A Clinical and Electrophysiological Study of the effects of 4-aminopyridine on Upper Limb Impairment in Multiple Sclerosis

Dr Marion Avril Simpson
ORCID ID 0000-0001-5395-3743

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Department of Medicine, Austin Health, University of Melbourne

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Abstract

Introduction: Multiple Sclerosis (MS) is a leading cause of disability in Australian adults. Recent decades have seen great advances in disease-modifying therapies for some forms of MS but options for symptomatic therapy remain limited and, in most cases, unsupported by randomized clinical trial evidence. Upper limb dysfunction, visual impairment and fatigue are common and disabling MS symptoms which lack proven symptomatic therapies.

Modified-release 4-aminopyridine (fampridine-MR) is licensed in Australia for the symptomatic treatment of walking disability in patients with MS. Its potential for use in other neurological domains, its mode of action, and the reasons for widely variable responses in treated patients, remain unknown.

Various clinical and electrophysiological measures can be used to objectively evaluate patients with MS. These include a collection of functional tests of upper limb strength, dexterity and sensation, most of which are seldom conducted as part of routine clinical evaluation. Objective electrophysiological evaluation of Central Nervous System (CNS) conduction can be performed using evoked potential (EP) testing and Transcranial Magnetic Stimulation (TMS). The effect of fampridine-MR on areas of disability other than walking and how this might be reflected by changes in CNS conduction has not been previously fully investigated in the context of blinded randomized controlled trials.

As a precursor to the original data, this thesis includes a comprehensive literature review of the diagnosis, pathophysiology and clinical features of MS; currently available disease-modifying and symptomatic treatments; objective clinical and electrophysiological tests used to evaluate of CNS conduction and MS symptoms, and the role of fampridine and related drugs in the treatment of MS symptoms.

The original research presented within this thesis will test the following hypotheses:

1. Fampridine-MR treatment is associated with improvements in upper limb impairment, vision and fatigue in patients with multiple sclerosis.
2. Objective electrophysiological measures differ between patients on and off treatment with fampridine, and can be used to predict clinical response.

Methods: These hypotheses were tested in two substudies. Study 1 was a small pilot study in patients taking fampridine-MR for walking disability; study 2 was a larger, randomized double-blind placebo-controlled trial in treatment-naïve patients with upper limb impairment. Clinical and
electrophysiological measures were made before, during and after the treatment period and compared
between groups. A group of healthy control participants was also included for validation of the
clinical and electrophysiological measures.

The primary clinical outcome measure was improvement in upper limb function using the 9-hole peg
test. Secondary clinical outcomes included grip strength, sensory discrimination capacity, visual
acuity and fatigue. Electrophysiological outcomes included peripheral nerve conduction, EP’s and
TMS parameters.

Results: Whilst patients differed from healthy control subjects on the majority of the clinical and
electrophysiological measures, treatment with fampridine-MR was not associated with improvement
in any of the clinical or electrophysiological measures used.

Conclusion: Fampridine-MR was not found to be an effective symptomatic treatment for upper limb
impairment, vision or fatigue in patients with MS. The significance of these findings in the context of
other published literature and the methodological strengths and limitations of the study are discussed.
Declaration

I, Marion Avril Simpson, declare that the attached thesis comprises only my original work towards the degree of Doctorate of Medical Science (University of Melbourne). I declare that due acknowledgement has been made to my collaborators (see Preface & Acknowledgements) and that all other authors’ ideas and data have been appropriately acknowledged within the References section.

The word count of this thesis is 49,754 words (excluding figures, appendices and references).

Dr Marion Avril Simpson
Preface & Acknowledgements

The study was designed by me, Dr Marion Simpson, in conjunction with my supervisor Professor Richard Macdonell. Approval for the study was obtained from the Human Research Ethics Committee of Austin Health. I was solely responsible for patient recruitment and consent. Patients were recruited from the MS clinic of the Austin Hospital, through referral from private neurologists via word of mouth, and from an advertisement placed with MS Research Australia. Healthy control subjects were recruited by word of mouth. I performed all of the electrophysiological testing. The clinical testing was also carried out by me and by Ms Elise Heriot and Ms Joanne Dimovitis, who are employees of the Neuroimmunology Clinical Research, Education & Support Service (NCRESS) of Austin Health. Statistical Analysis was performed in conjunction with Professor Leonid Churilov, Florey Neurosciences Institute. I was solely responsible for writing the manuscript for the thesis and works submitted for publication, with editorial input from Professor Richard Macdonell, Professor Helen Dewey and Mr Gavin Simpson.

In addition to the above-mentioned collaborators, I would also like to acknowledge the contribution of Ms Belinda Bardsley, Research Manager of NCRESS, who provided assistance with unblinding of patients after completion of treatment and editorial input into works submitted for publication. Mr Michael Ching, Clinical Trials Pharmacist at Austin Health, was responsible for blinding of treatments. Mr Mark Edmonds, Senior Scientist, Neurosciences Laboratory, Austin Health, provided training in the conduct of Evoked Potential testing and Transcranial Magnetic Stimulation, as well as technical support in the conduct of electrophysiological testing.

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3. Poster presentation, ANZAN Neurophysiology Workshop, Gold Coast 2015. The Fampridine Upper Limb Study: Baseline Clinical and Electrophysiological Data. Simpson, Marion; Dimovitis, Joanne; Heriot, Elise; Bardsley, Belinda; Carey, Leeanne; Yiannikas, Con; Macdonell, Richard.
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Chapter 1: Introduction

Multiple sclerosis (MS) is a common and disabling disease of the central nervous system, affecting an estimated 13,000 Australians and with an increasing incidence [1, 2]. The mean age of symptom onset and diagnosis is in the mid-thirties, and consequently the disease has a marked social and economic impact [3, 4]. MS can have a relapsing-remitting course (RRMS), with discrete attacks of central nervous system inflammation and associated symptoms followed by complete or incomplete clinical recovery, or it can be progressive from the onset with a gradual decline in function and accumulation of disability (primary progressive MS, PPMS). In both cases, permanent disability results from a combination of central nervous system demyelination and axonal transection. The majority of patients initially presenting with RRMS will eventually develop secondary progressive MS (SPMS), where progressive neurological decline continues to occur in the absence of discrete relapses. MS is an important cause of disability with only a modest effect on life expectancy [5-8] and therefore patients often have ongoing symptoms which are present for many years of their lives. Although recent decades have seen great advances in available disease-modifying therapies for MS [9, 10], treatment options for the symptoms and disability associated with MS remain very limited.

By its nature, MS can affect most areas of the central nervous system and thus produce a wide variety of symptoms, such as visual loss in optic neuritis, ataxia and diplopia in brainstem/cerebellar disease, or limb weakness, sensory symptoms and sphincter dysfunction in spinal cord disease. Impairment in motor function is a common symptom and is often a central focus for medical and allied health practitioners, which is reflected in the widespread use of the Expanded Disability Status Scale [11] (EDSS; see Appendix 2) as a tool for clinical monitoring in MS practice and a major outcome measure in MS clinical trials. This is a 20-point scale ranging from 0 (no symptoms due to MS) to 10 (death due to MS) composed of sub-scores in the domains of vision, brainstem function, pyramidal tract (motor) function, sensory impairment, cerebellar function, bowel/bladder function, cognitive function and ambulation. It is heavily weighted towards motor function, and for EDSS > 3, particularly towards ambulation. Therefore mobility impairment (and walking in particular) has become a central outcome measure in drug trials for new disease-modifying therapies in MS. Much less emphasis is placed in terms of the EDSS on the non-motor domains, which are more difficult to examine objectively, despite their potentially great impacts on patient quality of life and function. In long term patient cohorts the time it takes for patients to reach an EDSS of 3 is highly variable but once this point is reached the rate of further disease progression appears to be remarkably consistent regardless of the time taken to reach EDSS 3[12].

Clinical assessment using the EDSS can be complemented by radiological assessment with Magnetic Resonance Imaging (MRI)[13] and various electrophysiological techniques. Evoked potentials (EPs)
are the primary electrophysiological tool and have been important in the diagnosis of MS and in predicting disease course [14], particularly in the era prior to the widespread availability of MRI. EPs permit non-invasive assessment of central nervous system function and are both reproducible and objective. As well as their role in diagnosis and prognostication, it has been suggested that evoked potentials and transcranial magnetic stimulation (TMS) could be important in the monitoring of treatment response, for both the disease-modifying treatments and symptomatic therapies, but the existing body of literature in this area remains relatively small[15].

The central body of this thesis concerns studies of the clinical and electrophysiological effects of a recently-available symptomatic therapy for MS, modified-release fampridine (4-aminopyridine; fampridine-MR). This drug is a voltage-gated potassium channel blocker which has recently been licensed for the treatment of MS-related walking impairment in Australia and other countries[16]. The effects of this medication on a number of MS symptoms will be explored, including upper limb motor function, somatosensory function, visual function and fatigue. In exploring the potential role of this therapy I will first address: (1) Existing symptomatic treatments for the impairments of motor function, somatosensory function, visual function and fatigue in patients with MS (PwMS) ; (2) The use of evoked potentials and transcranial magnetic stimulation in the evaluation of these and other symptoms of MS (including cognitive impairment, sphincter dysfunction and affective disorders); (3) Existing clinical techniques used in the objective evaluation of upper limb motor and sensory function, visual function and fatigue; and (4) The mode of action and expected consequences of fampridine and related symptomatic therapies. By way of introduction, I will discuss some general information relating to MS epidemiology, pathophysiology, diagnosis and treatment.
Chapter 2: What is Multiple Sclerosis?

In this chapter, general information will be presented relating to the pathophysiology, diagnosis, classification and treatment options in Multiple Sclerosis at the current time and in the past.

Epidemiology of MS

Multiple Sclerosis (MS) is an acquired disorder affecting the central nervous system (CNS; brain and spinal cord) which is characterised pathophysiologically by areas of inflammation leading to stripping of the myelin sheath which covers CNS neurones, a process known as demyelination, and subsequent loss of axons due to inflammatory damage. MS can present at any age, including the paediatric age group, but most commonly affects adults in their third and fourth decades and shows a female: male predominance of 3:1. No single cause has been identified for the development of MS, but most theories favour a combination of genetic factors with environmental triggers. Epstein-Barr Virus (EBV) infection and vitamin D levels being thought to play important roles in pathophysiology.

A number of genes have been identified as a result of twin studies and other genetic analyses which have been shown to increase the risk of developing MS. The risk in first-degree relatives of affected individuals is estimated to be slightly elevated above the background population level. Large population-based studies such as those of Ascherio [17, 18] have supported the idea that EBV infection, most often in early adulthood, is essential to the development of MS, such that prior infection is a prerequisite for the condition to manifest.

Since the earliest epidemiological studies of MS were reported in the 1920’s [19, 20], a striking geographical variation has been noted, such that the disease becomes markedly more prevalent with increasing latitudes away from the equator. Its prevalence is lowest in equatorial regions and it becomes increasingly common towards each pole. Even within individual countries such as Australia and the UK, the same direction of prevalence is reported, such that MS is significantly more common in Tasmania than Queensland [21] and in the northernmost Shetland Isles compared to the South of England [22, 23]. This unusual geographical distribution gave rise to the theory that sunlight (ultraviolet) exposure and/or vitamin D levels which increase in response may be important in the development of MS [24, 25]. It is unknown whether sunlight exposure or vitamin D supplementation can be of therapeutic use to prevent or alter the course of MS when established. At the time of writing, trials are under way in Australia and New Zealand to determine whether treatment with vitamin D alters the risk of developing MS on an individual patient level after a first episode of CNS demyelination, a so-called Clinically Isolated Syndrome (CIS) [26]. It has also been noted that the
map of areas of highest MS risk comprises mostly those countries which were invaded by the Vikings in the 8th-10th centuries, leading to speculation that it may be more strongly related to genetic factors and patterns of migration than to environmental exposure, sunlight levels and vitamin D per se [27]. A combination of factors seems likely.

**History of MS Diagnostic Criteria**

As is the case for many neurological disorders, it was Jean-Martin Charcot who is said to have described the first case of MS in 1868 in one of his housemaids who displayed the triad of ataxia, intention tremor and scanning speech [28, 29] (although it has since been suggested that she might have had Friedrich’s ataxia rather than MS!), and this was probably the earliest attempt at forming clinical diagnostic criteria for the disease. Other early descriptions included those of the German neurologist Otto Marburg [30] who identified the combination of optic disc pallor with pyramidal signs and absent abdominal reflexes as being characteristic of MS, giving an early indication of the importance of what would later be termed “dissemination in space” or pathology affecting more than one location in the CNS. In the years that followed, there have been multiple different and continuously evolving diagnostic criteria for MS and its subtypes, and with the advent of supportive investigations such as cerebrospinal fluid (CSF) analysis, evoked potentials and MRI, these are being constantly refined and updated.

Probably the earliest formal diagnostic criteria were those of the Welsh neurologist Sydney Allison in 1931, who developed the following clinical diagnostic criteria for what was then called Disseminated Sclerosis (DS): (a) typical cases of DS; (b) early cases in which DS is a probable diagnosis; (c) history of cases deceased or untraceable, and (d) doubtful cases of DS; symptoms and signs inconclusive [31]. In 1954, the same author in conjunction with a colleague, Millar, published more specific diagnostic criteria which included typical symptoms of MS such as “transitory uniocular blindness, double vision and vertigo, pins and needles, or transient weakness or numbness of one limb”; these authors also made the distinction between clinical symptoms, resolved or still present, and physical signs, and noted the importance of multifocal CNS involvement in making this diagnosis[32].

Validation of diagnosis was clearly very difficult in the pre-MRI era and was beset by the lack of a “gold standard” against which clinical diagnoses could be compared. Poser, who was later to be highly influential in the area of diagnostic criteria for MS, conducted a survey in 1965 among 190 neurologists in 52 different countries in which he provided clinical information regarding 25 cases of autopsy-proven MS and three non-MS cases, and asked neurologists to classify as probable, possible or definite. Overall, there was approximately a 60% agreement with pathological diagnoses, but accuracy varied widely (from 2.8% to 98.2%) for cases of autopsy-proven MS, reflecting the
difficulty of accurate diagnosis in the era before sophisticated imaging and other supportive investigations [33].

The Swedish neurologist Tore Broman first captured the dual importance of dissemination in time and space of neurological symptoms which was later to become central to more refined diagnostic criteria [34]. He highlighted the features of age of onset, multiplicity of lesions, number of “bouts” and the results of examination of CSF protein, a precursor of the CSF oligoclonal bands which were to become important in the modern-day diagnostic criteria.

During the early years of clinical trials of potential MS therapies, it became necessary to have a set of standardised diagnostic criteria which could be used for patient inclusion in such clinical trials. The National Institute of Neurological Diseases and Blindness, part of the US National Institutes of Health (NIH), established a task force led by George Schumacher. This led to publication of the fairly stringent eponymous criteria in 1965 [35]. The key features were as follows: objective evidence of neurological dysfunction on examination (history of relapsing symptoms alone without observable clinical signs did not suffice); evidence of dissemination in space with examination findings consistent with at least two distinct lesions; predominantly white-matter damage (as opposed to lesions suggestive of damage to cerebral cortex or grey matter); time course suggestive of either multiple relapses, each lasting >24 hours, or slow and gradual progression over at least 6 months; absence of any other definable causes for neurological signs and symptoms; and age of onset between 10 and 50 years old. These criteria were adopted across the world, with the exception of certain Asian countries where there is known to be a particularly high prevalence of Neuromyelitis Optica (NMO), formerly known as Devic’s Disease, a condition distinct from MS where inflammation affects predominantly the spinal cord and optic nerves. The early Japanese diagnostic criteria of Kuroiwa [36] made allowances for this. In the era before MRI and antibody testing for aquaporin-4, the serological hallmark of NMO, reliable clinical distinction of NMO from MS would have been more or less impossible, and therefore the inclusion of patients from NMO-prevalent areas has the potential to muddy the waters in clinical and epidemiological studies of MS; furthermore, treating NMO patients with MS therapies has the potential to cause harm, although this may not have been known at the time.

Later, the Scottish neurologist Douglas McAlpine, in his highly influential book “Multiple Sclerosis”, proposed that a reliable history of transient symptoms compatible with MS (i.e. a relapse with complete recovery) in association with persisting objective clinical abnormalities elsewhere in the nervous system on examination should be sufficient evidence for dissemination in time and space, allowing diagnosis to be made earlier in the disease course or in milder cases [37]. He also
emphasised the fact that “definite MS” could not be reliably diagnosed without pathological evidence (from biopsy or autopsy), and classified cases as only probable, possible or latent.

Over time, the accuracy of supportive laboratory investigations has improved dramatically, ultimately leading to their inclusion in published diagnostic criteria. Poser [38] led a group of neurologists from the USA, Canada and the UK to formulate set of widely-used criteria which included both clinical and “paraclinical” features, specifically evoked potentials, computed tomography (CT) imaging and MRI. Using these, patients could be classified as “clinically definite” (two clinical attacks and evidence of two separate lesions, or one clinical attack and paraclinical evidence of a second lesion); “laboratory-supported definite” (two attacks and clinical or paraclinical evidence of one lesion, or one attack with clinical evidence of two separate lesions, or one attack and paraclinical evidence of a separate lesion, with unmatched oligoclonal bands in the CSF in each case); “clinically probable” – as above but without the demonstration of oligoclonal bands; and “laboratory-supported probable” with two clinical attacks and positive oligoclonal bands, but without the requirement for persisting clinical signs. In addition, the upper limit for age of onset was raised to 59 years. Once again, these criteria moved towards earlier diagnostic certainty without the requirement for fixed disability, a point which later became important in enabling the development of early effective disease-modifying therapy administration.

More recently, the New Zealand neurologist Ian McDonald led an international committee of neurologists which produced the highly influential McDonald criteria [39], the first to formally incorporate MRI findings, and which also included criteria for the Primary Progressive MS (PPMS) subtype. The first incarnation of the McDonald criteria allowed a diagnosis of Clinically Definite MS (CDMS) to be made on the basis of either clinical findings alone (such as two or more attacks and objective clinical evidence of two or more lesions), or a combination of clinical and paraclinical findings (such as one clinical attack, clinical evidence of only one neurological lesion but MRI evidence of dissemination in time and space fulfilling the Barkhof criteria[40], discussed below). Finally, an insidious onset with progression over at least 1 year (or shorter if radiological progression could be confirmed on MRI) but without a history of discrete clinical attacks, and with supportive evidence from CSF analysis plus either MRI or evoked potentials, could fulfil the diagnostic criteria for PPMS. The McDonald criteria have remained highly influential and are still widely used as inclusion criteria for clinical trials of therapeutic agents for MS, although they have been refined in line with advances in technology [41, 42] in order to provide further clarification on the definition of an “attack” and the nature of the MRI abnormalities required for diagnosis. In their current form the criteria allow dissemination in time to be proven on a single MRI scan which shows the simultaneous presence of contrast-enhancing lesions (reflecting recent inflammatory disease activity) with non-enhancing (older) lesions. It would appear likely that future revisions will be required in order to take
into account advances in MRI technology and other supportive information from paraclinical biomarkers.

Thus, over time, refinements to formal diagnostic criteria have allowed diagnosis to be made earlier and with greater certainty by incorporating information from both clinical symptoms and examination findings with supportive findings from radiological, biochemical and electrophysiological testing. As well as permitting earlier diagnosis and treatment, this may have had some impact on disease incidence and prevalence which have progressively continued to rise.

**Diagnosis and Diagnostic criteria in 2016**

**Clinical Features**

MS can affect any part of the CNS, and as a result can produce a variety of neurological symptoms, although some clinical syndromes are considered highly typical. These will now be described.

**Transverse Myelitis (TM)**

Inflammation of the spinal cord or transverse myelitis (TM) is probably the commonest presenting syndrome of MS, with resultant sensory or motor symptoms being present in up to half of all patients at the time of initial presentation[43]. The combination of sensorimotor symptoms and sometimes sphincter disturbance can vary according to the site of the lesion, with symptoms being present below the level of the lesion and (again, depending on location) unilateral or bilateral and often asymmetrical. MS spinal lesions show a predilection for the cervical or thoracic spinal cord and lesions in the cervical spinal cord are typically associated with Lhermitte’s Phenomenon, a sensation of electric shocks traversing the body on flexion of the cervical spine. In RRMS-associated TM, symptoms are typically mild to moderate and evolve over a period of days; dramatically severe or acute presentations are more typical of NMO spectrum disorders, a mimic of MS.

**Optic Neuritis**

MS also has a predilection for the optic nerves and optic neuritis (ON) is another common initial presentation of the disease, although it can be present in a number of other conditions which are mimics of MS. MS-related ON typically produces unilateral, painful, gradual-onset loss of visual acuity associated with loss of colour vision, with pain usually present on eye movement. Improvement typically occurs over a period of weeks and usually a good clinical recovery is made (although ON may leave traces which are permanently detectable electrophysiologically by visual evoked potential (VEP) in spite of clinical improvement). By contrast, ischaemic optic neuropathy is
typically hyperacute in onset and often does not recover well at all. NMO-related ON is often more severe than MS-related ON and usually bilateral [44].

Acute ON is predominantly a clinical diagnosis (based on findings of decreased visual acuity, pain on eye movement, central scotoma, decreased colour vision and relative afferent papillary defect) with supporting evidence from visual evoked potentials and sometimes increased T2 signal on MRI of the optic nerve. Autopsy studies in MS patients have detected evidence of optic nerve demyelination in 95-99% of all patients [45, 46]. The optic nerve is commonly used as a site to assess neural damage in Patients with Multiple Sclerosis (PwMS) using techniques such as evoked potentials and optical coherence tomography (OCT, measuring retinal nerve fibre layer thickness).

**Brainstem/Cerebellar Syndrome**

Another common syndrome of MS results from involvement of the white matter tracts of the brainstem or cerebellum, producing symptoms such as diplopia due to involvement of the 6th nerve nucleus or internuclear ophthalmoplegia (commonly bilateral) due to involvement of the medial longitudinal fasciculus in the pons. Trigeminal neuralgia or trigeminal sensory loss due to involvement of the 5th nerve nucleus in the pons is another reported, though not especially common, MS symptom. Ataxia can occur for a variety of reasons, either due to cerebellar or brainstem involvement or sometimes as a result of spinal cord damage producing a sensory ataxia.

**Other Clinical Presentations**

Although the above clinical syndromes account for the majority of presentations of CNS demyelination, a number of other presenting symptoms are possible and can include cognitive dysfunction and fatigue, either alone or (more commonly) in conjunction with other physical symptoms. Larger cortical/subcortical lesions (sometimes termed tumefactive demyelination, if large enough, because of their ability to mimic cerebral tumours in presentation) can produce focal deficits such as aphasia or hemiparesis and, in rare cases, seizures and encephalopathy.

**Heat-Sensitive Symptoms**

Many PwMS report heat-sensitive symptoms, with exacerbation of symptoms such as blurred vision or sensorimotor disturbance in hot temperatures, such as in the shower, during fever or after exercise. The pathophysiological explanation is that an increase in core body temperature shortens the duration of action potentials, contributes to desynchronous axonal conduction and conduction block, producing a transient and reversible exacerbation of symptoms which typically resolves on return to normal core temperature. This linkage of symptoms with body temperature is known as Uhthoff’s Phenomenon, named after the German ophthalmologist who first described it in a patient with optic nerve
demyelination [47]. Heat-sensitive symptoms are typically associated with demyelination rather than any of its mimics.

**MS Disease Course**

MS disease course is heterogeneous and often unpredictable on an individual patient level at the time of diagnosis. A number of disease phenotypes are recognised and patients are classified accordingly on the basis of the pattern of their clinical course. As is the case with MS diagnosis, there has been no universally standardised definition of what constitutes each of the recognised disease subtypes, and efforts have been made in recent years towards a standardised definition to aid the selection of patients for clinical trials of new therapeutic agents. In 1996, Lublin and co-authors published the results of an international survey of MS clinicians relating to clinical course and proposed standardised definitions of RRMS, PPMS, SPMS and Progressive-Relapsing MS (PRMS), as well as definitions relating to severity (“Benign” and “Malignant” MS) [48] which are, however, seldom used in current practice. A re-examination of these definitions by the same author in 2013 led to an updated version. The severity descriptions were dropped, but replaced by a classification of “active” or “not active” which was applied to both relapsing and progressive forms of MS; some indication of whether patients were worsening or stable; and the removal of the PRMS subtype and the addition of CIS and Radiologically Isolated Syndrome (RIS) [49].

Currently, and reflecting the recommendations made in this document, classification is largely based on clinical criteria, with some supporting evidence from serial MRI imaging, and there is no readily available single biomarker which can accurately differentiate one disease phenotype from another. The timeframe of development and resolution or otherwise of clinical symptoms is also important in the classification of MS disease subtype.

**Clinically Isolated Syndrome (CIS)**

CIS refers to a first presentation of neurological symptoms which are deemed to be due to CNS demyelination, but which do not yet fulfil criteria for dissemination in time [50, 51]. At the time of presentation with CIS, the risk of subsequent conversion to MS can be predicted by the presence and number of asymptomatic demyelinating brain lesions present on MRI brain imaging as well as the presence or absence of oligoclonal bands in the CSF (see below, “Supporting Laboratory Investigations”). The most recent incarnation of the McDonald criteria [42] allows some patients to be diagnosed with CDMS after a single clinical episode, if imaging findings give appropriate evidence of dissemination in time, thus reducing the number of patients with “true” CIS. There is now evidence that institution of disease-modifying treatments at the time of presentation with CIS can reduce the risk of subsequent conversion to CDMS and delay its development in patients who do go on to develop CDMS [52-55].
Radiologically Isolated Syndrome (RIS)

This term refers to the presence of typical brain lesions on MRI suggestive of MS but without any clinical symptoms or abnormal examination findings to support this diagnosis [56]. The overall risk of developing MS after the discovery of RIS is difficult to quantify, but some radiological findings are deemed higher-risk than others in this regard, notably spinal cord lesions and gadolinium-enhancing lesions; positive oligoclonal bands in the CSF in this setting also increase the risk for future MS diagnosis. Treatment of RIS in the absence of clinical symptoms is not currently recommended as beneficial, as there is no clinical trial evidence suggesting that treatment of these patients is beneficial in terms of reducing the proportion who eventually develop MS. It is recommended that such patients are kept under regular surveillance and treatment may be instituted if appropriate clinical symptoms develop over time.

RRMS

RRMS is the most common phenotype of MS, affecting approximately 80% of patients at the time of diagnosis. RRMS is characterised by discrete attacks (“relapses”) where patients develop new neurological symptoms which persist typically for days to weeks and are followed by complete or incomplete neurological recovery, without progression of disability between or in the absence of relapses.

There is consensus on the diagnosis of a clinical relapse based on the recommendations of the International Panel on the Diagnosis of Multiple Sclerosis [57]: “patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the CNS, current or historical, with a duration of at least 24 hours, in the absence of fever or infection. It should be documented by contemporaneous neurological examination, but some historical events with symptoms and evolution characteristic for MS, but for which no objective neurological findings are documented, can provide reasonable evidence of a prior demyelinating event”. If neurological examination at the time of symptoms being present is not possible, for example in the case of resolved historical symptoms (which must last longer than 24 hours), a definite diagnosis of MS can still be made according to these criteria when at least one attack is corroborated by supporting evidence of neurological dysfunction from VEP (in the case of prior visual symptoms) or MRI showing a lesion which accords with the reported symptoms.

The symptoms of relapse depend on the region of the nervous system which is affected, and can include, for example, unilateral blurred vision (in a relapse producing ON), double vision and unsteadiness (in a relapse affecting the brainstem and/or cerebellum), sensory or sensorimotor symptoms in the legs, sometimes with associated sphincter disturbance (reflecting TM) and weakness
of a limb or of both limbs on one side of the body (reflecting an inflammatory lesion in the cerebral hemisphere, subcortical white matter or perhaps spinal cord). The tempo of onset is typically over hours to days in episodes of demyelination due to RRMS, with improvement over weeks to months. In PPMS, symptoms typically evolve slowly over a period of months, without remission or improvement of symptoms. Hyperacute onset of symptoms is more suggestive of an ischaemic aetiology (such as posterior circulation stroke in the case of acute-onset ataxia, or spinal cord infarction in the case of acute-onset paraparesis). If sufficiently severe as to cause functional disability, relapses are typically treated with a course of intravenous steroids (methylprednisolone or adrenocorticotropic hormone, ACTH), which has been demonstrated to hasten recovery, although is unable to improve the final extent of neurological recovery after a relapse [58]. Typically, patients at earlier stages of the disease will manifest a better recovery from relapses, although this is not always the case, but certainly a greater number of relapses over time is associated with poorer recovery, accumulating levels of disability and a poorer overall outcome [59]. As well as being the most common type of MS at diagnosis, RRMS is also the type which is associated with a response to currently available disease-modifying therapies, and there is a move towards increasingly early initiation of these treatments (see below) in patients in whom diagnosis has been confirmed [60].

SPMS

Data from historical studies of the natural history of MS have shown that, over time, the majority of patients with RRMS eventually develop secondary progressive disease. This phenotype is characterised by an initial RRMS phase, often lasting years, which is then superseded by slow, gradual and irreversible decline in function (and particularly in mobility) in the absence of clinical relapses or MRI evidence of inflammatory disease activity. Pathophysiologically, SPMS has been thought to be characterised by progressive axonal loss with limited remyelination or repair, as a consequence of earlier inflammatory damage, rather than ongoing inflammatory demyelination and remyelination [61, 62]. The limited pathological evidence available suggests that there may also be a chronic, low-level inflammatory process ongoing in the CNS in patients with secondary progressive disease [63].

The age of onset of MS (and consequently disease duration) is the strongest predictor of the transition from RRMS to SPMS. The latter is much more common in patients with a young age of onset and the median time to conversion from first symptoms to SPMS is 10-20 years, as reported in various cohort studies [64-68]. However, the majority of these studies of natural history predate the development and widespread availability of effective therapies for RRMS, and the influence of these treatments on either the incidence of SPMS or the time taken for its manifestation are unknown. At the time of writing, treatment options for SPMS are very limited, and include high-dose Biotin, which has limited supporting evidence [69]. Oral methotrexate is also used but the limited evidence which exists
suggested a modest benefit [70]. Mitoxantrone is also used in cases of rapidly worsening SPMS despite the absence of robust supporting trial evidence [71].

**PPMS**

Most often, patients with PPMS demonstrate disease progression as increasing symptoms, particularly walking difficulty, from onset, without ever manifesting clear clinical relapses. Progression does not always occur at the same rate, and temporary plateaus or even minor improvements can occur. PPMS can be associated with radiological evidence of disease progression but typically lesion load is much lower and MRI progression occurs at a lower rate, and certainly not necessarily in line with clinical progression [72, 73]. Historically, no therapy has been shown to alter prognosis or to improve outcome or level of disability in patients with PPMS. However, in recent times, there has been some evidence from the ORATORIO study to suggest that a newly available therapy, Ocrelizumab (a close relative of the anti-CD20+ antibody rituximab), may have some potential benefits in reducing disease progression [74, 75]. As is the case for SPMS, it is believed that the pathophysiological hallmark of PPMS is axonal loss rather than inflammatory demyelination driven by the systemic immune system. It may therefore be surprising that a powerful anti-inflammatory therapy which depletes systemic B cells can show some benefit in this condition. It is thought that PPMS may not be a homogeneous disease, however, and the relative contributions of inflammatory and degenerative disease activity may differ between patients. Initial subgroup analysis of the ORATORIO study, however, did not reveal an effect of baseline inflammatory disease activity (as indicated by Gadolinium-enhancing (Gd+) lesions) on the primary outcome measure [76].

**Supporting Laboratory Investigations**

To date, no biomarkers within the peripheral blood have been identified which can support or confirm a diagnosis of MS. Blood tests do have a role, however, in excluding other conditions which can mimic MS, specifically other autoimmune diseases (for example aquaporin-4 antibody to exclude NMO, anti-double stranded DNA antibody to exclude systemic lupus erythematosus, or anticardiolipin antibody and lupus anticoagulant testing to exclude the antiphospholipid antibody syndrome), vitamin deficiencies (both vitamin B12 and vitamin E deficiency can produce TM), nutritional indices (copper deficiency and zinc excess can both produce a myelopathy, sometimes with white matter changes on spinal cord MRI), infections (Lyme disease in endemic areas can mimic cerebral demyelination) and serum angiotensin-converting enzyme level (together with appropriate changes on chest x-ray or CT, elevations of this enzyme can suggest a diagnosis of sarcoidosis). Blood tests are also performed routinely in MS patients prior to commencement of immunotherapies, particularly the newer and more immunosuppressive agents with which opportunistic infections may occur (therefore patients are tested at baseline for anti-JC virus (JCV) antibody, varicella zoster
antibody, tuberculosis, viral hepatitides, Human Immunodeficiency Virus (HIV) and routine biochemistry and haematology prior to commencing immunosuppressive therapy).

Unmatched oligoclonal bands in the CSF, by contrast, are a well-established biomarker and are present in 90% of patients with multiple sclerosis. Their presence was first established in the 1950’s [77] and they have formed part of the Poser [38] and McDonald criteria [39, 41] for the diagnosis of MS, although in the current version of the McDonald criteria they are cited as being supportive of PPMS but not required for a diagnosis of RRMS [42]. They also have a predictive role in patients presenting with CIS, in whom their presence confers at least a 2-fold increased risk of subsequently going on to develop CDMS [78]. Matched oligoclonal bands (i.e. present in CSF with identical bands in the serum) can be present in a variety of systemic inflammatory or autoimmune diseases and are not of predictive or diagnostic value in the setting of MS.

There has been growing interest in a number of other potential biomarkers for diagnosis and prognostication in suspected MS, but to date these are not in clinical use and are currently confined to the research setting. These include markers of neurodegeneration such as neurofilaments [79, 80] and glial fibrillary acidic protein [81, 82] as well as markers of CNS and peripheral neuroinflammation such as the monocyte/macrophage specific membrane marker sCD163 [83], the glial activation marker YKL-40 (also known as Chitinase 3-like 1) [84, 85] or the B-cell chemoattractant CXCL13 which has been postulated as a biomarker for both diagnosis and inflammatory disease activity in MS [86, 87]. Other proposed biomarkers include Measles, Rubella and Varicella Zoster antibodies, mRNA and miRNA from peripheral blood mononuclear cells, serum and CSF, myelin-reactive T-cells, KIR4.1 antibodies, the T-cell marker serum osteopontin and microbiome-associated lipopeptides; however, none of these are close to being validated for use in routine clinical practice [88].

In the current era of “precision medicine” there is growing interest in genome-wide association studies which have the potential to identify a plethora of genetic variants which may influence the development or disease course in MS and a variety of other neurological diseases [89, 90]. The idea that these polymorphisms may inform treatment choice and allow individualisation of appropriate treatment in the future is certainly both interesting and attractive, but has not yet been translated into practice.

Supporting Radiological Investigations
As discussed above, MRI has revolutionised the accuracy of MS diagnosis and the stage at which a diagnosis of CDMS can be made, having the capacity to exclude mimics of MS and to demonstrate subclinical lesions which have not produced neurological symptoms or signs but which nevertheless
can demonstrate dissemination in time and space and allow earlier and more robust diagnosis. Over time, in line with the clinical criteria discussed above, there have also been multiple and continuously evolving sets of radiological criteria for MS diagnosis.

