the regions demonstrating increased activation are very important, as some regions may provide a mitigating effect on fatiguability. In block four of intrinsic alertness the HC handgrip first group had increased reaction time in the final block of the task, indicating increased fatiguability. This behavioural performance was associated with increased activation in the pFC, basal ganglia, cerebellum, thalamus, inferior temporal gyrus and orbital frontal gyrus. These results may suggest that these regions exert a mitigating effect on fatigue.

The multiple cortical loops associated with the basal ganglia, indicates that it can serve multiple functions including motor, emotions and behaviour. The focus in this thesis is with the function in goal-directed behaviour. The basal ganglia network is associated with motivation. It has strong connections to the striatum, limbic system, through the thalamus (Haber and Calzavara, 2009), and projects to frontal cortices (Alexander, 1986; Chaudhuri and Behan, 2000). The widespread connections indicate that the basal ganglia network incorporates information from the limbic system with the dopaminergic, striatal, and then projects to the frontal cortex for decision making in goal-directed behaviour. These neuroanatomical projections are consistent with the increased activation in the pFC and thalamus in the current study. Furthermore, studies have posited that the orbital frontal cortex is involved in goal-directed behaviour through its reward functions (Hollerman *et al.*, 2000; Murray *et al.*, 2007; Schoenbaum *et al.*, 2011; Valentin *et al.*, 2007).

This may indicate a form of hierarchy between the basal ganglia and the orbital frontal cortex. Where the basal ganglia can signal the possibility or expectation of reward, and the orbital frontal cortex can determine the most rewarding behaviour from multiple outcomes. Chaudhuri and Behan (2000) have hypothesised that through its motivation function the basal ganglia is implicated in fatigue. Together with the decrease in behavioural performance in this group, the current result trends are suggestive the basal ganglia is implicated in fatigue or fatiguability, even in an HC group.

The HC imagery first group, similarly, had increased activation in block four of the task compared to the first block, in the middle temporal gyrus, parahippocampal gyrus, precuneus, medial frontal gyrus and inferior frontal gyrus. This group exhibited no behavioural differences across the paradigm, indicating no increased fatiguability. Furthermore, the regions of increased activation differ. The middle temporal gyrus has been associated with both verbal and non-verbal semantic processing (Chertkow *et al.*, 1997; Hoffman *et al.*, 2007; Visser *et al.*, 2012), multi-modal sensory integration (Binder *et al.*, 2009; Mesulam, 1998) and lexical retrieval (Baldo *et al.*, 2013; DeLeon *et al.*, 2007; Grossman *et al.*, 2004). Given its widespread function, it is difficult to determine its specific function without considering the other regions activated at the same time. Studies have demonstrated that the precuneus is involved in recollection and episodic memory (Fletcher *et al.*, 1995; Henson *et al.*, 1999; Shallice *et al.*, 1994; Wagner *et al.*, 2005), source memory (Lundstrom *et al.*, 2003; 2005) and familiarity (Vilberg and Rugg, 2008). A meta-analysis conducted by Skinner and Fernandez (2007) showed that the precuneus is associated with both memory recollection and familiarity. Similarly, the parahippocampal gyrus is part of the medial temporal lobe (Squire *et al.*, 2004), which is well known to subserve memory functions. Furthermore, the parahippocampal gyrus has been associated with the processing of working memory (Duzel *et al.*, 2003), specifically when the memories were later successfully retrieved (Wagner *et al.*, 1998). Together the activation trends of the middle temporal gyrus, precuneus and parahippocampal gyrus, along with the higher order functions of the frontal lobes, may be suggestive of a beneficial effect of practise on behavioural performance in a HC group. This trend is further evidenced by the multiple regression that demonstrates the middle temporal gyrus is negatively correlated with fatigue scores, indicating that increased activation of this region may reduce the effect of fatiguability. The trends in the current results may suggest that through learning and practise task performance becomes easier, and less effortful.

The trends noted in the direct comparison between the HC handgrip first and HC imagery first groups showed further evidence suggesting that the memory regions, specifically the precuneus and parahippocampal gyrus may underlie the learning effect in the HC imagery first group. The behavioural analysis revealed that the HC handgrip first group had significantly increased reaction times in block four of intrinsic alertness compared to the HC imagery first group. This behavioural difference was accompanied by activation of the orbital frontal gyrus, cuneus, and inferior temporal gyrus. Whereas, in the HC imagery first group, increased activation in the parahippocampal gyrus and precuneus was observed. The cuneus is specifically involved in attention-orienting behaviour (Corbetta and Shulmann, 2002; Simpson *et al.*, 2011). The orbital frontal cortex is associated with goal-directed behaviour (Hollerman *et al.*, 2010; Murray *et al.*, 2007; Schoenbaum *et al.*, 2011; Valentin *et al.*, 2007). The inferior temporal gyrus has been implicated in a relay of information processing between visual, frontal and limbic areas (Miyashita, 1993; Riches *et al.*, 1991). The increased activation of these three regions together suggests that the HC handgrip first group recruited regions that simply orient attention and then relay information to frontal regions. The increased activation of the precuneus and the parahippocampal gyrus in the HC imagery first group demonstrates that this group recruits memory systems to a greater extent than the group that completed the handgrip condition first. Given the behavioural difference, it is possible that the neuronal shift towards memory systems may enhance performance, thereby producing a learning effect in behavioural performance, rather than the fatiguing effect initially hypothesised by the inclusion of the mental imagery condition.

Increased neuronal activation, in clinical populations, has been proposed to be associated with fatigue (Lange *et al.*, 2005). In a healthy population, the increased activation could be argued as brain regions that may actually provide a mitigating effect of fatiguability. The HC handgrip first group showed increased reaction time due to increased fatiguability, which was associated with increased activation in the basal ganglia, supporting the hypothesis that the basal ganglia is implicated in fatigue. Whereas the group that completed the mental imagery condition first showed no behavioural evidence of fatiguability, which was associated with increased activation in regions associated with memory retrieval. Furthermore, the same regions showed increased activation in this group when comparing between the two groups. This indicates that the increased recruitment of memory regions may enhance behavioural performance, despite the

increased metabolic demand. Thereby demonstrating that the hypothesis that increased neural activation is implicated in fatigue is too general.

7.4.4 Fatiguability in the MS group

The results discussed in this section did not survive the multiple comparisons threshold. As such the interpretations are made cautiously. The results are indicative of trends that may be seen when adequately powered, however inferences cannot be drawn from the uncorrected results. Similarly to the HC groups, both the MS handgrip first and MS imagery first groups showed increased activation in the last block of the alertness-motor paradigm during both intrinsic and extrinsic alertness. Here the trends are more consistent than in the HC split groups, this makes sense as the healthy volunteers do not suffer from significant fatigue as the individuals with MS do. The results further suggest that the additional regions of recruitment do not necessarily enhance the perception of fatigue as proposed by Lange *et al.* (2005). But some regions may mitigate the effect of fatiguability on performance. Specifically, the basal ganglia seems to mitigate the effect of fatiguability. During the intrinsic alertness task, both the MS handgrip first and MS imagery first group showed increased activation in the parahippocampal gyrus, middle temporal gyrus, pFC, and basal ganglia. The increased activation of the basal ganglia further suggests that this region is implicated in fatigue (Bruno *et al.*, 1998; Chaudhuri and Behan, 2000; 2004; Friedman and Friedman, 1993; Roelcke *et al.*, 1997; van Hilten *et al.*, 1993). Together with the activation of the pFC, this specifically implicates the associative cortical loop in MS fatigue. Furthermore, both groups demonstrate increased recruitment of memory regions (Baldo *et al.*, 2013; Binder *et al.*, 2009; Chertkow *et al.*, 1997; DeLeon *et al.*, 2007; Grossman *et al.*, 2004; Hoffman *et al.*, 2007; Mesulam, 1998; Visser *et al.*, 2012) towards the end of the task. However, the behavioural patterns between the groups differ. The trends emerging from the direct comparison between the two groups provides some suggestions to disentangle the relationship. The MS imagery first group, who did not show increased fatiguability, had increased activation of the basal ganglia and frontal cortex compared to the MS handgrip first group. This trend is suggestive that not only is the basal ganglia implicated in fatigue, but it may actually provide a mitigating effect of fatiguability/fatigue on behavioural performance. Again, both the pFC and the basal ganglia exhibited increased BOLD signal providing further evidence that the associative cortical loop is implicated. The basal ganglia network activation may be involved in mitigating the effect of fatiguability but is not always successful in producing a positive outcome behaviourally, as seen in the MS handgrip first group. This seems consistent with

the motivation functions of the basal ganglia, as reduced motivation or reward expectation may lead to reluctance to act. This is further supported by studies that have shown damage to the basal ganglia in diseases with increased fatigue such as Parkinson's disease (Friedman and Friedman, 1993; van Hilten *et al.*, 1993), post-polio fatigue (Bruno *et al.*, 1998) and MS (Roelcke *et al.*, 1997). It is possible that the increased activation of memory regions is observed as the MS group grow more familiar with the task and recollect information regarding the task. But this activation does not lead to the learning effect as noted in the HC group.

Both the MS handgrip first and imagery first groups revealed increased activation in the last block of the intrinsic alertness task. Unlike previous studies (Lange *et al.*, 2005), the current trends of increased activation does not necessarily mean increased fatigue. The MS handgrip first group showed no increased activation compared to the MS imagery first group, but they did have significantly decreased reaction time. This suggests that the functional pattern of activation recruited by the MS imagery first group may provide a mitigating effect of fatiguability. This mitigation of fatigue may be largely driven by the associative cortical loop involving the basal ganglia and the pFC. This supports the hypothesis that the basal ganglia is implicated in fatigue, but specifically indicates that it may play a role in mitigating the effect of fatigue.

The trends of activation emerging from the extrinsic alertness task provide further support that the basal ganglia may mitigate the effect of fatiguability. The MS handgrip first group showed no increased reaction time across the task. This behavioural pattern was associated with increased activation in the basal ganglia, parahippocampal gyrus, thalamus, temporal cortex, frontal cortex and parietal lobe. The increased activation of the basal ganglia provides further evidence for its involvement in the mitigation of fatiguability. This would be consistent with findings that found disruption to the basal ganglia is evident in disorders characterised by increased fatigue (Bruno *et al.*, 1998; Friedman and Friedman, 1993; Roelcke *et al.*, 1997; van Hilten *et al.*, 1993). Together with increased frontal cortex activation, implicates the associative cortical loop in the mitigation of fatiguability. Furthermore, in the extrinsic alertness task, the multiple regression demonstrates a negative correlation between basal ganglia activation and FSS scores. Supporting the idea that increased basal ganglia activation may mitigate the effect of fatiguability. Furthermore, the activation in the latter part of the extrinsic alertness task seems more consistent with the frontal-parietal-brainstem-thalamic network reported in some previous studies (Mottaghy *et al.*, 2006; Perin *et al.*, 2010; Sturm *et al.*, 1999; 2004).

The fact that this group recruit the frontal-parietal-brainstem-thalamic network towards the end of the task, together with the precuneus activation, may suggest that the MS handgrip first group are becoming more familiar with the task (Fletcher *et al.*, 1995; Henson *et al.*, 1999; Shallice *et al.*, 1994; Wagner *et al.*, 2005) and are therefore able to recruit higher order structures involved in extrinsic alertness. However, this does not change the finding that the MS group have significantly disrupted extrinsic alertness.

For the MS imagery first group, the behavioural results indicated an increased reaction time across the task and in terms of neuroimaging showed increased activation in regions including the basal ganglia, parahippocampal gyrus, temporal cortex, frontal cortex, and precuneus. The activations provide further evidence that the associative cortical loop of the basal ganglia is involved in fatigue. However, in this group its activation does not successfully mitigate fatiguability. Moreover, this group show increased memory recruitment towards the end of the task (Binder *et al.*, 2009; Baldo *et al.*, 2013; Chertkow *et al.*, 1997; DeLeon *et al.*, 2007; Duzel *et al.*, 2003; Fletcher *et al.*, 1995; Grossman *et al.*, 2004; Henson *et al.*, 1999; Hoffman *et al.*, 2007; Mesulam, 1998; Shallice *et al.*, 1994; Skinner and Fernandez, 2007; Villberg and Rugg, 2008; Visser *et al.*, 2012; Wagner *et al.*, 1998; 2005) but again this does not successfully mitigate the effect of fatiguability in the extrinsic alertness task.

When comparing between the two groups the opposite is observed than in intrinsic alertness. Namely only the MS handgrip first group showed increased activation. The increase was observed in the temporal gyri, precentral gyrus, parahippocampal gyrus, basal ganglia and frontal cortex. The resulting trends from this comparison may suggest that during extrinsic alertness in the MS groups, both the recruitment of the associative cortical loop of the basal ganglia and the recruitment of memory regions may mitigate the influence of fatiguability.

The consistent trend of increased activation in the last block of the task, in both the HC and MS groups, is noteworthy. Several studies have argued that increased activation leads to increased neural resources, such as glucose and oxygen, being required and that this may provide an explanation for the increased fatigue (Casereas *et al.*, 2006; Cook *et al.*, 2007; Lange *et al.*, 2005). However, the results of the current study demonstrate the opposite. The increased activation does not necessarily cause an increase in mental effort, which is consistent with Penner *et al.* (2003) that showed increased activation in MS that

did not increase with the severity of fatigue. But rather the results indicate that some of these regions are compensatory in an attempt to reduce the effect of fatiguability.

In the present study, the trend towards recruitment of memory regions, perhaps induced by the practise of mental imagery, and the associative loop of the basal ganglia is associated with the mitigation of fatigue. In the HC group, the recruitment of memory regions had a larger impact on the influence of fatiguability on behavioural performance. This demonstrates that the HC group can overcome the effect of fatiguability through learning and practise. This result is not surprising as the HC group do not suffer from significant fatigue as the MS group do. In the MS group, the associative cortical loop of the basal ganglia is more consistently associated with the mitigation of fatiguability. Interestingly, all MS groups, regardless of behavioural performance, recruited the basal ganglia to a greater extent in block four than block one of both intrinsic and extrinsic alertness. Given that the current results indicate the basal ganglia may mitigate the influence of fatiguability. It is possible to propose that the MS group recruit the basal ganglia to mitigate fatigue, however, are not always able to do this successfully. This may be due to the structural atrophy, noted in Chapter 4 (page 71) and multiple studies (Bisecco *et al.*, 2018; Calabrese *et al.*, 2010; Derache *et al.*, 2013; Finke *et al.*, 2015; Lansley *et al.*, 2013; Prinster *et al.*, 2005; Riccitelli *et al.*, 2012; Sepulcre *et al.*, 2006; van de Pavert *et al.*, 2014; Zhang *et al.*, 2017) disrupting the function of the basal ganglia.

The results demonstrate that the paradigm employed in the current study does allow for some detection of a neurobiological fatigue marker. However, there are several complex comparisons that, when simplified may allow for a better measure of fatigue. Moreover, these complex interpretations are likely contributing to the low statistical power of the fmri comprisons. During the design of the study, we hypothesised that the mental imagery condition may increase the fatiguability due to the increased task duration. The results somewhat indicate that the opposite is true, and that the mental imagery condition produces a learning effect instead. As such an experiment without the mental imagery condition but increasing the duration of the handgrip condition may provide a better objective measure of fatigue. This is discussed further in Chapter 9 (page 180).

7.4.5 Conclusion

In conclusion, the results suggest that intrinsic alertness requires only a basic form of response readiness for attention, whereas extrinsic alertness requires higher order cognitive functions. As such, the MS group adequately recruit mechanisms that underlie

intrinsic alertness, with some compensatory mechanisms, but these cannot overcome the behavioural deficits. The MS group have a consistent decreased activation of the pFC, which strongly indicates a deficit of higher order cognition in MS. This is further supported by the lack of warning effect observed in the MS group during the extrinsic alertness task.

In the HC group through learning, likely evidenced by a trend towards the recruitment of memory regions, task performance becomes easier. Therefore, the reduced effect of fatiguability following the learning effect is appropriate. Whereas, in the MS group, the associative cortical loop of the basal ganglia is consistently associated with the mitigation of fatiguability. But, when disrupted it cannot function optimally.

However, many of the results have only survived a less stringent threshold uncorrected for multiple comparisons and as such interpretations are made cautiously. In this chapter, the fMRI data is analysed using a GLM approach, which detects differences between groups or tasks. This specific analysis cannot determine how a network interacts. Complementary connectivity analyses may further elucidate this relationship.

Chapter 8 The impact of fatigue on global network topology and basal ganglia functional connectivity in multiple sclerosis.

8.1 Introduction

Multiple studies examining brain function have implicated the basal ganglia in MS fatigue (Bonzano *et al.*, 2017; DeLuca *et al.*, 2008; Fillipi *et al.*, 2002; Finke *et al.*, 2015; Genova *et al.*, 2013; Pravata *et al.*, 2016; Roelcke *et al.*, 1997; Rocca *et al.*, 2016a). Chaudhuri and Behan (2000; 2004) proposed that through the association with the dopamine system, dysfunction to the basal ganglia and its connections can lead to disruption of motivation, which may play a role in central fatigue. The exact nature of the association between MS fatigue and the basal ganglia remains unknown. In Chapter 7 (page 125) the results indicate that the basal ganglia may mitigate the effect of fatiguability on behavioural performance. Due to the system level nature of the general linear model (GLM) used in fMRI analysis, it cannot make inferences about how the system integrates. The human brain is a complex network of regions that interact through connections both structurally in white matter and functionally in grey matter and as such understanding connectivity is a vital part of understanding brain function.

Converging evidence from neuroimaging studies have indicated there may be a connectivity disruption in MS. Studies using DTI to examine the white matter tracts, showed that MS groups have increased mean diffusivity and decreased fractional anisotropy (Bammer *et al.*, 2000; Cercignani *et al.*, 2000; Ciccarelli *et al.*, 2001; Droogan *et al.*, 1999; Filippi, 2001; Filippi *et al.*, 2000; Guo *et al.*, 2001; Rocca *et al.*, 2000) suggesting a disruption in the main structural fibre tracts. Similarly, studies of functional connectivity demonstrated that individuals with MS have different connectivity to healthy individuals in multiple brain regions (Bonnet *et al.*, 2010; Cader *et al.*, 2006; Cerasa *et al.*, 2013; Duong *et al.*, 2005; Forn *et al.*, 2012; Hawellek *et al.*, 2011; Hulst *et al.*, 2015; Jaeger *et al.*, 2018; Ranjeva *et al.*, 2006; Rocca *et al.*, 2012; Roosendaal *et al.*, 2010; Schoonheim *et al.*, 2015a ; Tona *et al.*, 2014). There is a large amount of heterogeneity in the findings as studies report contradicting results of both increased and decreased functional connectivity in the same regions (Schoonheim *et al.*, 2015b).

Tools which allow for complex system analysis have now been developed (Bullmore and Sporns, 2009). One such tool is graph theory (refer to section 2.2.2 page 44). This technique allows for both differences of brain network topology to be identified and allows the functional connectivity of a distinct region to the whole network to be measured. This

technique has been successfully used to demonstrate several functional and structural connectivity disruptions in disorders such as dementia, amyotrophic lateral sclerosis, and schizophrenia (Filippi *et al.*, 2013).

There have only been a small number of studies that have examined MS using the graph theoretical approach both structurally (Fleisher *et al.*, 2017; He *et al.*, 2009; Kocevar *et al.*, 2016; Li *et al.*, 2013; Muthuraman *et al.*, 2016; Shu *et al.*, 2011;) and functionally (Abidin *et al.*, 2017; Gamboa *et al.*, 2014; Schoonheim *et al.*, 2012). For structural connectivity, He *et al.* (2009) created structural cortical networks based on cortical thickness measurements. The results demonstrated that the structural networks exhibited small-world properties, but that network efficiency was decreased with increased white matter lesion load. Furthermore, they showed that regional efficiency was decreased specifically in the insula, precentral gyrus, prefrontal cortex and temporal cortex. Similarly, Shu *et al.* (2011) indicated that both MS and HC exhibited small-world properties but both the global and local efficiency was significantly disrupted in the MS group, specifically in the sensorimotor, visual, default-mode and language regions. Furthermore, the decreased network efficiency was associated with increased disability scores, disease duration, and lesion load. Li *et al.* (2013) observed disruptions to efficiency in the frontal cortex, hippocampal gyrus, parahippocampal gyrus, motor region and occipital lobe in the MS group. Furthermore, they also demonstrated compensatory increased efficiency in the corpus callosum and cingulum. Individuals with MS also had increased characteristic path length (Kocevar *et al.*, 2016), clustering coefficient (Fleisher *et al.*, 2017; Muthuraman *et al.*, 2016), modularity (Fleisher *et al.*, 2017; Muthuraman *et al.*, 2016) and decreased global efficiency (Kocevar *et al.*, 2016). The functional connectivity studies, using resting state fMRI, observed global decreased path length (Rocca *et al.*, 2016b), global efficiency (Rocca *et al.*, 2016b), clustering coefficient (Rocca *et al.*, 2016b) and increased modularity (Abidin *et al.*, 2017; Gamboa *et al.*, 2014). Interestingly Schoonheim *et al.*, (2012) demonstrated decreased functional connectivity and network efficiency, but only in male participants. Furthermore, there were differences in local network properties. Abidin *et al.*, (2017), similarly found increased local efficiency in MS compared to HC in the parietal lobe, but also increased local efficiency in the inferior frontal gyrus. Rocca *et al.* (2016b) observed that MS participants had decreased hubs in the superior frontal gyrus, precuneus and anterior cingulate, specifically in the left hemisphere and a differential lateralisation of basal ganglia hubs. Basal ganglia hubs were in the left hemisphere in HC, but the right hemisphere in MS. Furthermore, the MS group had hubs in the left temporal pole and

cerebellum not present in the HC group and decreased nodal degree in the right cerebellum and bilateral caudate. Currently, there have been no studies that have used graph theoretical approaches in task-based fMRI data in MS.

The primary aim of the current chapter was to examine the relationship between fatiguability and both whole brain network topology and functional connectivity of the basal ganglia during an alertness-motor paradigm. The secondary aim was to explore whether whole brain network topology and basal ganglia functional connectivity differs between HC and MS groups. Some differences in network topology between HC and MS groups are expected. If the functional connectivity of the basal ganglia changes in the groups that experience increased fatiguability it may be that the basal ganglia increases the effect of fatiguability on behavioural performance. Whereas, if the connectivity of the basal ganglia changes in the groups that do not experience fatiguability the basal ganglia may mitigate the effect of fatigue.

8.2 Methods

8.2.1 Participants

A total of 40 MS participants and 40 healthy individuals were recruited according to the procedure described in the general methods section. 3 MS and 4 HC outliers were removed (refer to section 2.6 page 52).

8.2.2 Neuropsychological questionnaires

All participants, in both the MS and HC groups, were screened for fatigue, using the Fatigue Severity Scale (FSS) (Krupp *et al*., 1989), to ensure that the MS did have significant fatigue and the HC did not. Furthermore, the scores from the FSS was used as a measure of fatigue and used in the analysis.

8.2.3 Alertness-motor paradigm

The full paradigm is explained in chapter 5 (section 5.2.3-5.2.5 page 90-92), here a brief summary is provided only to comprehend the reaction time data used in the discussion of the current chapter. The paradigm consisted of interleaved periods of three different tasks, sensorimotor, intrinsic alertness and extrinsic alertness, requiring the participant to exert a certain force (low, medium, high). Each task was repeated four times, resulting in four blocks containing each of the three tasks at each of the three force levels. The order of tasks was pseudoransomised (Figure 8.1). Furthermore, there were two conditions to the paradigm, physical handgrip, and mental imagery, every participant completed both

conditions. The group of participants that completed the handgrip condition before the mental imagery condition is referred to as the handgrip first group. The group of participants that completed the mental imagery condition before the handgrip condition is referred to as the imagery first group. In the physical condition, participants performed the full paradigm whilst squeezing a hand dynamometer at the required force. In the mental imagery, task participants performed the full paradigm but were asked to only imagine squeezing the hand dynamometer at the required force.

The sensorimotor control task was a self-paced task and as such, no reaction time or force data was extracted from this task. Furthermore, it was a control task and as such is not analysed in this chapter. During the physical handgrip condition, for each of the tasks, participants were instructed to squeeze at the force indicated by the task: low force; medium force or high force. A schematic of the complete paradigm is represented in Figure 8.2 .

During the intrinsic alertness task (T2), participants would view a white fixation cross at the centre of the screen for varying durations (1750, 1780 or 1810 ms). When the white square appeared, participants were instructed to squeeze the handgrip at the required force. In the extrinsic alertness task (T3) the white fixation cross was displayed for a fixed duration (1500 ms). The white cross was followed by the presentation of a black screen, warning stimulus, which was displayed for varying durations (250, 280 or 310 ms). After the warning cue, the white square was displayed, and participants had to squeeze at the required force. The difference between onset of force grip and the onset of the white square was used as the participant's reaction time (refer to section 5.2.3-5.2.5 page 90-92).

Block 1	T3			T1			T2		
	Low	Medium	High	High	Medium	Low	Low	High	Medium
Block 2	T2			T1			T3		
	High	Low	Medium	High	Medium	Low	High	Medium	Low
Block 3	T3			T1			T2		
	Medium	High	Low	Low	Medium	High	Medium	Low	High
Block 4	T2			T1			T3		
	Low	Medium	High	Low	Medium	High	Low	High	Medium

Figure 8.1 Task design. *Note: T1= sensorimotor task. T2= intrinsic alertness task. T3= extrinsic alertness task.*

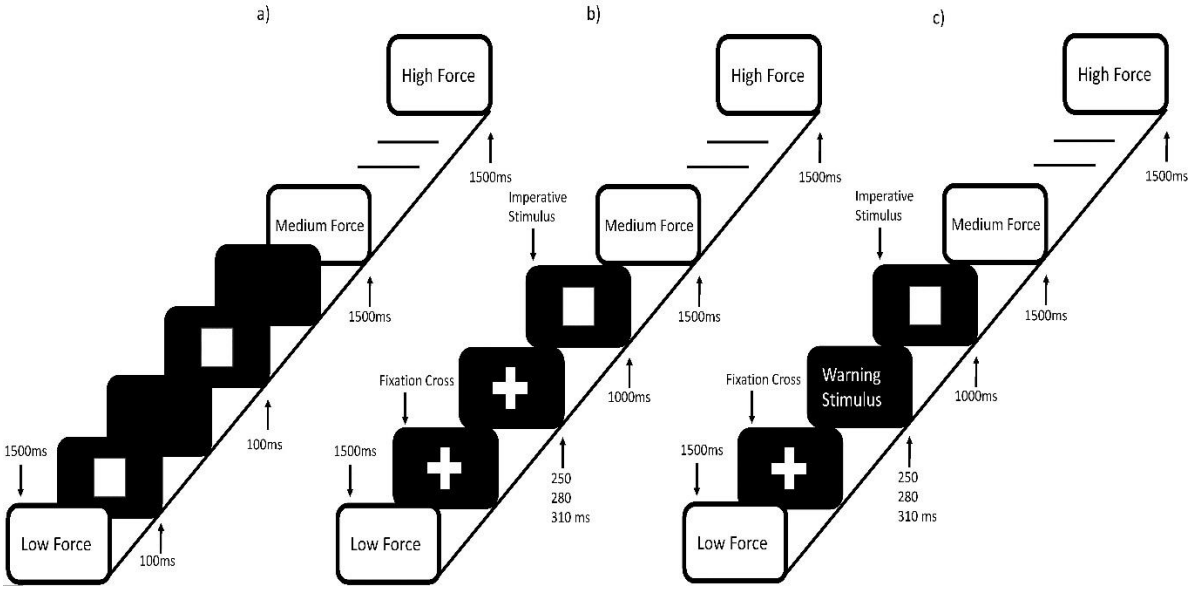


Figure 8.2 Schematic of alertness-motor paradigm. *a) sensorimotor control. b) intrinsic alertness. c) extrinsic alertness.*

8.2.4 Pre-processing and network analysis

MRI images were acquired by the steps detailed in section 2.7 (page 52). All the functional images were pre-processed in SPM12 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm). Images were realigned and resliced to correct for head movements; co-registered and segmented to normalise images into standard space based on the MNI template, this allowed for group level analysis. Gaussian smoothing was applied using 8mm full-width half-maximum (FWHM) Gaussian kernel, to account for residual inter-subject differences. The default SPM12 steps were used, except during the normalisation step, where the voxel size was set to 2x2x2 and the bounding box was changed. This was done to ensure that the data matched the automated anatomical labelling atlas (AAL) (Tzourio-Mazoyer *et al.*, 2002) used to define the ROIs. There are several different methods used to define ROIs (refer to section 2.2.2 page 44). The AAL is a brain parcellation based on anatomical brain regions and contains a total of 116 regions. Following the pre-processing steps, noise from white matter, cerebrospinal fluid, and movement signals were regressed out using least squares multiple regression, from each individual voxel. Furthermore, a bandpass filter (0.01-0.08Hz) to remove low and high frequency noise was applied. A mean time series was then extracted from each of the 116 ROIs. Because the extracted time-series contained information across all three tasks of the paradigm (T1, T2, T3), the time series were split according to the timings of each task and then concatenated to represent each of the tasks in the paradigm.

All analyses were performed in MATLAB 2015a (Mathworks Inc., Natick, MA, USA). The functional connectivity between ROIs was measured using the Pearson's correlation coefficient, resulting in a 116 x 116 connectivity matrix for each participant. Due to the ambiguity of selecting thresholds (De Vico Fallani *et al*, 2014; Garrison *et al*., 2015; Jalili, 2016; Rubinov and Sporns, 2010; 2011; Zalesky *et al*., 2012), weighted networks were used. To increase the normality and standardise the data for better group comparison a Fisher z-transform was conducted.

Three network measures were computed from the weighted connectivity matrices, namely, small world propensity (SWP) (Muldoon *et al*., 2016), modularity (Newman, 2004; Rubinov and Sporns, 2010), and global efficiency (Latora and Marchiori, 2001; Rubinov and Sporns, 2010). Watts and Strogatz (1998) first described the concept of a small-world network, using unweighted networks. A simple network was studied where each node of the network was connected to its four neighbouring nodes (lattice). This results in a highly clustered network, but a high characteristic path length as reaching the opposite end of the lattice would require many short-range connections. They noted that when randomly rewiring some of the lattice edges, to create long-range connections between distant nodes, both the characteristic path length and clustering decreased. Thus representing a more random network. They showed that having only a small number of long-range connections in the lattice network reduced the characteristic path length without affecting the clustering (small-world network). Muldoon *et al*. (2016) provide a measure of SWP, which quantifies the extent to which a network displays small-world characteristics. The SWP is defined by:

$$\phi = 1 - \sqrt{\frac{\Delta_C^2 + \Delta_L^2}{2}},$$

Where

$$\Delta_C = \frac{C_{latt} - C_{obs}}{C_{latt} - C_{rand}},$$

And

$$\Delta_L = \frac{L_{obs} - L_{rand}}{L_{latt} - L_{rand}}.$$

In the formula ϕ (SWP) represents the deviation of the clustering coefficient, C_{obs}, and characteristic path length, L_{obs}, from both lattice (C_{latt}, L_{latt}) and random (C_{rand}, L_{rand}) networks with the identical number of nodes and connections, where ΔC is the fractional deviation of the clustering coefficient (C_{obs}) from its null network (random or lattice) and ΔL is the fractional deviation of the characteristic path length (L_{obs}) from its null network (random or lattice). Networks are considered small-world if they have small-world propensity $0.4 < \phi \leq 1$.

Brain networks can be subdivided into non-overlapping groups of nodes, where the number of within-group edges is large, and that between group edges is small (Fortunato, 2009; Girvan and Newman, 2002). Modularity is a measure of segregation that quantifies the degree to which a brain network is divided into such clearly separate groups. The algorithms for modularity are generally optimization algorithms rather than calculating the exact value because calculating the largest modularity value is a computationally hard problem. This is a trade-off between accuracy and computational speed. The modularity of weighted networks is defined as follows (Newman, 2004):

$$Q = \frac{1}{2m} \sum_{ij} \left[A_{ij} - \frac{k_i k_j}{2m} \right] \delta(c_i, c_j).$$

In the formula Q (modularity) represents the extent to which the network is divided into distinct groups; A_{ij} is the weight of the connection between *i* to *j*; $\frac{kikj}{2m}$ is the probability of an edge existing between ROIs *i* and *j*, where k_i is the degree of ROI *i*. The δ-function $\delta(c_i c_j)$ is 1 if $c_i = c_j$ and 0 otherwise, where c_i is the community to which a ROI *i* is assigned; and *m* is the number of edges in the network, where $m = \frac{1}{2}\sum_{\{i,j\}} A_{ij}$.

Global efficiency is a measure of integration which uses the average inverse shortest path length. Unlike the characteristic path length, which is defined as the average shortest path

between all pairs of nodes, global efficiency can be calculated on disconnected networks. If there is no path between two nodes the path length between them is infinite and the associated global efficiency is 0. The global efficiency is defined by (Latora and Marchiori, 2001)

$$E = \frac{1}{n}\sum_{i\in N}\frac{\sum_{j\in N, j\neq i}\left(d_{ij}\right)^{-1}}{n-1}.$$

In the formula, E is the global efficiency, d_{ij} is the length of the shortest path, where the path length between two nodes is the inverse of their edge's weight, characteristic path length is $\frac{1}{N(N-1)}\Sigma_{i\neq j}\, d_{ij}$, where N is the number of nodes the and clustering coefficient is $\frac{1}{N}\Sigma_i C_i$, where C_i is the number of edges.

As well as calculating values for each of the three network measures, the functional connectivity of the basal ganglia with all other ROIs was computed by taking the average functional connectivity of the right and left putamen, pallidum and caudate across all 116 ROIs. To compare between MS and HC groups, independent sample t-tests were conducted for each of the three network measures, as well as for the global functional connectivity of the basal ganglia. For all independent sample t-tests, Levene's test for equality of variances (Levene, 1960) was used to assess violations to homogeneity of variance. If this assumption was violated the associated values were used.

The impact of fatigue was analysed in several different ways. Partial correlations, controlling for age, were used to correlate modularity, SWP and global efficiency with fatigue scores. For all correlations Spearman's rank correlations (Appendix L) were also conducted, due to outliers, however this did not alter the results. As such Pearson's correlation coefficients are reported. Furthermore, paired t-tests, for all three network measures and basal ganglia functional connectivity, were conducted to examine the differences between the first and last block of the tasks in both the HC and MS groups. Participants were split into two groups depending on whether they completed the mental imagery or handgrip condition first.

8.3 Results

8.3.1 Reaction time performance

The HC handgrip first group had increased reaction in the last block of intrinsic alertness ($p < .05$), but no reaction time differences between block 1 and 4 for extrinsic alertness ($p > .05$). For the HC imagery first group there were no reaction time differences between the beginning and the end of the paradigm for intrinsic alertness ($p > .05$) or extrinsic alertness ($p > .05$). The MS handgrip first group had increased reaction time in the last block of intrinsic alertness ($p < .01$) but no reaction time differences between block 1 and 4 for extrinsic alertness ($p > .05$). For the MS imagery first group the opposite was observed, there was an increased reaction time in block 4 for extrinsic alertness ($p < .05$), and no reaction time differences between the beginning and the end of the paradigm for intrinsic alertness ($p > .05$). The MS group had increased fatiguability compared to the HC group ($p < .05$).

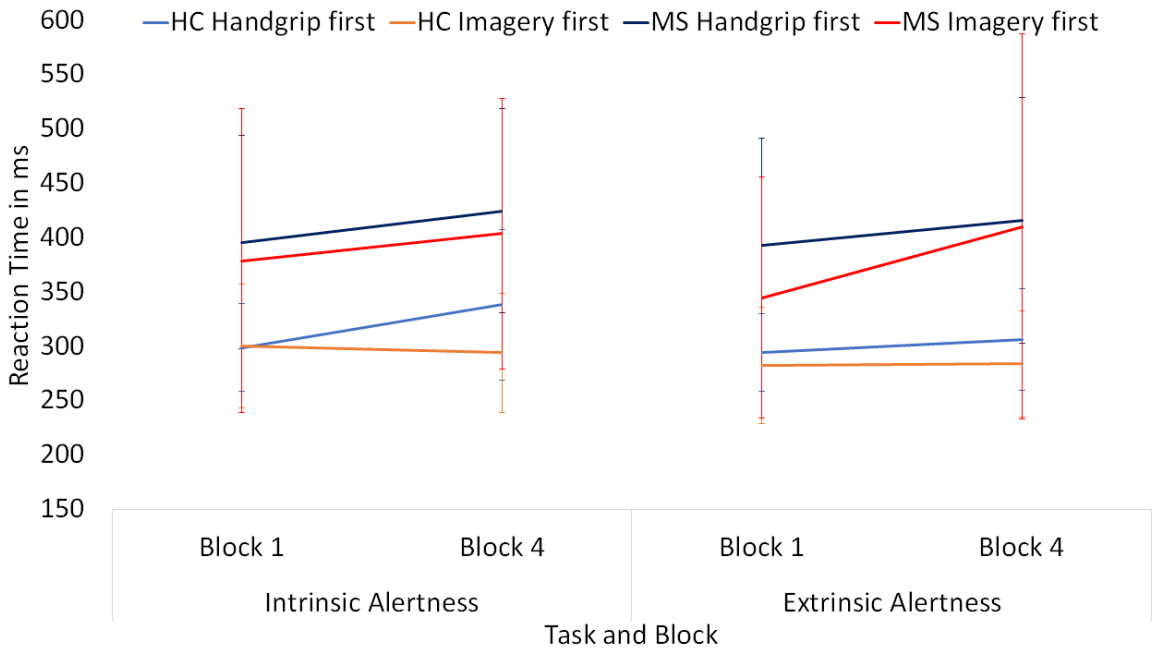


Figure 8.3 Line graph of reaction time in ms. *The HC handgrip first group are displayed in blue, the HC imagery first group are displayed in orange, the MS handgrip first group are displayed in navy and the MS imagery first group are displayed in red. The error bars represent one standard deviation.*

8.3.2 Network analysis of split HC groups during intrinsic alertness

For the HC handgrip first group paired t-tests between the first and last block of the intrinsic alertness task revealed a significant decrease in global efficiency in block 4 ($t(18) = 2.15$, $p < .05$). Furthermore there were no significant differences on the other two network

measures, SWP (t(18)= 1.75, p= .10), modularity (t(18)= -1.47, p= .16), and no functional connectivity differences in any of the basal ganglia regions; left caudate (t(18)= -.38, p= .71), right caudate (t(18)= -.06, p= .95), left putamen (t(18)= -.01, p= .99), right putamen (t(18)= -.79, p= .44), left pallidum (t(18)= -.47, p= .64), right pallidum (t(18)= -.88, p= .39) and average basal ganglia functional connectivity (t(18)= -.46, p= .65) between the first and last block.

During the intrinsic alertness task, the HC imagery group exhibited significantly increased functional connectivity for the right caudate (t(16)= -2.26, p= .04) and the left putamen (t(16)= -2.21, p= .04) in the last block. There were no significant differences for any of the network measures, SWP (t(16)= -1.23, p= .22), modularity (t(16)= .60, p= .56), global efficiency (t(16)= -.54, p= .60), or any of the other four basal ganglia regions left caudate (t(16)= .19, p= .85), right putamen (t(16)= -1.12, p= .28), left pallidum (t(16)= -1.38, p= .19), right pallidum (t(16)= -.84, p= .41), and no significant difference for average basal ganglia functional connectivity (t(16)= -1.80, p= .09), between the first and last block.

A direct comparison between the HC handgrip first and imagery first groups, during the last block of intrinsic alertness revealed that there were no significant differences between the groups on any of the network measures; SWP 9t(34) = -.71, p= .48, d= .33), modularity (t(34) = .68, p= .50, d= 0), global efficiency (t(34) = -.38, p= .0.70, d= .28). Furthermore, there were no significant functional connectivity differences in any of the basal ganglia regions; left caudate (t(34) = .17, p= .86, d= .07), right caudate (t(34) = -.50, p= .62, d= .17), left putamen (t(34) = -1.17, p= .25, d= .38), right putamen (t(34) = -1.28, p= .21, d= .41), left pallidum (t(26.78) = .14, p= .89, d= .0), right pallidum (t(34) = -.11, p= .91, d= .06) or for average basal ganglia functional connectivity (t(34) = -.53, p= .60, d= .13). Bar charts visually representing the data are displayed in Figure 8.4 and Figure 8.5.

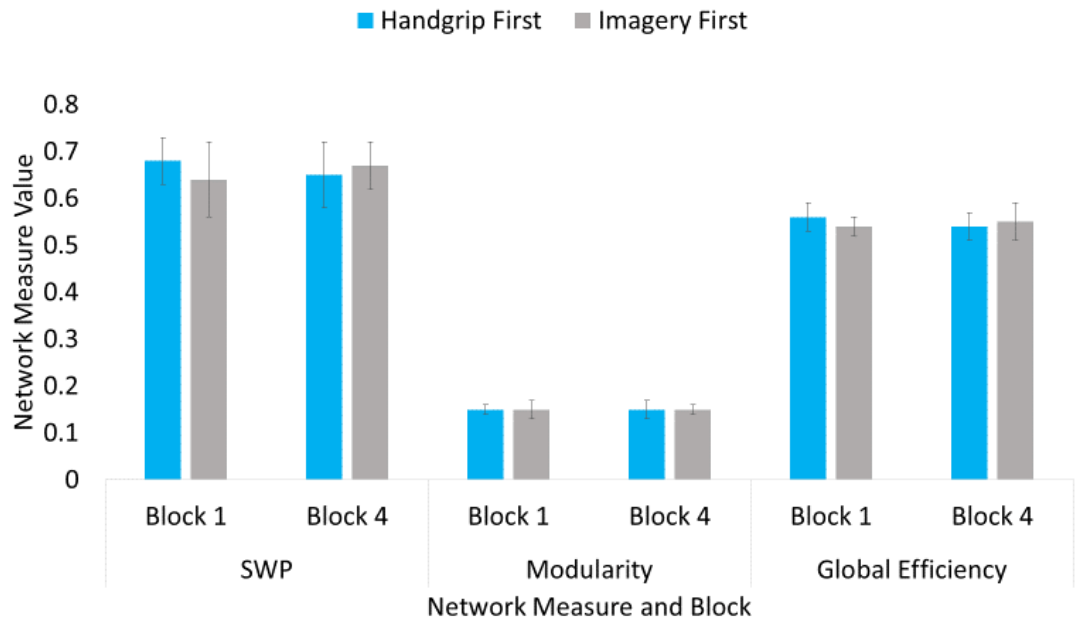


Figure 8.4 Bar chart of network measure for split HC groups during intrinsic alertness task. *The handgrip first group are displayed in cyan, and the imagery first group are displayed in grey. The error bars represent one standard deviation.*

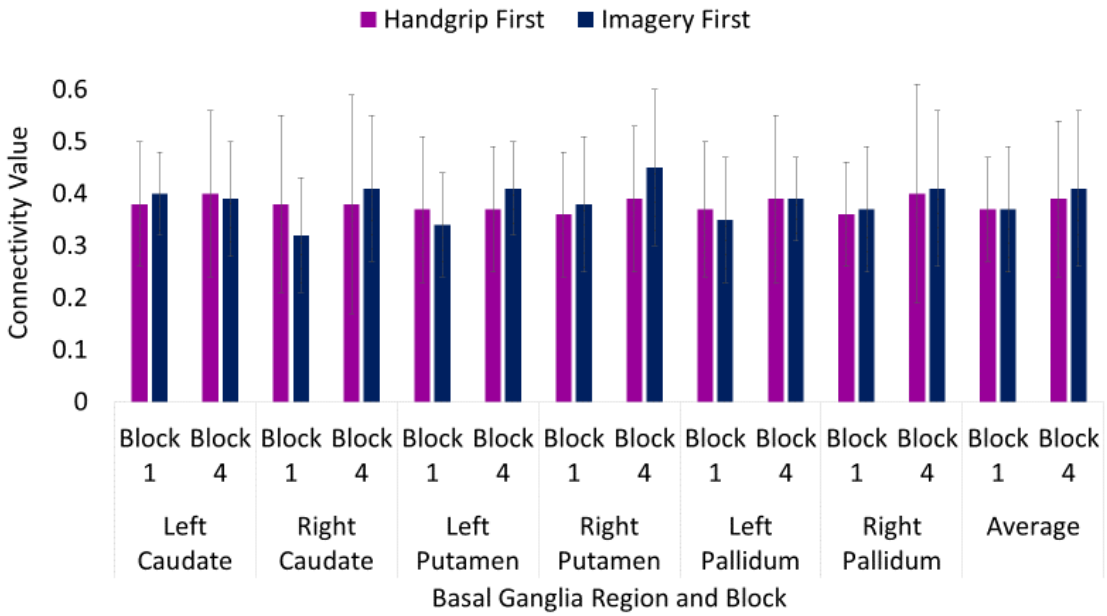


Figure 8.5 Bar chart of basal ganglia functional connectivity for split HC groups during intrinsic alertness task. *The handgrip first group are displayed in magenta, and the imagery first group are displayed in navy. The error bars represent one standard deviation. The connectivity value is Pearson's R.*

8.3.3 Network analysis of split HC groups during extrinsic alertness

Once the HC group was split into the handgrip first and imagery first groups there were no behavioural differences in within or between group performance, and as such the

network analysis was not included. Without a behavioural difference, further analyses do not provide any interpretable information.

