DEEP GREY MATTER AND FATIGUE IN

MULTIPLE SCLEROSIS

GRAHAM NIEPEL, MBChB MRCP (UK)

Thesis submitted to the University of Nottingham

for the degree of Doctor of Philosophy

July 2012

Abstract

Fatigue is a common and major symptom in multiple sclerosis (MS). A number of potential mechanisms exist as to the cause of MS-fatigue. These include that it is an immune-mediated symptom or that it is due to neuroendocrine or autonomic dysfunction. Studies have shown reduced activity in cortical and deep grey matter regions and disruption of cortico-subcortical circuits has been theorised. This may lead to difficulty in the planning or pre-movement stage of activity with compensatory overactivity contributing to fatigue. Finally, dysfunction of the hypocretin system, deficiency of which occurs in narcolepsy, has also been suggested. A number of deep grey matter structures, including the basal ganglia, thalamus and hypothalamus, are implicated in these mechanisms and the work presented in this thesis explores their role.

Conventional magnetic resonance imaging (MRI) techniques whilst crucial in diagnosis and monitoring disease activity are generally felt to correlate poorly with disability and symptomatology. Quantitative MRI techniques have been shown to provide a more comprehensive evaluation of the extent of MS pathology and correlate better with clinical deficit. T1 relaxation time measurement is one such quantitative MRI technique and has been shown to demonstrate abnormalities in small structures such as the pyramidal tracts and correlate with disability.

Firstly, we measured the T1 relaxation times of the thalamus and basal ganglia in a cohort of MS patients and assessed for any relationship with fatigue severity. Secondly, in view of its key role in the autonomic, neuroendocrine and hypocretin pathways, we performed the same measurement in the hypothalamus of a cohort of patients and again assessed for any relationship to fatigue.

Subsequently, to further evaluate any possible contribution from the hypocretin system we measured cerebrospinal fluid (CSF) hypocretin-1 levels in patients with a number of neurological diseases including a cohort of MS patients and evaluated for any relationship with severity of self-reported fatigue and hypersomnolence.

Studies in MS-fatigue, including those undertaken by our group, traditionally rely on self-reported measures of fatigue severity. These questionnaire-based measures are subject to a number of drawbacks including rater bias and lack of definition of fatigue. In the final study, we assessed the effectiveness of the wakefulness-promoting drug, modafinil, in MS patients with and without fatigue by assessing its effect on objective measures of alertness and vigilance, including neurophysiological and laboratory-based measures. In addition, in this study we evaluated any potential role of the autonomic system in MS-fatigue.

We found significantly higher T1 relaxation times in a number of deep grey matter structures including the thalamus, putamen and latterly the hypothalamus in MS patients as compared to controls. The T1 relaxation time of the thalamus was higher in fatigued patients as compared to non-fatigued patients and it correlated with fatigue severity.

We found lower CSF hypocretin-1 levels in patients with MS and inflammatory disorders as compared to non-inflammatory conditions and this was significant in the inflammatory cohort. However, we found no relationship with fatigue or hypersomnolence severity. We did, however, detect a significant difference on a sympathetic cardiovascular reflex test between fatigued and non-fatigued patients. Finally we noted a significant improvement with modafinil, as compared to placebo, in a number

of objective measures of alertness in patients with MS-fatigue and notably this was not a class-effect.

To this extent, the findings from this thesis provide evidence for the potential involvement of pathology in the thalamus in the mechanism of MS-fatigue, possibly through disruption of cortico-subcortical circuits. In addition, in a separate cohort of patients there was evidence of a relationship between autonomic disturbance and fatigue. We have however found no evidence of a relationship between the hypocretin system and fatigue in MS. Finally we have demonstrated supportive evidence for a role for modafinil in the treatment of fatigue, a symptom for which, despite its frequency and severity, there is often a paucity of treatment options available for MS specialists.

Publications

Niepel GG, Tench CR, Morgan PS, Evangelou N, Auer DP, Constantinescu CS. Deep grey matter and fatigue in MS: a T1 relaxation time study. Journal of Neurology. 2006 Jul; 253(7): 896 – 902.

Zellini F*, Niepel GG*, Tench CR, Constantinescu CS. Hypothalamic involvement assessed by T1 relaxation time in patients with relapsing-remitting multiple sclerosis. Multiple Sclerosis. 2009 Dec; 15(12): 1442 – 1449.
*Joint first author

Constantinescu CS, Niepel GG, Patterson M, Judd A, Braitch M, Fahey AJ, Harikrishnan S, Edwards LJ, Tench CR, Bennett GW, Ghatei M. Orexin A (hypocretin 1) levels are not reduced while Cocaine/amphetamine regulated transcript levels are increased in the cerebrospinal fluid of patients with multiple sclerosis: no correlation with fatigue and sleepiness. Journal of the Neurological Sciences. 2011 Aug; 307(1-2): 127 – 131.

Acknowledgements

Special thanks to the patients and volunteers that participated in the studies without whom this research would not have been possible. Finally, I would like to thank my wife, Louise, who despite all evidence to the contrary, never wavered in her belief.

Table of Contents

Abbreviations

ACTH	adrenocorticotropic hormone
ADEM	acute disseminated encephalomyelitis
BDI	Beck depression inventory
BP	blood pressure
CFFF	critical flicker fusion frequency
CFS	chronic fatigue syndrome
CIS	clinically isolated syndrome
CMRGlu	cerebral metabolic rate of glucose
CNS	central nervous system
CPAP	continuous positive airways pressure
CRH	corticotrophin releasing hormone
CRT	choice reaction time
CSF	cerebrospinal fluid
DT	diffusion-tensor
EDS	excessive daytime sleepiness
EDSS	expanded disability status scale
EEG	electroencephalography
EMG	electromyography
ESS	Epworth sleepiness scale
EWN	Edinger-Westphal nuclei
F	fatigue
FLAIR	fluid-attenuated inversion recovery

FLASH	fast low angle shot
fMRI	functional MRI
FS	functional system
FSS	fatigue severity scale
GAD	gadolinium
GBS	Guillain-Barre syndrome
^{1}H-MRS	proton magnetic resonance spectroscopy
HPA	hypothalamic-pituitary-adrenal axis
ICSD-2	international classification of sleep disorders-2
IFN-γ	interferon-γ
IL	interleukin
IQR	interquartile range
LC	locus coeruleus
MFIS	modified fatigue impact scale
MHE	minimal hepatic encephalopathy
MRI	magnetic resonance imaging
MRT	motor reaction time
MS	multiple sclerosis
MS-FS	MS-specific fatigue scale
MSLT	multiple sleep latency test
MT	magnetization transfer
MTR	magnetization transfer ratio
MVC	maximum voluntary contraction

MVF	maximal voluntary force
MWT	maintenance of wakefulness test
NAA	N-acetylaspartate
NAGM	normal-appearing grey matter
NAWM	normal-appearing white matter
NF	non-fatigue
OSAHS	obstructive sleep apnoea-hypopnoea syndrome
PD	proton density
PET	positron emission tomography
PPMS	primary-progressive MS
PSG	polysomnogram
PST	pupillographic sleepiness test
PUI	pupillary unrest index
QoL	quality of life
REM	rapid eye movement
ROI	region of interest
RRMS	relapsing-remitting MS
RRT	recognition reaction time
SLE	systemic lupus erythematosus
SOREMP	sleep-onset REM periods
SPMS	secondary-progressive MS
SSS	Stanford sleepiness scale
$T_{1/4}$	25% pupil dilatation time

$T_{3/4}$	75% pupil recovery time
TE	echo time
TBI	traumatic brain injury
TMN	tuberomamillary nucleus
TMS	transcranial magnetic stimulation
TNFα	tumour necrosis factor α
TR	repetition time
VAS	visual analogue scale
VEPs	visual evoked potentials
VLPO	ventrolateral preoptic nucleus
VP	vasopressin

List of Figures

List of Tables

CHAPTER 1

Chapter 1.   INTRODUCTION

1.1   Multiple sclerosis

Multiple sclerosis (MS) is a, predominantly T-cell-mediated, inflammatory disease of the central nervous system (CNS). Pathologically it is characterized by destruction of myelin within plaques that typically follow the course of blood vessels. Axonal loss occurs to varying degrees in patients.

The history of MS begins principally in the 1860s with Charcot's lectures at the Salpetriere and what was then termed *sclerose en plaques*. However, earlier descriptions can be found. A possible case of MS is that of Saint Lidwina of Scheidam who is documented as having developed disability in 1396. The diary of Augustus d'Este provides a personal account of MS over 2 decades beginning with an attack of bilateral optic neuritis in 1822. Ollivier d'Angers in 1824 described a likely case in a text on spinal cord disorders. The pathologists, Cruveilhier and Carswell both presented illustrations of MS lesions in the 1830s. The German physician Frerichs named the condition *Hirnsklerose* in 1849. Furthermore, Rindfleisch in 1863 described the perivascular predominance of plaques.

Clinical features of MS vary greatly, especially at the outset, indeed with a significant proportion of patients being polysymptomatic. Physical features with motor, co-ordination and sensory symptoms as well as specific syndromes localizing to the brainstem and spinal cord and cognitive features such as memory impairment and emotional lability occur frequently. MS-related fatigue, as I will detail, can be a physical and/or mental complaint in individual patients.

1.1.1   Pathophysiology

The characteristic feature of MS is the demyelinated plaque. The classic lesion is sharply demarcated with absent myelin and preserved axons within a matrix of glial scar tissue. However some plaques, 'shadow plaques' do have, as well as demyelinated axons, axons with abnormally thin myelin sheaths. These are felt to be areas of remyelination. In chronic lesions there is often only a small rim of thinly myelinated fibres of remyelination around the edge of the plaque. Also in chronic lesions an absence of oligodendrocytes is usual whilst in early, acute disease, oligodendrocyte numbers are preserved and remyelination is pronounced. The pattern seen in primary progressive MS is different. Here oligodendrocytes are destroyed in a small ring outside of the plaque edge suggesting that the primary process is that of oligodendrocyte destruction with secondary demyelination[1].

Inflammation is present within the plaque but also within 'normal-appearing' white matter (NAWM) and grey matter. The inflammatory infiltrate consists mainly of T lymphocytes. Macrophages are also present and, in low numbers, B lymphocytes too.

Axons are relatively but not absolutely preserved and axonal density is reduced in most plaques. The extent of axonal loss varies significantly between patients although is typically similar within different lesions in the same patient. Acute axonal pathology, such as axon interruption and swelling, as well as axonal loss is also seen but most commonly in acute, active MS. Axonal loss is important in that it is irreversible and it is suggested that it underlies the progressive stage of the disease. On neuroimaging, atrophy is the marker of axonal loss and certainly brain and spinal cord atrophy is felt to

correlate better with disability than lesion load. The cause for axonal loss is uncertain. Consequent to demyelination, loss of trophic support or increased susceptibility to immune attack or cytotoxics, such as free radicals, are plausible suggestions[2].

MS is traditionally considered a white matter disease. Plaques are typically distributed in the periventricular white matter and close to the brain surface, in the area between the cerebral cortex and the sub-cortical white matter. Other common sites include the optic nerves, cerebellar peduncles, pons, medulla and cervical spinal cord. As with the pathological involvement of NAWM, the grey matter is also frequently affected and I will now detail this.

1.1.2   Grey matter involvement in MS

The presence of lesions within the grey matter has long been recognized. Brownell and Hughes in 1962 reported the distribution of over 1500 cerebral plaques in the brains of 22 MS patients[3]. They described how 9% of plaques were contained within grey matter with 5% cortical lesions and 4% central grey matter. It was noted, however, that the cortical plaques were almost all from a single case.

More recently, further evidence has accumulated of grey matter involvement in MS from histopathological studies confirming both cortical and deep grey matter lesions as well as microscopic abnormalities in normal-appearing tissue, such as neuronal loss. One study evaluated an MS brain histologically and with imaging[4]. It was found to have 14 (4.3%) cortical lesions. Magnetic resonance imaging (MRI) however only picked up two of these pathologically identified lesions. In the same study a further 12 brains were evaluated solely histologically. This arm of the study, found 106

cortical lesions with a further 372 involving both cortex and subcortical white matter. Peterson et al looked at tissue blocks from 50 MS patients specifically to identify cortical lesions of which 112 were detected[5]. Cortical lesions were noted to be hypocellular compared to white matter lesions. The hypothalamus was investigated by Huitinga et al and found grey matter lesions in 3 of 17 hypothalami[6]. A separate study looked at the thalami of 10 MS patients and estimated a 35% neuronal loss as compared to controls[7].

As indicated above by the study of Kidd et al conventional MRI techniques, such as T2-weighted imaging, are more likely to identify white matter than grey matter lesions. More advanced MRI sequences are better at identifying grey matter disease. Fluid-attenuated inversion recovery (FLAIR) MRI is more sensitive to detecting cortical lesions[8]. Proton magnetic resonance spectroscopy (^{1}H-MRS) of cortical grey matter demonstrated reduced N-acetylaspartate (NAA)[9]. Low NAA has been correlated with axonal loss or dysfunction[10]. Focal, as well as more global, cortical atrophy was demonstrated in MS patients, even those with only short disease duration[11, 12]. Magnetization transfer (MT) and diffusion-tensor (DT) imaging of grey matter has demonstrated varied results. A study involving MT imaging of both the basal ganglia and cortical grey matter revealed significantly reduced magnetization transfer ratio (MTR) in MS patients[13]. Another study, however, found no significant difference between MS patients and controls[14], whilst a further study showed significantly increased fractional anisotropy in the deep grey matter and reduced mean diffusivity in the putamen[15]. Previous studies also employed ^{1}H-MRS to investigate the deep grey matter. MS patients, relative to controls, demonstrated a reduction in NAA concentration, in the thalamus, of between 11 and 19%, as well as a 17 – 25% lower thalamic volume[7, 16]. These results

were supported by post-mortem findings that revealed significant thalamic neuronal loss. Positron emission tomography (PET) studies also demonstrated abnormalities with reductions in the cerebral glucose metabolic rate (CMRGlu) in the grey matter of MS sufferers[17]. This has been demonstrated both in the cortex as well as the thalamus, putamen and head of the caudate nucleus.

1.2   Fatigue

Fatigue is an ill-defined complaint forwarded by patients with a wide variety of medical conditions. In-fact, it is a frequent complaint amongst the general working population. In one study of the primary care population, up to 20% of men and 25% of women complain of always feeling tired[18]. Within the medical population, fatigue is a major feature of, amongst other diseases; chronic fatigue syndrome, sleep disorders, depression, infectious diseases, autoimmune and endocrine disorders, neuromuscular diseases, epilepsy, Parkinson's disease and malignancy.

Both patients and health professionals use the term fatigue, within these conditions, to refer to a variety of concepts including; lethargy, tiredness, sleepiness, weakness, lack of motivation as well as reduced mental and physical endurance. This imprecision ensures that the study of fatigue as a symptom remains difficult. This should not however detract from its importance.

1.3   MS-related fatigue

As previously described, MS has both physical and cognitive effects. Indeed, fatigue in MS, as in other medical conditions, has been characterised in both mental and physical

terms. Various MS investigators have defined fatigue as a feeling of weakness, tiredness out of proportion to the level of exertion, lack of capacity to generate sufficient muscle force and inability to sustain mental performance.

To provide consensus, in 1998 the Multiple Sclerosis Council for Clinical Practice Guidelines published a definition of fatigue as *a subjective lack of physical and/or mental energy that is perceived by the patient or caregiver to interfere with usual and desired activities*[19].

The panel also sought to distinguish acute from chronic fatigue. Acute episodes of fatigue are commonly associated with relapses, concomitant infections and acute changes in weather and may of course be managed differently. It labeled chronic fatigue as *that present for any amount of time on 50% of the days for more than 6 weeks* whilst acute fatigue was *a new or significant increase in feelings of fatigue in the previous 6 weeks*[19].