The 2001 McDonald criteria discussed above were the first to formally incorporate MRI criteria and drew on the studies of Barkhof [40] and Tintore [91] which the panel felt were more sensitive and specific than those of other authors [92-94]. The radiological element of the 2001 McDonald criteria required 3 out of the following 4 features: either one Gadolinium-enhancing lesion or at least 9 T2 hyperintense lesions if there is no Gadolinium-enhancing lesion; at least 1 infratentorial lesion; at least 1 juxtacortical lesion; at least 3 periventricular lesions. They also noted that one spinal cord lesion could be substituted for one brain lesion.

Various revisions to the MRI criteria have been proposed over the years, including those of the international MAGNIMS (Magnetic Resonance Imaging in MS) collaborative which produced a set of recommendations after a workshop held in 2007 [95] which were ultimately published and used to inform the 2010 revision of the McDonald criteria [57]. This group proposed an algorithm for the confirmation of MS diagnosis after presentation with CIS, and suggested that diagnosis could be confirmed in either of the following ways: (1) serial MRI scans demonstrating dissemination in time and space; (2) single MRI scan demonstrating dissemination in time and space. Dissemination in space was redefined as the presence of at least one lesion in at least two of the following regions: periventricular, juxtacortical, infratentorial and spinal cord. Dissemination in time was defined as either a new lesion on a subsequent scan (regardless of timing of scan) or simultaneous enhancing and non-enhancing lesions, thereby demonstrating dissemination in time and space without the requirement for interval imaging.

Most recently, the MAGNIMS working group have produced an updated set of guidelines to take into account recent improvements in MRI technology and updated data [96]. This group of experts in the field has suggested that the number of periventricular lesions be increased back from 1 to 3 (reflecting the relative lack of specificity for diagnosis of demyelination over other causes of white matter lesions, such as cerebral ischaemia, in this brain region) and that in addition to lesions in infratentorial, juxtacortical and spinal cord regions, the optic nerve be included as an additional site of involvement (which most likely reflects advances in MRI imaging such that optic nerve lesions can now be demonstrated more readily). The previous stipulation of “juxtacortical” has now been replaced with “cortical/juxtacortical”, reflecting the increasing recognition that MS lesions can occur in brain cortex in a significant proportion of patients, even at the early stage of CIS [97]. The group also emphasise that no distinction needs be made between symptomatic and asymptomatic lesions, as this is of no clear diagnostic or prognostic significance. Furthermore, they discuss the possibility of
including “black holes” (which reflect old CNS damage) as a criterion for demonstrating dissemination in time on a single MRI scan, but dismiss this possibility on the basis of data which does not show this to be of additional predictive value in patients who have had a single clinical attack [98]. A possible exception is in paediatric populations, in whom it may be useful to differentiate MS from Acute Disseminated Encephalomyelitis (ADEM), a monophasic illness which can mimic MS [99]. The contribution of ultra-high field MRI scanners (i.e. 3 Tesla and 7 Tesla, in comparison to standard and widely available 1.5 Tesla MRI) was also discussed and while these scanners might be expected to provide additional information regarding the specificity of lesions to distinguish demyelinating lesions from those due to other pathologies, they are unlikely to result in earlier diagnosis at the current time and are certainly not required for routine diagnosis as per current criteria.

**Principles of Treatment**

MS treatments can be divided into symptomatic and disease-modifying treatments; the former will be discussed in detail in the following chapter. Regarding disease-modifying treatment options, recent decades have seen an explosion in the number and type of therapies available, with several further treatment options likely to become available in subsequent years. At the time of writing, there is no licensed effective therapy for PPMS or SPMS, and all disease-modifying treatments have proven use only in patients with RRMS. These will now be discussed briefly.

*Oral and Intravenous Non-Specific Immunosuppressive Therapies*

Prior to the development of MS-specific therapies, a number of non-specific oral immunosuppressants were used in treating the disease. Most of these are also used in the treatment of a wide variety of systemic and neurological autoimmune diseases. These include drugs such as azathioprine, methotrexate, mycophenolate mofetil and cyclophosphamide, all of which have been demonstrated to have at least some modest beneficial effects on inflammatory disease activity [100] but all of which carry serious potential side effects such as opportunistic infection and malignancy, particularly with long-term use or high-dose treatment. As a result they have been replaced by more efficacious and less toxic MS-specific therapies. One drug which is still used occasionally is mitoxantrone, an antineoplastic agent originally used in the treatment of acute myeloid leukaemia and prostate cancer, and has been shown to have efficacy in clinical trials in MS patients with highly active disease and rapidly progressive disability in the Mitoxantrone in Multiple Sclerosis trial [101]. It is given intravenously, typically in eight cycles administered at 3-monthly intervals. Unfortunately, despite reasonable efficacy in a population with rapidly progressive disease, it carries significant long-term risks of haematological malignancy, acute myeloid leukaemia [102] and acute or delayed cardiotoxicity with cardiac failure [103], meaning that treated patients require surveillance blood work.
and echocardiography for up to 5 years following the course of treatment. It may also produce premature ovarian failure [104].

Injectable Therapies

(i) Beta-interferon (IFN-β)

The first therapy to be licensed specifically for the treatment of RRMS was injectable IFN-β, which is an immunomodulator with effects on a variety of immune functions such as antigen presentation, T-cell proliferation and expression of cytokines and metalloproteinases. It is not known which one of these actions in particular mediates its efficacy in the treatment of MS. Injectable beta-interferon has been proven in a number of placebo-controlled randomised controlled trials to reduce relapse rate [105-107] and inflammatory disease activity as seen on MRI [108-110] and is licensed for the treatment of RRMS in many countries worldwide, including Australia. The drug has also shown efficacy in reduction of relapse rates in SPMS, but not in preventing disability progression [111] hence its license is not extended to patients with this disease subtype. IFN-β is available in a number of forms (intramuscular and subcutaneous injection) and with dosing regimens varying between fortnightly, weekly, three times a week and alternate daily administration. It is associated with a number of predictable side-effects, including flu-like symptoms after injection, headache, depression, injection site reactions and development of haematological or liver function abnormalities as well as neutralising antibodies which may result in treatment resistance [100]. However, long-term safety data have given reassuring evidence that there is no apparent increase of malignancy or other long-term safety issues, with at least 20 years of clinical use to date [112].

(ii) Glatiramer Acetate (GA)

GA is another molecule with a slightly obscure mode of action, being a compound of the amino acids glutamic acid, lysine, alanine and tyrosine. Interestingly it was originally developed in an attempt to induce the inflammatory CNS demyelinating disease, experimental autoimmune encephalomyelitis (EAE), a partial mimic of MS in rats, but instead was observed to reduce this and cerebral demyelination. It has subsequently proven to have comparable efficacy to IFN-β in head-to-head clinical trials in PwMS [111, 113] with proven reduction in both relapse rate [114] and MRI evidence of disease activity [115]. It is available in daily and three-times-weekly [116] preparations via subcutaneous injection, and has a more favourable side-effect profile, with injection site reactions being the commonest reported side effect and no increased incidence of flu-like symptoms. It does not produce the development of neutralising antibodies and, like IFN-β, has a well-established track record with over 20 years of safety data. Furthermore, it has a rating for use in pregnancy in Australia of category B1 (no increased frequency of foetal malformations or other harmful effects observed in
human or animal studies) and is therefore considered a safe therapy option for women of childbearing age or those actively trying to conceive [117].

Oral MS-Specific Therapies

(i)  **Fingolimod**

Fingolimod was the first oral therapy licensed in Australia for the treatment of RRMS and remains a popular therapy option. It exerts its action by modulation of the sphingosine-1-phosphate receptors which are present on a variety of cells including lymphocytes, CNS white matter and the cardiovascular system, resulting in a sequestration of peripheral blood lymphocytes in lymph nodes and most likely reducing lymphocyte traffic into the CNS. It has been demonstrated in large placebo-controlled trials to reduce relapse rate [118, 119] and has also been demonstrated to be superior to injectable weekly IFN-$\beta$ in this regard [120]; MRI endpoints in these trials also demonstrated a significant reduction in inflammatory disease activity [120, 121]. A number of predictable adverse effects have been identified, most notably cardiac conduction defects (with a peculiar finding of first-dose bradycardia, meaning that the first dose of drug requires to be given under medical supervision and the drug should be avoided in patients with pre-existing cardiac conduction defects), macular oedema (meaning that surveillance ophthalmological examination is recommended before and after drug commencement to prevent the development of visual disturbance), deranged liver function tests and lymphopenia. There is also an increased rate of infections seen with fingolimod treatment, most notably herpetic infection. Following two deaths in the TRANSFORMS study due to invasive zoster infection and herpes encephalitis in patients treated with the higher dose therapy of 1.25mg, it is mandated in Australia that all patients who are not immune to varicella zoster virus be vaccinated prior to commencement of drug as a precautionary measure. Furthermore, a small number of cases of the serious brain infection Progressive Multifocal Leucoencephalopathy (PML) have now been reported in fingolimod-treated patients [122], although this is thought to be much rarer as a complication of fingolimod therapy compared to natalizumab treatment. Evidence to date has not suggested an increased risk of malignancy in association with fingolimod therapy, however the drug is in its relative infancy and long-term safety data is lacking. Fingolimod has a pregnancy category in Australia of C, meaning that although there is no clear evidence of foetal malformations in humans, there is some evidence from animal studies to suggest at least a theoretical increased risk and use in women who are trying to conceive is not recommended [123].

(ii)  **Dimethyl Fumarate (DMF)**
Dimethyl Fumarate is another drug whose precise mode of action remains unknown, but is believed to involve the Nuclear Factor-Like2 (NRF-2) pathway which is involved in cellular oxidative stress, as well as producing a modest degree of lymphopenia. A closely related compound has been used historically in the treatment of psoriasis, and DMF has been licensed for the treatment of RRMS in Australia since 2013. It has been demonstrated in two large randomised controlled trials to have beneficial effects both in terms of relapse rate reduction and MRI evidence of disease activity [124, 125], with an apparently reasonable safety profile. Unfortunately, recent years have seen the reports of a small handful of PML cases [126, 127], notably in patients who have developed a profound lymphopenia while on treatment with the drug. Predictable side effects include gastrointestinal upset and flushing, both of which are more prevalent in the first few months after initiation of the treatment and both of which tend to settle over time in most cases. Like GA, it has a category B1 rating in pregnancy, although only small volumes of registry data are currently available regarding real-world experience in pregnant women [128].

(iii) Teriflunomide

Teriflunomide is an inhibitor of pyrimidine synthesis in circulating lymphocytes and is a close relative of leflunomide, which has been used historically in the treatment of rheumatoid arthritis. It has been demonstrated to reduce relapse rate and inflammatory activity on MRI [129] with comparable efficacy to the injectable immunomodulators. Common side-effects include hair thinning, deranged liver function and gastrointestinal upset, as well as (rarely) peripheral neuropathy. The drug is listed as category X in terms of its pregnancy rating in Australia, signifying that it should not be prescribed to women of childbearing potential or indeed men who are planning to father a child. On discontinuation (or unintended pregnancy) it is necessary to perform a drug washout with cholestyramine to avoid any potential long-term adverse effects of the drug. Although the long-term safety profile remains unknown, long-term use of the closely related leflunomide is not thought to increase the risk of any particular complications such as malignancy or opportunistic infection.

Intravenous MS-Specific Therapies

(i) Natalizumab

Natalizumab is a humanised monoclonal antibody against the α-4 integrin receptor present on lymphocytes which is licensed for the treatment of RRMS. Its mode of action is thought to involve prevention of migration of peripheral lymphocytes across the blood-brain barrier into the CNS and it is administered by intravenous infusion on a monthly basis. It is a highly effective therapy for RRMS, demonstrating a consistent and impressive reduction in relapse rate, sustained disability progression
and MRI evidence of inflammatory disease activity [130]. Although generally well-tolerated, it carries a risk of infusion reactions in approximately 4% of treated patients (serious in around 1%), and particularly with longer durations of use (>2 years) there is an increasing recognition of the risk of PML in patients who test positive for the JC virus. Because of the high efficacy of the drug, great efforts have been made in the field of PML risk mitigation, and it is now known that the risk of developing this condition is substantially elevated (up to 1%) in patients who are so-called “triple-positive”, i.e. JCV antibody index titre >1.5, duration of therapy in excess of 2 years and use of prior immunosuppressants [131]. The risk of PML is extremely low in patients who test JCV antibody negative, although it is possible for patients to display seroconversion. Therefore, repeated testing is required for patients on long-term treatment, formal vigilance programmes with regular MRI imaging should be used, and screening for early symptoms of PML is recommended in at-risk patients[132]. Finally, it is possible for Natalizumab-treated patients to develop neutralising antibodies, which can be transient or persistent and, if the latter, can be associated with reduced drug efficacy. Analysis of the pivotal clinical trial data suggests this occurs with a frequency of up to 9% of treated patients [133].

(ii) Alemtuzumab

The newest MS therapy in Australia at the time of writing, Alemtuzumab was first licensed for the treatment of RRMS in 2015 and, like Natalizumab, is an effective therapy used in patients with highly active disease. The drug is a monoclonal antibody against the CD52 antigen which is present on the surface of lymphocytes, and has been used historically in the treatment of chronic lymphocytic leukaemia and certain subtypes of lymphoma. It has been demonstrated in clinical trials using IFN-β as an active comparator to have high efficacy in both treatment-naïve patients with active disease and patients who have shown ongoing evidence of disease activity despite other treatments [134, 135]. It is administered by intravenous infusion for five consecutive days with a further treatment given for three consecutive days 1 year later. This can produce lasting clinical remission for many years in some patients. Like all powerful therapies, it has potentially serious side-effects, which are generally related to the development of other autoimmune diseases, most notably autoimmune thyroid dysfunction, idiopathic thrombocytopenic purpura and Goodpasture’s Disease of the kidney. As these complications can be early or delayed, surveillance blood and urine testing is mandated for all patients for 5 years after their most recent treatment. To date, there have been no cases of PML in alemtuzumab-treated MS patients, although there have been historical case reports of PML in patients treated with this drug for haematological malignancy [136]. The drug is not thought to be teratogenic but it is recommended that women avoid pregnancy for 4 months following their last treatment, and it carries a pregnancy category rating of C, meaning that data are too limited for a risk to reliably be excluded.
Pipeline Therapies

At the time of writing, a number of other agents have shown efficacy for the treatment of RRMS but have not yet been licensed for administration in Australia. These include the monoclonal antibodies daclizumab (anti-CD25) and ocrelizumab (anti-CD20). The latter has also been shown to have at least some impact in reducing disability progression in patients with PPMS, as outlined above [74]. Other oral agents in development include laquinimod and cladribine, with substantial further advances likely to occur in the next decade.

Autologous Haematopoietic Stem Cell Transplantation (AHSCT)

Recent years have seen tremendous interest from patients and clinicians in the use of AHSCT for the treatment of aggressive RRMS. To date, the evidence in favour of this therapy has predominantly been derived from case series and observational trials, with no randomised placebo-controlled studies in existence. The available data does suggest high efficacy in patients with active inflammatory MS in chronic progressive disease [137, 138]. Therapy regimes vary according to local protocols, but a typical treatment regimen would comprise a course of myeloablative high-dose chemotherapy followed by reinfusion of previously harvested haematopoetic stem cells to permit reconstitution of the immune system. Published evidence suggests that this treatment can produce prolonged remission in patients who have active inflammatory disease and who are otherwise fit enough to withstand the treatment, with treatment-related mortality (predominantly from sepsis) in the present day being estimated at 1-2% [139]. The relative efficacy of AHSCT as compared to the infusion therapies, Natalizumab and Alemtuzumab, is unknown and no head-to-head trials have been conducted to date. Effects on other parameters of disease progression such as brain atrophy are uncertain, but the available evidence suggests that, while there may be an initial acceleration in brain atrophy, probably as a result of treatment-related toxicities, there is eventual normalisation of atrophy to the rate associated with physiological ageing [140, 141].

In summary, recent decades have seen tremendous advances in the field of MS, particularly in the area of imaging, diagnosis and disease-modifying therapy for relapsing forms of the disease. While these have undoubtedly contributed to improvements in patient care for many, there are still great lacunae in existing knowledge regarding pathogenesis (particularly for progressive MS) and great limitations upon the options for symptomatic therapies. These will be discussed in the next chapter.
Chapter 3: Symptoms and Symptomatic Treatments in Multiple Sclerosis

In this chapter, the current literature regarding existing symptomatic therapies for patients with MS will be reviewed.

“A little is a lot where there is little else.”[142]

- Motor dysfunction: spasticity, ataxia, tremor, gait disturbance, upper limb impairment
- Somatosensory dysfunction: sensory loss, pain
- Visual disturbance
- Fatigue
- Cognitive Dysfunction
- Mood Disturbance
- Bowel/bladder and sexual dysfunction

Motor Dysfunction in MS

Motor dysfunction is a common symptom in MS, occurring in up to 90% of patients at some stage in the disease course [143]. This can take a variety of forms including spasticity, weakness, ataxia and tremor, all of which can contribute to gait disturbance, upper limb impairment and disability. A variety of symptomatic therapies have been evaluated for the treatment of aspects of motor dysfunction and these will now be discussed.

Symptomatic Treatment for Spasticity

Spasticity is defined as a velocity-dependent increase in tonic muscle stretch reflexes [144, 145] and results from damage to the descending inhibitory neurones carried in the corticospinal tracts in the white matter of the brain, brainstem or spinal cord. It is associated with evidence on neurological examination of increased tone, clonus and hyperreflexia and, if severe, can result in pain, loss of dexterity and muscle contractures. Spasticity may be generalised or restricted to one limb or region, and may not always be detrimental to function. In some patients, a degree of spasticity may mask underlying muscle weakness and in fact treatments which reduce spasticity may result in an overall detriment in motor function. Therefore, evaluation of spasticity should take into account the overall physical functioning of the patient, and should be directed at improving function rather than normalising examination findings. Drug treatment of spasticity is most effective when combined with targeted physical therapies.
**Drug Therapies**

Most drugs for the treatment of spasticity are prescribed on a trial-and-error basis and there is a paucity of evidence from randomised trials supporting any particular therapy. Furthermore, objective study of spasticity is confounded by the lack of an accepted definition for research [146] and the lack of a standardised means of assessment. The most widely used measure currently is the Ashworth scale [147], a 5-point scale based on the examiner’s judgement of the degree of spasticity present. This does not include any assessment of associated symptoms or effects on quality of life, despite the stated importance of these features in determining whether drug treatment of spasticity is appropriate or effective.

A wide variety of drug types with vastly different modes of action are currently used in the treatment of spasticity and these have been summarised in a Cochrane Review [148]. This comprised 26 placebo-controlled trials and 13 comparative studies using ten different agents, with a variety of outcomes including routine clinical tests, scores on the Ashworth scale, instrumental measures of stiffness and electrophysiological measures (such as electromyography (EMG) activity and H-reflex latency). The drugs included baclofen, dantrolene, tizanidine, benzodiazepines, botulinum toxin and cannabinoids. Positive results were reported for individual placebo-controlled trials of oral baclofen and intramuscular botulinum toxin, but no consistent outcome measures were used and numbers were too small to permit robust conclusions to be drawn or any meaningful comparison between agents to be made. More recently, interest has developed in the synthetic cannabinoids as agents for the treatment of spasticity and nabiximols (*Sativex*), an oromucosal spray containing cannabidiol and Δ9-tetrahydrocannabinol, has received approval for the treatment of MS-related spasticity in a number of countries worldwide [149] but is not available in Australia at the time of writing.

Baclofen is the drug most commonly used in the treatment of spasticity in clinical practice, despite the lack of supporting trial evidence and despite a variety of potential side-effects which can include worsening fatigue, somnolence and a reduction in the seizure threshold. For patients whose spasticity is not controlled with oral medications, or with particularly severe lower limb spasticity, continuous intrathecal baclofen via a pump delivery system is sometimes used, although this has attendant risks related to pump placement including infection in the central nervous system. In bedbound patients with severe spasticity, chemical or surgical rhizotomy offers a longer-lasting alternative to intrathecal baclofen. Intrathecal steroid administration has also been reported to be effective in non-randomised case series [150, 151].

**Non-pharmacological Therapies**
A variety of non-pharmacological therapies have been evaluated either alone or in conjunction with drug therapies, including physical therapies, transcranial magnetic stimulation (TMS) and electromagnetic therapies. The rationale for potential benefit from TMS comes from its ability to modulate excitability of descending corticospinal inputs. There is some evidence that it can produce clinical changes in this group of patients; a single study using the modified Ashworth Scale as the primary clinical outcome and evaluating patients using a placebo-controlled paradigm with sham TMS [152] and suggested modest clinical benefits. However, on the whole, studies of non-pharmacological therapies for spasticity have been beset with similar methodological issues to the drug studies [153] and the Cochrane Review on this subject was able to reach no firm conclusions as to their absolute or relative efficacy.

**Symptomatic Treatment for Ataxia and Tremor**

Ataxia in MS, defined as an impairment in co-ordination of movement, can have a variety of origins, the most common being cerebellar dysfunction which produces a variable combination of ataxia, dysmetria (difficulty with measuring movements or estimating distances), dysarthria (difficulty with the motor aspects of articulation, producing slurred speech) and dysdiadochokinesis (difficulty with rapidly alternating movements). Cerebellar ataxia is frequently associated with a tremor described as intention tremor which can further interfere with motor planning and voluntary movements. Ataxia can also result from impaired sensory function and proprioceptive loss, usually associated with disease in the dorsal columns of the spinal cord. It would be expected that ataxia caused by these different mechanisms might respond to different symptomatic treatments. A variety of agents are used clinically in the treatment of tremor, including benzodiazepines, primidone and gabapentin, but supporting clinical trial evidence for their use is lacking.

A recent Cochrane review [154] identified a total of ten randomised controlled trials including a total of 172 patients which examined symptomatic therapies for tremor and ataxia (presumably this was mostly cerebellar ataxia, though it is not clearly specified). Six of these were placebo-controlled trials of drug therapies (isoniazid, pyridoxine, cannabinoids and baclofen) and four were comparative non-drug studies involving neurosurgery for severe tremor (stereotactic thalamotomy vs deep brain stimulation) or rehabilitation interventions for gait ataxia. Once again, all studies were beset by methodological difficulties and lack of comparability, and no firm conclusions were reached by the review. However, the pharmacological data were largely negative with no convincing benefits associated with any of the study drugs [155-157]. For neurosurgical intervention, impressive results were achieved in tremor reduction immediately after surgery but unfortunately these were not maintained at 6-month follow-up and general disability scores did not improve over the study period [158].
Symptomatic Treatment of Gait Disturbance

Gait disturbance is common in MS, being estimated to affect up to 80% of patients at some stage in the disease [159]. It can be multifactorial in origin, arising as a result of weakness, tremor, ataxia, spasticity or other factors. It is an important determinant of MS-related disability [11] and treatment has been tried with a number of symptomatic therapies. In contrast to most others there is a current treatment which is supported by clinical trial evidence; modified-release fampridine (4-aminopyridine). This has been licensed in recent years in several countries including Australia for the symptomatic therapy of walking difficulty in MS. Fampridine is a potassium channel blocker which is thought to improve conduction along demyelinated axons, and has been evaluated in this context in two large double-blind placebo-controlled randomised trials including a total of 541 patients [160, 161]. Both of these studies used performance on the timed 25-foot walk test as the primary clinical endpoint, and defined clinical responders as patients who had a consistently faster walking speed on at least 3 out of 4 on-drug assessments, compared with the maximum speed from any of five off-drug assessments (the magnitude of the change in speed was not specified). They reported a responder rate of 35% (vs 8% in placebo group [160]) and 42.9% (vs 9.3% in placebo group [161]) respectively in fampridine-treated patients. It was not clear from these data exactly which of the multifactorial aspects of gait (e.g. leg strength, stamina, co-ordination etc.) were being specifically improved. Both studies reported a significant improvement in lower extremity manual muscle strength testing in fampridine responders, suggesting at least some of the improvement was due to improved muscle strength, but other parameters such as co-ordination and sensory function were not formally evaluated. Furthermore, the clinical and laboratory features which differentiated responders from non-responders were not clearly identified. The data surrounding fampridine and its potential uses will be further discussed in chapter 6.

Non-pharmacological Therapies for Gait Disturbance

Functional electrical stimulation of the common peroneal nerve via TMS over the motor cortex has been evaluated in the treatment of gait disturbance related to foot drop. Everaert et al [162] evaluated this rehabilitation intervention in 36 patients with neurological disorders (24 had MS) and foot drop and compared clinical and electrophysiological findings before and after several months’ use of the functional electrical stimulator. Patients were further classified as having progressive neurological disorders (including MS) or non-progressive (e.g. stroke). Following use of the stimulator, they reported increased MEP amplitudes and increased maximum voluntary contraction forces in both groups, but to a greater extent in the non-progressive disorders group. Walking speed increased by
24% in the “non-progressive” group and 7% in the “progressive” (including MS) group. The authors concluded that these improvements were most likely due to improvements in corticospinal connections. However, the study was unblinded and uncontrolled and the extent of placebo effect or spontaneous improvement was therefore unclear. Subsequent research has attempted to further evaluate this technology, with no conclusive evidence from randomised trials of benefit to date [163-165]. Other physical therapy interventions are discussed in detail below (see “Rehabilitation and Physical Therapies”).

**Symptomatic Treatment of Upper Limb Dysfunction**

Upper limb dysfunction may be almost as common as gait disturbance, affecting an estimated 75% of MS patients and producing loss of hand dexterity with attendant functional limitations [166]. However, despite the high prevalence and associated disability, limited evidence exists regarding the use of drug therapies in this context. Following on from the success of fampridine in gait disturbance, this drug has been evaluated for its effects on upper limb function, but the majority of studies published to date which have suggested any kind of benefit have been uncontrolled [167-170]. One small double-blinded study showed no effect on upper limb function [171]. These data will be discussed in more detail in chapter 6. Only a small body of literature exists evaluating physiotherapy-based motor retraining programs [172] or robotic technology [173] in this group of patients, and none of these were of sufficient methodological quality to adequately answer the research question.

**Conclusion**

In summary, disturbances of motor function are extremely common in MS patients and can include tremor, ataxia and spasticity, all of which can contribute to gait disturbance and disability, as well as upper limb dysfunction. Several drug treatments are used clinically in the treatment of spasticity despite an absence of robust supporting evidence from clinical trials. Oral baclofen is most commonly used in clinical practice, but has efficacy limitations and side-effects. Any pharmacological therapy for spasticity should be combined with physiotherapy, and the decision to treat spasticity with pharmacological means should weigh up the detrimental effects of spasticity symptoms (including pain and contractures) against the potential detrimental effects on overall physical function. Tremor is difficult to treat and symptomatic therapies are largely modest in terms of efficacy [174]. Some patients with gait disturbance respond to fampridine but its mode of action and the reason for differential response rates between patients is unclear, and further research is required.
Rehabilitation and Physical Therapies

Optimal management of patients with MS-related motor dysfunction of all types typically involves a multidisciplinary approach with rehabilitation therapy being used alongside medications in an attempt to reduce disability. The varied nature of MS symptoms means that rehabilitation interventions must also be varied and tailored to the needs of individual patients. Rehabilitation can be delivered in various settings, including inpatient units, outpatient facilities and home-based programmes, and can be delivered by a variety of practitioners including physiotherapists, occupational therapists, speech therapists, medical practitioners and nurses, with multidisciplinary rehabilitation interventions (that is, involving practitioners from more than one discipline) being the optimal option for most patients. Some attempts have been made at formal evaluation of specific rehabilitation interventions in this population, although these have been beset by difficulties relating to heterogeneity of treatment variables and outcome measures.

The American Academy of Neurology assembled an expert panel in order to systematically evaluate the enormous body of published literature pertaining to this subject and performed a meta-analysis including 142 well-conducted controlled trials of physical rehabilitation interventions which had definable objective outcome measures and which were not trials of drug therapy or electrical stimulation. Despite the large pool of published literature, the majority of the included studies were beset by methodological flaws, and there was very little high-quality evidence in favour of any of the interventions examined, with the exception of 8 weeks of weekly home-based or outpatient physiotherapy which was shown to improve gait but not upper limb function[175].

More recently, Khan et al [176] published a meta-analysis of 39 systematic reviews examining the subject of rehabilitation in MS, assessing the published literature for methodological quality and performing a grading of evidence. This group concluded that high-quality evidence was lacking for most rehabilitation interventions in MS, but also agreed that there was high-quality evidence supporting the use of both physical therapy (with the aim of improving both functional outcomes such as muscle strength, aerobic capacity and mobility level as well as fatigue and quality of life), and comprehensive fatigue management programs for patient-reported fatigue. Evidence in favour of physical therapy for improving the specific outcomes of balance was judged to be low-quality, and the same was true for a multitude of other interventions such as hyperbaric oxygen therapy, occupational therapy and upper limb rehabilitation programs, telerehabilitation and programs directed specifically at managing MS-related spasticity. Evidence for a number of further interventions (including hippotherapy and dietary modifications, particularly vitamin D supplementation) was judged to be inconclusive [175].
In summary, although there is evidence suggesting benefit from physical therapy in particular and various other forms of rehabilitation in MS patients, the published literature is beset by methodological issues including heterogeneity of outcome measures and lack of comparability between specific interventions or between interventions and drugs. It is common practice to offer physical therapies alongside pharmacological interventions where possible.

**Somatosensory Impairment and Pain in MS**

The EDSS is heavily weighted towards motor function and ambulation in particular, and therefore these symptoms are given particular importance in the evaluation of new therapies for MS. In fact, sensory symptoms are at least as common and often surprisingly disabling. The presence of pain and altered sensation as a feature of MS was first described by Charcot in 1872 [177] and these symptoms are estimated to affect around 85% of patients at some stage in the course of disease [143]. Somatosensory dysfunction in MS can take various forms, including paraesthesiae, numbness, hyperalgesia, allodynia, pain, sensory ataxia and Lhermitte’s phenomenon, the shock-like sensation experienced on neck flexion which is almost pathognomonic of cervical spinal demyelination.

By their nature, sensory symptoms are harder to evaluate objectively and as a result have been much less extensively studied than motor or visual dysfunction. Only a small body of literature exists on the subject. Rae-Grant and colleagues [178] conducted a study of 224 patients with MS and 93 age- and sex-matched controls, and reported a prevalence of 43% for sensory symptoms as the presenting feature of MS, with 34% of patients rating sensory disturbance as the “worst” MS symptom. Furthermore, there was a significant correlation between the number of sensory symptoms and the degree of reported disability (although the measure of disability used was not reported).

Beiske [179] studied a cohort of 142 patients with MS and reported a prevalence of 73.9% for pain and/or unpleasant sensory disturbance, which frequently interfered with level of function. Sensory disturbance affected patients widely regardless of demographics, disease course or level of disability, and only a small minority of patients were on treatment with analgesics (15.1%), antidepressants (3.2%), anticonvulsants (9.7%) or anti-spasticity agents (30.1%), suggesting that under-recognition of somatosensory symptoms is accompanied by lack of adequate treatment.

Pain is estimated to affect around 65% of PwMS at some stage in their disease course and may be acute or chronic [180]. Causes vary from acute inflammation in RRMS (both ON and TM can produce pain early in the disease course) to neuropathic pain and dysesthesiae (typically from spinal cord or thalamic damage) to pain associated with spasticity and increased muscle tone. Pain may be paroxysmal or persistent. Paroxysmal pain and other phenomena such as tonic spasms typically
respond well to anticonvulsant therapy (e.g. carbamazepine, phenytoin) whereas neuropathic pain typically requires treatment with pain-modulating medications such as gabapentin, pregabalin, amitryptiline and duloxetine. Severe or multifactorial pain may require multidisciplinary management including psychological therapy and sometimes opioids in addition to the above measures [174].

Despite a paucity of randomised trial evidence, a number of different pharmacological agents are routinely used to ameliorate painful dysaesthetic somatosensory symptoms in MS, and these will now be reviewed.

**Tricyclic Antidepressants**

Drugs of the tricyclic antidepressant class, including amitryptiline, nortryptiline and duloxetine, have been used for many years in the treatment of neuropathic pain in a variety of neurological conditions including MS. These are typically used at doses much lower than in the treatment of depression. Potential side-effects include sedation, dry mouth, blurred vision, urinary retention and constipation. Despite their ubiquity, there have been no randomised controlled trials in the MS population which support their use or favour a particular dosing regimen [181].

**Anticonvulsants**

Almost all of the available anticonvulsants have been employed in the treatment of MS-related neuropathic pain, and although there are some clinical trials supporting their use, these have generally been small, unblinded or uncontrolled which renders them highly liable to placebo effects. These included an open-label trial of gabapentin in 25 patients with central neuropathic pain in which 15 reported benefit and 11 withdrew due to adverse effects [182], and an open-label trial of pregabalin in which 9 of 16 patients reported improvement and 3 withdrew due to adverse effects [183]. Despite the lack of supporting evidence from larger or better-conducted studies, both drugs are routinely used in clinical practice. Other drugs which are also used include lamotrigine, phenytoin, levetiracetam, carbamazepine [181] and topiramate [184] but supporting evidence is lacking in all cases. Although on the whole this group of drugs appears to be well-tolerated, potential side-effects include sedation, deranged liver function (with phenytoin in particular), interactions with other drugs and cognitive slowing (with topiramate).

**Cannabinoids**

Perhaps the best-evaluated and the least widely-used therapies are the cannabinoids, which have at least been subjected to more rigorous randomised blinded trials of their efficacy. A placebo-controlled double-blind trial of Δ⁹-THC, a cannabis extract, in 66 patients with MS and central pain syndromes reported improved outcomes in terms of reduced pain intensity; however, unacceptable
cognitive side-effects and effects on long-term memory have limited the clinical utility of the drug [185]. A much larger study in 630 patients was designed to investigate the efficacy of cannabinoids in the treatment of spasticity, but used pain as a secondary outcome, and the researchers reported a net benefit overall as measured by a visual analogue scale, although in around 20% of cases pain worsened while on treatment [186]. It is not clear whether there might be a sub-group of responders to cannabinoids which has yet to be identified.

Conclusion

In summary, despite the high prevalence of somatosensory impairment and sensory symptoms in PwMS, this area has been relatively poorly studied and the existing literature is small. Cannabinoids have the most robust evidence but their use is limited predominantly by cognitive side-effects and legislation surrounding access. Unfortunately there are no effective therapies for sensory loss or numbness (as distinct from pain) in MS patients. Furthermore, there are some ethical barriers to placebo-controlled trials of therapies for pain, which may explain the paucity of objective research in this area.

Visual Disturbance in MS

Optic neuritis (ON) is a common symptom in PwMS, accounting for 15-20% of initial presentations and occurring clinically in up to 50% of patients at some point in the disease [187]. Although ON-associated visual deficits often recover well, persistent visual complaints have been reported to be present in around 1/3 of all PwMS, more often in patients with PPMS and those with previous clinical episodes of ON [188]. Despite the high prevalence of visual impairment in multiple sclerosis, visual functions are seldom formally evaluated as clinical trial outcomes and almost no symptomatic therapies have been developed. The visual component of the EDSS score (based largely on visual acuity) makes up a tiny component of the overall score. It is weighted towards severe visual field defects (which are not particularly common among PwMS) and may underestimate the functional impact of the more subtle problems that typically occur after one ON attack but can still have a significant impact on quality of life, including the ability to drive and work.