8.3.4 Network analysis of split MS groups during intrinsic alertness

In the MS handgrip first group there were no significant differences between the first and last block of the task on any network measure or any of the basal ganglia regions; SWP ($t(18)= 1.20$, $p= .25$), modularity ($t(18)= -.79$, $p= .44$) global efficiency ($t(18)= -.20$, $p= .84$) left caudate ($t(18)= -1.05$, $p= .31$) right caudate ($t(18)= -.64$, $p= .53$), left putamen ($t(18)= .21$, $p= .84$), right putamen ($t(18)= -.59$, $p= .56$), left pallidum ($t(18)= .26$, $p= .79$), right pallidum ($t(18)= -.85$, $p=.40$), and average basal ganglia functional connectivity ($t(18)= -.74$, $p= .47$).

For the MS imagery first group there was increased functional connectivity in the left caudate ($t(35) = 1.08$, $p= .05$) and right pallidum ($t(35) = 1.77$, $p= .03$). There were no significant differences on any of the network measures, or the other four basal ganglia regions; SWP ($t(17)= -1.70$, $p= .11$), modularity ($t(17)= -.85$, $p= .41$) global efficiency ($t(17)= .43$, $p= .67$), right caudate ($t(17)= -.44$, $p= .66$), left putamen ($t(17)= -.84$, $p= .42$) right putamen ($t(17)= .19$, $p= .85$), left pallidum ($t(17)= -.97$, $p= .35$) and average basal ganglia functional connectivity ($t(17)= -.45$, $p= .66$).

A direct comparison, during the last block of the intrinsic alertness task, demonstrated that there were no significant differences between the MS handgrip first and imagery first groups on any of the network measures; SWP ($t(35) = -1.89$, $p= .70$, $d= .72$), modularity ($t(35) = .55$, $p= .59$, $d= 0$) global efficiency ($t(35) = -.20$, $p= .0.99$, $d= 0$). Furthermore, there were no significant functional connectivity differences in any of the basal ganglia regions; left caudate ($t(35) = .58$, $p= .56$, $d= .29$) right caudate ($t(35) = .43$, $p= .67$, $d= .18$) left putamen ($t(35) = .02$, $p= .99$, $d= .0$) right putamen ($t(35) = .61$, $p= .55$, $d= .22$) left pallidum ($t(35) = .31$, $p= .76$, $d= .09$) right pallidum ($t(35) = .97$, $p= .34$, $d= .32$) and average basal ganglia functional connectivity ($t(35) = .69$, $p= .50$, $d= .13$). Bar charts visually representing the data are displayed in Figure 8.6 and Figure 8.7.

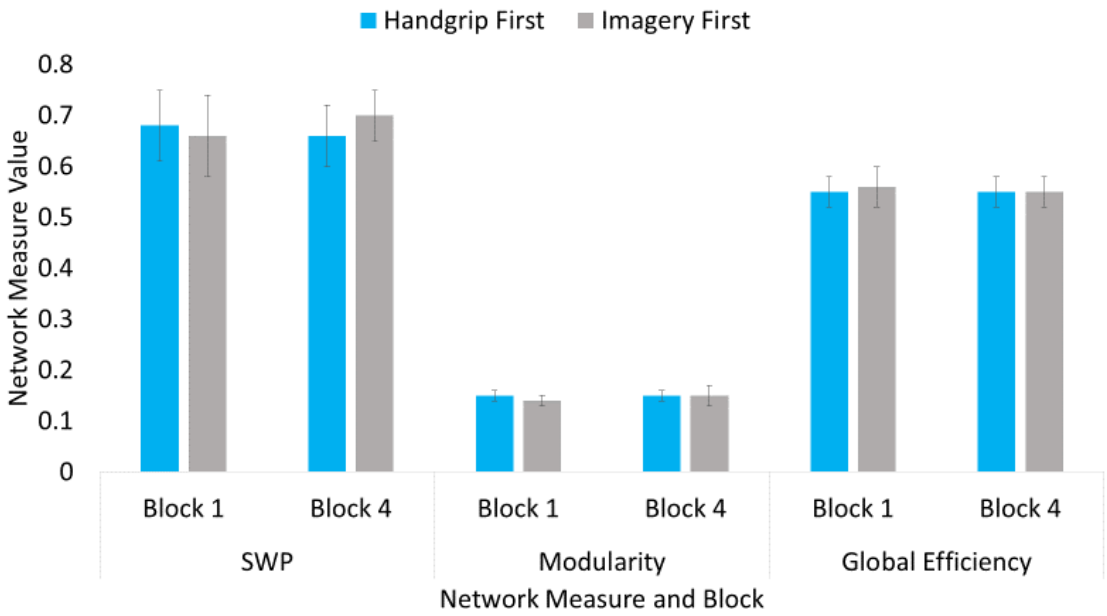


Figure 8.6 Bar chart for split MS groups during intrinsic alertness task. *The handgrip first group are displayed in cyan, and the imagery first group are displayed in grey. The error bars represent one standard deviation.*

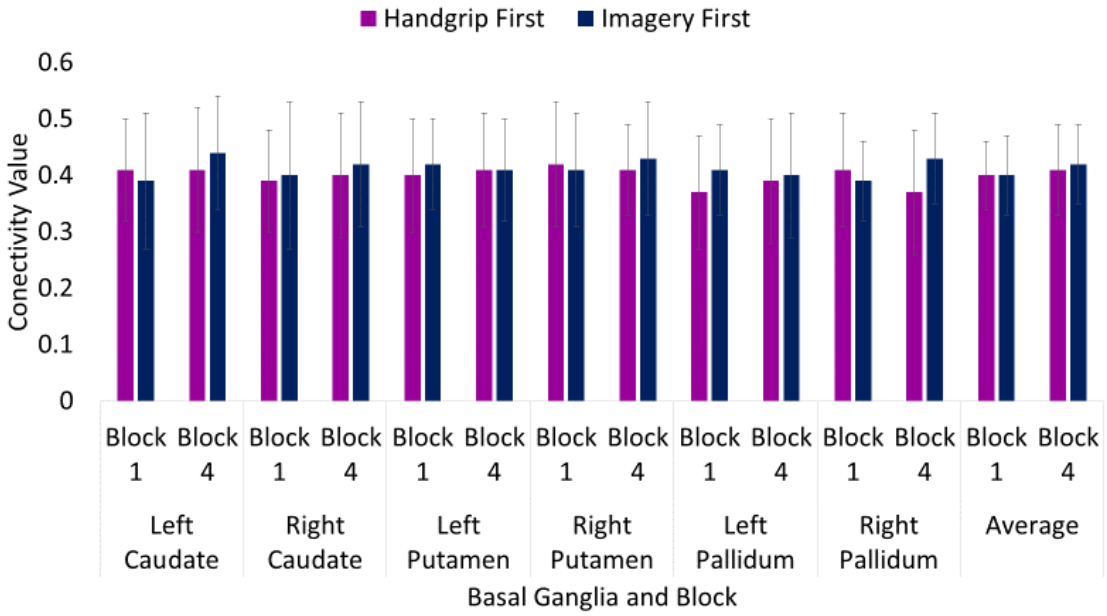


Figure 8.7 Bar chart of basal ganglia functional connectivity for split MS groups during intrinsic alertness task. *The handgrip first group are displayed in magenta, and the imagery first group are displayed in navy. The error bars represent one standard deviation. The connectivity value is Pearson's R.*

8.3.5 Network analysis of split MS groups during extrinsic alertness

The MS handgrip first group showed no significant differences between the first and last block on any network measure; SWP ($t(18)= .67$, $p= .51$) modularity ($t(18)= .57$, $p= .58$), global efficiency ($t(18)= -1.12$, $p= .28$). They demonstrated significant increased functional connectivity in the right putamen ($t(18)= -2.25$, $p= .04$) and the right pallidum ($t(18)= -$

2.08, p= .05). There were no functional connectivity differences in the other 4 regions; left caudate (t(18)= -.76, p= .46), right caudate (t(18)= -1.42, p= .17), left putamen (t(18)= -1.16, p= .26), left pallidum (t(18)= -.33, p= .75), and average basal ganglia functional connectivity (t(18)= -1.76, p= .10).

For the MS imagery first group there were no significant differences between the first and the last block of extrinsic alertness, on any network measure; SWP (t(17)= -.75, p= .47), modularity (t(17)= 1.01, p= .31), global efficiency (t(17)= .09, p= .93). Similarly, there were no functional connectivity differences in any of the basal ganglia regions; left caudate (t(17)= -.61, p= .55), right caudate (t(17)= -1.10, p= .29), left putamen (t(17)= .98, p= .34), right putamen (t(17)= -.26, p= .80), left pallidum (t(17)= .94, p= .36), right pallidum (t(17)= 1.13, p= .27), and average basal ganglia functional connectivity (t(17)= .03, p= .98) between the first and last block of the task.

The direct comparison between the MS handgrip first and imagery first groups revealed no significant differences between groups on any network measure in block 4 of the extrinsic alertness task; SWP (t(35) = -1.42, p= .17, d= .15), modularity (t(35) = -1.70, p= .10, d= .50), and global efficiency (t(35) = 1.01, p= .29, d= .36). Furthermore, there were no significant functional connectivity differences in any of the basal ganglia regions; left caudate (t(35) = .10, p= .92, d= .07), right caudate (t(35) = -.33, p= .74, d= .08), left putamen (t(23.16) = 1.24, p= .23, d= .46), right putamen (t(35) = 1.74, p= .09, d= .61), left pallidum (t(27.51) = 1.03, p= .31, d= .38), right pallidum (t(24.61) = 1.75, p= .09, d= .61), and average basal ganglia functional connectivity (t(26.24) = 1.19, p= .24, d= .33). Bar charts visually representing the data are displayed in Figure 8.8 and Figure 8.9.

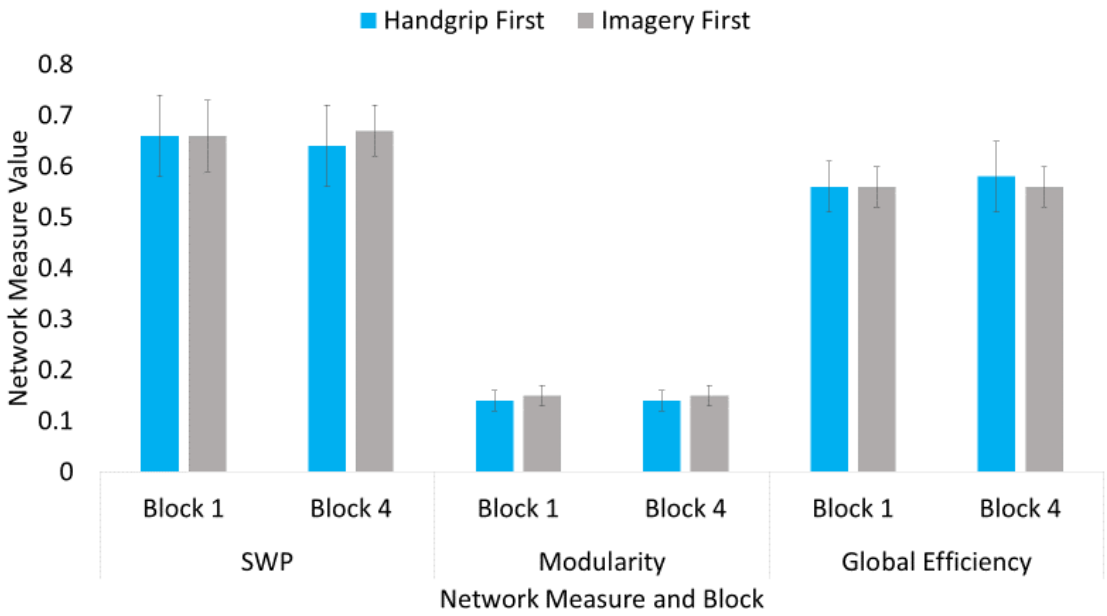


Figure 8.8 Bar chart for split MS groups during the extrinsic alertness task. *The handgrip first group are displayed in cyan, and the imagery first group are displayed in grey. The error bars represent one standard deviation.*

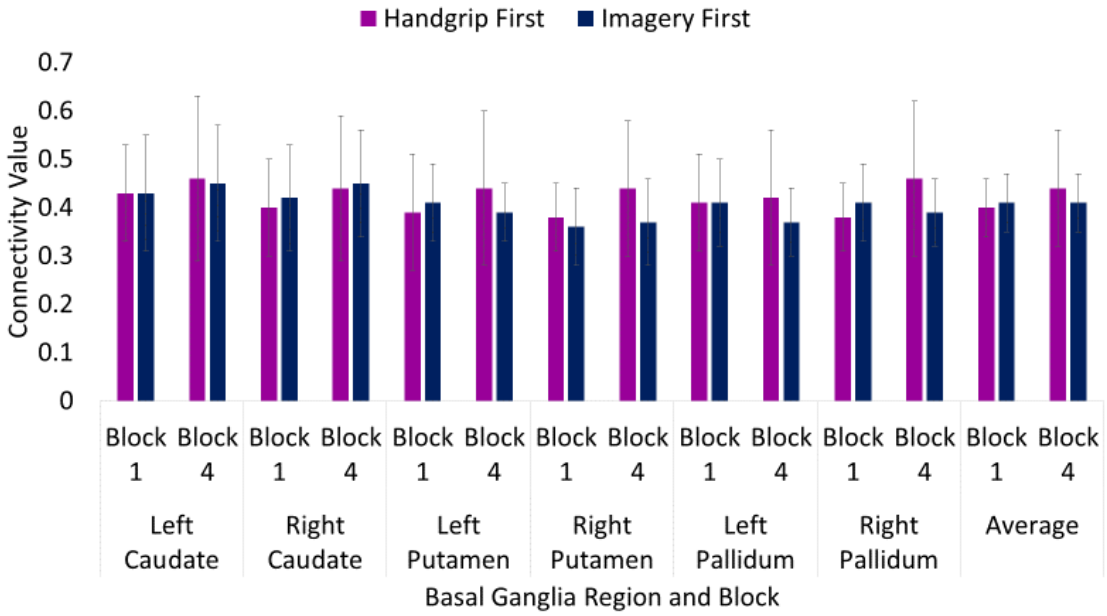


Figure 8.9 Bar chart of basal ganglia functional connectivity for split MS groups during the extrinsic alertness task. *The handgrip first group are displayed in magenta, and the imagery first group are displayed in navy. The error bars represent one standard deviation. The connectivity value is Pearson's R.*

8.3.6 Global task comparison between the MS and HC groups

During the intrinsic alertness task an independent sample t-test revealed no significant differences between HC and MS groups on any of the three network measures; SWP (t(71)= -1.50, p= .1, d=.35), modularity (t=(1,71)= .53, p= .60, d= .12), global efficiency (t(71)= .04, p= .97, d= 0). Furthermore, no significant differences on any of the six basal ganglia regions;

left caudate (t(71)= -1.61, p= .11, d= .33), right caudate (t(71)= -1.05, p= .30, d= .25), left putamen (t(54.41)= .45, p= .66, d= .13), right putamen (t(71)= -.13, p= .90, d= 0), left pallidum (t(54.71)= -.05, p= .96, d= 0), right pallidum (t(71)= -.47, p= .64, d= .15), and no significant difference on average basal ganglia activation (t(71)= -.65, p= .52, d= 0) between the HC and MS groups.

During the extrinsic alertness task an independent sample t-test revealed a significant increase in the functional connectivity of the left putamen (t(58.71)= -2.37, p= .02, d= .59) and right putamen (t(60.94)= -2.53, p= .01, d= .65) in the MS group compared to the HC group. There were no significant differences between the HC and MS groups on any of the three network measures; SWP (t(71)= .57, p= .57, d=.15), modularity (t=(71)= 1.35, p= .18, d= .32), global efficiency (t(71)= .36, p= .72, d= .08). Furthermore, no significant differences on any of the other four basal ganglia regions; left caudate (t(71)= 1.17, p= .25, d=.27), right caudate (t(58.37)= -1.00, p= .31, d= .28), left pallidum (t(71)= -.30, p= .76, d= .06), right pallidum (t(71)= -1.02, p= .31, d= .19), and no significant difference on average basal ganglia functional connectivity (t(56.47)= -1.45, p= .15, d= .42) between HC and MS groups. The descriptive statistics of network measures are displayed in Figure 8.10 (page 172) and for basal ganglia functional connectivity in Figure 8.11 (page 173).

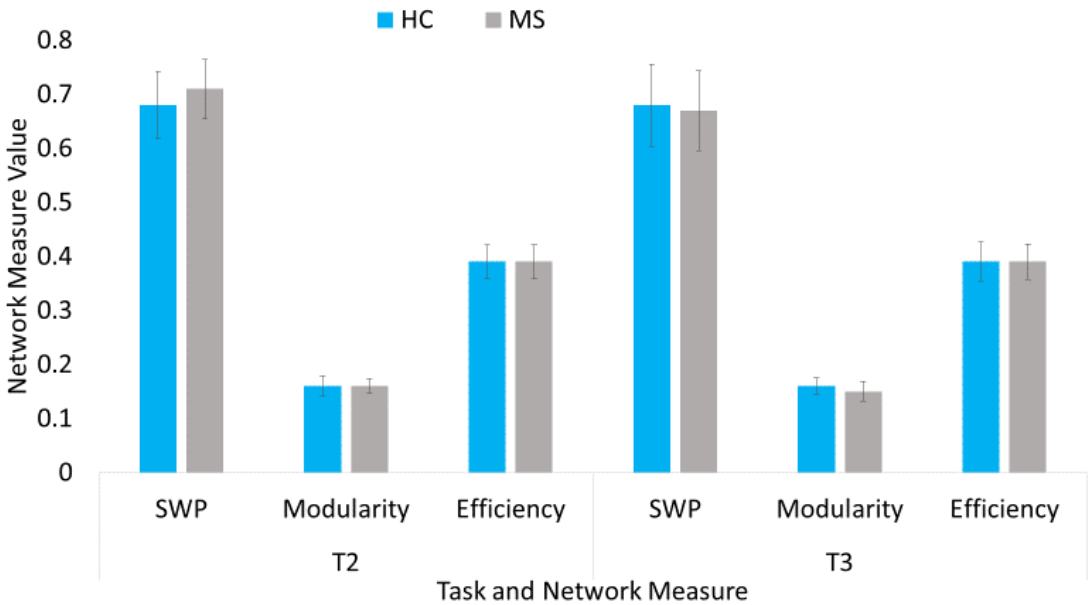


Figure 8.10 Bar chart of network measures across tasks and groups. *HC group displayed in cyan, MS group displayed in grey. The error bars represent one standard deviation.*

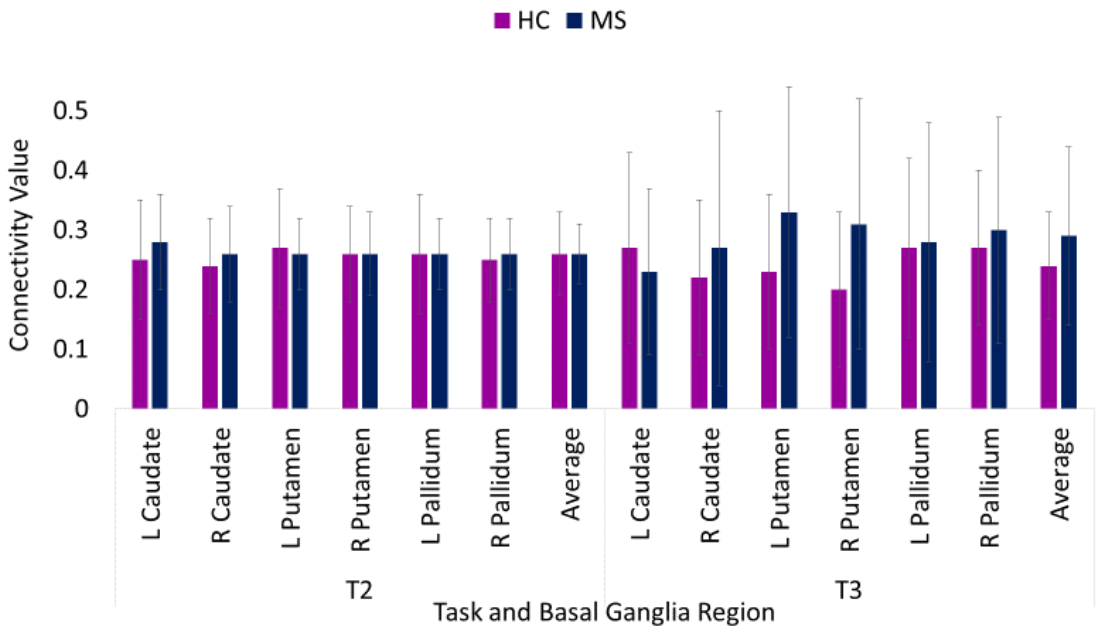


Figure 8.11 Bar chart of functional connectivity of the basal ganglia across tasks and groups. *HC group displayed in magenta, MS group displayed in navy. The error bars represent one standard deviation. The connectivity value is Pearson's R.*

8.3.7 Correlates of fatigue scores

Partial correlations, during the intrinsic alertness task, revealed no significant correlations between FSS scores and any of the three network measures in either the HC group (SWP R= .16, p= .35; modularity R= -.16, p= .35; global efficiency R= -.01, p= .94) or the MS group (SWP R= -.30, p= .08; modularity R= .21, p= .21; global efficiency R= -.15, p= .39). The scatterplot is displayed in Figure 8.12.

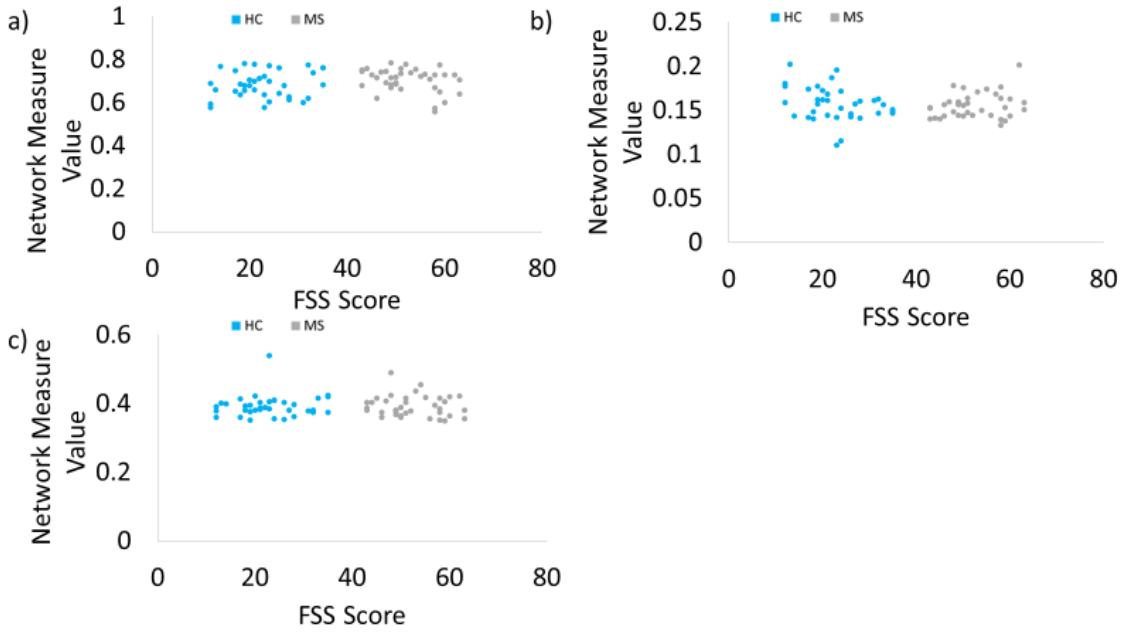


Figure 8.12 Scatterplot for the correlation between FSS and network measures for intrinsic alertness task*. a) SWP, b) modularity, c) global efficiency. HC values are displayed in cyan, MS values are displayed in grey. Each circle represents a participant.*

During the extrinsic alertness task, partial correlations revealed no significant correlations between FSS scores and any of the three network measures in either the HC group (SWP R= .15, p= .38; modularity R= -.02, p= .89; global efficiency R= .02, p= .90) or the MS group (SWP R= .17, p= .33; modularity R= -.09, p= .59; global efficiency R= .12, p= .49). The scatterplot is displayed in Figure 8.13.

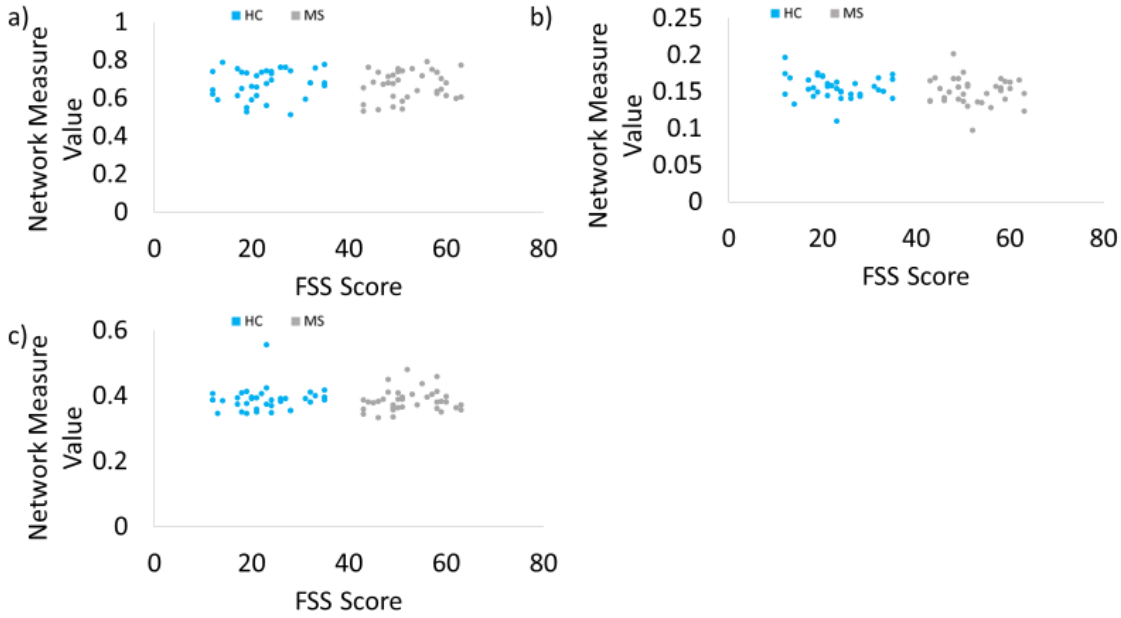


Figure 8.13 Scatterplot for the correlation between FSS and network measures for extrinsic alertness task*. a) SWP, b) modularity, c) global efficiency. HC values are displayed in cyan, MS values are displayed in grey. Each circle represents a participant.*

8.4 Discussion

The primary aim of the current chapter was to examine the relationship between fatiguability and both whole brain network topology and functional connectivity of the basal ganglia during an alertness-motor paradigm in both an HC and MS population.

8.4.1 Fatiguability in the HC group

In the HC group decreased global efficiency may enhance the perception of fatiguae or the increased fatiguability experienced may reduce the global efficiency. The HC handgrip first group demonstrated increased reaction time in block 4 of the intrinsic alertness task, compared to block 1. This decrease in behavioural performance was associated with decreased global efficiency. Global efficiency is inversely associated with the topological distance between ROIs and can be used as a measure of the capacity for information transfer and processing (Bullmore and Sporns, 2009). This result leads to two possible interpretations. The first is that the reduced global efficiency in this group may contribute to the enhanced fatiguability. This may suggest that whole brain network topology can

have an impact on cognitive function, even in a healthy population. It is also possible that the enhanced fatiguability impacts the global efficiency of the network. Disentangling this relationship is very difficult.

Whereas, increased functional connectivity of the basal ganglia seemingly mitigates the influence of fatiguability on behavioural performance. For the HC imagery first group there was some increased functional connectivity in the basal ganglia network in the last block of the intrinsic alertness task compared to the first block. This group did not have increased reaction time in the last block of the intrinsic alertness task, as with the HC handgrip first group. This finding provides evidence for the interpretation in Chapter 7 (page 125) that not only is the basal ganglia involved in fatigue, but the basal ganglia network may actually mitigate the effect of fatiguability and thereby maintain cognitive performance. Chaudhuri and Behan (2000; 2004) have hypothesised that through the motivation function the basal ganglia is implicated in fatigue. The current results indicated some support for this hypothesis as the increased functional connectivity of the basal ganglia network may have contributed to the preservation of cognitive function, measured by reaction time. Despite the within group differences, there were no differences between the groups on any network measures or in basal ganglia network functional connectivity. This finding suggests that the change in whole brain network topology or functional connectivity of the basal ganglia, that influences behavioural performance within groups, is small and is thereby not evident when comparing between the two groups.

In the HC group, the results make it difficult to disentangle the relationship between fatiguability and behavioural performance. As both decreased global efficiency and increased functional connectivity may be influencing behavioural performance. It is important to remember that the HC group do not suffer from persistent fatigue, as in the MS group. As such the results in the MS group may provide a clearer understanding.

8.4.2 Fatiguability in the MS group

In the MS group, the results are more consistent and strongly suggest that increased functional connectivity of the basal ganglia may mitigate the effect of fatiguability on behavioural performance. During the intrinsic alertness task, the MS handgrip first group showed increased reaction time across the alertness-motor paradigm, which was associated with no differences in network topology or functional connectivity of the basal ganglia. Whereas, the MS imagery first group showed increased functional connectivity of

the basal ganglia and no change in reaction time across the alertness-motor paradigm. This result indicates that the increased functional connectivity of the basal ganglia mitigates fatiguability. Because the group that did not have increased functional connectivity in the basal ganglia network had increased fatiguability, as indicated by the increased reaction time. During the extrinsic alertness task, the MS handgrip first showed increased functional connectivity of the basal ganglia network in block 4 compared to block 1 of the alertness-motor paradigm. This increase was associated with no change in behavioural performance across the alertness-motor paradigm. Whereas, the MS imagery first group did not show any difference in network topology or functional connectivity of the basal ganglia, again, associated with increased fatiguability evidenced by the increased reaction time in block 4 compared to block 1. These results are consistent with the hypothesis that the basal ganglia network is implicated in fatigue (Chaudhuri and Behan, 2000; 2004). Similarly, two recent studies have suggested that the basal ganglia to occipital lobe connections (Boissoneault *et al.,* 2018) and thalamo-cortical connections (Hidalgo de la Cruz *et al.*, 2018) are associated with MS fatigue. However, neither of these studies included measures of fatigue over the time, as in the current thesis. The current results provide evidence that this network may mitigate the effect of fatiguability through increased functional connectivity, thereby maintaining cognitive performance. When directly comparing between the two split MS groups, the results revealed no significant differences. This indicates that although increased functional connectivity of the basal ganglia may mitigate the impact of fatiguability within a group, the increase is not noticeable between groups. This is further evidenced by no differences in reaction times between the MS handgrip first and imagery first groups. The current results are consistent with the motivation functions of the basal ganglia, as reduced motivation or reward expectation, may lead to a reluctance to act. Furthermore, it is supported by multiple studies that found basal ganglia atrophy (Derache *et al.*, 2013; Finke *et al.*, 2015; Genova *et al.*, 2013) and abnormal functioning (Bonzano *et al.*, 2017; DeLuca *et al.*, 2008; Fillipi *et al.*, 2002; Finke *et al.*, 2015; Genova *et al.*, 2013; Pravata *et al.*, 2016; Roelcke *et al.*, 1997; Rocca *et al.*, 2016a) to be related to MS fatigue. It is possible that increased functional connectivity of this region increases the motivation, thereby reducing the effect of fatiguability.

Across both the HC and MS groups, the group that did not have increased reaction time, thereby indicating no fatiguability, demonstrated increased functional connectivity of the basal ganglia. As such, there is strong evidence to suggest that this increased functional

connectivity of the basal ganglia may mitigate the effect of fatiguability, in both an MS group that suffer from persistent fatigue and in an HC group. This may be because of its function in the motivation system, where increased functional connectivity increases the motivation, which in turn reduces the effect of fatiguability of reaction time. Despite the within group differences, there were no differences between the groups. It is possible that the changes in functional connectivity of the basal ganglia network may affect within group performance, but not change enough to create between group differences.

The secondary aim of this chapter was to explore whether whole brain network topology and basal ganglia network functional connectivity differs between the HC and MS groups. The MS group demonstrated stable whole brain network topology and exhibited similar efficient small-world properties in a whole brain network during an alertness-motor paradigm. The results of the comparison between the MS and HC groups showed no differences in small-world propensity, modularity, global efficiency or basal ganglia functional connectivity for the intrinsic alertness task. For extrinsic alertness, the MS group demonstrated significantly increased functional connectivity of the basal ganglia network, but no differences for small-world propensity, modularity or global efficiency. There have been no previous studies that have examined brain network topology in task-based data in MS. But, studies of resting state functional connectivity have consistently found increased modularity in MS (Abidin *et al.*, 2017; Gamboa *et al.*, 2014; Schoonheim *et al.*, 2012). Despite the differences between resting state and task-based functional connectivity it would still have been expected to observe some differences between HC and MS groups. It is possible that the use of a whole brain network may have impacted these results. Unlike in resting state fMRI, in task-based fMRI, there are functionally specific regions involved in task performance. Perhaps using a smaller number of ROIs, that are specifically chosen based on the task being completed, would yield different results. Despite this, the results from the current analysis showed that both the HC and MS groups exhibited efficient small-world properties in a whole brain network during an alertness-motor paradigm.

The comparison between the MS and HC groups provides further support that the basal ganglia network is implicated in fatigue (Chaudhuri and Behan *et al.*, 2000; 2004). It is possible that the basal ganglia network mitigates the effect of fatiguability, but that when disrupted, as in MS, it cannot function optimally. For intrinsic alertness, there were no differences in basal ganglia network functional connectivity between the HC and MS groups, but there was increased basal ganglia network functional connectivity in the MS

group during the extrinsic alertness task. Despite the increase in basal ganglia functional connectivity the MS group still showed a significant increase in reaction time compared to the HC group. When exploring the effect of fatiguability over time, the results, in the MS group specifically, demonstrated that increased functional connectivity of the basal ganglia network was associated with the preservation of cognitive performance, as measured by reaction time. The groups that did not have increased functional connectivity of the basal ganglia network showed increased reaction time, indicating an increased effect of fatiguability. The MS group exhibited similar functional connectivity to the HC group in the intrinsic alertness task but have increased functional connectivity in the extrinsic alertness task. This may be due to the fact that the MS group have intact intrinsic alertness, but have a significant deficit in extrinsic alertness, evidenced by the lack of warning effect in reaction time (see section 6.3.1 page 111). Therefore, there is a greater need for the increased functional connectivity of the basal ganglia to mitigate the effect of fatiguability in the extrinsic alertness task, where the MS group have a significant disruption. Despite, the increased functional connectivity in this network, the MS group still exhibited a significantly increased reaction time compared to the HC group. It is possible that the increased functional connectivity of the basal ganglia is recruited to mitigate the effect of fatiguability, as seen consistently in the final block of the alertness-motor paradigm, but that it can only compensate for a limited amount. This may explain why there are several within group differences, but that the basal ganglia network does not compensate enough to create between group differences.

8.4.3 Conclusions

The analyses from the current chapter revealed some inconsistent results of the impact of fatiguability in the HC group. This makes sense as the HC group do not have fatigue. They did exhibit some evidence that increased functional connectivity of the basal ganglia network may mitigate the impact of fatiguability. In the MS group, the effect of fatiguability is more consistent. During both the intrinsic and extrinsic alertness tasks the groups that demonstrated increased functional connectivity of the basal ganglia network showed no differences in cognitive performance, measured by reaction time. This provides strong evidence that increased functional connectivity of the basal ganglia network may mitigate the impact of fatiguability on behavioural performance. Both the HC and MS groups exhibited efficient small-world properties in a whole brain network during an alertness-motor paradigm. However, the results may differ when using a smaller more task specific network compared to the whole brain network employed here. There is

increased functional connectivity of the basal ganglia network in the MS group during the extrinsic alertness task. Despite this increase in functional connectivity the MS group have significantly increased reaction time compared to the HC group. This suggests that the basal ganglia cannot function optimally when disrupted, as noted in the MS group in Chapter 4 (page 71).

Chapter 9 General Discussion

9.1 Introduction

Multiple sclerosis (MS) is a disease of the central nervous system that results in widespread plaques in the brain and spinal cord. Several attempts have been made to understand the aetiology and pathophysiology of MS (see section 1.3.3 page 5 and 1.3.5 page 9). This has been difficult, potentially due to the heterogeneity of the disease. MS represents a significant health burden both financially and socially. Over 90% of individuals with MS reported fatigue as one of their most debilitating symptoms (Branas *et al.*, 2000; Flachenecker *et al.*, 2002). Fatigue leads to impaired quality of life (Janardhan and Bakshi, 2002; Krupp *et al.*, 1988; Mitchell *et al.*, 2005), mental health (Fisk *et al.*, 1994; Schwartz *et al.*, 1996), physical health (Fisk *et al.*, 1994), increased pain (Schwartz *et al.*, 1996) and is the main reason for loss of employment in MS (Smith and Arnett, 2005; van der Hielie *et al.*, 2015). Despite both pharmacological and non-pharmacological interventions for MS fatigue being proposed, none have provided a reliable and effective treatment. This may in part be due to the heterogeneity of MS but can also be due to the lack of adequate measurement of MS fatigue. There are several self-reported measures of fatigue available (for review see Dittner *et al.*, 2004), many of which are reliable and valid for MS (see section 1.4.3 page 17). However, these measures often yield inconsistent results when examining the relationship between fatigue and another construct, such as cognition (Bailey *et al.*, 2007; Bryant *et al.*, 2004; Claros-Salinas *et al.*, 2010; Jennekens-Schinkel *et al.*, 1988; Johnson *et al.*, 1997; Krupp and Elkins, 2000; Kujala *et al.*, 1995; Paul *et al.*, 1998; Schwid *et al.*, 2003). Where objective measures of fatigue provide more reliable results (DeLuca, 2005; Krupp and Elkins, 2000). Therefore, the development of a reliable and objective measurement of fatigue is essential.

Cognitive fatigue can be measured with reaction time on cognitive tasks across time, such as a prolonged alertness task (Boksem *et al.*, 2005; Helton and Russell, 2011). Through its dopaminergic influences, motivation plays a role in prolonged alertness. Individuals who are less motivated will be less alert compared to individuals with high motivation (Oken *et al.*, 2006). A specific brain structure involved in motivation is the basal ganglia, due to its strong dopaminergic inputs. Chaudhuri and Behan (2000; 2004) have proposed that the non-motor function of the basal ganglia may be implicated in central fatigue. Therefore, the alertness mechanism may be uniquely suited to providing an objective measure of performance related fatigue.

In Chapter 3 (page 54) a systematic review, examining multiple different alertness paradigms, a simple reaction time task conducted by Perin *et al.* (2010) was deemed most appropriate for the current thesis. This paradigm allowed for the measurement of reaction time, for both intrinsic and extrinsic alertness, over the whole paradigm. As such, the effect of fatiguability over time could be objectively measured, through reaction time. The inclusion of both intrinsic and extrinsic alertness tasks could elucidate subtle differences between the two forms of alertness. Specifically, as the extrinsic alertness task contains a warning cue that the intrinsic alertness task does not.

9.2 Thesis aims

The relationship between fatigue and cognition remains inconclusive. Furthermore, studies have proposed that the basal ganglia may be implicated in mental fatigue associated with various neurological disorders, but the exact nature of this relationship is unknown. To this end, an alertness-motor paradigm was employed to induce fatiguability and provide an objective measure of fatigue. The three main thesis aims were:

1. to use an alertness-motor paradigm to establish how fatiguability affects cognition in MS;
2. to determine whether the alertness-motor paradigm provides an objective measure of fatigue in MS;
3. to investigate whether the basal ganglia is involved in fatiguability in an MS population.

In this chapter, the specific evidence for each of these aims will be discussed. Followed by a discussion of the limitations of the thesis and proposals for future research.

9.3 Impact of fatiguability on cognition

Fatiguability has a detrimental effect on cognition. A healthy population can overcome the impact of increased fatiguability. Where an MS population, suffering from persistent fatigue, cannot, to the same extent. The reaction time results over time on the alertness-motor paradigm indicated that both the HC and MS groups showed some decreased behavioural performance due to increased fatiguability. This indicates that acutely induced fatiguability can disrupt cognition, even in a healthy population. But the results on the neuropsychological tests showed that the HC group completely overcome the influence of fatiguability and exhibit a learning effect when completing the attention tests a second time. Previous studies have demonstrated a learning effect between baseline and subsequent test performance in HC (Agarwal *et al.*, 2007; Carrier and Pashler, 1992;

McLeod *et al.*, 2004; Roediger and Butler, 2011; Roediger and Karpicke, 2006a; 2006b; Wheeler and Roediger, 1992; Wrisley, 2005), even when the subsequent testing was preceded by prolonged task performance, inducing fatiguability (Claros-Salinas *et al.*, 2010; Johnson *et al.*, 1997; Krupp and Elkins, 2000; Schwid *et al.*, 2003).

The MS group do not show a learning effect, but they also do not show a fatiguing effect as there was no significant difference in baseline and post scan performance. This demonstrates that the MS group can overcome the effect of fatiguability to some extent, but not to the extent seen in the HC group. It is possible that the fatigue experienced by the MS group inhibits a learning effect. But as there is no fatiguing effect, it is possible to suggest that the MS group do have strategies to overcome mental fatigue. This may be due to the functional reorganisation of the brain (Mainero *et al.*, 2006). Mainero *et al.* (2006) proposed that the MS brain undergoes a process of functional reorganisation to compensate for neurodegeneration. Such a compensatory mechanism could explain why the MS group are able to overcome the effects of fatiguability to some extent.