A number of features are felt to distinguish MS-related fatigue from that which is experienced by the normal population[19]. These are:

- Comes on easily
- Exacerbated by heat
- Prevents sustained physical activity
- Interferes with responsibilities
- Interferes with physical functioning
- Causes frequent problems

This contrasts with a number of features that are felt to be unhelpful in differentiating MS-related fatigue to that experienced by the normal population. These features include exacerbation with stress or depression, diurnal variation i.e. worse in the afternoon and improvement with rest, sleep or positive experiences.

1.3.1   Epidemiology

Fatigue is undoubtedly a common and major symptom. Fatigue is reported by 78 – 86% of MS patients[20, 21]. In studies it has been found to be the single most commonly reported symptom above all other physical or mental complaints[20]. It frequently is the presenting symptom[21].

Fatigue is ranked as one of the three worst symptoms by two thirds of MS patients[21]. It is a frequent cause of unemployment[22]. It interferes with activities of daily living. It can affect other symptoms of MS and has a significant impact in quality of life studies[23, 24].

1.4   Measuring fatigue

Multiple techniques exist to measure MS-fatigue in both a subjective and an objective fashion.

1.4.1   Subjective measures

These methods rely on patients interpreting their level of fatigue and its impact. They consist predominantly of questionnaires and rating scales. Numerous validated self-report

tools exist within the literature. They vary from simple, single-item visual analogue scales to 83-item questionnaires.

The Fatigue Severity Scale (FSS), I propose, is the most widely used fatigue-self-report measure used in MS studies[23]. It is a nine-item scale with each item being graded between 1 – 7 whereby 1 indicates strong disagreement and 7 strong agreement. The result is presented as either the mean or sum score. It is routinely suggested that the questions apply to your perception of fatigue over the preceding two weeks. Generally as a guide a mean score greater than 4 indicates fatigue whilst a score of 5 indicates severe fatigue. It has shown high reliability and validity and has been applied in both cross-sectional and longitudinal studies[23].

The benefits of using such a scale are that it is brief as well as easy to comprehend and employ for both health professionals and patients alike. Drawbacks include rater bias, fluctuation and lack of a definition of fatigue. In addition, there is a potential ceiling effect that could become particularly apparent in longitudinal studies.

1.4.2   Objective measures

These are performance-related measures that aim to quantify the level of fatigue. Tools have been applied in studies to assess both mental and physical fatigue.

Physical fatigue has been assessed in a variety of manners. One such example is recording maximal isometric voluntary contraction of certain muscles for a set period and calculating a so-called Fatigue Index, which is the decay in maximal force during exercise[25]. An alternative method involves repetitive electric and transcranial magnetic supramaximal stimulation of certain muscles during sustained exercise and

observing for any change in the force of the response[26]. Motor evoked potentials have been used as a further neurophysiological method of quantifying physical fatigue[27].

Assessment of cognitive fatigue has been undertaken in a variety of ways. Cognitive function as measured by a wide-ranging neuropsychological assessment has been undertaken in MS patients with assessment performed before and after a continuously, cognitively-effortful task[28]. Neurophysiological methods have been applied. Measuring evoked brain potentials in response to certain stimuli for example an auditory target-detection task has been used[27].

1.5   Pathophysiology of MS-fatigue

MS-related fatigue can be further defined according to the presumed pathophysiology as primary and secondary fatigue.

1.5.1   Secondary MS-fatigue

Secondary fatigue is that which is attributable to other MS-related factors. A number of factors have been proposed. Deconditioning, the idea that fatigue is due, in part, to impaired muscle performance due, for example, to muscle changes secondary to upper motor neuron involvement or muscle disuse with disability. Certainly exercise programs are commonly used in the management of fatigue. Specific respiratory muscle weakness has been identified as a potential cause of reduced exercise capacity[29, 30]. Pharmacological agents used to treat other MS-related symptoms could very well contribute to fatigue. Similarly pain or poor sleep due to any number of factors could also contribute to secondary MS fatigue

1.5.2   Primary MS-fatigue

This term is used to describe that which is felt to be directly attributable to the disease process. Investigators may use the term central fatigue too. There are multiple proposed mechanisms for the underlying cause of primary MS-related fatigue and I will detail them in the following paragraphs.

1.5.3   Immune disorder

The strongest evidence for this is that fatigue is a common and disabling symptom amongst autoimmune disorders, for example, systemic lupus erythematosus (SLE)[31]. As with MS, fatigue can signify the onset of an SLE relapse. A number of laboratory-based studies have looked for evidence that immune system activity has a role in MS-related fatigue and have revealed inconsistent results.

Heesen et al looked at 15 MS patients with fatigue and 15 without, as determined by FSS scores[32]. They measured serum levels of production of pro-inflammatory cytokines, tumour necrosis factor α (TNFα) and interferon-γ (IFN-γ), as well as the anti-inflammatory cytokine interleukin (IL)-10. Both TNFα and IFN-γ were significantly higher in the fatigue group. In addition, both cytokines correlated with a self-reported fatigue score when corrected for disability, disease duration, disease severity and progression, depression and treatment with interferon. A similar study by Flachenecker et al assessed the mRNA expression of the same cytokines in 26 patients with MS-related fatigue and 11 patients without[33]. TNFα mRNA levels were significantly higher in the fatigue cohort. It is of note that there is some evidence that

31

TNFα has been shown to be higher in patients with active MS as compared to those with stable disease[34].

Another study evaluated different inflammatory markers, serum C-reactive protein and soluble intercellular adhesion molecule-1 as well as urinary neopterin excretion, in 38 MS patients[35]. There was no correlation of any of these laboratory markers with fatigue scores as determined by the FSS.

A small study by Bertolone et al reports an improvement in fatigue scores in MS patients paralleling a decrease in serum levels of IL-1β, soluble IL-2 receptor and IL-6[36]. A further study also found higher levels of serum markers of immune activity in MS patients with fatigue as compare to those non-fatigue patients[37]. The markers were B2 microglobulin, soluble IL-2 receptor and soluble CD8. Rudick and Barna, however, reported no association of IL-2 and soluble IL-2 receptor with fatigue in 8 MS patients[38].

Also of note is the randomized, double-blind, placebo-controlled, crossover trial by Wingerchuk et al of high-dose aspirin in which the response of MS-fatigue was assessed[39]. Fatigue scores were found to be lower during aspirin treatment. One potential mechanism for this improvement is the effectiveness of aspirin in treating cytokine-mediated processes.

In addition, aspirin as well as other non-steroidal agents and, at times, corticosteroids are used for deteriorations associated with the administration of immune-modifying treatment, interferon-beta, in MS. These deteriorations, termed flu-like episodes, often feature prominent fatigue, are possibly related to increased cytokine levels

and provide further evidence for an immune process being involved in MS-related fatigue.

1.5.4 Neuroendocrine

It has been felt that neuroendocrine disturbance particularly of the hypothalamic-pituitary-adrenal (HPA) axis plays some role in the pathophysiology of MS-related fatigue. Studies in chronic fatigue syndrome (CFS), a condition characterized by disabling fatigue, have shown low levels of cortisol and hypoactivity of the adrenal gland to stresses[40]. In addition, patients with other autoimmune inflammatory conditions, such as rheumatoid arthritis, have been shown to have a hyperactivity of the HPA axis and a poor response to stress[41, 42].

A study of 52 MS patients evaluated the HPA axis with a battery of tests and found evidence of central upregulation of HPA activity that may be associated with active inflammation[43]. In addition, in the secondary-progressive MS sub-group, there was a reduction in the HPA response to stress that may be a consequence of the disease or chronic activation by corticosteroids. However, the presence or absence of fatigue was not found to be associated with any hormonal measures.

Heesen et al, within the trial mentioned earlier, evaluated the HPA axis activity of 15 patients with MS fatigue and 15 without[32]. There was no difference found between the groups although, there was a trend for a correlation between baseline cortisol level and FSS score. However, another study looked at 31 MS patients of which 15 patients had fatigue and 16 had no fatigue. This group found a significant association between the presence of fatigue and hyperactivity of the HPA axis[44].

Of note is that one study also evaluated the hypothalamic-pituitary-gonadal axis amongst its battery of investigations[43]. They found that 24% of male MS patients (age 32 – 40 years) had a low serum testosterone levels. Fatigue is a common symptom in patients with testosterone deficiency.

1.5.5 Autonomic nervous system dysfunction

MS causes autonomic nervous system dysfunction with the most common reported symptoms being that of bladder and sexual dysfunction. However, other systems are affected. In one study of 63 MS patients, an abnormality, other than bladder, gastrointestinal and sexual disturbance, was found in 56% of patients on autonomic investigations[45]. Twenty-nine percent of patients had an abnormal cardiovascular test with 18% classified as having autonomic dysfunction, defined as abnormalities found on two or more autonomic tests (not including bladder function). Another study found autonomic cardiovascular dysfunction in 27% of MS patients[46]. This is despite only a much smaller proportion of MS patients actually complaining of specific cardiovascular symptoms such as syncope. There has been some suggestion that there is an increase in autonomic dysfunction with increasing disability and also with chronic progressive MS however this correlation has not seen in all studies[45, 46].

The observation that symptoms of autonomic dysfunction, for example, weakness, exhaustion and cognitive difficulties and the relationship of such features to environmental factors, such as heat, has led investigators to hypothesise that autonomic dysfunction may contribute to fatigue. In addition, autonomic dysfunction has been

identified in CFS patients and treatment of orthostatic hypotension improves fatigue symptoms in CFS sufferers[47].

Studies of fatigue and autonomic dysfunction in MS patients have typically shown conflicting results. Merkelbach et al looked at 84 MS patients, 64% of whom suffered from fatigue[46]. There was no significant difference in fatigue scores between patients with and without cardiovascular autonomic dysfunction. Flachenecker et al, on the other hand, in a study of 60 MS patients, 45% of whom suffered from fatigue, found that cardiovascular autonomic dysfunction only occurred in those with MS-related fatigue[48]. There was evidence of significant differences in both parasympathetic and sympathetic tests between patients with and without fatigue. On further analysis, there appeared to be a more specific association between fatigue and sympathetic dysfunction.

1.5.6   CNS mechanisms

There is evidence from several studies of a central or CNS origin to fatigue, separate to the proposed neuroendocrine and autonomic nervous system dysfunction already discussed. I will detail evidence and hypotheses now and in the following sub-chapter.

Investigators have employed a variety of neurophysiological techniques. Leocani et al studied 32 MS patients using electroencephalography (EEG) with electromyography (EMG) during a simple motor action[49]. Results suggested impairment of cortical structures involved in motor planning in patients with MS-related fatigue. It was theorized that compensatory overactivity may occur in certain regions, such as frontal structures, and this in turn may lead to central fatigue.

Sheean et al employed transcranial magnetic stimulation (TMS) with EMG in 21 patients[26]. A decline in maximal voluntary force (MVF) during isometric muscle contraction was found and was paralleled by a decline in central activation. TMS immediately post-exercise revealed that the stimulated twitch force in patients was the same as pre-exercise. In controls, the MVF also declined but less rapidly and the post exercise stimulated twitch force of the muscle was significantly reduced. This suggested that fatigue in MS was central as opposed to the peripheral, muscular, fatigue of normal subjects.

TMS was also employed by Perretti et al in 41 MS patients[50]. Normally, after fatiguing exercise, the motor evoked potential produce by TMS decreases in amplitude. In this study, however, this decrease in amplitude did not occur in the MS group. This result was felt to support an intracortical dysfunction on the basis that this phenomenon has an intracortical origin.

Finally, Sandroni et al performed cerebral and spinal root magnetic stimulation in 10 MS patients with each patient being tested in a 'normal' and 'fatigued' state[27]. No significant difference was found in the central motor conduction time (difference in latency between the motor evoked potential produced by cerebral and root stimulation). Additional assessment of reaction times and brain evoked potentials to an auditory target, in the same two states, were also performed. Again evoked potentials did not differ between rested and fatigued states, however, reaction times during the task became significantly prolonged in the fatigued state. This suggested that the difficulty in the fatigued state was due to dysfunction in the planning or pre-movement stage rather than the stimulus evaluation or the initiation of movement stages.

MRI has also been used to investigate for a potential central mechanism in MS fatigue. Conventional MRI techniques, in particular T2-weighted imaging, have traditionally focused on white matter abnormalities and have become the main paraclinical tool for diagnosing MS. Studies have demonstrated that abnormalities detectable on proton density- (PD) or T2-weighted imaging have, at best, a limited role in the pathogenesis of MS-related fatigue. Colombo et al found a significant correlation between fatigue severity and MRI lesion burden on PD-weighted imaging[51]. Other studies, however, have detected no association between fatigue and T2 hyperintense or T1 hypointense lesion loads and nor has the number of gadolinium (GAD)-enhancing lesions been shown to be an important factor[17, 52, 53]. Furthermore, no correlation has been found with either any regional or global atrophy measure[17, 52].

1.5.7   Deep grey matter involvement

The potential role of the deep grey matter in the pathogenesis of fatigue has been demonstrated in other pathologies. An MRI based study of post-polio-fatigue patients revealed a significant correlation between fatigue severity and the presence of hyperintense areas within the white matter and grey matter, including the putamen[54]. Fatigue and somnolence, often transient, are reported side effects of stereotaxic surgery involving the thalamus and the globus pallidus[55, 56]. A meta-analysis of reports of various lesions, including cerebrovascular insults and tumours, affecting the basal ganglia revealed that the commonest disturbance subsequent to damage of the caudate nucleus was abulia[57].

The hypothalamus is also implicated as having a potential role in MS-related fatigue through a variety of mechanisms. These have been discussed in some detail above including via the neuroendocrine axis and autonomic dysfunction. A further potential mechanism is that of hypersomnia and this will be discussed in more detail in a future sub-chapter.

Investigators have used different neuroimaging techniques to assess the deep grey matter in MS patients. In a cohort of relapsing-remitting MS subjects, Filippi et al employed functional MRI (fMRI) and found that, during a simple motor task, patients with fatigue showed significantly lower, relative activation of the ipsilateral cerebellar hemisphere and rolandic operculum and the contralateral middle frontal gyrus and thalamus[58]. In addition, there was a significant inverse correlation between fatigue severity and the activity in the contralateral thalamus and ipsilateral rolandic operculum. A PET study by Roelcke et al revealed reductions in CMRGlu in MS patients with severe fatigue, as compared to those without fatigue, in several areas[17]. This CMRGlu reduction was evident in the prefrontal cortex, premotor cortex and supplementary motor area as well as the putamen and head of the caudate nucleus. Significantly lower CMRGlu was also seen in the internal capsule and white matter adjacent to the prefrontal cortex. The results of both these studies appeared to imply a role for the deep grey matter structures in the pathophysiology of MS-related fatigue. A purported mechanism is the disruption of cortico-subcortical circuits linking cortical grey matter with the basal ganglia and thalamus[58]. This dysfunction may lead to over-activity in alternative pathways to enable motor programming with this compensatory mechanism contributing to fatigue.

1.6 Excessive daytime sleepiness

Sleepiness is a high physiological drive toward sleep. It obviously is a normal experience for most individuals. Excessive daytime sleepiness (EDS), however, is abnormal and is that which interferes with normal activities of daily living. Patients suffering from EDS will often describe either a constant feeling of sleepiness or an irresistible propensity to sleep attacks.

EDS is a common complaint of people suffering from insufficient or disrupted sleep or for those suffering from a primary sleep disorder. The International Classification of Sleep Disorders (ICSD-2) describes the wide variety of diagnoses that may have hypersomnia as a prominent feature[59]:

I. Insomnias

II. Sleep-related breathing disorders

III. Hypersomnias of central origin

IV. Circadian rhythm sleep disorders

V. Parasomnias

VI. Sleep related movement disorders

These categories include relatively common conditions such as narcolepsy with or without cataplexy, obstructive sleep apnoea-hypopnoea syndrome (OSAHS) and restless legs syndrome.