In the acute setting, visual disturbance related to ON can be treated with corticosteroids, with supporting evidence of a reduction in duration of symptoms (but no evidence of improved overall recovery) coming from the Optic Neuritis Treatment Trial [189], which recently published its 15-year follow-up data. At this time point, the prevalence of visual impairment in the originally affected eye was 35-38% (depending on the measure used) and this correlated with worse quality of life scores for patients with persisting visual impairment. A number of other drugs have been evaluated in the context of preventing or reducing long-term visual loss when given in the acute stages of ON, including memantine, phenytoin and a variety of disease-modifying therapies.
Memantine

The NMDA-receptor antagonist memantine was evaluated for its ability to prevent axonal loss when used for 2 weeks early after onset of ON symptoms, following on from evidence from in vivo animal studies using the MS rat model of experimental autoimmune encephalomyelitis (EAE). Sixty patients were randomised to memantine or placebo and although treatment was associated with a reduction in Retinal Nerve Fibre Layer (RNFL) loss, this was not associated with any improvement in outcome as measured by visual fields, visual acuity or VEP p100 latency [190].

Phenytoin

More recently, there has been growing interest in the potential use of the anticonvulsant phenytoin as a neuroprotectant therapy given in the acute stage of ON. A large phase 2 study in patients with acute ON (excluding those with a likely diagnosis of NMO as evidenced by positive Aquaporin-4 antibodies) suggested a beneficial effect of the drug in preserving RNFL thickness at 6 months when given in the acute setting, but was not powered to assess outcomes in terms of longer-term visual function [191].

Disease-Modifying Therapies

Recent years have seen the inclusion of visual function as a secondary outcome measure in the evaluation of some disease-modifying therapies, including the AFFIRM trial of Natalizumab versus placebo in RRMS. This reported both a non-significant trend towards improved high-contrast acuity and a significant improvement in low-contrast acuity in the treatment group [192]. Similar but less marked differences between groups were reported in the SENTINEL trial which compared Natalizumab plus interferon-beta 1a to interferon-beta 1a alone [193]. A sub-analysis within the randomised trial of alemtuzumab versus interferon-beta 1a reported an improvement in contrast sensitivity in both groups but to a significantly greater extent in the alemtuzumab group [194]. However, these effects presumably reflect the preventative and anti-inflammatory effects of the drugs in question rather than symptomatic benefit.

4-aminopyridine and Modified-Release Fampridine (Fampridine-MR)

The only drugs which have been systematically evaluated in the context of symptomatic therapy for patients left with residual visual dysfunction following an attack of ON are the aminopyridines. 4-aminopyridine, a lipophilic older and shorter-acting version of fampridine-MR has been evaluated in the treatment of MS-related visual dysfunction. The investigators from a small and non-randomised study looking at various MS symptoms reported a beneficial effect on visual function and a decrease in VEP latency in 11 out of 13 subjects treated [195]. A double-blind, randomised placebo-controlled trial of 22 patients who were evaluated with low-contrast visual acuity, VEP and measurement of
RNFL thickness using Optical Coherence Tomography (OCT) [196] showed a non-significant trend towards improved visual acuity in the fampridine group and a significant between-group difference in P100 latency (1.57ms longer in the placebo group, p=0.004), particularly seen in patients with a greater degree of preserved RNFL thickness. The authors hypothesised that in patients with reduced RNFL thickness, the damage was too severe to be ameliorated by therapy. However, the magnitude of latency change reported is unlikely to be clinically meaningful, and the small numbers in the study and high dropout rate means that further research is required before use of the drug for visual symptoms in practice could be recommended. It may be the case that, as for ambulation, fampridine-MR could be of benefit in a subset of patients with MS-related visual impairment using RNFL thickness to define a subset more likely to respond. These data are discussed further in chapter 6.

Non-pharmacological therapies

There are few data regarding non-drug symptomatic therapies for vision, but a trial is currently in progress to evaluate a computer-based vision retraining program involving daily light-based therapy in patients with visual impairment secondary to optic neuritis; data are pending [197].

Conclusion

Visual symptoms are common in MS, may be under-recognised by clinicians and form a tiny component of the EDSS score, despite their functional consequences. Detection of MS-related visual impairment requires measures other than standard high-contrast acuity testing, and valuable pathophysiological information can be obtained by combining clinical data with VEP and OCT findings. Data regarding neuroprotectant therapy in acute optic neuritis shows some promise but have not yet been translated into clinical practice[191]. There are no currently available effective symptomatic therapies for MS-related visual dysfunction and while fampridine-MR may have some effect on electrophysiological measurements of optic nerve function, this has not so far been shown to be clinically meaningful.

Fatigue in MS

fatigue [n]: weariness from bodily or mental exertion [198]

The dictionary definition of fatigue is familiar, but no universally accepted definition of the term exists within the medical literature [199]. Fatigue is perhaps the most common single symptom in PwMS, with an estimated prevalence of up to 90% [200, 201] and up to 40% of patients cite it as their most troublesome symptom [202]. Previous work in the domain of sleep apnoea has identified a wide discrepancy in the definitions and use of terms such as fatigue, sleepiness and lack of energy among
patients and physicians [203], creating problems with the objective study of fatigue as a symptom and its response to potential treatments.

Several pathophysiological mechanisms have been proposed to account for MS-related fatigue. It has been suggested that the inflammatory activity itself produces a sensation of fatigue [204]. In support of this hypothesis is the fact that fatigue can be a very early or even presenting symptom of MS, and, like other MS symptoms, can be exacerbated by ambient high temperatures [205]. Fatigue can also vary, typically peaking during clinical relapses [206]. However, fatigue is not confined to patients with relapsing and remitting disease, and is equally (if not more) prevalent in patients with primary or secondary progressive disease, possibly implicating axonal loss or damage to specific brain regions rather than intensity of inflammation in the generation of fatigue [207, 208]. Furthermore, fatigue may be a result of poor sleep hygiene, compounded by comorbid symptoms such as depression, pain or spasticity. Sleep disorders such as Obstructive Sleep Apnoea or Restless Legs Syndrome may coexist with MS and a formal sleep study may be required to accurately identify these issues in selected patients [209].

A small number of symptomatic therapies have been used to treat fatigue in MS patients and the supporting evidence will now be reviewed.

Amantadine

Amantadine is probably the best-studied and most accepted therapy for treatment of fatigue in MS. Originally developed as an antiviral agent for the treatment of influenza, this drug also affects dopaminergic neurotransmission and is still used in the treatment of Parkinson’s Disease. A number of small randomised controlled trials (RCT’s) [210-213] and a Cochrane Library Systematic Review [214] have evaluated its efficacy in the treatment of MS-related fatigue. Five RCT’s including a total of 272 patients with MS and fatigue were included in the Cochrane Review of 2009. Comparison of these was beset by wide variation in outcome measures, ranging from “the preferred treatment period by patient and physician” [211, 212] to more specific fatigue scales such as Fatigue Severity Scale (FSS) and MS-specific Fatigue Scale (MS-FS). Overall the methodological quality of included studies was judged to be poor, largely due to paucity of information regarding methods and high risk of bias. Any benefits reported from individual trials were small and no definite conclusion regarding efficacy could be reached. However, the ready accessibility, cheap price and good tolerability of the drug mean that it continues to be used in clinical practice despite the lack of supporting evidence.

Modafinil

This non-amphetamine central nervous system stimulant is widely used to treat fatigue and excessive daytime somnolence associated with narcolepsy and other sleep disorders. Its precise mechanism of
action is unknown, and it has been shown to affect several neurotransmitters including dopamine, histamine, hypocretin, adrenaline and possibly glutamate or GABA [215]. Its mechanism of action is distinct from other stimulants in that it promotes normal cortical activity in the frontal lobe and thus encourages normal wakefulness, rather than acting as a true stimulant. Thus it is thought to have a lower potential for abuse than other symptomatic therapies.

A small number of prospective, blinded, randomised controlled trials (RCT’s) have evaluated the symptomatic benefit of modafinil in the treatment of MS-related fatigue, with mixed results. Stankoff [216] randomised 115 patients with MS and fatigue to variable doses of modafinil or placebo and measured outcomes as improvement by 15 points or more in modified Fatigue Impact Scale (mFIS) score after 35 days of treatment. No significant difference was found between patients treated with drug and placebo in either the overall mFIS score or the subscores for physical and cognitive fatigue symptoms. Lange [217] randomised 21 patients with MS and fatigue to modafinil or placebo and measured the outcome as Fatigue Severity Scale score as well as the d2 alertness test, the 9-hole peg test (9HPT) and cortical excitability as measured by TMS. After 8 weeks of treatment, statistically significant improvements were seen in the modafinil group on a variety of parameters, including FSS score, 9HPT time, d2 alertness score and motor evoked potential (MEP) amplitude, suggesting an increase in cortical excitability during drug treatment. It is unclear whether this electrophysiological change is due to direct brain effects of modafinil on specific cerebral neurotransmitters or whether these findings are due to a more general effect on wakefulness, which is in turn known to modulate cortical excitability. However, in absolute terms the clinical improvements were modest (10 point decrease in FSS and 6% improvement in time for 9HPT, well below the threshold for minimal clinically important difference for 9HPT [218] and probably also below the threshold for clinically important difference for FSS (which is undetermined in PwMS, but in patients with rheumatoid arthritis has been estimated to be around 20 points [219]).

**Pemoline**

This dopaminergic CNS stimulant was originally used in the treatment of attention deficit hyperactivity disorder and narcolepsy. Krupp [210] randomised 119 patients with MS and severe fatigue (rated as per the Fatigue Severity Scale) to treatment with amantadine (100mg bd), pemoline (titrated up to 56.25mg daily) or placebo for six weeks. Although all groups improved over the course of the study, only amantadine showed superiority to placebo. Pemoline has since been withdrawn from the commercial market due to its hepatotoxicity.

**4-aminopyridine**

Rossini [220] conducted a 12-month placebo-controlled crossover trial during which 49 patients completed the 1-year follow-up, and no significant difference was identified between groups, either
on the clinical outcome or various electrophysiological measures of CNS conduction (VEP, MEP, SSEP). Subgroup analysis based on serum levels of 4-aminopyridine identified a significant effect of 4-aminopyridine on fatigue in the “high” level group, but this produced only a modest difference of 2 points on the FSS, which is unlikely to be of clinical significance.

L-carnitine

This amino acid compound is important in the generation of mitochondrial energy and has been used as a symptomatic treatment for fatigue in Chronic Fatigue Syndrome and fibromyalgia. As well as its role in mitochondrial energy production, it may also have functions as an antioxidant or anti-inflammatory, and studies have shown reduced serum levels of carnitine in fatigued patients with MS on immunosuppressive therapies relative to untreated patients and healthy controls [221]. The effects of L-carnitine supplementation have also been evaluated by a Cochrane Review [222], which identified only one completed RCT. This was a crossover study comparing L-carnitine to amantadine and reported similar efficacy, but given the lack of placebo arm and lack of robust evidence regarding the efficacy of the latter treatment, this was not judged to be a clinically significant result. More recently, a double-blind RCT with crossover design showed no effect of L-carnitine in comparison to placebo [223].

Aspirin

Aspirin has also been evaluated as a symptomatic therapy for fatigue, following on from largely anecdotal evidence of benefit in individual patients using the drug for other purposes. A small randomised controlled crossover trial [224] showed a modest but statistically significant difference in mFIS scores (absolute between-group difference 4.4 points) in patients on aspirin compared to placebo and at baseline, but confidence intervals were wide and the clinical significance of a change of this magnitude is questionable. High-dose aspirin has also been compared directly to amantadine with similar reported benefits between groups, using the FSS as an outcome, but again improvements were small and of uncertain clinical significance [225].

Conclusion

Fatigue is a common and disabling symptom in MS, and despite extensive study its pathogenesis remains unclear. Study of fatigue is hampered by inadequate definitions which limit comparability of studies and treatments. A number of pharmacological agents have been studied in relation to the symptomatic treatment of fatigue in MS. All treatments can have powerful placebo effects on this subjective complaint and therefore randomised placebo-controlled trials are required to evaluate their efficacy. Amantadine and modafinil are used in clinical practice with mixed results, but neither drug
is supported by large, high-quality RCT evidence at the current time. Mood disturbance is a common confounding factor and depression in particular may contribute to or exacerbate fatigue, sometimes requiring treatment in its own right.

Cognitive Impairment in Multiple Sclerosis

Another common and probably under-recognised symptom of MS is cognitive impairment, which can affect patients across all types and stages of the disease and is estimated to affect between 40-65% of all MS patients, sometimes presenting at the time of diagnosis or preceding physical disability [226, 227]. Cognitive impairment in MS can take a variety of forms, but in adults typically affects information processing speed, learning, episodic and working memory (in children with MS, language functions such as verbal memory and fluency can also be affected, likely reflecting the critical stage in cognitive development at which MS typically develops in the paediatric population) [228]. The nature of cognitive dysfunction in PwMS is predominantly a “subcortical” picture with impairment in information processing and speed of thinking, but also affecting anterograde episodic memory [229]. Cognitive impairment can negatively affect quality of life and social functioning in PwMS independently from physical disability [230, 231], and commonly co-exists with other symptoms which potentially have a cortical origin such as mood disorder and fatigue [232]. Furthermore, it is not limited to patients with advanced disease and can be objectively demonstrated in patients with low EDSS or short disease duration [233].

It is increasingly recognised that cognitive dysfunction in MS is associated with involvement of the cortical grey matter, a site which has historically been difficult to image with readily available MRI techniques in practice [234]. Modern MRI is more capable of detecting grey matter atrophy, and robust associations between cognitive function and atrophy in specific cortical brain regions have been demonstrated, including the prefrontal, temporal, parietal and insular cortices [235]. White matter lesion volume has also been shown to correlate with cognitive function [236] but it is increasingly believed that grey matter metrics are a better reflection of the effects of MS on cognition.

Cognitive dysfunction is difficult to treat with either disease-modifying or symptomatic treatments, and once established is often progressive. A number of therapies have been trialled in this domain and these will now be discussed.

Disease-Modifying Treatments (DMT’s) and Cognitive Function

Many of the pivotal trials of therapies for RRMS have examined cognition amongst several secondary outcome measures, although have primarily been focused on relapse rate and MRI metrics as the primary outcomes. For the most part these have been relatively short studies (with follow-up duration around 2 years) and most have used a single cognitive outcome measure, such as the Paced Auditory
Serial Addition Test (PASAT) component of the MS Functional Composite (MSFC, discussed in more detail in chapter 5). A number of observational studies have been conducted examining cognition in treated patients in more detail.

The IFNB in MS Study Group’s trial of 372 patients with RRMS examined cognitive outcomes in a subset of 30 [237] patients treated with IFN-β-1b. Despite the small numbers, they were able to demonstrate a benefit of the drug in the domain of delayed recall (non-verbal memory) which was independent of effects on MRI lesion load or EDSS score [238]. Similar findings were reported from the BENEFIT study, in which patients with CIS were randomised to IFN-β-1b (early or late) or placebo; treatment was associated with a significant improvement in MSFC score, predominantly driven by improvements in PASAT [239] and earlier treatment produced greater benefit.

The pivotal trial of intramuscular weekly IFN-β-1a also reported cognitive benefits in the subset of treated patients who were examined, as evidenced by a delay in time for cognitive deterioration on PASAT, although there was no absolute benefit shown after 2 years’ follow-up [240]. The IMPACT study of intramuscular weekly IFN-β-1a in SPMS showed no benefit over a 2-year follow-up period [241]. The original studies of subcutaneous IFN-β-1a did not measure cognitive endpoints; however COGIMUS, a 3-year open-label study using this drug, reported the absence of the cognitive deterioration as well as the decline in social functioning and quality of life which would normally be expected during this population over this period [242, 243]. The pivotal trial of Glatiramer Acetate showed improvements in cognitive function in both drug- and placebo-treated groups, with no significant additional benefit from Natalizumab over and above what was seen with IFN-β-1a monotherapy [247]. Similarly, evidence is lacking in favour of the oral therapies for their effects on cognition. The pivotal trial of fingolimod [120] reported a significant benefit of drug treatment on the MSFC, but it was not clear whether this was due to improvements in the cognitive or physical components of the score or both. A study of cognitive outcomes in Alemtuzumab-treated patients is in progress at the time of writing [248].

For ethical reasons, it is now not likely that further placebo-controlled studies of new DMT’s will be conducted for RRMS therapies, although cognitive endpoints will almost certainly be included in
trials of new agents for progressive MS [249, 250] and may be of even greater relevance in this population where the prevalence of cognitive dysfunction is even higher [251].

**Symptomatic Treatment for Cognitive Dysfunction**

Various classes of symptomatic therapies have been evaluated, mostly drawing on therapies which have been documented to be useful in patients with Alzheimer’s Disease (AD), with limited benefits reported in PwMS. These findings were summarised in a Cochrane Review [252] which included a total of 7 placebo-controlled RCT’s in 625 patients. The drugs trialled were heterogeneous and included the acetylcholinesterase inhibitors donepezil [253, 254] and rivastigmine [255], the NMDA receptor antagonist memantine [256] and the herbal extract ginkgo biloba [257, 258], all of which have been used in the treatment of AD. Due to heterogeneity in drugs and outcome measures, meta-analysis was not possible, but the conclusion of the review was that evidence of benefit was lacking for all drugs tested.

Two small double-blind studies using single doses of the psychostimulants methylphenidate and L-amphetamine (both used in the treatment of ADHD) have shown improvements in cognitive function in patients with MS. A single dose of methylphenidate improved performance on PASAT in patients with MS and attentional deficits [259], although this finding has not been confirmed in a longer-duration treatment trial. Likewise, a single dose of L-amphetamine improved performance on the Symbol Digit Modality Test (SDMT) [260] but this was not borne out in a larger and longer-duration trial, nor were benefits on PASAT demonstrated [261], although there were positive results shown in the secondary outcome measures of verbal learning and visual memory. A small randomised trial of 4-aminopyridine showed a non-significant trend towards improved cognitive function, but this has not been evaluated in larger studies [262].

Recent years have seen great interest in the use of cannabinoids for the treatment of various symptoms in MS, predominantly pain and spasticity; however, it is known that cannabinoids can negatively affect cognitive function in healthy individuals using the drug on a recreational basis. Studies of Cannabis-Based Medicinal Extract on spasticity in PwMS did not show any detrimental outcomes (nor any cognitive benefits) [263], but these studies were short in duration and presumably unlikely to capture the longer-term cognitive and psychiatric effects of cannabinoids in this group. Two small cross-sectional studies comparing PwMS who were and were not using “street cannabis” showed a significantly greater prevalence of cognitive dysfunction in the former group [264, 265].

Non-pharmacological treatments for cognitive dysfunction have included cognitive rehabilitation programs, with increasing interest in the possible delivery of such programs through smartphones and computerised devices [266, 267]. Randomised trials of exercise therapies in MS have shown
improvements in information processing speed, although not in learning or memory functions specifically [268, 269].

**Conclusion**

Despite the high prevalence and significant functional impact of cognitive impairment in MS, no effective symptomatic therapy exists for this potentially debilitating symptom. In patients with RRMS, early initiation of DMTs may have protective effects against cognitive decline in addition to their beneficial effects on physical disability.

**Mood Disorder in Multiple Sclerosis**

Like cognitive impairment, mood disorder is extremely common in PwMS and is likely to be under-recognised by clinicians managing the disease [270]. Depression was a major symptom of one of Charcot’s first described cases of MS in the mid-19th century [271] and it has been estimated that as many as 95% of patients with MS have some kind of neuropsychiatric symptoms, ranging from major depression to euphoria to apathy [272]. Of these symptoms, major depression is the most common, with a lifetime prevalence of 50% in PwMS as a group [273, 274], three times as high as the general population [275, 276] and almost twice as high as sufferers of chronic disease in general [277]. This raises the possibility of an underlying neurobiological basis to depressive symptoms rather than depression developing simply as a reaction to chronic illness. As well as impacting on quality of life [278], major depression can adversely affect adherence to treatment [279], possibly also relapse rate [280], carer burden [281] and can certainly increase suicide risk [282], with an estimated risk which is 7.5 times that of the general population [283]. The overlap between the vegetative symptoms of depression (e.g. poor sleep, fatigue) and the physical symptoms of MS may contribute towards underdiagnosis and prevent access to appropriate treatment.

Other mood disorders and personality changes common in PwMS include anxiety and euphoria. Anxiety has an estimated lifetime prevalence of 33% in PwMS, [284] and may often coexist with depression. The *Euphoria Sclerotica* state was first described by Charcot as a “cheerful indifference without cause” [285]; modern neuropsychiatric studies have estimated its prevalence to be around 9%, using the euphoria/disinhibition subset score from the Neuropsychiatric Inventory [286]. The related phenomenon of pathological laughing and crying is thought to be present in as many as 10% of MS patients [287]. Psychotic symptoms in association with MS are rare, although they may be slightly more common than dictated by chance [288]. Most evidence comes from single case reports only.

The pathophysiology of affective disorders in MS is uncertain, although a number of attempts have been made to try to elucidate this using neuroimaging techniques. Early studies in the pre-MRI era suggested, unsurprisingly, a much greater prevalence of depression in patients with demyelinating
brain lesions than in those with lesions confined to the spinal cord [289]. Larger MRI-based studies have not identified a direct relationship between lesion load overall and psychiatric morbidity [290], but have suggested some correlation between affective disorder and lesions in the temporoparietal region. Correlations have also been identified between depression and lesion load in the medial prefrontal cortex and the dominant temporal lobe [291]. In an MRI-based study comparing 21 PwMS who demonstrated evidence of depression (where the depression did not predate the MS diagnosis) versus 19 matched non-depressed MS patients, Feinstein [292] reported that around 40% of the variance between groups could be accounted for by left inferior medial prefrontal cortex T2 lesion volume and left temporal atrophy, implicating these specific brain regions as being of potential importance in the pathogenesis of mood disorder.

Symptomatic Treatment of Mood Disorders in MS

Principles of treatment are similar to primary mood disorders, with a combination of pharmacological therapy and psychological support such as Cognitive Behavioural Therapy [293] being favoured in the majority of patients. In contrast to most other patients with mood disorders, there may be barriers to psychological therapy (such as communication difficulty or cognitive impairment) specific to MS patients, and concomitant medications and symptoms may impact on the most appropriate choice of antidepressant or anxiolytic therapy. As for many symptomatic treatments, robust supporting evidence is lacking. A Cochrane Review [294] identified only two RCTs with a total of 70 included patients using paroxetine or desipramine, with only non-significant trends towards benefit seen with either drug.

In practice, Selective Serotonin Reuptake Inhibitors (SSRIs) are the first line of treatment [275, 295], although side effects of insomnia or sexual dysfunction may exacerbate existing MS symptoms. Alternatively, tricyclic antidepressants may be used, with the additional potential benefit that some drugs of this class (e.g. duloxetine, amitryptiline) may have pain-modulating benefits and may therefore be useful in patients with co-morbid neuropathic pain issues. Anticholinergic side effects may also be useful in patients with insomnia or bladder dysfunction due to detrusor hyperactivity [174]. In comparison to primary psychiatric disease, it has been suggested that MS-related mood disorders may have a slightly better prognosis, possibly due to the absence of a psychiatric history in many patients [275].

Disease-Modifying Treatments and Affective Disorders in MS

Historically, disease-modifying treatment with beta-interferon has been associated with an increased risk of depression [275], particularly in patients with a premorbid history of mood disorder. This was first suggested by the findings from the CHAMPS study of intramuscular interferon-β 1a, indicating an increased risk of depression in patients on drug (20%) versus placebo (13%) [52]. However, more
recent evidence has not corroborated these findings, with no difference in rates of depression between patients on interferons and glatiramer acetate [296], and no increase in depressive symptoms (despite disability progression) in patients before and after 1 year of treatment with interferon-β [297]. No increased risk of mood disorder has been reported with the newer oral or infusable disease-modifying treatments to date[298].

Conclusion

Affective disorders are common in PwMS, although the pathogenesis is uncertain. Symptomatic management is identical to that of primary psychiatric disease, though the impact of treatment side-effects on MS physical symptoms must be borne in mind.

**Bowel/Bladder and Sexual Dysfunction in MS**

Disturbance of sphincter and sexual functions are also common in MS and under-recognised and under-treated in many patients. The prevalence of bladder dysfunction has been estimated to be as high as 96% in patients who have had MS for 10 years or more [299]. This can manifest in various ways, including urinary frequency, urgency, nocturia, hesitancy, retention and incontinence, reflecting a variability in the underlying pathophysiological mechanism. Neurogenic bladder symptoms commonly occur due to disruption in the descending inhibitory autonomic pathways of the spinal cord, producing hyper-reflexia of the detrusor muscle (with resultant frequency, urgency and decreased bladder emptying), but can also occur due to interruption of the detrusor muscle reflex as a consequence of damage in the conus medullaris, producing detrusor hyporeflexia. This results in inability to contract the bladder adequately, manifesting as hesitancy and retention, sometimes with overflow. Detrusor-sphincter dyssynergia can also occur, with inco-ordination between the detrusor muscle and the bladder; in many cases, symptoms are multifactorial in origin. It is important to identify the major likely contributor in order to tailor symptomatic treatment appropriately. However, symptoms alone are often a poor indicator as to the underlying pathophysiological process (for example, urinary incontinence may occur due to detrusor hyper- or hypo-activity, and treatment for one problem may exacerbate the other). Formal urodynamic studies are the best means to identify the primary contributor but these may be difficult to access expeditiously. PwMS are also vulnerable to urinary tract infections, particularly if there is poor bladder emptying, which can further compound symptoms of bladder dysfunction.

Symptomatic treatments for detrusor hyperactivity (all of which lack supporting RCT evidence) predominantly consist of anticholinergic therapies. These include oxybutynin, tolterodine and solifenacin, which vary in their selectiveness for the acetylcholine receptor, and carry the standard
battery of anticholinergic side effects (blurred vision, constipation, urinary retention and in some cases sedation or cognitive effects [300]) to greater or lesser degrees. In patients who have a combination of detrusor over- and under-activity, these agents may cause more problems than benefits, and such patients may often require intermittent or permanent catheterisation to ensure adequate bladder emptying [174]. In recent years, intravesical injection of Botulinum toxin has emerged as a promising therapy for patients whose symptoms are refractory to oral anticholinergics or in whom systemic side-effects are unacceptable[301]. Detrusor hypoactivity and detrusor-sphincter dyssynergia typically respond less well to oral therapies and catheterisation is frequently the only solution for this type of bladder dysfunction. Lifestyle modifications such as moderating fluid intake, avoiding caffeine and other diuretics, scheduled bladder emptying and double-voiding can also be useful in some patients.

Constipation is a common MS symptom, either occurring chronically or alternating with diarrhoea and/or faecal urgency, and as mentioned above may be exacerbated by medications used to treat bladder dysfunction. Pathophysiologically, the mechanisms may include a combination of slowed colonic transit time, abnormal rectal function and reduced perineal sensation. Management consists predominantly of lifestyle and dietary modifications such as improving fibre intake, hydration, scheduled bowel emptying and exercise, with non-specific medical therapies such as stool softeners, laxatives and enemas being required in some patients [174].

Sexual dysfunction is estimated to occur in up to 75% of patients with MS [302] and can be multifactorial in origin, result from a combination of impairments including loss of sensation, motor weakness and spasticity together with other symptoms such as fatigue and depression. Symptoms in males can include erectile dysfunction, delayed ejaculation and loss of libido and are generally treated with symptomatic therapies such as phosphodiesterase inhibitors (sildenafil), together with treatment of comorbid depression, fatigue or pain. Symptoms in women typically include decreased vaginal lubrication and anorgasmia, and are typically treated with topical lubricants, with some evidence of benefit from phosphodiesterase inhibitors in females also [303].

Conclusion

Sphincter dysfunction and sexual dysfunction are common and under-recognised in PwMS, and symptomatic management options are again limited and largely unsupported by clinical trial evidence.

The most common MS symptoms requiring symptomatic management have been introduced and the currently available limited treatment options for these have been discussed. The forthcoming chapters will discuss how these symptoms can be objectively evaluated in the research setting using electrophysiological measures and clinical tests.
Chapter 4: Electrophysiological Evaluation of MS Symptoms

In this chapter, the existing literature regarding the role of electrophysiological techniques in the evaluation of some common MS symptoms will be discussed, including: motor and sensory impairment, visual dysfunction, fatigue, cognitive impairment, sphincter dysfunction, sexual dysfunction and affective disorders.

Electrophysiological Testing: Role in Diagnosis and Evaluation of MS Symptoms

- Electrophysiological testing in diagnosis
- Electrophysiological study of motor dysfunction
- Electrophysiological testing in evaluating somatosensory symptoms
- Electrophysiological testing in evaluating visual disturbance
- Electrophysiological testing in evaluating fatigue
- Electrophysiological testing in evaluating cognition and mood disturbance
- Electrophysiological testing in evaluating bowel/bladder dysfunction

Electrophysiological Testing in Diagnosis and Prognostication

MS cannot be diagnosed on the basis of clinical features alone but rather on a combination of clinical history and examination findings with supporting evidence from paraclinical tests. Evoked potentials (EPs) are electrophysiological measures of conduction in the CNS, whereby a stimulus is applied at a peripheral site and recorded over the cerebral cortex (in the case of afferent EPs) or vice versa (in efferent EPs). Demyelination results in slowing of conduction speeds in the CNS and can be identified using various types of EPs depending on the pathway to be interrogated. The most frequently used are visual evoked potentials (VEP), followed by somatosensory evoked potentials (SSEP) and motor evoked potentials (MEP). In patients with relevant symptoms, brainstem auditory evoked potentials (BAEP) [304] or vestibular evoked myogenic potentials (VEMP) [305, 306] may also be useful.

Abnormal VEPs were included in the initial version of the McDonald criteria for the diagnosis of PPMS [39], and remain in the current criteria as corroborative evidence of demyelination in patients with prior visual symptoms suggestive of optic neuritis. In comparison to MRI, EPs have much less precise lesion-localising abilities, and are unable to discriminate between active and inactive regions of demyelination. Nevertheless, they do still have a place in the evaluation of subclinical disease and may have potential roles in the prediction of MS disease course [307-309] and in the objective evaluation of symptomatic or disease-modifying therapies [196, 310].
Building on early research on the utility of individual evoked potentials in the diagnosis of MS, interest has grown in recent years in combining the results of various types of evoked potentials to aid specificity of diagnosis and in evaluating symptoms and response to therapy [311].

Multimodal evoked potentials have been evaluated as a tool for assessing disability in patients with RRMS. O’Connor [312] et al used a combination of VEP, SSEP and BAEP to derive a composite score in patients with RRMS. These investigators demonstrated a robust correlation with this score and level of disability, a correlation more than twice as high with degree of disability than with MRI findings. More recently, similar findings have been described in a group of patients with PPMS [313].

Multimodal EPs have also been evaluated for their ability to predict prognosis, with several longitudinal studies providing evidence that a composite EP score at presentation can reliably predict disease progression on EDSS over time, with greater certainty than MRI [314-317]. This appears to be true in patients who present with early stage disease including CIS [318, 319] with less prognostic ability in patients who present later with higher degrees of disability.

Multimodal EPs can also be applied to the evaluation of drug therapies, such as in a recent study of fingolimod-treated patients where a composite score of MEP, SSEP and VEP correlated well with pre and post treatment EDSS progression and could thus be used as a surrogate marker alongside clinical and radiological data [320]. EPs can also be used in the evaluation of specific symptoms. This will now be reviewed.

**Electrophysiological Evaluation of Motor Dysfunction in MS**

Most PwMS will develop weakness to a greater or lesser degree at some point during the course of the disease and the motor systems of the pyramidal tracts are probably the pathways in which electrophysiological study has the widest application. Various parameters can be measured with different purposes.

**Central Motor Conduction Time (CMCT)**

The large myelinated fibres which comprise the motor pathways of the CNS are often involved in MS. They can be interrogated using transcranial magnetic stimulation (TMS) in order to measure motor evoked potentials (MEP) and central motor conduction time (CMCT) [321], the latter reflecting conduction velocity in the CNS. TMS was introduced in the mid-1980’s as a painless, non-invasive and better-tolerated alternative to electrical stimulation of the motor system [322].
During TMS, a large, brief current is passed through a coil placed on the scalp which induces a strong but short-lasting magnetic field at right angles to the coil. This in turn excites cortical interneurons triggering an action potential which spreads trans-synaptically to corticomotorneurons and then along the corticospinal pathways to anterior horn cells located in the spinal cord with the resultant motor evoked potential (MEP) response recorded in peripheral muscles (Figure 1: TMS recording apparatus).

**How to Measure CMCT using TMS**

In practical terms, CMCT is measured by first stimulating the contralateral motor cortex with the magnetic coil and then by placing the coil over the spine using the pulse to stimulate proximal nerve roots and recording the response (MEP) from the same peripheral muscle after each stimulus. The difference in latencies of the MEP between stimulations approximates the time taken for the pulse to travel through the CNS. Before performing this assessment, the resting motor threshold (MT) must be determined, defined as the minimum stimulus intensity (expressed as a percentage of the maximum output of the stimulator) which produces a response of a certain peak-to-peak amplitude (typically 100μV in at least 50% of 10 trials). Once the MT has been found, a supra-threshold stimulation (typically 120% of threshold) is applied over the motor cortex and the response measured in the periphery. Stimulation is then applied over the cervical spinal cord at C6 for upper limb MEP or over the lumbar spine at L4 for lower limb MEP, and the resultant response recorded from the same peripheral muscle (usually first dorsal interosseous or abductor pollicis brevis in the upper limb or flexor or adductor hallucis brevis in the lower limb). Latency rather than amplitude is typically recorded, being the more consistently reproducible of the two measures [323]. Normal values are established within each laboratory and both the absolute value and side-to-side comparison can be useful in detecting pathology.

**Pathophysiology of CMCT Prolongation**

The prolongation of CMCT latency seen in PwMS is likely to reflect a combination of slowed conduction (due to CNS demyelination), conduction block and temporal dispersion (reflecting failure or reduction of transmission down particular axons), but dissecting out the relative contributions of each can be difficult. In electrophysiological study of the peripheral nervous system (PNS), stimulating at two (proximal) sites and recording over a (distal) muscle allows inferences to be made regarding the velocity of conduction between the two stimulation sites, without an intervening synapse. The amplitude of the compound muscle action potential (CMAP) is a reflection of the number of fibres firing, and can reliably detect conduction block. In studying CNS conduction, stimulation with TMS over the cortex and recording in the peripheral musculature requires the
impulse to cross multiple synapses. TMS also produces not just one but multiple descending volleys of several indirect descending waves as a result of transynaptic neurotransmission [324, 325]. For this reason, prolonged central motor conduction time may reflect (1) true reduction in conduction speed of fast large myelinated fibres, typically producing a marked delay in CMCT; (2) insufficient activation of (normal) spinal motoneurones by (abnormal) cortical motoneurones, typically producing only a modest increase in CMCT; or (3) a reduction in size of the initial descending volley meaning that a discharge cannot be produced without temporal summation with later descending volleys [325]; this latter point may also explain why there is greater variability or “jitter” in CMCT latency in MS patients relative to healthy controls.

The main limitation of CMCT latency is that it measures only the fastest-conducting fibres, which have the shortest latency. Although less reproducible and less widely-studied, abnormalities can also be seen in the amplitude and duration of MEP responses. These may reflect a reduction in number or a temporal dispersion of conduction in descending fibres, or a decrease in excitability of motoneurones in response to the cortical stimulus [325]. Modifications of this technique have been used to further study motor tracts in MS patients. Using a variant of the “collision technique” developed in the study of peripheral nerve conduction [326] and a paired-pulse TMS protocol, Firmin examined the distribution patterns for motor conduction time in healthy controls [327] and PwMS [328] and demonstrated that conduction patterns in MS patients were more variable with a widening of distribution patterns but with preservation of the bimodal pattern seen in healthy controls. Although not as well-validated as CMCT measurement, it might be expected that this technique would be more sensitive for detecting abnormal patterns of conduction in MS patients for its ability to show abnormalities in slower- as well as faster-conducting fibres.