In MS, previous studies are inconsistent with only one demonstrating a learning effect in MS (Sumowski *et al.*, 2010). In contrast, Krupp and Elkins (2000) demonstrated that MS participant had a significant decline in performance following a continuous performance task. However, Sumowski *et al.*, (2010) did not examine the effect of fatiguability, but only whether a learning effect was present in MS. Krupp and Elkins (2000) did examine the effect of fatiguability but did not examine a fatigued MS group. As such their result is not representative of an MS population with fatigue. Moreover, they report the difference between MS and HC at second administration of the tasks, rather than the within group change between first and second administration of the neuropsychological tests or the difference between the two groups. However, given that there was no difference between the HC and MS groups at baseline and a significant difference was present at second administration it is possible that the decline would be significant in within group performance. A further difference was the use of neuropsychological tests. Krupp and Elkins (2000) employed memory tests, whereas this thesis used tests of attention. Multiple studies have shown that memory is generally disrupted in MS where attention remains intact (Benedict *et al.*, 2006; Rao *et al.*, 1991a; 1991b). This may suggest that the memory tests employed by Krupp and Elkins (2000) were more difficult than the tests of attention employed in the current study. There were multiple differences between the two previously reported studies (Sumovski *et al.,* 2010; Krupp and Elkins, 2000), which makes comparison difficult and may explain the different pattern of results observed. Future

studies could explore both the attention and memory domain to determine whether the fatiguing effect is exclusively in the memory domain, or whether it is more related to task difficulty (see section 9.7 page 190).

9.4 Objective measure of fatigue

The results from the current thesis demonstrated that the alertness-motor paradigm can provide some very interesting insights into the impact of fatiguability on cognition. In the current study there is a consistent dissocaiation between perceived fatigue and performance measures of fatiguability in the MS group. There is an association in the HC group suggesting that the performance measures used are appropriate. Therefore the discrepancy stems from an overperception of fatigue in MS. This is supported by previous studies showing a similar dissociation (Middleton *et al.*, 2006; Roberg *et al.*, 2012), but also provides a potential explanation for the inconsistencies in the relationship between fatigue and cognition noted in various studies (Bailey *et al.*, 2007; Bryant *et al.*, 2004; Claros-Salinas *et al.*, 2010; Jennekens-Schinkel *et al.*, 1988; Johnson *et al.*, 1997; Krupp and Elkins, 2000; Kujala *et al.*, 1995; Paul *et al.*, 1998; Schwid *et al.*, 2003).

During the study design phase, it was hypothesised that the mental imagery condition would increase the effect of fatiguability. This was true for the perception of fatigue, as evidenced by the ratings on the Borg scale. But, this was not the case for the performance measure of fatiguability. In fact, in multiple groups the mental imagery condition produced a learning effect, which was an unexpected result. Previous studies have similarly shown a beneficial outcome from mental imagery rehabilitation programmes following stroke (Braun *et al.*, 2006; Sharma *et al.,* 2006; Ietswaart *et al.*, 2011) and in MS (Bovend'Eerdt *et al.*, 2010; Seebacher *et al.,* 2017; 2018). It is difficult to disentangle whether the learning effect is produced by practise, as the mental imagery condition was identical to the handgrip condition. Or whether the learning effect is directly related to the mental imagery process, which in itself has a beneficial outcome (Bovend'Eerdt *et al.*, 2010; Seebacher *et al.,* 2017; 2018). A potential way to clarify this learning effect is proposed in section 9.7 (page 190).

9.5 Basal ganglia and fatiguability

Chaudhuri and Behan (2000; 2004) were one of the first to suggest that the non-motor functions of the basal ganglia may be implicated in central fatigue. Interestingly, they did not provide any direct evidence for this but given the function and neuroanatomy of the basal ganglia, the interpretation seemed appropriate. The current thesis provides strong

evidence that the basal ganglia is implicated in fatigue. More specifically, the current results demonstrated that the basal ganglia may, in fact, mitigate the effect of fatiguability, especially, in an MS population. In the fMRI analysis (Chapter 7 page 125) consistently increased activation of the basal ganglia was observed at the end of both the intrinsic and extrinsic alertness task in the MS groups. This finding provides strong evidence that the basal ganglia is implicated in fatiguability and fatigue perception during cognition. This increased activation of the basal ganglia was even greater in the MS groups that did not show increased fatiguability across the alertness-motor paradigm. Thereby, indicating that the basal ganglia activation may mitigate the effect of fatiguability on cognition. Moreover, basal ganglia activation was also associated with reduced fatigue severity scale (FSS) scores in the multiple regression, providing further evidence for the idea that the basal ganglia mitigates the impact of fatiguability on performance. On the other hand, in the HC group the recruitment of memory regions had a larger impact on the influence of fatiguability on behavioural performance than basal ganglia recruitment. This demonstrates that the healthy population can overcome the effect of fatiguability through learning and practise. This result is not surprising as the HC group do not suffer from significant fatigue as the MS group do. Furthermore, the fMRI analysis revealed activation of the basal ganglia and pFC in conjunction in the HC group. This strongly implicates the associative cortical loop in fatigue. The connectivity analysis (Chapter 8 page 157) provides further evidence that the basal ganglia mitigates the impact of fatiguability on cognition. Both the HC and MS groups showed that increased functional connectivity of the basal ganglia network was associated with no fatiguability across the alertness-motor paradigm for both intrinsic and extrinsic alertness tasks. It is possible that the increased functional connectivity allows for activation of the basal ganglia, which in turn provides the mitigating effect of fatiguability on cognition. The successful recruitment of the basal ganglia to reduce the impact of fatiguability on cognition may explain why there was no fatiguing effect on neuropsychological test performance in the MS group.

The structural morphometry analysis (Chapter 4 page 71) showed reduced grey matter volume in the MS group in the thalamus, basal ganglia and prefrontal cortex. This provides strong evidence for a cortical-subcortical disruption in MS, specifically in the associative cortical loop. As such it is possible that despite the MS group demonstrating increased recruitment of the basal ganglia, this region is atrophied and therefore does not produce the same effect as noted in the HC group. This interpretation is supported by the findings in both chapter 7 (page 125) and 8 (page 157). During the extrinsic alertness task the MS

group do show increased functional connectivity of the basal ganglia compared to the HC group, but this did not result in better behavioural performance. This indicates that although the MS group recruit the basal ganglia to reduce the influence of fatiguability, it does not function optimally. However, perhaps through the process of functional reorganisation, the MS group can still recruit a compensatory mechanism to overcome the effect of fatiguability somewhat. Therefore, the MS group, do not show a fatiguing effect on the neuropsychological test but also cannot perform at the same level as the HC and do not show the learning effect. Similarly, the fMRI revealed that the HC group consistently exhibited increased activation of the pFC compared to the MS group. This makes sense when the role of the basal ganglia through its dopaminergic activity and role in motivation is considered. Energy is expended when performing any tasks. Before a task is executed a choice is made based on the expected reward and expected energy expenditure. The executive functions of the pFC is related to decision making (Buchsbaum *et al*., 2005; Engle *et al*., 1999; Fuster, 1997; Goldman- Rakic, 1996; Knight *et al*., 1995; Laird *et al*., 2005; Rossi *et al*., 2009; Stuss and Benson, 1986; Yuan and Raz 2014). In MS fatigue perceived exertion is high, perhaps due to disruption to the pFC leading to incorrect decisions about expected reward and expected energy expenditure being made. This may lead to decreased motivation due to the imbalance between reward and expenditure. Equally, motivation may be decreased, due to disruption to the basal ganglia, resulting in expected energy expenditure always being higher than the expected reward. Thereby altering the way in which the pFC makes decisions about which actions to complete. Chaudhuri and Behan (2000) support the second explanation. They postulate that the atrophy of the associative loop will result in a reduced dopaminergic drive, from the basal ganglia, through the loop and ultimately suppress frontal activation. Figure 9.1 illustrates each of these potential disruptions along with a healthy associative loop.

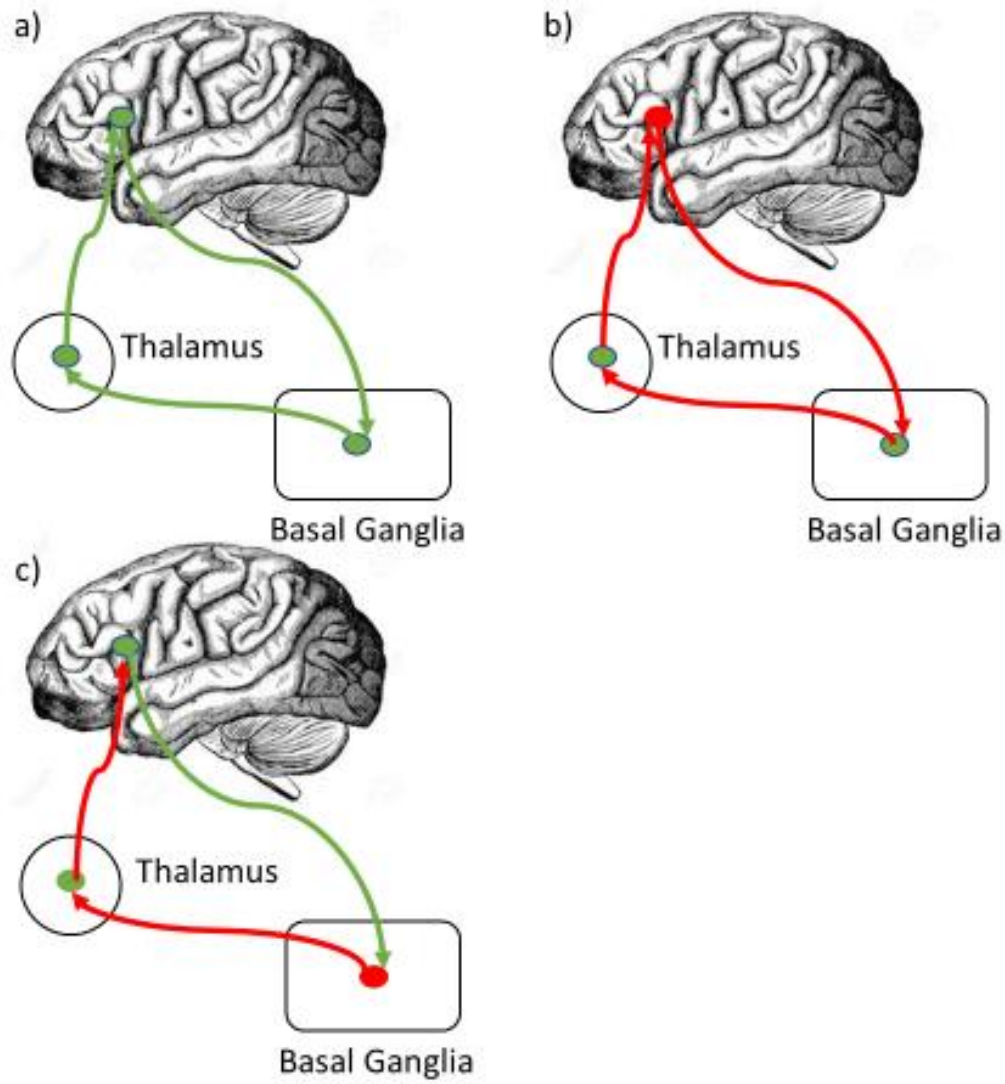


Figure 9.1 Potential associative loop disruptions. *a) healthy associative loop. b) associative loop with driving deficit in pFC. c) associative loop with driving deficit in the basal ganglia.*

The current thesis provides strong evidence that the basal ganglia is implicated in fatigue. Specifically, the basal ganglia may mitigate the effect of fatiguability, but due to structural atrophy of the associative loop in MS, it cannot function optimally. There are multiple studies that support both the disruption to the basal ganglia (Bisecco *et al.*, 2018; Calabrese *et al.*, 2010; Derache *et al.*, 2013; Finke *et al.*, 2015; Lansley *et al.*, 2013; Prinster *et al.*, 2005; Riccitelli *et al.*, 2012; Sepulcre *et al.*, 2006; van de Pavert *et al.*, 2014; Zhang *et al.*, 2017) and its involvement in fatigue (Bonzano *et al.*, 2017; DeLuca *et al.*, 2008; Fillipi *et al.*, 2002; Genova *et al.*, 2013; Rocca *et al.*, 2016; Roelcke *et al.*, 1997). Some also implicate the associative loop specifically (Bonzano *et al.,* 2017; DeLuca *et al.,* 2008; Fillipi *et al.*, 2002; Roelcke *et al.*, 1997). This thesis is the first to use a performance measure of fatiguability across time that allows for distinguishing between whether the basal ganglia induces or mitigates fatigue. Moreover, the current thesis highlights the importance of understanding grey matter pathology in MS symptomology. MS was traditionally

proposed to be a white matter disease (Poser *et al.*, 1983; Prins *et al.*, 2015). There is a growing body of evidence which suggests that grey matter is also affected in MS (Audoin *et al.*, 2006; Battaglini *et al.*, 2009; Bendfeldt *et al.*, 2012; Bisecco *et al.*, 2018; Grothe *et al.*, 2016; Lansley *et al.*, 2013; Onu *et al.*, 2015; Parisi *et al.*, 2014; Prinster *et al.*, 2005; Riccitelli *et al.*, 2012; Sepulcre *et al.*, 2006; van de Pavert *et al.*, 2014; Zhang *et al.*, 2017). Trapp *et al.* (2018) recently identified a new subtype of MS where no white matter demyelination is present, only grey matter damage has been reported. This research further evidences that MS is not exclusively a disease of brain white matter.

9.6 Limitations

A limitation of the current thesis relates to the use of medication by the MS group. Due to the widespread symptomology of MS, individuals are often taking a variety of different medications such as antidepressants, anxiolytics and disease modifying therapies. Studies have shown that immunosuppressive therapies, specifically interferon-β may enhance fatigue (Hadjimichael *et al.*, 2008; Iriarte *et al.*, 2000). Similarly, fatigue is a common side effect of anxiolytics and anti-depressant drugs which are often prescribed to MS individuals. A recent study by Thelen *et al.* (2014) indicated that polypharmacy in MS was associated with increased fatigue and subjective complaints of cognitive impairment. Medication in MS may, therefore, have an impact on fatigue perception in MS. In the present sample, medication use was not examined. Given that medication may impact fatigue in MS, it presents a confound in the current sample. However, it would be unethical to ask participants to stop medication to take part in the study. Moreover, because of the widespread use of drugs among patients excluding patients taking medication would mean the sample would no longer be representative of the patient population. As a result, the use of medication was not excluded from the study. Given a varied and large enough sample future studies could examine both patients on and without medication. This would determine whether medication is indeed a confounding variable.

Moreover, the very nature of relapsing-remitting MS poses a problem for research. Given the severe effect of a relapse, it is likely that MS participants do not take part in research during a relapse but rather during the remission phase of the disease. During the remission phase, the symptoms experienced by individuals with MS is less severe and they may even have undergone some remyelination leading to both structural and functional differences in the brain. Although this allows for the examination of the effect of neural plasticity and functional reorganisation, it does pose a problem for determining the effect

of a symptom on brain behaviour. Unfortunately, there is no easy solution to this, and rather results should be interpreted accordingly.

There is great difficulty when examining an MS population due to the disease heterogeneity. There are varying aetiologies such as genetics, vitamin D and post infection (see section 1.3.4 page 7). Furthermore, MS is comprised of several phenotypes, each of which have a slightly different neurological profile (see section 1.3.1 page 2). In the present thesis, the results showed that there were no differences related to disease phenotype or disease duration in terms of reaction time or force grip during the alertness-motor paradigm. Suggesting that they can be treated as one group for the purpose of this study, as such it is not considered a limitation in the present sample. Furthermore, the symptomology varies across individuals with MS, although the vast majority report fatigue as their most debilitating symptom (Branas *et al*., 2000; Schapiro, 2002, some do not suffer from fatigue. Similarly, some, but not all, individuals with MS suffer from anxiety, depression and cognitive impairments. All these factors make it very difficult to examine MS as a single disorder. Given that MS patients often suffer from comorbidities such as depression and anxiety (Minden *et al*., 1987; Feinstein, 2006) the sample in the present study may represent only a subtype of the MS population. However, given the effect of mood disorders on cognition (Burt *et al*., 1995; Austin *et al*., 2001; De Luca *et al*., 2004; Castaneda *et al*., 2008; Hammar *et al*., 2009; Rosier *et al*., 2012) and fatigue (Christensen & Duncan, 1995; Serretti *et al*., 1999; Targum & Fava, 2011) it was decided to exclude patients with comorbidities. Future studies may consider collecting a larger sample allowing for the comparison between MS phenotypes and even examining the effect of comorbidities.

The alertness-motor paradigm employed in the current study has revealed some interesting results, however, it may have been too complex to allow the adequate power initially calculated with the given sample size. The power calculation (see section 2.5 page 50) revealed that 40 participants, per group, would allow for at least 80% power to detect an effect of size .95 s.d. in the anterior cingulate cortex, .98 s.d. in the inferior parietal lobe, .64 in the prefrontal cortex and .65 in the thalamus. These regions were chosen based on the findings from a previous study (Perin *et al*., 2010). In the present thesis 40 participants in each group were recruited, however, due to some performance disruptions (see section 6.2.1 page 106), the final sample consisted of 36 HC and 37 MS participants. As shown in chapter 7 (page 125), many of the brain regions determined in the analyses did not survive a multiple comparisons threshold. This suggests that the power of the study was not adequate. Interestingly, during preliminary analyses of smaller samples,

results did survive the multiple comparisons threshold. It is possible that with small sample sizes false positives are detected, with medium sample sizes, as in the current sample, false positives are reduced, however, the sample is not large enough to detect true effects. Collecting large sample sizes, especially in neuroimaging, is very expensive and time consuming. As such, it may be that research needs to move towards multicentre or consortia studies that can pool resources. However, this does have its own limitations, as this type of research requires significant collaboration and scanner parameter checks periodically during the study. Most specifically these larger sample collections often focus on anatomical scans making it very difficult to ask specific neurocognitive questions. Low power is a major concern in neuroscience research. Ioannidis (2005) was one of the early articles to suggest that published research findings may be false. Button *et al*. (2013) highlighted that low power is pervasive in the neuroscience literature, where the median power was only 21%. The authors, further suggested that small sample sizes reduced the likelihood of detecting true results, increased the likelihood of false positives, and inflated positive effect sizes. However, Nord *et al*. (2017) argued that although low power was a concern in neuroscience, the power estimates vary substantially across the different subfields. A more recent study specifically examined fMRI research (Poldrack *et al*., 2017) showed that although the number of large sample sized studies was increasing, the average sample size per group in 2015 was 19 participants. This sample size is remarkably low. Poldrack *et al*. (2017) also suggest that working in consortia would be beneficial (for example the Human Connectome Project, www.humanconnectome.org). They specifically suggest that simply increasing the funding to single centres would be detrimental to junior researchers. Journals are now also becoming sensitive to the issue of low power and for example, a recent editorial in the Journal of Neuroscience suggested that preregistration may provide a useful method to increase the reproducibility and reliability of neuroimaging studies (Picciotto, 2018). Cremers *et al*. (2017) suggest a few possible ways in which to overcome low power in a study. The authors suggest that increasing the sample size, using less stringent thresholds or focusing on a particular region of interest will allow for increased power. However, each of these have some major disadvantages. Increasing the sample size is often too costly and time-consuming. Furthermore, using less stringent thresholds leads to a large amount of Type I and Type II errors. Focusing on a particular region of interest is difficult given the complex organisation of the brain, specifically when examining patient populations.

In this thesis the threshold was lowered, but only when behavioural results were significant. This was because it was not possible to increase the sample size or conduct region specific analysis. It would be impractical to conduct a region specific analysis in an MS sample due to the heterogeneity and widespread nature of the disease. There are multiple possible explanations for the low power observed in the current study. Firstly, the *a priori* power calculation does not account for whole brain activation, only in specific regions, which were not the main regions identified by the present analysis. Furthermore, the power calculation was computed based on a comparison between the MS and HC groups but did not take into account that these groups would need to be further subdivided into handgrip first and imagery first groups.

An alternative is to increase the signal-to-noise ratio. The signal-to-noise ratio is a measure that compares background noise to the desired signal. In MRI, this is often determined by the hardware, imaging protocols and acquisition sequences (Redpath, 1998; Welvaert and Rosseel, 2013). Hence the introduction of higher field strengths as the signal-to-noise ratio increases almost linearly with increased field strength (Redpath, 1998). However, this does come with its own limitations. As such, great care must be taken when setting up imaging sequences. Furthermore, it is possible to increase the signal-to-noise ratio by acquiring more images, often achieved through increased TR, and by increasing the number of events sampled, by introducing more trials (Amaro and Barker, 2006).

9.7 Future directions

As a future direction for the current study design it is suggested that the mental imagery condition is not employed, but rather the duration of the physical handgrip condition is extended. In the current study design completing the mental imagery condition confounded the results slightly, by producing a learning effect (see section 9.4 page 183). By dropping the mental imagery condition and increasing the duration of the handgrip condition, the results would not be confounded by the learning effect as a result of the mental imagery condition. As such, if a learning effect were still apparent, then the learning effect is produced due to practice of a task. If no learning effect was observed, the learning was related to the mental imagery process itself and then the mental imagery process may provide a potential rehabilitation and coping strategy for MS fatigue. Furthermore, this slight alteration would have multiple improvements. The increased duration of the handgrip condition would allow for more samples of event of interest, thereby increasing the signal-to-noise ratio (Armato and Barker, 2006). Moreover, the

increased duration may enhance the fatiguability induced by the paradigm. If the results were consistent with those in the present thesis increased basal ganglia activation in the groups without increased reaction time would be observed. The increased duration may also allow for the investigation of the difference between more blocks. As such, one could examine the functional pattern of activation at the beginning of the paradigm, the onset of fatiguability, and at the end of the paradigm. This could further elucidate whether the basal ganglia can sustain its mitigating effect.

In the current thesis, only the functional connectivity of the basal ganglia to the whole brain network was examined. In future, both the structural and functional connectivity in the associative loop, specifically between the pFC, thalamus, and basal ganglia, together with how this associative loop integrates with the rest of the brain may elucidate a specific connectivity deficit. It may be that the disruption to the associative loop is driven by a specific region as proposed in Figure 9.1. This could potentially highlight whether there is a specific region to target for treatment. Or it may be more related to how the disrupted associative loop integrates with the rest of the network. Thereby, indicating that the focus should be on the whole associative loop.

A further area of study could be to use the alertness-motor paradigm to evaluate mental imagery as a potential rehabilitation tool for MS. It is proposed that the mental imagery condition is altered to a mental imagery task that is not identical to the handgrip condition, but still related to intrinsic and extrinsic alertness. This could clarify whether the mental imagery process in itself produces a beneficial outcome. The paradigm would allow for the effect of mental imagery practise to be evaluated on both force grip and mental fatigue. If the mental imagery task is unrelated to the handgrip condition and still produces a learning effect, then the mental imagery process may provide a potential rehabilitation and coping strategy for MS fatigue. If the unrelated mental imagery task does not produce a learning effect, then the learning effect is produced due to practice of a task.

To further understand whether the fatiguing effect is specific to different tasks. Future studies could include memory tests and attention test with varying cognitive load (difficulty). If a fatiguing effect was only noted in the memory domain, it may be that where a greater deficit is present in MS memory (Benedict *et al.*, 2006; Rao *et al.*, 1991a; 1991b), they are less able to overcome the effect of fatiguability. If the fatiguing effect was noted in the tasks with higher cognitive load, it may be that task difficulty is what

determines whether the effect of fatiguability can be overcome. If no fatiguing effect is observed, it may be that MS participants are able to successfully overcome the effects of fatiguability on a variety of tasks, perhaps due to functional reorganisation of the brain (Mainero *et al.,* 2006).

9.8 Conclusions

Fatiguability has a detrimental effect on cognition. A healthy population can overcome the impact of increased fatiguability, to some extent. Where an MS population, suffering from persistent fatigue, cannot. The results from multiple neuroimaging modalities are complementary and indicate that the basal ganglia may mitigate the effect of fatiguability on cognition, predominantly through its associative loop that projects to the pFC. However, due to atrophy in the basal ganglia and pFC in the MS group, the basal ganglia network is not able to perform optimally. This may lead to both the increased fatigue perception and the increased effect of fatiguability on cognition noted in this group. MS was traditionally seen as a white matter disease, however, the findings from this thesis suggest that understanding the grey matter function may provide a better comprehension of disease symptomology. The alertness-motor paradigm employed by the current thesis has revealed some very interesting results and provides a good performance measure of fatigue. With further refinements to determine the parameters for most effective inducement of fatiguability, this alertness-motor paradigm could provide an objective measure of fatigue and the underlying neural substrates. Currently, there are no true objective measures of fatigue and none that may elucidate the neurobiological markers of fatigue. The development of such an objective measure would radically improve the understanding of fatigue with important implications for both research and clinically. In research, such a tool would allow for the effectiveness of fatigue treatments to be objectively measured. Potentially certain neurobiological profiles may respond to treatments differently, thus clinically, it would allow for individuals exhibiting a certain neurological fatigue profile to be referred to the appropriate treatment more efficiently.

References

Abercrombie, E.D., DeBoer, P. and Heeringa, M.J. (1997). Biochemistry of somatodendritic dopamine release in substantia nigra: an in vivo comparison with striatal dopamine release. *Advances in Pharmacology*, 42, pp. 133-136.doi: 10.1016/S1054-3589(08)60713-9

Abidin, A.Z., Chockanathan, U., DSouza, A.M., Inglese, M. and Wismüller, A. (2017). Using large-scale Granger causality to study changes in brain network properties in the Clinically Isolated Syndrome (CIS) stage of multiple sclerosis. *Medical Imaging 2017: Biomedical Applications in Molecular, Structural, and Functional Imaging*, 10137, A101371B. doi: 10.1117/12.2254395.

Agarwal, P.K., Karpicke, J.D., Kang, S.H., Roediger III, H.L., and McDermott, K.B. (2007). Examining the testing effect with open- and closed-book tests. *Applied Cognitive Psychology,* 22, pp. 861-876. doi: 10.1002/acp.1391.

Albin, R.L., Young, A.B. and Penney, J.B. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences*, 12, pp.366-375.doi: 10.1016/0166-2236(89)90074-X

Alexander, G. E., DeLong, M. R. and Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review Neurosciences*, 9, pp. 357-381. doi: 10.1146/annurev.ne.09.030186.002041

Allen, D.N., Goldstein, G., Heyman, R.A. and Rondinelli, T. (1998). Teaching memory strategies to persons with multiple sclerosis. *Journal of Rehabilitation, Research and Development*, 39, pp. 405-410. Retrieved from: http://staff.unak.is/not/andy/NursResearchMethods0708/jrrd/teachingmemorystrategies.pdf

Allen, D.N., Longmore, S. and Goldstein, G. (1995). Memory training and multiple sclerosis: a case study. *International Journal of Rehabilitation and Health,* 1, pp.189-202.doi: 10.1007/BF02214731

Allen, I.V. and McKeown, S.R. (1979). A histological, histochemical and biochemical study of the macroscopically normal white matter in multiple sclerosis. *Journal of the Neurological Sciences,* 41, pp.81-91.doi: 10.1016/0022-510X(79)90142-4

Allen, I.V., McQuaid, S., Mirakhur, M. and Nevin, G. (2001). Pathological abnormalities in the normal-appearing white matter in multiple sclerosis. *Neurological Sciences*, 22, pp.141-144.doi: 10.1007/s100720170012

Alonso, A., Jick, S.S., Olek, M.J., and Hernan, M.A. (2007). Incidence of multiple sclerosis in the United Kingdom. *Journal of Neurology*, 254, pp. 1736-1741.doi: 10.1007/s00415-007-0602-z

Alter, M., Kahana, E., Zilber, N., Miller, A. and Israeli MS Study Group. (2006). Multiple sclerosis frequency in Israel's diverse populations. *Neurology,* 66, pp.1061-1066.doi: 10.1212/01.wnl.0000204194.47925.0d

Alvarez, J. A., and Emory, E. (2006). Executive function and the frontal lobes: a meta-analytic review. *Neuropsychology Review*, 16, pp. 17-42. doi: 10.1007/s11065-006-9002-x.

Amaro Jr, E. and Barker, G.J. (2006). Study design in fMRI: basic principles. *Brain and Cognition*, 60, pp.220-232.doi: 10.1016/j.bandc.2005.11.009

Amato, M.P., Portaccio, E., Goretti, B., Zipoli, V., Iudice, A., Pina, D.D., Malentacchi, G., Sabatini, S., Annunziata, P., Falcini, M. and Mazzoni, M. (2010). Relevance of cognitive deterioration in early relapsing-remitting MS: a 3-year follow-up study. *Multiple Sclerosis Journal*, *16*, pp. 1474-1482. doi:10.1177/1352458510380089.

Amato, M.P., Zipoli, V., and Portaccio, E. (2008). Cognitive changes in multiple sclerosis. *Expert Review of Neurotherapeutics*, 8, pp. 1585–1596. doi:10.1586/14737175.8.10.1585.

Andreasen, A.K., Splijd, P.E., Andersen, H., and Jakobsen, J. (2010). Fatigue and processing speed are related in multiple sclerosis. European Journal on Neurology, 17, pp. 212-218. doi: 10.1111/j.1468-1331.2009.02776.x.

Appelhans, B. M., & Luecken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Review of General Psychology*, *10*(3), 229. doi: 10.1037/1089-2680.10.3.229.

Armutlu, K., Korkmaz, N. C., Keser, I., Sumbuloglu, V., Akbiyik, D. I., Guney, Z., and Karabudak, R. (2007). The validity and reliability of the Fatigue Severity Scale in Turkish multiple sclerosis patients. *International Journal of Rehabilitation Research*, 30, pp. 81-85. doi: 10.1097/MRR.0b013e3280146ec4.

Arnason, B.G.W., Jacobs, G., Hanlon, M., Clay, B.H., Noronha, A.B.C., Auty, A., Davis, B., Nath, A., Bouchard, J.P., Belanger, C. and Gosselin, F. (1999). TNF neutralization in MS-Results of a randomized, placebo-controlled multicenter study. *Neurology*, 53, pp.457-465. doi: 10.1212/01.wnl.0000406608.85830.82

Arnett, P.A. (2005). Longitudinal consistency of the relationship between depression symptoms and cognitive functioning in multiple sclerosis. *CNS Spectrums*, 10, pp.372-382.doi: 10.1017/S1092852900022744

Arnett, P.A., Barwick, F.H. and Beeney, J.E. (2008a). Depression in multiple sclerosis: review and theoretical proposal. *Journal of the International Neuropsychological Society*, 14, pp.691-724.doi: 10.1017/S1355617708081174

Arnett, P.A., Rao, S.M., Bernardin, L., Grafman, J., Yetkin, F.Z. and Lobeck, L. (1994). Relationship between frontal lobe lesions and Wisconsin Card Sorting Test performance in patients with multiple sclerosis. *Neurology,* 44, pp.420-420.doi: 10.1212/WNL.44.3_Part_1.420

Arnett, P.A., Smith, M.M., Barwick, F.H., Benedict, R.H. and Ahlstrom, B.P. (2008b). Oral motor slowing in multiple sclerosis: Relationship to neuropsychological tasks requiring an oral response. *Journal of the International Neuropsychological Society*, 14, pp.454-462.doi: 10.1017/S1355617708080508.

Ascherio, A. and Munger, K.L. (2007a). Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Annals of Neurology*, 61, pp.288-299.doi: 10.1002/ana.21117

Ascherio, A. and Munger, K.L. (2007b). Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. *Annals of Neurology*, 61, pp.504-513.doi: 10.1002/ana.21141

Ashburner, J., and Friston, K. J. (2000). Voxel-based morphometry—the methods. *NeuroImage*, 11, pp. 805-821. doi: 10.1006/nimg.2000.0582.

Aston-Jones, G. (2005). Brain structures and receptors involved in alertness. *Sleep medicine*, 6, S3-S7. doi: 10.1016/S1389-9457(05)80002-4.

Attarian, H.P., Brown, K.M., Duntley, S.P., Carter, J.D. and Cross, A.H. (2004). The relationship of sleep disturbances and fatigue in multiple sclerosis. *Archives of Neurology*, 61, pp.525-528.doi: 10.1001/archneur.61.4.525

Audoin, B., Davies, G.R., Finisku, L., Chard, D.T., Thompson, A.J. and Miller, D.H. (2006). Localization of grey matter atrophy in early RRMS. *Journal of Neurology*, 253, pp.1495-1501.doi: 10.1007/s00415-006-0264-2

Audoin, B., Ibarrola, D., Ranjeva, J.P., Confort-Gouny, S., Malikova, I., Ali-Chérif, A., Pelletier, J. and Cozzone, P. (2003). Compensatory cortical activation observed by fMRI during a cognitive task at the earliest stage of multiple sclerosis. *Human Brain Mapping*, 20, pp.51-58.doi: 10.1002/hbm.10128

Audoin, B., Van Au Duong, M., Ranjeva, J.P., Ibarrola, D., Malikova, I., Confort-Gouny, S., Soulier, E., Viout, P., Ali-Chérif, A., Pelletier, J. and Cozzone, P.J. (2005). Magnetic resonance study of the influence of tissue damage and cortical reorganization on PASAT performance at

the earliest stage of multiple sclerosis. *Human Brain Mapping*, 24, pp.216-228.doi: 10.1002/hbm.20083

Auger, C., Montplaisir, J. and Duquette, P. (2005). Increased frequency of restless legs syndrome in a French-Canadian population with multiple sclerosis. *Neurology*, 65, pp.1652-1653.doi: 10.1212/01.wnl.0000184519.47689.c4

Aulchenko, Y.S., Hoppenbrouwers, I.A., Ramagopalan, S.V., Broer, L., Jafari, N., Hillert, J., Link, J., Lundström, W., Greiner, E., Sadovnick, A.D. and Goossens, D. (2008). Genetic variation in the KIF1B locus influences susceptibility to multiple sclerosis. *Nature Genetics*, 40, p.1402.doi: 10.1038/ng.251

Austin, M.P., Mitchell, P., and Goodwin, G.M. (2001). Cognitive deficits in depression: possible implications for functional neuropathology. *British Journal of Psychiatry*, 178, pp. 200-206. doi: 10.1192/bjp.178.3.200

Australia and New Zealand Multiple Sclerosis Genetics Consortium. (2009). Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. *Nature Genetics*, 41, p.824.doi: 10.1038/ng.396

Babbe, H., Roers, A., Waisman, A., Lassmann, H., Goebels, N., Hohlfeld, R., Friese, M., Schröder, R., Deckert, M., Schmidt, S. and Ravid, R. (2000). Clonal expansions of CD8+ T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *Journal of Experimental Medicine*, 192, pp.393-404.doi: 10.1084/jem.192.3.393

Bagert, B., Camplair, P. and Bourdette, D. (2002). Cognitive dysfunction in multiple sclerosis. *CNS Drugs*, 16, pp.445-455.doi: 10.2165/00023210-200216070-00002

Bailey, A., Channon, S. and Beaumont, J.G. (2007). The relationship between subjective fatigue and cognitive fatigue in advanced multiple sclerosis. *Multiple Sclerosis Journal*, 13, pp.73-80. doi: 10.1177/1352458506071162

Balashov, K.E., Rottman, J.B., Weiner, H.L. and Hancock, W.W. (1999). CCR5+ and CXCR3+ T cells are increased in multiple sclerosis and their ligands MIP-1α and IP-10 are expressed in demyelinating brain lesions. *Proceedings of the National Academy of Sciences*, 96, pp.6873-6878.doi: 10.1073/pnas.96.12.6873

Baldo, J.V., Arévalo, A., Patterson, J.P. and Dronkers, N.F. (2013). Grey and white matter correlates of picture naming: evidence from a voxel-based lesion analysis of the Boston Naming Test. *Cortex*, 49, pp.658-667. doi: 10.1016/j.cortex.2012.03.001.

Bamford, N.S., Robinson, S., Palmiter, R.D., Joyce, J.A., Moore, C. and Meshul, C.K. (2004). Dopamine modulates release from corticostriatal terminals. *Journal of Neuroscience*, 24, pp.9541-9552.doi: 10.1523/JNEUROSCI.2891-04.2004

Bammer, R., Augustin, M., Strasser-Fuchs, S., Seifert, T., Kapeller, P., Stollberger, R., Ebner, F., Hartung, H.P. and Fazekas, F. (2000). Magnetic resonance diffusion tensor imaging for characterizing diffuse and focal white matter abnormalities in multiple sclerosis. *Magnetic Resonance in Medicine*, 44, pp.583-591. doi: 10.1002/1522-2594(200010)44:4<583::AID-MRM12>3.0.CO;2-O

Barak, Y. and Achiron, A. (2002). Effect of interferon-beta-1b on cognitive functions in multiple sclerosis. European *Neurology,* 47, pp.11-14. doi: 10.1159/000047940

Baranzini, S.E., Wang, J., Gibson, R.A., Galwey, N., Naegelin, Y., Barkhof, F., Radue, E.W., Lindberg, R.L., Uitdehaag, B.M., Johnson, M.R. and Angelakopoulou, A. (2008). Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Human Molecular Genetics*, 18, pp.767-778.doi: 10.1093/hmg/ddn388

Barkhof, F., Brück, W., De Groot, C.J., Bergers, E., Hulshof, S., Geurts, J., Polman, C.H. and van der Valk, P. (2003). Remyelinated lesions in multiple sclerosis: magnetic resonance image appearance. *Archives of Neurology*, 60, pp.1073-1081.doi: 10.1001/archneur.60.8.1073

Barkhof, F., Filippi, M., Miller, D.H., Scheltens, P., Campi, A., Polman, C.H., Comi, G., Ader, H.J., Losseff, N. and Valk, J. (1997). Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. *Brain: a Journal of Neurology,* 120, pp.2059-2069.doi: 10.1093/brain/120.11.2059

Barnes, K. A., Cohen, A. L., Power, J. D., Nelson, S. M., Dosenbach, Y. B., Miezin, F. M., Petersen, S.E., and Schlaggar, B. L. (2010). Identifying basal ganglia divisions in individuals using resting-state functional connectivity MRI. *Frontiers in Systems Neuroscience,* 4, A18. doi: 10.3389/fnsys.2010.00018.

Barnes, K. A., Nelson, S. M., Cohen, A. L., Power, J. D., Coalson, R. S., Miezin, F. M., Vogel, A.C., Dubis, J.W., Church, J.A., Petersen, S.E., and Schlaggar, B. L. (2011). Parcellation in left lateral parietal cortex is similar in adults and children. *Cerebral Cortex*, 22, pp. 1148-1158. doi: 10.1093/cercor/bhr189.

Barnett, M.H. and Prineas, J.W. (2004). Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Annals of neurology*, 55, pp.458-468.doi: 10.1002/ana.20016

Barnett, M.H., Parratt, J.D., Pollard, J.D. and Prineas, J.W. (2009). MS: is it one disease. *International MS Journal*, 16, pp.57-65. Retrieved from: https://www.researchgate.net

Bartes – Serrallonga, M., Adan, A., Sole-Casals, J., Caldu, X., Falcon, C., Perez-Pamies, M., Bargallo, N., Serra-Grabulosa, J.M. (2014). Cerebral networks of sustained attention and working memory: a functional magnetic resonance imaging study based on the continuous performance test. *Revista de Neurologia,* 58, pp. 289-295. Retrieved from: https://www.neurologia.com/

Battaglini, M., Giorgio, A., Stromillo, M.L., Bartolozzi, M.L., Guidi, L., Federico, A. and De Stefano, N. (2009). Voxel-wise assessment of progression of regional brain atrophy in relapsing-remitting multiple sclerosis. *Journal of the Neurological Sciences*, 282, pp.55-60.doi: 10.1016/j.jns.2009.02.322

Beatty, W.W., Blanco, C.R., Wilbanks, S.L., Paul, R.H. and Hames, K.A. (1995). Demographic, clinical, and cognitive characteristics of multiple sclerosis patients who continue to work. *Journal of Neurologic Rehabilitation,* 9, pp.167-173.doi: 10.1177/154596839500900306

Beatty, W.W., Goodkin, D.E., Hertsgaard, D., Monson, N. (1990). Clinical and demographic predictors of cognitive performance in multiple sclerosis. Do diagnostic type, disease duration, and disability matter? *Archives of Neurology*, 47, pp. 305-308. doi: 10.1001/archneur.1990.00530030081019

Beatty, W.W., Wilbanks, S.L., Blanco, C.R., Hames, K.A., Tivis, R. and Paul, R.H. (1996). Memory disturbance in multiple sclerosis: reconsideration of patterns of performance on the selective reminding test. *Journal of Clinical and Experimental Neuropsychology*, 18, pp. 56-62. doi: 10.1080/01688639608408262.

Belenky, G., Wesensten, N.J., Thorne, D.R., Thomas, M.L., Sing, H.C., Redmond, D.P., Russo, M.B. and Balkin, T.J. (2003). Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: A sleep dose-response study. *Journal of Sleep Research*, 12, pp.1-12.doi: 10.1046/j.1365-2869.2003.00337.x

Bendfeldt, K., Blumhagen, J.O., Egger, H., Loetscher, P., Denier, N., Kuster, P., Traud, S., Mueller-Lenke, N., Naegelin, Y., Gass, A. and Hirsch, J. (2010). Spatiotemporal distribution pattern of white matter lesion volumes and their association with regional grey matter volume reductions in relapsing-remitting multiple sclerosis. *Human Brain Mapping*, 31, pp.1542-1555. doi: 10.1002/hbm.20951.

Bendfeldt, K., Hofstetter, L., Kuster, P., Traud, S., Mueller-Lenke, N., Naegelin, Y., Kappos, L., Gass, A., Nichols, T.E., Barkhof, F. and Vrenken, H. (2012). Longitudinal gray matter changes in multiple sclerosis—Differential scanner and overall disease-related effects. *Human Brain Mapping*, 33, pp.1225-1245.doi: 10.1002/hbm.21279

Benedict, R.H., Cookfair, D., Gavett, R., Gunther, M., Munschauer, F., Garg, N. and Weinstock-Guttman, B. (2006). Validity of the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). *Journal of the International Neuropsychological Society,* 12, pp.549-558.doi: 10.1017/S1355617706060723

Benedict, R.H., Wahlig, E., Bakshi, R., Fishman, I., Munschauer, F., Zivadinov, R., and Weinstock-Guttman, B. (2005). Predicting quality of life in multiple sclerosis: accounting for physical disability, fatigue, cognition, mood disorder, personality, and behavior change. *Journal of Neurological Sciences*, 231, pp. 29-34. doi: 10.1016/j.jns.2004.12.009

Berardelli, A., Hallett, M., Rothwell, J.C., Agostino, R., Manfredi, M., Thompson, P.D. and Marsden, C.D. (1996). Single–joint rapid arm movements in normal subjects and in patients with motor disorders. *Brain*, 119, pp.661-674. doi: 10.1093/brain/119.2.661.

Bergendal, G., Fredrikson, S., and Almkvist, O. (2007). Selective decline in information processing in subgroups of multiple sclerosis: An 8-year longitudinal study. *European Neurology*, 57, pp. 193–202. doi:10.1159/000099158.

Bernat, J. L. (2006). Chronic disorders of consciousness. *The Lancet*, 367, pp. 1181-1192.doi: 10.1016/S0140-6736(06)68508-5

Bigland-Ritchie, B., Jones, D. A., Hosking, G. P. and Edwards, R. H. (1978). Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. *Clinical Science and Molecular Medicine,* 54, pp. 609-614.doi: 10.1042/cs0540609

Binder, J.R., Desai, R.H., Graves, W.W. and Conant, L.L. (2009). Where is the semantic system? A critical review and meta-analysis of 120 functional neuroimaging studies. *Cerebral Cortex*, 19, pp.2767-2796.doi: 10.1093/cercor/bhp055

Birnboim, S., and Miller, A. (2004). Cognitive rehabilitation for multiple sclerosis patients with executive dysfunction. *Journal of Cognitive Rehabilitation*, 22, pp.8-11. Retrieved from: http://www.jofcr.com/

Bisecco, A., Stamenova, S., Caiazzo, G., d'Ambrosio, A., Sacco, R., Docimo, R., Esposito, S., Cirillo, M., Esposito, F., Bonavita, S. and Tedeschi, G. (2018). Attention and processing speed performance in multiple sclerosis is mostly related to thalamic volume. *Brain Imaging and Behavior*, *12*, pp.20-28.doi: 10.1007/s11682-016-9667-6

Blandini, F., Nappi, G., Tassorelli, C. and Martignoni, E. (2000). Functional changes of the basal ganglia circuitry in Parkinson's disease. *Progress in Neurobiology*, 62, pp.63-88. doi: 10.1016/S0301-0082(99)00067-2.