1.7 Measuring EDS

Similar to measurements of fatigue, there are both objective and subjective tools available.

1.7.1 Subjective measures

As with fatigue measures, these consist predominantly of questionnaires and similarly there are numerous published tools. Examples include the Stanford Sleepiness Scale (SSS) and the Epworth Sleepiness Scale (ESS).

The ESS is the most commonly used questionnaire both experimentally and clinically[60]. It rates sleep propensity in eight various, sedentary situations during recent times. Each item is rated between 0 – 3 whereby 0 is 'would never doze' and 1, 2 and 3 indicate slight, moderate and high chance of dozing respectively. A distinction is made between dozing and simply feeling tired. Scores, therefore, range from 0 – 24. An ESS score > 10 indicates hypersomnolence whilst > 16 indicates a high level of EDS.

It has been shown to be valid and reliable and to significantly distinguish normal subjects from patients with variety of conditions including narcolepsy and OSAHS[60, 61]. It allows monitoring of treatment effects for example improvement in patients with OSAHS when administered nasal continuous positive airways pressure (CPAP)[61]. It may even allow the clinician to distinguish moderate or severe OSAHS from mild OSAHS although this idea is disputed by other studies[60, 62]. Scores have been shown to correlate significantly with sleep latency as measured during the Multiple Sleep Latency Test (MSLT) although this again has been questioned by other studies[60, 62, 63].

Other benefits include that it is brief and simple to administer. It has also been suggested it may overcome the component of daytime sleepiness that may be more temporary, for example, related to the time-of-day or possibly the effects of drugs. Drawbacks again include rater bias as well as possible gender bias with a study having suggested that men may not report subjective sleepiness as often as women[63].

The SSS provides an immediate assessment of how alert you are feeling[64]. The subject chooses which of seven descriptions currently represents their level of alertness. It is obviously very quick to administer and provides an immediate indication which may be usefully in studies to assess immediate responses to treatments. However, it is susceptible to time-of-day variation and rater bias. In addition it may be less applicable clinically when assessing overall response to treatments.

1.7.2   Objective measures

The MSLT is the gold-standard measure of the degree of sleepiness on the day of assessment[65]. It consists of a series of four-to-five, 20-minute daytime naps in conditions that are conducive to sleep. The latency to sleep onset is recorded. Unfortunately it is expensive as well as time-consuming for both patient and investigator. A normal sleep pattern has to be maintained for one-week prior to testing. In addition, a polysomnogram (PSG) should be performed the night before testing to look for causes that may lead to EDS and confirm adequate sleep is obtained. An MSLT latency of < 8 minutes is consistent with EDS whilst a latency of < 5 minutes indicates severe EDS. Other measures are also taken including the number of sleep-onset REM periods (SOREMP) with ≥ 2 SOREMPs supportive, although not alone diagnostic, of narcolepsy.

A variant of this is the Maintenance of Wakefulness Test (MWT)[66]. In this investigation the patient is asked to remain awake for 40 minutes in dim light and in a semi-reclined position. Again it remains relatively time-consuming and expensive. The MWT is less frequently used although it is sometimes the choice investigation for example by employers such as airlines.

Critical Flicker Fusion Frequency (CFFF) is a tool employed quite frequently in psychopharmacology[67]. During the investigation the subject determines the frequency at which a flickering light appears to be a continuous steady light. It is felt to assess the level of tonic CNS alertness and vigilance.

Pupillography is a further neurophysiological investigation to assess alertness and will be discussed in detailed in the following sub-chapters.

1.8   Pupillography

In subjects with decreasing alertness there is a characteristic, spontaneous pupillary behaviour in darkness. Whilst alert the pupil remains relatively large and stable with minimal oscillations in diameter. These changes in diameter are typically less than 0.3mm with a frequency of approximately 1 Hz. However with drowsiness the pupil size gradually diminishes and there are slow oscillations, less than 0.5 Hz, of diameter that are of gradually increasing amplitude and which can reach several millimetres. These have traditionally been termed 'fatigue waves' although some authors have suggested sleepiness waves as being more accurate[68].

This observation is the basis for the Pupillographic Sleepiness Test (PST) in which a patient has their pupil size constantly monitored via infrared video

pupillography whilst in a quiet, dark environment[69]. Typically three measurements are

derived, the total power of fluctuations (a measure of the total amplitude of low

frequency pupillary movements), the mean pupil diameter for a data segment and the

pupillary unrest index (PUI). The PUI is the cumulative distance moved by the pupil

margin per minute. Both the PUI and power will increase with diminishing alertness

whilst the mean pupil diameter will decrease

.

1.8.1   Pathophysiological basis of fatigue waves

Pupil constriction occurs due to contraction of the circular smooth-muscle fibres of the

sphincter pupillae via activation of the parasympathetic system. For example in the light

reflex, impulses travel from the retina, via the optic nerve to the optic tract. From there

they diverge toward the midbrain to synapse in the pretectal nuclei and then to both

Edinger-Westphal nuclei (EWN) in which the cell bodies of the parasympathetic

preganglionic neurons lie. From here the impulses travel to synapse, in the ciliary

ganglion within the orbital apex, with the postganglionic parasympathetic fibres which

travel to the sphincter pupillae. It is of note that the sphincter provides a much greater

force on pupillary size than the pupillary dilator muscle.

Pupillary dilatation occurs via two processes; activation of peripheral

sympathetic innervation of radial muscle fibres of the dilator papillae and central

inhibition of the EWN. This central inhibition is currently felt to be mediated by two

pathways which project from brainstem nuclei to the EWN. The first is a GABA-ergic

pathway from the A1/A5 nuclei to the EWN which passes by way of the hypothalamus.

The second is a noradrenergic pathway from the locus coeruleus (LC) to the EWN. It is

of note that, amongst other sites, hypocretin-containing neurons project from the hypothalamus to the LC.

A dynamic equilibrium normally maintains pupillary size. With sleepiness, central inhibition of the EWN reduces and there is an unstable drift of central sympathetic activation. This leads to increasing miosis and the instability underlies the pupillary fatigue waves.

1.8.2   Pupillography and sleepiness

The question of whether pupillography can be used as an indicator of sleepiness has been investigated in a number of studies both in normal individuals and patients.

Danker-Hopfe and colleagues, employed pupillography, the SSS questionnaire and a modified MSLT at 2-hour intervals throughout a 16-hour day in 12 healthy, non-sleep-deprived individuals[70]. The study demonstrated that, for time-of-day variations in sleepiness, the PUI and mean pupil diameter correlated with sleep latency. The physiological assessments did not, however, correspond with the individual's subjective assessment of their sleepiness.

A study by Wilhelm et al involved performing pupillography and completing the SSS questionnaire 2-hourly in a cohort of 13 healthy but sleep-deprived subjects[71]. Over the course of 10 hours the PUI, power of fluctuations and subjective rating all increased significantly whilst the mean pupil diameter became significantly smaller. Whilst the SSS values also increased over the study period, there was, however, no correlation between the SSS scores and the pupil data.

A further study by the same group involved the same investigations in healthy controls repeated 2-hourly over a 30-hour period of extended wakefulness and demonstrated a similar pattern of PUI values and pupil diameter over the course of the investigation[72]. This study did also show a significant correlation of subjective scales with pupillography results in that higher SSS scores were associated with higher PUI values.

Regen et al also looked at pupillography in healthy subjects and found that changes in PUI correlated with distinct changes in the waking EEG, when both investigations were performed 2-hourly, during a period of extended wakefulness[73].

Pupillography has also been studied in small numbers of patients with a variety of conditions. Merritt and colleagues assessed cohorts of narcoleptics, patients with OSAHS and healthy controls with pupillography and concurrent EEG[74]. Only the mean pupil diameter was measured on pupillography. They found that there was a significant increase in the amount of slow-wave activity with increasing pupillary miosis in sleep-disordered patients although not controls.

McLaren et al studied patients with narcolepsy and idiopathic hypersomnia, as well as healthy controls, with pupillography and MSLT[75]. They found that sleep latency was significantly correlated with PUI. Grouping patients according to the severity of their sleepiness, based on the shortness of their sleep latency, revealed that the median PUI was greatest in the patients with severe sleepiness. Wilhelm et al also performed pupillography in hypersomniacs (narcolepsy and OSAHS) and seven controls[76]. There was a significant difference in PUI and power between patients and controls.

Pupillography has the benefit of being a quick, relatively simple objective investigation which is closely related to the gold-standard investigation of sleepiness, the MSLT. In fact it is felt to reflect the same physiological aspect of sleepiness as the MSLT. It has been shown to detect differences between hypersomniacs and controls and can also document the effect of treatments[75-77].

1.8.3 Pupillography and MS

There has been limited use of pupillography in studies involving MS patients. Egg and colleagues investigated whether pupillography variables, principally as measures of autonomic instability, were related to MS fatigue[78]. The cohort of 51 MS patients included those with a single clinical attack, relapsing-remitting, primary- and secondary-progressive MS patients. It is of note that seven patients were suffering from an acute relapse at the time of the study and 18 patients were on disease modifying or immunosuppressant treatment. There was also a cohort of 22 healthy volunteers although not sex-matched. The investigation protocol involved questionnaires including the SSS, Modified Fatigue Impact Scale (MFIS) and FSS as well as pupillography to determine the PUI, their preferred outcome. Thirty-four (67%) of the MS patients had severe fatigue according to the FSS as compared to six (27%) of the control group. None of the patients or controls suffered from hypersomnolence as determined by the SSS. The study found that the mean PUI value tended to be higher in MS patients than controls but this did not reach significance. Unexpectedly they found a significant inverse correlation in MS patients between PUI and fatigue as determined by the FSS and MFIS. This would seem to suggest that fatigue in MS patients was associated with higher levels of alertness which

seems at odds with what would be expected. The investigators, however, preferred to conclude that autonomic instability was not associated with fatigue in MS.

A further study by the same group involved 61 MS patients and 42 controls[79]. This again included a number of patients with a single attack or undergoing a relapse. Patients underwent the PST as well as completed the ESS and SSS. No fatigue assessment was performed as the investigators were primarily evaluating hypersomnolence in MS. No difference was found between patients and healthy volunteers in terms of the PUI and the questionnaire-based measures. It was noted that, after correction for age and sex, there was a significantly smaller mean pupil diameter in the MS group. No correlation was found between the PUI and ESS or SSS scores. It is of note that there were a higher number of healthy volunteers (38%), as compared to the MS cohort (26%), with an ESS score indicative of EDS, although this was not statistically significant. It was concluded that there was no pupillographic evidence of EDS in MS.

1.9   EDS and fatigue in hypersomnolent conditions

The idea of an association between fatigue and sleepiness is contentious. Some authors feel it is clear that somnolent patients suffer with fatigue. Others however disagree and maintain that fatigue is a common, non-specific complaint that should be differentiated from sleepiness and may be more likely to signify depression.

Evidence does exist however to support the former argument that fatigue is a component of hypersomnolent conditions. OSAHS, a condition characterised by brief episodes of asphyxia during sleep with associated oxygen desaturation secondary to obstruction of the pharynx, is classically associated with EDS. However, a study of 190

patients with OSAHS revealed that reports of fatigue, lack of energy and tiredness were more frequent than sleepiness[80]. In fact lack of energy was considered by the group as the major problem they faced.

Narcolepsy is the classical hypersomnia disorder and will be discussed in more detail later. Patients with narcolepsy in one study complained of significantly greater fatigue than controls[81]. In addition, narcoleptics report significantly low levels of energy and vitality on quality of life questionnaires[82]. Modafinil, a wake-promoting drug, is commonly used as first choice treatment for narcolepsy in the United Kingdom. Trials of the use of modafinil for EDS in narcolepsy patients have also shown a significant improvement in vitality scores[83, 84].

1.10   Narcolepsy

Narcolepsy is a condition first described by Westphal in 1877 and is characterized by a tetrad of clinical features. EDS is the principal symptom. It is constant although will fluctuate in severity and improve after a nap. Cataplexy is the term for episodic bilateral loss of striated muscle tone provoked by emotion, most typically laughter. As with EDS the threshold for provoking these attacks varies. The third feature is hypnagogic hallucinations which are vivid, dream-like episodes occurring at the onset of sleep. They are short-lived, typically frightening, visual experiences sometimes with auditory and tactile elements. Finally, sleep paralysis which is the sensation of complete or partial inability to move either at the onset of sleep or awakening. They are also typically frightening and may be associated with the hallucinations.

Other symptoms such as disturbed nocturnal sleep with brief frequent awakenings and automatic behaviour, whereby brief, semi-purposeful activity whilst drowsy and of which the patient is amnestic, are also frequently seen in narcoleptics.

1.10.1   Pathophysiology of narcolepsy

The majority of cases of narcolepsy are sporadic whilst 1% are familial with a typically autosomal dominant pattern of inheritance. Genetic factors do however play a role in sporadic cases. The clearest example of this is the association with a specific HLA subtype on chromosome 6. There is a strong association between narcolepsy and HLA DQB1*0602 and the risk for developing narcolepsy is even higher in patients who are homozygous for this DNA subtype.

Traditionally all diseases associated with specific HLA subtypes are autoimmune diseases. Therefore the hypothesis considered most probable is that of an autoimmune process affecting an area of the brain central to sleep regulation and leading to narcolepsy. There has, however, been little laboratory evidence to support this. Studies have shown no cerebrospinal fluid (CSF) abnormalities such as oligoclonal bands or abnormal cell counts although a recent study did suggest the presence of an IgG autoantibody in the serum of narcoleptics[85, 86].

1.10.2   Hypocretin and the hypothalamus

In 1998, two independent groups described two hypothalamic, excitatory neuropeptides that each named either hypocretin-1 and -2 or orexin-A and -B[87, 88]. It soon became clear that these separately-termed peptides were one and the same.

In humans, hypocretin-containing neurons are localized in the posterior and lateral hypothalamic nuclei but project widely throughout the brain and spinal cord. Their dendrites secret hypocretin-1 and -2 that interact with two hypocretin receptors (hcrtr-1 and -2). These receptors are spread widely including the cerebral cortex, amygdala, hippocampus, thalamic and subthalamic nuclei as well as brainstem structures, including the reticular formation, dorsal raphe nuclei and LC.

Hypocretin is felt to have a number of important roles including energy metabolism, autonomic function and may activate the HPA axis. However, it became apparent in the late 1990s that hypocretin played a key role in the development of narcolepsy.

Canine narcolepsy was detected in a number of breeds in the 1970s. In 1999 it was identified that mutations in hcrtr-2 gene and subsequent reduction in the function of hcrtr-2 led to familial canine narcolepsy but without alteration in the number of hypocretin neurons or reduction in the CSF hypocretin-1 levels. Sporadic canine narcolepsy, on the other hand, does involve loss of hypocretin neurons and consequently low CSF hypocretin-1 levels. A murine narcolepsy model was also developed with hypocretin knockout mice lacking both hypocretin-1 and -2[89].

Evidence for the role of hypocretin in human narcolepsy followed on. An early study found that CSF hypocretin-1 levels were undetectable in seven of nine narcoleptics investigated[90]. Hypocretin-1 and -2 have been found to be absent in brain tissue (cortex and pons) of six narcoleptics in a separate study[91]. In the same study, hypocretin mRNA was absent in sections of hypothalamus of two narcoleptics whilst a different hypothalamic peptide mRNA was intact. A further study showed a marked

reduction of 85-95% in the number of hypocretin-containing neurons in the hypothalamic tissue of four narcoleptics[92]. There was also some suggestion of neurodegeneration with an increase in the number of astrocytes in the hypothalamus. It is therefore postulated that the hypocretin neurons are reduced due to some autoimmune process.