The Role of CMCT in Diagnosis

Early electrophysiological studies evaluated the diagnostic utility of CMCT in PwMS. These showed fairly consistent results, with prolongation of CMCT in MS patients relative to controls being evident in up to 90% of patients with clinically weak muscles and around 50% of MS patients without signs of clinical weakness or spasticity [329-332]. The diagnostic reliability of these parameters as compared to the contemporaneous Poser criteria [38] has been calculated at 0.83 for prolonged CMCT [333]. It is well-recognised that there may be significant disparity between the clinical findings in MS patients and the extent of demonstrable electrophysiological abnormalities of CMCT [323, 329] such that the most marked delay in CMCT does not equate with the most marked clinical weakness. Hence this technique may be used to detect clinically silent lesions and provide a useful adjunct to clinical examination findings. Prolongation of CMCT latency may also be associated with impaired motor function in the absence of weakness. Van der Kamp [334] demonstrated a strong negative correlation between CMCT latency and hand dexterity (measured using the Purdue peg test) in patients without
clinical evidence of hand muscle weakness, which may reflect the relatively greater importance of the corticospinal pathways in manual dexterity and fine motor control compared to their importance in crude muscle strength or tone; tests of finger dexterity are a sensitive clinical index of corticospinal tract integrity.

The relative sensitivity and specificity of visual evoked potential (VEP) and CMCT in MS diagnosis have been debated [333, 335-339]. VEP latency appears to be significantly more sensitive than CMCT alone (89% vs. 58%)[339], and clinically silent lesions of the optic nerves are much more common than those of the pyramidal tracts (85% for optic nerves vs. 10% for pyramidal tracts). However, when MEP measurement takes into account a wider variety of abnormalities than just raw CMCT latency (i.e. area, amplitude and side-to-side differences) it increases in sensitivity for detecting clinically silent lesions [340]. In comparison to VEP, measurement of CMCT is technically more difficult, slightly harder for patients to tolerate, and in some patients with inexcitable cerebral cortices, impossible. Furthermore, there is a much higher concordance rate between MEP and MRI than between VEP and MRI – and therefore a much lower chance of uncovering useful complementary information by the addition of this test.

**CMCT in the Assessment of Disease Severity and Prognosis**

CMCT has been compared to clinical findings in an attempt to determine a correlation with disability and disease severity. Kalkers [341] reported a correlation between CMCT and clinical assessment of leg (although not hand) function, as well as EDSS, in patients with RRMS and SPMS (but not PPMS). Similar findings were reported by Kidd [342], supporting the idea that CMCT from leg muscles may be more robustly associated with EDSS than that of upper limb muscles, which is unsurprising given the heavy weighting of the EDSS score towards ambulatory function.

Prognostication in MS remains challenging, with both MRI and clinical parameters having limited accuracy in predicting clinical course. Measurement of CMCT has therefore been evaluated in this regard. Feuillet [343] performed a 6-month longitudinal study of 15 patients with early RRMS (mean EDSS 2.0) and reported a positive correlation between MEP abnormalities at baseline (either prolonged CMCT or increased MEP amplitude ratio) and disability (EDSS) at 6 months, with a robust association between 6-month motor function score and number of TMS abnormalities at baseline. Other authors have suggested that amplitude ratio (i.e. MEP to peripheral CMAP amplitude) may be more sensitive than CMCT, including CIS [344] as well as CDMS [345].

Various studies have evaluated the predictive value of CMCT in conjunction with other variables. Bejarano [346] evaluated CMCT among other prognostic markers in a prospective cohort and reported that while baseline EDSS, grey matter volume and CMCT were each weakly correlated with clinical progression, the combination of these three variables in a mathematical model had a
reasonable accuracy (80%) for predicting EDSS change over 2 years. Along similar lines, Fuhr [14] prospectively evaluated 30 patients with RRMS or SPMS longitudinally over 2 years and demonstrated a robust correlation between EDSS score and MEP latencies as well as EDSS score and the number of abnormal evoked potentials, with a more robust correlation when a formula was applied which combined both EP scores. Leocani’s [309] multimodal EP score comprising MEP and SSEP in a longitudinal study demonstrated a significant positive correlation between baseline combined EP score and likelihood of disease progression over time. More recently, a composite score derived from MEP and VEP latencies has been shown in a prospective study to be predictive of disability over a 20-year follow-up period in a group of patients with RRMS and SPMS [347, 348]. The relationship between prognosis and MRI is weaker for PPMS than RRMS [349] but even in this group a multimodal EP score encompassing VEP, SSEP and MEP was shown to be predictive of disability over a 3-year period [350].

**CMCT in Longitudinal Disease Assessment**

The potential use of CMCT as an objective tool for monitoring disease progression and responses to therapy has also been evaluated, with mixed results. Kandler [351] studied a cohort of 100 patients with MS and demonstrated a robust correlation between CMCT and degree of motor dysfunction according to the pyramidal FS subscore of the EDSS. A subgroup of 11 clinically stable patients was re-examined over the following 3 months, and a further 27 patients who suffered a clinical relapse were examined during and after steroid treatment. Despite small numbers, there was a statistically significant correlation between clinical findings and CMCT, in that patients who clinically responded to steroid treatment had a robust reduction in CMCT. Non-improvers had no change, and results from patients without relapse were stable across two readings [351]. Improvement in CMCT has also been documented as mirroring clinical improvement in response to physiotherapy [352] and in patients who improve without treatment for a relapse [353], making a specific effect of corticosteroids on neural excitability less likely. Kidd [342] studied a group of 20 patients with progressive MS (both SPMS and PPMS) longitudinally over a 12-month period, attempting to correlate clinical findings with MRI parameters and CMCT measurement. They showed that clinical progression over the study period was seldom associated with progressive prolongation in CMCT, unless there were also new spinal cord lesions which developed over the same timeframe. One explanation for this is that CMCT is a better reflection of inflammatory demyelinating damage rather than the axonal degeneration thought to be responsible for the majority of the clinical deterioration in progressive MS.

**CMCT in the Classification of MS Disease Subtype**

The clinical determination of progressive MS, whether primary or secondary early in its course, is challenging, and an objective marker of disease classification is highly desirable both to minimise the unnecessary use of immunotherapies and in order to identify suitable patients for clinical trials of
neuroprotective agents. It might be supposed that a more functional measure of motor system activity (such as MEP/TMS) might provide complementary information to MRI and might aid in diagnosing progressive MS. To this end, Humm [354] studied 141 patients with RRMS, PPMS and SPMS and measured CMCT with a number of other parameters. This study reported a marked increase in CMCT in the group with progressive MS, independent of disease duration, number of spinal lesions or clinical motor deficit. Facchetti [355] also demonstrated a significant increase in CMCT, correlating with EDSS and pyramidal FS score, as well as a higher incidence of abnormal SSEP, MEP and BAEP in patients with SPMS when compared to those with RRMS and to healthy controls. These data have not been further validated with defined cut-off points, nor have they translated into routine practice at the time of writing.

Other uses of TMS

Corticospinal Excitability Using MEP Recruitment Curves

In addition to measurement of CMCT, TMS can be used in the construction of MEP recruitment curves which are believed to reflect the excitability of cortical motoneurones themselves (as distinct from interneurones, which are better reflected in the paired-pulse TMS paradigms discussed below). This technique uses increasing suprathreshold stimulations and measures the resultant mean MEP amplitudes.

Stimulation is applied via a TMS coil over the motor cortex and recordings are made from a peripheral muscle (typically first dorsal interosseous or abductor pollicis brevis in the upper limb). MT is determined first in the same manner as for the measurement of CMCT, and a series of randomly distributed suprathreshold stimuli (typically ten each at 110%, 120%, 130% and 140% of threshold) is applied. Mean peak-to-peak MEP amplitude is calculated at each stimulus intensity and expressed as a proportion of the maximum CMAP amplitude obtained by supramaximal stimulation of the nerve in the periphery to the same muscle. In subjects where resting motor threshold is high, full recruitment curves cannot be obtained because 130% or 140% of threshold exceeds the maximum output of the stimulator. A physiological pattern of recruitment shows a progressive increase in MEP relative to CMAP amplitude with progressively increasing stimulus intensity (figure 2, physiological MEP recruitment curve).

It might reasonably be expected that patterns of MEP recruitment would be altered in PwMS, though the limited data available suggest that this may not be the case. Abnormalities in MEP recruitment curves can be seen after limb immobilisation following fracture [356] but have not been reproducibly identified in patients after stroke [357] or in patients with MS or neurodegenerative disorders [358]. There is evidence that various anticonvulsant drugs (for example lorazepam and lamotrigine) can alter patterns of MEP recruitment [359]. Steroids when used to treat MS relapses have no effect [360] but
apart from this no other data exist regarding the effects of MS symptomatic or disease-modifying therapies on patterns of MEP recruitment.

_Intracortical Excitability Using Paired-Pulse TMS_

A more commonly used application of TMS uses paired-pulse paradigms (PPTMS) to study cortical excitability at the level of interneurons using paired conditioning and test stimuli. This is believed to reflect interactions between cortical interneurons and motoneurones at the level of the intracortical circuits.

Two of the best-studied parameters are intracortical inhibition (ICI) and facilitation (ICF), which are studied using paired pulses of conditioning and test stimuli given at variable interstimulus intervals (ISIs). Short-latency ISIs (typically 1-15ms) are used to study the phenomenon of short latency intracortical inhibition (SICI) and intracortical facilitation (ICF). As for CMCT above, the MT must first be determined using the method previously outlined. The conditioning stimulus is typically delivered at 80% of MT and the suprathreshold test stimulus is typically delivered at 120%. The peak-to-peak MEP amplitude is measured for each ISI and expressed as a ratio compared to the mean MEP amplitude when the test stimulus is delivered alone (without a preceding conditioning stimulus). In normal individuals, at short ISIs (1-5ms) there is inhibition of the test response compared with the baseline unconditioned response (termed SICI) whereas at longer ISIs of 7-15ms the test response is facilitated compared with the unconditioned response (termed ICF). (Figure 3 – physiological PPTMS curve).

The effect of MS on PPTMS parameters has been previously studied by a small number of investigators. They have described abnormalities of short-latency ICI (specifically, reduction in ICI) and ICF [361, 362] in PwMS, particularly SPMS. Data have suggested that there may be some correlation between the degree of abnormality and level of disability [363-365]. To date, there have been no systematic studies of the effects of symptomatic or disease-modifying therapies on PPTMS parameters in this population, other than one small study of 3,4-diaminopyridine in PwMS which showed decreased ICI, increased ICF and no change in CMCT in drug-treated patients [366].

In conclusion, motor dysfunction is a symptom which lends itself particularly well to objective study using MEP’s, although to date there is limited literature on either the expected findings in PwMS, the prognostic implications of abnormalities detected or the response of such abnormalities to currently available MS therapies.

_Electrophysiological Evaluation of Somatosensory Impairment in MS_
Sensory symptoms are difficult to evaluate objectively, being prone to vary depending on mood, pain threshold and a variety of other unquantifiable factors. SSEPs have been evaluated as a tool for objectively quantifying sensory impairments in MS patients. These are relatively easy to perform, non-invasive and generally well-tolerated. Like other EPs, they may be used to detect clinically silent lesions, demonstrating dissemination in space and sometimes contributing to diagnostic certainty [367].

In practical terms, SSEPs are performed by stimulating over a peripheral nerve (typically the median nerve for upper limb SSEPs and the tibial nerve for lower limb SSEPs) and recording with EEG surface electrodes along the trajectory of the pathway through the nervous system, from the periphery (e.g. Erb’s point in the upper limb, the popliteal fossa in the lower limb), to the spinal cord (e.g. C6 and C2 in upper limb SSEP’s, L1 and L3 in the lower limb) and cerebral cortex (positions C3’ and C4’ in the 10-20 EEG electrode system, above the position of the somatosensory cortex). Because of the small amplitude of the potentials to be recorded and the liability of muscle artefact to interfere with the recording process, 200-300 stimuli are applied and the responses averaged. Several potentials are recorded, typically including (for the upper limb) the N9 response (Erb’s point), N11 (cervical spine at C6), N13 (cervical spine at C2) and N20 (somatosensory cortex; the P25 response is also recorded at this location and the N20-P25 interpeak is used to calculate the amplitude of cortical response in addition to its latency). In the lower limb, the N8 response is recorded at the popliteal fossa, the Lumbar Point (LP) response at L1 and the N37 response at the cerebral cortex (with the P40 response to enable calculation of amplitude). The contribution of the peripheral nervous system (typically not affected in MS) is removed by calculating the N20-N13 or N37-LP interpeak latencies to give a value for the Central Conduction Time (CCT). As with motor studies, lower limb studies are more sensitive than upper limb studies for detecting evidence of demyelination [368].

Standard SSEPs predominantly reflect function in the dorsal columns (the structures conveying sensations of vibration and proprioception, mediated via Aβ non-nociceptive fibres), and are less sensitive for detecting abnormalities of the spinothalamic sensory pathways (pain and temperature) [369]. Perhaps unsurprisingly, impaired proprioception (which is a relatively cruder measure of sensory function than vibration sense) is most strongly correlated with SSEP abnormalities.

Laser-evoked potentials (LEPs; whereby painful, hot stimuli are applied via laser to stimulate the spinothalamic pathways via Aδ nociceptive fibres) can be used to complement information gained from SSEPs [370, 371]. Available data suggest that LEPs may be more sensitive than SSEPs in showing abnormalities in PwMS, reflecting a greater propensity for disease involvement of the spinothalamic tracts than the dorsal columns [372].

Leocani [308] compared SSEPs to quantitative sensory testing and standard clinical examination in a group of 19 PwMS and demonstrated that upper and lower limb SSEPs correlated well with findings
of impaired vibration sense on clinical examination and were more sensitive than quantitative testing of vibration. In keeping with the studies of spinothalamic sensory function discussed above, quantitative testing of thermal detection correlated poorly with SSEP findings and was more sensitive than standard SSEP or clinical examination.

Nociti [373] compared SSEP findings with performance on a functional test of upper limb function (9HPT time) and self-reported upper limb function (DASH questionnaire) and reported a convincing association between SSEP abnormality and functional performance (subjective and objective). These findings could be explained by SSEP acting as a generic marker of CNS damage or could reflect the impact of sensory nervous system pathways on motor function; correlation with MEP findings or sensory clinical examination might shed some light on this but was not performed in this study.

SSEPs have also been applied to the objective evaluation of pain, a symptom which is hard to quantify and liable to variation in perception secondary to a number of confounding factors. Truini [374] compared neurophysiological findings (SSEP and LEP) in patients with neuropathic pain of the extremities and Lhermitte’s phenomenon, and demonstrated that the former group had a greater prevalence of abnormalities detectable on LEP, whereas the latter had more frequent abnormalities on standard SSEP. They also reported an association between disease severity (measured by EDSS), progressive (rather than relapsing) disease and the presence of neuropathic pain. In all patients with pain, there was also evidence of cervical or thoracic spinal lesions, and the authors hypothesised that spinothalamic damage was strongly implicated in the generation of neuropathic pain in this group.

In summary, although SSEPs may have a role in the diagnosis of MS and the demonstration of clinically silent abnormalities, their poor correlation with symptoms (abnormal SSEPs are found in up to 80% of PwMS without sensory symptoms or signs [375]) means that their role in objective evaluation of sensory symptoms may be limited.

**Electrophysiological Evaluation of Vision in MS**

Visual evoked potentials (VEPs) are the most widely-used electrophysiological technique in clinical practice in MS patients, and are often used to demonstrate dissemination in space or time, which can be important in making a diagnosis in patients presenting with demyelination elsewhere in the nervous system. As is the case for MEPs and SSEPs, VEP latency may be markedly abnormal in the absence of any reported disturbance of visual function [376].

In practical terms, VEPs are recorded from each eye in turn with the subject seated in a comfortable chair at a fixed distance (1m) from a high-contrast black and white chequerboard with square size 17mm in the central part of the visual field [377]. The chequerboard flashes such that the black and
white squares change places at a set frequency (typically 2Hz) and the subject is instructed to fixate on a red spot in the centre of the screen. Recording electrodes are positioned at O1, O2 and Oz with additional electrodes at Pz, Fz and Cz (ground) according to the 10-20 electrode placement system. 100-200 responses are recorded and the traces averaged. The potential of greatest relevance is the P100 for which latency and amplitude can both be measured. Normal values are established within each laboratory and the side-to-side difference as well as the absolute numbers for latency and amplitude are noted.

Although both latency and amplitude of VEPs can be recorded, latency is the more reproducible and clinically useful marker [368]. The diagnostic sensitivity is high, with early longitudinal studies estimating a prevalence of abnormality in over 90% of patients who eventually come to be diagnosed with CDMS [378]. However, the degree of sensitivity may depend on the paradigm used, including the speed of pattern reversal of the chequerboard [368] and the degree of contrast between black and white squares on the chequerboard (with low-contrast paradigms appearing to be more sensitive for detecting subclinical optic nerve damage) [379]. Multifocal VEPs are less widely used in clinical practice but show promise in terms of superior sensitivity and specificity in both clinically affected and unaffected eyes, and demonstrate robust correlation with clinical and radiological correlates of optic nerve function[380].

There is frequently discordance between VEP abnormalities and standard visual acuity, such that patients may manifest clinical recovery from an episode of optic neuritis (or indeed have abnormal findings in the absence of any prior history of visual symptoms) [381] and have abnormalities of VEP latency or amplitude which persist for years [382] or are permanent [378]. When a more sensitive measure of optic nerve function, contrast sensitivity rather than Snellen Chart visual acuity, is compared to VEP latency, there is a more robust correlation [379].

VEP parameters are thought to be more variable in PwMS than in healthy controls and can be affected by temperature, particularly in patients with heat-sensitive symptoms (Uhthoff’s phenomenon). Persson and Sachs [383] compared VEP latency and amplitude between PwMS with and without Uhthoff’s phenomenon (temperature-sensitive decrease in vision) and healthy controls. Exercise (and resultant increase in core temperature) was associated with a marked decrease in VEP amplitude (and correspondingly visual acuity) in the Uhthoff group, with only a small increase in latency in these patients and no change in either parameter in the other groups. Physiologically, this can be explained by temperature-induced conduction block in the optic nerve of patients with heat-sensitive symptoms. The corresponding decrease in visual acuity in this group would be in keeping with the observations that conduction block, not latency prolongation by itself, is related to clinical symptoms.

The utility of VEP as a tool for monitoring MS longitudinally over time has been investigated by a small number of authors. Latencies have been reported both to improve [384] and to remain static
In patients who have improved clinically (by contrast, SSEP latencies have not been shown to change longitudinally [387-390] in patients who improve clinically).

In summary, there is a limited correlation between VEP latency (and even less so for amplitude) and visual acuity, which may limit the utility of these parameters in objective assessment of visual function at a single point in time. However, VEP parameters may change over time [198, 382] and in response to drug therapies [196] and hence may have a role alongside clinical measurements in longitudinal disease assessment and evaluation of new therapies. The current primary role of VEP’s is in the detection of subclinical optic nerve involvement and it would seem likely that this non-invasive and well-tolerated test will continue to provide useful complementary information alongside MRI and clinical findings [57].

**Electrophysiological Evaluation of Fatigue in MS**

For subjective symptoms like fatigue, an objective biomarker would be highly desirable in the evaluation of therapies and for longitudinal monitoring, but no such biomarker is currently validated for use in clinical practice. There has been interest in the potential uses of TMS in this context, based on the premise that cortical neuronal pathways may be important in the generation of fatigue. Liepert [391] studied a small number (n=16) of PwMS with and without fatigue and compared both groups to healthy controls. Participants were required to perform a fatiguing motor task, and TMS measurements were performed before and afterwards. The fatigued group displayed a significant reduction in ICI prior to exercise relative to the other groups, and there was a positive correlation between fatigue severity and the time taken for return to baseline of the motor threshold after completion of the motor task (motor threshold increased post-exercise in all subjects) although this did not reach significance. It was not clear from these data whether the apparent disinhibition of the primary motor cortex on the SICI was causal or an effect of fatigue, and a similar study failed in any case to corroborate these findings [392]. Colombo [393] reported a trend towards a greater prevalence of CMCT prolongation in patients with fatigue than without, but did not control for the confounding factors of lesion load or level of disability, both of which were positively correlated with the score on the Fatigue Severity Scale (FSS).

Several TMS studies have been conducted with the aim of investigating the pathophysiology of fatigue in MS, and most investigators appear to be in agreement that patterns of corticomotor excitability differ between PwMS and control groups with patients displaying greater increase in amplitude of cortical MEP’s in response to a fatiguing motor task and a prolonged cortical silent period [394-396], although the extent to which this reflects altered excitability in MS *per se* rather than being specific to fatigued patients specifically is unclear. These findings support the idea of
fatigue as a centrally-generated phenomenon [397], which certainly fits with the clinical impression of fatigue being a common feature of many CNS pathologies including stroke and Parkinson’s Disease as well as MS [208].

EEG has also been evaluated as an objective biomarker in this context. Leocani [398] compared EEG patterns of cortical activation during a simple voluntary motor task (repeated thumb extension) between patients with MS with and without fatigue and healthy controls. They identified abnormal patterns in event-related synchronization immediately following the motor task in fatigued MS patients, believed to reflect a failure of cortical inhibitory processes in the fatigued group. It is unclear how this translates pathophysiologically to fatigue. One possible mechanism would be a maladaptive cortical reorganisation following damage to motor regions, although in this particular study patients were not disabled (EDSS <1.5) and had not accumulated evident motor damage. This study has not since been replicated in more disabled patients to test whether the abnormal patterns are more marked.

**Electrophysiological Evaluation of Cognitive and Affective Disorders in MS**

Although there has been tremendous interest in TMS in the investigation and particularly the treatment of depression in the absence of MS [399, 400], there is no significant body of literature relating to diagnostic (or therapeutic) TMS, other evoked potentials or EEG for affective disorders in the MS population.

**Electrophysiological Evaluation of Bowel and Bladder Dysfunction in MS**

Bowel, bladder and sexual dysfunction may be amenable to evaluation using objective electrophysiological techniques, most notably pudendal nerve SSEPs. This technique can interrogate the afferent pathways between the genitourinary tract and the CNS and requires peripheral stimulation in the genital region and recording over the sensory regions of the cerebral cortex, permitting measurement of response latency [401, 402]. Abnormalities have been documented in a high proportion of PwMS regardless of the presence of genitourinary symptoms [403, 404]. Similar findings have been reported in the presence of bowel dysfunction [405, 406] as well as sexual dysfunction in males [407] and females [408]. There is no existing literature regarding longitudinal electrophysiological assessment of sphincter or sexual dysfunction, nor any data regarding alterations in objective tests in response to symptomatic or disease-modifying therapies.
Thus there is a reasonable body of literature relating to the use of electrophysiological techniques and particularly EPs in the diagnosis of MS and a smaller body of literature relating to their use in the evaluation of specific clinical symptoms or response to drug therapies. The complementary clinical evaluation techniques which can be used in this context will now be discussed further.
Chapter 5: Objective Clinical Evaluation of MS Symptoms

In this chapter, the techniques, issues and pitfalls surrounding the objective clinical evaluation of ambulation, upper limb function, vision, somatosensory function, fatigue and cognition in PwMS will be discussed, and the reasons for selection of the particular tools used in the present study will be explored.

Objective monitoring of disease course is vital in treating patients with MS, both as a means of assessing response to therapy and predicting future prognosis. Clinical trials of new symptomatic and disease-modifying therapies for MS also require an objective measurement of disability and disease progression to permit evaluation of efficacy. By far the most widely-used tool is the Expanded Disability Status Scale (EDSS) [11], which has a number of limitations despite its ubiquity both in clinical practice and clinical research. The EDSS (Appendix 2) is a 20-point non-linear ordinal rating scale where 0 is “normal neurological examination” and 10 is “death due to MS”. The scale assesses the following clinical domains or Functional Systems (FS): vision, pyramidal function, brainstem function, cerebellar function, sensation, bowel/bladder function, memory/cognition and ambulation. By definition, some domains can be assessed more objectively than others, for example the visual or pyramidal FS are easier to quantify than sensation or cognitive function, but attempts are made to standardise assessment of all domains, with assessments being carried out only by certified assessors, according to universal published criteria.

Attempts have been made to assess the inter- and intra-rater reliability of the EDSS. Noseworthy [409] studied the variability between two clinicians’ contemporaneous assessments of the same 168 PwMS. Perfect agreement in the EDSS score, other than that which would be expected by chance, occurred in only 62% of cases, with the highest degree of agreement in the overall EDSS step and the pyramidal FS score and the lowest agreement in the cerebellar and sensory FS scores. However, agreement within 1 step occurred in 92% of cases. The implication of this finding is that caution should be applied in interpreting changes below this threshold level, particularly in those FS which rely on patient-reported parameters or assessment by different clinicians. Goodkin [410] compared inter- and intra-rater reliability in the assessment of patients with EDSS 1.0-3.5 inclusive (at lower levels of disability there is greater variability than in more disabled patients, where total score is dictated predominantly by ambulatory function rather than individual FS scores [411]), and perhaps unsurprisingly found a higher degree of variability between compared to within individual EDSS raters. In practical terms, it may be difficult to ensure that serial assessments are always performed by the same assessor, thus clinical or trial outcomes must allow for this degree of variability.

A sustained increase in EDSS score on serial longitudinal assessment suggesting disability progression is a commonly-used endpoint in clinical trials of disease-modifying MS therapies. For patients who have only had a single assessment at one time point, the MS Severity Score (MSSS) was
developed to give some index of the rate of accumulation of disability, by adjusting the EDSS at one time point for the duration of disease [412]. This score can be used to make broad comparisons between groups of patients, but because of wide variability of disease over time in individual patients is of limited prognostic value in predicting future disease course on the individual patient level.

The non-linearity of the EDSS makes statistical analysis challenging and the scale can be relatively insensitive to clinical deterioration, particularly at the mid-to-higher end of the scale. In practice, there is a bimodal distribution of scores, with patients clustered primarily in either the 1.0-3.5 or the 6.0-6.5 groups [413], and the rate of progression through the EDSS steps varies greatly from patient to patient, and according to what step applies at presentation (or entry into a clinical trial). The use of the EDSS as a primary outcome measure presents particular challenges in assessment of disease-modifying therapies for patients with progressive MS, who by definition typically have later-stage disease. The tool may also not be sufficiently sensitive in the detection of real clinical deterioration which does not fulfil the threshold (usually related to ambulation) for advancing 0.5 or 1 points on the score, but which is nevertheless of functional importance to the patient.

Another limitation of the EDSS is its heavy weighting towards ambulation and relative neglect of other potentially disabling MS symptoms, such as cognition, bladder/bowel function and upper limb function. A complementary tool which is often used alongside EDSS in clinical trials is the Multiple Sclerosis Functional Composite Measure (MSFC) [414]. This score comprises quantitative measures of three domains: ambulation (using the timed 25 foot walk test, T25WT), upper limb function (using the 9-hole peg test, 9HPT) and cognition (using the Paced Auditory Serial Addition Test, PASAT). Scores on each of these three measures are compared to a reference group and converted to standard scores (z-scores), where a score of +1 indicates that the subject has scored 1 Standard Deviation (SD) better than the reference population and a score of -1 that they have scored 1 SD worse. The MSFC has been evaluated for inter- and intra-rater reliability and has been found to be highly reproducible, with both values between 95%- 97%, although with practice effects which are generally evident in the first 4 trials of the test [415]. These practice effects are much more marked for the 9HPT and PASAT than for the T25WT [416].

The MSFC has been shown to correlate with other measures of disability, including the EDSS itself. Correlation is particularly good for patients with progressive forms of MS [417], although MSFC change correlates poorly with EDSS change over time [418]. There is also good correlation with functional measures such as the Short Form Health Survey-36 (SF-36) score [419], Guy’s Neurological Disability Score [420] and the MS Impact Scale-29 (MSIS-29) [421]. Patients with progressive forms of MS typically score worse than those with RRMS [422]. Limitations include the lack of normative values or an accepted clinically meaningful difference in scores (although some attempt has been made to establish these for the 9HPT and T25W [218]), and the lack of inclusion of...
measures of visual function or mood [422]. Correlation of MSFC with radiological findings is relatively poor [416, 417], although this simply serves to highlight the need for complementary clinical measurement alongside radiological monitoring, particularly in the progressive MS patient group.

Literature relating to more detailed assessments of specific MS symptoms will now be reviewed.

**AMBULATION**

Gait dysfunction is a common symptom of multiple sclerosis, affecting an estimated 75% of patients at some stage in the disease. Typically, it becomes more prevalent with increasing disease duration and severity [143], although it can certainly be present in patients with early stage or mild disease [423, 424]. It can occur for a variety of reasons, such as pyramidal tract dysfunction leading to weakness or spasticity, sensory tract dysfunction leading to impaired proprioception and sensory ataxia, cerebellar dysfunction leading to impaired balance and cerebellar ataxia, and fatigue leading to lack of stamina during ambulation. Furthermore, more than one factor leading to gait impairment is frequently present in the same patient. As outlined above, objective measurement of gait is important in establishing the EDSS step, which is widely used in clinical assessment and clinical trials. Gait is perceived as an important physical function by the majority of PwMS [425] and gait dysfunction has adverse effects on both quality of life [426] and economic parameters including employment status [427] in this population. A number of validated measures of gait are commonly used in assessing PwMS and these will now be discussed.

**Timed 25 Foot Walk Test (T25FW)**

Perhaps the most widely used instrument is the T25W, which was not in fact developed specifically for use in MS patients, but which has been validated in this context and forms part of the MSFC (discussed above). In practical terms, subjects are timed walking the measured distance of 25 feet as fast as possible, although safely, and with the use of an assistive device where appropriate as judged by the treating neurologist. Two trials are performed, with a rest period of up to 5 minutes between the two. Unsurprisingly, given the heavy weighting of the EDSS score towards ambulation, there is a robust positive correlation between T25FW and EDSS [428]. Furthermore, there is a correlation between T25FW score and disease subtype, with longer times being seen in patients with PPMS than SPMS or RRMS. As well as being convenient, easy to administer, rapid to perform and suitable for testing patients with a relatively wide spectrum of walking abilities, the minimal clinically meaningful change for T25FW time has been well-established and repeatedly found to be around 20% [218, 429, 430]. There are a number of variants on this test in existence, including variations in distance (timed
10m and 30m walk tests), variations in pace (“comfortable” vs “maximum safe” pace) or starting position (static vs dynamic), although the standard T25FW is by far the most widely used and the relative utility of these other parameters remains unknown.

**6-Minute Walk Test (6MW)**

Originally developed for measuring ambulation in patients with cardiorespiratory disease, the 6MW has been modified slightly and evaluated in patients with MS. Subjects are asked to walk as far and as fast as possible during the allotted time of 6 minutes [431]. The test is typically performed in a long corridor with cones at each end, and subjects are instructed to pivot rapidly around each cone. The 6MW can reliably distinguish MS patients from healthy controls, has excellent inter- and intrarater reliability (ICC 0.95 and 0.91, respectively [431]) and has been shown to correlate negatively with EDSS score and positively with subjective measures of ambulation (MS Walking Scale; MSWS) and fatigue (mFIS physical subsection). As well as being useful in quantifying ambulation, it can give some indication of the impact of fatigue on physical function, and appears to be more sensitive to the detection of ambulatory impairment in patients with mild disease than the T25FW. However, this test may be extremely demanding for patients with significant fatigue or walking disability and may limit the conduct of further testing. In addition, it requires more space than the T25FW, as subjects require sufficient room for walking and turning repeatedly and without interruption. A shorter version of this (2MW) has also been used [432] but has not been as widely validated in the MS population.

**Timed Up and Go Test (TUG)**

The TUG is simple and easily performed in confined spaces, requiring subjects to stand up from a chair, walk 3m, turn around and return to the chair again in the shortest possible time. Originally developed for evaluating gait in the elderly [433], it has also been evaluated in PwMS and compared to the more commonly used timed walking tests over a longer distance (10m and 30m, in this case). The TUG correlates reasonably well (r=0.85) with these other measures, particularly in patients with more significant MS-related disability (EDSS >4) [434]. An obvious limitation is that functions other than ambulation are tested including balance and turning, although this might give a more accurate reflection of real-life ambulatory function.

**Six spot step test**

This test evaluates balance and co-ordination as well as ambulation *per se* and requires subjects to walk as fast as possible through a rectangular area with six cylindrical blocks inserted at pre-set locations, and to kick each block out of its circular marker, with a complete test requiring subjects to perform the sequence twice with each leg [435]. It has been shown to correlate well with both EDSS (r=0.80) and T25FW (r=0.92), although less well with a patient-reported scale of walking disability,
the MSWS-12 (r=0.69) [435]. Test-retest reliability is good, although the minimally important clinical difference has not been quantified.

**Motion Analysis**

Walking speed is only one aspect of walking, and it is possible for interventions such as rehabilitation or pharmacological therapies to have perceived benefits in the domain of ambulation without improving walking speed. Therefore, attempts have been made to capture these improvements in other ways. Computerized gait analysis systems (e.g. GAITrite [436]) have been used in an attempt to capture some of the nuances of gait impairment in PwMS. At present their use is limited to the research setting by time and resource constraints.

**Real-World Gait Measurement**

Various measures have been attempted to capture aspects of ambulation in real life outside the laboratory as a more realistic index of functional capacity. Modern technology such as pedometers [437] and GPS devices [438] can be given to patients for use at home, although the extent to which they reflect physical activity rather than ambulation specifically is uncertain. In addition, cost and factors related to patient compliance might prohibit their widespread use.

**Self-reported Gait Measures**

The MSWS-12 is a disease-specific 12-point questionnaire using Likert-type responses which was developed with the aim to capture aspects of ambulation and its resultant impact on quality of life over the fortnight prior to the questionnaire being administered [439]. It has been shown to correlate reasonably strongly with EDSS scores (r=0.8), particularly across the lower range of scores from 1-4.5, and to correlate fairly well with objective measure of step count with a mobile accelerometer (r=0.65) [440]. In addition, it appears to be more responsive to change than several other commonly-used instruments, being able to demonstrate improvement in walking in patients who have received steroids after a relapse, and may therefore be of use longitudinally in the clinical trial setting [439].

In conclusion, there are a number of means of quantifying ambulation in patients with MS. Of these, the T25FW is the most widely used in clinical practice and research, forming one third of the MSFC. It is favoured because of its ubiquity, as well as being cheap, rapid to perform, and usable across a
range of disabilities, although it may lack sensitivity in detecting ambulatory disability in patients with lesser degrees of disability in particular.

**UPPER LIMB FUNCTION**

Although not always reflected in the EDSS score, upper limb function is an important determinant of the ability to carry out activities of daily living and the potential for independence. Furthermore, upper limb impairment is common in PwMS, even in the early stages of the disease. A prevalence of 50% has been reported in a cohort of patients with mean EDSS of 3.5, and, unsurprisingly, a higher prevalence in patients with higher levels of disability [441]. Compared to ambulation, upper limb function has been less intensively studied and there is a relative paucity of clinical tools for its objective study.