Bloch, F. (1946). Nuclear induction. *Physical Review,* 70, pp. 460- 474. doi: 10.1103/PhysRev.70.460.

Blumensath, T., Jbabdi, S., Glasser, M. F., Van Essen, D. C., Ugurbil, K., Behrens, T. E., and Smith, S. M. (2013). Spatially constrained hierarchical parcellation of the brain with resting-state fMRI. *NeuroImage*, 76, pp. 313-324. doi: 10.1016/j.neuroimage.2013.03.024.

Bö, L., Geurts, J.J.G., Mörk, S.J. and Valk, P. (2006). Grey matter pathology in multiple sclerosis. *Acta Neurologica Scandinavica*, 113, pp.48-50. doi: 10.1111/j.1600-0404.2006.00615.x.

Bobholz, J.A., and Rao, S.M. (2003). Cognitive Dysfunction in multiple sclerosis: a review of recent developments. *Current Opinions in Neurology*, 16, pp. 283-288.doi: 10.1097/01.wco.0000073928.19076.84

Bodini, B., Cercignani, M., Khaleeli, Z., Miller, D.H., Ron, M., Penny, S., Thompson, A.J. and Ciccarelli, O. (2013). Corpus callosum damage predicts disability progression and cognitive dysfunction in primary-progressive MS after five years. *Human Brain Mapping*, 34, pp.1163-1172. doi: 10.1002/hbm.21499.

Boissoneault, J., Sevel, L., Robinson, M.E. and Staud, R. (2018). Functional brain connectivity of remembered fatigue or happiness in healthy adults: Use of arterial spin labeling. *Journal of Clinical and Experimental Neuropsychology*, 40, pp.224-233.doi: 10.1080/13803395.2017.1329407

Boksem, M. A., Meijman, T. F., and Lorist, M. M. (2005). Effects of mental fatigue on attention: an ERP study. *Cognitive Brain Research*, 25, pp. 107-116. doi: 10.1016/j.cogbrainres.2005.04.011.

Bolkan, S.S., Stujenske, J.M., Parnaudeau, S., Spellman, T.J., Rauffenbart, C., Abbas, A.I., Harris, A.Z., Gordon, J.A. and Kellendonk, C. (2017). Thalamic projections sustain prefrontal activity during working memory maintenance. *Nature Neuroscience*, 20, pp.987-996. doi: 10.1038/nn.4568.

Bonetti, B. and Raine, C.S. (1997). Multiple sclerosis: Oligodendrocytes display cell death–related molecules in situ but do not undergo apoptosis. *Annals of Neurology*, 42, pp.74-84.doi: 10.1002/ana.410420113

Bonnet, M.C., Allard, M., Dilharreguy, B., Deloire, M., Petry, K.G. and Brochet, B. (2010). Cognitive compensation failure in multiple sclerosis. *Neurology*, 75, pp.1241-1248. doi: 10.1212/WNL.0b013e3181f612e3.

Bonzano, L., Pardini, M., Roccatagliata, L., Mancardi, G.L. and Bove, M. (2017). How people with multiple sclerosis cope with a sustained finger motor task: A behavioural and fMRI study. *Behavioural Brain Research*, 325, pp.63-71.doi: 10.1016/j.bbr.2017.02.008

Booss, J., Esiri, M.M., Tourtellotte, W.W. and Mason, D.Y. (1983). Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. *Journal of the Neurological Sciences*, 62, pp.219-232.doi: 10.1016/0022-510X(83)90201-0

Borg, G.A.V. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise,* 14, pp. 377-381. doi: 10.1249/00005768-198205000-00012

Bovend'Eerdt, T.J., Dawes, H., Sackley, C., Izadi, H. and Wade, D.T. (2010). An integrated motor imagery program to improve functional task performance in neurorehabilitation: a single-blind randomized controlled trial. *Archives of Physical Medicine and Rehabilitation*, 91, pp.939-946.doi: 10.1016/j.apmr.2010.03.008

Bowman, E.M., Aigner, T.G. and Richmond, B.J. (1996). Neural signals in the monkey ventral striatum related to motivation for juice and cocaine rewards. *Journal of Neurophysiology*, 75, pp.1061-1073.doi: 10.1152/jn.1996.75.3.1061

Boyacioğlu, R., Schulz, J., Koopmans, P. J., Barth, M., and Norris, D. G. (2015). Improved sensitivity and specificity for resting state and task fMRI with multiband multi-echo EPI compared to multi-echo EPI at 7T. *NeuroImage*, 119, 352-361. doi: 10.1016/j.neuroimage.2015.06.089.

Braley, T.J. and Chervin, R.D. (2010). Fatigue in multiple sclerosis: mechanisms, evaluation, and treatment. *Sleep*, 33, pp.1061-1067.doi: 10.1093/sleep/33.8.1061

Branas, P., Jordan, R., Fry-Smith, A., Burls, A. and Hyde, C. (2000). Treatments for fatigue in multiple sclerosis: a rapid and systematic review. *Health Technology Assessment*, 4, pp. 1-61. doi:10.3310/hta4270

Braun, S.M., Beurskens, A.J., Borm, P.J., Schack, T., and Wade, D.T. (2006). The effects of mental practice in stroke rehabilitation: a systematic review. *Archives of Physical Medicine and Rehabilitation*, 87, pp. 842-852. doi: 10.1016/j.apmr.2006.02.034.

Brefczynski, J.A and DeYoe, E.A. (1999). A physiological correlate on the 'spotlight' of visual attention. *Nature Neuroscience,* 2, pp. 370- 374. doi: 10.1038/7280.

Brett, M., Penny, W. and Kiebel, S. (2003). Introduction to random field theory. *Human Brain Function*, 2, pp. 867-879.doi: 10.1016/B978-012264841-0/50046-9

Breuer, F. A., Blaimer, M., Mueller, M. F., Seiberlich, N., Heidemann, R. M., Griswold, M. A., and Jakob, P. M. (2006). Controlled aliasing in volumetric parallel imaging (2D CAIPIRINHA). *Magnetic Resonance in Medicine*, 55, pp. 549-556. doi: 10.1002/mrm.20787.

Brex, P.A., Ciccarelli, O., O'riordan, J.I., Sailer, M., Thompson, A.J. and Miller, D.H. (2002). A longitudinal study of abnormalities on MRI and disability from multiple sclerosis. *New England Journal of Medicine*, 346, pp.158-164.doi: 10.1056/NEJMoa011341

Bruce, J.M., Bruce, A.S. and Arnett, P.A. (2007). Mild visual acuity disturbances are associated with performance on tests of complex visual attention in MS. *Journal of the International Neuropsychological Society*, 13, pp.544-548.doi: 10.10170S1355617707070658

Bruce, J.M., Bruce, A.S., and Arnett, P.A. (2010a). Response variability is associated with self-reported cognitive fatigue in multiple sclerosis. *Neuropsychology,* 24, pp. 77-83. doi: 10.1037/a0015046.

Bruce, J.M., Bruce, A.S., Hancock, L., and Lynch, S. (2010b). Self-reported memory problems in multiple sclerosis: influence of psychiatric status and normative dissociative experiences. *Archives of Clinical Neuropsychology,* 25, pp. 39-48. doi: 10.1093/arclin/acp092

Brück, W. (2017). Pathology of MS subtypes (RIS, CIS, RRMS, PMS, Balo disease). *Journal of the Neurological Sciences*, 381, pp.5-6. doi: 10.1016/j.jns.2017.08.042

Bruno, R.L., Creange, S.J. and Frick, N.M. (1998). Parallels between post-polio fatigue and chronic fatigue syndrome: a common pathophysiology?. *The American Journal of Medicine*, 105, pp.66S-73S. doi: 10.1016/S0002-9343(98)00161-2.

Bryant, D., Chiaravalloti, N.D. and DeLuca, J. (2004). Objective Measurement of Cognitive Fatigue in Multiple Sclerosis. *Rehabilitation Psychology*, 49, pp.114-122.doi: 10.1037/0090-5550.49.2.114

Buchsbaum, B.R., Greer, S., Chang, W.L., and Berman, K.F. (2005). Meta-analysis of neuroimaging studies of the Wisconsin card-sorting task and component processes. *Human Brain Mapping,* 25, pp. 35-45.doi: 10.1002/hbm.20128

Bullmore, E., and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nature Reviews. Neuroscience*, 10, pp. 186-198. doi: 10.1038/nrn2575.

Burt, D.B., Zembar, M.J., and Niederehe, G. (1995). Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. *Pschology Bulletin*, 117, pp. 285-395. doi: 10.1037/0033-2909.117.2.285

Buschman, T.J. and Miller, E.K. (2014). Goal-direction and top-down control. *Philosophical Transactions of the Royal Society B*, 369, p.20130471. doi: 10.1098/rstb.2013.0471.

Butler, S., Chalder, T., Ron, M. and Wessely, S. (1991). Cognitive behaviour therapy in chronic fatigue syndrome. *Journal of Neurology, Neurosurgery and Psychiatry*, 54, pp.153-158.doi: 10.1136/jnnp.54.2.153

Button, K.S., Ioannidis, J.P., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S. and Munafò, M.R., (2013). Power failure: why small sample size undermines the reliability of neuroscience. *Nature Reviews Neuroscience*, 14, pp.365-376.doi: 10.1038/nrn3475

Cader, S., Cifelli, A., Abu-Omar, Y., Palace, J. and Matthews, P.M. (2005). Reduced brain functional reserve and altered functional connectivity in patients with multiple sclerosis. *Brain*, 129, pp.527-537.doi: 10.1093/brain/awh670

Caine, E.D., Bamford, K.A., Schiffer, R.B., Shoulson, I. and Levy, S. (1986). A controlled neuropsychological comparison of Huntington's disease and multiple sclerosis. *Archives of Neurology*, 43, pp.249-254.doi: 10.1001/archneur.1986.00520030039009

Calabrese, M., Magliozzi, R., Ciccarelli, O., Geurts, J.J., Reynolds, R. and Martin, R. (2015). Exploring the origins of grey matter damage in multiple sclerosis. *Nature Reviews Neuroscience*, 16, pp.147-158. doi:10.1038/nrn3900.

Calabrese, M., Rinaldi, F., Grossi, P., Mattisi, I., Bernardi, V., Favaretto, A., Perini, P. and Gallo, P. (2010). Basal ganglia and frontal/parietal cortical atrophy is associated with fatigue

in relapsing—remitting multiple sclerosis. *Multiple Sclerosis Journal*, 16, pp.1220-1228.doi: 10.1177/1352458510376405

Calabrese, P. (2006). Neuropsychology of multiple sclerosis. *Journal of Neurology*, 253, pp. 10–15. doi:10.1007/s00415-006-1103-1.

Campbell, G.R., Ziabreva, I., Reeve, A.K., Krishnan, K.J., Reynolds, R., Howell, O., Lassmann, H., Turnbull, D.M. and Mahad, D.J. (2011). Mitochondrial DNA deletions and neurodegeneration in multiple sclerosis. *Annals of Neurology*, 69, pp.481-492.doi: 10.1002/ana.22109

Canadian MS Research Group. (1987). A randomized controlled trial of amantadine in fatigue associated with multiple sclerosis. *Canadian Journal of Neurological Sciences*, 14, pp.273-278.doi: 10.1017/S0317167100026603

Cannella, B. and Raine, C.S. (1995). The adhesion molecule and cytokine profile of multiple sclerosis lesions. *Annals of Neurology*, 37, pp.424-435.doi: 10.1002/ana.410370404

Carpenter, M.B., Carleton, S.C., Keller, J.T. and Conte, P. (1981). Connections of the subthalamic nucleus in the monkey. *Brain Research*, 224, pp.1-29.doi: 10.1016/0006-8993(81)91113-6

Carr, H. Y., and Purcell, E. M. (1954). Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Physical Review*, 94, pp. 630–638. doi: 10.1103/PhysRev.94.630.

Carrier, M., and Pashler, H. (1992). The influence of retrieval on retention. *Memory & Cognition,* 20, pp. 633-642. doi: 10.3758/BF03202713

Carswell, R. (1838). *Pathological anatomy: illustrations of the elementary forms of disease*. Longman, Orme, Brown, Green and Longman.

Carton, H., Vlietinck, R., Debruyne, J., De Keyser, J., D'hooghe, M.B., Loos, R., Medaer, R., Truyen, L., Yee, I.M. and Sadovnick, A.D. (1997). Risks of multiple sclerosis in relatives of patients in Flanders, Belgium. *Journal of Neurology, Neurosurgery & Psychiatry,* 62, pp.329-333.doi: 10.1136/jnnp.62.4.329

Caseras, X., Mataix-Cols, D., Giampietro, V., Rimes, K. A., Brammer, M., Zelaya, F., Chalder, T. and Godfrey, E.L. (2006). Probing the working memory system in chronic fatigue syndrome: a functional magnetic resonance imaging study using the n-back task. *Psychosomatic Medicine,* 68, pp. 947-955.doi: 10.1097/01.psy.0000242770.50979.5f

Castaneda, A.E., Suvisaari, J., Marttunen, M., Perala, J., Saarni, S.I., Aalto-Setala, T., Aro, H., Koskinen, S., Lonnqvist, J., and Tuulio-Henriksson, A. (2008). Cognitive functioning in a population-based sample of young adults with a history of non-psychiartic unipolar depressive disorder without psychiatric comorbidity. *Journal of Affective Disorders,* 110, pp. 36-45.doi: 10.1016/j.jad.2007.12.239

Castle, M., Aymerich, M.S., Sanchez-Escobar, C., Gonzalo, N., Obeso, J.A. and Lanciego, J.L. (2005). Thalamic innervation of the direct and indirect basal ganglia pathways in the rat: Ipsi-and contralateral projections. *Journal of Comparative Neurology*, 483, pp.143-153.doi: 10.1002/cne.20421

Catalan, M., De Michiel, A., Bratina, A., Mezzarobba, S., Pellegrini, L., Marcovich, R., Tamiozzo, F., Servillo, G., Zugna, L., Bosco, A. and Sartori, A. (2011). Treatment of fatigue in multiple sclerosis patients: a neurocognitive approach. *Rehabilitation research and practice*, A 670537. doi: 10.1155/2011/670537

Cauley, S. F., Polimeni, J. R., Bhat, H., Wald, L. L., and Setsompop, K. (2014). Interslice leakage artifact reduction technique for simultaneous multislice acquisitions. *Magnetic Resonance in Medicine*, 72, pp. 93-102. doi: 10.1002/mrm.24898.

Ceccarelli, A., Rocca, M.A., Pagani, E., Colombo, B., Martinelli, V., Comi, G. and Filippi, M. (2008). A voxel-based morphometry study of grey matter loss in MS patients with different clinical phenotypes. *NeuroImage*, 42, pp.315-322. doi: 10.1016/j.neuroimage.2008.04.173.

Cerasa, A., Valentino, P., Chiriaco, C., Pirritano, D., Nistico, R., Gioia, C.M., Trotta, M., Del Giudice, F., Tallarico, T., Rocca, F. and Augimeri, A. (2013). MR imaging and cognitive correlates of relapsing–remitting multiple sclerosis patients with cerebellar symptoms. *Journal of Neurology*, 260, pp.1358-1366. doi: 10.1007/s00415-012-6805-y.

Cercignani, M., Iannucci, G., Rocca, M.A., Comi, G., Horsfield, M.A. and Filippi, M. (2000). Pathologic damage in MS assessed by diffusion-weighted and magnetization transfer MRI. *Neurology*, 54, pp.1139-1144.doi: 10.1212/WNL.54.5.1139

Chalder, T., Berelowitz, G., Pawlikowska, T., Watts, L., Wessely, S., Wright, D. and Wallace, E.P. (1993). Development of a fatigue scale. *Journal of Psychosomatic Research*, 37, pp.147-153.doi: 10.1016/0022-3999(93)90081-P

Chang, A., Tourtellotte, W.W., Rudick, R. and Trapp, B.D. (2002). Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. *New England Journal of Medicine*, 346, pp.165-173.doi: 10.1056/NEJMoa010994

Charcot, M. (1868). Histologie de la sclerose en plaque. *Gaz. Hôspital.*, 41, pp.554-556.

Chaudhuri, A. and Behan, P.O. (2000). Fatigue and basal ganglia. *Journal of the neurological sciences*, 179, pp.34-42.doi: 10.1016/S0022-510X(00)00411-1

Chaudhuri, A. and Behan, P.O. (2004). Fatigue in neurological disorders. *The Lancet*, 363, pp.978-988.doi: 10.1016/S0140-6736(04)15794-2

Chen, C.C.G. and Parker, C.R. (2004). Adrenal androgens and the immune system. *Seminars in Reproductive Medicine,* 22, pp. 369-377. doi: 10.1055/s-2004-861553

Chertkow, H., Bub, D., Deaudon, C. and Whitehead, V. (1997). On the status of object concepts in aphasia. *Brain and Language*, 58, pp.203-232.doi: 10.1006/brln.1997.1771

Chesson, A., Hartse, K., McDowell, W.A., Davila, D., Johnson, S., Littner, M., Wise, M. and Rafecas, J. (2000). Practice parameters for the evaluation of chronic insomnia. *Sleep*, 23, pp.237-242.doi: 10.1093/sleep/23.2.1k

Chiaravalloti, N.D. and DeLuca, J. (2008). Cognitive impairment in multiple sclerosis. *The Lancet Neurology*, 7, pp.1139-1151.doi: 10.1016/S1474-4422(08)70259-X

Chiaravalloti, N.D., DeLuca, J., Moore, N.B. and Ricker, J.H. (2005a). Treating learning impairments improves memory performance in multiple sclerosis: a randomized clinical trial. *Multiple Sclerosis Journal*, 11, pp.58-68.doi: 10.1191/1352458505ms1118oa

Chiaravalloti, N.D., Hillary, F.G., Ricker, J.H., Christodoulou, C., Kalnin, A.J., Liu, W.C., Steffener, J. and DeLuca, J. (2005b). Cerebral activation patterns during working memory performance in multiple sclerosis using FMRI. *Journal of Clinical and Experimental Neuropsychology*, 27, pp.33-54.doi: 10.1080/138033990513609

Chiaravalloti, N.D., Stojanovic-Radic, J., and DeLuca, J. (2013). The role of speed versus working memory in predicting learning new information in multiple sclerosis. *Journal of Clinical and Experimental Neuropsychology*, 35, pp. 180-191. doi: 10.1080/13803395.2012.760537.

Chica, A. B., Thiebaut de Schotten, M., Toba, M., Malhotra, P., Lupiáñez, J. and Bartolomeo, P. (2012). Attention networks and their interactions after right-hemisphere damage. *Cortex*, 48, pp. 654-663. doi: 10.1016/j.cortex.2011.01.009

Chilcot, J., Norton, S., Kelly, M.E. and Moss-Morris, R. (2016). The Chalder Fatigue Questionnaire is a valid and reliable measure of perceived fatigue severity in multiple sclerosis. *Multiple Sclerosis Journal*, 22, pp.677-684.doi: 10.1177/1352458515598019

Cho, S.H., Kim, D.G., Kim, D.S., Kim, Y.H., Lee, C.H. and Jang, S.H. (2007). Motor outcome according to the integrity of the corticospinal tract determined by diffusion tensor

tractography in the early stage of corona radiata infarct. *Neuroscience Letters*, 426, pp.123-127. doi: 10.1016/j.neulet.2007.08.049.

Christensen, L., and Duncan, K. (1995). Distinguishing depressed from nondepressed individuals using energy and psychosocial variables. *Journal Consult Clinical Psychology*, 63, pp. 495–498. doi: 10.1037/0022-006X.63.3.495

Christodoulou, C., MacAllister, W.S., McLinskey, N.A. and Krupp, L.B. (2008). Treatment of cognitive impairment in multiple sclerosis. *CNS Drugs*, 22, pp.87-97. doi: 10.2165/00023210-200822020-00001

Christodoulou, C., Melville, P., Scherl, W.F., MacAllister, W.S., Elkins, L.E. and Krupp, L.B. (2006). Effects of donepezil on memory and cognition in multiple sclerosis. *Journal of the Neurological Sciences*, 245, pp.127-136. doi: 10.1016/j.jns.2005.08.021

Ciccarelli, O., Werring, D.J., Wheeler–Kingshott, C.A.M., Barker, G.J., Parker, G.J.M., Thompson, A.J. and Miller, D.H. (2001). Investigation of MS normal-appearing brain using diffusion tensor MRI with clinical correlations. *Neurology*, 56, pp.926-933.doi: 10.1212/WNL.56.7.926

Ciftci, K. (2011). Minimum spanning tree reflects the alterations of the default mode network during Alzheimer's disease. *Annals of Biomedical Engineering*, 39, pp. 1493-1504. doi: 10.1007/s10439-011-0258-9.

Claros-Salinas, D., Bratzke, D., Greitemann, G., Nickisch, N., Ochs, L. and Schröter, H. (2010). Fatigue-related diurnal variations of cognitive performance in multiple sclerosis and stroke patients. *Journal of the Neurological Sciences*, 295, pp.75-81.doi: 10.1016/j.jns.2010.04.018

Cleare, A.J. (2003). The neuroendocrinology of chronic fatigue syndrome. *Endocrine Reviews*, 24, pp.236-252. doi: 10.1210/er.2002-0014

Clemens, B., Zvyagintsev, M., Sack, A.T., Heinecke, A., Willmes, K, Sturm, W. (2011). Revealing the functional neuroanatomy of intrinsic alertness using fMRI: methodological peculiarities. *PLos One*, 6, e25453. doi: 10.1371/journal.pone.0025453.

Clemens, B., Zvyagintsev, M., Sack, A.T., Heinecke, A., Willmes, K, Sturm, W. (2013). Comparison of fMRI activation patterns for test and training procedures of alertness and focused attention. *Restorative Neurology and Neuroscience,* 31, pp. 311-336. doi: 10.3233/RNN-120266.

Cohen, A. L., Fair, D. A., Dosenbach, N. U., Miezin, F. M., Dierker, D., Van Essen, D. C., Schlaggar, B.L., and Petersen, S. E. (2008). Defining functional areas in individual human brains using resting functional connectivity MRI. *NeuroImage*, 41, pp. 45-57. doi: 10.1016/j.neuroimage.2008.01.066.

Cohen, R.A. and Fisher, M. (1989). Amantadine treatment of fatigue associated with multiple sclerosis. *Archives of Neurology*, 46, pp.676-680.doi: 10.1001/archneur.1989.00520420096030

Cole, M. W., Reynolds, J. R., Power, J. D., Repovs, G., Anticevic, A., and Braver, T. S. (2013). Multi-task connectivity reveals flexible hubs for adaptive task control. *Nature Neuroscience*, 16, pp. 1348-1355. doi: 10.1038/nn.3470.

Comabella, M., Craig, D.W., Camiña-Tato, M., Morcillo, C., Lopez, C., Navarro, A., Rio, J., Montalban, X., Martin, R. and BiomarkerMS Study Group. (2008). Identification of a novel risk locus for multiple sclerosis at 13q31. 3 by a pooled genome-wide scan of 500,000 single nucleotide polymorphisms. *PloS One*, 3, p.e3490.doi: 10.1371/journal.pone.0003490

Comi, G. and Leocani, L. (2002). Assessment, pathophysiology and treatment of fatigue in multiple sclerosis. *Expert Review of Neurotherapeutics*, 2, pp.867-876.doi: 10.1586/14737175.2.6.867

Compston, A. (1988). The 150th anniversary of the first depiction of the lesions of multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry*, 51, pp.1249-1252.doi: 10.1136/jnnp.51.10.1249

Compston, A., and Coles, A. (2002). Multiple sclerosis. *Lancet*, 359, pp. 1221-1231. doi: 10.1016/S0140-6736(02)08220-X

Compston, A., and Coles, A. (2008). Multiple sclerosis. *Lancet,* 372, pp. 1502-1517. doi: 10.1016/S0140-6736(08)61620-7.

Confavreux, C., Vukusic, S. and Adeleine, P. (2003). Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. *Brain*, 126, pp.770-782.doi: 10.1093/brain/awg081

Confavreux, C., Vukusic, S., Moreau, T. and Adeleine, P. (2000). Relapses and progression of disability in multiple sclerosis. *New England Journal of Medicine*, 343, pp.1430-1438.doi: 10.1056/NEJM200011163432001

Cook D. B., O'Connor P. J., Lange G., Steffener J. (2007). Functional neuroimaging correlates of mental fatigue induced by cognition among chronic fatigue syndrome patients and controls. *NeuroImage* 36, pp. 108–122.doi: 10.1016/j.neuroimage.2007.02.033

Corbetta, M. and Shulman, G.L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience*, 3, pp.201-215.doi: 10.1038/nrn755

Corley, S.M., Ladiwala, U., Besson, A. and Yong, V.W. (2001). Astrocytes attenuate oligodendrocyte death in vitro through an α6 integrin-laminin–dependent mechanism. *Glia,* 36, pp.281-294.doi: 10.1002/glia.1116

Coull, J.T., Jones, M.E., Egan, T.D., Frith, C.D. and Maze, M. (2004). Attentional effects of noradrenaline vary with arousal level: selective activation of thalamic pulvinar in humans. *NeuroImage*, 22, pp.315-322.doi: 10.1016/j.neuroimage.2003.12.022

Craddock, R. C., James, G. A., Holtzheimer, P. E., Hu, X. P., and Mayberg, H. S. (2012). A whole brain fMRI atlas generated via spatially constrained spectral clustering. *Human Brain Mapping*, 33, pp. 1914-1928. doi: 10.1002/hbm.21333.

Cremers, H.R., Tor, D., and Yarkoni, T. (2017). The relation between statistical power and inference in fMRI. *Plos One,* 12, e0184923.doi: 10.1371/journal.pone.0184923.

Cruz-Gomez, Á.J., Campos, N.V., Belenguer, A., Ávila, C. and Forn, C. (2013). Regional brain atrophy and functional connectivity changes related to fatigue in multiple sclerosis. *PLoS One*, 8, p.e77914. doi: 10.1371/journal.pone.0077914.

Dalton, C.M., Brex, P.A., Miszkiel, K.A., Hickman, S.J., MacManus, D.G., Plant, G.T., Thompson, A.J. and Miller, D.H. (2002). Application of the new McDonald criteria to patients with clinically isolated syndromes suggestive of multiple sclerosis. *Annals of Neurology*, 52, pp.47-53.doi: 10.1002/ana.10240

Daumann, J., Wagner, D., Heekeren, K., Neukirch, A., Thiel, C. M., and Gouzoulis-Mayfrank, E. (2010). Neuronal correlates of visual and auditory alertness in the DMT and ketamine model of psychosis. *Journal of Psychopharmacology*, 24, pp. 1515-1524. doi: 10.1177/0269881109103227.

De Vico Fallani, F., Richiardi, J., Chavez, M., and Achard, S. (2014). Graph analysis of functional brain networks: practical issues in translational neuroscience. *Philosophical Transactions of The Royal Society B Biological Sciences*, 369, 20130521. doi: 10.1098/rstb.2013.0521.

Deale, A., Chalder, T., Marks, I. and Wessely, S. (1997). Cognitive behavior therapy for chronic fatigue syndrome: a randomized controlled trial. *American Journal of Psychiatry*, 154, pp.408-414.doi: 10.1176/ajp.154.3.408

DeGangi, G. and Porges, S., (1990). Neuroscience foundations of human performance. Rockville, MD: *American Occupational Therapy Association Inc.*

DeJager, P.L., Jia, X., Wang, J., De Bakker, P.I., Ottoboni, L., Aggarwal, N.T., Piccio, L., Raychaudhuri, S., Tran, D., Aubin, C. and Briskin, R. (2009). Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. *Nature Genetics*, 41, p.776.doi: 10.1038/ng.401

DeLeon, J., Gottesman, R.F., Kleinman, J.T., Newhart, M., Davis, C., Heidler-Gary, J., Lee, A. and Hillis, A.E. (2007). Neural regions essential for distinct cognitive processes underlying picture naming. *Brain*, 130, pp.1408-1422. doi: 10.1093/brain/awm011.

DeLong, M.R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends in Neurosciences,* 13, pp.281-285.doi: 10.1016/0166-2236(90)90110-V

DeLuca, G.C., Yates, R.L., Beale, H. and Morrow, S.A., (2015). Cognitive impairment in multiple sclerosis: clinical, radiologic and pathologic insights. *Brain Pathology*, 25, pp.79-98. doi:10.111/bpa.12220.

DeLuca, J. (2005). *Fatigue, cognition, and mental effort*. Fatigue as a Window to the Brain. MIT Press, Cambridge, MA, USA, pp.37-57.

DeLuca, J., Barbieri-Berger, S. and Johnson, S.K. (1994). The nature of memory impairments in multiple sclerosis: acquisition versus retrieval. *Journal of Clinical and Experimental Neuropsychology*, 16, pp.183-189.doi: 10.1080/01688639408402629

DeLuca, J., Chelune, G.J., Tulsky, D.S., Lengenfelder, J. and Chiaravalloti, N.D. (2004). Is speed of processing or working memory the primary information processing deficit in multiple sclerosis? *Journal of Clinical and Experimental Neuropsychology,* 26, 550-62. doi: 10.1080/13803390490496641

DeLuca, J., Gaudino, E.A., Diamond, B.J., Christodoulou, C. and Engel, R.A. (1998). Acquisition and storage deficits in multiple sclerosis. *Journal of Clinical and Experimental Neuropsychology*, 20, pp.376-390.doi: 10.1076/jcen.20.3.376.819

DeLuca, J., Genova, H.M., Capili, E.J. and Wylie, G.R. (2009). Functional neuroimaging of fatigue. *Physical Medicine and Rehabilitation Clinics*, 20, pp.325-337. doi: 10.1016/j.pmr.2008.12.007.

DeLuca, J., Genova, H.M., Hillary, F.G. and Wylie, G. (2008). Neural correlates of cognitive fatigue in multiple sclerosis using functional MRI. *Journal of the Neurological Sciences*, 270, pp.28-39.doi: 10.1016/j.jns.2008.01.018

DeLuca, J., Leavitt, V.M., Chiaravalloti, N. and Wylie, G. (2013). Memory impairment in multiple sclerosis is due to a core deficit in initial learning. *Journal of Neurology*, 260, pp.2491-2496. doi: 10.1007/s00415–013-6990-3

Denney, D.R., and Lynch, S.G. (2009). The impact of multiple sclerosis on patients' performance on the Stroop Test: Processing speed versus interference. *Journal of the International Neuropsychological Society*, 15, pp. 451– 458. doi:10.1017/S1355617709090730.

Denney, D.R., Lynch, S.G., Parmenter, B.A., and Horne, N. (2004). Cognitive impairment in relapsing and primary progressive multiple sclerosis: Mostly a matter of speed. *Journal of the International Neuropsychological Society*, 10, pp. 948–956. doi: 10.10170S1355617704107030.

Denney, D.R., Sworowski, L.A., and Lynch, S.G., (2005). Cognitive impairment in three subtypes of multiple sclerosis. *Archives of Clinical Neuropsychology,* 20, pp. 967-981. doi: 10.1016/j.acn.2005.04.012.

Derache, N., Grassiot, B., Mézenge, F., Dugué, A.E., Desgranges, B., Constans, J.M. and Defer, G.L. (2013). Fatigue is associated with metabolic and density alterations of cortical and deep gray matter in relapsing-remitting-multiple sclerosis patients at the earlier stage of the disease: A PET/MR study. *Multiple Sclerosis and Related Disorders*, 2, pp.362-369. doi: 10.1016/j.msard.2013.03.005.

Descarries, L., Watkins, K.C., Garcia, S., Bosler, O. and Doucet, G. (1996). Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: a quantitative autoradiographic and immunocytochemical analysis. *Journal of Comparative Neurology*, 375, pp.167-186. doi: 10.1002/(SICI)1096-9861(19961111)375:2<167::AID-CNE1>3.0.CO;2-0

DeSousa, E.A., Albert, R.H. and Kalman, B. (2002). Cognitive impairments in multiple sclerosis: a review. *American Journal of Alzheimer's Disease & Other Dementias*, 17, pp.23-29.doi: 10.1177/153331750201700104

Devor, A., Tian, P., Nishimura, N., Teng, I.C., Hillman, E.M., Narayanan, S.N., Ulbert, I., Boas, D.A., Kleinfeld, D., and Dale, A.M. (2007). Supressed neuronal activity and concurrent arteriolar vasoconstriction ma explain negative blood oxygenation level-dependent signal. *Journal of Neuroscience,* 27, pp. 4452-4459. doi: 10.1523/JNEUROSCI.0134-07.2007.

Di Fabio, R.P., Soderberg, J., Choi, T., Hansen, C.R. and Schapiro, R.T. (1998). Extended outpatient rehabilitation: its influence on symptom frequency, fatigue, and functional status for persons with progressive multiple sclerosis. *Archives of Physical Medicine and Rehabilitation*, 79, pp.141-146.doi: 10.1016/S0003-9993(98)90290-8

Dinges, D.F., Pack, F., Williams, K., Gillen, K.A., Powell, J.W., Ott, G.E., Aptowicz, C. and Pack, A.I. (1997). Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4–5 hours per night. *Sleep*, 20, pp.267-277.doi: 10.1093/sleep/20.4.267

Dittner, A.J., Wessely, S.C. and Brown, R.G. (2004). The assessment of fatigue: a practical guide for clinicians and researchers. *Journal of Psychosomatic Research*, 56, pp.157-170.doi: 10.1016/S0022-3999(03)00371-4

Doniger, G.M., Beeri, M.S., Bahar-Fuchs, A., Gottlieb, A., Tkachov, A., Kenan, H., Livny, A., Bahat, Y., Sharon, H., Ben-Gal, O. and Cohen, M. (2018). Virtual reality-based cognitive-motor training for middle-aged adults at high Alzheimer's disease risk: A randomized controlled trial. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*, 4, pp.118-129. doi: 10.1016/j.trci.2018.02.005.

Dosenbach, N. U., Fair, D. A., Miezin, F. M., Cohen, A. L., Wenger, K. K., Dosenbach, R. A., Fox, M.D., Snyder, A.Z., Vincent, J.L., Raichle, M.E., Schlaggar, B. L., and Petersen, S.E. (2007). Distinct brain networks for adaptive and stable task control in humans. *Proceedings of the National Academy of Sciences*, 104, pp. 11073-11078. doi: 10.1073/pnas.0704320104.

Drew, M., Tippett, L.J., Starkey, N.J., and Isler, R.B. (2008). Executive dysfunction and cognitive impairment in a large community – based sample with multiple sclerosis from New Zealand: A descriptive study. *Archives of Clinical Neuropsychology*, 23, pp. 1–19. doi:10.1016/j.acn.2007.09.005.

Droogan, A.G., Clark, C.A., Werring, D.J., Barker, G.J., McDonald, W.I. and Miller, D.H. (1999). Comparison of multiple sclerosis clinical subgroups using navigated spin echo diffusion-weighted imaging. *Magnetic Resonance Imaging*, 17, pp.653-661.doi: 10.1016/S0730-725X(99)00011-9

Dujmovic, I. (2011). Cerebrospinal fluid and blood biomarkers of neuroaxonal damage in multiple sclerosis. *Multiple Sclerosis International*, 2011, 767083. doi: 10.1155/2011/767083.

Dulau, C., Deloirre, M., Diaz, H., Saubusse, A., Charre-Morin, J., Prouteau, A. and Brochet, B. (2017). Social cognition according to cognitive impairment in different clinical phenotypes of multiple sclerosis. *Journal of Neurology,* 264, pp. 740-748. doi: 10.1007/s00415-017-8417-z.

Duong, M.V.A., Audoin, B., Boulanouar, K., Ibarrola, D., Malikova, I., Confort-Gouny, S., Celsis, P., Pelletier, J., Cozzone, P.J. and Ranjeva, J.P. (2005). Altered functional connectivity related to white matter changes inside the working memory network at the very early stage of MS. *Journal of Cerebral Blood Flow & Metabolism*, 25, pp.1245-1253. doi:10.1038/sj.jcbfm.9600122

Dutta, R., McDonough, J., Yin, X., Peterson, J., Chang, A., Torres, T., Gudz, T., Macklin, W.B., Lewis, D.A., Fox, R.J. and Rudick, R. (2006). Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Annals of Neurology*, 59, pp.478-489.doi: 10.1002/ana.20736

Duzel, E., Habib, R., Rottel, M., Guderian, S., Tulving, E., and Heinze, H.J. (2003). Human hippocampal and parahippocampal activity during visual associative recognition memory for spatial and non-spatial stimulus configurations. *The Journal of Neuroscience*, 23, pp. 9439-9444. doi: 10.1523/JNEUROSCI.23-28-09439.2003.

Dyment, D.A., Ebers, G.C. and Sadovnick, A.D. (2004). Genetics of multiple sclerosis. *The Lancet Neurology*, 3, pp.104-110.doi: 10.1016/S1474-4422(03)00663-X

Ebers, G. and Bulman, D. (1986). The geography of MS reflects genetic susceptibility. *Neurology*, 36, pp.108-108. Retrieved from: http://www.neurology.org/

Elian, M. and Dean, G. (1987). Multiple sclerosis among the United Kingdom-born children of immigrants from the West Indies. *Journal of Neurology, Neurosurgery & Psychiatry*, 50, pp.327-332.doi: 10.1136/jnnp.50.3.327

Engle, R. W., Tuholski, S. W., Laughlin, J. E., and Conway, A. R. A. (1999). Working memory, short-term memory, and general fluid intelligence: A latent-variable approach. *Journal of Experimental Psychology: General*, 128, pp. 309–331. doi: 10.1037/0096-3445.128.3.309

Engle, C., Greim, B., and Zettl, U.K. (2007). Diagnostics of cognitive dysfunctions in multiple sclerosis. *Journal of Neurology*, 254, pp. 30–34. doi: 10.1007/s00415-007-2009-2

Engstrom, M., Flensner, G., Landtblom, A.M., Ek, A.C. and Karlsson, T. (2013). Thalamo-striato-cortical determinants to fatigue in multiple sclerosis. *Brain and Behavior*, 3, pp.715-728. doi: 10.1002/brb3.181.

Enoka, R. M., & Stuart, D. G. (1992). Neurobiology of muscle fatigue. *Journal of Applied Physiology*, 72, 1631-1648.doi: 10.1152/jappl.1992.72.5.1631

Erro, M.E., Lanciego, J.L. and Giménez-Amaya, J.M. (2002). Re-examination of the thalamostriatal projections in the rat with retrograde tracers. *Neuroscience Research*, 42, pp.45-55. doi: 10.1016/S0168-0102(01)00302-9

Esiri, M.M. (1980). Multiple sclerosis: a quantitative and qualitative study of immunoglobulin-containing cells in the central nervous system. *Neuropathology and Applied Neurobiology*, 6, pp.9-21.doi: 10.1111/j.1365-2990.1980.tb00199.x

Faber, L. G., Maurits, N. M., and Lorist, M. M. (2012). Mental fatigue affects visual selective attention. *PloS One*, 7, e48073. doi: 10.1371/journal.pone.0048073.

Fan, J., McCandliss, T.B.D., Fossella, J., Flombaum, J.I., and Posner, M.I. (2005). The activation of attentional networks. *NeuroImage*, 26, pp. 471 – 479. doi: 10.1016/j.neuroimage.2005.02.004.

Fawcett, J.W. and Asher, R.A. (1999). The glial scar and central nervous system repair. *Brain Research Bulletin*, 49, pp.377-391.doi: 10.1016/S0361-9230(99)00072-6

Fazekas, F., Offenbacher, H., Fuchs, S., Schmidt, R., Niederkorn, K., Horner, S. and Lechner, H. (1988). Criteria for an increased specificity of MRI interpretation in elderly subjects with suspected multiple sclerosis. *Neurology*, 38, pp.1822-1822.doi: 10.1212/WNL.38.12.1822

Feaster, H.T. and Bruce, J.M. (2011). Visual acuity is associated with performance on visual and non-visual neuropsychological tests in multiple sclerosis. *The Clinical Neuropsychologist*, 25, pp.640-651.doi: 10.1080/13854046.2011.565075

Feinberg, D. A., Moeller, S., Smith, S. M., Auerbach, E., Ramanna, S., Glasser, M. F., Miller, K.L., Ugurbil, K., and Yacoub, E. (2010). Multiplexed echo planar imaging for sub-second whole brain FMRI and fast diffusion imaging. *PloS One*, 5, e15710. doi: 10.1371/journal.pone.0015710.

Feinstein, A. (2006). Mood disorders in multiple sclerosis and the effects on cognition. *Journal of the neurological sciences*, 245, pp.63-66.doi: 10.1016/j.jns.2005.08.020

Ferris, C. F., Febo, M., Luo, F., Schmidt, K., Brevard, M., Harder, J. A., Kulkarni, P., Messenger, T., and King, J. A. (2006). Functional magnetic resonance imaging in conscious animals: a new tool in behavioural neuroscience research. *Journal of Neuroendocrinology*, 18, pp. 307-318. doi: 10.1111/j.1365-2826.2006.01424.x.

Filippi, M. (2001). Magnetic resonance imaging findings predicting subsequent disease course in patients at presentation with clinically isolated syndromes suggestive of multiple sclerosis. *Neurological Sciences*, 22, pp.S49-S51.doi: 10.1007/s100720100033

Filippi, M. and Rocca, M.A. (2004). Cortical reorganisation in patients with MS. *Journal of Neurology, Neuroscience and Psychiatry,* 75, pp. 1087-1089. doi: 10.1136/jnnp.2004.036020

Filippi, M., Iannucci, G., Cercignani, M., Rocca, M.A., Pratesi, A. and Comi, G. (2000). A quantitative study of water diffusion in multiple sclerosis lesions and normal-appearing white matter using echo-planar imaging. *Archives of Neurology*, 57, pp.1017-1021.doi: 10.1001/archneur.57.7.1017

Filippi, M., Rocca, M.A., Ciccarelli, O., De Stefano, N., Evangelou, N., Kappos, L., Rovira, A., Sastre-Garriga, J., Tintorè, M., Frederiksen, J.L. and Gasperini, C. (2016). MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. *The Lancet Neurology*, 15, pp.292-303.doi: 10.1016/S1474-4422(15)00393-2

Filippi, M., Rocca, M.A., Colombo, B., Falini, A., Codella, M., Scotti, G. and Comi, G. (2002). Functional magnetic resonance imaging correlates of fatigue in multiple sclerosis. *NeuroImage*, 15, pp.559-567.doi: 10.1006/nimg.2001.1011

Filippi, M., van den Heuvel, M.P., Fornito, A., He, Y., Pol, H.E.H., Agosta, F., Comi, G. and Rocca, M.A. (2013). Assessment of system dysfunction in the brain through MRI-based connectomics. *The Lancet Neurology*, 12, pp.1189-1199.doi: 10.1016/S1474-4422(13)70144-3

Finke, C., Schlichting, J., Papazoglou, S., Scheel, M., Freing, A., Soemmer, C., Pech, L.M., Pajkert, A., Pfüller, C., Wuerfel, J.T. and Ploner, C.J. (2015). Altered basal ganglia functional connectivity in multiple sclerosis patients with fatigue. *Multiple Sclerosis Journal*, 21, pp.925-934.doi: 10.1177/1352458514555784

Fischer, J.S. (1988). Using the Wechsler Memory Scale-Revised to detect and characterize memory deficits in multiple sclerosis. *The Clinical Neuropsychologist*, 2, pp.149-172.doi: 10.1080/13854048808520097

Fischer, J.S., Priore, R.L., Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., Richert, J.R., Salazar, A.M., Goodkin, D.E., Granger, C.V. and Simon, J.H. (2000). Neuropsychological effects of interferon β-1a in relapsing multiple sclerosis. *Annals of Neurology*, 48, pp.885-892.doi: 10.1002/1531-8249(200012)48:6<885::AID-ANA9>3.0.CO;2-1

Fischer, M.T., Wimmer, I., Höftberger, R., Gerlach, S., Haider, L., Zrzavy, T., Hametner, S., Mahad, D., Binder, C.J., Krumbholz, M. and Bauer, J. (2013). Disease-specific molecular events in cortical multiple sclerosis lesions. *Brain*, 136, pp.1799-1815.doi: 10.1093/brain/awt110

Fisk, J.D., Pontefract, A., Ritvo, P.G., Archibald, C.J. and Murray, T.J. (1994). The impact of fatigue on patients with multiple sclerosis. *Canadian Journal of Neurological Sciences*, 21, pp.9-14.doi: 10.1017/S0317167100048691

Flachenecker, P., Bihler, I., Weber, F., Gottschalk, M., Toyka, K.V. and Rieckmann, P. (2004). Cytokine mRNA expression in patients with multiple sclerosis and fatigue. *Multiple Sclerosis Journal*, 10, pp.165-169.doi: 10.1191/1352458504ms9910a

Flachenecker, P., Kümpfel, T., Kallmann, B., Gottschalk, M., Grauer, O., Rieckmann, P., Trenkwalder, C. and Toyka, K.V. (2002). Fatigue in multiple sclerosis: a comparison of different rating scales and correlation to clinical parameters. *Multiple Sclerosis Journal*, 8, pp.523-526.doi: 10.1191/1352458502ms8390a

Flandin, G., and Friston, K. J. (2016). Analysis of family-wise error rates in statistical parametric mapping using random field theory. *Human Brain Mapping.* doi: 10.1002/hbm.23839

Fleischer, V., Gröger, A., Koirala, N., Droby, A., Muthuraman, M., Kolber, P., Reuter, E., Meuth, S.G., Zipp, F. and Groppa, S. (2017). Increased structural white and grey matter network connectivity compensates for functional decline in early multiple sclerosis. *Multiple Sclerosis Journal*, 23, pp.432-441. doi: 10.1177/1352458516651503

Fletcher, P.C., Frith, C.D., Baker, S.C., Shallice, T., Frackowiak, R.S. and Dolan, R.J. (1995). The mind's eye—precuneus activation in memory-related imagery. *NeuroImage*, 2, pp.195-200. doi: 10.1006/nimg.1995.1025.