Quantitative MRI has been employed to investigate further. Lodi et al used ^{1}H-MRS in 23 narcoleptics[93]. They applied a volume of interest to include bilateral hypothalami and evaluated for reduction in NAA. The study found a significant reduction in NAA content in narcoleptics as compared to controls. In addition, NAA content was lower in narcoleptics with cataplexy than those without. Other than the presence of cataplexy there was no relationship between the ^{1}H-MRS results and PSG or MSLT results. A separate study applied ^{1}H-MRS to the brainstem (the ponto-medullary junction) of narcoleptics and found no difference between patient and control groups[94].

Another group used an alternative quantitative MRI technique, voxel-based morphometry, to assess the cortical and sub-cortical grey matter including specific regions of interest such as the lateral hypothalamus and amygdala in 12 narcoleptics with cataplexy[95]. This study found a significant reduction in cortical grey matter volume in narcoleptic patients as compared to controls. This reduction was mainly in the inferior-temporal and frontal regions. Sub-cortical structures including the hypothalamus did not show any significant difference as compared to controls.

1.11   Hypocretin and narcolepsy symptomatology

The mechanism of how hypocretin deficiency leads to narcolepsy is uncertain. Hypocretin neurons are most active during wakefulness and less active in non-REM

sleep. Inhibition of hypocretin neurons seems crucial for sleep initiation. A study has shown that local administration of hypocretin-1 into the LC suppressed REM sleep in a dose-dependent fashion. In addition, an antibody that neutralizes hypocretin could block this suppression of REM sleep.

Hypocretin neurons interact with a spectrum of neurotransmitters for example cholinergic cells in the basal forebrain and dopaminergic cells in the brainstem. It is postulated that hypocretin neurons act as a stabilizer between maintenance of wakefulness and promotion of sleep. Subsequently it is felt that narcolepsy symptoms are most likely secondary to neurotransmitter imbalance due to an altered signal from hypocretin-containing neurons.

EDS is felt to be secondary to reduced dopaminergic transmission. Hallucinations are a manifestation of REM sleep in drowsiness whilst cataplexy and sleep paralysis are manifestations of REM sleep during wakening with REM sleep characterized by muscle atonia. Certainly muscle atonia is induced by activity of cholinergic receptors and inhibition of motor neurons in the brainstem[89].

1.12   CSF hypocretin-1 levels and hypersomnia

Since the initial study, there have been a number of further studies evaluating the levels of CSF hypocretin-1 in a variety of hypersomnolent as well as inflammatory and degenerative neurological conditions[96-109].

A large study revealed 99% specificity and 87% sensitivity between low or undetectable CSF levels and narcolepsy with cataplexy[96]. Normal levels were typically associated with mild narcolepsy, atypical or no cataplexy, and even HLA DQB1*0602 or

DR2 negativity. In addition, studies have shown that undetectable CSF hypocretin-1 levels were significantly associated with shorter latency to sleep onset and a higher frequency of SOREMPs on MSLT and therefore associated with more severe EDS[97].

One study reported that 25% of patients with idiopathic hypersomnia have moderately low CSF hypocretin-1 levels[98]. A single patient (3% of cohort) with OSAHS in a further study was also found to have a low level[96].

Case reports also reveal low CSF hypocretin-1 levels in a variety of secondary hypersomnia conditions. A patient developed narcolepsy with cataplexy, with a moderately low hypocretin-1 level, presumed to be as a consequence of the diencephalic stroke he sustained post-surgical removal of a craniopharyngioma[99]. Low hypocretin-1 has also been reported in other cases including hypothalamic tumours and Prader-Willi syndrome[100, 101].

A number of groups have also looked at other neurological conditions. Studies have looked at groups of patients suffering from Guillain-Barre Syndrome (GBS) in the context of this being an immune-mediated condition. These found that between 18 – 64% of patients have low or undetectable levels, with a higher percentage of patients being affected in the Japanese cohorts[102-104]. There is some suggestion that low levels may be associated with rapidly progressive GBS[103]. Patients with Miller-Fisher Syndrome, a variant of GBS, also had a low hypocretin-1 level in 42% of cases[103]. Another study revealed low CSF hypocretin-1 levels in a small group of patients with SLE[105].

Investigators have also looked at patients with conditions where EDS or sleep-wake disorders are common complaints. A study of six patients with myotonic

dystrophy who suffered from EDS concluded that a single patient had a CSF level within the narcolepsy range whilst three further patients had moderately low levels[106]. The levels did not correlate with disease severity, ESS score or MSLT severity. One group looked at a cohort of 19 patients with advanced Parkinson's disease and found undetectable levels in nine and moderately low levels in ten patients[107]. Levels did correlate with disease severity but not ESS score. A smaller study, however, revealed normal levels in each of three Parkinson's disease patients who complained of EDS[108]. Baumann et al investigated 44 patients who had suffered traumatic brain injury (TBI) and reported low or undetectable levels in 37 patients with moderate-severe TBI but not in those with mild TBI[109].

1.13   CSF hypocretin-1 levels in CNS demyelinating disorders

There are certainly a number of case reports of patients with MS who develop hypersomnia as their manifestation of a relapse. A 22-year old woman with MS developed acute hypersomnia and was found to have new bilateral-hypothalamic lesions on MRI[110]. Her CSF hypocretin-1 level was undetectable. Similarly a 45-year old woman, again with a history of MS, developed hypersomnia and was also found to have bilateral hypothalamic lesions on MRI[111]. CSF Hypocretin-1 level was undetectable. Neither case reported other features of narcolepsy. Interestingly the latter case reports the fact that prior to her relapse and whilst asymptomatic, in terms of somnolence, she was found to have a new lesion only in the right hypothalamus. This may be consistent with the human neuro-pathological studies, whereby patients showed an 85 – 95% reduction in

hypocretin-containing neurons in narcolepsy[92]. Is there a point, in terms of loss or dysfunction of hypocretin-containing neurons, at which patients become symptomatic?

Gledhill et al report a case of secondary narcolepsy without cataplexy in a patient with acute disseminated encephalomyelitis (ADEM)[112]. ADEM is a para-infectious or post-vaccination monophasic CNS demyelinating disorder presenting typically with a degree of encephalopathy and multifocal neurological signs. The patient presented with acute hypersomnia, disorientation and brainstem signs. MRI imaging revealed lesions involving, amongst other areas, the hypothalami and CSF analysis revealed a low hypocretin-1 level.

A number of studies have investigated hypocretin-1 levels in cohorts of MS patients without specifically looking at EDS. Kanbayashi et al obtained CSF from eight MS patients amongst a larger study involving patients with inflammatory (predominantly GBS) and non-inflammatory (Parkinson's disease, stroke, epilepsy, dementia) neurological disorders[102]. Although patients with GBS had significantly lower hypocretin-1 levels than patients with non-inflammatory disorders, MS patients did not. MS patient's CSF hypocretin-1 levels were within normal range.

A further study looked at 44 patients with MS, again within the context of a larger study of patients with a wide variety of neurological as well as psychiatric disorders[105]. These included; infective CNS diseases, benign intracranial hypertension, normal-pressure hydrocephalus and conversion disorders. The MS patients' results were within control range however it was noted that low levels were present in some patients in all of the groups.

A study by Knudsen et al investigated 23 MS patients and 25 patients with optic neuritis[113]. It was noted that the majority of patients in each group were undergoing an acute attack and the specific aim was to compare patients in remission from those in an acute phase. They found normal CSF hypocretin-1 levels in all patients with no difference between the attack and remission groups. Thirty-six patients also completed the ESS with five patients having scores indicative of EDS. No analysis was reported on whether hypocretin levels were different in patients with hypersomnolence nor any correlation analysis of ESS scores and CSF levels.

1.14   EDS in MS

As reported above, hypersomnia can occur in MS patients as a symptom of an acute MS relapse. Studies also show that MS patients who suffer from fatigue may also complain of EDS. One small study involving 30 MS patients revealed that 60% of those with MS-related fatigue had hypersomnia as determined by the ESS. This compared to 13% of those without fatigue[114].

Furthermore studies have investigated the complaint of EDS in patients with MS-related fatigue by looking at whether sleep disturbance or circadian rhythm disorders may contribute to either of these complaints. Conflicting results have emerged. Taphoorn et al employed actigraphy and MSLT in a study of 16 MS patients with fatigue. Actigraphy involves a wrist-worn motion-detector that can monitor and store activity during sleep and wake for prolonged periods[115]. It is able to detect sleep disturbances and circadian rhythm disorders. The investigators found no evidence of a circadian

rhythm disorder in MS patients and no difference in actigraphy between patients and controls.

A further small study evaluated six MS patients with fatigue, but without EDS as measured by the ESS and MSLT, for circadian rhythm disorder by using body core temperature and PSG[116]. The investigations revealed normal sleep-wake rhythm and sleep structure.

On the other hand, Attarian et al compared 15 patients with and 15 patients without MS-related fatigue again using actigraphy as well as the ESS and fatigue questionnaires[114]. Two patients from the fatigue group had a circadian rhythm abnormality and a further 10 had disrupted sleep. This study found a significantly high probability of a relationship between the presence of fatigue and abnormal sleep cycle or disturbed sleep.

1.15   Treatment of hypersomnolence in narcolepsy

Lifestyle changes are of value in the management of narcolepsy, such as planned daytime naps and avoiding large carbohydrate meals and alcohol consumption. However, pharmacological treatment is the mainstay with CNS stimulants having been the traditional approach. These include methamphetamine, dextroamphetamine, methylphenidate and pemoline. Side effects such as tachycardia, irritability, agitation and headache as well as tolerance and the potential for abuse are significant drawbacks.

Modafinil is a non-stimulant, wake-promoting drug that has, over recent years, become the most common, certainly in the UK, first-line agent introduced. It has fewer side effects than stimulants and less risk of abuse.

Modafinil was studied in a large multi-centre, double-blind, placebo-controlled trial involving 240 narcoleptics who were randomized to one of two doses, 200mg or 400mg daily, or placebo[117]. After the nine-week study period, there was a significant improvement with either dose in ESS scores as well as MWT and MSLT mean sleep latency as compared to baseline. There was a significant improvement in MSLT mean sleep latency in the larger dose cohort as compared to placebo. Both treatment groups showed a significant improvement compared to placebo in ESS scores and MWT mean sleep latency.

Its mode-of-action is unclear. It is hypothesized that it likely interacts with a complex network within the hypothalamus and brainstem. A number of wakefulness-promoting neurons have been identified including; the hypocretin-containing neurons of the posterior and lateral (perifornical) hypothalamus; the histamine-containing neurons of the tuberomamillary nucleus (TMN) in the posterior hypothalamus; the noradrenergic neurons of the LC in the pons and dopaminergic neurons within the ventral tegmental area of the midbrain. Potential sleep-promoting neurons include the GABA-ergic neurons of the ventrolateral preoptic nucleus (VLPO) of the hypothalamus. Interconnection between these cell groups is thought to form an arousal system which itself projects widely through the cerebral cortex.

In animal studies, modafinil has been shown to activate a number of these areas. Scammell et al conducted a study that involved assessing the expression of the transcription factor Fos (this being an indicator of neuronal activation) in the brain after administering modafinil[118]. Firstly, it was noted that modafinil promoted wakefulness in the rats. In addition, there was evidence of increased neuronal activation with

modafinil, as compared to a control substance, in hypothalamic areas, namely the TMN and the perifornical area. Other areas including the LC also showed significant activation. With double immunohistochemistry staining, it was also shown that it was indeed hypocretin-containing neurons activated by modafinil in the perifornical area. When modafinil was infused during what would be the normal sleeping period, it was also demonstrated that there was a significant decrease in activation within the VLPO as compared to the control substance.

Furthermore, in human studies some evidence has also been provided for the activation of the LC by modafinil. Modafinil showed an apparent opposite effect on alertness and autonomic testing to the drug clonidine in healthy controls[119]. The LC is particularly sensitive to the effects of clonidine with so-called switching-off occurring. Therefore modafinil has been shown to activate wake-promoting neurons and suppress sleep promoting neurons' activity but it is not clear whether this is through a direct or indirect mechanism on these centres.

1.16 Management of MS-fatigue

Obviously treatment of any factor leading to secondary fatigue is essential. For example, neuropathic analgesia or anti-spasticity agents in patients with disturbed sleep due to pain or spasms. Appropriate management of depression is also essential in view of its role as a potential confounding factor in MS-related fatigue. Further specific interventions for primary MS fatigue will now be detailed.

1.16.1   Non-pharmacological management

A number of approaches have been proposed and investigated for MS-fatigue. Energy conservation courses are often employed by occupational therapists and a study by Mathiowetz et al has supported its role. A six-session, two-hour per week course was shown to significantly improve fatigue scores in a group of 54 MS patients[120]. Quality-of-life scores also improved including vitality subscales. Energy conservation methods commonly employed include; addressing the importance of rest periods; ergonomic principles regarding one's own body, as well as the work or home environment; priority setting; recognizing personal limits and living a balanced lifestyle.

Exercise programs are another method employed by therapists to improve MS-related fatigue with studies having confirmed its value. One such study looked at a six-month period of weekly yoga classes or weekly aerobic exercise with a stationary bicycle or no active intervention[121]. There was a significant improvement with either treatment in both fatigue measures and again quality-of-life vitality scores. A shorter-term exercise program of only 12-weeks duration, as part of a multi-disciplinary, holistic, outpatient rehabilitation program, again showed a significant improvement in fatigue, quality of life and also depression[122].

Cooling garments or cooling water baths are often advised to fatigue sufferers in view of the clear association of increased MS-fatigue with higher body temperatures.

1.16.2   Pharmacological treatment

A number of agents have been evaluated and employed for MS-related fatigue.

60

1.16.3   Amantadine

Amantadine was introduced as an anti-viral agent before then finding a role in the management of Parkinson's disease, particularly the treatment of motor dyskinesias. Its mode of action in Parkinson's is likely via its effect on glutamate receptors. Its mode of action in fatigue is unknown.

Rosenberg and Appenzeller studied the effect of amantadine 200mg daily for one week on fatigue in 10 MS patients in a randomized, double-blind, placebo-controlled, crossover trial[123]. Six subjects reported a subjective improvement in fatigue with amantadine as compared to one who felt an improvement with placebo.

Another study used a similar randomized, double-blind, placebo-controlled, crossover design to evaluate the effect of four-weeks treatment with amantadine 200mg daily in 29 patients[124]. Subjectively, there was a significant improvement with amantadine, as compared to placebo, in a number of areas including; energy level; concentration/memory function; overall well-being and ability to solve problems, although not in an overall fatigue score. During treatment with amantadine there was also a significant improvement in a task requiring sustained attention, the Stroop Interference Test.

A double-blind, parallel group, six-week study was reported of 119 MS patients randomized to amantadine 200mg daily, pemoline (building up to 56.25mg daily) or placebo[125]. Two subjective fatigue scales were used to evaluate response. The amantadine group showed a significant improvement in fatigue as compared to placebo in one scale, the MS-specific Fatigue Scale (MS-FS). There was however no significant

difference in FSS scores between the groups although scores did improve compared to baseline

Amantadine has subsequently received somewhat of an endorsement by the National Institute for Clinical Excellence which suggested, whilst not to be used routinely, patients should be informed of a potential benefit for fatigue[126]. A Cochrane Review, on the other hand, concluded that whilst generally well tolerated its efficacy is poorly documented, with poor quality trials open to bias and only small and inconsistent improvements in fatigue seen[127].

1.16.4   Pemoline

Pemoline is a CNS stimulant which was withdrawn from the UK in 1997 because of concerns regarding hepatotoxicity although continued to be used in North America up to 2005. As well as the study described above, a second, randomized, double-blind, placebo-controlled, crossover trial in 46 MS patients has been reported[128]. Four-weeks treatment with an escalating dose of pemoline (up to 75mg daily) was evaluated with a visual analogue fatigue scale. No significant effect was seen although a trend for improvement with pemoline was noted at the higher dosage.