**9 Hole Peg Test (9HPT)**

The most widely used tool used for quantifying upper limb dysfunction in patients with MS is the 9HPT [442, 443], which is one component of the MSFC. Subjects are required to insert 9 pegs individually into 9 holes and remove them again, in the shortest possible time, with an allotted maximum of 300 seconds. Two trials are administered for each hand and the times recorded. As for other parts of the MSFC, Z-scores are then calculated against established norms which are corrected for age, sex and hand dominance [414]. The 9HPT is easy and quick to perform, and has been demonstrated to have excellent inter- and intra-rater reliability [416]. It has established normative values [444] and an established minimal clinically important difference of 20% [218]. It has also been shown to correlate well with measures of overall activity and independence [445]. The 9HPT is predominantly a test of fine motor function, and does not make any assessment of proximal limb function, gross motor function or bimanual coordination. One disadvantage of the test is that many patients with severely impaired upper limb function may be unable to complete even one trial of the test, limiting its use as a longitudinal comparator in the later stages of the disease.

**Action Research Arm Test (ARAT)**

The ARAT is a 19-item test requiring subjects to handle objects of varying sizes and perform gross movements of the upper limb. It is divided into four subcategories (grasp, grip, pinch, gross movements) and each section is scored on an ordinal scale from 3 (normal) to 0 (unable to perform the movement). It is relatively easy and quick to perform, with a maximum time of 10 minutes per
arm, and provides a comprehensive assessment of proximal and distal function as well as gross and fine motor skills [446]. It has been extensively studied in patients with stroke [447, 448] and has been demonstrated to have excellent inter- and intra-rater reliability [449], and the minimal clinically important difference has been established [450], although it is relatively less well-validated in patients with MS. It is fairly insensitive to mild degrees of abnormality, potentially limiting its use in early-stage or mild disease.

**Box and Blocks Test (BBT)**

The BBT requires subjects to move as many 2.5cm cuboidal blocks from one box to another using one hand at a time within the allotted timeframe of 60 seconds. Like the ARAT, it has been validated particularly in stroke patients [451] but also in MS patients [452] and there are published normative values [453]. It has excellent inter-rater reliability both in healthy controls [453] and in patients with neurological causes of upper limb dysfunction [454]. Being slightly easier to perform than the 9HPT, it can be used across a wider spectrum of disability, and gives some indication of both fine motor functions and proximal movements.

**Jebsen Hand Function Test (JHFT)**

The JHFT measures the subject’s ability to perform seven commonly used functional tests (e.g. writing a sentence, card-turning, simulated feeding) in as rapid a time as possible, with a maximum test time of 120 seconds for each hand and task. The test measures only the time taken to perform each task (with higher scores reflecting more severe disability) without rating the quality of the movement. It is easy and relatively quick to perform, as well as giving a more tangible index of real-life upper limb functional impairment and evaluating a variety of types of movement. Normative values have been established [455] and inter-rater reliability is reasonably good (0.84[456]) but the minimal clinically important difference has not been established and the required equipment for testing may be expensive or difficult to obtain.

**Purdue Pegboard Test (PPBT)**

The PPBT requires subjects to remove as many pegs from a cup as possible in 30 seconds and place them in individual holes [457]. It was originally developed for use in patients with Parkinson’s Disease and has not been completely validated in the MS population, nor has its reliability or minimal
clinically important difference been established. Hence, it offers little benefit in comparison to the more widely-used 9HPT.

**Test d’Evaluation des Membres Supérieurs de Personnes Agées (TEMPA)**

The TEMPA is a functional test of nine manual ADL-like tasks which assess various domains of upper limb function including fine motor skills, proximal and distal limb movement [458]. It has similar properties to the ARAT but is somewhat longer to perform and requires a reasonably good degree of upper limb function. Therefore, it is of limited applicability to more severely disabled patients.

**Self-reported outcome measures**

A number of patient-centred questionnaire-based outcome measures are also in use, including the Disabilities of Arm, Shoulder and Hand scale (DASH [459]), ABILHAND [460], Manual Ability Measure-36 [461] and the Motor Activity Log [462]. None of these have been widely adopted in PwMS.

Of these various outcome measures, the 9HPT is by far the most widely used in the MS population, has the best documented psychometric properties including reliability (although only in the context of deterioration of function, and not for improvement [446]), and is a reasonably good screening test. Like gait, however, upper limb impairment may be complex and multifactorial, and the relative contributions of weakness, spasticity, ataxia, sensory loss and fatigability to the ultimate end result of clinical test performance is not known.

**VISUAL DYSFUNCTION**

The prevalence of clinically measurable visual dysfunction depends largely on the measurement tool used, but visual disturbance is a common symptom and one which is perceived to be of high importance to PwMS [463]. Despite this, the measure of visual function in the EDSS is relatively crude, resulting in likely underrepresentation of the prevalence and functional impact of visual symptoms. There is no measure of visual function in the current incarnation of the MSFC, meaning that this potentially important symptom has been relatively neglected in objective measurements for MS clinical trials [422]. Recent years have seen the addition of visual parameters as secondary
outcome measures in some clinical trials. It is highly likely that visual outcome measures will remain important in the future assessment of potential neuroprotective agents, largely because of the relative accessibility of the optic nerves and the variety of clinical, radiological and electrophysiological measures which can be used to assess optic nerve function to provide a more complete picture of CNS pathology. As with upper limb function and ambulation, there are a number of different methods for quantifying vision in PwMS.

The Snellen chart is the most widely-used measure of high-contrast visual acuity and forms the main part of assessment of the visual FS score in the EDSS. This is a simple test to administer, requiring subjects to read a line of black letters printed on a white background from a fixed distance (typically 6m/20 feet) with each eye individually and with both eyes together, and the score is calculated from the lowest (and smallest) line which can be read at this distance and reported as the patient’s visual acuity relative to that of healthy individuals (6/6 being normal and 6/9 or poorer reflecting reduced visual acuity). In PwMS, high-contrast acuity is a relatively insensitive test [464], mainly because optic neuritis typically affects low-contrast acuity and colour vision, and PwMS report reduced vision-related quality of life without discernible reduction in high-contrast acuity on the Snellen chart [465].

Low-contrast visual acuity or contrast sensitivity testing can be performed via similar means but using grey letters of progressively more faint contrast on a white background. The Sloan and Pelli-Robson charts are the most widely used and are both simple to administer. For the Sloan test, participants are required to read the chart from a fixed distance at 100% contrast (for measurement of Snellen visual acuity), 2.5% and 1.25%, with the letters becoming progressively smaller with each line down, and the score is calculated as the number of letters identified correctly. In the Pelli-Robson chart, all letters are the same size but the type becomes progressively more faint as the subject reads down the chart, and the score is calculated as the logarithm of the score for the lowest line which can be read at a fixed distance. In PwMS the measurement of low-contrast visual acuity has been shown to be a more sensitive measure of visual dysfunction [466] with the ability to detect visual dysfunction in patients with acuity of 20/20 or better [467] and to correlate better with reported real-world symptoms such as difficulties with reading, facial recognition and driving [468] as well as vision-related quality of life [469, 470]. Low-contrast visual acuity testing has already been incorporated into clinical trials of MS therapies, including the AFFIRM study of natalizumab [130, 193], where it demonstrated a reduction in loss of visual acuity over a longitudinal study in treated patients. This measure has the advantages of being simple and easily reproducible, provided conditions (distance, illumination, and correction for refractive error) are kept constant. However, the minimal clinically important difference has not been completely established. It has been estimated at 10 letters [471], although a deterioration of 5 letters may be clinically significant for patients with good visual acuity [472], with 7 letters being the cut-off point for two standard deviations from the norm.
Another benefit of low-contrast acuity testing is its relatively robust correlation with other paraclinical measures of visual function, such as MRI lesion burden, visual evoked potential latency and retinal nerve fibre layer thickness as measured by optical coherence tomography [473]. The relative accessibility of the optic nerve and the robust nature of these correlations make the visual system an attractive target for using as a model for testing new agents for neuroprotection and neural repair [191]. Incorporation of low-contrast acuity testing in the visual FS score of the EDSS or inclusion of a measure of contrast sensitivity in the MSFC might improve the sensitivity of these measures in detecting visual pathology. Clearly there are limitations to the testing of even low-contrast acuity, particularly for patients who have visual difficulties as a result of oculomotor disturbance rather than optic nerve pathology, meaning that even sensitive testing of acuity may not give a true reflection of visual symptoms.

FATIGUE

The objective evaluation of fatigue represents a particularly great challenge for the MS clinician and researcher, and one which is beset by difficulties relating to the subjectivity of the symptom and the lack of a standardised definition for either nature or severity. Nevertheless, it is a challenge which requires to be overcome, given that fatigue is a near-ubiquitous symptom in the MS patient population, with an estimated 80% of MS patients suffering from “pathological fatigue” (fatigue which is sufficiently severe as to cause functional impairment [474]) and the majority of patients citing this as their most disabling symptom. Various definitions of fatigue are used, including “a subjective lack of physical and/or mental energy, perceived by the individual or caregiver to interfere with usual and desired activity”; “reversible motor and cognitive impairment with reduced motivation and desire to rest”; or “difficulty with initiation of or sustaining voluntary activities that does not correlate with muscle weakness, depression, or muscle fatigue”[475, 476]. Vucic and colleagues [397] make the distinction between the “psychological” sense of fatigue, i.e. “a subjective lack of physical or mental energy that is perceived by the individual or caregiver to interfere with activities of daily living” [477] and the strictly “muscular” sense of fatigue, defined as “increasing inability to generate, sustain or repeat a [muscular] contraction”. The extent of contribution from both of these factors is unclear, further muddying the waters of this area of study.

Various questionnaire-based tools are used in the assessment of fatigue for clinical trial purposes, including the 9-item Fatigue Severity Scale (FSS) [201], the 40-item Fatigue Impact Scale (FIS) [200] the 21-item modified Fatigue Impact Scale (mFIS) [478], the 5-item Fatigue Description Scale [479] and the 10-point Visual Analogue Scale [480]. Each is weighted towards a different dimension of fatigue (for example physical, cognitive or psychosocial impacts of fatigue). Some are concerned with the symptom of fatigue itself and others with the disability experienced as a result. Most scales
use the more subjective or psychological rather than the muscular definition of fatigue; for example, the mFIS begins “Fatigue is a feeling of physical tiredness and lack of energy that many people experience from time to time, but people who have medical conditions like MS experience stronger feelings of fatigue and more often and with greater impact than others”. The mFIS also makes some attempt to assess the multi-dimensional nature of fatigue, with separate subscores being calculable for physical, cognitive and psychosocial domains [200].

Although all of the above-mentioned scales have been validated for use in patients with MS, the lack of a standardised definition of fatigue makes comparison between these different tools difficult, and there is no current consensus that one tool is of much greater validity than the others. Studies of symptomatic treatments for fatigue have used a variety of outcome measures, limiting their comparability and making pooled meta-analysis challenging. A Cochrane review of the effects of amantadine on MS-related fatigue included 5 randomised controlled trials, each with a different measure of fatigue (and perhaps unsurprisingly with inconclusive results overall) [214]. Various outcome measures have been employed in studies of the non-amphetamine stimulant modafinil [481, 482], limiting comparability, and once again the magnitude of benefit remains uncertain.

Although fatigue is included in the EDSS it is seldom given much weight in this context because of its resistance to objective measurement, and the presence of fatigue generally does not impact on the cognitive FS score in most clinical trial protocols. There is interest in using objective electrophysiological tools [483, 484] in the adjunctive measurement of fatigue, but this has not yet translated into the clinical trial setting or to clinical practice.

**SOMATOSENSORY DYSFUNCTION**

Although sensory impairment is common among MS patients, being present in around 25% of patients at disease presentation [485] and up to 70% of all MS patients [486], its subjective nature and liability to be influenced by other factors (such as mood, fatigue and motor impairment) make objective and reproducible sensory testing extremely difficult. Objective measurement of sensory function is desirable because of the prevalence and morbidity associated with sensory dysfunction in its own right, as well as the possibility that sensory dysfunction can impact on motor function and thus affect performance on objective tests of motor parameters [487] and thus on the EDSS or MSFC score. Experimental work in the peripheral nerve arena has suggested that impairment of median nerve cutaneous sensation (by infiltration of local anaesthetic around the median nerve) can impair functional performance (writing) without affecting motor strength [488] and it is well-accepted that adequate motor control is reliant to some extent on at least some sensory feedback. A small study in a group of MS patients found that performance on the 9HPT correlated significantly with light-touch
pressure sensation on the thumb and index fingers (measured using Semmes-Weinstein Monofilaments), two-point discrimination of the index finger (using an aesthesiometer) and the strength of elbow extension (measured using a dynamometer) [489].

Various methods can be used to try to quantify sensory parameters, including monofilament testing (where participants are touched with progressively greater forces in order to ascertain when they first feel a tactile stimulus), vibration testing using measurable tuning forks, object recognition testing and two-point discrimination testing. For example, the Vibatron device (a quantitative assessment of vibration sense, using a rod with variable intensity of vibration) has been validated in the MS patient population [490] and shown to correlate with EDSS in a group of patients with RRMS, and to be more sensitive in detecting sensory dysfunction than the sensory FS score from the EDSS. Quantitative testing of sensory function should include testing of dorsal column modalities (i.e. vibration and proprioception) [491] and spinothalamic sensory function (pain and temperature) [492], although in neither case is there a particularly robust correlation with clinical symptoms, suggesting that quantitative testing is fairly insensitive for both groups of modalities [308].

A large body of literature exists in relation to sensory impairment after stroke, which affects an estimated 50 to 85% of stroke survivors [493]. In this group of patients, as in the MS population, sensory impairment is known to adversely affect quality of life and functional outcomes, but is often neglected in the clinical and rehabilitation settings [493, 494]. Within the stroke rehabilitation setting, a number of quantitative clinical tests have been developed which can reliably assess the multidimensional aspects of sensory function in the upper limbs. Those which were included in this study will now be discussed in more detail.

The Wrist Position Sense Test (WPST) [495] is a tool for the quantitative measurement of proprioception. This test was developed to service an unmet need among stroke patients, that is, a test of proprioception which did not rely on having a contralateral normal limb for comparison (such as the Kinesthetic Acuity Test [496] or the Kinesthesiometer Test [497]) or sufficient motor function for the patient to reposition a limb unaided (such as in the Kinesthesia Test [498]). The WPST requires the subject to place their hand and wrist in a splint device behind a curtain (to remove visual cues). The splint is positioned directly above a scale for the measurement of degrees. The wrist is then passively moved by the examiner through a variety of positions, and the subject has to indicate (either verbally or by moving a pointer) the position in which they perceive their wrist to be (see figure 4). The mean error and standard deviations are then compared to normative values for healthy control arms.

The Tactile Discrimination Test (TDT) has also been validated in patients with stroke as part of the Sensory Discrimination Capacity [493]. Not only is this modality important in a functional sense in its own right (for example, feeling for coins in a trouser pocket), there is evidence that impaired tactile
sensation is strongly associated with impaired motor performance [488]. As for the WPST, although a variety of tests exist which can be used in the assessment of tactile discrimination [499], most of them are confounded by the difficulties of testing on patients with impaired upper limb motor function, making it difficult to ascertain the reason for poor test performance. The TDT [500] circumvents this issue by using similar apparatus to the WPST described above (see figure 5). Again, participants place their hand behind a curtain to eliminate visual cues, and their hand is placed over a series of ridged gratings which vary in the width of their ridges. Participants are asked to indicate which of three gratings is the “odd one out” and whether it is rougher or smoother than the other two. Published normative values exist, the test has been validated for reliability and clear differences exist between healthy controls and stroke survivors.

Cortical sensory function has an obvious functional importance and is not reliably assessed by the measures described above. Arguably it may be more relevant to patients with stroke (with a predominantly cortical lesion) than MS, although cortical dysfunction can and certainly does occur in PwMS. Recognition of objects by touch requires both tactile exploration of the object and cortical sensory processing or gnosis in order to recognise the object. The Functional Tactical Object Recognition Test (fTORT) [501] has also been validated in stroke survivors as part of the Sensory Discrimination Capacity, and requires subjects to identify an object based on touch from a poster depicting 30 potential options (see figure 6). Objects may vary in terms of weight, size, material and other factors, and participants are scored according to how accurately they are able to identify the presented objects. None of the three aspects of the Sensory Discrimination Capacity has yet been validated in the MS patient population.

In summary, although somatosensory dysfunction is very common in patients with MS, it is often not measured quantitatively, and as a result its impact may not be reliably detected. As well as being an important and potentially disabling symptom in its own right, it can also impact on motor function and as such should be quantified where possible in the clinical research setting. The above objective tests have been validated in stroke patients but have not to date been tested in the MS population.

**COGNITION AND MOOD**

Cognitive function and mood are also difficult to evaluate objectively, but are important correlates of functional disability and do warrant objective measurement. These functions are challenging to measure as part of the standard neurological examination and are poorly reflected in the EDSS score. Furthermore, patients’ subjective reports of cognitive function are by definition likely to be unreliable and highly likely to be confounded by mood, fatigue and other subjective factors. The objective measurement of cognition is likely to assume an even greater importance in the evaluation of potential therapeutic agents for progressive MS, which is more strongly associated with cortical dysfunction.
and thus more likely to affect cognition and memory. Again, a variety of tools exist for the objective clinical evaluation of cognition in MS patients and these will now be discussed.

**Paced Auditory Serial Addition Test (PASAT)**

The PASAT is probably the most widely used test of cognition in patients with MS and forms one third of the MSFC, along with the T25W and 9HPT. The test requires subjects to listen to a recording where one number is read aloud every 3 seconds. Participants are required to add the number to the one heard previously. The PASAT is relatively time-consuming and fairly unpopular among MS patients [502], being perceived as difficult and requiring some level of mathematical ability. Furthermore, it has only a weak correlation with EDSS [503] and is strongly affected by practice effects [416, 504, 505], but has been demonstrated to correlate with MRI evidence of current disease activity as indicated by gadolinium enhancement [506].

**Symbol Digit Modalities Test (SDMT)**

The SDMT has been proposed as a viable alternative to PASAT and it has been suggested that it might more appropriately form part of the MSFC [503]. This is a briefer and easier test to administer, requiring subjects to write down the symbols corresponding to a series of numbers in a short space of time. SDMT has been demonstrated to correlate better with MRI white matter lesion burden [236] and is preferred by patients over the alternative PASAT [502]. However, on functional imaging, it appears to test a different sphere of cognitive function, with a greater reliance on subcortical over cortical networks (PASAT is associated with objective evidence of bifrontal cortical activation on fMRI [507]). SDMT is liable to confounding by visual dysfunction, which is less the case with PASAT.

**Brief Repeatable Battery (BRB)**

The SDMT and PASAT both form part of the BRB, a more in-depth neuropsychological assessment battery which was specifically developed for use in the MS patient population [508] and viewed as something of a “gold standard” in this setting. In addition to the SDMT and PASAT, it comprises the Selective Reminding Test, the 10/36 Spatial Recall Test and the Word List Generation test. Normative data exist, as well as corrections for practice effects [509]. However, its relatively time consuming nature (taking around 45 minutes to administer) makes it impractical for widespread use in the clinical trial setting where cognition is only one of several components assessed.

**Mood Disorder Screening**

There are a number of tools which can be used for screening for the presence of mood disorder in PwMS and have been specifically validated for use in this population, although most are
questionnaire-based and, possibly due to their time-consuming nature, are seldom used in clinical practice. They include the DSM-IV assessment and Beck Depression Inventory [510-512], both of which are widely used in patients without neurological disease. There is some evidence that a very brief 2-question tool [513] may be just as sensitive as the more in-depth evaluation, and much more straightforward to administer [514]. Assessment of suicidality is increasingly being used in MS clinical trials [515] but limited data are available regarding longitudinal assessment of MS patients using the above tools, sensitivity to change over time and assessment of mood disorders other than depression.

**BOWEL AND BLADDER DYSFUNCTION**

Tools such as the EDSS [11] and Guy’s Neurological Disability Scale [516] do include some assessment of bladder and bowel function, although no objective tests are carried out and data is based on patient self-report. It has been demonstrated that for bladder function in particular, self-reported data correlate poorly with objective measurement (post-micturition residual volume) [517] and are therefore an unreliable indicator of the true prevalence and severity of bladder dysfunction, particularly at the milder end of the scale. Despite this inadequacy, there is a paucity of published literature regarding longitudinal assessment of bladder and bowel function in this population, and in current practice monitoring is limited to asking patients regarding symptoms at serial visits.

**Conclusion**

There are limitations associated with the currently-used objective tests used to quantify function in PwMS. However, clinical assessment in the context of normal outpatient attendances and clinical trials is limited by time and resources. The currently-used tools of the EDSS and MSFC are particularly limited in their capacity to quantify visual impairment, fatigue, cognition, mood and sphincter functions, and it may be a requirement for future clinical trials to more thoroughly assess these domains, particularly in patients with progressive MS. In addition, there is growing interest in the potential use of objective paraclinical measures, including electrophysiological and radiological biomarkers such as those discussed in the previous chapter, which can complement the objective assessment of these domains and which may provide useful adjunctive information in trials of disease-modifying and symptomatic treatments.
Chapter 6: The role of fampridine and related drugs in symptomatic treatment in MS

In this chapter, the symptomatic therapy fampridine which is licensed for the treatment of ambulatory dysfunction, its mode of action and related drugs and the existing literature regarding their use in the treatment of symptoms of MS will be discussed.

Fampridine and Related Drugs: Mechanism of Action

The key pathophysiological event in MS is demyelination, where inflammation results in damage to the myelin sheaths of central nervous system neurones, with varying degrees of axonal loss occurring most likely as a secondary phenomenon. In healthy neurones, the myelin sheath functions as an electrical insulator, improving conduction along the axons. Demyelination causes conduction block and symptomatic neurological dysfunction, likely related to alterations in the function and distribution of voltage-gated ion channels at the site of the demyelinating lesion [518]. The hypothesis that overcoming conduction block in demyelinated axons could improve symptoms of MS led to the trial of the potassium channel blocking aminopyridines, 4-aminopyridine (4-AP; Fampridine) and its close relative 3,4-diaminopyridine (3,4-DAP), in the symptomatic treatment of MS.

How do the aminopyridines work in MS patients?

In order to understand the rationale for the use of these treatments, it is first necessary to understand a little of the physiology of action potential propagation and conduction along myelinated nerves under physiological conditions. As with all excitable cells, communication within and between neurones requires the generation of action potentials, defined as momentary changes in the surface potential of the cell. In neurones, these are generated by voltage-gated ion channels embedded within the cell membrane. These are pores which selectively allow the passage of ions such as sodium, potassium, chloride and calcium in and out of the cell. Voltage-gated sodium channels are present in high concentrations at the Nodes of Ranvier and facilitate the generation of action potentials. Voltage-gated potassium channels are clustered in the nodal and paranodal regions, beneath the myelin sheath, and act as modulators of neuronal excitability [519].

Under physiological conditions, an ATP-dependent sodium-potassium (Na⁺/K⁺) pump maintains the gradient between the intracellular and extracellular environment. In response to changes in the resting membrane potential, activation of voltage-gated ion channels causes a conformational change resulting in the passage of ions along the existing electrochemical gradient. At the onset of the action potential, sodium channels open and allow an influx of sodium into the cell, producing a rise in the membrane potential. This causes more ion channels to open until the membrane potential has reached its maximum value (around 55mV with all available channels open). At this point the reverse in polarity of the local cell membrane results in closure of sodium channels, active transport of sodium out of the cell in exchange for potassium using the energy consuming Na⁺/K⁺ pump and opening of
potassium channels with efflux of potassium from the cell causing return of the membrane potential to first hyperpolarization and then back to its resting state (around -70mV for an axon). In general, voltage-gated sodium and calcium channels cause a depolarization of the cell membrane and are therefore excitatory. Voltage-gated potassium channels typically move the cell towards repolarization and are therefore inhibitory.

In MS, symptoms of neurological dysfunction occur because of impaired conduction of action potentials along axons whose myelin sheaths have been damaged by an attack of inflammatory demyelination or due to axonal transection with the result that axons can no longer transmit action potentials to the desired destination. In demyelinated axons, the structural and functional relationships of ion channels on the neuronal membrane are affected [520] by an impedance mismatch brought about by the abrupt change in surface area at the junctions between myelinated and demyelinated parts of the axon. As a result, the resting membrane potential of the cell is decreased, and a greater inward current is required in order to generate an action potential. This may be partly mediated by circulating inflammatory cytokines or antibodies [519] which can block sodium channels at the Nodes of Ranvier, resulting in defective action potential generation [521], or possibly by exposure of K+ channels situated underneath the myelin sheath.

Blockade of potassium channels has relatively little impact on conduction in healthy, myelinated, nerve fibres, but because of the dense packing of K+ channels beneath myelin, areas of demyelination result in exposure of these channels and for this reason they are believed to be a key mediator of MS-related neuronal dysfunction [520]. It might therefore be expected that selective blockade of potassium channels could promote depolarization and therefore improve conduction along the axon. The aminopyridines are non-specific blockers of voltage-gated potassium (K+) channels; they differ in terms of their potency with 3,4-DAP having the highest affinity for the K+ channel. The hypothesis that such drugs could improve conduction in demyelinated axons was tested initially in vitro by Bostock who studied the effect of 4-AP on experimentally demyelinated axons (treated with diphtheria toxin). He demonstrated a prolongation and increase in amplitude of the action potential in demyelinated and unmyelinated nerve fibres, reversal of conduction block in demyelinated axons and an increase in the temperature at which conduction block occurred [522, 523]. This finding has subsequently been replicated by other authors [524].

Restoration of conduction via modulation of ion flux and maintenance of the resting membrane potential is only one mechanism by which the aminopyridines might exert their effect. There is also reason to believe that these drugs have the capacity to augment neuromuscular transmission [525]; for this reason, 3,4-DAP is used in the treatment of the Lambert-Eaton Myasthenic Syndrome and botulism (where there is a defect in pre-synaptic acetylcholine release [526]). The relative contribution of these two actions is still debated. Bostock’s original in vitro experiments were
performed in peripheral nerves, whereas MS is a disorder of CNS demyelination. Further, the relative concentrations of 4-aminopyridine used for in vitro experiments on demyelinated axons were 1000-5000 times higher than that used in clinical practice in humans, where the dose of 4-AP is limited by its dose-dependent pro-convulsant effect [527]. With this in mind, Smith [527] attempted to replicate the earlier work of Sherratt and Bostock using more clinically relevant lower doses and experimentally demyelinated rat central (rather than peripheral) nervous system axons, and in doing so failed to show a benefit from 4-AP in terms of restoring conduction (although when higher doses were used, the findings of earlier experiments from peripheral nerves could be replicated [527]. They also assessed the effect of 4-AP on synaptic transmission as measured by the H-reflex and dorsal root reflex, and demonstrated a marked and dose-related increase in amplitude of both reflexes in treated nerves. In addition, there was a dose-related increase in twitch tension of the muscle response to sciatic nerve stimulation in 4-AP treated nerves. Notwithstanding potential differences between the animal model and the human subjects, this would add weight to the notion that 4-AP exerts at least some of its beneficial effects via the augmentation of synaptic transmission, and not simply by overcoming conduction block in demyelinated axons. Consistent with this is the observation of symptomatic benefit from the aminopyridines in patients with non-demyelinating disorders of the central nervous system, such as patients with incomplete spinal cord injury where axonal injury is likely to be a greater contributor than focal demyelination [528, 529], the clear benefit in patients with disorders of neuromuscular transmission [526] and the lack of apparent benefit in peripheral nervous system disorders which are clearly demyelinating, such as Guillain-Barre Syndrome and Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) [530, 531].

Interestingly, there appear to be differences in the response of sensory and motor nerves to the administration of 4-AP. Kocsis [532] studied the effects of 4-AP on individual demyelinated axons removed from the ventral (motor) and dorsal (sensory) nerve roots in rats, and reported differential patterns of response. Motor nerves responded with an increase in duration of a single action potential, whereas sensory nerves responded with a burst of firing in response to a single stimulus. The reason for this differential response is not known, but the hyperexcitability seen in sensory fibres may be a plausible explanation for the frequency of paraesthesias and dysesthesias which are often reported clinically after drug administration in humans. Although MS is a disease of the central nervous system, there are clear differences in PwMS relative to healthy controls in patterns of peripheral nerve axonal excitability, possibly due to up-regulation of K⁺ channel expression in response to axonal injury [533] (similar changes are also reported in other CNS lesions such as spinal cord injury [534]). These can readily be measured by non-invasive techniques in vivo, and there is some evidence that 4-AP may have the ability to normalise patterns of peripheral nerve excitability in PwMS [535]. In a small study of 13 PwMS, these changes in peripheral nerve excitability correlated with an
improvement in grip strength and a self-reported (though not objective) improvement in walking ability.

It has also been suggested that 4-AP may have some immunomodulatory action. This hypothesis has arisen from the fact that the drug acts not only on potassium channels involved in transynaptic neurotransmission but also on potassium channels situated on non-excitible, immunologically active cells such as T cells, B cells, macrophages, astrocytes and dendritic cells, all of which are known to be important in the pathogenesis of MS [536]. Certainly, there is evidence from other autoimmune diseases of an upregulation of potassium channel expression on immunologically activated T-cells [537, 538], and similar findings have been described in pathological studies of brain tissue and CSF from MS patients[539]. In vitro experiments using an animal model of the inflammatory demyelinating peripheral neuropathy Guillain-Barré Syndrome (Experimental Allergic Neuritis) have provided evidence that the potassium channel blocker quinidine could improve physical function and decrease inflammatory infiltrate around the nerve [540]. Similarly, upregulation of potassium channel expression has also been described on B-cells [541], dendritic cells [539, 542] and microglia [543].

While these data are clearly interesting, the idea of 4-AP as an immunomodulatory agent would not sit well with the observed clinical benefits of the drug. The majority of available MS therapies are immunomodulatory in nature, and exert their effect in the relapsing-remitting, inflammatory phase of the disease. Fampridine-MR by contrast has been shown to be effective across the subtypes of MS, including in patients with progressive disease [160], when axonal degeneration rather than inflammatory activity is thought to be responsible for the accumulation of disability. Furthermore, the effects of fampridine-MR in responders are relatively rapid in onset and quickly reversible on cessation of the drug [544], arguing against a mode of action which is primarily immunological in nature.

Pharmacokinetics of Fampridine

4-AP is highly lipophilic and easily crosses the blood-brain barrier (BBB), in contrast to 3,4-DAP, which is poorly lipid-soluble and as a result does not easily cross the BBB, exerting most of its effects in the PNS. The primary mode of excretion of 4-AP is renal; pharmacokinetic studies have indicated a significant correlation with degree of renal impairment for both plasma 4-AP concentrations and half-life [545]; renal impairment is therefore a relative contraindication to use of this drug. The drug is absorbed rapidly when given via the oral route with peak plasma concentrations achieved at around 20-60 minutes after drug ingestion in the immediate-release form of fampridine, with a serum half-life of 1-3 hours [546]. For fampridine-MR, the time-to-peak after oral administration is around 5 hours and the serum half-life 5.2 hours [547]. The optimal dosing interval for steady-state plasma concentrations using the modified-release form is twice daily, 12 hours apart [548], and the recommended dose is 10mg bd. Seizures are much more likely to occur above a dose of 20mg bd.
This narrow therapeutic window is most likely a reflection of the relatively poor selectivity of the drug as a potassium channel blocker. Furthermore, higher doses of drug are not associated with greater likelihood of response [550]. By contrast, adverse effects do appear to be a dose-related phenomenon, with paraesthesias and dysaesthesias reliably appearing at plasma concentrations of 0.5mg/kg, and seizures occurring in humans at around 0.8mg/kg [551, 552].

**Adverse Effects of Fampridine**

4-AP was originally developed as an avicide, with the observation that only a small number of birds need to ingest the drug and they would then become confused and emit a distress cry “which touches a deep-rooted instinct in other members of the flock to avoid undesirable places” [553]. Information regarding the toxicity of 4-AP comes from animal data and case reports of effects on humans when the drug is taken in overdose. Intravenous administration of 4-AP in overdose to animal subjects produces symptoms of toxicity within 15 minutes. These include seizures, ataxia, tremors, dyspnoea, excessive salivation and pupillary dilatation [554]. The Lethal Dose 50 varies widely between species, with the majority of deaths in animal experiments being due to seizures [554].

Early case reports described a group of healthy young factory workers who took high doses of 4-AP in error, mistaking it for the aphrodisiac “Spanish fly”. They each consumed “a pinch” of 99% pure 4-AP with morning coffee and within minutes, all three developed a bad taste, abdominal pain and a burning of the throat. One individual self-induced vomiting but the other two developed seizures and obtundation, but recovered with supportive therapy [553]. Another early report described a body-builder who consumed toxic doses, believing it to be an anabolic steroid. He was afflicted by similar side-effects [555]. Seizures have also been described in PwMS on fampridine, although the doses and formulation taken were unknown [556]. Seizures are thought to be a dose-related phenomenon, being rare at the clinically used dose of 10mg bd, unless there is associated renal impairment [548].

A small number of cases of deliberate overdose of fampridine in MS patients have been reported. As expected, seizures occur when the drug is given in overdose, along with tremors, autonomic instability, abulia, cognitive deficits, dystonias, chorea, cardiac arrhythmias and effects on skeletal muscle [551, 557, 558]. In fact, there is documentation of MRI evidence of T2/FLAIR hyperintensities in the mesial temporal lobes, in a pattern most in keeping with limbic encephalitis, in a patient with 4-AP overdose [557], probably because of direct neurotoxic effects. Management of overdose is supportive, and consists predominantly of anticonvulsants (chiefly benzodiazepines, but also phenytoin which is a sodium channel blocker and may be of particular use in stabilising membrane potentials in this context) with respiratory and cardiovascular support as required [551].

In terms of side-effects seen at standard doses in humans, paraesthesiae (likely to be explained by drug-induced hyperexcitability of sensory nerves as discussed above [532]) and dizziness are the most
common non-serious side-effects, whereas seizures are the most potentially serious side-effect. Sensory side-effects are far more commonly reported with immediate-release preparations than fampridine-MR [559].

Clinical Benefits of the Aminopyridines in MS Patients

A number of studies have evaluated the aminopyridines in the symptomatic treatment of various MS symptoms and these will now be reviewed. Benefits have been reported in the domains of ambulation [161, 548], motor function [544], vision [195] and fatigue [560].

Early reports of possible symptomatic benefits from 4-AP were generally from small-scale, uncontrolled trials and therefore prone to bias and placebo effects, which is a particular issue with studies of symptomatic treatments. Investigators from two small studies which used evoked potentials as a more objective outcome measure have reported changes in electrophysiological outcomes [561, 562], although these have not always been correlated with clinical benefits.

3,4-DAP has also been reported to improve clinical signs and symptoms in PwMS [563]. In comparison to 4-AP, however, 3,4-DAP is much less lipophilic and as a result has a much poorer ability to cross the blood-brain barrier, with documented levels of drug in CSF being around 10% of serum concentrations [563]. However, the BBB is likely to be damaged and more permeable in patients with MS, and 3,4-DAP is a more potent potassium channel blocker than 4-AP, hence the rationale for its being trialled in this population. Polman [564] compared the two agents directly in a crossover trial in 24 patients with MS, 10 of whom were proven responders to 4-AP and 14 non-responders. They reported a greater incidence of side effects of paraesthesias and abdominal pain in the 3,4-DAP group. In terms of efficacy, there were no patients who responded to 3,4-DAP who had not responded to 4-AP, and the frequency and magnitude of responses were lower in the 4-AP responders when treated with 3,4-DAP. Consequently, only 4-AP has been further used in PwMS.