Folstein, M.F., Robins, L.N. and Helzer, J.E. (1983). The mini-mental state examination. *Archives of General Psychiatry*, 40, pp.812-812. doi: 10.1001/archpsyc.1983.01790060110016.

Forn, C., Rocca, M.A., Valsasina, P., Boscá, I., Casanova, B., Sanjuan, A., Ávila, C. and Filippi, M. (2012). Functional magnetic resonance imaging correlates of cognitive performance in patients with a clinically isolated syndrome suggestive of multiple sclerosis at presentation: an activation and connectivity study. *Multiple Sclerosis Journal*, 18, pp.153-163.doi: 10.1177/1352458511417744

Fortunato, S. (2010). Community detection in graphs. *Physics Reports*, 486, pp. 75-174.doi: 10.1016/j.physrep.2009.11.002

Fox, P.T., and Raichle, M.E. (1986). Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proceedings of the National Academy of Sciences of the United States of America,* 83, pp. 1140–1144. doi: 10.1073/pnas.83.4.1140.

Franciotta, D., Salvetti, M., Lolli, F., Serafini, B. and Aloisi, F. (2008). B cells and multiple sclerosis. *The Lancet Neurology,* 7, pp.852-858.doi: 10.1016/S1474-4422(08)70192-3

Frank, M.J. (2005). Dynamic dopamine modulation in the basal ganglia: a neurocomputational account of cognitive deficits in medicated and nonmedicated Parkinsonism. *Journal of Cognitive Neuroscience*, 17, pp.51-72.doi: 10.1162/0898929052880093

Friedman, J. and Friedman, H. (1993). Fatigue in Parkinson's disease. *Neurology*, 43, pp.2016-2016. doi: 10.1212/WNL.43.10.2016.

Frischer, J.M., Bramow, S., Dal-Bianco, A., Lucchinetti, C.F., Rauschka, H., Schmidbauer, M., Laursen, H., Sorensen, P.S. and Lassmann, H. (2009). The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain*, 132, pp.1175-1189.doi: 10.1093/brain/awp070

Friston, K.J., Harrison, L., and Penny, W. (2003). Dynamic causal modelling. *NeuroImage,* 19, pp. 1973-1302. doi: 10.1016/S1053-8119(03)00202-7.

Fuster, J.M. (1997). *The Prefrontal Cortex: Anatomy, Physiology and Neuropsychology of the Frontal Lobe*. Lippincott-Raven: Philadelphia.

Gale, C.R. and Martyn, C.N. (1995). Migrant studies in multiple sclerosis. *Progress in Neurobiology*, 47, pp.425-448.doi: 10.1016/0301-0082(95)80008-V

Galetta, S.L., Markowitz, C. and Lee, A.G. (2002). Immunomodulatory agents for the treatment of relapsing multiple sclerosis: a systematic review. *Archives of Internal Medicine,* 162, pp.2161-2169.doi: 10.1001/archinte.162.19.2161

Galiero, A., Fratini, F., Mataragka, A., Turchi, B., Nuvoloni, R., Ikonomopoulos, J. and Cerri, D. (2016). Detection of Mycobacterium avium subsp. paratuberculosis in cheeses from small ruminants in Tuscany. *International Journal of Food Microbiology,* 217, pp.195-199.doi: 10.1016/j.ijfoodmicro.2015.10.029

Gamboa, O.L., Tagliazucchi, E., von Wegner, F., Jurcoane, A., Wahl, M., Laufs, H. and Ziemann, U. (2014). Working memory performance of early MS patients correlates inversely with modularity increases in resting state functional connectivity networks. *NeuroImage*, 94, pp.385-395. doi: 10.1016/j.neuroimage.2013.12.008

Garg, A., Schwartz, D. and Stevens, A.A. (2007). Orienting auditory spatial attention engages frontal eye fields and medial occipital cortex in congenitally blind humans. *Neuropsychologia*, 45, pp.2307-2321.doi: 10.1016/j.neuropsychologia.2007.02.015

Garrison, K.A., Scheinost, D., Finn, E.S., Shen, X., Constable, R.T. (2015). The (in)stability of functional brain network measures across thresholds. *NeuroImage*, 118, pp. 651-661. doi: 10.1016/j.neuroimage.2015.05.046.

Genova, H.M., Rajagopalan, V., DeLuca, J., Das, A., Binder, A., Arjunan, A., Chiaravalloti, N. and Wylie, G. (2013). Examination of cognitive fatigue in multiple sclerosis using functional magnetic resonance imaging and diffusion tensor imaging. *PloS One*, 8, p.e78811. doi:10.1371/journal.pone.0078811

Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z.V.I., Chase, T.N., Monsma, F.J. and Sibley, D.R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, 250, pp.1429-1432.doi: 10.1126/science.2147780

Girvan, M. and Newman, M.E. (2002). Community structure in social and biological networks. *Proceedings of the National Academy of Sciences*, 99, pp.7821-7826.doi: 10.1073/pnas.122653799

Gitelman, D. R., Nobre, A. C., Parrish, T. B., LaBar, K. S., Kim, Y. H., Meyer, J. R., and Mesulam, M. M. (1999). A large-scale distributed network for covert spatial attention: further anatomical delineation based on stringent behavioural and cognitive controls. *Brain*, 122, pp. 1093-1106. doi: 10.1093/brain/122.6.1093.

Gobbi, C., Rocca, M.A., Riccitelli, G., Pagani, E., Messina, R., Preziosa, P., Colombo, B., Rodegher, M., Falini, A., Comi, G. and Filippi, M. (2014). Influence of the topography of brain damage on depression and fatigue in patients with multiple sclerosis. *Multiple Sclerosis Journal*, 20, pp.192-201. doi: 10.1177/1352458513493684.

Goldacre, M.J., Seagroatt, V., Yeates, D. and Acheson, E.D. (2004). Skin cancer in people with multiple sclerosis: a record linkage study. *Journal of Epidemiology & Community Health*, 58, pp.142-144.doi: 10.1136/jech.58.2.142

Goldman – Rakic, P.S. (1998). The prefrontal landscape: implications of function architecture for understanding human mentation and the central executive. In: Roberts, A.C., Robbins, T.W., and Weiskranz, L. (eds). *The Prefrontal Cortex: Executive and Cognitive Functions*. Oxford University Press, pp. 87-102.

Gonon, F. (1997). Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum in vivo. *Journal of Neuroscience*, 17, pp.5972-5978. doi: 10.1523/JNEUROSCI.17-15-05972.1997

Good, K., Clark, C.M., Oger, J., Paty, D. and Klonoff, H. (1992). Cognitive impairment and depression in mild multiple sclerosis. *Journal of Nervous and Mental Disease*. 180, pp.730-732.doi: 10.1097/00005053-199211000-00009

Gore, J. C. (2003). Principles and practice of functional MRI of the human brain. *Journal of Clinical Investigation*, 112, pp. 4-9. doi: 10.1172/JCI200319010.

Gottschalk, M., Kümpfel, T., Flachenecker, P., Uhr, M., Trenkwalder, C., Holsboer, F. and Weber, F. (2005). Fatigue and regulation of the hypothalamo-pituitary-adrenal axis in multiple sclerosis. *Archives of Neurology*, 62, pp.277-280.doi: 0.1001/archneur.62.2.277

Grahn, J.A. and Manly, T. (2012). Common neural recruitment across diverse sustained attention tasks. *PLos One,* 7, e49556. doi: 10.1371/journal.pone.0049556.

Greenhouse, S.W., and Geisser, S. (1959). On methods in the analysis profile data. *Psychometrika,* 24, pp. 95-112. doi: 10.1007/BF02289823.

Grossman, M., McMillan, C., Moore, P., Ding, L., Glosser, G., Work, M. and Gee, J. (2004). What's in a name: voxel-based morphometric analyses of MRI and naming difficulty in Alzheimer's disease, frontotemporal dementia and corticobasal degeneration. *Brain*, 127, pp.628-649. doi: 10.1093/brain/awh075.

Grothe, M., Lotze, M., Langner, S. and Dressel, A. (2016). The role of global and regional gray matter volume decrease in multiple sclerosis. *Journal of Neurology*, 263, pp.1137-1145. doi: 10.1007/s00415-016-8114-3.

Grothe, M., Lotze, M., Langner, S. and Dressel, A. (2017). Impairments in Walking Ability, Dexterity, and Cognitive Function in Multiple Sclerosis Are Associated with Different Regional Cerebellar Gray Matter Loss. *The Cerebellum*, 16, pp.945-950. doi: 10.1007/s12311-017-0871-8.

Guo, A.C., Jewells, V.L. and Provenzale, J.M. (2001). Analysis of normal-appearing white matter in multiple sclerosis: comparison of diffusion tensor MR imaging and magnetization transfer imaging. *American Journal of Neuroradiology*, 22, pp.1893-1900.doi: 10.1148/radiol.2223010311

Gustavsson, A., Svensson, M., Jacobi, F., Allgulander, C., Alonso, J., Beghi, E., Dodel, R., Ekman, M., Faravelli, C., Fratiglioni, L., Gannon, B., Jones, D.H., Jennum, P., Jordanova, A., Jonsson, L., Karampampa, K., Knapp, M., Kobelt, G., Kurth, T., Lieb, R., Linde, M., Musayev, A., Norwood, F., Preisig, M., Pugliatti, M., Rehm, J., Salvador-Carulla, L., Schlehofer, B., Simon, R., Steinhausen, H.C., Stovner, L.J., Vallot, J.M., Van den Bergh, P., van Os, J., Vos, P., Xu, W., Wittchen, H.U., Jonsson, B., and Olesen, J. (2011). Cost of disorders of the brain in Europe 2010. European Neuropsychopharmacology, 21, pp. 718–779. doi: 10.1016/j.euroneuro.2011.08.008

Haber, S.N. and Calzavara, R. (2009). The cortico-basal ganglia integrative network: the role of the thalamus. *Brain Research Bulletin*, 78, pp.69-74. doi: 10.1016/j.brainresbull.2008.09.013.

Hadjimichael, O., Vollmer, T. and Oleen-Burkey, M. (2008). Fatigue characteristics in multiple sclerosis: the North American Research Committee on Multiple Sclerosis (NARCOMS) survey. *Health and Quality of Life Outcomes*, 6, pp.100-110.doi: 10.1186/1477-7525-6-100

Hahn, E. L. (1950). Spin echoes. *Physical Review*, 80, pp. 580-594. doi: 10.1103/PhysRev.80.580.

Haider, L., Simeonidou, C., Steinberger, G., Hametner, S., Grigoriadis, N., Deretzi, G., Kovacs, G.G., Kutzelnigg, A., Lassmann, H. and Frischer, J.M. (2014). Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron. *Journal of Neurology, Neurosurgery and Psychiatry*, 85, pp.1386-1395. doi: 10.1136/jnnp-2014-307712.

Halsband, U., Ito, N., Tanji, J., and Freund, H. J. (1993). The role of premotor cortex and the supplementary motor area in the temporal control of movement in man. *Brain*, 116, pp. 243-266.doi: 10.1093/brain/116.1.243.

Hammar, A., and Ardal, G. (2009). Cognitive functioning in major depression- a summary. *Frontiers of Human Neuroscience,* 3, pp. 1-7.doi: 10.3389/neuro.09.026.2009

Hammond, S.R., English, D.R. and McLeod, J.G. (2000). The age-range of risk of developing multiple sclerosis: evidence from a migrant population in Australia. *Brain*, 123, pp.968-974.doi: 10.1093/brain/123.5.968

Hanken, K., Eling, P., Kastrup, A., Klein, J. and Hildebrandt, H. (2015). Integrity of hypothalamic fibers and cognitive fatigue in multiple sclerosis. *Multiple Sclerosis and Related Disorders*, 4, pp.39-46.doi: 10.1016/j.msard.2014.11.006 2211-0348

Hassan-Smith, G. and Douglas, M.R. (2011). Epidemiology and diagnosis of multiple sclerosis. *British Journal of Hospital Medicine,* 72, pp.M146-M151.doi: 10.12968/hmed.2011.72.Sup10.M146

Hawellek, D.J., Hipp, J.F., Lewis, C.M., Corbetta, M. and Engel, A.K. (2011). Increased functional connectivity indicates the severity of cognitive impairment in multiple sclerosis. *Proceedings of the National Academy of Sciences*, 108, pp.19066-19071. doi: 10.1073/pnas.1110024108.

Hawkes, C.H. and Macgregor, A.J. (2009). Twin studies and the heritability of MS: a conclusion. *Multiple Sclerosis Journal,* 15, pp.661-667.doi: 10.1177/1352458509104592

Hayasaka, S. and Nichols, T.E. (2003). Validating cluster size inference: random field and permutation methods. *NeuroImage*, 20, pp.2343-2356.doi: 10.1016/j.neuroimage.2003.08.003

Hayashi, T., Morimoto, C., Burks, J.S., Kerr, C. and Hauser, S.L. (1988). Dual-label immunocytochemistry of the active multiple sclerosis lesion: Major histocompatibility complex and activation antigens. *Annals of Neurology*, 24, pp.523-531.doi: 10.1002/ana.410240408

He, Y., and Evans, A. (2010). Graph theoretical modeling of brain connectivity. Current Opinion in Neurology, 23, pp. 341-350. doi: 10.1097/WCO.0b013e32833aa567.

He, Y., Dagher, A., Chen, Z., Charil, A., Zijdenbos, A., Worsley, K. and Evans, A. (2009). Impaired small-world efficiency in structural cortical networks in multiple sclerosis associated with white matter lesion load. *Brain*, 132, pp.3366-3379. doi: 10.1093/brain/awp089

Heesen, C., Nawrath, L., Reich, C., Bauer, N., Schulz, K.H. and Gold, S.M. (2006). Fatigue in multiple sclerosis: an example of cytokine mediated sickness behaviour?. *Journal of Neurology, Neurosurgery & Psychiatry*, 77, pp.34-39.doi: 10.1136/jnnp.2005.065805

Hefti, F., Enz, A. and Melamed, E. (1985). Partial lesions of the nigrostriatal pathway in the rat: acceleration of transmitter synthesis and release of surviving dopaminergic neurones by drugs. *Neuropharmacology,* 24, pp.19-23.doi: 10.1016/0028-3908(85)90090-5

Helton, W. S., and Russell, P. N. (2011). Working memory load and the vigilance decrement. *Experimental Brain Research*, 212, pp. 429-437. doi: 10.1007/s00221-011-2749-1.

Henderson, A.P., Barnett, M.H., Parratt, J.D. and Prineas, J.W. (2009). Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. *Annals of Neurology*, 66, pp.739-753.doi: 10.1002/ana.21800

Henson, R.N., Rugg, M.D., Shallice, T., Josephs, O. and Dolan, R.J. (1999). Recollection and familiarity in recognition memory: an event-related functional magnetic resonance imaging study. *Journal of Neuroscience*, 19, pp.3962-3972. doi: 10.1523/JNEUROSCI.19-10-03962.1999.

Hernandez-Echeagaray, E., Galarraga, E. and Bargas, J. (1998). 3-α-Chloro-imperialine, a potent blocker of cholinergic presynaptic modulation of glutamatergic afferents in the rat neostriatum. *Neuropharmacology*, 37, pp.1493-1503.doi: 10.1016/S0028-3908(98)00131-2

Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H. and Surmeier, D.J. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-Type Ca2+ currents and excitability vía a novel PLCβ1–IP3–calcineurin-signaling cascade. *Journal of Neuroscience,* 20, pp.8987-8995. doi: 10.1523/JNEUROSCI.20-24-08987.2000

Hidalgo de la Cruz, M., d'Ambrosio, A., Valsasina, P., Pagani, E., Colombo, B., Rodegher, M., Falini, A., Comi, G., Filippi, M. and Rocca, M.A. (2018). Abnormal functional connectivity of thalamic sub-regions contributes to fatigue in multiple sclerosis. *Multiple Sclerosis Journal*, 24, pp.1183-1195.doi: 10.1177/1352458517717807

Hikida, T., Kimura, K., Wada, N., Funabiki, K. and Nakanishi, S. (2010). Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron*, 66, pp.896-907.doi: 10.1016/j.neuron.2010.05.011

Hikosaka, O. (2007). Basal ganglia mechanisms of reward-oriented eye movement. *Annals of the New York Academy of Sciences,* 1104, pp.229-249.doi: 10.1196/annals.1390.012

Hikosaka, O., Sakamoto, M. and Usui, S. (1989). Functional properties of monkey caudate neurons. III. Activities related to expectation of target and reward. *Journal of Neurophysiology*, 61, pp.814-832.doi: 10.1152/jn.1989.61.4.814

Hilti, C.C., Jann, K., Heinemann, D., Federspiel, A., Dierks, T., Seifritz, E., Cattapan-Ludewig, K. (2013). Evidence for a cognitive control network for goal-directed attention in simple sustained attention. *Brain and Cognition*, 81, pp. 193–202. doi: 10.1016/j.bandc.2012.10.013.

Hirsch, R.L., Panitch, H.S. and Johnson, K.P. (1985). Lymphocytes from multiple sclerosis patients produce elevated levels of gamma interferon in vitro. *Journal of Clinical Immunology*, 5, pp.386-389.doi: 10.1007/BF00915335

Hochmeister, S., Grundtner, R., Bauer, J., Engelhardt, B., Lyck, R., Gordon, G., Korosec, T., Kutzelnigg, A., Berger, J.J., Bradl, M. and Bittner, R.E. (2006). Dysferlin is a new marker for leaky brain blood vessels in multiple sclerosis. *Journal of Neuropathology & Experimental Neurology,* 65, pp.855-865.doi: 10.1097/01.jnen.0000235119.52311.16

Hoffman, P., Pobric, G., Drakesmith, M. and Lambon Ralph, M.A. (2012). Posterior middle temporal gyrus is involved in verbal and non-verbal semantic cognition: Evidence from rTMS. *Aphasiology*, 26, pp.1119-1130.doi: 10.1080/02687038.2011.608838

Hojsgaard, C.H., Schreiber, K., Magyari, M., Ammitzboll, C., Börnsen, L., Romme, C.J., Ratzer, R., Soelberg, S.P., and Sellebjerg, F. (2018). Progressive multiple, sclerosis, cognitive function, and quality of life. *Brain Behaviour*, 5, e00875.doi: 10.1002/brb3.875.

Hollerman, J.R., Tremblay, L. and Schultz, W. (2000). Involvement of basal ganglia and orbitofrontal cortex in goal-directed behavior. In *Progress in Brain Research.* 126, pp. 193-215. doi: 10.1016/S0079-6123(00)26015-9.

Holman, D.W., Klein, R.S. and Ransohoff, R.M. (2011). The blood–brain barrier, chemokines and multiple sclerosis. *Biochimica et Biophysica Acta*, 1812, pp.220-230.doi: 10.1016/j.bbadis.2010.07.019

Honarmand, K., and Feinstein, A. (2009). Validation of the Hospital Anxiety and Depression Scale for use with multiple sclerosis patients. *Multiple Sclerosis Journal*, 15, pp. 1518-1524. doi: 10.1177/1352458509347150.

Hong, S. and Hikosaka, O. (2011). Dopamine-mediated learning and switching in cortico-striatal circuit explain behavioral changes in reinforcement learning. *Frontiers in Behavioral Neuroscience*, 5, A15.doi: 10.3389/fnbeh.2011.00015

Howes, D. and Boller, F. (1975). Simple reaction time: evidence for focal impairment from lesions of the right hemisphere. Brain, 98, pp. 317-332. doi: 10.1093/brain/98.2.317.

Huang, D., Han, Y., Rani, M.S., Glabinski, A., Trebst, C., Sørensen, T., Tani, M., Wang, J., Chien, P., O'bryan, S. and Bielecki, B. (2000). Chemokines and chemokine receptors in inflammation of the nervous system: manifold roles and exquisite regulation. *Immunological Reviews*, 177, pp.52-67.doi: 10.1034/j.1600-065X.2000.17709.x

Huang, Y. and Mucke, L. (2012). Alzheimer mechanisms and therapeutic strategies. *Cell*, 148, pp.1204-1222.doi: 10.1016/j.cell.2012.02.040

Hughes, A.J., Daniel, S.E., Kilford, L. and Lees, A.J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *Journal of Neurology, Neurosurgery & Psychiatry*, 55, pp.181-184.doi: 10.1136/jnnp.56.8.938

Hughes, L.E., Smith, P.A., Bonell, S., Natt, R.S., Wilson, C., Rashid, T., Amor, S., Thompson, E.J., Croker, J. and Ebringer, A. (2003). Cross-reactivity between related sequences found in Acinetobacter sp., Pseudomonas aeruginosa, myelin basic protein and myelin oligodendrocyte glycoprotein in multiple sclerosis. *Journal of Neuroimmunology,* 144, pp.105-115.doi: 10.1016/S0165-5728(03)00274-1

Hulst, H.E., Schoonheim, M.M., Van Geest, Q., Uitdehaag, B.M., Barkhof, F. and Geurts, J.J. (2015). Memory impairment in multiple sclerosis: relevance of hippocampal activation and hippocampal connectivity. *Multiple Sclerosis Journal*, 21, pp.1705-1712. doi:10.1177/1352458514567727

Huolman, S., Hämäläinen, P., Vorobyev, V., Ruutiainen, J., Parkkola, R., Laine, T. and Hämäläinen, H. (2011). The effects of rivastigmine on processing speed and brain activation in patients with multiple sclerosis and subjective cognitive fatigue. *Multiple Sclerosis Journal*, 17, pp.1351-1361.doi: 10.1177/1352458511412061

Hyncicova, E., Vyhnálek, M., Kalina, A., Martinkovič, L., Nikolai, T., Lisý, J., Hort, J., Meluzínová, E. and Laczó, J. (2017). Cognitive impairment and structural brain changes in patients with clinically isolated syndrome at high risk for multiple sclerosis. *Journal of Neurology*, 264, pp.482-493. doi: 10.1007/s00415-016-8368-9.

Ietswaart, M., Johnston, M., Dijkerman, H.C., Joice, S., Scott, C.L., MacWalter, R.S., and Hamilton, S.J. (2011). Mental practice with motor imagery in stroke recovery: randomised controlled trial of efficacy. *Brain*, 132, pp. 1373-1386.doi: 10.1093/brain/awr077

International Multiple Sclerosis Genetics Consortium & The Wellcome Trust Case Control Consortium 2. (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*, 476, p.214.doi: 10.1038/nature10251

International Multiple Sclerosis Genetics Consortium. (2007). Risk alleles for multiple sclerosis identified by a genome wide study. *New England Journal of Medicine*, 357, pp.851-862.doi: 10.1056/NEJMoa073493

Ioannidis, J.P. (2005). Why most published research findings are false. *PLoS Medicine*, 2, p.e124.doi: 10.1371/journal.pmed.0020124

Iriarte, J., Subirá, M.L. and de Castro, P. (2000). Modalities of fatigue in multiple sclerosis: correlation with clinical and biological factors. *Multiple Sclerosis Journal*, 6, pp.124-130.doi: 10.1177/135245850000600212

Italian REMS Study Group , Manconi, M., Ferini-Strambi, L., Filippi, M., Bonanni, E., Iudice, A., Murri, L., Gigli, G.L., Fratticci, L., Merlino, G., and Terzano, G. (2008). Multicenter case-control study 10.5665/sleep/31.7.944 on restless legs syndrome in multiple sclerosis: the REMS study. *Sleep*, 31, pp.944-952.doi: 10.5665/sleep/31.7.944

Jaeger, S., Paul, F., Scheel, M., Brandt, A., Heine, J., Pach, D., Witt, C.M., Bellmann-Strobl, J. and Finke, C. (2018). Multiple sclerosis–related fatigue: Altered resting-state functional connectivity of the ventral striatum and dorsolateral prefrontal cortex. *Multiple Sclerosis Journal*, 1, A1352458518758911. doi: 10.1177/1352458518758911.

Jakkula, E., Leppä, V., Sulonen, A.M., Varilo, T., Kallio, S., Kemppinen, A., Purcell, S., Koivisto, K., Tienari, P., Sumelahti, M.L. and Elovaara, I. (2010). Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene. *The American Journal of Human Genetics*, 86, pp.285-291.doi: 10.1016/j.ajhg.2010.01.017

Jalili, M. (2016). Functional brain networks: does the choice of dependency estimator and binarization method matter. *Scientific Reports,* 6, A29780. doi: 10.1038/srep29780.

Janardhan, V. and Bakshi, R. (2002). Quality of life in patients with multiple sclerosis: the impact of fatigue and depression. *Journal of the Neurological Sciences*, 205, pp.51-58.doi: 10.1016/S0022-510X(02)00312-X

Jennekens-Schinkel, A., Sanders, E.A.C.M., Lanser, J.B.K. and Van der Velde, E.A. (1988). Reaction time in ambulant multiple sclerosis patients: Part I. Influence of prolonged cognitive effort. *Journal of the Neurological Sciences,* 85, pp.173-186.doi: 10.1016/0022-510X(88)90154-2

Johns, M.W. (1991). A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep,* 14, pp. 540-545. doi: 10.1093/sleep/14.6.540

Johnson, S.K., Lange, G., DeLuca, J., Korn, L.R. and Natelson, B. (1997). The effects of fatigue on neuropsychological performance in patients with chronic fatigue syndrome, multiple sclerosis, and depression. *Applied Neuropsychology*, 4, pp.145-153.doi: 10.1207/s15324826an0403_1

Junker, A. and Brück, W. (2012). Autoinflammatory grey matter lesions in humans: cortical encephalitis, clinical disorders, experimental models. *Current Opinion in Neurology*, 25, pp.349-357. doi: 10.1097/WCO.0b013e3283534a8a.

Kahana, E., Alter, M. and Zilber, N. (2008). Environmental factors determine multiple sclerosis risk in migrants to Israel. *Multiple Sclerosis*, 14, pp. S69-S70. Retrieved from: http://journals.sagepub.com/home/msj

Kaiser, M. (2011). A tutorial in connectome analysis: topological and spatial features of brain networks. *NeuroImage*, 57, pp. 892-907. doi: 10.1016/j.neuroimage.2011.05.025.

Kalmar, J.H., Gaudino, E.A., Moore, N.B., Halper, J. and DeLuca, J. (2008). The relationship between cognitive deficits and everyday functional activities in multiple sclerosis. *Neuropsychology*, 22, pp.442-449.doi: 10.1037/0894-4105.22.4.442

Katsuki, F. and Constantinidis, C. (2014). Bottom-up and top-down attention: different processes and overlapping neural systems. *The Neuroscientist*, 20, pp.509-521.doi: 10.1177/1073858413514136

Kawagoe, K.T., Garris, P.A., Wiedemann, D.J. and Wightman, R.M. (1992). Regulation of transient dopamine concentration gradients in the microenvironment surrounding nerve terminals in the rat striatum. *Neuroscience*, 51, pp.55-64.doi: 10.1016/0306-4522(92)90470-M

Kawagoe, R., Takikawa, Y. and Hikosaka, O. (1998). Expectation of reward modulates cognitive signals in the basal ganglia. *Nature Neuroscience*, 1, pp.411-416.doi: 10.1038/1625

Kawaguchi, Y., Wilson, C.J., Augood, S.J. and Emson, P.C. (1995). Striatal interneurones: chemical, physiological and morphological characterization. *Trends in Neurosciences*, 18, pp.527-535. doi: 10.1016/0166-2236(95)98374-8

Kaynak, H., Altintaş, A., Kaynak, D., Uyanik, Ö., Saip, S., Ağaoğlu, J., Önder, G. and Siva, A. (2006). Fatigue and sleep disturbance in multiple sclerosis. *European Journal of Neurology*, 13, pp.1333-1339. doi: 10.1111/j.1468-1331.2006.01499.x

Kellerman, T., Reske, M., Jansen, A., Satrapi, P., Shah, N.J., Schneider, F., and Habel. U. (2011). Latencies in BOLD response during visual attention processes. *Brain Research,* 1386, pp. 127-138. doi: 10.1016/j.brainres.2011.02.023.

Kendall, A.P., Kautz, M.A., Russo, M.B. and Killgore, W.D. (2006). Effects of sleep deprivation on lateral visual attention. *International Journal of Neuroscience*, 116, pp.1125-1138.doi: 10.1080/00207450500513922.

Kim, S. G., and Bandettini, P. A. (2011). Principles of BOLD functional MRI. In Faro, S., Mohamed, F., Law, M., and Ulmer, J. (eds). *Functional Neuroradiology*, pp. 293-303. US: Springer. doi: 10.1007/978-1-4419-0345-7_16.

Kinomura, S., Larsson, J., Gulya´s, B., and Roland, P. E. (1996). Activation by attention of the human reticular formation and thalamic intralaminar nuclei. *Science,* 271, pp. 512–515. doi: 10.1126/science.271.5248.512.

Kirk, J., Plumb, J., Mirakhur, M. and McQuaid, S. (2003). Tight junctional abnormality in multiple sclerosis white matter affects all calibres of vessel and is associated with blood–brain barrier leakage and active demyelination. *Journal of Pathology,* 201, pp.319-327.doi: 10.1002/path.1434

Knight, R. T., Grabowecky, M. F., and Scabini, D. (1995). Role of human prefrontal cortex in attention control. *Advances in Neurology*, 66, pp. 21-36. Retrieved from: https://www.researchgate.net/

Kobelt, G., Berg, J., Atherly, D. and Hadjimichael, O. (2006). Costs and quality of life in multiple sclerosis a cross-sectional study in the United States. *Neurology*, 66, pp.1696-1702.doi: 10.1212/01.wnl.0000218309.01322.5c

Kocevar, G., Stamile, C., Hannoun, S., Cotton, F., Vukusic, S., Durand-Dubief, F. and Sappey-Marinier, D. (2016). Graph theory-based brain connectivity for automatic classification of multiple sclerosis clinical courses. *Frontiers in Neuroscience*, 10, A478.doi: 10.3389/fnins.2016.00478

Korteweg, T., Tintoré, M., Uitdehaag, B., Rovira, A., Frederiksen, J., Miller, D., Fernando, K., Filippi, M., Agosta, F., Rocca, M. and Fazekas, F. (2006). MRI criteria for dissemination in space in patients with clinically isolated syndromes: a multicentre follow-up study. *The Lancet Neurology*, 5, pp.221-227.doi: 10.1016/S1474-4422(06)70353-2

Kroencke, D.C., Lynch, S.G. and Denney, D.R. (2000). Fatigue in multiple sclerosis: relationship to depression, disability, and disease pattern. *Multiple Sclerosis Journal*, 6, pp.131-136.doi: 10.1177/135245850000600213

Krupp, L.B. and Elkins, L.E. (2000). Fatigue and declines in cognitive functioning in multiple sclerosis. *Neurology,* 55, pp.934-939.doi: 10.1212/WNL.55.7.934

Krupp, L.B., Alvarez, L.A., LaRocca, N.G. and Scheinberg, L.C. (1988). Fatigue in multiple sclerosis. *Archives of Neurology*, 45, pp.435-437.doi: 10.1001/archneur.1988.00520280085020

Krupp, L.B., Christodoulou, C., Melville, P., Scherl, W.F., MacAllister, W.S. and Elkins, L.E. (2004). Donepezil improved memory in multiple sclerosis in a randomized clinical trial. *Neurology*, 63, pp.1579-1585. doi: 10.1212/01.WNL.0000142989.09633.5A

Krupp, L.B., Coyle, P.K., Doscher, C., Miller, A., Cross, A.H., Jandorf, L., Halper, J., Johnson, B., Morgante, L. and Grimson, R. (1995). Fatigue therapy in multiple sclerosis Results of a double-blind, randomized, parallel trial of amantadine, pemoline, and placebo. *Neurology*, 45, pp.1956-1961.doi: 10.1212/WNL.45.11.1956

Krupp, L.B., LaRocca, N.G., Muir-Nash, J., and Steinberg, A.D. (1989). The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Archives of Neurology,* 46, pp. 1121-1123. doi: 10.1001/archneur.1989.00520460115022

Kujala, P., Portin, R., Revonsuo, A. and Ruutiainen, J. (1995). Attention related performance in two cognitively different subgroups of patients with multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry,* 59, pp.77-82.doi: 10.1136/jnnp.59.1.77

Kuramoto, E., Furuta, T., Nakamura, K.C., Unzai, T., Hioki, H. and Kaneko, T. (2009). Two types of thalamocortical projections from the motor thalamic nuclei of the rat: a single neuron-tracing study using viral vectors. *Cerebral Cortex*, 19, pp.2065-2077. doi: 10.1093/cercor/bhn231.

Kurtzke, J.F. (1993). Epidemiologic evidence for multiple sclerosis as an infection. *Clinical Microbiology Reviews*, 6, pp.382-427.doi: 10.1128/CMR.6.4.382

Laird, A.R., McMilliam, K.M., Lancaster, J.L., Kochunov, P., Turkeltaub, P.E., Pardo, J.V., and Fox, P.T. (2005). A comparison of label-based review and ALE meta-analysis in the Stroop task. *Human Brain Mapping,* 25, pp. 6-21. doi: 10.1002/hbm.20129

Lanciego, J.L., Luquin, N. and Obeso, J.A. (2012). Functional neuroanatomy of the basal ganglia. *Cold Spring Harbor Perspectives in Medicine*, p.a009621.doi: 10.1101/cshperspect.a009621

Landro, N.I., Celius, E.G. and Sletvold, H. (2004). Depressive symptoms account for deficient information processing speed but not for impaired working memory in early phase multiple sclerosis (MS). *Journal of the Neurological Sciences,* 217, pp.211-216.doi: 10.1016/j.jns.2003.10.012

Lange, G., Steffener, J., Cook, D.B., Bly, B.M., Christodoulou, C., Liu, W.C., De Luca J. and Natelson, B.H. (2005). Objective evidence of cognitive complaints in Chronic Fatigue

Syndrome: A BOLD fMRI study of verbal working memory. *NeuroImage*, 26, 513-524.doi: 10.1016/j.neuroimage.2005.02.011

Lansley, J., Mataix-Cols, D., Grau, M., Radua, J. and Sastre-Garriga, J. (2013). Localized grey matter atrophy in multiple sclerosis: a meta-analysis of voxel-based morphometry studies and associations with functional disability. *Neuroscience & Biobehavioral Reviews*, 37, pp.819-830. doi: 10.1016/j.neubiorev.2013.03.006.

Larocca, N., Kalb, R., Scheinberg, L., and Kendall, P. (1985). Factors associated with unemployment of patients with multiple sclerosis. *Journal of Chronic Disease*, 38, pp. 203-210. doi: 10.1016/0021-9681(85)90093-1

Lassmann, H. (2018). Multiple sclerosis pathology. *Cold Spring Harbor Perspectives in Medicine*, 8, p.a028936. doi: 10.1101/cshperspect.a028936.

Lassmann, H., Brück, W. and Lucchinetti, C.F. (2007). The immunopathology of multiple sclerosis: an overview. *Brain Pathology*, 17, pp.210-218.doi: 10.1111/j.1750-3639.2007.00064.x

Latora, V., and Marchiori, M. (2001). Efficient behaviour of small-world networks. *Physical Review*, 5, A198701.doi: 10.1103/PhysRevLett.87.198701

Lauwereyns, J., Watanabe, K., Coe, B. and Hikosaka, O. (2002). A neural correlate of response bias in monkey caudate nucleus. *Nature*, 418, pp.413-417.doi 10.1038/nature00892

Lawrence, N.S., Ross, T.J., Hoffmann, R., Garavan, H., and Stein, E.A. (2003). Multiple neuronal networks mediate sustained attention. *Journal of Cognitive Neuroscience,* 15, pp. 1028-1038. doi: 10.1162/089892903770007416.

Leavitt, V.M., Totso, G., Riley, C.S. (2018). Cognitive phenotypes in multiple sclerosis. *Journal of Neurology*, 265, pp. 562-566. doi:10.1007/s00415-018-8747-5.

Lee, K.H., Hashimoto, S.A., Hooge, J.P., Kastrukoff, L.F., Oger, J.J.F., Li, D.K.B. and Paty, D.W. (1991). Magnetic resonance imaging of the head in the diagnosis of multiple sclerosis: A prospective 2-year follow-up with comparison of clinical evaluation, evoked potentials, oligoclonal banding, and CT. *Neurology,* 41, pp.657-660.doi: 10.1212/WNL.41.5.657

Lee, M., Reddy, H., Johansen-Berg, H., Pendlebury, S., Jenkinson, M., Smith, S., Palace, J. and Matthews, P.M. (2000). The motor cortex shows adaptive functional changes to brain injury from multiple sclerosis. *Annals of Neurology*, 47, pp.606-613. doi: 10.1002/1531-8249(200005)47:5<606::AID-ANA8>3.0.CO;2-L.

Leisman, G., Melillo, R. and Carrick, F.R. (2013). 'Clinical motor and cognitive neurobehavioral relationships in the basal ganglia. In Barrios, F.A. and Bauer, C. (eds.*) Basal ganglia-An integrative view*. IntechOpen, DOI: 10.5772/55227

Lengenfelder, J., Bryant, D., Diamond, B.J., Kalmar, J.H., Moore, N.B., and DeLuca, J. (2006). Processing speed interacts with working memory efficiency in multiple sclerosis. *Archives of Clinical Neuropsychology,* 21, pp. 229-238. doi: 10.1016/j.acn.2005.12.001.

Leo, G.J. and Rao, S.M. (1988). Effects of intravenous physostigmine and lecithin on memory loss in multiple sclerosis: report of a pilot study. *Journal of Neurologic Rehabilitation*, 2, pp.123-129. doi: 10.1177/136140968800200305

Levene, H. (1960). Robust tests for equality of variances. In: Olkin, I. (ed). *Contributions to Probability and Statistics*, Palo Alto: Stanford University Press, pp. 278-92. doi: 10.2307/2285659.

Lewin, S., Friedman, L., Wu, D., Miller, D.A., Thompson, L.A., Klein, S.K., Wise, A.L., Hedera, P., Buckley, P., Meltzer, H., Friedland, R.P., Duerk, J.L. (1996). Cortical localization of human sustained attention: detection with functional MR using a visual vigilance paradigm. *Journal of Computer Assisted Tomography*, 20, pp. 695-701. doi: 10.1097/00004728-199609000-00002.

Li, Y., Jewells, V., Kim, M., Chen, Y., Moon, A., Armao, D., Troiani, L., Markovic-Plese, S., Lin, W. and Shen, D. (2013). Diffusion tensor imaging based network analysis detects alterations

of neuroconnectivity in patients with clinically early relapsing-remitting multiple sclerosis. *Human Brain Mapping*, 34, pp.3376-3391. doi: 10.1002/hbm.22158

Lincoln, N.B., Dent, A., Harding, J., Weyman, N., Nicholl, C., Blumhardt, L.D. and Playford, E.D. (2002). Evaluation of cognitive assessment and cognitive intervention for people with multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry*, 72, pp.93-98.doi: 10.1136/jnnp.72.1.93

Litvan, I., Grafman, J., Vendrell, P., Martinez, J.M., Junque, C., Vendrell, J.M., and Barraquer-Bordas, L. (1988). Multiple memory deficits in patients with multiple sclerosis exploring the working memory system. *Archives of Neurology*, 45, pp. 607-610. doi: 10.1001/archneur.1988.00520300025012.

Liu, J. Z., Shan, Z. Y., Zhang, L. D., Sahgal, V., Brown, R. W. and Yue, G. H. (2003). Human brain activation during sustained and intermittent submaximal fatigue muscle contractions: an FMRI study. *Journal of Neurophysiology*, 90, pp. 300-312. doi: 10.1152/jn.00821.2002

Liu, J. Z., Zhang, L., Yao, B., Sahgal, V. and Yue, G. H. (2005). Fatigue induced by intermittent maximal voluntary contractions is associated with significant losses in muscle output but limited reductions in functional MRI-measured brain activation level. *Brain Research*, 1040, pp. 44-54.doi: 10.1016/j.brainres.2005.01.059

Logothetis, N.K. (2003). The underpinnings of the BOLD functional magnetic resonance imaging signal. *Journal of Neuroscience,* 23, pp. 3963-3971. doi: 10.1523/JNEUROSCI.23-10-03963.2003.

Logothetis, N.K., and Wandell, B.A. (2004). Interpreting the BOLD signal. *Annual Review of Physiology,* 66, pp. 735-769. doi: 10.1146/annurev.physiol.66.082602.092845.

Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., Oeltermann, A. (2001). A neurophysiological investigation of the basis of the fMRI signal. *Nature,* 412, pp. 150-157. doi: 10.1038/35084005.

López-Góngora, M., Querol, L. and Escartín, A. (2015). A one-year follow-up study of the Symbol Digit Modalities Test (SDMT) and the Paced Auditory Serial Addition Test (PASAT) in relapsing-remitting multiple sclerosis: an appraisal of comparative longitudinal sensitivity. *BMC Neurology*, 15, A40.doi: 10.1186/s12883-015-0296-2

Lovera, J., Bagert, B., Smoot, K., Morris, C.D., Frank, R., Bogardus, K., Wild, K., Oken, B., Whitham, R. and Bourdette, D. (2007). Ginkgo biloba for the improvement of cognitive performance in multiple sclerosis: a randomized, placebo-controlled trial. *Multiple Sclerosis Journal*, 13, pp.376-385. doi: 10.1177/1352458506071213

Lu, X., and Ashe, J. (2005). Anticipatory activity in primary motor cortex codes memorized movement sequences. *Neuron,* 45, pp. 967-973. doi: 10.1016/j.neuron.2005.01.036.