1.16.5   Aminopyridines

As mentioned earlier, there is a convincing association between fatigue and body temperature and it is known that higher temperatures leads to increased intermittent conduction block along demyelinated nerve fibres. Frequency-dependent conduction block is one physiological mechanism for this intermittent conduction block. Partially

demyelinated nerve fibres may conduct single or low frequency impulses faithfully but are unable to transmit trains of impulses at high frequency. It has been therefore hypothesized that fatigue may be a temporary weakness due to functional blockade of conduction. In view of this, aminopyridines, as potassium channel blockers that prolong the duration of the action potential and potentiate synaptic transmission, have been studied as a potential treatment.

Studies of 3,4-diaminopyridine in small numbers of MS patients have shown subjective improvements in fatigue as well as increased activation on fMRI[129]. In addition, an electrophysiological measure of fatigue during a simple maximal voluntary isometric contraction also demonstrated a significant improvement with treatment[130].

1.16.6   Modafinil

Three studies have been undertaken to evaluate the potential effect of modafinil on MS-related fatigue. Zifko et al looked at 50 MS patients with chronic fatigue in an open-label, non-placebo-controlled, two-centre study of modafinil, up to a maximum dose of 400mg daily, for three months[131]. Tools used to evaluate response included the ESS and FSS. Of note mean FSS score at baseline was 30.3 +/-8.5 indicating the group as a whole suffered from severe fatigue. On the other hand, mean ESS score was 9.7 +/- 3.9 suggesting that hypersomnolence was not a significant problem for the group. There was a significant improvement in both measures' scores with treatment (median daily dose 100mg).

Rammohan et al conducted a nine-week, single-blind, cross-over study involving 72 MS patients with severe fatigue as assessed by the FSS[132]. All patients received placebo for weeks 1-2 and 7-9 and modafinil 200mg for weeks 3-4, titrating to 400mg daily for weeks 5-6. Again FSS and ESS were used, amongst a number of other measures. Although some patients did report hypersomnolence, as assessed by the ESS, the mean ESS score at baseline was only 9.5 (range 1-20). There was a significant improvement in FSS score with the lower dose as compared to placebo but not modafinil 400mg daily. Other subjective fatigue scales showed similar results. The ESS scores did, however, significantly improve with both treatment doses as compared to placebo.

A five-week, double-blind, placebo-controlled, parallel-group study was reported by Stankoff et al[133]. It involved 115 patients with chronic fatigue randomized to modafinil titrating up to 400mg or placebo. The MFIS (primary outcome), a fatigue visual analogue scale and the ESS were employed in the study. There was no significant difference in any of the measurements with modafinil as compared to placebo.

1.17   Summary and aims

Fatigue is a common and major symptom in MS. A number of potential mechanisms have been proposed as to its pathogenesis. The preceding review has illustrated the involvement of deep grey matter structures in several of these models. One of the primary aims of this thesis was to investigate any association between deep grey matter pathology and MS-fatigue.

Conventional MRI techniques are an important tool in the diagnosis of MS and monitoring disease activity. However, it is evident that quantitative MRI techniques

can demonstrate abnormalities in normal appearing tissue thereby providing a more comprehensive picture of the disease burden. It is felt that these non-conventional measures hold significant promise in offering a better correlation with clinical features.

T1 mapping is one such technique and has previously demonstrated pathology in small structures such as the pyramidal tracts and a correlation with disability. To this end, we employed T1 mapping in the thalamus and basal ganglia to investigate the potential role of disruption of cortico-subcortical circuits in contributing to MS-fatigue.

Conventional MRI techniques have identified lesions occurring in the hypothalamus but quantitative MRI has not been previously employed to assess this deep grey matter structure in MS[110, 111]. As described in this introduction, it is implicated in MS-fatigue through its role in the autonomic and neuroendocrine system as well as the hypocretin pathway. To further evaluate this we performed T1 mapping on the hypothalamus, measured CSF hypocretin-1 levels in a further cohort of patients as well as undertook autonomic investigations.

Management of MS-fatigue requires a multi-disciplinary approach as well as pharmacological agents. Studies on the efficacy of treatments for fatigue are complicated by the difficulty in defining fatigue and the reliance on determining response with subjective questionnaires. The wakefulness-promoting agent, modafinil has been assessed previously, in a limited number of studies, using these measures. Its likely mode-of-action involves activation of a number of structures, including nuclei within the hypothalamus and brainstem, which are components of the arousal system. The final aim of this thesis is to further address the potential role for modafinil in MS-fatigue through

the application of objective measures of alertness and vigilance to establish a more accurate picture of its effect.

CHAPTER 2

Chapter 2.   T1 RELAXATION TIMES OF THE DEEP GREY MATTER AND FATIGUE IN MS

2.1 Introduction

2.1.1 T1 relaxation time studies in MS

Conventional MRI techniques, in particular T2-weighted imaging, have traditionally focused on white matter abnormalities and have become the main paraclinical tool for diagnosing MS. However, there is generally a poor correlation with clinical deficit and disability. T1 relaxation time, or T1 mapping, is a quantitative MRI-derived measure and has been used to demonstrate abnormalities in lesions and NAWM and provides better correlation with clinical deficit. Studies have shown that, in MS lesions, T1 relaxation time correlates with degree of hypointensity and accordingly the T1 relaxation time of hypointense lesions, on T1-weighted images, has correlated significantly with disability[134-136]. T1 relaxation time measurements are prolonged in the NAWM of MS patients[135, 136]. Pathology within NAWM may correlate more strongly with clinical status and progression in disability than T2 lesion volume[137, 138]. Studies looking at the cervical spinal cord and the pyramidal tracts found significant correlations between T1 relaxation times and disability measures[139, 140]. In NAWM, at least, it has been suggested that T1 relaxation time measurement appears to be more sensitive than MT, in detecting the subtle pathological abnormalities[14].

2.1.2 Grey matter and T1 relaxation time

T1 relaxation times have been measured in both cortical and deep grey matter but with diverse results. One investigation found significantly prolonged T1 relaxation time in the frontal cortex in MS subjects[14]. However, another study showed no significant

68

differences in cortical grey matter between patients and controls[141]. With regard to the deep grey matter, a study looking at the thalamus found a significantly prolonged T1 relaxation time in secondary progressive MS (SPMS) patients, as compared to relapsing-remitting MS (RRMS) or controls[136]. A further study found only an apparent trend for higher T1 values in the deep grey matter, whilst no significant difference was found in a third study[14, 141].

2.1.3 Aims

Therefore to explore further the possible central component of fatigue in MS, we measured the T1 relaxation time of deep grey matter structures in a cohort of RRMS patients. The specific aims of this study were: (i) to assess any relationship between the mean T1 relaxation times (T1 values) of the thalamus, putamen and caudate nucleus and fatigue severity, (ii) to compare these T1 values with a group of age- and sex-matched healthy volunteers, (iii) to evaluate any relationship between T1 values of the deep grey matter structures and clinical features or T2 hyperintense lesion volume.

2.2 Materials and methods

2.2.1 Subjects

Fifty-two patients with RRMS as defined by McDonald's criteria were recruited[142, 143]. The group consisted of 12 males and 40 females with a median age of 39 years (interquartile range [IQR], 32 – 43). Median disease duration was 9 years (IQR, 3 – 14) and the median Expanded Disability Status Scale (EDSS) score was 2.5 (IQR, 2 –

3.5)[144]. All subjects were relapse- and corticosteroid-free for at least one month. Eight patients had been receiving glatiramer acetate treatment for approximately six years. No patients suffered from significant medical or psychiatric conditions that could confound the study.

The normal control cohort consisted of 19 subjects (5 male and 14 female) with a median age of 33 years (IQR, 28 – 40). The control and patient groups were matched in terms of gender and age. The controls had no history of neurological disease or other significant medical condition.

Approval was obtained from the Nottingham Research Ethics Committee. All subjects gave informed consent.

2.2.2   Fatigue assessment

Fatigue was quantified using the FSS in 48 of the RRMS patients[23]. The FSS was ascertained within twenty-four hours of the MRI and at the same time as the patients underwent a full neurological examination by an experienced observer blinded to the scan results. The 48 patients consisted of 11 males and 37 females; median age was 39 years (IQR, 32 – 43), median disease duration 9 years (IQR, 3 – 14) and median EDSS 2.5 (IQR, 2 – 3).

For analysis relating fatigue severity to MRI and clinical parameters 14 patients, who were receiving medication that could potentially affect fatigue, were excluded. The remaining sub-group of 34 patients consisted of 7 males and 27 females. Their median age was 38 years (IQR, 32 – 42), median disease duration 9 years (IQR, 3 – 13) and median EDSS 2.5 (IQR, 2 – 3). Two groups of MS patients were identified:

fatigued patients (F) (FSS score ≥ 5.0 [n = 20]) and non-fatigued patients (NF) (FSS score ≤ 4.0 [n = 11]). The three outstanding patients, with an FSS score of between 4.1 and 4.9, were not included in the F and NF group comparisons but their data was used in correlation analyses.

2.2.3   MRI acquisition

All scans were performed on a 1.5 Tesla, Vision MR scanner (Siemens Medical, Erlangen, Germany). For the calculation of T1 maps of the brain, image data was obtained using a 3-D inversion recovery, fast low-angle shot (FLASH) sequence. The sequence and method for calculation of the T1 maps was performed as described in previous studies by our group[139]. The following sequence parameters were applied: echo time (TE) 3ms, flip angle 10°, repetition time (TR) 4.8ms (per FLASH line), TR 5000ms (between inversion pulse) and 488Hz bandwidth per point. A 256 x 256 matrix was used and twenty-four 3-D partitions were acquired with a voxel size of 1 x 1 x 3mm. Seven inversion times were used ranging from 100 to 1800 ms. Total acquisition time was 14 minutes.

PD- and T2-weighted images were obtained with a turbo spin echo sequence with the following parameters: TR 4100ms, TE (PD) 22ms, TE (T2) 90ms, matrix 256 x 256, 46 interleaved 3mm axial slices.

2.2.4   MRI analysis

All MRI analysis was performed on, in-house developed, software by an experienced operator blinded to the subject's clinical details. T1 relaxation times were determined by

defining regions of interest (ROIs) bilaterally, on axial T1 map slices, in three deep grey matter areas: the thalamus, putamen and caudate nucleus. The ROIs were outlined on all consecutive slices which demonstrated the structures. A semi-automated technique using the Sobel operator and non-maximal suppression was employed (Fig 2.1)[145].

Briefly, the image intensity gradient is measured in the transverse plane for each voxel by the Sobel operator. The gradient magnitude is highest for partially volumed voxels. This allows for detection of the boundary between grey matter and CSF or white matter. Therefore, the method does not depend directly on the voxel intensities but requires a difference in intensity to delineate the edge. The boundary may be localized precisely by eliminating all voxels except those where the gradient magnitude is maximal in the direction of the gradient. This process of identifying the voxels is called non-maximal suppression.

At some points, the boundary between grey and white matter was not defined clearly. This occurred most frequently at the lateral edge of the thalamus. Conservative manual correction of this was then required. The original T1 map images, prior to the application of the Sobel operator and non-maximal suppression, were used as reference images to help confirm the edges. To further avoid partial volume effects from surrounding white matter and CSF, tissue the width of one voxel beneath the ROI outline was also automatically removed from histogram analysis. Together this allowed for the largest volume of the grey matter structures to be considered while avoiding contamination. ROI histograms were then generated from the T1 map images.

Lesion volume was determined on T2-weighted images using a semi-automated, seed growing technique. PD-weighted scans were used to confirm lesions.

Figure 2.1 (A) T1 image axial slice of a healthy volunteer after application of the Sobel operator and non-maximal suppression to automatically detect the images. (B) the same axial slice showing ROIs defined by the detected edges and some manual editing where necessary
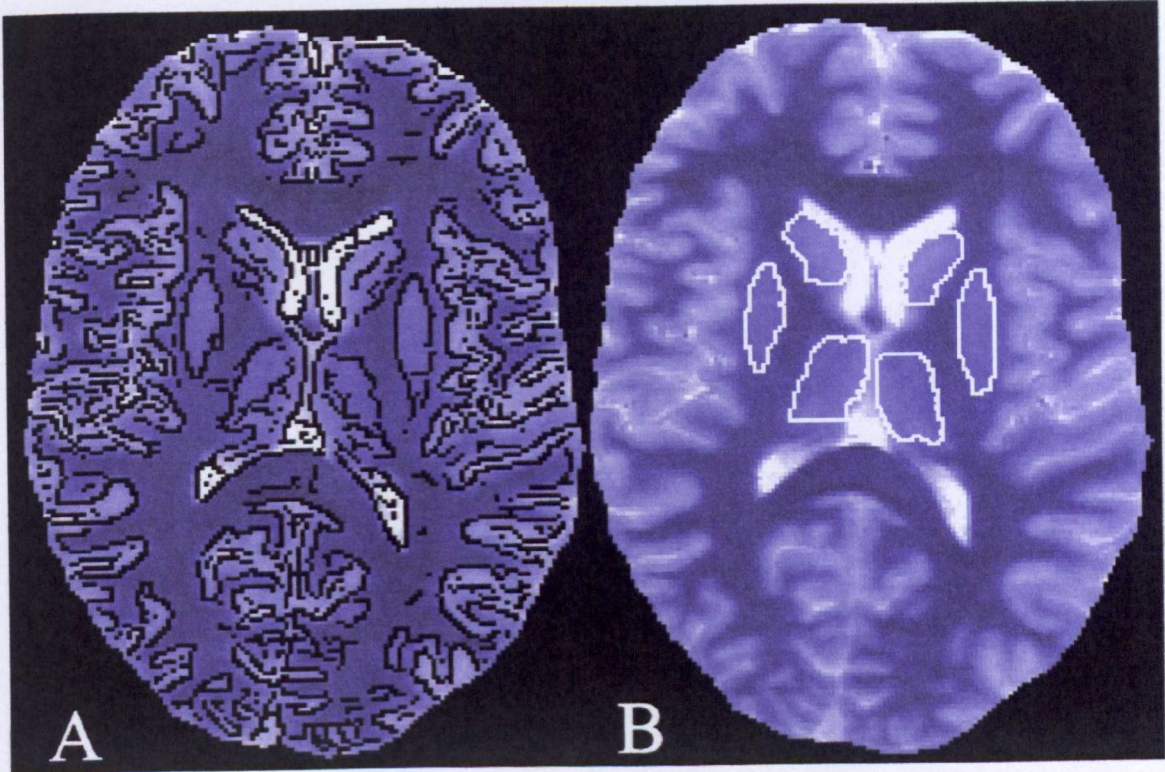
2.2.5 Reproducibility

Intra-observer variation was determined by redefining the ROIs for all three deep grey matter structures on 10, randomly-selected, patient's scans. These repeat measurements were obtained a minimum of 4 weeks after the original set. The semi-automatic edge detection method was again applied and histograms generated from the T1 maps.

2.2.6 Statistical analysis

The Mann-Whitney U-test was applied to compare the T1 values of the MS patient group and the healthy controls. This was also used to perform comparisons between the F and NF MS patient cohorts. Spearman's rank correlation coefficient (rho) was determined to assess correlations between the clinical and MRI-derived parameters. A p value of less than 0.05 was considered to be significant. Intra-observer error was expressed by the coefficient of variation between the repeated measurements.

2.3 Results

2.3.1 Patients versus controls

The T1 values of the thalamus, putamen and caudate nucleus are reported in Table 2.1 and Fig 2.2. The median T1 of the thalamus and the putamen were significantly higher in the MS patients than in the controls. In the caudate nucleus, the median T1 was also higher in patients as compared to controls, however this did not reach statistical significance. This result was also reproduced when examining only the subgroup of 34 patients not receiving medication that could potentially contribute to fatigue.