General Improvement

A double-blind cross-over study randomised 70 patients with MS to fampridine or placebo for 12 weeks each [565]. The primary outcome measure was an improvement of 1 point on the EDSS score or a positive subjective response from the patient during treatment with active drug. Secondary outcomes included VEPs and eye movement registrations (EMR). EEG’s were recorded in all subjects. 10 of 70 patients improved by one point or more in EDSS during fampridine treatment (due to improvements in “pyramidal function” in all cases), while none improved during the placebo
period. Eighteen patients reported a subjective improvement during fampridine treatment, compared to only one during placebo treatment. Conversely, two patients reported a subjective deterioration with fampridine compared to 15 with placebo. A significant decrease of VEP latency (-3.81ms) was reported, but without any corresponding improvement in visual function. There was a high incidence of minor side effects (such as paraesthesiae and dizziness) associated with fampridine treatment. No seizures were reported although one patient did have asymptomatic epileptiform activity measured on EEG during drug treatment. Thus a response rate of 29.5% was reported for the group as a whole, with largely acceptable side effects. Responders were more likely to report temperature-sensitive symptoms at baseline (Uhthoff’s phenomenon). Both fampridine and a normalisation of core temperature in patients with heat-sensitive symptoms would be expected to reduce hyperpolarisation, prolong the action potential duration and ameliorate conduction block. It has not been established whether a combination of these two strategies might produce greater benefits than either in isolation.

A Cochrane Library Review in 2002 [566] concluded that there was insufficient evidence to support the clinical use of aminopyridines in the symptomatic therapy of MS. Publication bias was cited as a major issue in this area, together with the problems caused by heterogeneous outcome measures and lack of selection of clinically meaningful outcomes. However, since this review was published, further literature has become available which has changed the landscape regarding the therapeutic use of fampridine, and this will now be discussed.

**Ambulation and Lower Limb Function**

Bever [567] studied 36 patients with MS in a double-blind trial and randomised them to placebo or 3,4-DAP. They reported a significant improvement in lower limb strength (as measured by dynamometry), but found that side-effects were treatment-limiting in nearly one quarter of patients with the doses used (an escalating dose paradigm of up to five times greater than the standard dose used in practice).

One of the earliest randomised controlled trials was a small double-blind crossover study carried out by Schwid [171] who randomised 10 patients with MS and stable but significant disability (mean EDSS 6.5) to oral 4-AP 17.5mg bd or placebo for a duration of 1 week. These authors reported a benefit in terms of gait in nine out of ten patients on drug as compared to placebo (with an improvement of mean gait speed for 8MWT from 35 seconds to 27.9 seconds) and a subjective overall improvement in global health for seven out of ten patients on drug compared to placebo which did not reach statistical significance. These authors noted a trend towards greater effects with an increased serum level of 4-AP.
The majority of the supporting evidence for fampridine-MR use in treating ambulatory disability comes from the work of Goodman [160] who conducted a double-blind placebo-controlled randomised controlled trial of fampridine-MR 10mg bd in 301 PwMS, using performance on T25FW as the primary outcome. “Responders” were defined as having a consistent improvement of walking speed (magnitude of improvement was not specified) in at least three out of the four follow-up visits; 35% of the drug treatment group and 8% of the placebo group (p<0.0001) met the criteria for “response” with a mean improvement in walking speed of 25%. Unsurprisingly, responders had better performances on the MSWS-12 score, a self-reported scale of ambulation-related disability, and both responders and non-responders on drug treatment recorded greater lower extremity manual muscle strengths than the placebo group. In terms of treatment-related adverse events, one drug-treated patient developed an anxiety attack and one had a focal seizure in the context of significant intercurrent illness. Otherwise the drug was well-tolerated with low rates of discontinuation. Responders could not be differentiated from non-responders on the basis of disease duration, course, symptoms or any other baseline clinical or demographic features.

The same authors conducted a subsequent study of 240 patients using the same criteria for clinical responders and replicated their original findings [161]. They reported a response rate of 42.9% in the fampridine-MR group and 9.3% in the placebo group, suggesting a number needed to treat of three to produce one favourable clinical response. The mean improvement in walking speed for responders was 24.7%. Measurement of plasma fampridine-MR concentrations revealed no significant difference between responders and non-responders.

A condition of the post-marketing regulation of fampridine-MR was the conduct of a further placebo-controlled study using a lower dose of fampridine (5mg bd) and comparing this to 10mg and placebo in a 3-arm blinded randomised trial. Rather than the assessment of multiple walking speed measurements averaged out as was used in the original studies by Goodman [160], the primary outcome measure used was a single-timepoint assessment of walking speed with the T25FW relative to baseline after 4 weeks of treatment, with secondary outcomes including the MSWS-12 and the 6MWT. 429 patients were randomised, and the study showed a dose-dependent trend for faster walking speeds in the treated group, which failed to reach statistical significance. Similar findings were reported for the MSWS-12 secondary endpoint. A post-hoc analysis which employed similar methods to the original pivotal trials (i.e. average of “on drug” performance rather than single timepoint) reported a significantly greater proportion of patients who improved by ≥20% on T25FW in the fampridine-MR 10mg group relative to the lower dose or placebo groups. Improvements in walking speed of ≥20% (regardless of treatment group) were also associated with significant improvements on the MSWS-12, corroborating the idea that this degree of change produces a discernible functional benefit [218]. In addition, the post-hoc analysis demonstrated a three-fold improvement (P<0.014) relative to placebo (with a non-significant trend towards improvement
compared to low-dose fampridine) in the 10mg bd arm with respect to distance achieved on the 6MWT. The authors suggested that the reason for this failure to achieve the primary endpoint was the limited test-retest reliability of the T25FW and the variability associated with this measure, which was accounted for by the use of averaging in the original trials. In addition, the measurement was made during a lengthy study visit on two occasions four hours apart, in order to compare the findings at peak and trough levels of drug, and this may have contributed to patient fatigue and variable performance on the test which was used.

A subsequent post-hoc analysis explored these findings in more detail, specifically in relation to the test of endurance (6MWT) [568]. These investigators reported a significant difference in change in 6MWT from baseline to 2 weeks in the 10mg group which was not seen in the lower dose group relative to placebo. Similarly, the proportion of patients who attained an improvement of ≥20% (used as the minimal clinically important difference, although this has not been established for this particular test and is extrapolated from the T25FW) was significantly greater with the fampridine 10mg group relative to the two other arms. Thus, although there is proven to be a good correlation between these two measures of walking in cohorts of patients with MS [569], it may be that the benefits of fampridine-MR are easier to detect with a more endurance-based assessment of walking, which may be more sensitive in this context.

The MOBILE study [570] was a double-blind randomised controlled trial in 132 PwMS with ambulatory dysfunction. In place of the T25FW, outcome measures included the MSWS-12, the timed up and go (TUG) test and the Berg Balance Scale. Treatment duration was 24 weeks and there was a trend towards improvement in all measures which was seen in the early weeks following treatment initiation and reversed on cessation of the drug, but which only reached statistical significance on a small number of measures (specifically, MSWS-12 thresholds and TUG test speed thresholds). These data warrant replication in a suitably powered study (currently in progress [571]) but provide evidence for the notion that walking speed may not be the only function which can reflect improved ambulatory function in drug-treated patients. In this study, the benefits were seen as early as two weeks post-treatment onset, suggesting that response can be determined during a relatively short trial period.

An open-label post-marketing study followed 120 PwMS who were being treated with fampridine-MR for ambulatory dysfunction and attempted to characterise the full profile of ambulatory benefits seen with drug treatment [168]. All participants received two weeks of treatment, which was then discontinued in non-responders; responders were classified according to a composite response criterion as having achieved an improvement of at least 15% in at least one of the following parameters: speed (T25FW), endurance (2MWT) or self-reported walking ability (MSWS-12). From the original group of 120, 112 were assessed at day 14 and of these 83 (74%) were classified as
responders (the high rate of “responders” may reflect the relative leniency of the composite response criteria, although even when the relatively stricter criterion of 20% improvement on T25FW alone was used, response rate was still higher at 50.9% than in the original placebo-controlled trials). Responders could not be predicted on the basis of demographics or disease characteristics. When responders were defined according to the relatively stricter criteria of 20% improvement in T25FW only, the population of responders also showed benefit in the measures of endurance (2MWT), self-reported walking ability (MSWS-12), gait cadence (steps per metre) and step length. Finally, the responder group showed benefits in all other outcomes assessed, specifically the 9HPT (albeit with a small magnitude of response, around 11%, below the minimal clinically important difference [218]), fatigue and quality of life measurements. Non-responders in terms of gait did not show benefit in any other domain except quality of life overall. The authors also made the point that interpretation of the T25FW should be different for patients with faster baseline times (specifically <8 seconds) as the test is prone to ceiling effects and may not detect clinically significant improvements in this group, being more sensitive in patients with T25FW times in excess of 8 seconds.

Some attempts have been made to characterise how fampridine exerts its effects on gait, and to differentiate responders and non-responders based on clinical and demographic parameters. A single-centre study examined 61 patients with MS and gait impairment and randomised them to fampridine 10mg bd or placebo in double-blind crossover design. A number of measures of gait were used, including T25FW, 6MWT, 3D motion analysis, balance scales and an accelerometer to measure general levels of physical activity, as well as questionnaire-based self-assessment of ambulatory function [572]. 31% of patients met the criteria for responding to treatment based on attaining faster speeds on T25FW (the majority of these also displayed improvement in 6MWT), although the magnitude of response in responders was lower than that which was reported during the original pivotal clinical trials, with an average 14% improvement in walking speed rather than 20-25%. Possibly because of the smaller magnitude of change, no benefit was discernible in the self-reported MSWS-12, however T25FW responders did show greater levels of physical activity based on accelerometer measurements (interpreted as a consequence of improved ambulation, rather than a cause, and likely reflecting the functional benefits associated with a beneficial effect of drug treatment). Analysis of gait pattern of responders at the individual level showed some heterogeneity in terms of how fampridine modulated the gait. For example, some subjects demonstrated clear improvements in range of motion at the knee, others more at the hip joint, and some showed more benefit in the more-affected limb while others showed greater improvements in the less-affected limb. So while gait pattern changes were documented, and did correlate with improvements in the T25FW and 6MWT, the nature of the pattern changes was inconsistent and no clear trends could be identified to determine which patterns of gait abnormality might respond best to this drug.

**Upper Limb Function**
A small body of literature exists regarding the potential benefits of fampridine on upper limb function, but most studies are small and uncontrolled. Most recently, Savin [170] examined upper limb function in an open-label study of 26 patients with MS, using the outcome measures of hand grip strength, 9HPT, functional capacity as measured by the Arthritis Hand Function Test (AHFT) and various questionnaires reflecting the functional impairment associated with the upper limb deficit. They reported an increase in hand grip and pinch strength only in participants’ dominant hands which was evident by one month of treatment and persisted after three months; in addition they reported a reduction in time to complete the 9HPT at one month (which was not statistically significant) and three months (which was, but which failed to reach the minimal clinically important difference of 20% [218]). In the AHFT of various real-world measures of upper limb function, participants showed faster times on the safety pin task, buttoning and shoelace tying, but not inserting coins.

Ambulation was also measured in the same study and around 25% of the group demonstrated a 20% increase in walking speed and were defined as responders for gait. For upper limb function, one third of participants were defined as responders based on an improvement of 20% on the 9HPT. However it was not reported whether the responder group for upper limb function were also the responders for ambulation. Unfortunately no placebo control group was included. These data suggested that ambulation was quicker to improve than upper limb function, with significant benefits being observable at 1 month (in contrast to 3 months for any of the upper limb function measures), which may reflect the size of the sample group (26 patients vs 21 for upper limb function), the relative magnitude of benefit in terms of ambulatory function or the complexity of performance of upper limb assessment tasks and requirement for practice in order to reflect improvements (however, no attempt was made to control for learning and practice effects because of the absence of a control group).

A small, blinded, placebo-controlled trial of ten patients [171] was conducted and assessed grip strength in addition to the primary outcome measure of ambulation, and demonstrated a significant impact on ambulation but only a non-significant trend towards improvement in upper limb function.

In a larger but uncontrolled open-label Danish study which was primarily concerned with ambulatory function [169], manual function was measured using the 9HPT in 108 patients treated with fampridine-MR. There was a significant improvement in 9HPT time at four weeks compared to baseline, but the magnitude of response was small (mean improvement of 4% on raw 9HPT time) and there was no placebo control group or any attempt made to control for learning effects associated with repeated performances.

The investigators of a similar French open-label study which was primarily concerned with ambulation also documented the clinical response of upper limb function. “Responders” were defined as those patients who improved on any one of three measures of ambulation, with the result that 74% of the group were classified as responders; responders for gait also showed significant improvements
in 9HPT times, although once again the magnitude of response was below the minimal clinically important difference and there was no attempt made to control for placebo effect.

A single-patient case report described a marked improvement in upper limb tremor in response to open-label treatment with 4-AP, with concomitant improvement in manual dexterity as measured by 9HPT. There was no attempt made in these trials to differentiate between different aspects of upper limb dysfunction, nor was it reported in any of them whether functional impairment occurred as a result of tremor, weakness, spasticity, sensory dysfunction or a combination of the above.

**Vision**

The effects of fampridine on visual functions (both visual acuity, contrast sensitivity and oculomotor function) have been evaluated in a small number of studies, many of which have been small or uncontrolled.

One of the earliest studies was by Jones [573] who examined visual function in five PwMS with temperature-sensitive visual symptoms, hypothesising that these patients would be more likely to show benefit from treatment than those with fixed neurological deficits. A comparison group of five PwMS with stable spastic paraparesis but without visual symptoms was also included, along with two healthy control subjects. Various measures of visual function were taken, including visual acuity (via Snellen chart), visual evoked potential latency and amplitude, luminance threshold (measuring ability to detect light of various intensities) and temporal resolution of vision (measuring ability to detect two stimuli separated by varying time intervals). Both the measures of visual function and the doses of drug varied between patients, and although the authors concluded that the drug could be of benefit because 75% of patients showed some degree of improvement in at least one of the many visual functions measured, issues with study design mean that the results are inconclusive overall.

Davis [574] administered single doses of fampridine at variable doses of 10-25 mg (n=15) or placebo (n=5), to 20 patients with MS and temperature-sensitive symptoms. No details were provided regarding treatment allocation but it is implied that randomisation of treatments or doses was not formally conducted. The researchers reported an improvement in “visual function” for 11 of 13 drug-treated patients tested, (only 13 fulfilled the inclusion criteria of temperature-sensitive visual deficits; all of these had prolonged VEP latencies), and for 0 of 5 patients treated with placebo. The aspects of visual function which improved were VEP latency and critical flicker-fusion frequency (which is a test of capacity to appreciate the intermittency of rapidly repeated light stimuli, and is a relatively non-localising test of damage to the visual pathway, being liable to produce abnormal results with lesions at various sites [575]). Notably, objective testing of visual acuity or quantitative perimetry did not demonstrate an improvement, although the authors report that “patients whose visual tests improved after 4-AP administration were also aware of a general improvement in vision”. Visual
acuity was also examined in tandem with motor deficits by Bever [576] who studied a group of eight MS patients with temperature-sensitive neurological deficits and used variable doses of fampridine titrated towards two different target peak serum concentrations, and reported significant improvements in contrast sensitivity at both dose ranges, but without demonstrable effects on VEP latency. Some motor parameters also improved, however one patient suffered a generalised seizure with a serum level just above the upper limit of the higher dose range, and another developed a confusional episode with an even greater serum drug level. These findings indicate that caution should be exercised in using the drug at higher doses in this group, and highlighting the potential for variability in pharmacokinetics between patients.

Another group of investigators reported benefits in a small non-randomised study (but which did include a healthy control group and a placebo group) studied twelve PwMS and temperature-sensitive symptoms after intravenous administration of 4-AP and reported beneficial effects in seven of twelve patients tested [550]. Positive outcomes were determined by objective evidence of improvement on videotaped neurological examination as assessed by a blinded assessing neurologist, but without quantitative assessment of visual acuity. High-quality double-blind, randomised controlled trials evaluating the effect of 4-AP on visual acuity are scarce, but two such studies have been conducted by Van Diemen [577, 578]. These investigators initially randomised 70 patients with stable MS symptoms to intravenous 4-AP or placebo and measured outcome in terms of subjective global clinical impression and objective measurement of VEP latency and eye movement registrations (EMRs). Although this study reported some subjective clinical improvements, overall the drug was poorly tolerated and it was felt that side-effects with this route of drug administration (particularly paraesthesiae and dizziness) would severely limit clinical utility in this setting. Small improvements were demonstrated in VEP latency and amplitude but were so small as to be of questionable significance. In the second study, the same authors randomised 70 patients to oral 4-AP or placebo and measured outcome in terms of the EDSS overall and its individual functional system (FS) scores, as well as a battery of neurophysiological measurements. No improvements occurred in visual parameters (visual acuity or contrast sensitivity), although VEP latency was significantly shorter in the treated group. Improvements were seen in EMR which were of uncertain clinical significance.

Probably the most rigorously-conducted study of the effects of fampridine-MR on visual function is the pilot study of Horton [196] who studied 22 patients with MS and objective evidence of optic neuropathy (as evidenced by reduction in retinal nerve fibre layer thickness (RNFL) as measured by optical coherence tomography (OCT) in one or both eyes) in a double-blind, placebo-controlled crossover study with two consecutive five-week treatment periods. Clinical outcomes were Snellen visual acuity and contrast sensitivity (the primary outcome was improvement of five letters on the 2.5% chart) and VEP latency was also measured. The study failed to meet its primary outcome measure, but there were a significantly greater number of responders in the drug arm (28.6%)
compared to placebo (10.7%). There was a significant difference between groups for VEP latency, being significantly shortened in the drug-treated group and related to RNFL thickness, such that patients who had relatively better preserved optic nerves (RNFL 60-80μm) responded best, with minimal response seen in patients with more severely damaged optic nerves (RNFL <60 μm). The size of the study was not sufficiently large to permit sub-analysis of the clinical primary outcome based on the magnitude of optic nerve damage as indicated by RNFL thickness. However, the implication of these findings is that there may be a subset of patients with less severe optic neuropathy, or predominantly demyelinating pathophysiology rather than axonal loss, who do have greater potential for clinical benefit. The authors report their intention to carry out a further investigation in this group of patients to explore this in more depth.

Most authors have focused on visual acuity rather than oculomotor function or other aspects of vision. Outside the domain of MS, 4-AP has been tested in a number of patients with downbeat nystagmus due to other neurological conditions, most often idiopathic or due to cerebellar degeneration, with reported benefits in terms of improving fixation and restoring gaze-holding ability [579, 580]. It has not been ascertained whether these benefits carry over to PwMS with oculomotor disturbance.

**Fatigue and Cognition**

Various authors have attempted to explore the effects of 4-AP on fatigue or cognition, either independently or in combination, although once again many studies have been small or uncontrolled. In a well-conducted pilot study by Smits [262] randomised twenty PwMS were randomised to either 4-AP or placebo using a double-blind crossover design, and measured cognitive outcomes using the Brief Repeatable Battery (BRB) at baseline and after 2 and 4 weeks of treatment. No significant difference was demonstrated according to treatment group. Magnin [581] reported improvements in verbal fluency in an open label study of 50 PwMS treated with fampridine-MR, with benefits which were independent of gait response, although no control arm was included to control for learning or placebo effects. Researchers in a similar uncontrolled prospective study [167] measured cognitive function using the PASAT and fatigue using the mFIS, and compared findings between gait responders (comprising 56% of the study population) and non-responders. This study reported significant subjective improvements in gait responders in the mFIS score overall and the cognitive and physical subsets, but failed to demonstrate objective improvement in cognition in the PASAT, highlighting the potential for differences in perceived and actual performance. Clearly the lack of control arm is a major confounding factor in a study with this degree of subjectivity of outcome measures. Investigators in another open-label study [168] reported significant reductions in fatigue (measured on FSS) in gait responders relative to non-responders, but once again lacked a control arm.

Bever [567] examined neuropsychological outcomes as a secondary outcome measure in their double-blind randomised controlled study which was predominantly concerned with gait and which included
36 patients with MS treated with 4-AP at variable doses up to 100mg/day. As discussed above, they documented improvements in leg strength, but no neuropsychological improvements were reported using the Brief Repeatable Battery (BRB) of cognitive tests.

Ruck [582] studied 52 PwMS and mobility impairment in an open-label study of fampridine-MR and reported improvements in the Fatigue Scale for Motor and Cognitive Functions and PASAT after 9-12 months of therapy, but without a placebo control group. Romani [583] studied 60 patients with MS, two thirds of whom were classified as being “fatigued” based on screening FSS score, with some attempts made to match between groups for age, sex, disease duration and severity but without a randomised trial design. Fatigued patients were treated with either 4-AP or fluoxetine for 8 weeks, and improvements were seen in both groups relative to baseline, but without a detectable difference between groups and without a placebo control arm. Sheean [560] studied eight fatigued PwMS patients before and after three weeks of open-label treatment with 4-AP and documented small improvements in FSS score which are of questionable significance given their small magnitude and the trial design.

Rossini [584] recognised the importance of a placebo control group in studying a subjective symptom of this kind and randomised 54 patients with progressive MS to either 4-AP or placebo for a duration of six months each in a crossover study design. The clinical primary outcome was the change in fatigue severity scale, with secondary outcomes including EDSS, cognitive function assessed using a battery of neuropsychological tests and evoked potentials (VEP, SSEP and MEP). The study was negative for the primary outcome, with improvements seen in fatigue levels in both groups (albeit larger in the 4-AP treated group) but no significant difference between drug and placebo groups overall. Likewise, no significant differences were seen on the secondary clinical or neurophysiological outcomes. A sub-analysis was performed in which serum levels of drug were measured and categorised as “high” or “low” (although all participants received the same dose) and when the “high” group was analysed alone, there was a significant benefit in terms of fatigue along with an apparent reduction in the variability of MEP latencies which correlated with the reduction in fatigue in the subgroup analysis.

**Funding of Fampridine-MR in Australia**

Largely on the basis of the evidence of benefit in ambulatory function, fampridine-MR has been approved for use by the Therapeutic Goods Administration (TGA) in Australia and a number of other countries for the symptomatic treatment of walking impairment in MS. It is not yet funded under the Pharmaceutical Benefits Scheme at the time of writing and as such is not widely used due to the cost to patients (approximately $200/month), particularly when it is considered that a substantial proportion of the patients most likely to benefit are no longer in paid employment. Furthermore, its
potential benefits on domains other than ambulation have not yet been fully explored, nor is there an existing way to identify which patients might respond and which are unlikely to do so.
Chapter 7: Methods

Introduction

There are numerous limitations regarding currently available symptomatic treatments for MS which have been discussed in the preceding chapters, and a number of considerations relating to the objective clinical and electrophysiological evaluation of PwMS and their specific symptoms have also been highlighted. Significant gaps in the existing body of literature relating to the efficacy of 4-AP in this context and to its precise mode of action have also been identified. The central body of this thesis seeks to address some of these limitations and gaps, and to test a number of hypotheses in relation to this subject.

Aims

The principal aims of this study are as follows:

1. To conduct a novel investigation into the potential benefits of fampridine-MR in the treatment of PwMS with impairments of upper limb function
2. To explore the potential benefits of fampridine-MR in the treatment of other MS symptoms, specifically vision and fatigue
3. To quantify sensorimotor changes in patients treated with fampridine-MR compared with placebo using a variety of electrophysiological and clinical methods
4. To correlate objective clinical and electrophysiological measures of improvement in order to better understand the mechanism of action of fampridine-MR and the reasons for differential clinical responses in the MS patient population, with the ultimate goal of being able to identify a subset of patients who are most likely to benefit from this therapy based on clinical and electrophysiological parameters.

Hypotheses

The following scientific hypotheses will be tested:
1. Fampridine-MR is associated with improvements in upper limb function in patients with upper limb functional impairment due to multiple sclerosis.
2. Objective electrophysiological measures of central conduction differ in patients on and off treatment with fampridine-MR, and can distinguish clinical responders and non-responders.

The data to be presented were obtained from a small pilot study (The Fampridine Withdrawal Study, FWS), conducted in a small group of patients who were already being treated with fampridine, and a larger double-blind randomised controlled trial (The Fampridine Upper Limb Study, FULS). The methodology of these studies is broadly similar and as such will be presented jointly in the following chapter. In addition to the results of the pilot FWS study and larger FULS study, details of the healthy control subject group and a comparison of clinical and electrophysiological findings between MS patients and healthy controls will also be presented.

**Study Population**

Patients were recruited from the MS Clinic at the Austin Hospital, by referral from private specialists and via an advertisement placed in the newsletter of MS Research Australia. Healthy controls were recruited by word of mouth at the Austin Hospital. Both clinical studies used the following inclusion and exclusion criteria for PwMS:

**Inclusion criteria**
- Clinically definite MS (of any duration or subtype) as per McDonald criteria [57]
- Age 18 or over (no upper age limit was stipulated)
- Ability to provide own informed consent for participation in the study
- Subjective and objective impairment of function in one or both upper limbs, such that the participant would consider taking a symptomatic therapy for this if available (FULS study only)
- Current therapy with fampridine-MR for the treatment of ambulatory dysfunction, and willingness to temporarily cease treatment for the purpose of the study (FWS study only)

**Exclusion criteria**
- Contraindications to fampridine-MR therapy: history of seizures or epilepsy at any time during life; moderate or severe renal impairment with eGFR <60 ml/min/1.73m²; hypersensitivity to fampridine; pregnancy
- Current or recent MS relapse within 60 days prior to randomisation
• Alternative likely cause for upper limb impairment (e.g. peripheral nerve lesion, musculoskeletal injury)
• Recent (within 60 days) addition of new treatment for MS including disease-modifying treatments and symptomatic therapies, or treatment with corticosteroids in the context of a presumed acute relapse

The use of other drug therapies with actions on the central nervous system was not stipulated as an exclusion criterion but their use had to be kept constant over the duration of the study period. Participants were informed of their right to withdraw from the study at any time and for any reason.

The following inclusion criteria were used for the healthy control group:
• Age 18 or over
• No history of MS or other disorder of the central or peripheral nervous system
• Ability to provide own informed consent for participation in the study

**Ethics Committee Approval**
Prior to commencement of the studies, Ethics Committee approval was obtained from the Human Research Ethics Committee (HREC) of Austin Health. The HREC was responsible for the monitoring of the study during its completion and required the submission of regular written progress reports.

**Study Design**

(1) **Fampridine Withdrawal Study (FWS)**
The FWS was a small prospective pilot study in which participants who were already taking fampridine were required to stop treatment for a period of >10 days without randomisation of treatments but with clinical and electrophysiological assessments being carried out by the same blinded assessors during the course of the study; patients were not blinded to the treatment being received. An independent staff member was responsible for informing patients regarding the timing of their visits relative to withdrawal or recommencement of treatment.

(2) **Fampridine Upper Limb Study (FULS)**
The FULS was a larger prospective randomised, double-blind placebo-controlled trial. Patients remained blinded until the completion of their final study visit, after which unblinding was performed by an independent staff member. Assessors remained blinded to all patients’ treatment allocations until after completion of the final testing on the final patient. Randomisation of treatment was carried out by a Clinical Trials Pharmacist from Austin Health who was not otherwise involved in the conduct of the study.
Both studies included a screening visit during which inclusion and exclusion criteria were checked, including the taking of blood for measurement of eGFR if this had not been done within the preceding 6 months, and the performance of a urine pregnancy test where there was considered to be a possibility of pregnancy.

At screening, the following baseline information was collected from patients:

- Age
- Sex
- Hand dominance
- Details of MS diagnosis, subtype and disease duration
- Details of current MS treatment including symptomatic and disease-modifying therapies
- Details of co-morbidities and concomitant medications
- eGFR

Subsequent to screening, the following visit schedules were used for each study:

(1) **FWS**
After screening, 3 separate study visits were required, separated in time by at least 2 weeks. At each study visit, the following assessments were made by a blinded assessor:

- 9HPT in dominant and non-dominant hands
- Hand grip strength by manual dynamometer in dominant and non-dominant hands
- Sensory Discrimination Capacity (see below) in dominant and non-dominant hands
- Modified Fatigue Impact Scale (mFIS)
- Visual acuity and contrast sensitivity using Sloan Chart
- Electrophysiological testing (see below)

(2) **FULS – patient group**
After screening 5 separate study visits were required at predetermined intervals. The following assessments were made at the visits as detailed below.

**Visit 1 (Baseline):**
- 9HPT in dominant and non-dominant hands
- Hand grip strength by manual dynamometer in dominant and non-dominant hands
- Sensory Discrimination Capacity (see below) in dominant and non-dominant hands
• Modified Fatigue Impact Scale (mFIS)
• Visual acuity and contrast sensitivity using Sloan Chart
• Electrophysiological testing (see below)

At visit 1, participants received a supply of study drug or placebo (see below) comprising 5 bottles of treatment (to last 4 weeks, with one spare bottle), to be commenced as soon as possible after the completion of visit 1.

Visit 2 (2 weeks after baseline):
• 9HPT in dominant and non-dominant hands
• Hand grip strength by manual dynamometer in dominant and non-dominant hands
• Sensory Discrimination Capacity (see below) in dominant and non-dominant hands
• Modified Fatigue Impact Scale (mFIS)
• Visual acuity and contrast sensitivity using Sloan Chart
• Questionnaire regarding subjective improvement, side effects and patient’s belief regarding treatment group allocation

Visit 3 (4 weeks after baseline):
• 9HPT in dominant and non-dominant hands
• Hand grip strength by manual dynamometer in dominant and non-dominant hands
• Modified Fatigue Impact Scale (mFIS)
• Visual acuity and contrast sensitivity using Sloan Chart
• Electrophysiological testing (see below)
• Questionnaire regarding subjective improvement, side effects and patient’s belief regarding treatment group allocation

At visit 3, participants were provided with a further supply of the same study drug or placebo comprising 4 bottles of treatment to last for the remaining 4 weeks of the study.

Visit 4 (8 weeks after baseline):
• 9HPT in dominant and non-dominant hands
• Hand grip strength by manual dynamometer in dominant and non-dominant hands
• Sensory Discrimination Capacity (see below) in dominant and non-dominant hands
• Modified Fatigue Impact Scale (mFIS)
• Visual acuity and contrast sensitivity using Sloan Chart
- Electrophysiological testing (see below)
- Questionnaire regarding subjective improvement, side effects and patient’s belief regarding treatment group allocation

At visit 4, participants were required to bring all remaining supplies of study drug or placebo and these were returned to pharmacy for disposal. After visit 4, participants were not required to take any further doses of study medication.

**Visit 5 (>2 weeks after visit 4):**

- 9HPT in dominant and non-dominant hands
- Hand grip strength by manual dynamometer in dominant and non-dominant hands
- Sensory Discrimination Capacity (see below) in dominant and non-dominant hands
- Modified Fatigue Impact Scale (mFIS)
- Visual acuity and contrast sensitivity using Sloan Chart
- Questionnaire regarding subjective improvement, side effects and patient’s belief regarding treatment group allocation

As detailed above, patients were unblinded following completion of visit 5 by an independent staff member.

**(3) FULS – healthy control group**

After screening, 2 separate study visits were required, separated in time by at least 2 weeks. The following assessments were made at each visit:

- 9HPT in dominant and non-dominant hands
- Hand grip strength by manual dynamometer in dominant and non-dominant hands
- Sensory Discrimination Capacity (see below) in dominant and non-dominant hands
- Electrophysiological testing (see below)

**Study Drug**

In FWS, participants had a pre-existing supply of medication and were instructed to randomly cease medication at least 10 days prior to one of the three visits and to recommence after the relevant “off” visit had been completed.
In FULS, participants were randomised to receive either Fampridine-MR 10mg bd or placebo of identical appearance. This dose of Fampridine-MR was selected based on the TGA approved dose of drug for the treatment of walking impairment and the work of previous authors [160, 548]. Drug and placebo were supplied by the sponsor of the study, Biogen Idec. Both drug and placebo were packaged in identically-appearing bottles containing 14 tablets (1 week’s supply) which were numbered in the order in which they were to be taken. The study drug and placebo were dispensed by the Clinical Trials Pharmacy of the Austin Hospital. Participants were instructed to keep empty medication bottles and to return these to the study investigator for subsequent disposal by the Clinical Trials Pharmacy and tablet-counting was performed to permit assessment of compliance with study medication.

Clinical Assessments

All clinical assessments were performed by one of three trained assessors who were blinded to the treatment allocation. Testing was performed in a quiet room with participants seated in a comfortable chair (or in their own wheelchair, if applicable). Where possible, testing was performed by the same assessor and at the same time of day for each of the visits. If deemed necessary by the investigator, hand-warming was performed prior to the commencement of testing.

9-hole peg test (9HPT)

The 9HPT was administered according to the instructions of the MS Functional Composite Manual [414]. A maximum of two successfully completed trials (maximum time for each 300 seconds) was performed in each hand, with the best performance recorded as the primary outcome.

Grip Strength

Grip strength was measured using a manual dynamometer (Jamar Plus) which measures grip strength in kilograms and which has published normative values for healthy subjects, adjusted for age, sex and hand dominance. A maximum of two successfully completed trials was performed in each hand and the best performance recorded.

Sensory Discrimination Capacity

The Sensory Discrimination Capacity comprises three parts and was conducted according to the methodology of Carey [495, 500, 501]. This battery of tests of somatosensory function was originally developed for use in patients with stroke and had not previously been validated in PwMS. It comprises the Wrist Position Sense Test (WPST, a quantitative test of proprioceptive function), the Texture Discrimination Tests (TDT, a quantitative test of tactile discrimination) and the Functional
Tactile Object Recognition Test (fTORT, a quantitative test of object recognition and cortical sensory function or stereognosis).

**WPST**

In the WPST, subjects are required to place each hand in turn in the testing apparatus *(see figure 4)* which comprises a forearm splint aligning the wrist and hand situated on the lower of two levels displaying identical protractor scales, indicating degree measurements to the nearest 1°. The lower level is hidden from the subject’s view, and the upper level is visible to both the subject and examiner. After an initial test trial, twenty stimuli are presented in a predetermined random sequence to the participant, each consisting of an externally imposed movement during which the examiner moves the participant’s covered hand by a predetermined number of degrees in one direction or another. The participant is required to indicate (to the nearest 1 degree, either verbally or by moving the dial on the upper protractor scale) the position to which the hand has been moved. The participant is encouraged to guess if they are unsure as to the precise measurement, such that an answer is always provided (if no answer is provided, the attempt is removed from subsequent calculations). An equal number of stimulus movements in wrist flexion and extension are presented. The process is then repeated for the non-dominant hand and the absolute error for each stimulus and average absolute error in each hand is calculated and used as the index of proprioceptive ability.

**TDT**

In the TDT, subjects are required to differentiate the “odd one out” from sets of three finely graded plastic ridged surfaces, using a three-alternative forced-choice design [500]. The testing apparatus comprises a polyamide sheet containing fifteen surfaces (five rows of three) with spatial intervals ranging from 1500μm-3000μm *(see figure 5)*. Each row of three contains two identical gratings and one which is different. Participants are separated from the grating by a curtain to remove visual cues. Either the index or the middle finger can be used, although the selected finger is kept constant between visits. For each hand in turn, participants are asked to feel the three surfaces in each row and to indicate which one is different; if they are unsure, guessing is encouraged such that an answer is always provided (if no answer is provided, the attempt is deemed incorrect). The gratings are presented in a predetermined random order and fifteen trials are performed for each hand. For some trials, the stimulus is more “difficult” than for others, in that the difference between the two identical plates (referred to as the Percentage Spatial Increase, PSI) and the “odd one out” plate is smaller. In addition, there is a 1/3 probability of obtaining a correct result due to chance on each trial.
Adjustment is therefore made for this in the scoring of the test, such that an area under the curve is calculated for each trial, and the probability of guessing the correct result due to chance alone is subtracted; the AUC score ranges from 100 (indicating complete accuracy) to -50 (indicating errors greater than those which would be expected due to chance).