Lublin, F.D. and Reingold, S.C. (1996). Defining the clinical course of multiple sclerosis: results of an international survey. *Neurology*, 46, pp.907-911.doi: 10.1212/WNL.46.4.907

Lublin, F.D., Reingold, S.C., Cohen, J.A., Cutter, G.R., Sørensen, P.S., Thompson, A.J., Wolinsky, J.S., Balcer, L.J., Banwell, B., Barkhof, F. and Bebo, B. (2014). Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology*, 83, pp.278-286.doi: 10.1212/WNL.0000000000000560

Lucchinetti, C., Brück, W., Parisi, J., Scheithauer, B., Rodriguez, M. and Lassmann, H. (1999). A quantitative analysis of oligodendrocytes in multiple sclerosis lesions: a study of 113 cases. *Brain*, 122, pp.2279-2295.doi: 10.1093/brain/122.12.2279

Lundstrom, B.N., Ingvar, M. and Petersson, K.M. (2005). The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. *NeuroImage*, 27, pp.824-834. doi: 10.1016/j.neuroimage.2005.05.008.

Lundstrom, B.N., Petersson, K.M., Andersson, J., Johansson, M., Fransson, P. and Ingvar, M. (2003). Isolating the retrieval of imagined pictures during episodic memory: activation of the

left precuneus and left prefrontal cortex. *NeuroImage*, 20, pp.1934-1943. doi: 10.1016/j.neuroimage.2003.07.017.

Luo, C., Jian, C., Liao, Y., Huang, Q., Wu, Y., Liu, X., Zou, D. and Wu, Y. (2017). The role of microglia in multiple sclerosis. *Neuropsychiatric Disease and Treatment*, 13, pp.1661-1667.doi: 10.2147/NDT.S140634

MacKenzie, I.S., Morant, S.V., Bloomfield, G.A., MacDonald, T.M., and O'Riordan, J. (2013). Incidence and prevalence of multiple sclerosis in the UK 1990–2010: a descriptive study in the General Practice Research Database. *Journal of Neurology, Neurosurgery and Psychiatry*, 85, pp. 76-84.doi: 10.1136/jnnp-2013-305450

MacKenzie-Graham, A., Kurth, F., Itoh, Y., Wang, H.J., Montag, M.J., Elashoff, R. and Voskuhl, R.R. (2016). Disability-specific atlases of gray matter loss in relapsing-remitting multiple sclerosis. *JAMA Neurology*, 73, pp.944-953.doi: 10.1001/jamaneurol.2016.0966

Mackworth, N.H. (1948). The breakdown of vigilance during prolonged visual search. *Quarterly Journal of Experimental Psychology*, 1, pp.6-21.doi: 10.1080/17470214808416738

Maes, M., Mihaylova, I. and De, M.R. (2005). Decreased dehydroepiandrosterone sulfate but normal insulin-like growth factor in chronic fatigue syndrome (CFS): relevance for the inflammatory response in CFS. *Neuro Endocrinology Letters,* 26, pp.487-492. Retrieved from: https://www.researchgate.net/

Magliozzi, R., Howell, O., Vora, A., Serafini, B., Nicholas, R., Puopolo, M., Reynolds, R. and Aloisi, F. (2007). Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain*, 130, pp.1089-1104. doi: 10.1093/brain/awm038

Maguire, E. A., Gadian, D. G., Johnsrude, I. S., Good, C. D., Ashburner, J., Frackowiak, R. S., and Frith, C. D. (1999). Navigation-related structural change in the hippocampi of taxi drivers. *Proceedings of the National Academy of Sciences*, 97, pp. 4398-4403. doi: 10.1073/pnas.07003959.

Mahad, D., Ziabreva, I., Lassmann, H. and Turnbull, D. (2008). Mitochondrial defects in acute multiple sclerosis lesions. *Brain*, 131, pp.1722-1735.doi: 10.1093/brain/awn105

Mainero, C., Caramia, F., Pozzilli, C., Pisani, A., Pestalozza, I., Borriello, G., Bozzao, L. and Pantano, P. (2004). fMRI evidence of brain reorganization during attention and memory tasks in multiple sclerosis. *NeuroImage*, 21, pp.858-867.doi: 10.1016/j.neuroimage.2003.10.004

Mainero, C., Pantano, P., Caramia, F., & Pozzilli, C. (2006). Brain reorganization during attention and memory tasks in multiple sclerosis: insights from functional MRI studies. *Journal of Neurological Sciences*, 245, pp. 93-98. doi: 10.1016/j.jns.2005.08.024.

Mallet, N., Ballion, B., Le Moine, C. and Gonon, F. (2006). Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. *Journal of Neuroscience*, 26, pp.3875-3884.doi: 10.1523/JNEUROSCI.4439-05.2006

Mallet, N., Le Moine, C., Charpier, S. and Gonon, F. (2005). Feedforward inhibition of projection neurons by fast-spiking GABA interneurons in the rat striatum in vivo. *Journal of Neuroscience*, 25, pp.3857-3869.doi: 10.1523/JNEUROSCI.5027-04.2005

Mansfield, P. (1977). Multi-planar imaging formation using NMR spin-echo. *Journal of Physics C: Solid State Physics,* 10, L55-L58. doi: 10.1088/0022-3719/10/3/004.

Manto, M. and Taib, N.O.B. (2010). Cerebellar nuclei: key roles for strategically located structures. *The Cerebellum,* 9, pp.17-21. doi: 10.1007/s12311-010-0159-8.

Marik, C., Felts, P.A., Bauer, J., Lassmann, H. and Smith, K.J. (2007). Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity?. *Brain*, 130, pp.2800-2815.doi: 10.1093/brain/awm236

Marrie, R.A. (2004). Environmental risk factors in multiple sclerosis aetiology. *The Lancet Neurology*, 3, pp.709-718.doi: 10.1016/S1474-4422(04)00933-0

Marrie, R.A., Fisher, E., Miller, D.M., Lee, J.C., and Rudick, R.A. (2005). Association of fatigue and brain atrophy in multiple sclerosis. *Journal of Neurological Sciences*, 228, pp. 161-166. doi: 10.1016/j.jns.2004.11.046

Martinelli-Boneschi, F., Esposito, F., Brambilla, P., Lindström, E., Lavorgna, G., Stankovich, J., Rodegher, M., Capra, R., Ghezzi, A., Coniglio, G. and Colombo, B. (2012). A genome-wide association study in progressive multiple sclerosis. *Multiple Sclerosis Journal*, 18, pp.1384-1394.doi: 10.1177/1352458512439118

Matejcikova, Z., Mareš, J., Vranová, H.P., Klosova, J., Sladkova, V., Dolakova, J., Zapletalová, J. and Kaňovský, P. (2015). Cerebrospinal fluid inflammatory markers in patients with multiple sclerosis: a pilot study. *Journal of Neural Transmission*, 122, pp. 273-277.doi: 10.1007/s00702-014-1244-9

Matesanz, F., González-Pérez, A., Lucas, M., Sanna, S., Gayán, J., Urcelay, E., Zara, I., Pitzalis, M., Cavanillas, M.L., Arroyo, R. and Zoledziewska, M. (2012). Genome-wide association study of multiple sclerosis confirms a novel locus at 5p13. 1. *PloS One*, 7, p.e36140.doi: 10.1371/journal.pone.0036140

Mattay, V.S., Fera, F., Tessitore, A., Hariri, A.R., Das, S., Callicott, J.H. and Weinberger, D.R. (2002). Neurophysiological correlates of age-related changes in human motor function. *Neurology*, 58, pp.630-635. doi: 10.1212/WNL.58.4.630.

McAlpine, D., Compston, N.D. and Lumsden, C.E. (1955). *Multiple sclerosis*, London, E. & S. Livingstone, 195.

McCarthy, M., Beaumont, J.G., Thompson, R. and Peacock, S. (2005). Modality-specific aspects of sustained and divided attentional performance in multiple sclerosis. *Archives of Clinical Neuropsychology*, 20, pp.705-718. doi: 10.1016/j.acn.2005.04.007

McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., McFarland, H.F., Paty, D.W., Polman, C.H., Reingold, S.C. and Sandberg-Wollheim, M. (2001). Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Annals of Neurology, 50, pp.121-127.doi: 10.1002/ana.1032

McKenna, J.T. and Vertes, R.P. (2004). Afferent projections to nucleus reuniens of the thalamus. *Journal of Comparative Neurology*, 480, pp.115-142.doi: 10.1002/cne.20342

McLeod, V., Tamara, C., Perrin, D.H., Guskiewicz, K.M., Shultz, S.J, Diamond, R., Gansneder, B.M. (2004). Serial administration of clinical concussion assessments and learning effects in healthy young athletes. *Clinical Journal of Sports Medicine,* 14, pp. 287-295. doi: 10.1097/00042752-200409000-00007

Menon, V. and Uddin, L.Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Structure and Function*, 214, pp. 655-667. doi: 10.1007/s00429-010-0262-0.

Menon, V., Adleman, N.E., White, C.D., Glover, G.H., and Reiss, A.L. (2001). Error-related brain activation during a Go/NoGo response inhibition task. *Human Brain Mapping*, 12, pp. 131-143. doi: 10.1002/1097-0193(200103)12:3<131::AID-HBM1010>3.0.CO;2-C.

Merlino, G., Fratticci, L., Lenchig, C., Valente, M., Cargnelutti, D., Picello, M., Serafini, A., Dolso, P. and Gigli, G.L. (2009). Prevalence of 'poor sleep'among patients with multiple sclerosis: an independent predictor of mental and physical status. *Sleep Medicine*, 10, pp.26-34.doi: 10.1016/j.sleep.2007.11.004

Mesaros, S., Rovaris, M., Pagani, E., Pulizzi, A., Caputo, D., Ghezzi, A., Bertolotto, A., Capra, R., Falautano, M., Martinelli, V. and Comi, G. (2008). A magnetic resonance imaging voxel-

based morphometry study of regional gray matter atrophy in patients with benign multiple sclerosis. *Archives of Neurology*, 65, pp.1223-1230. doi: 10.1001/archneur.65.9.1223.

Mesulam, M.M. (1998). From sensation to cognition. *Brain*, 121, pp.1013-1052. doi:10.1093/brain/121.6.1013.

Michiels, V., de Gucht, V., Cluydts, R. and Fischler, B. (1999). Attention and information processing efficiency in patients with Chronic Fatigue Syndrome. *Journal of Clinical and Experimental Neuropsychology*, 21, pp. 709-729.doi: 10.1076/jcen.21.5.709.875

Middleton, L. S., Denney, D. R., Lynch, S. G., and Parmenter, B. (2006). The relationship between perceived and objective cognitive functioning in multiple sclerosis. *Archives of Clinical Neuropsychology*, 21, pp. 487-494.doi: 10.1016/j.acn.2006.06.008

Miller, D.H., Hammond, S.R., McLeod, J.G., Purdie, G. and Skegg, D.C. (1990). Multiple sclerosis in Australia and New Zealand: are the determinants genetic or environmental? *Journal of Neurology, Neurosurgery & Psychiatry,* 53, pp.903-905.doi: 10.1136/jnnp.53.10.903

Milo, R. and Kahana, E. (2010). Multiple sclerosis: geoepidemiology, genetics and the environment. *Autoimmunity Reviews*, 9, pp. A387-A394.doi: 10.1016/j.autrev.2009.11.010

Minagar, A., Barnett, M.H., Benedict, R.H., Pelletier, D., Pirko, I., Sahraian, M.A., Frohman, E. and Zivadinov, R. (2013). The thalamus and multiple sclerosis Modern views on pathologic, imaging, and clinical aspects. *Neurology*, 80, pp.210-219. doi: 10.1212/WNL.0b013e31827b910b.

Minden, S.L., Oray, J., and Reich, P. (1987). Depression in multiple sclerosis. *General Hospital Psychiatry*, 9, pp. 426-434.doi: 10.1016/0163-8343(87)90052-1

Mitchell, A. J., Benito-León, J., González, J. M. M., and Rivera-Navarro, J. (2005). Quality of life and its assessment in multiple sclerosis: integrating physical and psychological components of wellbeing. *The Lancet Neurology*, 4, pp. 556-566.doi: 10.1016/S1474-4422(05)70166-6

Mitchell, D. G. (1999). *MRI principles*. WB Saunders Company.

Miyashita, Y. (1993). Inferior temporal cortex: where visual perception meets memory. *Annual Review of Neuroscience*, 16, pp.245-263. doi: 10.1146/annurev.ne.16.030193.001333.

Moeller, S., Xu, J., Auerbach, E.J., Yacoub, E., and Ugurbil, K. (2012). Signal leakage (l-factor) as a measure of parallel imaging performance among simultaneously multi-slice (sms) excited and acquired signals. *Proceedings of the Annual Meeting of the International Society of Magnetic Resonance in Medicine*, 519. Retrieved from: https://www.ismrm.org

Moeller, S., Yacoub, E., Olman, C. A., Auerbach, E., Strupp, J., Harel, N., and Uğurbil, K. (2010). Multiband multislice GE-EPI at 7 tesla, with 16-fold acceleration using partial parallel imaging with application to high spatial and temporal whole-brain fMRI. *Magnetic Resonance in Medicine*, 63, pp. 1144-1153. doi: 10.1002/mrm.22361.

Mogenson, G.J., Ciriello, J., Garland, J. and Wu, M. (1987). Ventral pallidum projections to mediodorsal nucleus of the thalamus: an anatomical and electrophysiological investigation in the rat. *Brain Research*, 404, pp.221-230.doi: 10.1016/0006-8993(87)91373-4

Montalban, X., Tintore, M., Swanton, J., Barkhof, F., Fazekas, F., Filippi, M., Frederiksen, J., Kappos, L., Palace, J., Polman, C. and Rovaris, M. (2010). MRI criteria for MS in patients with clinically isolated syndromes. *Neurology,* 74, pp.427-434.doi: 10.1212/WNL.0b013e3181cec45c

Morariu, M.A. and Linden, M. (1980). Multiple sclerosis in American blacks. *Acta Neurologica Scandinavica*, 62, pp.180-187.doi: 10.1111/j.1600-0404.1980.tb03020.x

Morgen, K., Sammer, G., Courtney, S.M., Wolters, T., Melchior, H., Blecker, C.R., Oschmann, P., Kaps, M. and Vaitl, D. (2006). Evidence for a direct association between cortical atrophy and cognitive impairment in relapsing–remitting MS. *NeuroImage*, 30, pp.891-898.doi: 10.1016/j.neuroimage.2005.10.032

Morton, S.M. and Bastian, A.J. (2006). Cerebellar contributions to locomotor adaptations during splitbelt treadmill walking. *Journal of Neuroscience*, 26, pp.9107-9116. doi: 10.1523/JNEUROSCI.2622-06.2006.

Mostert, S. and Kesselring, J. (2002). Effects of a short-term exercise training program on aerobic fitness, fatigue, health perception and activity level of subjects with multiple sclerosis. *Multiple Sclerosis Journal*, 8, pp.161-168.doi: 10.1191/1352458502ms7790a

Mottaghy, F.M., Willmes, K., Horwitz, B. Muller,H.W., Krause, B.J., and Sturm, W. (2006). Systems level modelling of a neuronal network subserving intrinsic alertness. *NeuroImage*, 29, pp. 225-233. doi: 10.1016/j.neuroimage.2005.07.034.

Muldoon, S.F., Bridgeford, E.W., and Bassett, D.S. (2016). Small-world propensity and weighted brain networks. *Scientific Reports*, 25, e. 6.22057.doi: 10.1038/srep22057

Multiple Sclerosis Council for Clinical Practice Guidelines, (1998). Fatigue and Multiple Sclerosis: Evidence-based Management Strategies for Fatigue in Multiple Sclerosis: *Clinical Practice Guidelines*. Washington D.C.

Mumford, J. A. and Nichols, T. E. (2008). Power calculation for group fMRI studies accounting for arbitrary design and temporal autocorrelation. *NeuroImage*, 39, 261-268. doi: 10.1016/j.neuroimage.2007.07.061

Mumford, J. A., Horvath, S., Oldham, M. C., Langfelder, P., Geschwind, D. H., and Poldrack, R. A. (2010). Detecting network modules in fMRI time series: a weighted network analysis approach. *NeuroImage*, 52, pp. 1465-1476. doi: 10.1016/j.neuroimage.2010.05.047

Murray, E.A., O'Doherty, J.P. and Schoenbaum, G. (2007). What we know and do not know about the functions of the orbitofrontal cortex after 20 years of cross-species studies. *Journal of Neuroscience*, 27, pp.8166-8169.doi: 10.1523/JNEUROSCI.1556-07.2007

Murray, T.J. (1985). Amantadine therapy for fatigue in multiple sclerosis. *Canadian Journal of Neurological Sciences*, 12, pp.251-254.doi: 10.1017/S0317167100047107

Muthuraman, M., Fleischer, V., Kolber, P., Luessi, F., Zipp, F. and Groppa, S. (2016). Structural brain network characteristics can differentiate CIS from early RRMS. *Frontiers in Neuroscience*, 10, A14. doi: 10.3389/fnins.2016.00014

Mycko, M.P., Papoian, R., Boschert, U., Raine, C.S. and Selmaj, K.W. (2003). cDNA microarray analysis in multiple sclerosis lesions: detection of genes associated with disease activity. *Brain*, 126, pp.1048-1057.doi: 10.1093/brain/awg107

Nakamura, K. and Hikosaka, O. (2006). Role of dopamine in the primate caudate nucleus in reward modulation of saccades. *Journal of Neuroscience*, 26, pp.5360-5369.doi: 10.1523/JNEUROSCI.4853-05.2006

Nambu, A. (2004). A new dynamic model of the cortico-basal ganglia loop. *Progress in Brain Research*, 143, pp. 461-466.doi: 10.1016/S0079-6123(03)43043-4

Nambu, A. (2005). A new approach to understand the pathophysiology of Parkinson's disease. *Journal of Neurology,* 252, pp.iv1-iv4.doi: 10.1007/s00415-005-4002-y

Nambu, A., Tokuno, H. and Takada, M. (2002). Functional significance of the cortico–subthalamo–pallidal 'hyperdirect'pathway. *Neuroscience Research*, 43, pp.111-117. doi: 10.1016/S0168-0102(02)00027-5

Nambu, A., Tokuno, H., Hamada, I., Kita, H., Imanishi, M., Akazawa, T., Ikeuchi, Y. and Hasegawa, N. (2000). Excitatory cortical inputs to pallidal neurons via the subthalamic nucleus in the monkey. *Journal of Neurophysiology*, 84, pp.289-300. doi: 10.1152/jn.2000.84.1.289

Nelson, A.B. and Kreitzer, A.C. (2014). Reassessing models of basal ganglia function and dysfunction. *Annual Review of Neuroscience*, 37, pp.117-135.doi: 10.1146/annurev-neuro-071013-013916

Nelson, S.M., Cohen, A.L., Power, J.D., Wig, G.S., Miezin, F.M., Wheeler, M.E., Velanova, K., Donaldson, D.I., Phillips, J.S., Schlaggar, B.L. and Petersen, S.E. (2010). A parcellation scheme for human left lateral parietal cortex. *Neuron*, 67, pp.156-170.doi: 10.1016/j.neuron.2010.05.025

Newman, M.E.J. (2004). Analysis of weighted networks. *Physical Review,* E 70, 056131.doi: 10.1103/PhysRevE.70.056131

Nichols, T. and Hayasaka, S. (2003). Controlling the familywise error rate in functional neuroimaging: a comparative review. *Statistical Methods in Medical Research*, 12, pp.419-446.doi: 10.1191/0962280203sm341ra

Nichols, T.E., Das, S., Eickhoff, S.B., Evans, A.C., Glatard, T., Hanke, M., Kriegeskorte, N., Milham, M.P., Poldrack, R.A., Poline, J.B. and Proal, E., (2016). Best practices in data analysis and sharing in neuroimaging using MRI. *bioRxiv*, p.054262.doi: 10.1038/nn.4500.

Niepel, G., Bibani, R.H., Vilisaar, J., Langley, R.W., Bradshaw, C.M., Szabadi, E. and Constantinescu, C.S. (2013). Association of a deficit of arousal with fatigue in multiple sclerosis: effect of modafinil. *Neuropharmacology*, 64, pp.380-388.doi: 10.1016/j.neuropharm.2012.06.036

Nischwitz, S., Cepok, S., Kroner, A., Wolf, C., Knop, M., Müller-Sarnowski, F., Pfister, H., Roeske, D., Rieckmann, P., Hemmer, B. and Ising, M. (2010). Evidence for VAV2 and ZNF433 as susceptibility genes for multiple sclerosis. *Journal of Neuroimmunology*, 227, pp.162-166.doi: 10.1016/j.jneuroim.2010.06.003

Nocentini, U., Bozzali, M., Spanò, B., Cercignani, M., Serra, L., Basile, B., Mannu, R., Caltagirone, C. and De Luca, J. (2014). Exploration of the relationships between regional grey matter atrophy and cognition in multiple sclerosis. *Brain Imaging and Behavior*, 8, pp.378-386. doi: 10.1007/s11682-012-9170-7.

Nord, C.L., Valton, V., Wood, J. and Roiser, J.P. (2017). Power-up: a reanalysis of 'power failure'in neuroscience using mixture modelling. *Journal of Neuroscience*, pp.3592-3616.doi: 10.1523/JNEUROSCI.3592-16.2017

Obeso, J.A., Rodríguez-Oroz, M.C., Benitez-Temino, B., Blesa, F.J., Guridi, J., Marin, C. and Rodriguez, M. (2008). Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease. *Movement disorders,* 23, pp.S548-S559.doi: 10.1002/mds.22062

Offenbacher, H., Fazekas, F., Schmidt, R., Freidl, W., Flooh, E., Payer, F. and Lechner, H. (1993). Assessment of MRI criteria for a diagnosis of MS. *Neurology*, 43, pp.905-905.doi: 10.1212/WNL.43.5.905

Ogawa, S., Lee, T. M., Kay, A. R., and Tank, D. W. (1990). Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proceedings of the National Academy of Sciences*, 87, pp. 9868-9872. doi: 10.1073/pnas.87.24.9868

Oken, B.S., Salinsky, M.C., and Elsas, S.M. (2006). Vigilance, alertness, or sustained attention: physiological basis and measurement. *Clinical Neurophysiology,* 117, pp. 1885-1901. doi: 10.1016/j.clinph.2006.01.017.

Onu, M., Adina, A., Victor, F. and Ovidiu, B. (2015). Gray Matter Changes in Demyelinating Disease: Correlations with Clinical Scores. *Maedica*, 10, pp. 319-324.Retrieved from: http://www.maedica.ro/

Ordidge, R.J., Mansfield, P., and Coupland, R.E. (1981). Rapid biomedical imaging by NMR. *British Journal of Radiology*, 54, pp. 850-855. doi: 10.1259/0007-1285-54-646-850

Ordidge, R.J., Mansfield, P., Doyle, M., and Coupland, R.E. (1982). Real time movie images by NMR. *British Journal of Radiology*, 55, pp. 729-733. doi: 10.1259/0007-1285-55-658-729

Ozawa, K., Suchanek, G., Breitschopf, H., Breck, W., Budka, H., Jellinger, K. and Lassmann, H. (1994). Patterns of oligodendroglia pathology in multiple sclerosis. *Brain*, 117, pp.1311-1322.doi: 10.1093/brain/117.6.1311

Pantano, P., Iannetti, G.D., Caramia, F., Mainero, C., Di Legge, S., Bozzao, L., Pozzilli, C. and Lenzi, G.L. (2002a). Cortical motor reorganization after a single clinical attack of multiple sclerosis. *Brain*, 125, pp.1607-1615. doi: 10.1093/brain/awf164.

Pantano, P., Mainero, C., Iannetti, G.D., Caramia, F., Di Legge, S., Piattella, M.C., Pozzilli, C., Bozzao, L. and Lenzi, G.L. (2002b). Contribution of corticospinal tract damage to cortical motor reorganization after a single clinical attack of multiple sclerosis. *NeuroImage*, 17, pp.1837-1843. doi: 10.1006/nimg.2002.1313.

Pardini, M., Uccelli, A., Grafman, J., Yaldizli, O., Mancardi, G., and Roccatagliata, L. (2014). Isolated cognitive relapses in multiple sclerosis. *Journal of Neurology, Neurosurgery, and Psychiatry*, 85, pp. 1035–1037. doi: 10.1136/jnnp2013-307275.

Pardo, J.V., Fox, P.T., Raichle, M.E. (1991). Localization of a human system for sustained attention by positron emission tomography. *Nature,* 349, pp.61-64. doi: 10.1038/349061a0

Parisi, L., Rocca, M.A., Mattioli, F., Riccitelli, G.C., Capra, R., Stampatori, C., Bellomi, F. and Filippi, M. (2014). Patterns of regional gray matter and white matter atrophy in cortical multiple sclerosis. *Journal of Neurology*, 261, pp.1715-1725. doi: 10.1007/s00415-014-7409-5.

Parry, A.M., Scott, R.B., Palace, J., Smith, S. and Matthews, P.M. (2003). Potentially adaptive functional changes in cognitive processing for patients with multiple sclerosis and their acute modulation by rivastigmine. *Brain*, 126, pp.2750-2760. doi: 10.1093/brain/awg284.

Patrick, E., Christodoulou, C., Krupp, L.B. and New York State MS Consortium. (2009). Longitudinal correlates of fatigue in multiple sclerosis. *Multiple Sclerosis Journal*, 15, pp.258-261.doi: 10.1177/1352458508097466

Patrikios, P., Stadelmann, C., Kutzelnigg, A., Rauschka, H., Schmidbauer, M., Laursen, H., Sorensen, P.S., Brück, W., Lucchinetti, C. and Lassmann, H. (2006). Remyelination is extensive in a subset of multiple sclerosis patients. *Brain*, 129, pp.3165-3172.doi: 10.1093/brain/awl217

Patsopoulos, N.A., Bayer Pharma MS Genetics Working Group, the Steering Committees of Studies Evaluating IFNβ-1b and a CCR1-Antagonist, ANZgene Consortium, GeneMSA, International Multiple Sclerosis Genetics Consortium and de Bakker, P.I. (2011). Genome-wide meta-analysis identifies novel multiple sclerosis susceptibility loci. *Annals of Neurology*, 70, pp.897-912.doi: 10.1002/ana.22609.

Pattyn, N., Neyt, X., Henderickx, D. and Soetens, E. (2008). Psychophysiological investigation of vigilance decrement: boredom or cognitive fatigue? *Physiology & Behavior*, 93, pp.369-378.doi: 10.1016/j.physbeh.2007.09.016

Paty, D.W., Oger, J.J.F., Kastrukoff, L.F., Hashimoto, S.A., Hooge, J.P., Eisen, A.A., Eisen, K.A., Purves, S.J., Low, M.D., Brandejs, V. and Robertson, W.D. (1988). MRI in the diagnosis of MS A prospective study with comparison of clinical evaluation, evoked potentials, oligoclonal banding, and CT. *Neurology*, 38, pp.180-180.doi: 10.1212/WNL.38.2.180

Paul, R.H., Beatty, W.W., Schneider, R., Blanco, C.R. and Hames, K.A. (1998). Cognitive and physical fatigue in multiple sclerosis: relations between self-report and objective performance. *Applied Neuropsychology*, 5, pp.143-148.doi: 10.1207/s15324826an0503_5

Pauling, L., and Coryell, C.D. (1936). The magnetic properties and structure of hemoglobin, oxyhemoglobin, and carbonmonoxyhemoglobin. *Proceedings of the National Academy of Sciences of the United States of America,* 22, pp. 210–216. doi: 10.1073/pnas.22.4.210

Pedersen, J.R., Johannsen, P., Bak, C.K., Kofoed, B., Saermark, K. and Gjedde, A. (1998). Origin of human motor readiness field linked to left middle frontal gyrus by MEG and PET. *NeuroImage*, 8, pp.214-220. doi: 10.1006/nimg.1998.0362.

Penner, I.K., Rausch, M., Kappos, L., Opwis, K. and Radü, E.W. (2003). Analysis of impairment related functional architecture in MS patients during performance of different attention tasks. *Journal of Neurology*, 250, pp.461-472.doi: 10.1007/s00415-003-1025-0

Pepper, C.M., Krupp, L.B., Friedberg, F., Doscher, C. and Coyle, P.K. (1993). A comparison of neuropsychiatric characteristics in chronic fatigue syndrome, multiple sclerosis, and major depression. *Journal of Neuropsychiatry and Clinical Neurosciences*, 5, pp.200-205.doi: 10.1176/jnp.5.2.200

Perin, B., Godefroy, O., and de Marco, G. (2010). Alertness in young healthy subjects: An fMRI study of brain region interactivity enhanced by a warning signal. *Brain and Cognition*, 72, pp. 271–281. doi: 10.1016/j.bandc.2009.09.010.

Petajan, J.H., Gappmaier, E., White, A.T., Spencer, M.K., Mino, L. and Hicks, R.W. (1996). Impact of aerobic training on fitness and quality of life in multiple sclerosis. *Annals of Neurology*, 39, pp.432-441.doi: 10.1002/ana.410390405

Petersen, S.E. and Posner, M.I. (2012). The attention system of the human brain: 20 years after. *Annual Review of Neuroscience*, 35, pp.73-89. doi: 10.1146/annurev-neuro-062111-150525

Philip, P., Sagaspe, P., Taillard, J., Moore, N., Guilleminault, C., Sanchez-Ortuno, M., Åkerstedt, T.B. and Bioulac, B. (2003). Fatigue, sleep restriction, and performance in automobile drivers: a controlled study in a natural environment. *Sleep*, 26, pp.277-280.doi: 10.1093/sleep/26.3.277

Picciotto, M. (2018). Analytical Transparency and Reproducibility in Human Neuroimaging Studies. *Journal of Neuroscience,* 38, pp. 3375-3376. doi: 10.1523/JNEUROSCI.0424-18.2018

Pinto, Y., van der Leij, A.R., Sligte, I.G., Lamme, V.A. and Scholte, H.S. (2013). Bottom-up and top-down attention are independent. *Journal of Vision*, 13, A16.doi: 10.1167/13.3.16

Piras, M.R., Magnano, I., Canu, E.D., Paulus, K.S., Satta, W.M., Soddu, A., Conti, M., Achene, A., Solinas, G., and Aiello, I. (2003). Longitudinal study of cognitive dysfunction in multiple sclerosis: neuropsychological, neuroradiological, and neurophysiological findings. *Journal of Neurology and Neurosurgery and Psychiatry*, 74, pp. 878-85. doi: 10.1136/jnnp.74.7.878

Plewes, D.B. and Kucharczyk, W. (2012). Physics of MRI: a primer. *Journal of Magnetic Resonance Imaging,* 35, pp. 1038- 1054. doi: 10.1002/jmri.23642

Pliskin, N.H., Hamer, D.P., Goldstein, D.S., Towle, V.L., Reder, A.T., Noronha, A. and Arnason, B.G.W. (1996). Improved delayed visual reproduction test performance in multiple sclerosis patients receiving interferon beta-1b. *Neurology*, 47, pp.1463-1468. doi: 10.1212/WNL.47.6.1463

Plohmann, A., Kappos, L., Ammann, W., Thordai, A., Wittwer, A., Huber, S., Bellaiche, Y., and Lechner-Scott, J. (1998). Computer assisted retraining of attentional impairments in patients with multiple sclerosis. *Journal of Neurology and Neurosurgery*, 64, pp. 455-462.doi: 10.1136/jnnp.64.4.455

Poldrack, R.A., Baker, C.I., Durnez, J., Gorgolewski, K.J., Matthews, P.M., Munafò, M.R., Nichols, T.E., Poline, J.B., Vul, E. and Yarkoni, T. (2017). Scanning the horizon: towards transparent and reproducible neuroimaging research. *Nature Reviews Neuroscience*, 18, pp.115-126. doi: 10.1038/nrn.2016.167

Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L. and Lublin, F.D. (2011). Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Annals of Neurology*, 69, pp.292-302.doi: 10.1002/ana.22366

Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., Hartung, H.P., Kappos, L., Lublin, F.D., Metz, L.M., McFarland, H.F., O'Connor, P.W. and Sandberg-Wollheim, M. (2005). Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Annals of Neurology,* 58, pp.840-846.doi: 10.1002/ana.20703

Ponomarev, E.D., Shriver, L.P., Maresz, K. and Dittel, B.N. (2005). Microglial cell activation and proliferation precedes the onset of CNS autoimmunity. *Journal of Neuroscience Research*, 81, pp.374-389. doi: 10.1002/jnr.20488

Popp, R.F., Fierlbeck, A.K., Knüttel, H., König, N., Rupprecht, R., Weissert, R. and Wetter, T.C. (2017). Daytime sleepiness versus fatigue in patients with multiple sclerosis: a systematic review on the Epworth sleepiness scale as an assessment tool. *Sleep Medicine Reviews*, 32, pp.95-108.doi: 10.1016/j.smrv.2016.03.004

Porro, C.A., Cettolo, V., Francescato, M.P. and Baraldi, P. (2000). Ipsilateral involvement of primary motor cortex during motor imagery. *European Journal of Neuroscience*, 12, pp.3059-3063.doi: 10.1046/j.1460-9568.2000.00182.x

Poser, C.M., Paty, D.W., Scheinberg, L., McDonald, W.I., Davis, F.A., Ebers, G.C., Johnson, K.P., Sibley, W.A., Silberberg, D.H. and Tourtellotte, W.W. (1983). New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Annals of Neurology,* 13, pp.227-231.doi: 10.1002/ana.410130302

Posner, M. (1975). The psychobiology of attention. In Gazzaniga, M.S. and Blakemore, C. (Eds.), *Handbook of Psychobiology*, Academic, New York, pp. 441-480.

Posner, M.I. and Petersen, S.E. (1990). The attention system of the human brain. *Annual Review of Neuroscience,* 13, pp.25-42.doi: 10.1146/annurev.ne.13.030190.000325

Post, M., Steens, A., Renken, R., Maurits, N. M., & Zijdewind, I. (2009). Voluntary activation and cortical activity during a sustained maximal contraction: an fMRI study. *Human Brain Mapping*, 30, 1014-1027.doi: 10.1002/hbm.20562

Poustchi-Amin, M., Mirowitz, S. A., Brown, J. J., McKinstry, R. C., and Li, T. (2001). Principles and applications of echo-planar imaging: a review for the general radiologist. *Radiographics*, 21, pp. 767-779.doi: 10.1148/radiographics.21.3.g01ma23767

Power, J.D., Cohen, A.L., Nelson, S.M., Wig, G.S., Barnes, K.A., Church, J.A., Vogel, A.C., Laumann, T.O., Miezin, F.M., Schlaggar, B.L. and Petersen, S.E. (2011). Functional network organization of the human brain. *Neuron*, 72, pp.665-678.doi: 10.1016/j.neuron.2011.09.006

Pravatà, E., Zecca, C., Sestieri, C., Caulo, M., Riccitelli, G.C., Rocca, M.A., Filippi, M., Cianfoni, A. and Gobbi, C. (2016). Hyperconnectivity of the dorsolateral prefrontal cortex following mental effort in multiple sclerosis patients with cognitive fatigue. *Multiple Sclerosis Journal*, 22, pp.1665-1675.doi: 10.1177/1352458515625806

Preibisch, C., Bührer, M., and Riedl, V. (2015). Evaluation of multiband EPI acquisitions for resting state fMRI. *PloS One*, 10, e0136961. doi: 10.1371/journal.pone.0136961

Prineas, J.W. and Connell, F. (1979). Remyelination in multiple sclerosis. *Annals of Neurology*, 5, pp.22-31.doi: 10.1002/ana.410050105

Prineas, J.W., Barnard, R.O., Kwon, E.E., Sharer, L.R. and Cho, E.S. (1993). Multiple sclerosis: Remyelination of nascent lesions: Remyelination of nascent lesions. *Annals of Neurology*, 33, pp.137-151.doi: 10.1002/ana.410330203

Prineas, J.W., Kwon, E.E., Cho, E.S., Sharer, L.R., Barnett, M.H., Oleszak, E.L., Hoffman, B. and Morgan, B.P. (2001). Immunopathology of secondary-progressive multiple sclerosis. *Annals of Neurology*, 50, pp.646-657.doi: 10.1002/ana.1255

Prineas, J.W., Kwon, E.E., Goldenberg, P.Z., Ilyas, A.A., Quarles, R.H., Benjamins, J.A. and Sprinkle, T.J. (1989). Multiple sclerosis. Oligodendrocyte proliferation and differentiation in fresh lesions. *Laboratory Investigation*, 61, pp.489-503. Retrieved from: https://www.researchgate.net/

Prins, M., Schul, E., Geurts, J., der Valk, P., Drukarch, B. and Dam, A.M. (2015). Pathological differences between white and grey matter multiple sclerosis lesions. *Annals of the New York Academy of Sciences*, 1351, pp.99-113. doi: 10.1111/nyas.12841.

Prinster, A., Quarantelli, M., Lanzillo, R., Orefice, G., Vacca, G., Carotenuto, B., Alfano, B., Brunetti, A., Brescia Morra, V. and Salvatore, M. (2010). A voxel-based morphometry study of disease severity correlates in relapsing—remitting multiple sclerosis. *Multiple Sclerosis Journal*, 16, pp.45-54. doi: 10.1177/1352458509351896.

Prinster, A., Quarantelli, M., Orefice, G., Lanzillo, R., Brunetti, A., Mollica, C., Salvatore, E., Morra, V.B., Coppola, G., Vacca, G. and Alfano, B. (2006). Grey matter loss in relapsing–remitting multiple sclerosis: a voxel-based morphometry study. *NeuroImage*, 29, pp.859-867.doi: 10.1016/j.neuroimage.2005.08.034

Purcell, E.M., Torrey, H.C., and Pound, R.V. (1946). Resonance absorption by nuclear magnetic moments in a solid. *Physical Review,* 69, pp. 37-38. doi: 10.1103/PhysRev.69.37

Pusswald, G., Mildner, C., Zebenholzer, K., Auff, E. and Lehrner, J. (2014). A neuropsychological rehabilitation program for patients with Multiple Sclerosis based on the model of the ICF. *NeuroRehabilitation*, 35, pp.519-527.doi:10.3233/NRE-141145

Putcha, D., Brickhouse, M., O'Keefe, K., Sullivan, C., Rentz, D., Marshall, G., Dickerson, B., and Sperling, R. (2011). Hippocampal hyperactivation associated with cortical thinning in Alzheimer's disease signature regions in non-demented elderly adults. *Journal of Neuroscience*, 31, pp. 17680–17688.doi: 10.1523/JNEUROSCI.4740-11.2011

Rae-Grant, A.D., Eckert, N.J., Bartz, S. and Reed, J.F. (1999). Sensory symptoms of multiple sclerosis: a hidden reservoir of morbidity. *Multiple Sclerosis Journal*, 5, pp.179-183.doi: 10.1177/135245859900500307

Ragsdale Jr, C.W. and Graybiel, A.M. (1991). Compartmental organization of the thalamostriatal connection in the cat. *Journal of Comparative Neurology*, 311, pp.134-167. doi: 10.1002/cne.903110110

Rahmann, A.U. (1986). *Nuclear magnetic resonance: basic principles*. New York: Springer.

Raine, C.S., Scheinberg, L. and Waltz, J.M. (1981). Multiple sclerosis. Oligodendrocyte survival and proliferation in an active established lesion. *Laboratory Investigation*, 45, pp.534-546.Retrieved from: https://www.nature.com/labinvest/

Rammohan, K.W., Rosenberg, J.H., Lynn, D.J., Blumenfeld, A.M., Pollak, C.P. and Nagaraja, H.N. (2002). Efficacy and safety of modafinil (Provigil®) for the treatment of fatigue in multiple sclerosis: a two centre phase 2 study. *Journal of Neurology, Neurosurgery and Psychiatry,* 72, pp.179-183.doi: 10.1136/jnnp.72.2.179

Ranjeva, J.P., Audoin, B., Duong, M.V.A., Confort-Gouny, S., Malikova, I., Viout, P., Soulier, E., Pelletier, J. and Cozzone, P.J. (2006). Structural and functional surrogates of cognitive impairment at the very early stage of multiple sclerosis. *Journal of the Neurological Sciences,* 245, pp.161-167.doi: 10.1016/j.jns.2005.09.019

Rao, S.M. (1986). Neuropsychology of multiple sclerosis: a critical review. *Journal of Clinical and Experimental Neuropsychology,* 8, pp.503-542.doi: 10.1080/01688638608405173

Rao, S.M., Leo, G.J. and Aubin-Faubert, P.S. (1989). On the nature of memory disturbance in multiple sclerosis. *Journal of Clinical and Experimental Neuropsychology,* 11, pp.699-712.doi: 10.1080/01688638908400926

Rao, S.M., Leo, G.J., Bernardin, L. and Unverzagt, F. (1991a). Cognitive dysfunction in multiple sclerosis.: I. Frequency, patterns, and prediction. *Neurology*, 41, pp.685-691.doi: 10.1212/WNL.41.5.685

Rao, S.M., Leo, G.J., Ellington, L., Nauertz, T., Bernardin, L. and Unverzagt, F. (1991b). Cognitive dysfunction in multiple sclerosis. II. Impact on employment and social functioning. *Neurology*, 41, pp.692-696.doi: 10.1212/WNL.41.5.692

Rauch, A., Rainer, G., and Logothetis, N.K. (2008). The effects of a serotonin-induced dissociation between spiking and perisynaptic activity on BOLD functional MRI. *Proceedings of the National Academy of Science,* 105, pp. 6759-6764. doi: 10.1073/pnas.0800312105

Raz, A. and Buhle, J. (2006). Typologies of attentional networks. *Nature Reviews Neuroscience*, 7, pp. 367-379.doi: 10.1038/nrn1903

Reddy, H., Narayanan, S., Arnoutelis, R., Jenkinson, M., Antel, J., Matthews, P.M. and Arnold, D.L. (2000). Evidence for adaptive functional changes in the cerebral cortex with axonal injury from multiple sclerosis. *Brain*, 123, pp.2314-2320. doi: 10.1093/brain/123.11.2314.

Redgrave, P., Rodriguez, M., Smith, Y., Rodriguez-Oroz, M.C., Lehericy, S., Bergman, H., Agid, Y., DeLong, M.R. and Obeso, J.A. (2010). Goal-directed and habitual control in the basal ganglia: implications for Parkinson's disease. *Nature Reviews Neuroscience*, 11, pp. 760-772.doi: 10.1038/nrn2915

Redpath, T.W. (1998). Signal-to-noise ratio in MRI. *The British Journal of Radiology*, 71, pp.704-707.doi: 10.1259/bjr.71.847.9771379

Riccitelli, G., Rocca, M.A., Forn, C., Colombo, B., Comi, G. and Filippi, M. (2011). Voxelwise assessment of the regional distribution of damage in the brains of patients with multiple sclerosis and fatigue. *American Journal of Neuroradiology*, 32, pp.874-879. doi: 10.3174/ajnr.A2412.

Riccitelli, G., Rocca, M.A., Pagani, E., Martinelli, V., Radaelli, M., Falini, A., Comi, G. and Filippi, M. (2012). Mapping regional grey and white matter atrophy in relapsing–remitting multiple sclerosis. *Multiple Sclerosis Journal*, 18, pp.1027-1037.doi: 10.1177/1352458512439239

Riches, I.P., Wilson, F.A. and Brown, M.W. (1991). The effects of visual stimulation and memory on neurons of the hippocampal formation and the neighboring parahippocampal gyrus and inferior temporal cortex of the primate. *Journal of Neuroscience*, 11, pp.1763-1779. doi: 10.1523/JNEUROSCI.11-06-01763.1991.

Ridderinkhof, K.R., Ullsperger, M., Crone, E.A., and Nieuwenhuis, M. (2004). The role of the medial frontal cortex in cognitive control. *Science*, 306, pp.443-447. doi: 10.1126/science.1100301.

Rindfleisch, E. (1863). Histologisches Detail zu der grauen Degeneration von Gehirn und Rückenmark. *Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin*, 26, pp.474-483. doi: 10.1007/BF01878008

Robbins, T. W. (1984). Cortical noradrenaline, attention and arousal. *Psychological Medicine*, 14, pp. 13–21. doi: 10.1017/S0033291700003032.

Roberg, B.L., Bruce, J.M., Lovelace, C.T., and Lynch, S. (2012). How patients with multiple sclerosis perceive cognitive slowing. *The Clinical Neuropsychologist,* 26, pp. 1278-1295. doi: 10.1080/13854046.2012.733413.