74

2.3.2　　　　Fatigued versus non-fatigued MS patients

Clinical parameters

The median FSS score of the F group was 6.1 (IQR 5.3 – 6.6) and of the NF group was

3.2 (IQR 2.7 – 3.6). Between the two groups there was no significant difference in age (F

39 years [IQR 31 – 43], NF 33 years [IQR 31 – 37]), sex (F 15% male, NF 18% male) or

disease duration (F 11 years [IQR 3 – 14], NF 7 years [IQR 2 – 12]). The F cohort had a

significantly higher median EDSS score (3.0 [IQR 2 – 4] vs. 2.0 [IQR 1.5 – 2.5]; p =

0.009).

T2 lesion load

Median T2 volume was not significantly different between the F 5.88 cm^3 [IQR 2.87 –

12.68] and NF 4.23 cm^3 [IQR 1.98 – 7.44] groups.

T1 relaxation times

The median T1 of the thalamus was significantly higher in the F group, as compared to

the NF (Table 2.2 and Fig 2.3). There was no difference between T1 of the putamen or

the caudate between the F and NF patients. This result was reproduced on including all

patients for whom FSS data was acquired.

2.3.3 Fatigue correlations in RRMS patients

<u>Clinical parameters</u>

Fatigue severity correlated significantly with EDSS score (rho = 0.533; p = 0.001). There was no significant correlation between fatigue severity and age (rho = 0.180; p = 0.31) or disease duration (rho = 0.163; p = 0.36).

<u>T2 lesion load</u>

Fatigue severity did not correlate with T2 volume (rho = 0.161; p = 0.36).

<u>T1 relaxation times</u>

The T1 of the thalamus correlated significantly with fatigue severity (rho = 0.418; p = 0.014) (Fig 2.4). There was no significant correlation between fatigue and the T1 of the caudate (rho = 0.276; p = 0.11) or the putamen (rho = 0.305; p = 0.079).

2.3.4 Conventional MRI and clinical correlations of T1 relaxation times

Age, disease duration, EDSS and T2 lesion volume did not correlate significantly with the mean T1 relaxation times of the individual deep grey matter structures.

2.3.5 Reproducibility

Intra-observer variability was 0.30% for the T1 value of the caudate nucleus, 0.40% for the putamen and 1.38% for the thalamus.

Table 2.1   Deep grey matter T1 values (ms) of patients and controls

	Controls, n = 19	RRMS, n = 52	p Value
Thalamus	927 (913 – 933)	942 (923 – 961)	0.012
Caudate	1041 (1013 – 1074)	1054 (1042 – 1077)	0.066
Putamen	952 (919 – 974)	969 (945 – 988)	0.027

Data are expressed as median (interquartile range)

RRMS = relapsing-remitting multiple sclerosis

Table 2.2   Deep grey matter T1 values (ms) of RRMS patients with and without fatigue

	F, n = 20	NF, n = 11	p Values
Thalamus	956 (932 – 973)	923 (893 – 949)	0.018
Caudate	1066 (1049 – 1092)	1057 (1050 – 1067)	0.13
Putamen	982 (963 – 1000)	969 (938 – 984)	0.22

Data are expressed as median (interquartile range)

F = patients with fatigue; NF = patients without fatigue
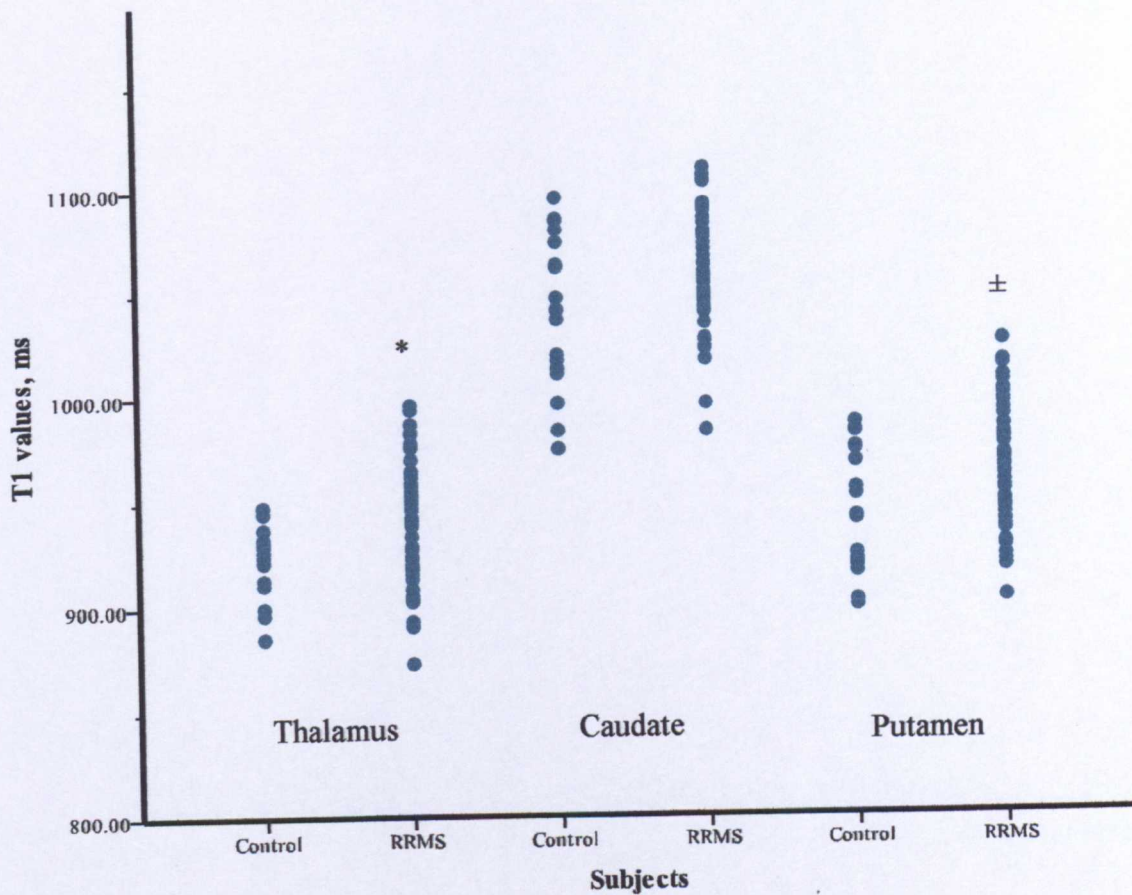
Fig 2.2   Scatterplot of deep grey matter T1 values of patients and controls

* Thalamus T1 value in RRMS patients compared to controls (p = 0.012)

± Putamen T1 value in RRMS patients compared to controls (p = 0.027)

RRMS = relapsing-remitting multiple sclerosis
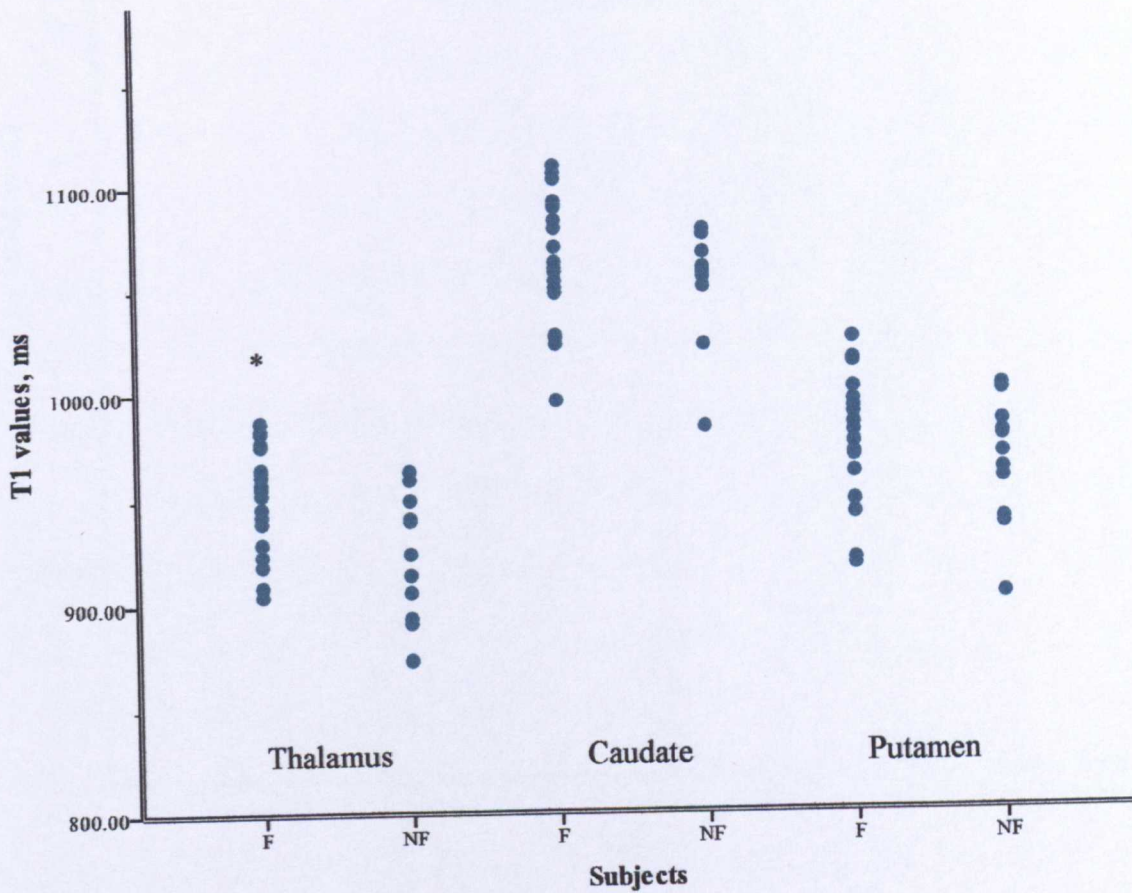
Fig 2.3   Scatterplot of deep grey matter T1 values of RRMS patients with and without fatigue

* Thalamus T1 value in those with fatigue compared to those without (p = 0.012)

F = patients with fatigue; NF = patients without fatigue
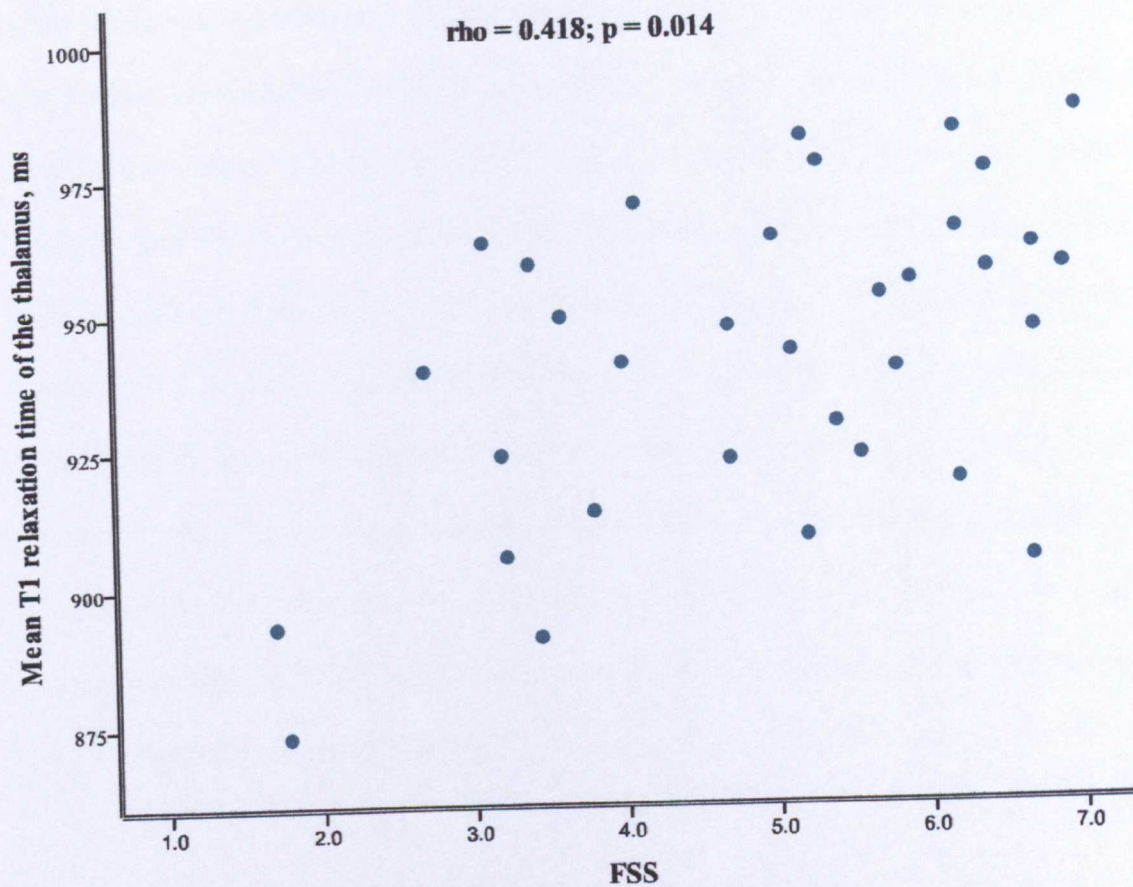
Figure 2.4   Scatterplot of FSS scores and T1 values of the thalamus in RRMS

FSS = Fatigue Severity Scale

2.4　　　Discussion

In this study, we measured T1 relaxation time in three deep gray matter structures. This quantitative parameter has been used previously to investigate MRI detectable changes in MS but, in common with other quantitative parameters, has shown varying sensitivity to the changes in the deep gray matter of RRMS patients. Previous studies applied small ROIs of various sizes, with areas of between 10 and 36 mm^2, centrally to each structure[14, 136, 141]. This can limit sample size and result in large variance on the mean T1 value. A potential limitation of our technique was the possibility of increased influence from partial volume effects. By employing an edge detection method and discounting the voxel directly beneath the edge we are confident the risk was minimized. Furthermore, we used a 3D acquisition to reduce image artifact. Our technique also showed a high level of reproducibility.

2.4.1　　　T1 relaxation times in patients and controls

Prolongation of T1 relaxation times has been shown to be due to gliosis or expansion of the extracellular space, which itself can be secondary to vasogenic oedema or axonal loss[146, 147]. Higher T1 values correlate with increasing degrees of hypointensity of MS lesions on T1 weighted images that in turn, histopathologically, have been associated with more severe axonal loss[148]. Studies also found that, in MS lesions, degree of hypointensity and increasing T1 relaxation time were highly correlated with reduction of the MTR[134]. Reduced MTR, in turn, correlates with higher degrees of axonal loss in postmortem samples[148]. In addition, ^{1}H-MRS of hypointense lesions demonstrated that reduction in NAA correlated strongly with prolongation of the T1 relaxation time[135].

As discussed before, axonal loss may occur as part of MS lesions within the deep grey matter structures[3, 6, 7], either due to the inflammatory activity or possibly the loss of trophic support[5]. Axonal loss may also be secondary to degeneration anterograde to a focal lesion outside of the grey matter. This pattern of Wallerian degeneration has been described in demyelinating conditions[149, 150]. Our study, notably, found no correlation between the deep grey matter T1 values and the T2 lesion volume.