\textit{fTORT}

In the fTORT, subjects are required to discern various everyday objects using touch without the benefit of vision. A curtain separates the participant from the object, and each hand in turn is placed behind the curtain. A poster displaying the potential objects which might be given is displayed nearby (see figure 6), and each object is grouped with two others; one which is similar, the “pair”, and one which is somehow related but quite different, the “distractor” (for example, a full milk bottle is grouped with a half-full milk bottle, the pair, and an empty soft drink bottle, the distractor); participants receive 3 points for each correct answer, 2 points if they select the paired object and 1 point if they select the distractor; zero points are scored if they select another object from a different group. After an initial test trial, six trials are performed with each hand and the score recorded as a percentage correct for each hand individually.

\textbf{Modified Fatigue Impact Scale (mFIS)}

The mFIS [200] is a self-reported 21-question scale during which participants are questioned regarding the impact of their fatigue over the preceding 4-week period, and are required to choose between five answers for each question (“never”, “rarely”, “sometimes”, “often” and “almost always”) with 0-4 points being assigned for each question accordingly. It can be divided into subsets of “physical”, “cognitive”, “psychosocial” and “total” levels of fatigue and has been validated for use in PwMS. Subjects were given the questionnaire to complete by themselves or, for subjects who were unable to write easily, one of the blinded assessors completed the questionnaire in consultation with the participant.

\textbf{Visual Acuity and Contrast Sensitivity}

Visual testing was performed with the patient seated in a comfortable chair at a fixed distance of 3m from the eye chart. Participants were instructed to wear corrective lenses if required and to read the chart from the top until it became illegible or until the chart was completed, whichever was sooner. An eye patch was applied to each eye in turn for testing of monocular vision and subsequently
binocular vision was tested. A Sloan visual acuity chart was used and the number of correct responses recorded at 3 different levels of contrast; 100%, 2.5% and 1.25%.

**Questionnaire regarding side-effects and treatment allocation**

At each visit after the initial visit, participants were asked to indicate whether they had noted any improvements while taking the study medication, and whether there were any adverse effects. In addition, they were asked to comment on whether they believed they were receiving drug or placebo.

**Electrophysiological Assessments**

All assessments were performed by the same blinded assessor at approximately the same time of day and using constant conditions. Measurements were made with subjects seated in a comfortable chair (or their own wheelchair, if applicable) in a quiet and electrically insulated room using a Synergy Electromyography Machine.

**Median Nerve Motor and Sensory Nerve Conduction Studies, F-wave responses**

As a precursor to the studies of central nervous system conduction, peripheral conduction was measured in each median nerve. If required, hands were warmed in water prior to commencement of recordings. Bipolar surface recording electrodes were applied to abductor pollicis brevis (APB) and progressively increasing stimulations were administered over the median nerve at the wrist and elbow until supramaximal responses were obtained. The median nerve distal motor latency and amplitude were recorded and conduction velocity was calculated from the measurement of distance between the proximal and distal stimulation sites. F-wave responses, reflecting conduction along the whole length of the median nerve, were obtained by administering ten supramaximal stimuli to the median nerve at the wrist while recording over APB. The shortest latency value and the persistence of responses was recorded for each upper limb. Sensory action potentials were recorded orthodromically for each upper limb using digital stimulating ring electrodes and a bar recording electrode situated over the median nerve at the wrist with progressively increasing magnitudes of stimulation being administered until a supramaximal response was achieved. The amplitude and conduction velocity (calculated from measurement of distance between stimulating and recording electrodes) were recorded. Traces were marked automatically by the Synergy software and the markings confirmed manually by the same blinded assessor for all of the above measurements.

**Visual Evoked Potentials (VEP)**
Pattern-shift VEPs were recorded in each eye independently. Recordings were made in a darkened room and the contralateral eye was covered using a gauze patch. Subjects were seated at a fixed distance of 100cm from the computer monitor with the centre of the screen adjusted to eye level in each case and were instructed to fixate on a red spot in the centre of the chequerboard. EEG recording electrodes were positioned at the following locations according to the 10-20 recording system: Fz, Cz (ground electrode), Pz, Oz, O1, O2. For each eye, two runs of 128 sweeps were recorded, with stimulation delivered at a frequency of 2Hz. The chequerboard consisted of 16x12 squares with a check size of 17mm. The channels recorded were Fz-Oz, Fz-Pz, Fz-O1 & Fz-O2, using a sweep of 300ms and a gain of 10µV/division. Responses were averaged using the Synergy EMG software and traces were manually marked by the blinded assessor. P100 latency and amplitude were recorded for each eye and were classified as normal or abnormal based on the normal values for our laboratory (P100 latency $\leq 120$ms; side-to-side difference $\leq 8$ms).

Somatosensory Evoked Potentials (SSEP)

SSEPs from each median nerve were recorded with the stimulating electrode positioned over the median nerve at the wrist. EEG and surface recording electrodes were positioned at the following locations according to the 10-20 recording system. Erb’s Point, cervical vertebrae C2 and C6, cortical sensory electrodes at C3’, C4’, with reference electrodes at Fz, A1 and A2 linked on the ears bilaterally and a ground electrode on the ipsilateral forearm. The channels recorded were: Fz-Erbs point, Fz-C6, (C2 was used if N13 was not easily seen) Fz-C3’, Fz-C4’ and A1/A2-C3’ or C4’ contralateral to the arm being stimulated, using a sweep of 200ms and a gain of 5µV per division. The stimulus intensity was increased until subjects were able to perceive a sensory stimulus in the distribution of the median nerve and a small motor response consisting of a thumb twitch was achieved. Subjects were instructed to relax while at least 2 sweeps of 150 stimuli were administered, and responses were averaged using the Synergy EMG software until at least 2 good-quality traces had been obtained. Traces were manually marked by the same blinded assessor and the following potentials were recorded: latency for N9 (Erb’s point), N11, N13 (cervical spine at C6 and C2), N20 (cortical sensory response), N20 and P25 and the peak-peak N20-P25 amplitude was measured. Central conduction time (CCT) reflecting conduction time in central somatosensory pathways from the upper limb was calculated by subtracting N13 latency from N20 latency. Results were classified as normal, delayed or absent based on the standard values for our laboratory (where ULN (mean +/-2 SD) values are: N9=11.3, N13=17.1, N20=24.3 and CCT=8.7ms).

Transcranial Magnetic Stimulation (TMS) – Resting Motor Threshold (MT)

All TMS recordings were made using a standard round coil and a Magstim 200 stimulator. The coil was placed tangential to the scalp centred at the vertex (Cz). An appropriate direction of coil current was used for each hemisphere, anticlockwise for the left and clockwise on the right when looked at.
from above. Surface bipolar recording electrodes were positioned over the contralateral APB muscle for each cerebral hemisphere in turn and subjects were instructed to relax the target muscle. MEPs were recorded on the Synergy EMG machine using band-pass filtering 10Hz – 5kHz, sweep speed 100 ms and gain 100 µV/division. Auditory EMG feedback was used to ensure relaxation of the target muscles. Resting MT (rMT) was obtained first as a precursor to the other TMS measurements. Stimulation commenced at 30% of maximum output and was increased in 5% increments until an MEP response became visible. The rMT was then established to the nearest 1% of stimulator output by changing the stimulus intensity by 1% in the required direction until the lowest stimulus intensity which yielded an MEP with peak-to-peak amplitude > 100 µV on 50% or more of 10 trials was obtained; this value was defined as the rMT and was used to calculate the stimulus intensity required for subsequent measurements using single or paired pulse TMS. For some subjects, rMT exceeded the maximum stimulator output for one or both hemispheres (this can occur due to physiological or pathological reasons). When this occurred no further TMS parameters could be calculated for that hemisphere. For these subjects, a value of >99% was recorded as rMT. For another subset of participants, the rMT was <99% but exceeded 72%, which was the value at which stimulations at 140% of MT would exceed the maximum output of the stimulator. For these subjects, it was possible to perform the stimulations required for the paired-pulse TMS measurements and the measurement of MEP latency and amplitude, but it was not possible to perform the MEP recruitment curve measurement (see below). In subjects with rMT >84%, stimulation at 120% of rMT would exceed the maximum stimulator output and further PPTMS testing for measurement of SICI and ICF could not be performed. For these subjects, the absolute value of the rMT was recorded but no further analysis could be undertaken.

**Motor Evoked Potentials (MEP)**

Cortical and cervical MEPs were recorded with surface recording electrodes positioned over the contralateral APB muscle with the subject at rest. For the cortical MEP, stimuli were delivered at 120% of rMT with the coil centred over Cz until at least two good-quality MEP traces were obtained; the shorter of these was selected for inclusion. For the cervical MEP, stimuli were delivered over the C6 cervical vertebra at 50% of the stimulator output until at least two MEP traces were obtained. Traces were manually marked by the same blinded assessor and the shortest MEP latency was recorded. Central Motor Conduction Time (CMCT) was calculated by subtracting the cervical MEP latency from the cortical MEP latency. Results were classified as normal, delayed or absent based on the normal values for our laboratory (ULN (mean +/- 2sd): Cortical =21.75ms, Cervical =13.35ms and CMCT =8.6ms).

**MEP Recruitment Curves**
For subjects in whom rMT was recordable and did not exceed 72% of the maximum stimulator output for that hemisphere, 40 stimuli were delivered in a predetermined random sequence with the coil centered over Cz with ten each at intensities of 110%, 120%, 130% and 140% of the rMT. Traces were manually marked by the same blinded assessor and the mean peak-to-peak MEP amplitude at each of the four stimulus intensities was established and expressed as a percentage of the maximal peripheral CMAP amplitude for each median nerve and plotted against stimulus intensity to allow the construction of recruitment curves.

**Paired pulse TMS (PPTMS)**

For subjects in whom rMT was recordable and did not exceed 84% of maximum stimulator output for that hemisphere, intracortical excitability and inhibition were characterised using PPTMS. Two Magstim 200 stimulators were linked with a Bistim unit allowing the delivery of two stimuli through the same coil. 10 single stimuli and 40 paired stimuli were delivered in a predetermined random sequence for each median nerve, with 10 paired stimuli at each of the following interstimulus intervals (ISI): 2ms, 4ms, (to measure short-latency intracortical inhibition, SICI) 10ms, 12ms (to measure short-latency intracortical facilitation, ICF). The single stimuli given without a conditioning stimulus were delivered at 120% of rMT. For each of the paired stimuli, a conditioning stimulus at 80% rMT was delivered followed by a test stimulus at 120% rMT. Traces were marked manually by the same blinded assessor. The ratio of the mean peak-to-peak amplitude at each ISI for the 10 paired responses to the mean peak-to-peak amplitude for the 10 single stimuli was calculated and expressed as a percentage and was represented graphically as a PPTMS response curve for each group.

**Statistical Analysis**

Statistical analysis was performed using STATA software. Comparison was made between MS patients and healthy control subjects using Fisher’s Exact Test and the Mann-Whitney Test. For the randomised controlled trial, the primary outcome measure was improvement in 9HPT scores at visit three (four weeks of treatment) in the drug-treated group relative to placebo-treated group. Persistence of this effect was measured at visit four (eight weeks of treatment). Z-scores were obtained for each patient by calculating the ratio of their 9HPT time for dominant and non-dominant hands at each visit to the published normative value [444] as determined for their age, sex and hand dominance. The Z-scores at 4 weeks were then compared to Z-scores at baseline for drug and placebo groups using random effects regression analysis. A similar calculation was performed for the secondary outcome of grip strength, whereby Z-scores were calculated for each patient based on published normative values for their age, sex and hand dominance [585] and were compared at 4 weeks to baseline, with persistence of effect at 8 weeks also being measured. For the remainder of the secondary outcome measures, absolute values were used (for latency and amplitude for the evoked
potential measures; for percentage correct for the Visual Acuity and Sensory Discrimination Capacity measures and the calculated ratios for the PPTMS measures).

**Sample Size Calculation**

The magnitude of effects of fampridine on upper limb function have not previously been systematically studied in patients with MS. The response of lower limb function in terms of effect on timed 25 foot walk time as measured by Goodman [160] was initially used as an index of possible effect size. Assuming a similar magnitude of effect size, it was estimated that in order to achieve a power of 80% with a significance level (α) of 0.05, 38 patients in each group (fampridine, placebo) would be needed to statistically identify differences in upper limb function between the groups. However, given that there may well be differences in response based on measures of upper limb and lower limb function, we deemed that a more relevant approach would be to base the calculation on the data of Erasmus, who assessed performance of the 9 hole peg test in patients with MS and controls [586]. Using this data as an index, 16 patients in each group would be required to reliably show a 40% improvement using a power of 80% and α of 0.05. This magnitude of improvement would represent approximately double the minimal clinically important difference for the 9HPT [218]. Taking into account an assumed attrition rate of 10% of the participants through the period of the study, these two approaches to sample size calculation and the feasibility of conducting a single centre study, we selected a sample size of 20 in each group on the basis that this would provide statistically significant findings.
Chapter 8: Results

1. Fampridine Withdrawal Study (FWS)

Demographic details of the 5 patients included in this pilot study are summarised in table 1.

Table 1: FWS Demographic details of included patients

<table>
<thead>
<tr>
<th>Sex</th>
<th>60% female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age</td>
<td>54.2 (IQR 45-63)</td>
</tr>
<tr>
<td>Median Disease Duration</td>
<td>14 years (IQR 11-23)</td>
</tr>
</tbody>
</table>
| Disease Subtype | 40% RRMS  
|               | 40% SPMS  
|               | 20% PPMS  |
| Median EDSS  | 6 (range 3.5-6.5) |

All patients were being prescribed fampridine for the treatment of ambulatory dysfunction associated with MS and all reported a beneficial response in this regard. Three of the five patients had subjective and objective dysfunction of one or both upper limbs; two had normal upper limb function.

Clinical Parameters Tested

Results from the one “OFF” visit were compared between individuals to the results from the two “ON” visits. At the “OFF” visit, four of five patients displayed prolongation of the 9-hole peg test time in one or both upper limbs; all five displayed reduced hand grip strength in one or both upper limbs and all subjects demonstrated abnormal scores on the mFIS scale. Statistical analysis of these findings and adjustments for learning effects could not be performed due to the small number of measurements, though it was noted that there was an apparent trend for drug treatment to be associated with small improvements on 9HPT times, grip strength, mFIS score and WPST performance (but not the other aspects of the Sensory Discrimination Capacity). For the Wrist Position Sense Test (WPST), results are reported as the median number of degrees of error in the subject’s estimate of wrist position. For the Tactile Discrimination Test (TDT), results are reported as the percentage correct responses, adjusted for the difficulty of the test as indicated by the Percentage Spatial Increase between stimuli. This is described as area under the curve (AUC). For the Functional Tactile Object Recognition Test (fTORT), results are reported as the median raw percentage of correct responses. Results are presented in table 2.
### Table 2: FWS Clinical outcome measures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median OFF (IQR)</th>
<th>Median ON (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9HPT time – dominant hand</td>
<td>28.6 sec (22.3-42.7)</td>
<td>26.3 sec (22.1-34.5)</td>
</tr>
<tr>
<td>9HPT time – non-dominant hand</td>
<td>29.7 sec (24.1-33.8)</td>
<td>28.4 sec (24.1-35.3)</td>
</tr>
<tr>
<td>Grip strength – dominant hand</td>
<td>22.1kg (20.1-28.7)</td>
<td>26.5kg (23.7-31)</td>
</tr>
<tr>
<td>Grip strength – non-dominant hand</td>
<td>18.9kg (14.4-37.7)</td>
<td>21.7kg (18.6-38.2)</td>
</tr>
<tr>
<td>mFIS score</td>
<td>40 (35-59)</td>
<td>38.5 (29-56.8)</td>
</tr>
<tr>
<td>WPST error</td>
<td>12° (8.4°-13.4°)</td>
<td>8.3° (5.6°-11.3°)</td>
</tr>
<tr>
<td>TDT AUC</td>
<td>63% (50-71%)</td>
<td>66% (53-73%)</td>
</tr>
<tr>
<td>tTORT correct</td>
<td>98% (88-100%)</td>
<td>96% (95-100%)</td>
</tr>
<tr>
<td>Vision at 100% contrast*</td>
<td>50 (49-53)</td>
<td>52 (48-54)</td>
</tr>
<tr>
<td>Vision at 2.5% contrast*</td>
<td>29 (23-35)</td>
<td>29 (25-35)</td>
</tr>
<tr>
<td>Vision at 1.25% contrast*</td>
<td>17 (10-25)</td>
<td>24 (14-28)</td>
</tr>
</tbody>
</table>

* Sloan number correct from a possible 60

*Electrophysiological Parameters Tested*

At the “OFF” visit, four of five patients displayed abnormal VEP latency in one or both eyes, three of five displayed abnormal SSEP latency in one or both upper limbs, and all five displayed prolonged MEP latency and CMCT in one or both upper limbs. Statistical analysis of these findings could not be performed due to the small number of measurements, though it was noted that there was an apparent trend for drug treatment to be associated with reduction in resting motor threshold and improvements in P100 latency and amplitude, though this was not observed for the other EP’s measured. Results are summarised in table 3.

### Table 3: FWS Electrophysiological outcome measures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median OFF (IQR)</th>
<th>Median ON (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting Motor Threshold</td>
<td>63% (53-74%)</td>
<td>60% (48-64%)</td>
</tr>
<tr>
<td>VEP P100 latency</td>
<td>123ms (105.3-134.4)</td>
<td>114.6ms (111.6-127.8)</td>
</tr>
<tr>
<td>VEP P100 amplitude</td>
<td>5.9µV (4.6-6.8)</td>
<td>7.4µV (4.5-9.2)</td>
</tr>
<tr>
<td>SSEP CCT</td>
<td>8.6ms (6.1-9.3)</td>
<td>9.4ms (9.3-9.8)</td>
</tr>
<tr>
<td>MEP CMCT</td>
<td>12 (8.7-14.3)</td>
<td>14.8 (13.8-14.9)</td>
</tr>
</tbody>
</table>

In summary, this pilot study demonstrated that the study protocols were acceptable and well-tolerated by participants. The numbers of participants included were too small to permit meaningful statistical analysis. Notably, this study was single-blinded and not placebo-controlled, and the patient group
studied was using fampridine for gait impairment rather than upper limb dysfunction. In this context, no conclusions can be drawn in relation to drug efficacy.

2. Fampridine Upper Limb Study (FULS): Baseline Clinical Testing

40 PwMS (95% right hand dominant) and 20 healthy controls (60% female; median age 53, IQR 41-62.5; 83% right hand dominant) were recruited according to the inclusion criteria described above. Demographic details of included patients are summarised in table 4 below. There were no significant differences between groups with regards to age (p=0.82), sex (p>0.9999) or hand dominance (p=0.249).

Table 4: FULS Demographic details of included patients and controls

<table>
<thead>
<tr>
<th>Patients (n=40)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median age</strong></td>
<td>52 years (IQR 46-63)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>60% female</td>
</tr>
<tr>
<td><strong>Right hand dominant</strong></td>
<td>95%</td>
</tr>
<tr>
<td><strong>Median disease duration</strong></td>
<td>13.5 years (IQR 7-21)</td>
</tr>
<tr>
<td><strong>Median EDSS</strong></td>
<td>6*</td>
</tr>
<tr>
<td><strong>Disease subtype</strong></td>
<td>RRMS 22.5%</td>
</tr>
<tr>
<td></td>
<td>SPMS 45%</td>
</tr>
<tr>
<td></td>
<td>PPMS 32.5%</td>
</tr>
</tbody>
</table>

*defined as ability to ambulate 100m with constant unilateral assistance [11].

For variables in which published normative values exist (9-HPT, hand grip strength, mFIS score, visual acuity), comparisons were made between patients and the published norms using unpaired t-tests; these results are summarised in table 5 below.

Table 5: FULS Baseline Clinical Findings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients Mean</th>
<th>Published normal Mean</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>9HPT right hand</td>
<td>34.3 seconds</td>
<td>18.8 seconds</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>9HPT left hand</td>
<td>43.7 seconds</td>
<td>19.5 seconds</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hand grip right</td>
<td>24.3kg</td>
<td>35.1kg</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hand grip left</td>
<td>20.4kg</td>
<td>30.3kg</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>mFIS (median)</td>
<td>49 (IQR 43-58)</td>
<td>11 (IQR 5-21) healthy; 33 (IQR 16-45) PwMS</td>
<td></td>
</tr>
</tbody>
</table>
As expected, patients demonstrated significantly prolonged times for the 9-HPT and significantly reduced grip strength in both hands relative to published normative values. Limited data exist in relation to normative values for the mFIS, but the largest published dataset to date suggests a median mFIS score of 33 (IQR 16-45) for MS patients and 11 (IQR 5-21.5) for healthy controls, with higher values (indicating greater fatigue levels) in SPMS than RRMS patients [587]. In our population, median mFIS score at baseline was 48.5 (IQR 42.5-57.5), indicating high levels of fatigue in this group.

PwMS demonstrated evidence of decreased visual acuity which was most marked at low contrast, with median scores of 50/60 at 100% contrast (IQR 42-54); 21/60 at 2.5% contrast (IQR 12-28) and 11/60 at 1.25% contrast (IQR 3-18).

**Sensory Discrimination Capacity**

Results of the Sensory Discrimination Capacity testing are summarised in table 6. For the Wrist Position Sense Test (WPST), results are reported as the median number of degrees of error in the subject’s estimate of wrist position. For the Tactile Discrimination Test (TDT), results are reported as the percentage correct responses, adjusted for the difficulty of the test as indicated by the Percentage Spatial Increase between stimuli. This is described as area under the curve (AUC). For the Functional Tactile Object Recognition Test (fTORT), results are reported as the median raw percentage of correct responses. Relative to controls, patients performed significantly worse on all aspects of the testing with the exception of fTORT (right hand) which showed no significant difference.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients Median (IQR)</th>
<th>Controls Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPST right hand</td>
<td>11.7° (6.95°-15.85°)</td>
<td>6.6° (5.41°-7.44°)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WPST left hand</td>
<td>10.25° (7.45°-14.1°)</td>
<td>5.71° (4.72°-6.58°)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>TDT right hand AUC</td>
<td>38% (19-56%)</td>
<td>67% (60-77%)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>TDT left hand AUC</td>
<td>47% (18-64%)</td>
<td>69% (56-78%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>fTORT right hand</td>
<td>100% (95-100%)</td>
<td>100% (98-100%)</td>
<td>0.82</td>
</tr>
<tr>
<td>fTORT left hand</td>
<td>95% (85-100%)</td>
<td>100% (95-100%)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

In summary, the MS patient group performed worse than healthy controls on all of the clinical tests of upper limb function (with the exception of the fTORT), vision and fatigue.

3. **FULS: Baseline Electrophysiological Testing**
**Visual Evoked Potential**

VEP results are summarised in table 7. Median VEP latencies were significantly longer for patients than controls; amplitudes were also lower in patients than controls but this reached statistical significance for the left eye only.

**Table 7: VEP measurement in patients and controls**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients Median (IQR)</th>
<th>Controls Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEP P100 Latency R</td>
<td>126.75ms (109.95-148.2)</td>
<td>105.4 ms (101-109.4)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>VEP P100 Latency L</td>
<td>123.3ms (111.75-143.4)</td>
<td>106.1ms (102.9-111.85)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>VEP P100 Amplitude R</td>
<td>6.45μV (5-9.2)</td>
<td>9.15 μV (6.35-10.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>VEP P100 Amplitude L</td>
<td>5.9μV (3.8-9.45)</td>
<td>6.75 μV (1.1-8.15)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Somatosensory and Motor Evoked Potentials**

SSEP and MEP (CMCT) results are summarised in table 8. Median SSEP latencies were significantly longer for patients than controls; N20-P25 amplitudes demonstrated no significant difference between groups. CMCT was significantly prolonged in patients relative to controls.

**Table 8: SSEP and MEP measurement in patients and controls**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients Median (IQR)</th>
<th>Controls Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSEP CCT R</td>
<td>8.55ms (6.2-12ms)</td>
<td>5.45ms (5.3-6.9ms)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SSEP CCT L</td>
<td>8.6ms (6.1-13.2ms)</td>
<td>6ms (5.15-6.6ms)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SSEP N20-P25 R</td>
<td>1.9μV (1.2-2.7 μV)</td>
<td>2.25μV (1.9-3.75 μV)</td>
<td>0.051</td>
</tr>
<tr>
<td>SSEP N20-P25 L</td>
<td>2.20μV (1.5-3.3 μV)</td>
<td>2.4μV (1.8-3.65 μV)</td>
<td>0.35</td>
</tr>
<tr>
<td>MEP CMCT R</td>
<td>10.51ms (9.13-13.85ms)</td>
<td>9.05ms (7.95-10.25ms)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEP CMCT L</td>
<td>11.05ms (8.5-14.25ms)</td>
<td>9.2ms (8.4-9.55ms)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Transcranial Magnetic Stimulation**

There was no significant difference in mean rMT between patients (57%, IQR 47-80% for right APB (left hemisphere), 59%, IQR 50-73% for left APB (right hemisphere) and controls (51%, IQR 47-57%
for right APB (left hemisphere, 54%, IQR 47-61% for left APB (right hemisphere) for either hemisphere; p=0.31 for right APB, p=0.23 for left APB.

For PPTMS, 33 of 40 patients and 20 of 20 controls had sufficiently low resting motor thresholds to permit further testing of SICI and ICF with suprathreshold stimulation (up to 120% of RMT). For those subjects who could be tested, there was no significant difference between patients and controls in the ratios of conditioning response to test response (CR:TR) at any of the four ISIs measured. Both groups showed the expected pattern of inhibition of the test response at short ISIs (2ms, 4ms) and facilitation of the test response at longer ISIs (10ms, 12ms). Results are summarised in table 9 below.

**Table 9: PPTMS findings in patients and controls**

<table>
<thead>
<tr>
<th>Median Nerve</th>
<th>ISI</th>
<th>Patients CR:TR (IQR)</th>
<th>Controls CR:TR (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RIGHT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2ms</td>
<td></td>
<td>0.54 (0.3-0.66)</td>
<td>0.38 (0.18-0.72)</td>
<td>0.37</td>
</tr>
<tr>
<td>4ms</td>
<td></td>
<td>0.51 (0.29-0.69)</td>
<td>0.38 (0.2-0.59)</td>
<td>0.49</td>
</tr>
<tr>
<td>10ms</td>
<td></td>
<td>1.18 (0.93-1.72)</td>
<td>1.45 (1.14-2.22)</td>
<td>0.08</td>
</tr>
<tr>
<td>12ms</td>
<td></td>
<td>1.32 (1.08-1.75)</td>
<td>1.52 (1.14-2.06)</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>LEFT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2ms</td>
<td></td>
<td>0.47 (0.32-0.82)</td>
<td>0.38 (0.22-0.57)</td>
<td>0.08</td>
</tr>
<tr>
<td>4ms</td>
<td></td>
<td>0.59 (0.39-0.93)</td>
<td>0.47 (0.26-0.81)</td>
<td>0.35</td>
</tr>
<tr>
<td>10ms</td>
<td></td>
<td>1.49 (1.01-1.95)</td>
<td>1.38 (1.1-1.64)</td>
<td>0.98</td>
</tr>
<tr>
<td>12ms</td>
<td></td>
<td>1.35 (1-1.76)</td>
<td>1.47 (1.05-1.83)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

For the MEP recruitment curves, 27 of 40 patients and 19 of 20 controls had sufficiently low resting motor thresholds to permit further testing of response to suprathreshold stimulation (up to 140% of RMT). For those subjects who could be tested, there were significant differences between patients and controls in the ratios of MEP amplitude: peripheral CMAP amplitude at stimulation intensities of 130% and 140% for the right APB (left hemisphere) and significant differences between patients and controls at all stimulation intensities tested for the left APB (right hemisphere). Results are summarised in table 10 and figure 7.
Table 10: MEP Recruitment Curves in patients and controls

<table>
<thead>
<tr>
<th>Median Nerve</th>
<th>% Stimulation</th>
<th>Patients MEP:CMAP (IQR)</th>
<th>Controls MEP:CMAP (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIGHT</td>
<td>110%</td>
<td>0.03 (0.01-0.07)</td>
<td>0.04 (0.03-0.06)</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>120%</td>
<td>0.04 (0.02-0.16)</td>
<td>0.08 (0.04-0.14)</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>130%</td>
<td>0.07 (0.04-0.16)</td>
<td>0.15 (0.08-0.23)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>140%</td>
<td>0.09 (0.05-0.29)</td>
<td>0.22 (0.13-0.29)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LEFT</td>
<td>110%</td>
<td>0.03 (0.02-0.05)</td>
<td>0.08 (0.05-0.12)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>120%</td>
<td>0.04 (0.02-0.07)</td>
<td>0.11 (0.07-0.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>130%</td>
<td>0.06 (0.03-0.1)</td>
<td>0.16 (0.11-0.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>140%</td>
<td>0.08 (0.05-0.14)</td>
<td>0.22 (0.16-0.33)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In summary, results for all of the evoked potentials tested were significantly different between patients and controls. Paired-pulse TMS testing showed no differences between patients and controls, however MEP recruitment curves differed between the two populations at most of the stimulus intensities tested.

4. FULS: Response to Drug Treatment vs Placebo

To ascertain the response to drug treatment, comparisons were made between drug- and placebo-treated patients after 4 weeks and 8 weeks of treatment and baseline values. Following the randomisation procedure, the demographic characteristics of patients in drug- and placebo-treated groups are summarised in table 11.

Table 11: demographic details of drug-treated and placebo groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Drug Treated (n=20)</th>
<th>Placebo (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age</td>
<td>53.5 (IQR 47-64)</td>
<td>51.5 (IQR 43.5-63)</td>
</tr>
<tr>
<td>Sex</td>
<td>60% F 40% M</td>
<td>60% F 40% M</td>
</tr>
<tr>
<td>EDSS category</td>
<td>Mild: 15%</td>
<td>Mild: 5%</td>
</tr>
<tr>
<td>(where mild = 0-3, moderate = 3.5-5.5, severe ≥ 6)</td>
<td>Moderate: 20%</td>
<td>Moderate: 55%</td>
</tr>
<tr>
<td></td>
<td>Severe: 65%</td>
<td>Severe: 40%</td>
</tr>
<tr>
<td>Disease Subtype</td>
<td>RRMS: 20%</td>
<td>RRMS: 25%</td>
</tr>
<tr>
<td></td>
<td>SPMS: 40%</td>
<td>SPMS: 50%</td>
</tr>
<tr>
<td></td>
<td>PPMS: 40%</td>
<td>PPMS: 25%</td>
</tr>
<tr>
<td>Hand Dominance</td>
<td>Right: 85%</td>
<td>Right: 80%</td>
</tr>
</tbody>
</table>
Clinical Outcome Measures

The primary outcome measure was performance on the 9HPT at 4 weeks, with persistence of effects at 8 weeks a secondary endpoint. Raw times were converted to Z-scores to permit comparison according to the methodology of Fischer [414] and to allow adjustment for age and sex according to published normative values [444]. Results are summarised in table 12. Linear regression analysis and adjustment for baseline input values was performed. There was no significant difference between drug and placebo groups at any time point.

Table 12: performance on 9HPT at baseline, 4 and 8 weeks by treatment group

<table>
<thead>
<tr>
<th>Hand</th>
<th>Drug (median Z/IQR)</th>
<th>Placebo (median Z/IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.99 (1.15-11.15)</td>
<td>2.98 (1.12-8.92)</td>
<td>0.9136</td>
</tr>
<tr>
<td>4W</td>
<td>3.36 (1.59-10.69)</td>
<td>1.89 (-0.03-9.23)</td>
<td>0.3385</td>
</tr>
<tr>
<td>8W</td>
<td>1.84 (0.86-7.72)</td>
<td>3.19 (-0.21-8.17)</td>
<td>0.6919</td>
</tr>
<tr>
<td>Non-dominant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.45 (-1.24-2.62)</td>
<td>1.48 (-2.12-5.26)</td>
<td>0.4713</td>
</tr>
<tr>
<td>4W</td>
<td>0.66 (-1.77-1.25)</td>
<td>0.33 (-5.98-2.52)</td>
<td>0.7063</td>
</tr>
<tr>
<td>8W</td>
<td>0.40 (-1.46-1.23)</td>
<td>0.79 (-5.98-2.8)</td>
<td>0.580</td>
</tr>
</tbody>
</table>

In all cases, performance on 9HPT at 4W and 8W was strongly correlated with performance at baseline (p>0.9999 for both groups and both hands). Baseline performance in turn was strongly correlated with EDSS category. The absence of effect of drug vs placebo held true after adjustment for disease duration and EDSS category. It also held true after adjustment for the presence of MEP abnormalities (prolonged CMCT) at baseline at both time points tested.

For the secondary clinical outcomes, results after 4W treatment and 8W treatment were compared to baseline using linear regression with random effects analysis, and adjustment was performed for the following: results at baseline, drug vs placebo group and hand dominance. Results are summarised in Table 13. For grip strength (via hand dynamometer), results were related to baseline performance but no effect was found for treatment group or hand dominance. This held true after adjustment for the presence of MEP abnormalities at baseline, at both time points tested. For proprioception (measured by WPST), tactile discrimination (measured by TDT) and object recognition (measured by fTORT), results were related to baseline performance but no effect was found for treatment group or hand dominance. Scores on the mFIS were measured at 4W and 8W and compared to baseline. Again, results at both time points were correlated with baseline scores but no effect was found according to treatment group. These findings held true after adjustment for the presence of SSEP abnormalities at
baseline (for WPST, TDT and fTORT) and for the presence of VEP abnormalities at baseline (for visual acuity and contrast sensitivity), at both time points tested.

**Questionnaire regarding side-effects and treatment allocation**

Of the forty patients included, 17 correctly identified which treatment arm they had received at the final study visit (five correctly identified that they had been given study drug, and twelve that they had been given placebo). Five patients incorrectly identified themselves as having been in the drug arm and 12 incorrectly identified themselves as receiving placebo; the remaining six were unsure after completion of the study.

**Electrophysiological Outcome Measures**

For the electrophysiological measures tested, results after 4W and 8W treatment were compared to baseline using linear regression with random effects analysis, and adjustment was performed for results at baseline and drug vs placebo group. Results are summarised in Table 14.

**Evoked Potentials and Peripheral Conduction**

For VEPs, both P100 latency and amplitude in each eye at 4W and 8W correlated with baseline values but no effect was found by treatment group. For F-wave latency (indicating conduction in the peripheral part of the median nerve), results at 4W and 8W in each hand correlated with baseline values but no effect was found by treatment group. For SSEPs (measured as Central Conduction Time and N20-P25 amplitude), results at 4W and 8W correlated with baseline values but no effect was found by treatment group in either hand. For CMCT and rMT, results at 4W and 8W correlated with baseline values but no effect was found by treatment group.

**Paired-Pulse TMS and MEP Recruitment Curves**

For PPTMS, results from conditioning response:test response at each of the 4 ISI’s were compared from baseline to 4W and 8W for both hands and interaction analysis was performed. Data were adjusted for age, sex, disease duration and EDSS category. There was no significant effect of treatment group at any of the time points or any of the ISI’s measured (drug vs placebo p=0.126). Results of the comparison between baseline and 4W are summarised in Figure 8.