Rocca, M.A., Bonnet, M.C., Meani, A., Valsasina, P., Colombo, B., Comi, G. and Filippi, M. (2012). Differential cerebellar functional interactions during an interference task across multiple sclerosis phenotypes. *Radiology*, 265, pp.864-873.doi:10.1148/radiol.12120216

Rocca, M.A., Cercignani, M., Iannucci, G., Comi, G. and Filippi, M. (2000). Weekly diffusion-weighted imaging of normal-appearing white matter in MS. *Neurology*, 55, pp.882-884.doi: 10.1212/WNL.55.6.882

Rocca, M.A., Falini, A., Colombo, B., Scotti, G., Comi, G. and Filippi, M. (2002). Adaptive functional changes in the cerebral cortex of patients with nondisabling multiple sclerosis correlate with the extent of brain structural damage. *Annals of Neurology,* 51, pp.330-339. doi: 10.1002/ana.10120.

Rocca, M.A., Meani, A., Riccitelli, G.C., Colombo, B., Rodegher, M., Falini, A., Comi, G. and Filippi, M. (2016a). Abnormal adaptation over time of motor network recruitment in multiple sclerosis patients with fatigue. *Multiple Sclerosis Journal*, 22, pp.1144-1153. doi: 10.1177/1352458515614407.

Rocca, M.A., Valsasina, P., Meani, A., Falini, A., Comi, G. and Filippi, M. (2016b). Impaired functional integration in multiple sclerosis: a graph theory study. *Brain Structure and Function*, 221, pp.115-131.doi: 10.1007/s00429-014-0896-4

Rodríguez, M., González, J., Sabaté, M., Obeso, J. and Pereda, E. (2003a). Firing regulation in dopaminergic cells: effect of the partial degeneration of nigrostriatal system in surviving neurons. *European Journal of Neuroscience,* 18, pp.53-60. doi: 10.1046/j.1460-9568.2003.02723.x

Rodriguez, M., Morales, I., Gonzalez-Mora, J.L., Gomez, I., Sabate, M., Dopico, J.G., Rodriguez-Oroz, M.C. and Obeso, J.A. (2007). Different levodopa actions on the extracellular dopamine pools in the rat striatum. *Synapse,* 61, pp.61-71.doi: 10.1002/syn.20342

Rodríguez, M., Pereda, E., González, J., Abdala, P. and Obeso, J.A. (2003b). How is firing activity of substantia nigra cells regulated? Relevance of pattern-code in the basal ganglia. *Synapse*, 49, pp.216-225.doi: 10.1002/syn.10233

Roediger III, H.L., and Butler, A.C. (2011). The critical role of retrieval practice in long-term retention. *Trends in Cognitive Sciences*, 15, pp. 20-27. doi: 10.1016/j.tics.2010.09.003

Roediger III, H.L., and Karpicke, J.D. (2006a). Test-enhanced learning: Taking memory tests improves long-term retention. *Psychological Sciences*, *17*, pp. 249-255. doi: 10.1111/j.1467-9280.2006.01693.x

Roediger III, H.L., and Karpicke, J.D. (2006b). The power of testing memory: Basic research and implications for educational practice. *Perspectives of Psychological Sciences*, 1, pp. 181 – 210. doi: 10.1111/j.1745-6916.2006.00012.x

Roehrs, T.G. and Karst, G.M. (2004). Effects of an aquatics exercise program on quality of life measures for individuals with progressive multiple sclerosis. *Journal of Neurologic Physical Therapy*, 28, pp.63-71.doi: 10.1097/01.NPT.0000281186.94382.90

Roelcke, U., Kappos, L., Lechner-Scott, J., Brunnschweiler, H., Huber, S., Ammann, W., Plohmann, A., Dellas, S., Maguire, R.P., Missimer, J. and Radii, E.W. (1997). Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue A 18F-fluorodeoxyglucose positron emission tomography study. *Neurology*, 48, pp.1566-1571.doi: 10.1212/WNL.48.6.1566

Roosendaal, S.D., Hulst, H.E., Vrenken, H., Feenstra, H.E., Castelijns, J.A., Pouwels, P.J., Barkhof, F. and Geurts, J.J. (2010). Structural and functional hippocampal changes in multiple sclerosis patients with intact memory function. *Radiology,* 255, pp.595-604.doi:10.1148/radiol.10091433

Rosati, G. (2001). The prevalence of multiple sclerosis in the world: an update. *Neurological Sciences*, 22, pp.117-139.doi: 10.1007/s100720170011

Rosenblum, D. and Saffir, M. (1998). Therapeutic and symptomatic treatment of multiple sclerosis. *Physical Medicine and Rehabilitation Clinics*, 9, pp.587-601.doi: 10.1016/S1047-9651(18)30251-1

Rosier, J.P., Elliorr, R., and Sahakian, B.J. (2012). Cognitive mechanisms of treatment in depression. *Neuropsychopharmacology*, 37, pp. 117-136.doi: 10.1038/npp.2011.183

Rossi, A.F., Pessoa, L., Desimone, R. and Ungerleider, L.G. (2009). The prefrontal cortex and the executive control of attention. *Experimental Brain Research*, 192, pp.489-497.doi: 10.1007/s00221-008-1642-z

Rosti-Otajärvi, E., Ruutianen, J., Huhtala, H., Hämäläinen, P. (2014). Cognitive performance profile in different phenotypes of MS with cognitive complaints. *Multiple Sclerosis and Related Disorders,* 3, pp. 436-472. doi: 10.1016/j.msard.2014.01.003.

Rubinov, M., and Sporns, O. (2010). Complex network measures of brain connectivity: uses and interpretations. *NeuroImage,* 52, pp. 1059-1069. doi: 10.1016/j.neuroimage.2009.10.003

Rubinov, M., and Sporns, O. (2011). Weight-conserving characterization of complex functional brain networks. *NeuroImage,* 56, pp. 2068-2079. doi: 10.1016/j.neuroimage.2011.03.069

Sanna, S., Pitzalis, M., Zoledziewska, M., Zara, I., Sidore, C., Murru, R., Whalen, M.B., Busonero, F., Maschio, A., Costa, G. and Melis, M.C. (2010). Variants within the immunoregulatory CBLB gene are associated with multiple sclerosis. *Nature Genetics*, 42, p.495.doi 10.1038/ng.584

Sastre-Garriga, J., Arévalo, M.J., Renom, M., Alonso, J., González, I., Galán, I., Montalban, X. and Rovira, A. (2009). Brain volumetry counterparts of cognitive impairment in patients with multiple sclerosis. *Journal of the Neurological Sciences*, 282, pp.120-124. doi: 10.1016/j.jns.2008.12.019.

Sato, F., Parent, M., Levesque, M. and Parent, A. (2000). Axonal branching pattern of neurons of the subthalamic nucleus in primates. *Journal of Comparative Neurology*, 424, pp.142-152. doi: 10.1002/1096-9861(20000814)424:1<142::AID-CNE10>3.0.CO;2-8

Sato, M., and Hikosaka, O. (2002). Role of primate substantia nigra pars reticulata in reward-oriented saccadic eye movement. *Journal of Neuroscience*, 22, pp.2363-2373.doi: 10.1523/JNEUROSCI.22-06-02363.2002

Sawcer, S., Goodfellow, P.N. and Compston, A. (1997). The genetic analysis of multiple sclerosis. *Trends in Genetics*, 13, pp.234-239.doi: 10.1016/S0168-9525(97)01140-2

Sbardella, E., Petsas, N., Tona, F., Prosperini, L., Raz, E., Pace, G., Pozzilli, C. and Pantano, P. (2013). Assessing the correlation between grey and white matter damage with motor and cognitive impairment in multiple sclerosis patients. *PLoS One*, 8, p.e63250. doi: 10.1371/journal.pone.0063250.

Schiff, N.D. (2008). Central thalamic contributions to arousal regulation and neurological disorders of consciousness. *Annals of the New York Academy of Sciences*, 1129, pp.105-118. doi: 10.1196/annals.1417.029.

Schoenbaum, G., Takahashi, Y., Liu, T.L. and McDannald, M.A. (2011). Does the orbitofrontal cortex signal value?. *Annals of the New York Academy of Sciences*, 1239, pp.87-99.doi: 10.1111/j.1749-6632.2011.06210.x

Scholtz, J., Klein, M. C., Behrens, T. E., and Johansen-Berg, H. (2009). Training induces changes in white-matter architecture. *Nature Neuroscience*, 12, pp. 1370-1371. doi: 10.1038/nn.2412

Schoonheim, M.M., Hulst, H.E., Brandt, R.B., Strik, M., Wink, A.M., Uitdehaag, B.M., Barkhof, F. and Geurts, J.J. (2015a). Thalamus structure and function determine severity of cognitive impairment in multiple sclerosis. *Neurology,* 84, pp.10-1212. doi:10.1212/WNL.0000000000001285

Schoonheim, M.M., Hulst, H.E., Landi, D., Ciccarelli, O., Roosendaal, S.D., Sanz-Arigita, E.J., Vrenken, H., Polman, C.H., Stam, C.J., Barkhof, F. and Geurts, J.J. (2012). Gender-related differences in functional connectivity in multiple sclerosis. *Multiple Sclerosis Journal,* 18, pp.164-173.doi: 10.1177/1352458511422245

Schoonheim, M.M., Meijer, K.A. and Geurts, J.J. (2015b). Network collapse and cognitive impairment in multiple sclerosis. *Frontiers in Neurology*, 6, A82.doi 10.3389/fneur.2015.00082

Schröder, K.F., Hopf, A., Lange, H. and Thörner, G. (1975). Morphometrical-statistical structure analysis of human striatum, pallidum and subthalamic nucleus. *Journal fur Hirnforschung*, 16, pp.333-350. Retrieved from: https://www.researchgate.net/

Schultz, W., Apicella, P., Scarnati, E. and Ljungberg, T. (1992). Neuronal activity in monkey ventral striatum related to the expectation of reward. *Journal of Neuroscience*, 12, pp.4595-4610.doi: 10.1523/JNEUROSCI.12-12-04595.1992

Schumacher, G.A., Beebe, G., Kibler, R.F., Kurland, L.T., Kurtzke, J.F., McDowell, F., Nagler, B., Sibley, W.A., Tourtellotte, W.W. and Willmon, T.L. (1965). Problems of experimental trials of therapy in multiple sclerosis: report by the panel on the evaluation of experimental

trials of therapy in multiple sclerosis. *Annals of the New York Academy of Sciences*, 122, pp.552-568.doi: 10.1111/j.1749-6632.1965.tb20235.x

Schwartz, C.E., Coulthard-Morris, L. and Zeng, Q. (1996). Psychosocial correlates of fatigue in multiple sclerosis. *Archives of Physical Medicine and Rehabilitation*, 77, pp.165-170.doi: 10.1016/S0003-9993(96)90162-8

Schwid, S.R., Tyler, C.M., Scheid, E.A., Weinstein, A., Goodman, A.D. and McDermott, M.P. (2003). Cognitive fatigue during a test requiring sustained attention: a pilot study. *Multiple Sclerosis Journal*, 9, pp.503-508.doi: 10.1191/1352458503ms9460a

Seebacher, B., Kuisma, R., Glynn, A. and Berger, T. (2017). The effect of rhythmic-cued motor imagery on walking, fatigue and quality of life in people with multiple sclerosis: A randomised controlled trial. *Multiple Sclerosis Journal*, 23, pp.286-296. doi: 10.1177/1352458516644058.

Seebacher, B., Kuisma, R., Glynn, A. and Berger, T. (2018). Exploring cued and non-cued motor imagery interventions in people with multiple sclerosis: a randomised feasibility trial and reliability study. *Archives of Physiotherapy*, 8, A6.doi: 10.1186/s40945-018-0045-0

Sepulcre, J., Masdeu, J.C., Goni, J., Arrondo, G., Velez de Mendizabal, N., Bejarano, B. and Villoslada, P. (2009). Fatigue in multiple sclerosis is associated with the disruption of frontal and parietal pathways. *Multiple Sclerosis Journal*, 15, pp.337-344. doi: 10.1177/1352458508098373.

Sepulcre, J., Sastre-Garriga, J., Cercignani, M., Ingle, G.T., Miller, D.H. and Thompson, A.J. (2006). Regional gray matter atrophy in early primary progressive multiple sclerosis: a voxel-based morphometry study. *Archives of Neurology*, 63, pp.1175-1180.doi: 10.1001/archneur.63.8.1175

Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E. and Aloisi, F. (2004). Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathology,* 14, pp.164-174. doi: 10.1111/j.1750-3639.2004.tb00049.x

Serretti, A., Jor,i M.C., Casadei, G., and Akiskal, H. (1999). Delineating psychopathologic clusters within dysthymia: A study of 512 outpatients without major depression. *Journal of Affective Disorders*, 56, pp. 17–25.doi: 10.1016/S0165-0327(99)00056-7

Setsompop, K., Gagoski, B.A., Polimeni, J.R., Witzel, T., Wedeen, V.J., and Wald, L.L. (2012). Blipped-controlled aliasing in parallel imaging for simultaneous multislice echo planar imaging with reduced *g*-factor penalty. *Magnetic Resonance in Medicine*, 67, pp. 1210-1224. doi: 10.1002/mrm.23097

Shallice, T., Fletcher, P., Frith, C.D., Grasby, P., Frackowiak, R.S. and Dolan, R.J. (1994) Brain regions associated with acquisition and retrieval of verbal episodic memory. *Nature*, 368, pp. 633–635. doi: 10.1038/368633a0.

Sharief, M.K. and Hentges, R. (1991). Association between tumor necrosis factor-α and disease progression in patients with multiple sclerosis. *New England Journal of Medicine*, 325, pp.467-472.doi: 10.1056/NEJM199108153250704

Sharma, N., Pomeroy, V.M., and Baron, J.C. (2006). Motor imagery: a backdoor to the motor system after stroke. *Stroke*, 37, pp. 1941-1952. doi: 10.1161/01.STR.0000226902.43357.fc

Shen, W., Flajolet, M., Greengard, P. and Surmeier, D.J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. *Science,* 321, pp.848-851.doi: 10.1126/science.1160575

Shiffrin, R.M. and Czerwinski, M.P. (1988). A model of automatic attention attraction when mapping is partially consistent. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, 14, pp.562-569.doi: 10.1037//0278-7393.14.3.562

Shmuel, A., Augath, M., Oeltermann, A., Logothetis, N.K. (2006). Negative functional MRI response correlates with decreases in neuronal activity in monkey visual area V1. *Nature Neuroscience,* 9, pp. 569-577. doi: 10.1038/nn1675

Shu, N., Liu, Y., Li, K., Duan, Y., Wang, J., Yu, C., Dong, H., Ye, J. and He, Y. (2011). Diffusion tensor tractography reveals disrupted topological efficiency in white matter structural networks in multiple sclerosis. *Cerebral Cortex,* 21, pp.2565-2577.doi: 10.1093/cercor/bhr039

Sillito, A.M., Cudeiro, J. and Jones, H.E. (2006). Always returning: feedback and sensory processing in visual cortex and thalamus. *Trends in Neurosciences*, 29, pp.307-316.doi: 10.1016/j.tins.2006.05.001

Simpson, G.V., Weber, D.L., Dale, C.L., Pantazis, D., Bressler, S.L., Leahy, R.M. and Luks, T.L. (2011). Dynamic activation of frontal, parietal, and sensory regions underlying anticipatory visual spatial attention. *Journal of Neuroscience*, 31, pp.13880-13889. doi: 10.1523/JNEUROSCI.1519-10.2011.

Skinner, E.I. and Fernandes, M.A. (2007) Neural correlates of recollection and familiarity: a review of neuroimaging and patient data. *Neuropsychologia*, 45, pp. 2163–2179. doi: 10.1016/j.neuropsychologia.2007.03.007.

Smith, C., Hale, L., Olson, K. and Schneiders, A.G. (2009). How does exercise influence fatigue in people with multiple sclerosis?. *Disability and Rehabilitation*, 31, pp.685-692.doi: 10.1080/09638280802273473

Smith, M.M. and Arnett, P.A. (2005). Factors related to employment status changes in individuals with multiple sclerosis. *Multiple Sclerosis Journal,* 11, pp.602-609.doi: 10.1191/1352458505ms1204oa

Smith, M.M. and Arnett, P.A. (2007). Dysarthria predicts poorer performance on cognitive tasks requiring a speeded oral response in an MS population. *Journal of Clinical and Experimental Neuropsychology*, 29, pp.804-812.doi: 10.1080/13803390601064493

Smith, Y., and Parent, A. (1986). Differential connections of caudate nucleus and putamen in the squirrel monkey (Saimiri sciureus). *Neuroscience*, 18, pp. 347–371. doi: 10.1016/0306-4522(86)90159-4

Smith, Y., Bennett, B.D., Bolam, J.P., Parent, A. and Sadikot, A.F. (1994). Synaptic relationships between dopaminergic afferents and cortical or thalamic input in the sensorimotor territory of the striatum in monkey. *Journal of Comparative Neurology*, 344, pp.1-19.doi: 10.1002/cne.903440102

Smith, Y., Hazrati, L.N. and Parent, A. (1990). Efferent projections of the subthalamic nucleus in the squirrel monkey as studied by the PHA-L anterograde tracing method. *Journal of Comparative Neurology*, 294, pp.306-323.doi: 10.1002/cne.902940213

Smolders, J., Peelen, E., Thewissen, M., Menheere, P., Tervaert, J.W.C., Hupperts, R. and Damoiseaux, J. (2009). The relevance of vitamin D receptor gene polymorphisms for vitamin D research in multiple sclerosis. *Autoimmunity Reviews*, 8, pp.621-626.doi: 10.1016/j.autrev.2009.02.009

Solanky, M., Maeda, Y., Ming, X., Husar, W., Li, W., Cook, S. and Dowling, P. (2001). Proliferating oligodendrocytes are present in both active and chronic inactive multiple sclerosis plaques. *Journal of Neuroscience Research,* 65, pp.308-317.doi: 10.1002/jnr.1155

Solari, A., Motta, A., Mendozzi, L., Pucci, E., Forni, M., Mancardi, G. and Pozzilli, C. (2004). Computer-aided retraining of memory and attention in people with multiple sclerosis: a randomized, double-blind controlled trial. *Journal of the Neurological Sciences*, 222, pp. 99-104.doi: 10.1016/j.jns.2004.04.027

Somers, D. C., Dale, A. M., Seiffert, A. E., and Tootell, R. B. (1999). Functional MRI reveals spatially specific attentional modulation in human primary visual cortex. *Proceedings of the National Academy of Sciences*, 96, pp. 1663–1668. doi: 10.1073/pnas.96.4.1663.

Squire, L.R., Stark, C.E. and Clark, R.E. (2004). The medial temporal lobe. *Annual Reviews of Neuroscience*, *27*, pp.279-306. doi: 10.1146/annurev.neuro.27.070203.144130.

Staffen, W., Mair, A., Zauner, H., Unterrainer, J., Niederhofer, H., Kutzelnigg, A., Ritter, A., Golaszewski, S., Iglseder, B., and Ladurner, G. (2002). Cognitive function and fMRI in patients with multiple sclerosis: evidence for compensatory cortical activation during an attention task. *Brain*, 125, pp. 1275-1282. doi: 10.1093/brain/awf125.

Stankoff, B.M.D.P., Waubant, E.M.D.P., Confavreux, C., Edan, G., Debouverie, M., Rumbach, L., Moreau, T., Pelletier, J., Lubetzki, C., Clanet, M. and French Modafinil Study Group. (2005). Modafinil for fatigue in MS A randomized placebo-controlled double-blind study. *Neurology,* 64, pp.1139-1143.doi: 10.1212/01.WNL.0000158272.27070.6A

Stanley, M. L., Moussa, M. N., Paolini, B. M., Lyday, R. G., Burdette, J. H., and Laurienti, P. J. (2013). Defining nodes in complex brain networks. *Frontiers in Computational Neuroscience*, 7, A169. doi: 10.3389/fncom.2013.00169

Stephan, K.E., Penny, W.D., Moran, R.J., Ouden, H.E.M., Daunizeau, J., and Friston, K.J. (2010). Ten simple rules for dynamic causal modelling. *NeuroImage,* 49, pp. 3099-3109. doi: 10.1016/j.neuroimage.2009.11.015

Stevens, A. A., Tappon, S. C., Garg, A., and Fair, D. A. (2012). Functional brain network modularity captures inter-and intra-individual variation in working memory capacity. *PloS One*, 7, e30468. doi: 10.1371/journal.pone.0030468

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology*, 18, pp. 643-662. doi: 10.1037/h0054651

Sturm W. and Willmes, K. (2001). On the functional neuroanatomy of intrinsic and phasic alertness. NeuroImage, 14, pp. 76-84. doi: 10.1006/nimg.2001.0839.

Sturm, W., de Simone, A., Krause, B.J., Specht, K., Hesselmann, V., Radermacher, I., Herzog, H., Tellmann, L., Muller-Gartner, W., and Willmes, K. (1999). Functional anatomy of intrinsic alertness: evidence for a fronto–parietal–thalamic-brainstem network in the right hemisphere. *Neuropsychologia*, 37, pp. 797-805.doi: 10.1016/S0028-3932(98)00141-9

Sturm, W., Longoni, F., Fimm, B., Dietrich, T., Weis, S., Kemna, S., Herzog, H., Willmes, K. (2004). Network for auditory intrinsic alertness: a PET study. *Neuropsychologia,* 42, pp. 563-568. doi: 10.1016/j.neuropsychologia.2003.11.004

Sturm, W., Schmenk, B., Fimm, B., Specht, K., Weis, S., Thron, A., and Willmes, K. (2006). Spatial attention: more than intrinsic alerting? *Experimental Brain Research*, 171, pp. 16–25. doi: 10.1007/s00221-005-0253-1.

Stuss, D. T. (2011). Functions of the frontal lobes: relation to executive functions. *Journal of the International Neuropsychological Society*, *17*, pp. 759-765. doi: 10.1017/S1355617711000695

Stuss, D. T., Floden, D., Alexander, M. P., Levine, B., and Katz, D. (2001). Stroop performance in focal lesion patients: dissociation of processes and frontal lobe lesion location. *Neuropsychologia*, *39*, pp. 771-786. doi: 10.1016/S0028-3932(01)00013-6

Stuss, D.T., and Benson, D.F. (1986). *The Frontal Lobes*. Raven Press: New York.

Stys, P.K., Zamponi, G.W., Van Minnen, J. and Geurts, J.J. (2012). Will the real multiple sclerosis please stand up?. *Nature Reviews Neuroscience*, 13, pp.507-514.doi: 10.1038/nrn3275

Sumovski, J.F., Chiaravalloti, N.D., DeLuca, J. (2010). Retrieval practise improves memory in multiple sclerosis: clinical application of the testing effect. *Neuropsychology,* 24, pp. 267-272. doi: 10.1037/a0017533.

Surmeier, D.J., Ding, J., Day, M., Wang, Z. and Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends in Neurosciences*, 30, pp.228-235.doi: 10.1016/j.tins.2007.03.008

Taillard, J., Moore, N., Claustrat, B., Coste, O., Bioulac, B. and Philip, P. (2006). Nocturnal sustained attention during sleep deprivation can be predicted by specific periods of subjective daytime alertness in normal young humans. *Journal of Sleep Research*, 15, pp.41-45.doi: 10.1111/j.1365-2869.2006.00505.x.

Takada, M., Inoue, K.I., Koketsu, D., Kato, S., Kobayashi, K. and Nambu, A. (2013). Elucidating information processing in primate basal ganglia circuitry: a novel technique for pathway-selective ablation mediated by immunotoxin. *Frontiers in Neural Circuits*, 7, pp.140.doi: 10.3389/fncir.2013.00140

Takikawa, Y., Kawagoe, R. and Hikosaka, O. (2002). Reward-dependent spatial selectivity of anticipatory activity in monkey caudate neurons. *Journal of Neurophysiology*, 87, pp.508-515.doi: 10.1152/jn.00288.2001

Tana, M.G., Montin, E., Cerutti, S, and Bianchi, A.M. (2010). Exploring cortical attentional system by using fMRI during a continuous performance test. *Computational Intelligence and Neuroscience,* 2010, 329213. doi: 10.1155/2010/329213.

Tang, M. Y., Chen, T. W., Zhang, X. M., and Huang, X. H. (2014). GRE T2-weighted MRI: principles and clinical applications. *BioMed Research International*, 2014, ID 312142. doi: 10.1155/2014/312142

Targum, S.D., and Fava, M. (2011). Fatigue as a residual symptom of depression. *Innovations in Clinical Neuroscience,* 8, 40-43. Retrieved from: http://innovationscns.com/

Tartaglia, M.C., Narayanan, S. and Arnold, D.L. (2008). Mental fatigue alters the pattern and increases the volume of cerebral activation required for a motor task in multiple sclerosis patients with fatigue. *European Journal of Neurology*, 15, pp.413-419.doi: 10.1111/j.1468-1331.2008.02090.x

Tas, M.W., Barkhol, F., Van Walderveen, M.A., Polman, C.H., Hommes, O.R. and Valk, J. (1995). The effect of gadolinium on the sensitivity and specificity of MR in the initial diagnosis of multiple sclerosis. *American Journal of Neuroradiology*, 16, pp.259-264. Retrieved from: https://pdfs.semanticscholar.org/caaa/fb208b8cbb29b7d51db545195193e3150cf0.pdf

Tassi, P., and Muzet, A. (2001). Defining the states of consciousness. *Neuroscience & Biobehavioral Reviews*, 25, pp. 175-191. doi: 10.1016/S0149-7634(01)00006-9.

Taus, C., Giuliani, G., Pucci, E., D'Amico, R. and Solari, A. (2003). Amantadine for fatigue in multiple sclerosis. *The Cochrane Database of Systematic Reviews*, 2, pp.CD002818-CD002818.doi: 10.1002/14651858.CD002818

Tellez, N., Comabella, M., Julià, E.V., Río, J., Tintoré, M.A., Brieva, L., Nos, C. and Montalban, X. (2006). Fatigue in progressive multiple sclerosis is associated with low levels of dehydroepiandrosterone. *Multiple Sclerosis Journal*, 12, pp.487-494.doi: 10.1191/135248505ms13220a

Tellez, N., Rio, J., Tintore, M., Nos, C., Galan, I. and Montalban, X. (2005). Does the Modified Fatigue Impact Scale offer a more comprehensive assessment of fatigue in MS?. *Multiple Sclerosis Journal*, 11, pp.198-202.doi: 10.1191/1352458505ms11480a

Testa, M.A. and Simonson, D.C. (2017). The Use of Questionnaires and Surveys. In *Clinical and Translational Science,* pp. 207-226. doi: 10.1016/B978-0-12-802101-9.00012-0.

Thelen, J.M., Lynch, S.G., Bruce, A.S., Hancock, L.M. and Bruce, J.M. (2014). Polypharmacy in multiple sclerosis: relationship with fatigue, perceived cognition, and objective cognitive performance. *Journal of Psychosomatic Research,* 76, pp.400-404.doi: 10.1016/j.jpsychores.2014.02.013

Thiel, C. M., and Fink, G. R. (2007). Visual and auditory alertness: modality-specific and supramodal neural mechanisms and their modulation by nicotine. *Journal of Neurophysiology*, 97, pp. 2758-2768. doi: 10.1152/jn.00017.2007.

Thiel, C.M., Zilles, K., and Fink, G.R. (2004). Cerebral correlates of alerting, orienting and reorienting of visuospatial attention: an event-related fMRI study. *NeuroImage,* 21, pp. 318–328. doi: 10.1016/j.neuroimage.2003.08.044.

Thirion, B., Dodel, S., and Poline, J. B. (2006). Detection of signal synchronizations in resting-state fMRI datasets. *NeuroImage*, 29, pp. 321-327. doi: 10.1016/j.neuroimage.2005.06.054

Thomas, P.W., Thomas, S., Kersten, P., Jones, R., Slingsby, V., Nock, A., Smith, A.D., Baker, R., Galvin, K.T. and Hillier, C. (2014). One year follow-up of a pragmatic multi-centre randomised controlled trial of a group-based fatigue management programme (FACETS) for people with multiple sclerosis. *BMC Neurology*, 14, A109.doi: 10.1186/1471-2377-14-109

Thomas, S., Thomas, P.W., Kersten, P., Jones, R., Green, C., Nock, A., Slingsby, V., Smith, A.D., Baker, R., Galvin, K.T. and Hillier, C. (2013). A pragmatic parallel arm multi-centre randomised controlled trial to assess the effectiveness and cost-effectiveness of a group-based fatigue management programme (FACETS) for people with multiple sclerosis. *Journal of Neurology, Neurosurgery and Psychiatry*, 84, pp. 1092-1099.doi: 10.1136/jnnp-2012-303816

Thompson, A.J., Kermode, A.G., Wicks, D., MacManus, D.G., Kendall, B.E., Kingsley, D.P.E. and McDonald, W.I., 1991. Major differences in the dynamics of primary and secondary progressive multiple sclerosis. *Annals of Neurology*, *29*(1), pp.53-62.doi: 10.1002/ana.410290111

Thompson, K.G., Biscoe, K.L. and Sato, T.R. (2005). Neuronal basis of covert spatial attention in the frontal eye field. *Journal of Neuroscience*, 25, pp.9479-9487.doi: 10.1523/JNEUROSCI.0741-05.2005

Thornton, A.E., Raz, N. and Tucker, K.A. (2002). Memory in multiple sclerosis: contextual encoding deficits. *Journal of the International Neuropsychological Society*, 8, pp.395-409.doi: 10.1017/S1355617702813200

Thulborn, K.R., Waterton, J.C., Matthews, P.M., and Radda, G.K. (1982) Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. *Biochimia et Biophysica Acta*, 714, pp. 265–270. doi: 10.1016/0304-4165(82)90333-6

Tintoré, M., Rovira, A., Martínez, M.J., Rio, J., Díaz-Villoslada, P., Brieva, L., Borrás, C., Grivé, E., Capellades, J. and Montalban, X. (2000). Isolated demyelinating syndromes: comparison of different MR imaging criteria to predict conversion to clinically definite multiple sclerosis. *American Journal of Neuroradiology,* 21, pp.702-706.doi: 10.1177/13524585010070060 3

Tintore, M., Rovira, A., Rio, J., Nos, C., Grive, E., Sastre-Garriga, J., Pericot, I., Sánchez, E., Comabella, M. and Montalban, X. (2003). New diagnostic criteria for multiple sclerosis application in first demyelinating episode. *Neurology*, 60, pp.27-30.doi: 10.1212/WNL.60.1.27

Todd, N., Moeller, S., Auerbach, E. J., Yacoub, E., Flandin, G., and Weiskopf, N. (2016). Evaluation of 2D multiband EPI imaging for high-resolution, whole-brain, task-based fMRI studies at 3T: Sensitivity and slice leakage artifacts. *NeuroImage*, 124, pp. 32-42. doi: 10.1016/j.neuroimage.2015.08.056

Toma, K., Matsuoka, T., Immisch, I., Mima, T., Waldvogel, D., Koshy, B., Hanakawa, T., Shill, H. and Hallett, M. (2002). Generators of movement-related cortical potentials: fMRI-constrained EEG dipole source analysis. *NeuroImage*, 17, pp.161-173. doi: 10.1006/nimg.2002.1165.

Tona, F., Petsas, N., Sbardella, E., Prosperini, L., Carmellini, M., Pozzilli, C. and Pantano, P. (2014). Multiple sclerosis: altered thalamic resting-state functional connectivity and its effect on cognitive function. *Radiology,* 271, pp.814-821.doi:10.1148/radiol.14131688

Tootell, R. B., Hadjikhani, N., Hall, E. K., Marrett, S., Vanduffel, W., Vaughan, J. T., and Dale, A. M. (1998). The retinotopy of visual spatial attention. *Neuron*, 21, pp. 1409-1422. doi: 10.1016/S0896-6273(00)80659-5.

Trapp, B.D., Vignos, M., Dudman, J., Chang, A., Fisher, E., Staugaitis, S.M., Battapady, H., Mork, S., Ontaneda, D., Jones, S.E. and Fox, R.J. (2018). Cortical neuronal densities and

cerebral white matter demyelination in multiple sclerosis: a retrospective study. *The Lancet Neurology*, epub. doi: 10.1016/S1474-4422(18)30245-X

Tsuchida, A., and Fellows, L. K. (2013). Are core component processes of executive function dissociable within the frontal lobes? Evidence from humans with focal prefrontal damage. *Cortex*, *49*, pp. 1790-1800. doi: 10.1016/j.cortex.2012.10.014

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B. and Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage*, 15, pp.273-289.doi: 10.1006/nimg.2001.0978

Valentin, V.V., Dickinson, A. and O'Doherty, J.P. (2007). Determining the neural substrates of goal-directed learning in the human brain. *Journal of Neuroscience*, 27, pp.4019-4026. doi: 10.1523/JNEUROSCI.0564-07.2007

Valko, P. O., Bassetti, C. L., Bloch, K. E., Held, U., and Baumann, C. R. (2008). Validation of the fatigue severity scale in a Swiss cohort. *Sleep*, 31, pp. 1601-1607. doi: 10.1093/sleep/31.11.1601

van de Pavert, S.H., Muhlert, N., Sethi, V., Wheeler-Kingshott, C.A., Ridgway, G.R., Geurts, J.J., Ron, M., Yousry, T.A., Thompson, A.J., Miller, D.H. and Chard, D.T. (2016). DIR-visible grey matter lesions and atrophy in multiple sclerosis: partners in crime?. *Journal of Neurology, Neurosurgery and Psychiatry*, 87, pp.461-467. doi: 10.1136/jnnp-2014-310142.

van den Heuvel, M. P., and Hulshoff Pol, H.E. (2010). Exploring the brain network: a review on resting-state fMRI functional connectivity. *European Neuropsychopharmacology*, 20, pp. 519-534. doi: 10.1016/j.euroneuro.2010.03.008

van der Hiele, K., van Gorp, D., Ruimschotel, R., Kamminga, N., Visser, L. and Middelkoop, H. (2015). Work participation and executive abilities in patients with relapsing-remitting multiple sclerosis. *PloS One*, 10, p.e0129228. doi:10.1371/journal.pone.0129228

van der Valk, P. and DeGroot, C.J.A. (2000). Staging of multiple sclerosis (MS) lesions: pathology of the time frame of MS. *Neuropathology and Applied Neurobiology*, 26, pp.2-10.doi: 10.1046/j.1365-2990.2000.00217.x

Van Dongen, H., Maislin, G., Mullington, J.M. and Dinges, D.F. (2003). The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep*, 26, pp.117-126.doi: 10.1093/sleep/26.2.117

van Hilten, J.J., Hoogland, G., Van Der Velde, E.A., Middelkoop, H.A., Kerkhof, G.A. and Roos, R.A. (1993). Diurnal effects of motor activity and fatigue in Parkinson's disease. *Journal of Neurology, Neurosurgery & Psychiatry*, 56, pp.874-877. doi: 10.1136/jnnp.56.8.874.

van Hoogmoed, D., Fransen, J., Repping-Wuts, H., Spee, L., Bleijenberg, G. and van Riel, P.L.C.M. (2013). The effect of anti-TNF-α vs. DMARDs on fatigue in rheumatoid arthritis patients. *Scandinavian Journal of Rheumatology, 42*, pp.15-19.doi: 10.3109/03009742.2012.709878.

van Kessel, K., Moss-Morris, R., Willoughby, E., Chalder, T., Johnson, M.H. and Robinson, E. (2008). A randomized controlled trial of cognitive behavior therapy for multiple sclerosis fatigue. *Psychosomatic Medicine*, 70, pp.205-213.doi: 10.1097/PSY.0b013e3181643065

Van Oosten, B.W., Barkhof, F., Truyen, L., Boringa, J.B., Bertelsmann, F.W., Von Blomberg, B.M.E., Woody, J.N., Hartung, H.P. and Polman, C.H. (1996). Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2. *Neurology*, 47, pp.1531-1534. doi: 10.1212/WNL.47.6.1531

Vejmelka, M., and Paluš, M. (2010). Partitioning networks into clusters and residuals with average association. *Chaos: An Interdisciplinary Journal of Nonlinear Science*, 20, 033103. doi: 10.1063/1.3460360

Veksler, B.Z. and Gunzelmann, G. (2018). Functional equivalence of sleep loss and time on task effects in sustained attention. *Cognitive Science*, 42, pp.600-632.doi: 10.1111/cogs.12489

Vendrell, P., Junqué, C., Pujol, J., Jurado, M. A., Molet, J., and Grafman, J. (1995). The role of prefrontal regions in the Stroop task. *Neuropsychologia*, 33, pp. 341-352.doi: 10.1016/0028-3932(94)00116-7

Vertes, R.P., Hoover, W.B. and Rodriguez, J.J. (2012). Projections of the central medial nucleus of the thalamus in the rat: node in cortical, striatal and limbic forebrain circuitry. *Neuroscience*, 219, pp.120-136. doi: 10.1016/j.neuroscience.2012.04.067.

Vgontzas, A.N., Zoumakis, E., Lin, H.M., Bixler, E.O., Trakada, G. and Chrousos, G.P. (2004). Marked decrease in sleepiness in patients with sleep apnea by etanercept, a tumor necrosis factor-α antagonist. *The Journal of Clinical Endocrinology & Metabolism,* 89, pp.4409-4413.doi: 10.1210/jc.2003-031929

Vilberg, K.L. and Rugg, M.D. (2008) Memory retrieval and the parietal cortex: a review of evidence from a dual-process perspective. *Neuropsychologia*, 46, pp. 1787–1799. doi: 10.1016/j.neuropsychologia.2008.01.004.

Visser, M., Jefferies, E., Embleton, K.V. and Lambon Ralph, M.A. (2012). Both the middle temporal gyrus and the ventral anterior temporal area are crucial for multimodal semantic processing: distortion-corrected fMRI evidence for a double gradient of information convergence in the temporal lobes. *Journal of Cognitive Neuroscience*, 24, pp.1766-1778.doi: 10.1162/jocn_a_00244

Viswanathan, A., and Freeman, R.D. (2007). Neurometabolic coupling in cerebral cortex reflects synaptic more than spiking activity. *Nature Neuroscience,* 10, pp. 1308-1312. doi: 10.1038/nn1977

Wagner, A.D., Schacter, D.L., Rotte, M., Koutstaal, W., Maril, A., Dale, A.M., Rosen, B.R., and Buckner, R.L. (1998). Building memories: remembering and forgetting of verbal experiences as predicted by brain activity. *Science*, 281, pp. 1188-1191. doi: 10.1126/science.281.5380.1188.

Wagner, A.D., Shannon, B.J., Kahn, I. and Buckner, R.L. (2005) Parietal lobe contributions to episodic memory retrieval. *Trends in Cognitive Sciences*, 9, pp. 445– 453. doi: 10.1016/j.tics.2005.07.001.

Wakana, S., Jiang, H., Nagae-Poetscher, L.M., Van Zijl, P.C. and Mori, S. (2004). Fiber tract–based atlas of human white matter anatomy. *Radiology*, 230, pp.77-87. doi: 10.1148/radiol.2301021640.

Wall, N.R., De La Parra, M., Callaway, E.M. and Kreitzer, A.C. (2013). Differential innervation of direct-and indirect-pathway striatal projection neurons. *Neuron*, 79, pp.347-360. doi: 10.1016/j.neuron.2013.05.014

Wang, X., Pathak, S., Stefaneanu, L., Yeh, F.C., Li, S. and Fernandez-Miranda, J.C. (2016). Subcomponents and connectivity of the superior longitudinal fasciculus in the human brain. *Brain Structure and Function,* 221, pp. 2075-2092.doi: 10.1007/s00429-015-1028-5

Warm, J.S., Parasuraman, R. and Matthews, G. (2008). Vigilance requires hard mental work and is stressful. *Human Factors*, 50, pp.433-441.doi: 10.1518/001872008X312152

Waterhouse, J., Fukuda, Y., and Morita, T. (2012). Daily rhythms of the sleep-wake cycle. *Journal of Physiological Anthropology*, 31, pp. 5. doi: 10.1186/1880-6805-31-5.

Watts, D.J., and Strogatz, S.H. (1998). Collective dynamics of small world networks. *Nature,* 393, pp. 440-442. doi: 10.1038/30918

Weinshenker, B.G., Penman, M., Bass, B., Ebers, G.C. and Rice, G.P.A. (1992). A double-blind, randomized, crossover trial of pemoline in fatigue associated with multiple sclerosis. *Neurology*, 42, pp.1468-1468.doi: 10.1212/WNL.42.8.1468

Weinstein, A., Schwid, S.I., Schiffer, R.B., McDermott, M.P., Giang, D.W. and Goodman, A.D. (1999). Neuropsychologic status in multiple sclerosis after treatment with glatiramer. *Archives of Neurology*, 56, pp.319-324.doi: 10.1001/archneur.56.3.319

Wellcome Trust Case Control (2007). Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nature Genetics*, 39, p.1329.doi: 10.1038/ng.2007.17

Welvaert, M. and Rosseel, Y. (2013). On the definition of signal-to-noise ratio and contrast-to-noise ratio for fMRI data. *PloS One*, 8, p.e77089.doi: 10.1371/journal.pone.0077089

Wheeler, M.A., and Roediger III, H.L. (1992) Disparate effects of repeated testing: Reconciling Ballard's (1913) and Bartlett's (1932) results. *Psychological Science*, 3, pp. 240–245.doi: 10.1111/j.1467-9280.1992.tb00036.x

Wingerchuk, D.M., Benarroch, E.E., O'Brien, P.C., Keegan, B.M., Lucchinetti, C.F., Noseworthy, J.H., Weinshenker, B.G. and Rodriguez, M. (2005). A randomized controlled crossover trial of aspirin for fatigue in multiple sclerosis. *Neurology*, 64, pp.1267-1269.doi: 10.1212/01.WNL.0000156803.23698.9A

Wishart, H.A., Saykin, A.J., McDonald, B.C., Mamourian, A.C., Flashman, L.A., Schuschu, K.R., Ryan, K.A., Fadul, C.E. and Kasper, L.H. (2004). Brain activation patterns associated with working memory in relapsing-remitting MS. *Neurology*, 62, pp.234-238.doi: 10.1212/01.WNL.0000103238.91536.5F

Wolkorte, R., Heersema, D. J., & Zijdewind, I. (2016). Reduced Voluntary Activation During Brief and Sustained Contractions of a Hand Muscle in Secondary-Progressive Multiple Sclerosis Patients. *Neurorehabilitation and Neural Repair*, 30, pp. 307-316.doi: 10.1177/1545968315593809

Wolswijk, G. (2000). Oligodendrocyte survival, loss and birth in lesions of chronic-stage multiple sclerosis. *Brain*, 123, pp.105-115.doi: 10.1093/brain/123.1.105

World Health Organization (2008). *Atlas: multiple sclerosis resources in the world 2008.* Geneva: WHO Press.

Wrisley, D.M., Stephens, M.J., Mosley, S., Wojnowski, A., Duffy, J., and Burkard, R. (2007). Learning effects of repetitive administrations of the sensory organisation test in healthy young adults. *Archives of Physical Medicine and Rehabilitation,* 8, pp.1049-1054. doi: 10.1016/j.apmr.2007.05.003.

Wurtz, R.H. and Albano, J.E. (1980). Visual-motor function of the primate superior colliculus. *Annual Review of Neuroscience*, 3, pp.189-226.doi: 10.1146/annurev.ne.03.030180.001201

Xu, J., Moeller, S., Auerbach, E.J., Strupp, J., Smith, S.M., Feinberg, D.A., Yacoub, E. and Uğurbil, K., 2013. Evaluation of slice accelerations using multiband echo planar imaging at 3 T. *NeuroImage*, *83*, pp.991-1001.doi: 10.1016/j.neuroimage.2013.07.055

Xuan, B., Mackie, M.A., Spagna, A., Wu, T., Tian, Y., Hof, P.R., and Fan, J. (2016). The activation of interactive attentional networks. *NeuroImage,* 129, pp. 308–319. doi: 10.1016/j.neuroimage.2016.01.017.