Our study demonstrated significantly longer T1 relaxation times of two deep gray matter structures, the thalamus and the putamen, in RRMS patients when compared to healthy volunteers. This is, to our knowledge, the first report of such a finding. A third structure, the caudate nucleus, also showed a longer T1 relaxation time in RRMS patients but did not reach statistical significance. Prolonged T1 relaxation time was previously reported in the thalamus of SPMS patients when compared with an RRMS group or healthy controls[136]. However, there was no significant difference between RRMS patients and the control group. A cohort of both RR- and SPMS patients was also used in another study, which found no significant difference with healthy controls[141]. Griffin et al investigated a group of RRMS patients and found only an apparent trend for higher T1 values in the deep grey matter[14]. Their research, however, was into early disease with a symptom duration of less than 3 years and mild disability, with an EDSS score of less than or equal to three. This would suggest that the pathology that leads to prolongation of the T1 relaxation time, in the deep grey matter of RRMS patients, is either a gradual process or simply a later phenomenon. Contrary to this notion, however, we found that the duration of symptoms did not correlate with the T1 values of the deep grey matter.

2.4.2 MS-related fatigue – clinical correlations

Our results reveal a correlation between fatigue severity and disability, which interestingly was despite a relatively low median EDSS score for the patient cohort. Earlier studies have also shown a correlation between fatigue and disability[151]. In addition, a study, which involved SPMS as well as RRMS patients, found a higher frequency of SPMS in the fatigued group that would suggest that as disability increases fatigue becomes a more frequent complaint[52]. This is, however, not a consistent finding with a number of investigations showing no association between FSS and EDSS scores[17, 21]. Disease duration has not been shown to be associated with fatigue severity and it is known that fatigue can be the first symptom of MS[17, 21, 52]. Our results also show no correlation between fatigue and disease duration.

2.4.3 MS-related fatigue – MRI correlations

In agreement with most published studies we found no correlation between T2 lesion volume and fatigue severity. This has been discussed in detail within the introduction. Our results, therefore, support the premise that abnormalities on conventional MRI have a limited, if any, role to play in the pathophysiology of fatigue.

Our study demonstrated that fatigue severity correlates with the T1 value of the thalamus, with a significantly higher T1 value of the thalamus in RRMS patients with fatigue as compared to those without. First of all this supports the concept that it is the spatial distribution of pathology in MS that is most relevant in terms of clinical features rather than the overall disease burden. Moreover, it may be that non-

conventional MRI techniques are necessary to provide solutions to the clinical-radiological paradox. In addition, it supports previous studies discussed in chapter 1, which have appeared to imply a role for the deep grey matter structures in the pathophysiology of MS-related fatigue.

An fMRI study found that, during a simple motor task, RRMS patients with fatigue showed significantly lower, relative activation of the thalamus as well as certain cortical areas including the rolandic operculum and the middle frontal gyrus[58]. A significant inverse correlation between fatigue severity and the activity in the contralateral thalamus and ipsilateral rolandic operculum was found. In addition, a PET study revealed reductions in CMRGlu in MS patients with severe fatigue, as compared to those without fatigue, in several areas including the prefrontal cortex, premotor cortex and supplementary motor area as well as the putamen and head of the caudate nucleus[17]. These areas are important in a number of tasks including motor programming and execution. The purported mechanism is the disruption of cortico-subcortical circuits linking cortical grey matter with the basal ganglia and thalamus. It is suggested that this dysfunction may lead to over-activity in alternative pathways to enable motor programming and it is this compensatory mechanism that contributes to fatigue. Our findings provide additional support for this hypothesis.

To summarise, this study found evidence of abnormal T1 relaxation times of the thalamus and putamen in RRMS. This supports previous studies that have demonstrated pathology within deep grey matter structures. Furthermore, this pathology within the thalamus was associated with fatigue severity and provides evidence for a role,

potentially through dysfunction of cortico-subcortical circuits, in the pathophysiology of MS-fatigue.

CHAPTER 3

Chapter 3.   T1 RELAXATION TIME MAPPING OF THE HYPOTHALAMUS AND FATIGUE IN MS

3.1   Introduction

3.1.1   T1 relaxation time studies in MS

As discussed in the previous chapter, T1 relaxation time is a quantitative MRI-derived measure and has been used to demonstrate abnormalities in MS lesions, NAWM and grey matter and has been shown to correlate better with clinical deficit. It is sensitive in detecting subtle pathological abnormalities. It has also been used in the preceding chapter and in previous studies in small structures such as the basal ganglia, thalamus and cervical spinal cord.

3.1.2   Hypothalamus and MS

Although not considered a typical site of involvement in MS, post mortem studies have previously shown the presence of both plaques and axonal damage in and around the hypothalamus[3, 6]. Evidence of involvement on MRI has been predominantly limited to case reports often of individual patients who have presented with symptoms suggestive of hypothalamic involvement[110, 111, 152]. There is a single quantitative MRI study which has demonstrated focal atrophy in, amongst a number of areas, the hypothalamus, in patients with a clinically isolated syndrome (CIS) and at risk of developing MS[153]. There are also a number of studies that have evaluated groups of patients with a combination of conventional MRI techniques and neuroendocrine investigations[43, 154, 155]. These have demonstrated neuroendocrine abnormalities although the results have varied between similar studies.

3.1.3   Hypothalamus and fatigue

A number of CNS mechanisms potentially involved in the pathophysiology of fatigue, including autonomic and neuroendocrine dysfunction, have been described in the introduction. There is a potential role for the hypothalamus in a number of these mechanisms due to its significant interconnections and its involvement in key homeostatic mechanisms. Descending autonomic neurons originate from hypothalamic nuclei including the paraventricular nuclei synapsing with preganglionic sympathetic and parasympathetic neurons. Emotional control on the autonomic system is thought to be mediated by hypothalamic-limbic pathways. Some of these limbic pathways, such as the mamillothalamic tract, pass via thalamic nuclei. Neurons from the supraoptic and paraventricular nuclei project to the posterior pituitary stimulating the secretion of oxytocin and vasopressin (VP). Additional neurons from other hypothalamic nuclei project to the median eminence of the infundibulum with activation leading to the release of peptides which in turn leads to hormone release from the anterior pituitary. In addition the potential factor of hypersomnolence in MS-fatigue also implicates the hypothalamic-hypocretin neurons.

3.1.4   Aims

Therefore to explore further the possible central component of fatigue in MS, we measured the T1 relaxation time of the hypothalamus in a cohort of RRMS patients. The specific aims of this study were: (i) to assess any relationship between the mean T1 relaxation time (T1 value) of the hypothalamus and fatigue severity, (ii) to compare the T1 value with a group of age-matched healthy volunteers, (iii) to evaluate any

relationship between T1 values of the hypothalamus and clinical features or T2 hyperintense lesion volume.

3.2 Materials and methods

3.2.1 Subjects

Thirty-eight patients with RRMS as defined by McDonald's criteria were recruited[142, 143]. The group consisted of 9 males and 29 females with a median age of 38 years (IQR, 32 – 41). Median disease duration was 9 years (IQR, 3 – 13) and the median EDSS score was 2.5 (IQR, 2 – 3)[144]. All subjects were relapse- and corticosteroid-free for at least one month. Seven patients had been receiving glatiramer acetate treatment for approximately six years. No patients suffered from significant medical or psychiatric conditions that could confound the study.

The normal control cohort consisted of 13 subjects (5 male and 8 female) with a median age of 35 years (IQR, 29 – 42). The control and patient groups were matched in terms of age. The controls had no history of neurological disease or other significant medical condition.

Approval was obtained from the Nottingham Research Ethics Committee. All subjects gave informed consent.

3.2.2 Fatigue assessment

Fatigue was quantified using the FSS in the patient group[23]. The FSS was ascertained within twenty-four hours of the MRI and at the same time as the patients underwent a full

neurological examination by an experienced observer blinded to the scan results. For analysis relating fatigue severity to MRI and clinical parameters 11 patients, who were receiving medication that could potentially affect fatigue, were excluded. The remaining sub-group of 27 patients consisted of 6 males and 21 females. Their median age was 36 years (IQR, 31 – 41), median disease duration 10 years (IQR, 3 – 13) and median EDSS 2.5 (IQR, 2 – 3). Two groups of MS patients were identified: fatigued patients (F) (FSS score $\geq$ 5.0 [n = 17]) and non-fatigued patients (NF) (FSS score $\leq$ 4.0 [n = 8]). The two outstanding patients, with a FSS score of between 4.1 and 4.9, were not included in the F and NF group comparisons but their data ˥ used in correlation analyses.

3.2.3   MRI acquisition

All scans were performed on a 1.5 Tesla, Vision MR scanner (Siemens Medical, Erlangen, Germany). For the calculation of T1 maps of the brain, image data was obtained using a 3-D inversion recovery, fast low-angle shot (FLASH) sequence. The sequence and method for calculation of the T1 maps was performed as previously described[139]. The following sequence parameters were applied: echo time (TE) 3ms, flip angle 10°, repetition time (TR) 4.8ms (per FLASH line), TR 5000ms (between inversion pulse) and 488Hz bandwidth per point. A 256 x 256 matrix was used and twenty-four 3-D partitions were acquired with a voxel size of 1 x 1 x 3mm. Seven inversion times were used ranging from 100 to 1800 ms. Total acquisition time was 14 minutes.

PD- and T2-weighted images were obtained with a turbo spin echo sequence with the following parameters: TR 4100ms, TE (PD) 22ms, TE (T2) 90ms, matrix 256 x 256, 46 interleaved 3mm axial slices.

3.2.4 MRI analysis

All MRI analysis was performed on in-house developed software by an experienced operator blinded to the subject's clinical details. T1 relaxation times were determined by defining ROIs bilaterally, on axial T1 map slices, in the hypothalamus. The ROIs were outlined on all consecutive slices which demonstrated the structures. A semi-automated technique using the Sobel operator and non-maximal suppression was employed as described in chapter 2(Fig 3.1)[145].

The medial aspect of the hypothalamus borders CSF, however at a number of points, the lateral boundary was not defined clearly. Conservative manual correction of this was then required. The original T1 map images, prior to the application of the Sobel operator and non-maximal suppression, were used as reference images to help confirm the edges. Similarly the rostral border of the hypothalamus had to be established and was done so by identifying the anterior and posterior commissures which signifies the superior margin of the nuclei. To further avoid partial volume effects from surrounding white matter and CSF, tissue the width of one voxel beneath the ROI outline was also automatically removed from histogram analysis. Together this allowed for the largest volume of the hypothalamus to be considered while avoiding contamination. ROI histograms were then generated from the T1 map images.

Figure 3.1 (A) T1 image axial slice of a healthy volunteer after application of the Sobel operator and non-maximal suppression to automatically detect the images. (B) the same axial slice showing ROIs defined by the detected edges and some manual editing where necessary
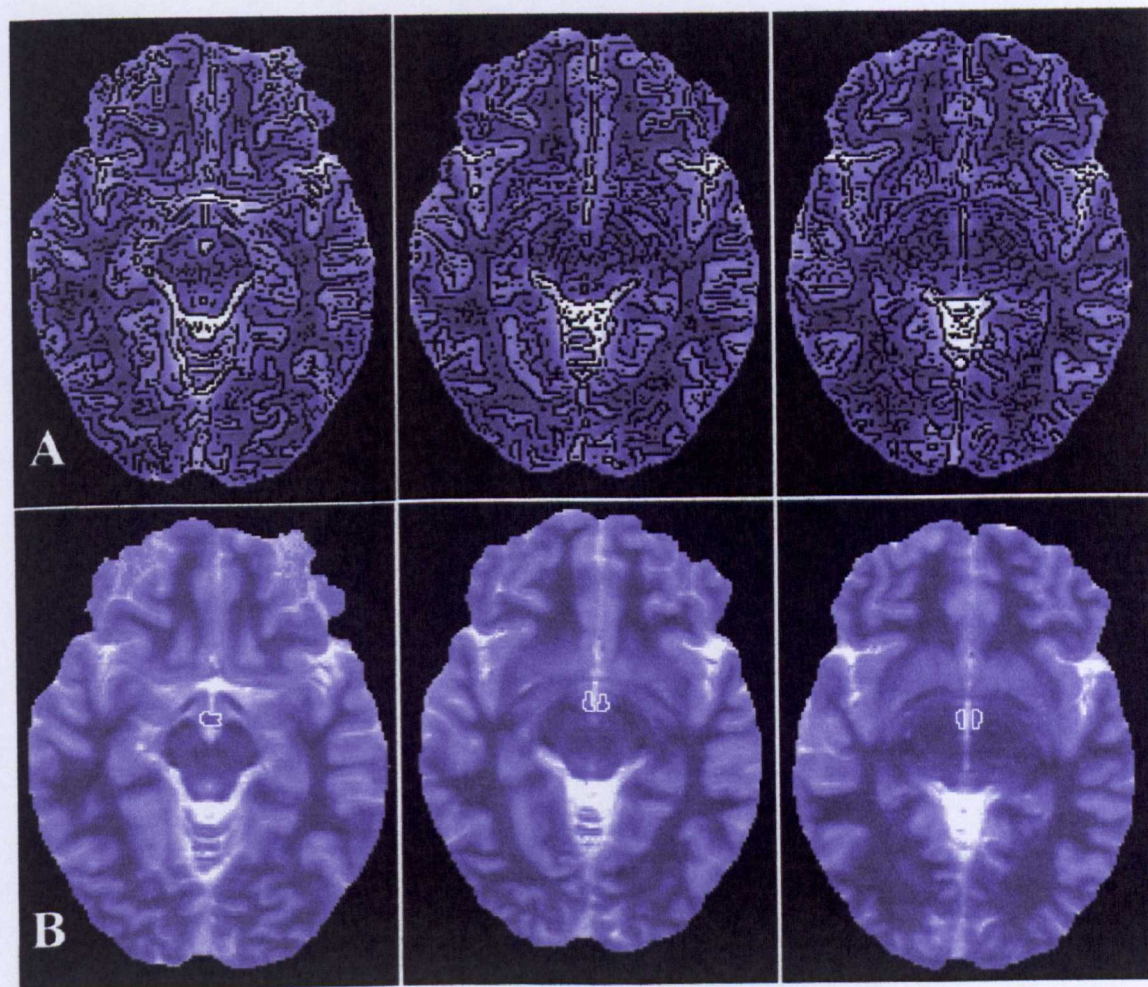
3.2.5 Reproducibility

Intra-observer variation was determined by redefining the ROIs for the hypothalamus on five, randomly-selected, patient's scans. These repeat measurements were obtained a minimum of four weeks after the original set. The semi-automatic edge detection method was again applied and histograms generated from the T1 maps.

3.2.6 Statistical analysis

The Mann-Whitney U-test was applied to compare the T1 values of the MS patient group and the healthy controls. This was also used to perform comparisons between the F and NF MS patient cohorts. Spearman's rank correlation coefficient (rho) was determined to assess correlations between the clinical and MRI-derived parameters. A p value of less than 0.05 was considered to be significant. Intra-observer error was expressed by the coefficient of variation between the repeated measurements.

3.3 Results

3.3.1 Patients versus controls

The median T1 of the hypothalamus is significantly higher in the MS patients than in the controls (Table 3.1 and Fig 3.2). This result was reproduced when examining the subgroup of 27 patients not receiving medication that could potentially contribute to fatigue (Fig 3.3).