For MEP recruitment curves, results of MEP amplitude:peripheral CMAP amplitude at each of the four stimulus intensities were compared from baseline to 4W and 8W and interaction analysis was performed. Data were adjusted for age, sex, disease duration and EDSS category. Again, there was no significant effect of treatment group at any of the time points or any of the stimulus intensities measured (drug vs placebo p=0.71). Results of the comparison between baseline and 4W are summarised in Figure 9.
Table 13 – clinical secondary outcome measures by treatment group at 4 weeks (4W) and 8 weeks (8W)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (BL) Median (IQR)</th>
<th>4W Median (IQR)</th>
<th>8W Median (IQR)</th>
<th>4W Drug/Placebo P-value</th>
<th>8W Drug/Placebo P-value</th>
<th>4W vs BL P-value</th>
<th>8W vs BL P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grip strength (Z)*</td>
<td>-1.22 (-1.18, -1.14)</td>
<td>-1.92 (-1.95, -1.8)</td>
<td>-0.61 (-0.41, -0.19)</td>
<td>0.863</td>
<td>0.796</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WPST*</td>
<td>10.95 (7.45-14.4)</td>
<td>11.75 (7.4-14.3)</td>
<td>10.7 (8.4-15.22)</td>
<td>0.285</td>
<td>0.826</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TDT*</td>
<td>44% (25-60%)</td>
<td>47% (27-68%)</td>
<td>42% (25-60%)</td>
<td>0.38</td>
<td>0.411</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>rTORT*</td>
<td>97% (86-100%)</td>
<td>100% (90-100%)</td>
<td>100% (95-100%)</td>
<td>1.0</td>
<td>1.0</td>
<td>&lt;0.0001</td>
<td>0.049</td>
</tr>
<tr>
<td>mFIS</td>
<td>48.5 (42.5-57.5)</td>
<td>44 (35-52)</td>
<td>43 (36-52)</td>
<td>0.167</td>
<td>0.582</td>
<td>0.013</td>
<td>0.021</td>
</tr>
<tr>
<td>Sloan 100%</td>
<td>149 (126.5-161.5)</td>
<td>154 (124-164)</td>
<td>151.5 (124-169)</td>
<td>0.158</td>
<td>0.269</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sloan 2.5%</td>
<td>64 (35-84)</td>
<td>69 (34-79)</td>
<td>66.5 (41.5-84)</td>
<td>0.06</td>
<td>0.26</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sloan 1.25%</td>
<td>34 (7-54)</td>
<td>18 (6-54)</td>
<td>26.5 (3-54)</td>
<td>0.585</td>
<td>0.654</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Adjusted for hand dominance
Table 14 – electrophysiological secondary outcome measures by treatment group at 4 weeks (4W) and 8 weeks (8W)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (BL) Median (IQR)</th>
<th>4W Median (IQR)</th>
<th>8W Median (IQR)</th>
<th>4W Drug/Placebo P-value</th>
<th>8W Drug/Placebo P-value</th>
<th>4W/BL P-value</th>
<th>8W/BL P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P100 lat.</td>
<td>126.6 (111.3-145.75)</td>
<td>124.95 (111.9-144.3)</td>
<td>125.85 (111.6-149.4)</td>
<td>0.958</td>
<td>0.533</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P100 amp.</td>
<td>6.2 (4.05-9.2)</td>
<td>5.95 (3.9-9.1)</td>
<td>6.1 (4.3-8.3)</td>
<td>0.344</td>
<td>0.644</td>
<td>&lt;0.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>F-wave lat.</td>
<td>27.38 (25.7-29.8)</td>
<td>27.4 (25.9-29.9)</td>
<td>28.3 (26.1-29.7)</td>
<td>0.351</td>
<td>0.101</td>
<td>&lt;0.0001</td>
<td>0.004</td>
</tr>
<tr>
<td>CCT</td>
<td>8.6 (6.2-12.1)</td>
<td>7.4 (5.8-10.55)</td>
<td>6.9 (5.55-9.15)</td>
<td>0.714</td>
<td>0.211</td>
<td>0.79</td>
<td>0.094</td>
</tr>
<tr>
<td>N20-P25</td>
<td>2 (1.25-3.05)</td>
<td>1.9 (1.2-3.2)</td>
<td>1.5 (1.2-3.1)</td>
<td>0.263</td>
<td>0.265</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>CMCT</td>
<td>10.68 (8.9-13.9)</td>
<td>11.1 (9.1-13.25)</td>
<td>11.4 (9.6-14.35)</td>
<td>0.671</td>
<td>0.894</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RMT</td>
<td>59% (48-79%)</td>
<td>56% (46-73%)</td>
<td>58% (49-74%)</td>
<td>0.401</td>
<td>0.173</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
In summary, while performances and measures of all variables at four weeks and eight weeks were strongly related to baseline measures, indicating internal consistency in the results, there was no effect of treatment group (drug vs placebo) found for any of the clinical or electrophysiological measures at any of the time points included.

**Withdrawals and Serious Adverse Events (SAEs)**

Study withdrawals and SAEs were recorded and the reviewing Ethics Committee notified during the course of the study. There were three withdrawals from the placebo arm and no withdrawals from the drug treatment arm. Withdrawals were for the following reasons: (1) lack of efficacy (after 14 days’ treatment) in two cases; (2) presumed side-effects (after 28 days’ treatment).

Three subjects were hospitalised during the study period; two were in the drug group and one in the placebo group. One patient (placebo group) was hospitalised due to vertigo. Symptoms were present prior to study commencement but worsened after entry into the study. The study drug was continued and symptoms abated. Another patient (drug group) was hospitalised after an episode of syncope and diarrhoea which developed within the first two weeks of commencement of the study. At the time, blinding was maintained and the patient was subsequently re-challenged with the same medication with no recurrence in symptoms, and went on to complete the required period of treatment with the study drug. The relationship of these events to the study drug was deemed uncertain. The final patient (drug group) was hospitalised with a urinary tract infection after 5 weeks of treatment with the study drug. In this context, he reported a global reduction in function and exacerbation of his usual MS symptoms. He had a previous history of multiple urinary tract infections with similar presentations. His symptoms improved with antibiotic treatment and the study drug was continued throughout. The relationship of the adverse event to the study drug was deemed uncertain.
Chapter 9: Discussion

This study represents a significant addition to the existing body of literature relating to the effects of fampridine-MR on upper limb function and in relation to the physiological effects of the drug in PwMS. It is the first study designed to address this research question using a rigorous double-blind randomised trial methodology. The study has both strengths and limitations, which will now be discussed.

The Fampridine Withdrawal Study (FWS)

The FWS was designed to explore the effects of drug treatment in a population of patients who were already taking the drug were most likely to be clinical responders in terms of ambulation, and thus might therefore be more liable to demonstrate improvements in electrophysiological responses or improvements in clinical responses in domains outside ambulation. This pilot study demonstrated that the study design was feasible and tolerable to participants, and that the required clinical and electrophysiological assessments could be conducted within a reasonable timeframe for study visits. Although statistical analysis could not be performed due to the small sample size, the results suggested that more formal evaluation in a double-blind trial setting was warranted.

The most significant methodological difficulty affecting recruitment was the lack of availability of the drug and prohibitive cost of private prescriptions, resulting in a small sample and prohibiting a meaningful formal statistical analysis. Of the five patients included, three were funding their own supply of drug and two were receiving ongoing supplies of drug as part of the extension phase of a previously-conducted phase IV clinical trial. Therefore these two patients had already been classified as “responders” in terms of their ambulatory function. For the purposes of the trial, response was defined as improvement in walking speed on the T25FW – though there was no specified minimum improvement required for inclusion – after 2 and 4 weeks treatment, with an associated improvement on the subjective MSWS-12 questionnaire reflecting self-rated ambulation. It is noteworthy that the proportion of defined “responders” in this open-label study was substantially greater than in the pivotal placebo-controlled trial of Goodman [160], reflecting the relatively less stringent criteria for response. Any degree of improvement counted towards response, regardless of whether this met the threshold for minimal clinically important difference. The open-label design made bias due to placebo effect also a real risk [160, 588]. The three patients who were funding their own private prescriptions had not been required to objectively demonstrate response. We did not stipulate that those patients who had already shown a good response demonstrate this a second time, as we felt this would further reduce our already-small pool of available patients and further limit recruitment. A number of patients were approached at screening and were unwilling to cease drug, even for the
required 10 day period for the purposes of measurements being made off therapy, and in fact it might be surmised that these patients were particularly good responders, although this could not be formally evaluated.

Another potential methodological issue was the absence of clinically evident upper limb dysfunction in a portion of this group of patients, again reflecting difficulties with recruitment and the small pool of available patients. Of the five included patients, two demonstrated no clinically evident upper limb dysfunction, meaning that the likelihood of demonstrating a clinical response in terms of the clinical outcome measures was extremely small. However, I deemed that it would still be valid to include these patients even in the absence of clinical signs and symptoms of upper limb impairment because of the objective electrophysiological tests which might not be dependent on the presence or absence of clinical upper limb dysfunction.

A third issue related to blinding; by the nature of the study, patients were not blinded to their treatment allocation for the study period. This may have affected the clinical tests performed, even though reasonable efforts were made to maintain blinding of the assessors. Again, this would not be expected to affect outcomes of the electrophysiological testing and given that this was an exploratory pilot study whose findings would subsequently be validated in a double-blind setting, this was not felt to be a major issue.

In summary, the findings of this pilot study suggested some trends towards improvement in patients when “on” was compared to “off” treatment, with small reductions in times for 9HPT and small improvements in grip strength, fatigue, wrist position sense and VEP latency. However, the small size of the study and the absence of a placebo control group means that caution must be applied in the interpretation of these findings. The methodological issues identified certainly had the potential to affect the validity of our findings in a number of ways, but in a climate where drug costs are high and access to drug supplies is limited, I did not feel it would be possible to improve recruitment enough to overcome the issue of sample size. Imposing an exclusion on patients without upper limb dysfunction would have further compounded this issue. Despite these issues, I felt it important to perform this pilot study in a population of patients who had the potential to be “higher yield” in terms of response rate, and the results certainly implied that it would be worthwhile exploring these research questions in a larger placebo-controlled study specifically including patients with upper limb impairment.

**The Fampridine Upper Limb Study (FULS)**

The central body of this work concerns the FULS, which is the first study of its kind designed to adequately address the question of upper limb response to fampridine in a placebo-controlled setting.
and with electrophysiological correlates. The inclusion criteria were selected such as to reflect the patient group who might be inclined to take this medication in a real-world setting, and the blinding and assessment process was rigorous enough to minimise the chance of placebo effects producing bias in the findings. The study results were internally consistent and it was clear that no significant effects of fampridine-MR on clinical or electrophysiological outcomes could be demonstrated in this population and using this assessment paradigm. The battery of assessments conducted was rigorous and comprehensive. As in the FWS, I have identified a number of methodological issues with FULS which may have affected the validity of my findings.

The first of these relates to sample size. Sample size was calculated on the basis of the primary outcome and the minimal clinically important difference for the 9HPT which has been estimated at 20% [218]. While we felt that our sample size was adequate to address this outcome, many of the secondary clinical and electrophysiological outcomes have unknown minimal clinically important differences, and therefore it might be assumed that the required sample size would be different, and potentially much greater numbers of participants would be required to demonstrate an effect.

The second methodological limitation relates to the heterogeneity in nature of the upper limb impairment as well as heterogeneity in terms of disease duration and severity demonstrated in our included patient population. Our population included patients with a variety of severity in upper limb impairment, and a variety in terms of its nature. Some patients had predominant muscle weakness, while others had spasticity, ataxia, tremor or sensory dysfunction. There was also a range of disease durations and disease courses. Numbers of included patients were too small to permit subgroup analysis according to the nature of upper limb impairment in particular patients. It might therefore be the case that the drug could be more beneficial for some types of upper limb dysfunction or some subtypes of MS than others. We did not specify the type of dysfunction for two reasons; one being that the original trials of fampridine-MR for gait disturbance did not specify a particular type of ambulatory dysfunction as a requirement for inclusion in the study, and the second being that the mode of action of drug is sufficiently unclear that we were unable to predict which patients might benefit most from its action. Furthermore, we sought to include patients who might be a realistic representation of those patients who would take a symptomatic therapy in the real world, and therefore did not wish to be too prohibitive in this regard. Future research in this area might consider the benefits of targeting therapy towards patients with particular types of upper limb dysfunction (for example weakness or spasticity) who might be more likely to benefit from this drug than patients with, for example, ataxia or sensory dysfunction.

Some authors have restricted their studies of fampridine-MR to patients with heat-sensitive symptoms, believing that these patients would be most likely to show benefit because of the presumed
reversibility of their neurological dysfunction. I felt that this would be unjustifiably prohibitive and would create major difficulties with achieving the projected sample size.

A third methodological issue relates to matching of groups at baseline. Although this was a randomised trial, and any variation between groups at baseline was therefore presumed due to chance, comparison of drug- and placebo-treated patients at baseline did identify a greater disease severity (measured by EDSS category) in the drug-treated patients, with 20% of the drug-treated group categorised as “moderate” severity and 65% as severe, as compared to 55% moderate and 40% severe in the placebo-treated group. Although this was adjusted for in analysis of our primary outcome measure, and therefore unlikely to have affected the results overall, unadjusted analysis of the multiple secondary outcome measures did not take this into account and it might be reasonable to suppose that this could have affected some of the secondary outcome measures.

A fourth methodological issue relates to withdrawals and missing data. As detailed above, there were three withdrawals during the course of the study, all from the placebo group, which had the potential to bias the results in either direction and which created further imbalance between the groups. Missing data resulted from a number of sources. Some patients had such severe disability that they were unable to complete some parts of the clinical testing. A small minority were unable to tolerate parts of the electrophysiological testing (somatosensory evoked potentials, in two cases). By the nature of transcranial magnetic stimulation, some patients and even healthy controls have an rMT which is so high as to preclude further suprathreshold stimulation, rendering either paired pulse TMS (which requires stimulation at 120% of rMT) or, more often, MEP recruitment curve measurement (which requires stimulation at 140% of resting motor threshold), impossible. It is expected that the randomised nature of the study design would have led to relatively comparable numbers of patients being affected by these issues in each group, but variations due to chance could not be eliminated in a study of relatively small sample size.

A final methodological issue relates to inter-rater variability in the clinical assessments performed. Strenuous efforts were made to ensure that serial clinical measurements were made by the same assessor, and this was achieved in over 90% of study visits. Whilst some measurements were made by other observers inter-observer variation is unlikely to have influenced the results. For the primary outcome measure, the 9HPT, inter-rater reliability is very high and has been estimated at >90% in healthy controls [444] and 86% in PwMS[586] Similarly, inter-rater reliability for the mFIS has been established in a cohort of PwMS as being around 95%[589] and for Sloan contrast sensitivity around 86-95% in PwMS and healthy controls[590]. At present there is no interobserver variability data for the Sensory Discrimination Capacity test. All three assessors were trained in the execution of this by
the same trainer and under the same conditions, to minimise the degree and impact of any inter-rater variability.

The study design ensured that placebo effect was minimised, as this was felt to have been a potential confounding factor. 17/40 patients correctly identified their treatment arm after the final visit; a further 17/40 were incorrect (with the majority of patients in both groups believing they had received placebo); the impact of patient’s beliefs regarding treatment allocation on their clinical performance is difficult to estimate but the blinded nature of the study would hopefully have reduced the impact of this on the study results.

A number of other authors have sought to evaluate the effects of 4-AP or fampridine-MR on upper limb function [167-171], vision [565, 573, 577] and fatigue [167, 168, 220, 582, 583] [560], and our findings are largely in accord with those reports.

Savin [170] conducted a small open-label pilot study in 26 patients examining a variety of clinical measures of upper limb function, and reported significant benefits in dominant hand grip strength as well as a reduction in dominant hand 9HPT time, the latter of which failed to reach the level of minimal clinically important difference. These results were reported in raw kg values and times rather than Z-scores (adjusted for age and sex, both of which are known to affect both of these parameters) and the study was not placebo-controlled, so that these findings should be interpreted with caution. Benefits were seen after four weeks of treatment, suggesting that the time course of our study would have been sufficient to detect any benefits which were present. Jensen measured manual function in a group of 108 treated patients in an open-label study which was predominantly concerned with ambulation, and reported a small but statistically significant decrease in raw time for 9HPT; again, raw times rather than Z-scores were reported and no placebo control group was included. Similar findings were reported by Allart [168]; in this study, small-magnitude decreases in raw 9HPT times were reported in a population defined as responders in terms of gait, without placebo control group. Pavsic [167] reported similar small benefits in 9HPT times alongside a multitude of other improvements in fampridine-MR responders (defined in terms of gait) in another open-label study of 30 patients.

Blinded trials in this area are few and far between. Schwid [171] conducted a blinded trial examining grip strength, and failed to demonstrate any statistically significant improvement in treated patients. To date, no other authors have published the findings from blinded trials examining grip strength as the primary endpoint, although we have identified a number of further studies in progress currently [591] [592].
No benefit of fampridine-MR on vision could be demonstrated in the current study. These findings are largely in accord with those of other authors, though again blinded and randomised trials are scarce. Van Diemen [565, 577] studied both intravenous and orally administered 4-AP and showed no improvement in visual acuity in a pair of blinded crossover studies. Davis [195] found no improvement in visual function in a group of 20 patients given various doses of 4-AP or placebo (n=5, though allocation procedures were not reported). Jones [573] reported improvement in various visual functions in an open-label study of five patients with temperature-sensitive symptoms using high-dose 4-AP, with a high rate of intolerable side effects within the group.

One well-conducted double-blind placebo-controlled study of the effects of fampridine-MR on vision [196] reported no benefit of drug treatment on the clinical primary outcome measure (improvement on Snellen visual acuity chart at 2.5% contrast), although there was a suggestion that RNFL thickness might be a factor which predicted improvement. It was therefore proposed to conduct a further study in a group of patients using RNFL as an inclusion criterion. Another placebo-controlled study [593] has reportedly been completed and the investigators failed to show benefit, although findings have not formally been published. Thus our findings are in accord with this well-conducted placebo-controlled study which failed to show a clinical benefit in terms of visual acuity. I have identified one further placebo-controlled clinical trial which is currently in progress in this area [594] and includes patients with a previous episode of optic neuritis due to MS.

In the current study, no benefit of fampridine-MR on fatigue could be demonstrated on the mFIS scale. Pavsic reported a significant benefit of drug on mFIS score in an open-label study, although the magnitude was small and no attempt was made to control for placebo effect [167]. Benefits have been reported in other small and open-label studies using various fatigue scales and include those of Allart [168] (using the Fatigue Severity Scale), Ruck (using the Fatigue Scale for Motor and Cognitive Functions) [582] and Sheean (using the Fatigue Severity Scale) [560], but none included a placebo control group and caution should therefore be applied in the interpretation of these findings. Romani (using the Fatigue Impact Scale) compared 4-AP to fluoxetine and demonstrated no difference in fatigue levels, but no placebo control arm was included [583]. The only study which did include a placebo group was that of Rossini [220], and overall the primary outcome (change in FSS) did not differ between groups, although there was a suggestion of small-magnitude benefit in patients who were treated with high-dose 4-AP. A dose-finding placebo-controlled study by Goodman [549] failed to demonstrate a benefit in terms of fatigue. Thus my findings are in accord with the existing published literature, with a placebo control arm being especially important in the evaluation of a purely subjective symptom of this nature. I was not able to identify any trials currently in progress in this domain.
Published literature relating to the electrophysiological effects of 4-AP is also scarce but again my results are broadly in accord with previously described findings.

Mainero [366] studied the response of a group of PwMS to a single dose of 20mg of 3,4-DAP. They reported no effect on CMCT, MEP latency, amplitude, or rMT (similar to our findings), but in contrast they did demonstrate a reduction in intracortical inhibition and an increase in intracortical facilitation in a PPTMS paradigm. Given the differences in the drug and dose, however, the comparability to our findings is uncertain. Sheean [560] studied a group of eight PwMS also using 3,4-DAP and found no effect in their chosen electrophysiological parameters (CMCT and rMT) after 3 weeks of treatment.

Fujihara studied six patients with stable spastic paraparesis related to MS and measured MEP’s before and after intravenous administration of 4-AP [561]. They reported a significant increase in MEP amplitude in response to drug, with no significant effect on MEP onset latency. Given the different route of administration, the different measurement made (MEP amplitudes are sufficiently variable as to be unreliable for repeated measurements) and the absence of a placebo group or other measures to control for MEP amplitude variability, the significance of these findings is uncertain.

Rossini [220] studied the response of 54 patients with progressive MS to high-dose oral 4-AP (32mg per day) in a placebo-controlled crossover design and measured MEPs, SSEPs and VEPs before and after treatment. They did not identify any effect of drug on latency or amplitude of responses, although they did report an improved synchronisation of MEP responses, contributing to an improvement in “MEP shape score” (not one of my measured parameters) which reportedly correlated with clinical benefit in terms of fatigue. Zeller studied CMCT for lower limb MEP’s in a population of 25 patients who were using fampridine-MR for walking disability; they reported a positive correlation between change in CMCT in response to drug and clinical response in terms of gait improvement [595]. Upper limb parameters were not included in their study. They proposed that abnormal CMCT at baseline was able to predict clinical response (in that none of their five patients with normal baseline lower limb CMCT were defined as responders, whereas nine of twenty patients with prolonged CMCT met the response criteria of 10% improvement in T25FW and timed 50m walk); this was not in accord with my own findings, where no baseline electrophysiological markers were predictive of clinical outcome.

A number of authors have investigated the effects of 4-AP on VEP latencies. Van Diemen [552] studied 70 patients using a placebo-controlled crossover design and studied the response to intravenous 4-aminopyridine at variable doses (titrated to the development of side effects) and demonstrated a statistically significant decrease in latency and increase in amplitude for one eye (left)
only, but without reported clinical improvement. The magnitude of latency reduction was small and the clinical significance is uncertain. Jones [573] administered high-dose oral 4-AP to two subjects with a prior history of optic neuritis for one week and reported no significant difference, despite a clinical response in terms of visual improvement in both cases (notably, this study was not placebo-controlled). Davis studied the response of 20 patients with temperature-sensitive symptoms to a single dose of oral 4-aminopyridine (or placebo) and reported a significant reduction in VEP latency following treatment which was not seen with placebo. The magnitude of the latency change was fairly large (mean 6.90ms with central field measurement) but this only correlated with clinical improvement in two of 20 patients tested [195].

Electrophysiological responses to 4-AP have also been described in populations of patients following spinal cord injury. Wolfe [596] reported a significant reduction in the rMT in a group of paraplegic and incomplete quadriplegic patients after 4-AP in a crossover study paradigm, as well as reductions in MEP latency, CMCT and increased MEP amplitudes, but without any effect on peripheral conduction parameters. The sample size was small (18 patients in total) but the population was relatively homogenous in terms of the site of the neurological lesion, which may account for the difference in findings from my own results. Similarly, Qiao [597] reported an improvement in MEP amplitude (not one of our measured parameters) along with a significant decrease in MEP latencies which were prolonged at baseline with no effect on peripheral nervous system conduction in a group of stable spinal cord injured patients after a single oral dose of 10mg 4-AP. Clinical effects were modest, with associated improvements being reported (anecdotally only, without formal measurement) in two of 13 patients. This is in accord with an animal study which examined the response of intravenous 4-AP in rats with graded spinal cord injury and demonstrated an improvement in amplitude.

In summary, my clinical and electrophysiological findings are mainly in accord with those in the existing published literature. Most authors who have studied the effects of 4-AP using an appropriate study design have not demonstrated any clinical benefit in the domains which were tested in the current study. Some authors have shown small effects on the electrophysiological parameters which I included but in general their results have not been directly comparable to my own.

The negative results with respect to the impact of fampridine on the electrophysiological measures means that no firm conclusions can be drawn regarding its mode of action in PwMS. It was hypothesised that (i) fampridine might exert its effects by increasing cortical excitability; (not evident comparing rMT or MEP recruitment curves in treated and untreated patients) or (ii) fampridine might improve central conduction times, (not evident comparing measurements of VEP, SSEP or CMCT before and on treatment). It is possible that the magnitude of these effects is not sufficient to detect in
an alternative explanation is that this sample size, or that there is too great a degree of heterogeneity in electrophysiological response to produce positive findings at the group level. It is also possible that fampridine may exert its clinical effects through another means, such as a synchronisation of motor unit firing or a reduction in variability of conduction times across axons, neither of which were fully evaluated in this study.

The principal advantage of the present study is therefore in its placebo control arm and double-blind treatment allocation and blinded outcome assessment, as many of the functions studied are liable to bias as a result of placebo effect. This study represents the first double-blind placebo-controlled trial evaluating 9HPT and other outcomes relating to upper limb function, and adds to a very small body of literature relating to the effects of drug on vision and fatigue. To my knowledge, one other placebo-controlled study in this domain is in progress [592] but has not been published at the time of writing.

Future research questions which remain to be addressed in this domain include whether there are subpopulations of patients, classified according to the nature of their upper limb impairment (e.g. weakness rather than ataxia or sensory loss), the site of their neurological lesions (e.g. spinal lesions rather than brain lesions), their disease course (e.g. RRMS vs progressive MS) or their clinical features such as temperature sensitivity, who might demonstrate a more favourable clinical response. The additional contribution of physical and occupation therapies in addition to drug therapy is also something which could be explored and might be expected to have additional impact on response to drug treatment. Finally, a more thorough electrophysiological evaluation of patients who are clear responders in terms of either gait or other neurological functions might also yield useful information regarding the mode of drug action or might allow the generation of hypotheses relating to predictors of clinical response in treated patients.
Chapter 10: Conclusions

Lack of effective symptomatic therapies for PwMS represents a large unmet need. Despite great strides forward in recent decades in the area of disease-modifying treatment for RRMS, there are only a very limited number of symptomatic therapies in current use, and appraisal of the evidence suggests that very few of these are supported by high-quality evidence from appropriately designed randomised controlled trials.

Fampridine-MR is one of the few symptomatic therapies which does have some robust supporting evidence, and is a potentially interesting therapy in terms of its mode of action and variability in terms of clinical response. Beyond its role in walking difficulty, many other potential benefits have been reported anecdotally and in open-label non-randomised studies, but these have generally not been formally tested in a double-blind randomised design. The present study has added to the small existing body of literature evaluating fampridine-MR in this context. The findings suggest that while individual patients may continue to report symptomatic benefits from the drug, on the whole such benefits are likely to be modest and caution should be applied in widening the clinical indications for the drug.

Evoked potentials and TMS are techniques of interest in the current climate where biomarkers to be used alongside clinical and radiological monitoring are highly desirable for diagnosis, prognostication and response to therapy. The findings from the present study support the ability of these objective biomarkers to differentiate between MS patients and healthy control subjects, with significant differences being demonstrated in most of the measured electrophysiological parameters. In the context of fampridine-MR, however, it does not appear likely that such biomarkers can adequately differentiate patients based on their likely response to drug or otherwise, and reliable differences in the measured parameters were not apparent after drug treatment and did not correlate with clinical findings. The findings from the present study provide good evidence that these techniques are reproducible, tolerable and acceptable to patients, and confirm the feasibility of using similar paradigms to study other drug therapies in future.
Figures

1. TMS recording apparatus using standard coil and recording from left abductor pollicis brevis
2. Physiological MEP Recruitment Curve

Figure 2: Physiological MEP recruitment curve

![Graph showing the MEP amplitude in relation to % stimulation above resting motor threshold.](Image)
3. Physiological PPTMS Curve

![Physiological PPTMS curve](image)

Figure 3: Physiological PPTMS curve
4. Wrist Position Sense Test apparatus (3 pictures)
5. Tactile Discrimination Test apparatus – surfaces with variable grating size
6. Functional Tactile Object Recognition Test apparatus – poster of included objects
7. MEP Recruitment Curves in Patients and Controls at Baseline
8. PPTMS curves by treatment group at Baseline (BL) and 4 weeks (4W)
9. MEP Recruitment Curves by treatment group at Baseline (BL) and 4 weeks (4W)

[Image: MEP Recruitment Curves diagram]
References


242. Patti, F., et al., *Quality of life, depression and fatigue in mildly disabled patients with relapsing-remitting multiple sclerosis receiving subcutaneous interferon beta-1a: 3-year...*


### Appendix 1: Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>APB</td>
<td>Abductor Pollicis Brevis</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
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<td>ADEM</td>
<td>Acute Disseminated Encephalomyelitis</td>
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<tr>
<td>AHSCT</td>
<td>Autologous Haematopoietic Stem Cell Transplantation</td>
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<td>ARAT</td>
<td>Action Research Arm Test</td>
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<tr>
<td>BAEP</td>
<td>Brainstem Auditory Evoked Potential</td>
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<td>BBB</td>
<td>Blood Brain Barrier</td>
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<td>BBT</td>
<td>Box and Blocks Test</td>
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<tr>
<td>bd</td>
<td>twice daily</td>
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<tr>
<td>CCT</td>
<td>Central Conduction Time</td>
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<tr>
<td>CDMS</td>
<td>Clinically Definite Multiple Sclerosis</td>
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<tr>
<td>CIDP</td>
<td>Chronic Inflammatory Demyelinating Polyradiculoneuropathy</td>
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<tr>
<td>CIS</td>
<td>Clinically Isolated Syndrome</td>
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<tr>
<td>CMCT</td>
<td>Central Motor Conduction Time</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>DASH</td>
<td>Disabilities of the Arm, Shoulder and Hand</td>
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<tr>
<td>DMF</td>
<td>Dimethyl Fumarate</td>
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<tr>
<td>EAE</td>
<td>Experimental Autoimmune Encephalomyelitis</td>
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<tr>
<td>EBV</td>
<td>Epstein-Barr Virus</td>
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<tr>
<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>EMR</td>
<td>Eye Movement Registration</td>
</tr>
<tr>
<td>ERP</td>
<td>Event-Related Potential</td>
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<tr>
<td>FIS</td>
<td>Fatigue Impact Scale</td>
</tr>
<tr>
<td>FS</td>
<td>Functional Systems (in EDSS)</td>
</tr>
<tr>
<td>FSS</td>
<td>Fatigue Severity Scale</td>
</tr>
<tr>
<td>fTORT</td>
<td>Functional Tactical Object Recognition Test</td>
</tr>
<tr>
<td>GA</td>
<td>Glatiramer Acetate</td>
</tr>
<tr>
<td>GNDS</td>
<td>Guy’s Neurological Disability Score</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>ICF</td>
<td>Intracortical Facilitation</td>
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<tr>
<td>IFN-β</td>
<td>Interferon-beta</td>
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ISI  Interstimulus Interval
JCV  John Cunningham Virus
JHFT  Jebsen Hand Function Test
LEP  Laser Evoked Potential
LP  Lumbar Point
MAGNIMS  Magnetic Resonance Imaging in Multiple Sclerosis
MEP  Motor Evoked Potential
mFIS  Modified Fatigue Impact Scale
MRI  Magnetic Resonance Imaging
MS  Multiple Sclerosis
MSFC  Multiple Sclerosis Functional Composite
MS-FS  Multiple Sclerosis-Specific Fatigue Score
MSIS-29  MS Impact Scale-29
MSSS  Multiple Sclerosis Severity Score
MSWS  Multiple Sclerosis Walking Scale
MT  Motor Threshold
NMO  Neuromyelitis Optica
OCT  Optical Coherence Tomography
ON  Optic Neuritis
NIH  National Institutes of Health
PASAT  Paced Auditory Serial Addition Test
PML  Progressive Multifocal Leucoencephalopathy
PNS  Peripheral nervous system
PPMS  Primary Progressive Multiple Sclerosis
PPT  Purdue Pegboard Test
PPTMS  Paired-Pulse Transcranial Magnetic Stimulation
PRMS  Progressive-Relapsing Multiple Sclerosis
PSI  Percentage Spatial Increase (in TDT)
PwMS  Patients with Multiple Sclerosis
RCT  Randomised Controlled Trial
RIS  Radiologically Isolated Syndrome
RNFL  Retinal Nerve Fibre Layer
RRMS  Relapsing and Remitting Multiple Sclerosis
SF-36  Short Form Health Survey-36
SICI  Short-Latency Intracortical Inhibition
SPMS  Secondary Progressive Multiple Sclerosis
SSEP  Somatosensory Evoked Potential

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<table>
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
</tr>
<tr>
<td>T25FW</td>
<td>Timed 25-foot Walk Test</td>
</tr>
<tr>
<td>TDT</td>
<td>Tactile Discrimination Test</td>
</tr>
<tr>
<td>TEMPA</td>
<td>Test d’Evaluation des Membres Supérieurs de Personnes Agées (TEMPA)</td>
</tr>
<tr>
<td>TM</td>
<td>Transverse Myelitis</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>TUG</td>
<td>Timed Up and Go test</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper Limit of Normal</td>
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<tr>
<td>VEMP</td>
<td>Vestibular Evoked Myogenic Potential</td>
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<tr>
<td>VEP</td>
<td>Visual Evoked Potential</td>
</tr>
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<td>WPST</td>
<td>Wrist Position Sense Test</td>
</tr>
<tr>
<td>2MW</td>
<td>2-minute Walk Test</td>
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<tr>
<td>3,4-DAP</td>
<td>3,4-Diaminopyridine</td>
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<td>4-AP</td>
<td>4-Aminopyridine</td>
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<tr>
<td>6MW</td>
<td>6-minute Walk Test</td>
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<tr>
<td>9HPT</td>
<td>9- Hole Peg Test</td>
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Appendix 2: Kurtzke Expanded Disability Status Scale (EDSS)

0 - Normal neurological exam (all grade 0 in Functional Systems (FS)).

1.0 - No disability, minimal signs in one FS (i.e., grade 1).

1.5 - No disability, minimal signs in more than one FS (more than on FS grade 1).

2.0 - Minimal disability in one FS (one FS grade 2, others 0 or 1).

2.5 - Minimal disability in two FS (two FS grade 2, others 0 or 1).

3.0 - Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three or four FS grade 2, others 0 or 1) though fully ambulatory.

3.5 - Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3; or five FS grade 2 (others 0 or 1).

4.0 - Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest 500 meters.

4.5 - Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance: characterized by relatively severe disability usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300 meters.

5.0 - Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work a full day without special provisions): (usual FS equivalents are one grade 5 alone, others 0 or 1: or combinations of lesser grades usually exceeding specifications for step 4.0).

5.5 - Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities: (usual FS equivalents are one grade 5 alone, others 0 or 1: or combination of lesser grades usually exceeding those for step 4.0).

6.0 - Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 meters with or without resting: (usual FS equivalents are combinations with more than two FS grade 3 +).

6.5 - Constant bilateral assistance (canes, crutches, braces) required to walk about 20 meters without resting (usual FS equivalents are combinations with more than two FS grade 3 +).

7.0 - Unable to walk beyond approximately 5 meters even with aid, essentially restricted to a wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day; (usual FS equivalents are combinations with more than one FS grad 4 +; very rarely pyramidal grade 5 alone).

7.5 - Unable to take more than a few steps; restricted to wheelchair, may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair; (usual FS equivalents are combinations with more than one FS grade 4 +).
8.0 - Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day, retains many self-care functions; generally has effective use of arms; (usual FS equivalents are combinations, generally grade 4+ in several systems).

8.5 - Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions; (usual FS equivalents are combinations generally 4+ in several systems).

9.0 - Helpless bed patient: can communicate and eat; (usual FS equivalents are combinations, mostly grade 4+).

9.5 - Totally helpless bed patient; unable to communicate effectively or eat/swallow; (usual FS equivalents are combinations, almost all grade 4+).

10.0 - Death due to MS.
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