Yaguez, L., Nagel, D., Hoffman, H., Canavan, A., Wist, E., and Homberg, V. (1998). A mental route to motor learning: improving trajectorial kinematics through imagery training. *Behavioural Brain Research*, 90, pp. 95- 106. doi: 10.1016/S0166-4328(97)00087-9.

Yuan, P., and Raz, N. (2014). Prefrontal cortex and executive functions in healthy adults: A meta-analysis of structural neuroimaging studies. *Neuroscience and Biobehavioural Review*, 42, pp.180-192. doi: 10.1016/j.neubiorev.2014.02.005

Yue, G., and Cole, K.J. (1992). Strength increases from the motor program: comparison of training with maximal voluntary and imagined muscle contractions. *Journal of Neurophysiology*, 67, pp. 1114-1123. doi: 10.1152/jn.1992.67.5.1114.

Zalesky, A., Cocchi, L., Fornito, A., Murray, M.M., and Bullmore, E. (2012). Connectivity differences in brain networks. *NeuroImage,* 60, pp. 1055-1062. doi: 10.1016/j.neuroimage.2012.01.068

Zhang, X., Zhang, F., Huang, D., Wu, L., Ma, L., Liu, H., Zhao, Y., Yu, S. and Shi, J. (2016). Contribution of gray and white matter abnormalities to cognitive impairment in multiple sclerosis. *International Journal of Molecular Sciences*, 18, 46. doi: 10.3390/ijms18010046.

Zhou, J., Zhou, C., Li, J., and Zhang, M. (2015). Cognitive style modulates conscious but not unconscious thought: Comparing the deliberation-without-attention effect in analytics and wholists. *Consciousness and Cognition*, 36, pp. 54-60. doi: 10.1016/j.concog.2015.05.018.

Ziemssen, T., Hoffman, J., Apfel, R. and Kern, S. (2008). Effects of glatiramer acetate on fatigue and days of absence from work in first-time treated relapsing-remitting multiple sclerosis. *Health and Quality of Life Outcomes,* 6, pp.67-72.doi: 10.1186/1477-7525-6-67

Zigmond, A.S., and Snaith, R.P. (1983). The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica*, 67, pp. 361-370. doi: 10.1111/j.1600-0447.1983.tb09716.x

Appendix A: Fatigue Severity Scale

Participant Number:

Fatigue Severity Scale

Please circle the number between 1 and 7 which you feel best fits the following statements. This refers to your usual way of life within the last week. 1 indicates "strongly disagree" and 7 indicates "strongly agree."

Read and circle a number	Strongly Disagree → Strongly Agree
1. My motivation is lower when I am fatigued.	1 2 3 4 5 6 7
2. Exercise brings on my fatigue.	1 2 3 4 5 6 7
3. I am easily fatigued.	1 2 3 4 5 6 7
4. Fatigue interferes with my physical functioning.	1 2 3 4 5 6 7
5. Fatigue causes frequent problems for me.	1 2 3 4 5 6 7
6. My fatigue prevents sustained physical functioning.	1 2 3 4 5 6 7
7. Fatigue interferes with carrying out certain duties and responsibilities.	1 2 3 4 5 6 7
8. Fatigue is among my most disabling symptoms.	1 2 3 4 5 6 7
9. Fatigue interferes with my work, family, or social life.	1 2 3 4 5 6 7

Appendix B: Hospital Anxiety and Depression Scale

Participant Number:

HADS

This questionnaire is designed to help describe how you feel. Please read each item and then place a cross in the box next to the reply that comes closest to how you have been feeling in the past week. Try to give your first reaction. This will probably be more accurate than spending a long time thinking about an answer.

Please cross only one box for each question ☒

1.1	I feel tense / wound up:	A	1.8	I feel as if I am slowed down:	D
	Most of the time	3 ☐		Nearly all of the time	3 ☐
	A lot of the time	2 ☐		Very often	2 ☐
	Occasionally	1 ☐		Sometimes	1 ☐
	Not at all	0 ☐		Not at all	0 ☐
1.2	I still enjoy things I used to:	D	1.9	I get a frightened feeling like 'butterflies' in my stomach:	A
	Definitely as much	0 ☐		Not at all	0 ☐
	Not quite as much	1 ☐		Occasionally	1 ☐
	Only a little	2 ☐		Quite often	2 ☐
	Hardly at all	3 ☐		Very often	3 ☐
1.3	I get a sort of frightened feeling as if something awful is about to happen:	A	1.10	I have lost interest in my appearance:	D
	Very definitely and quite badly	3 ☐		Definitely	3 ☐
	Not too badly	2 ☐		I don't take as much care as I should	2 ☐
	A little, but it doesn't worry me	1 ☐		I may not take quite as much care	1 ☐
	Not at all	0 ☐		I take just as much care as ever	0 ☐
1.4	I can laugh and see the funny side of things:	D	1.11	I feel restless as if I have to be on the move:	A
	As much as I ever could	0 ☐		Very much indeed	3 ☐
	Not quite as much now	1 ☐		Quite a lot	2 ☐
	Definitely not so much	2 ☐		Not very much	1 ☐
	Not at all	3 ☐		Not at all	0 ☐
1.5	Worrying thoughts go through my mind:	A	1.12	I look forward with enjoyment to things:	D
	A great deal of the time	3 ☐		As much as I ever did	0 ☐
	A lot of the time	2 ☐		Rather less than I used to	1 ☐
	From time to time	1 ☐		Definitely less than I used to	2 ☐
	Only occasionally	0 ☐		Hardly at all	3 ☐
1.6	I feel cheerful	D	1.13	I get sudden feelings of panic:	A
	Not at all	3 ☐		Very often indeed	3 ☐
	Not often	2 ☐		Quite often	2 ☐
	Sometimes	1 ☐		Not very often	1 ☐
	Most of the time	0 ☐		Not at all	0 ☐
1.7	I can sit at ease and feel relaxed:	A	1.14	I can enjoy a good book, radio or TV programme:	D
	Definitely	0 ☐		Often	0 ☐
	Usually	1 ☐		Sometimes	1 ☐
	Not often	2 ☐		Not often	2 ☐
	Not at all	3 ☐		Very seldom	3 ☐

Appendix C: Ethics Approval.

Health Research Authority

South West - Frenchay Research Ethics Committee

Level 3, Block B

Whitefriars

Lewins Mead,

Bristol BS1 2NT

Email: nrescommittee.southwest-frenchay@nhs.net

18 April 2016

Miss Christelle Van Antwerpen

CRICBristol

60 St Michaels Hill

Bristol

BS2 8DX

Dear Miss van Antwerpen

Study title:	**Evaluation of a cognitive-motor system for an objective and quantitative measurement of fatigue with MRI in patients with Multiple Sclerosis**
REC reference:	**16/SW/0059**
Protocol number:	**2593**
IRAS project ID:	**201594**

Thank you for your letter of 30 March 2016, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Natasha Bridgeman, nrescommittee.southwest-frenchay@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

Appendix C

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Healthy Control Recruitment Email 201594]	1	10 February 2016
Copies of advertisement materials for research participants [MS Poster Advertisment 201594]	2	30 March 2016
Copies of advertisement materials for research participants [Healthy Control Poster Advertisment 201594]	2	30 March 2016
Covering letter on headed paper [Cover Letter 201594]	1	10 February 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Insurance Letter 201594]	1	10 February 2016
IRAS Checklist XML [Checklist_30032016]		30 March 2016

Participant consent form [Multiple Sclerosis Consent Form 201594]	1	10 February 2016
Participant consent form [Healthy Control Consent Form 201594]	1	10 February 2016
Participant information sheet (PIS) [MRI Information Sheet 201594]	1	19 November 2015
Participant information sheet (PIS) [Multiple Sclerosis PIS 201594]	2	30 March 2016
Participant information sheet (PIS) [Healthy Control PIS 201594]	2	30 March 2016
REC Application Form [REC_Form_15022016]		15 February 2016
Research protocol or project proposal [Study Protocol 201594]	1	20 January 2016
Summary CV for Chief Investigator (CI) [Summary CV van Antwerpen 201594]	1	28 January 2016
Summary CV for supervisor (student research) [Supervisors Summary CVs]	1	10 February 2016
Summary, synopsis or diagram (flowchart) of protocol in non technical language [Study Summary 201594]	1	30 March 2016
Validated questionnaire [Neuropsychological Battery of validated questionnaires Version 2 201594]	2	30 March 2016

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Appendix C

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:

http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

16/SW/0059	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

[redacted]

Ms Wendy Bertram Vice Chair

Email:nrescommittee.southwest-frenchay@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Mrs Anna Brooke*

Appendix D: Initial MRI screening form.

NAME OF PARTICIPANT..... Sex: M / F
Date of birth…… Weight in kg/stones/lbs…… Height in cm/ft……
Please read the questions on the screening form CAREFULLY. Your safety in the magnetic environment is our primary concern. THIS IS VERY IMPORTANT. For a very small number of individuals, being scanned can be uncomfortable, or endanger health or even life. The purpose of these questions is to make sure that you are not such a person. The information you provide will be treated as strictly confidential and will be held in secure conditions. If you are unsure of the answer to any of the questions, please ASK the person who gave you this form or the person who will be performing the scan. Definitions of some of the more technical terms are given overleaf.

I wish to be screened by the same gender YES/NO*

Please answer all questions	*Circle answer*
1. Have you been fitted with a pacemaker, artificial heart valve, cochlear implant or any other implanted device?	YES/NO
2. Have you any surgical clips, aneurysm clips, shunts or stents in your body?	YES/NO
3. Have you ever had any metal fragments in your eyes?	YES/NO
4. Have you been exposed in your life to metal debris as a result of welding, grinding, filing, sawing or drilling of metal either occupationally or recreationally?	YES/NO
5. Do you wear a hearing aid?	YES/NO
6. Have you ever had any metal fragments, e.g. shrapnel in any other part of your body?	YES/NO
7. Have you any surgically implanted metal in any part of your body (e.g. joint replacement or bone reconstruction)?	YES/NO
8. Have you ever had any surgery that might have involved metal implants of which you are not aware?	YES/NO
9. Is there any possibility that you might be pregnant?	YES/NO
10. Do you have a contraceptive coil (IUD) installed?	YES/NO
11. Have you been sterilised using clips?	YES/NO
12. Do you have **any** dental work (including dentures, crowns, bridgework, braces) in your mouth, other than simple fillings?	YES/NO
13. Have you ever suffered from any of: epilepsy, diabetes or thermoregulatory problems?	YES/NO
14. Have you ever suffered from any heart disease?	YES/NO
15. Do you have any tattoos? Do you have any permanent eye makeup?	YES/NO
16. Are you wearing any skin patches? (eg. Nicotine)	YES/NO

I have read and understood the questions above and have answered them correctly.
SIGNED…… DATE……

In the presence of …….. (Name) …….. (Signature)

Definition of Technical Terms

PACEMAKER: An electronic device that is surgically placed in the patient's body and connected to the heart to regulate the heartbeat. ***The safe operation of a pacemaker can be temporarily or permanently disrupted if a person with a pacemaker goes near an MRI scanner***.

COCHLEAR IMPLANT: An electronic medical device that bypasses damaged structures in the inner ear and directly stimulates the auditory nerve, allowing some deaf individuals to learn to hear and interpret sounds and speech.

ANEURYSM CLIP: A surgically implanted metal clip used to cut off blood flow through the neck of an aneurysm. An aneurysm is a deformity of a blood vessel in the body, which can swell and burst causing a haemorrhage.

SHUNT: A surgically implanted connector, which allows passage of fluid between two parts of the body. A common use of a shunt is to allow fluid to drain away from the brain, thus reducing pressure in the brain. May also describe a tube which allows blood to be moved from one part of the body to another.

STENT: A surgical implanted device that is inserted into a blood vessel to provide support, keep the vessel open and promote unblocked and enhanced blood flow. It is also sometimes used in other fluid carrying vessels in the body such as bile ducts etc.

THERMOREGULATORY PROBLEMS: Thermoregulation is the body's in-built ability to keep all parts of your body at their correct temperature. Some illnesses prevent the person from properly controlling the temperature of their body. If you think you may have such an illness, please answer "YES" and discuss it with the person who gave you the form, or the person who is in charge of the scan.

Appendix E: Stroop neuropsychological test group

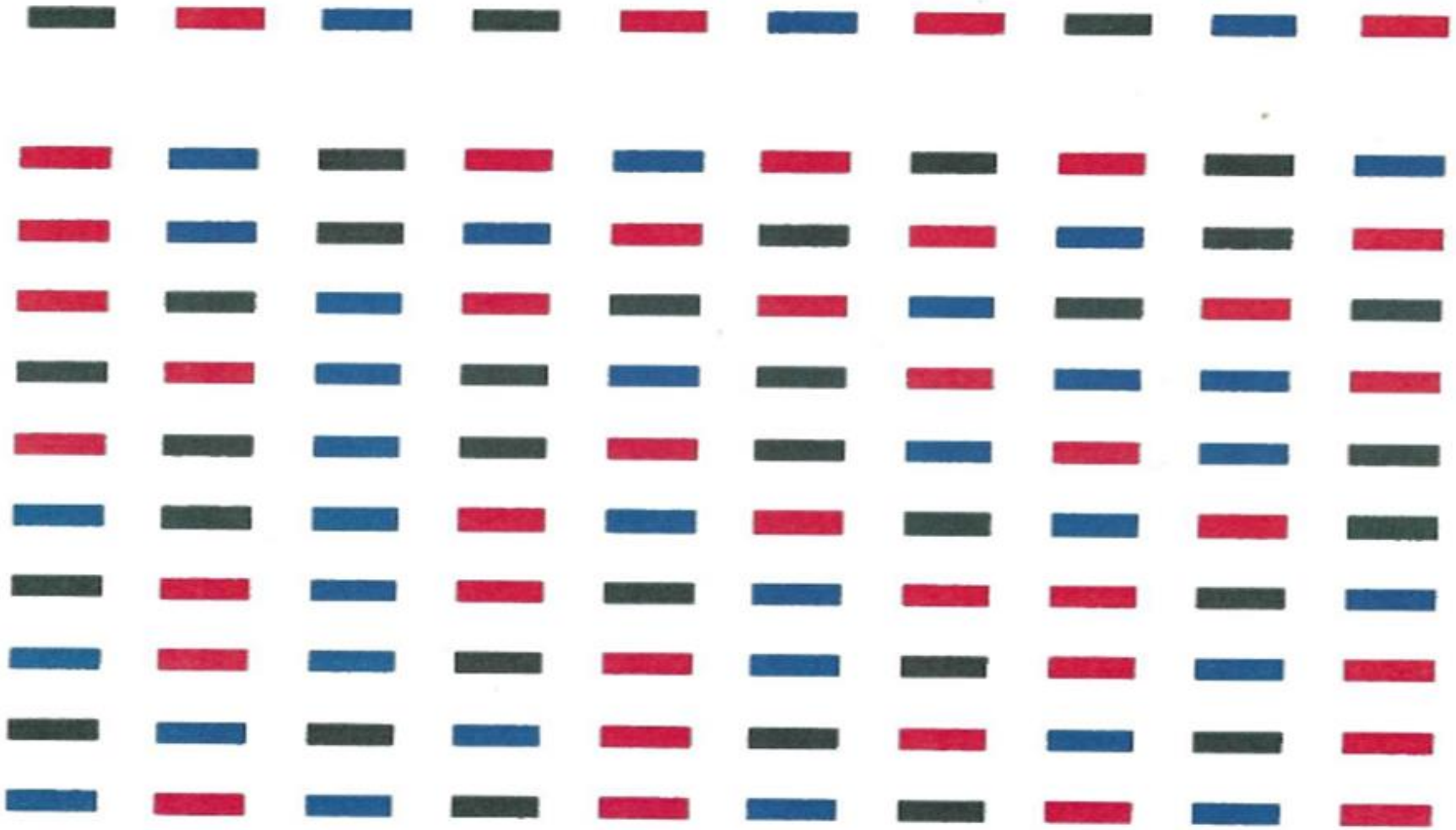


Figure E.1 Colour Naming Task.

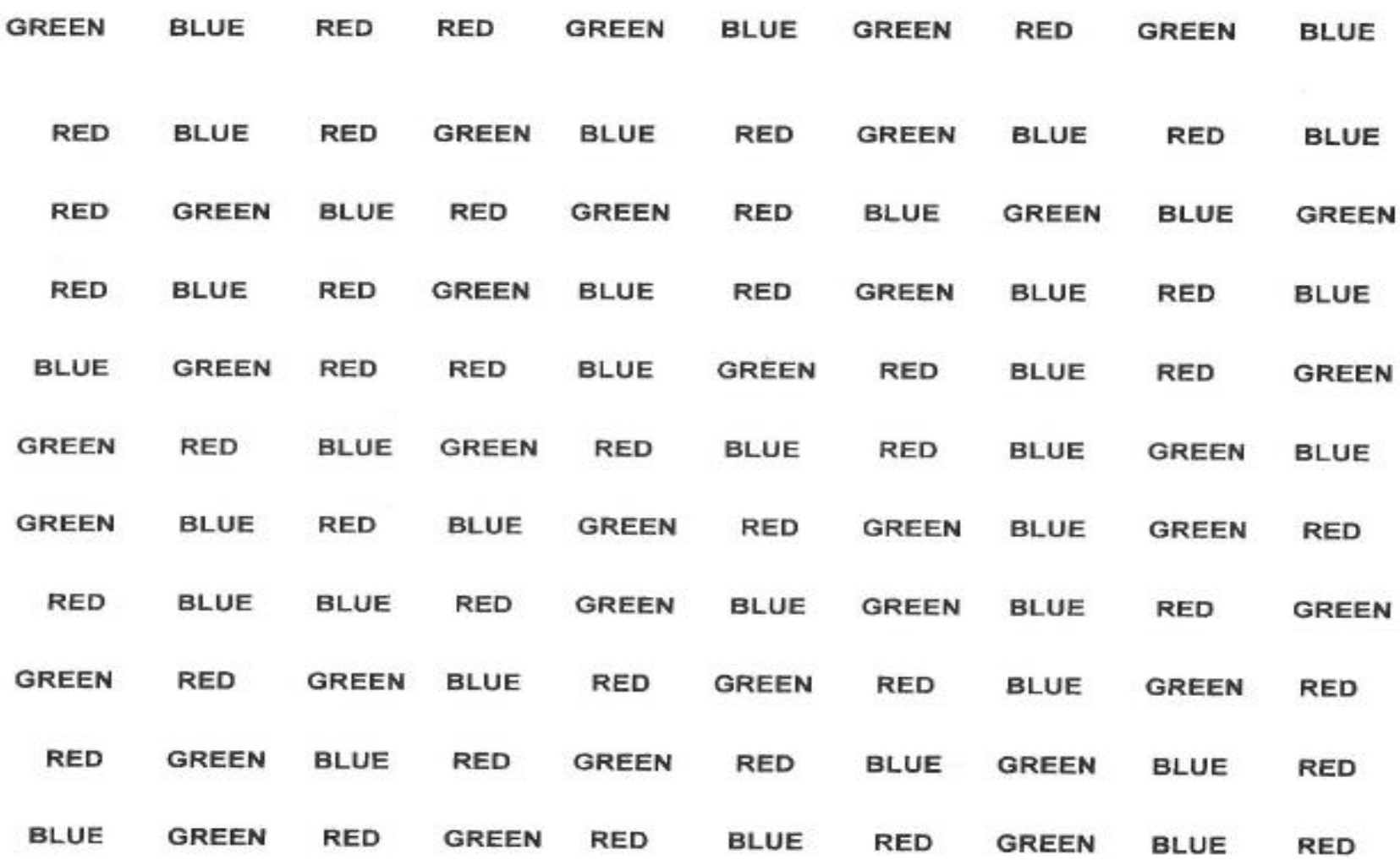


Figure E.2 Colour Naming Task.

Figure E.3 Interference Task

Appendix F: Trail making neuropsychological test group.

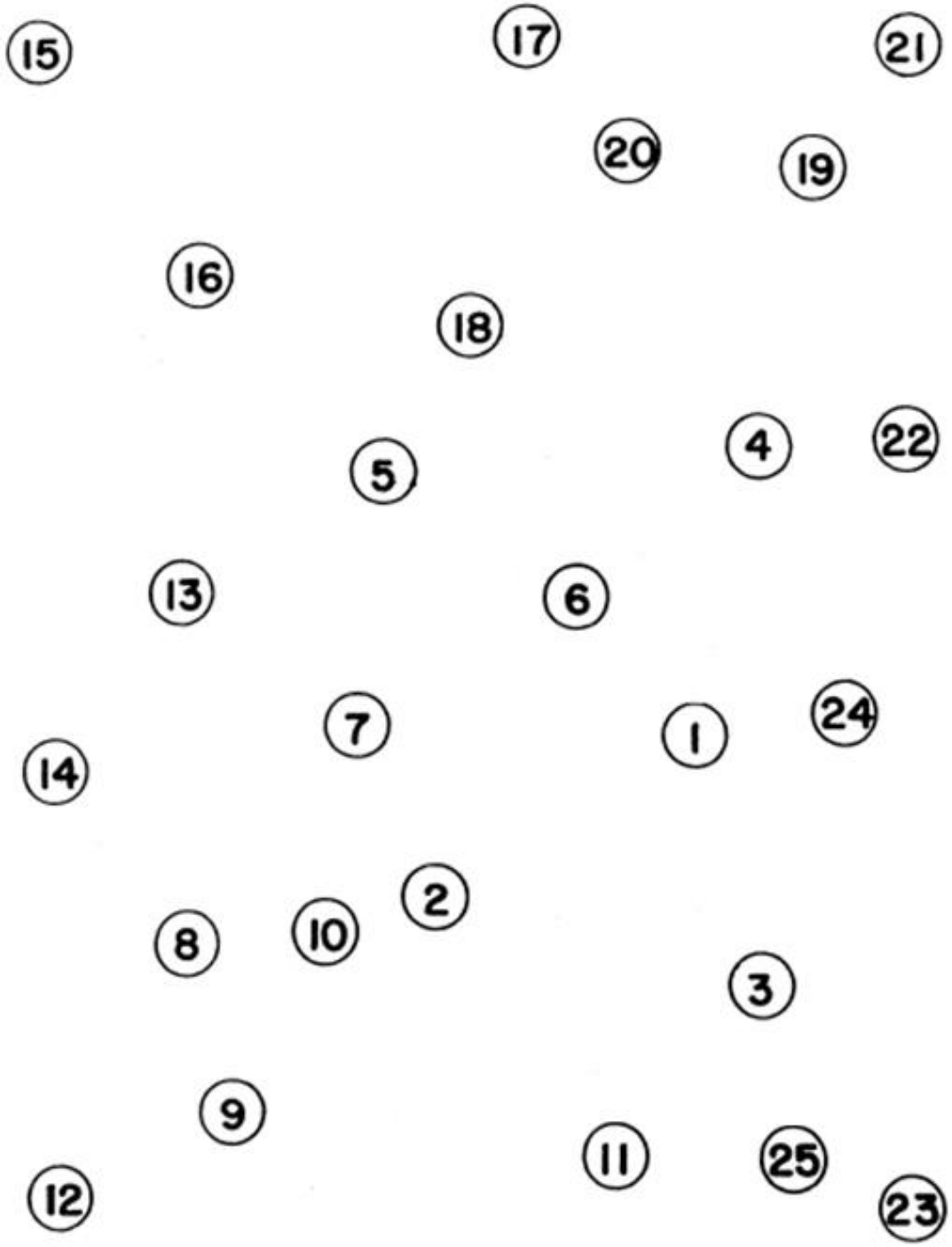


Figure F.1 Trail making A task.

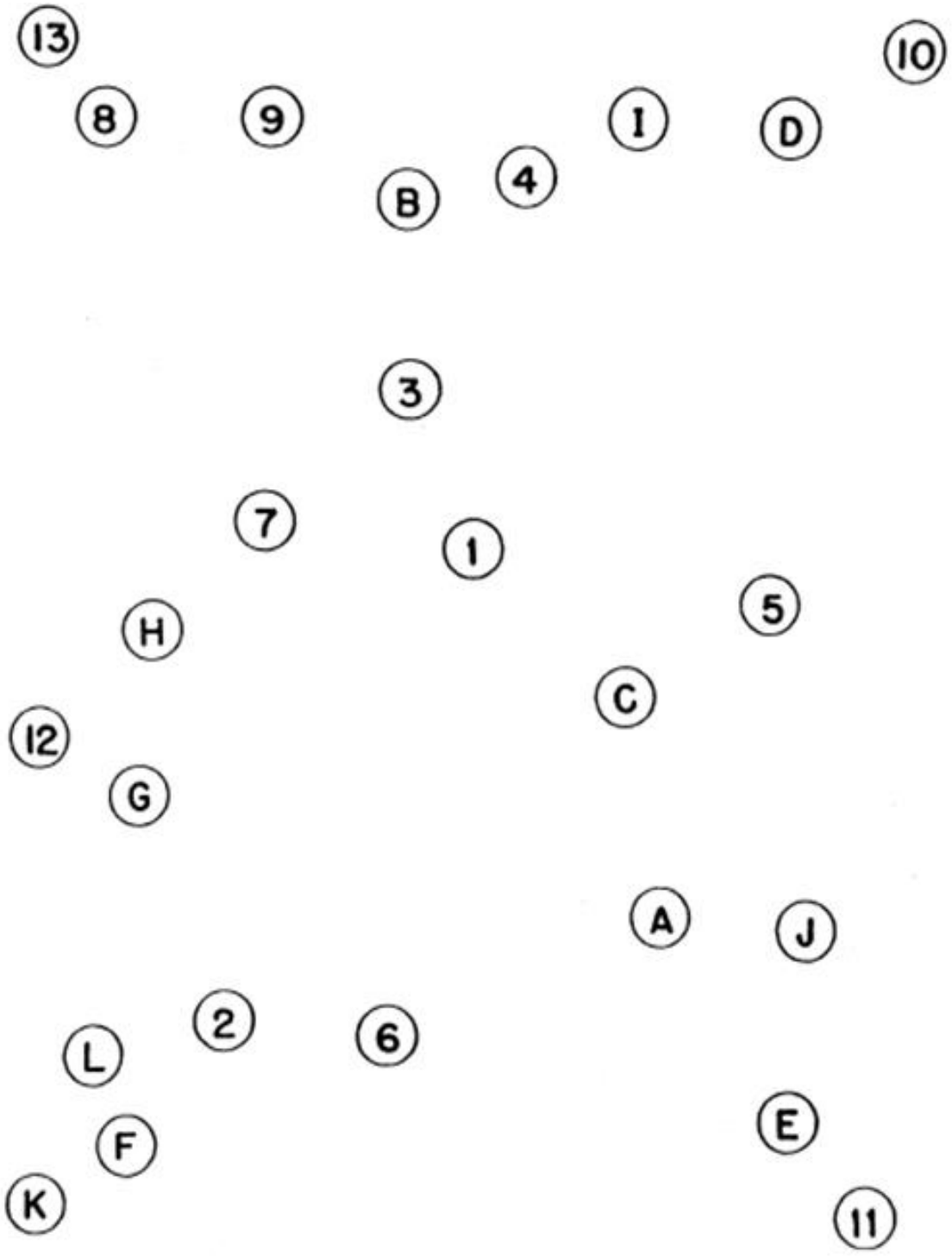
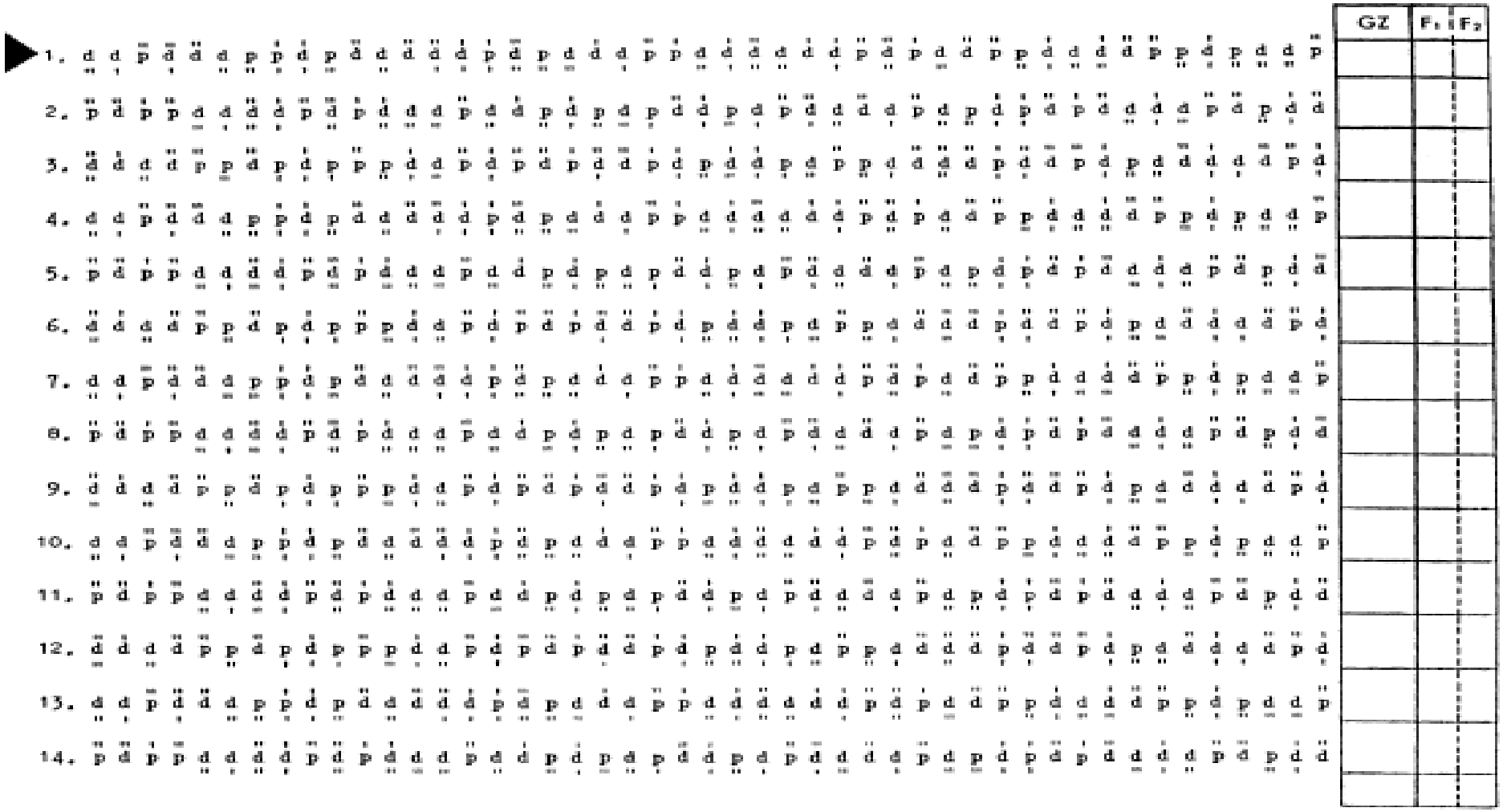


Figure F.2 Trail making B task.

Appendix G: D2 task.



Appendix H: Second MRI screening form.

This form should be completed and signed immediately before your scan, after removal of all jewellery or other metal objects and (if required by the operator) changing your clothes.

NAME OF PARTICIPANT ..... Date of birth..... Sex: M / F

Please read the following questions CAREFULLY and provide answers. For a very small number of individuals, being scanned can endanger comfort, health or even life. The purpose of these questions is to make sure that you are not such a person.
You have the right to withdraw from the screening and subsequent scanning if you find the questions unacceptably intrusive. The information you provide will be treated as strictly confidential and will held in secure conditions.

Before you are taken through for your scan it is essential that you remove all metal objects including: Watches, Pens, Loose Change, Keys, Hair clips, All Jewellery, brassieres with metal fasteners, metallic cosmetics, Cash/debit cards

Please answer all questions	Circle your answer
1. Are you wearing or carrying any metal items such as those listed above?	YES/NO
2. Have your answers to any of the questions in the initial screening form changed? (The initial screening form must be shown to you before you answer this question.)	YES/NO
3. Have you been fitted with a pacemaker, artificial heart valve, cochlear implant or any other implanted device?	YES/NO
4. Is there any possibility that you might be pregnant?	YES/NO
5. Are you currently feeling unwell (colds, flu etc.) or have you been unwell in the last week?	YES/NO

I have read and understood the questions above and have answered them correctly.

SIGNATURE..... **DATE**.....

FOR STAFF USE:
I certify that the initial screening form and the consent form have been completed by the person named above and I have attached them to this form. The volunteer has been given the standard information sheet about MRI scans, together with any necessary scan-specific information, and has been given an opportunity to ask questions. I am satisfied that the volunteer is adequately informed and understands the content of the consent form. I have taken adequate steps to ensure that the volunteer has no ferro-magnetic metal in or on his/her person and I am satisfied that the scan can proceed.

SIGNATURE..... **NAME (print)** .....

Appendix I: Systematic Review Protocol

PROSPERO International prospective register of systematic reviews

Review title and timescale

Review title

Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review:

Understanding the neural substrates of the alertness mechanism: A systematic review of functional magnetic resonance imaging (fMRI).

Original language title

For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

Anticipated or actual start date

Give the date when the systematic review commenced, or is expected to commence.

22/01/2016

Anticipated completion date

Give the date by which the review is expected to be completed.

01/08/2017

Stage of review at time of this submission

Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started √

Review stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	No	No

Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

Review team details

Named contact

The named contact acts as the guarantor for the accuracy of the information presented in the register record.

Christelle van Antwerpen

Named contact email

Enter the electronic mail address of the named contact.

Cv13365@bristol.ac.uk

Named contact address

Enter the full postal address for the named contact.

Clinical Research & Imaging Centre (CRiCBristol), University of Bristol, 60 St Michael's Hill, Bristol BS2 8DX

Named contact phone number

Enter the telephone number for the named contact, including international dialing code.

+441173421505

Organisational affiliation of the review

Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

University of Bristol

Website address:

www.bristol.ac.uk

Review team members and their organisational affiliations

Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation

Miss	Christelle	Van Antwerpen	University of Bristol
Dr	Jade	Thai	University of Bristol
Dr	Naoki	Masuda	University of Bristol
Prof	Giovanni	De Marco	CeRSM laboratory, University of Paris Ouest
Ms	Souhir	Daly	University of Paris Ouest

Funding sources/sponsors

Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.

University of Bristol.

Conflicts of interest

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.

Are there any actual or potential conflicts of interest?

None known

Collaborators

Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title	First name	Last name	Organisation details

Review methods

Review question(s)

State the question(s) to be addressed / review objectives. Please complete a separate box for each question.

To establish the neural substrates of the alertness mechanism based on evidence from functional MRI studies.

To determine the paradigms used in order to assess alertness.

Searches

Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.

We will search the following electronic bibliographic databases: MEDLINE, SCOPUS, Psychoinfo and EMBASE. Studies will be searched from date of first use successful use of fMRI in 1991. We will hand search reference lists of eligible studies, key journals will be individually searched as well as grey literature: internet resources, theses and conferences. Duplications will be removed. Papers before conception of Blood Oxygen level dependant BOLD contrast in MRI for functional brain mapping in 1991, will be removed.

URL to search strategy

If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.

I give permission for this file to be made publicly available

Yes

Condition or domain being studied

Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.

The alertness mechanism.

Participants/population

Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.

Inclusions:

All studies using fMRI to assess the alertness mechanism in adults (participants aged >18).

Exclusions:

Studies using children (participants aged < 18).

Studies after the onset of the review.

Studies before 1991.

Studies using other imaging techniques than fMRI.

Intervention(s), exposure(s)

Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed

None.

Comparator(s)/control

Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).

Not applicable.

Types of study to be included initially

Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.

Studies that employ fMRI techniques that use experimental, quantitative analysis or meta-analysis of brain.

Context

Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

Several neuroimaging techniques can be used in order to assess the neural substrates of the alertness mechanism, however we limit the review to fMRI techniques. The conception of Blood Oxygen level dependant BOLD contrast in MRI for functional brain mapping was first reported in 1991, therefore no studies prior to this are included.

Primary outcome(s)

Give the most important outcomes.

Evaluate the paradigm used to test the alertness mechanism.

Identify the neural substrates that underlie the alertness mechanism.

Secondary outcomes

List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.

None

Give information on timing and effect measures, as appropriate.

Data extraction, (selection and coding)

Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.

Two reviewers will independently assess titles and abstracts of identified records to determine eligibility using pre-specified inclusion and exclusion criteria. Those titles that are irrelevant will be discarded. Full text articles of potentially relevant references (as deemed by at least one reviewer) will be retrieved. The retrieved full text articles will be assessed independently by two reviewers based on pre-

stated inclusion criteria. Using an iterative approach papers will be read and re-read to determine if they contain data useful to the aims of this review. Disagreements between reviewers will be resolved via discussion and if no consensus is reached by a third party. The following details about each study will be extracted by one reviewer: Study (Author, date), Country, alertness paradigm used, number of participants, demographics of participants, aim of paper, type of magnet strength, data analysis method, neural substrates of alertness mechanism, type of treatment if any, behavioural measures used and results of the behavioural measures..

Risk of bias (quality) assessment

State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.

fMRI studies with magnet strength less than 1.5 will not be included. We will access bias of MRI studies by best practice criteria set by Nichols, T.E., Das, S., Eickhoff, S.B., Evans, A.C., Glatard, T., Hanke, M., Kriegeskorte, N., Milham, M.P., Poldrack, R.A., Poline, J.-B., Proal, E., Thirion, B.,Van Essen, D.C., White, T., and Yeo, B.T.T. (2015). Best Practices in Data Analysis and Sharing in Neuroimaging using MRI. bioRxiv. http://dx.doi.org/ 10.1101/054262.

Strategy for data synthesis

Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.

A narrative synthesis and the following data will be extracted alertness paradigm, study population, control group or none, fMRI data analysis method, magnet strength, neural substrates of alertness mechanism, behavioural measures used and results of the behavioural measures. If there is sufficient data a meta-analysis will be conducted

Analysis of subgroups or subsets

Give any planned exploration of subgroups or subsets within the review. ‘None planned’ is a valid response if no subgroup analyses are planned.

None

Review general information

Type of review

Select the type of review from the drop down list.

Other

Qualitative Synthesis

Language

Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.

English

Will a summary/abstract be made available in English?

Yes

Country

Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.

England

Other registration details

List places where the systematic review title or protocol is registered (such as with the Campbell Collaboration, or The Joanna Briggs Institute). The name of the organisation and any unique identification number assigned to the review by that organization should be included.

Reference and/or URL for published protocol

Give the citation for the published protocol, if there is one.

Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

I give permission for this file to be made publicly available

Yes

Dissemination plans

Give brief details of plans for communicating essential messages from the review to the appropriate audiences.

Results will be written up as part of a PhD thesis. Additionally, we will publish our results in a peer reviewed journal, present data at scientific conferences and disseminate the results through the MS Research, Treatment and Education charity.

Do you intend to publish the review on completion?

Yes

Keywords

Give words or phrases that best describe the review. (One word per box, create a new box for each term)

Alertness

Alerting

Phasic

Tonic

Intrinsic

Extrinsic

Arousal

Wakefulness

Readiness

Vigilence

Sustained Attention

Functional Magnetic resonance imaging

fMRI

functional MRI

functional MR imaging

BOLD

Blood Oxygen level dependant (signal)

Details of any existing review of the same topic by the same authors

Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

Current review status

Review status should be updated when the review is completed and when it is published.

On-going.

Any additional information

Provide any further information the review team consider relevant to the registration of the review.

Details of final report/publication(s)

This field should be left empty until details of the completed review are available. Give the full citation for the final report or publication of the systematic review.

Give the URL where available.

Appendix J: Borg Ratings of Perceived Effort

BORG RATING OF PERCEIVED EXERTION (RPE) SCALE

Number rating	Verbal rating	Example
6		No effort at all. Sitting and doing nothing.
7	Very, very light	Your effort is just notice-able.
8		
9	Very light	Walking slowly at your own pace.
10		Light effort.
11	Fairly light	Still feels like you have enough energy to continue exercising.
12		
13	Somewhat hard	
14		Strong effort needed.
15	Hard	
16		Very strong effort needed.
17	Very hard	You can still go on but you really have to push yourself. It feels very heavy and you're very tired.
18		
19	Very, very hard	For most people, this is the most strenuous exercise they have ever done. Almost maximal effort.
20		Absolute maximal effort (highest possible). Exhaus-tion.

Appendix K: Statistical analysis for global task performance of split groups.

Table K.1 Descriptive statistics for global task reaction time (ms) for handgrip first and imagery first groups for both HC and MS.

		T2 RT	T3 RT	p - value
HC	Handgrip First	320.15 (45.98)	310.74 (43.30)	0.34
	Imagery First	303.96 (54.37)	283.67 (50.94)	0.10
MS	Handgrip First	399.21 (69.90)	398.74 (78.91)	0.92
	Imagery First	395.46 (144.17)	378.66 (162.39)	0.63

Note: Mean (s.d.)

Appendix L: Spearmans rank analyses for correlations with fatigue scores.

Table L.1 Spearmans Rho coefficients between neuropsychological test performance and disease duration.

	Colour Naming	Colour Words	Interference	Trail Making A	Trail Making B	D2 Speed	D2 Error	D2 Ratio
Disease Duration	-.19	.01	-.18	.01	.17	-.01	.46**	.47**

Note: * p= .05; ** p= .01. For pearsons correlation coefficient see section 4.3.2.

Table L.2 Spearmans Rho coefficients between FSS score and neuropsychological test performance.

	FSS Score	
	MS	HC
Colour Naming	.01	.12
Colour Words	.08	.27
Interference Task	.01	.34*
Trail Making A	.09	.21
Trail Making B	.29	.16
D2 Speed	-.09	-.2
D2 Error	.29	.37*
D2 Ratio	.31	.35*

Note: * p= .05; ** p= .01. For pearsons correlation coefficient see section 4.3.4.

Table L.3 Spearmans Rho coefficients between both fatigue and fatiguablity scores and tissue volume.

	FSS Score		Intrinsic RT		Extrinsic RT	
	MS	HC	MS	HC	MS	HC
GMV	-.17	-.17	-.17	-.11	.36*	-.08
WMV	-.05	.02	-.19	-.13	.18	.40
CSF	-.08	-.12	-.25	.04	.14	.05

Note: * p= .05; ** p= .01. For pearsons correlation coefficient see section 5.3.1.

Table L.4 Spearmans Rho coefficients scores between fatiguability scores and disease duration.

	MS Global	Handgrip First	Imagine First
Intrinsic RT	-.13	.08	.46
Extrinsic RT	-.16	.16	.40
Intrinsic Low	-.29	.08	-.04
Intrinsic Medium	-.17	.16	-.08
Intrinsic High	-.09	.37	.09
Extrinsic Low	-.28	.29	.11
Extrinsic Medium	-.20	.26	.04
Extrinsic High	-.09	.46	.17

Note: * p= .05; ** p= .01. For pearsons correlation coefficient see section 6.3.4, 6.3.6,

Table L.5 Spearmans Rho coefficients between fatigue and fatiguablity scores.

	FSS Score	
	MS	HC
Intrinsic RT	.03	.41**
Extrinsic RT	-.10	.52**
Intrinsic Low	-.03	-.1
Intrinsic Medium	-.07	-.24
Intrinsic High	-.12	-.39*
Extrinsic Low	-.05	-.13
Extrinsic Medium	-.13	-.23
Extrinsic High	-.14	-.32*

Note: * p= .05; ** p= .01. For pearsons correlation coefficient see section 6.3.8 & 6.3.9.

Table L.6 Spearmans Rho coefficients between fatigue scores and whole brain network topology.

		FSS Score	
		MS	HC
T2	SWP	-.30	.14
	Modularity	.21	-.23
	Global Efficiency	-.15	.18
T3	SWP	.17	.23
	Modularity	-.09	-.13
	Global Efficiency	.12	.11

Note: * p= .05; ** p= .01. For pearsons correlation coefficient see section 8.3.9.