93

Table 3.1   Hypothalamus T1 values (ms) of patients and controls

	Controls,	RRMS,	*p* Value
Hypothalamus	1047 (1028 – 1080)	1114 (1057 – 1147)	0.001
	n = 13	n = 38	
Hypothalamus	1047 (1028 – 1080)	1120 (1068 – 1147)	0.001
(sub-group)	n = 13	n = 27	

Data are expressed as median (interquartile range)

RRMS = relapsing-remitting multiple sclerosis

Table 3.2   Hypothalamic T1 values (ms) of RRMS patients with and without fatigue

	F, n = 17	NF, n = 8	*p* Values
Hypothalamus	1131 (1068 – 1164)	1098 (1070 – 1134)	0.288

Data are expressed as median (interquartile range)

F = patients with fatigue; NF = patients without fatigue
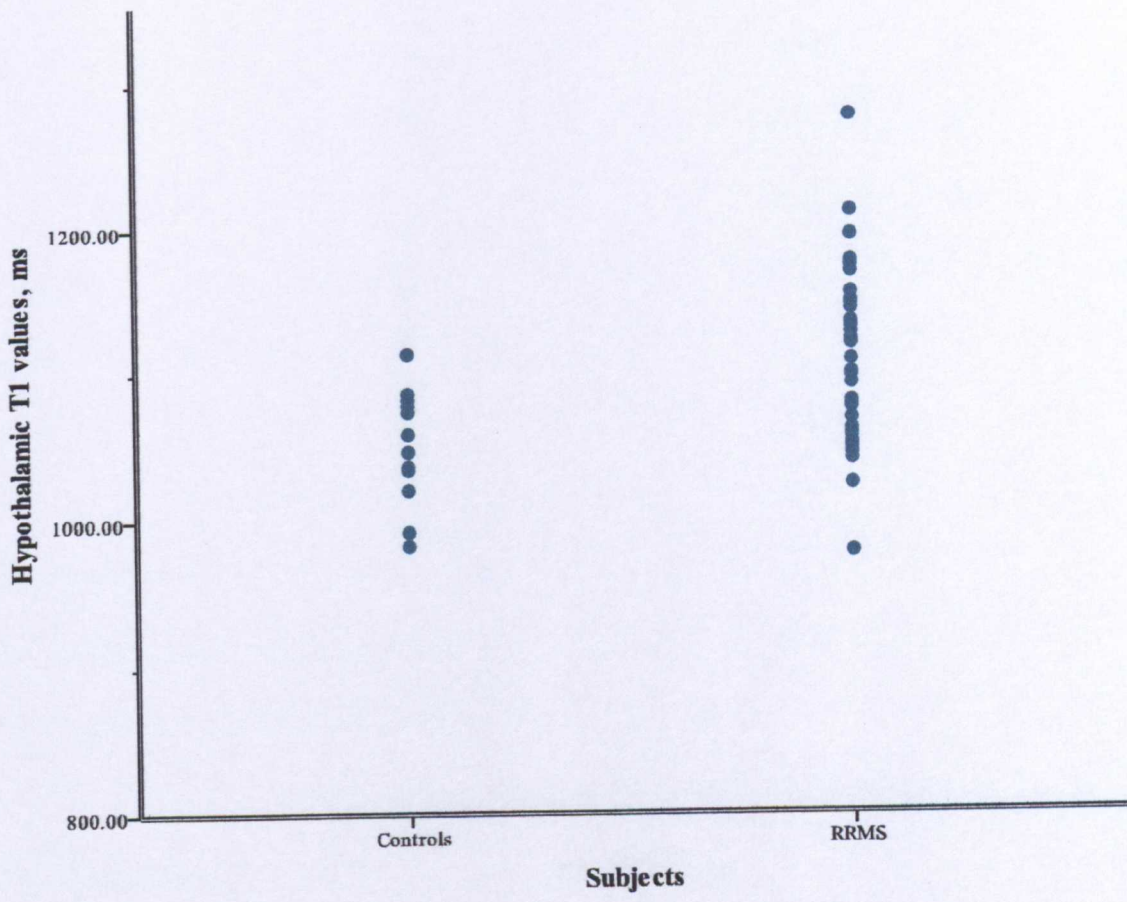
Figure 3.2   Scatterplot of hypothalamic T1 values in RRMS patients and controls

(p = 0.001)

RRMS = relapsing-remitting multiple sclerosis
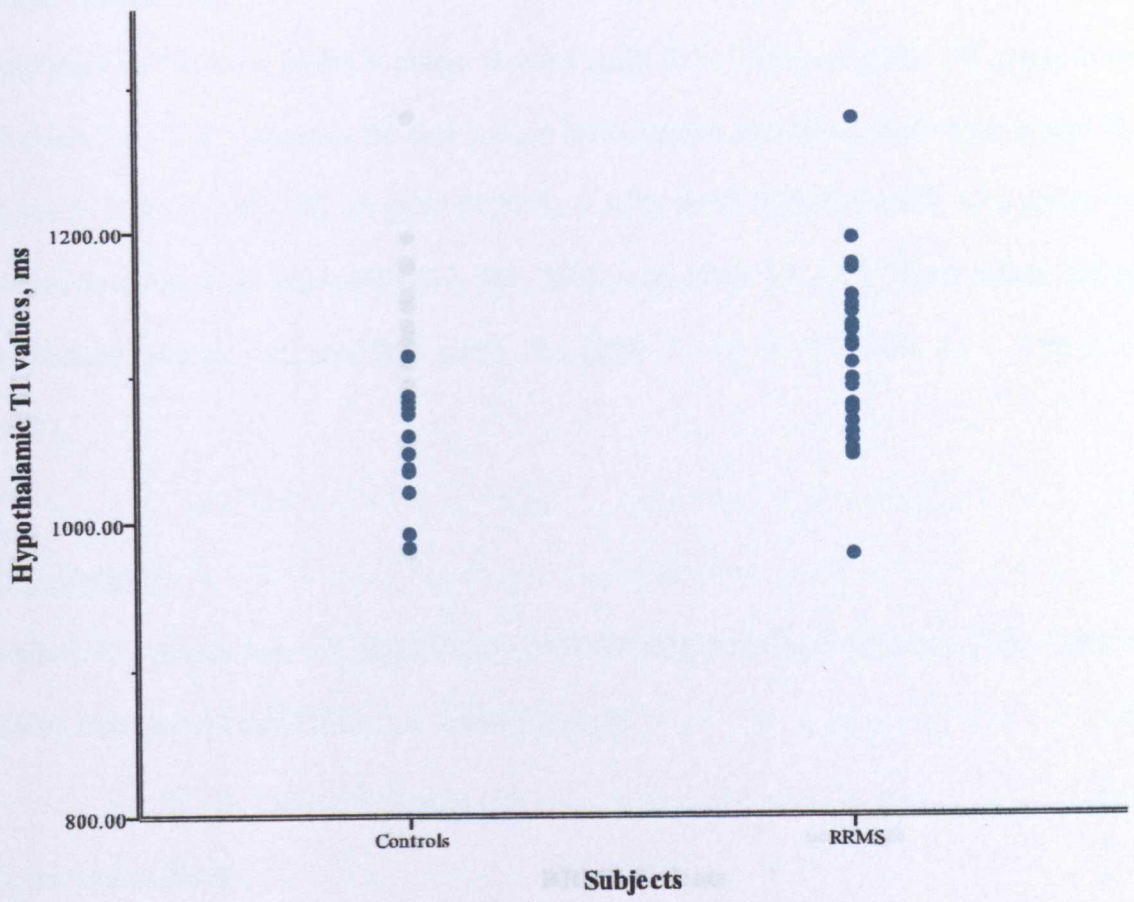
Figure 3.3   Scatterplot of hypothalamic T1 values in the subgroup of RRMS patients

not receiving medication that could affect fatigue and controls ($p = 0.001$)

RRMS = relapsing-remitting multiple sclerosis
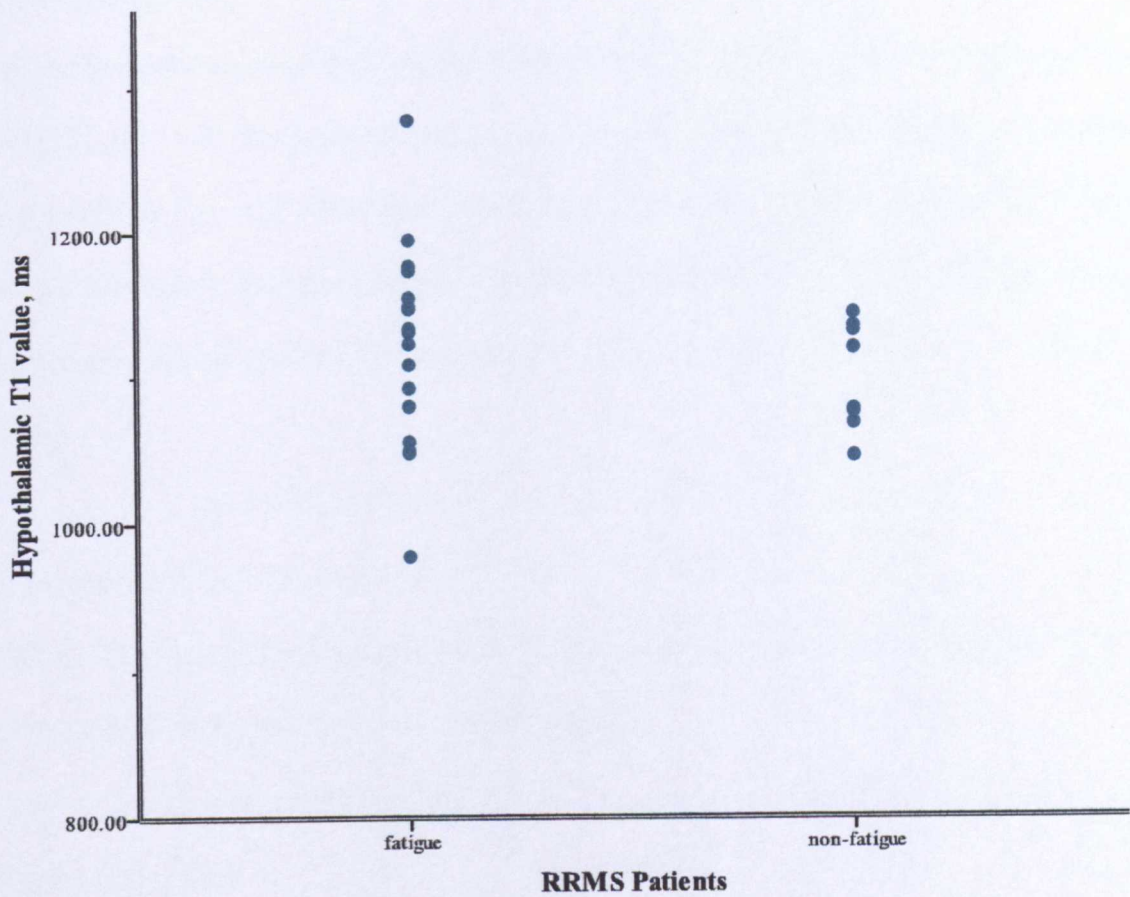
Figure 3.4   Scatterplot of hypothalamic T1 values in RRMS patients with and without

fatigue ($p = 0.288$)

RRMS = relapsing-remitting multiple sclerosis

3.3.2   Fatigued versus non-fatigued MS patients

Clinical parameters

The median FSS score of the F group was 6.2 (IQR 5.4 – 6.6) and of the NF group was

3.3 (IQR 2.8 – 3.8). Between the two groups there was no significant difference in age (F

39 years [IQR 31 – 42], NF 33 years [IQR 31 – 37]), sex (F 13% male, NF 12% male) or

disease duration (F 11 years [IQR 4 – 13], NF 8 years [IQR 2 – 13]). The F cohort had a

significantly higher median EDSS score (2.5 [IQR 2 – 4] vs. 2.0 [IQR 1.5 – 2.5]; p =

0.003).

T2 lesion load

Median T2 volume was not significantly different between the F 5.93 cm^3 [IQR 2.96 –

15.66] and NF 3.54 cm^3 [IQR 1.53 – 4.68] groups.

T1 relaxation times

The median T1 of the hypothalamus was not significantly different between the F 1131

ms [IQR 1068 – 1164] and NF 1098 ms [IQR 1070 – 1134] groups (Table 3.2 and Fig

3.4). This was reproduced on including all patients for whom FSS data was acquired.

3.3.3   Fatigue correlations in RRMS patients

Clinical parameters

Fatigue severity correlated significantly with EDSS score (rho = 0.683; p < 0.001). There

was no significant correlation between fatigue severity and age (rho = 0.091; p = 0.65) or

disease duration (rho = 0.029; p = 0.89).

T2 lesion load

Fatigue severity did not correlate with T2 volume (rho = 0.302; p = 0.13).

T1 relaxation times

There was no significant correlation between fatigue and the T1 of the hypothalamus (rho = 0.197; p = 0.32).

3.3.4 Conventional MRI and clinical correlations of T1 relaxation times

T2 lesion volume did correlate significantly with the mean T1 relaxation times of the hypothalamus (rho = 0.617; p = 0.001). In addition, there was a significant correlation between disease duration and T1 values (rho = 0.423; p = 0.028). Age and EDSS did not correlate significantly with T1 values.

3.3.5 Reproducibility

Intra-observer variability was 1.9% for the T1 value of the hypothalamus.

3.4 Discussion

In this study, we measured T1 relaxation time in the hypothalamus, a technique that has not been previously applied to this structure in MS patients. As with the study of the thalamus and basal ganglia, we aimed to measure the T1 value of the entire structure employing an edge detection method and discounting the voxel directly beneath the edge to minimize the risk of partial volume effects. The main challenges with investigating the

hypothalamus with MRI are its small size and difficulty in detecting certain borders. However, by applying an edge detection method that has been used before in assessing both small and deep grey matter structures as well as good anatomical knowledge with identification of markers such as the anterior and posterior commissures, artifact was minimized whilst allowing for the maximum amount of hypothalamus to be included in the analysis. Our technique showed a high level of reproducibility.

3.4.1   T1 relaxation times in patients and controls

This is to our knowledge the first study to employ a quantitative MRI technique to investigate the hypothalamus in a cohort of MS patients. As discussed in the previous chapters, more advanced MRI techniques are now readily used in MS studies to try and answer what is commonly known as the clinical-radiological paradox. In previous studies T1 relaxation times have been measured in plaques, NAWM and grey matter.

Our study demonstrated significantly prolonged T1 relaxation time of the hypothalamus in patients with RRMS as compared to age-matched controls. As discussed in chapter 2, prolongation of T1 relaxation times has been shown to be due to gliosis, vasogenic oedema or axonal loss. Previous histopathological studies in MS have revealed abnormalities in the hypothalamus.

Brownell and Hughes, in their pathological study of the distribution of cerebral plaques in 22 unselected MS patients from the 1960s documented involvement, albeit rare, of the hypothalamus[3]. Huitinga et al have reported the pathological findings on 17 MS patients in a study to specifically investigate whether the hypothalamus was involved[6]. Two of the patients had primary progressive (PP) MS and the remainder

were in the secondary progressive phase, prior to autopsy. As is often the case in post-mortem studies, the mean disease duration was long at 21 years (range 8 – 49 years) and the majority of patients were chair- or bed-bound. Lesions were found in hypothalamic grey matter in three patients. In four patients, axonal damage such as axonal swelling, loss of axonal density and amyloid precursor protein deposition was evident in the sections of hypothalamus. Overall 16 of the patient group had evidence of MS lesions in the hypothalamus or within white matter structures, such as the anterior commissure, internal capsule or optic system, adjacent to the hypothalamus. In addition, a high proportion of these lesions were classified as active lesions.

Plaques have been previously reported in the hypothalamus of MS patients but none were identified on T2-weighted or PD imaging in our cohort of patients. Certainly, considering the number of interconnections hypothalamic neurons have with other structures, such as the thalamus, limbic system and brainstem nuclei, Wallerian degeneration anterograde to a focal lesion could account for axonal loss. The patients in this study do represent a proportion of the patients from the study in chapter 2. Therefore, the widespread interconnections and a mechanism such as Wallerian degeneration could potentially account for diffuse subtle pathological abnormalities affecting deep grey matter structures. In this regard, T2 lesion volume did correlate significantly with T1 relaxation times in our study and may support this mechanism. Similarly a significant correlation was found between hypothalamic T1 relaxation time and disease duration. This was something not demonstrated in the previous study of the thalamus and basal ganglia but had been suggested by earlier studies of T1 relaxation times of the deep grey matter in patients with early disease[14, 136].

MRI based papers regarding involvement of the hypothalamus in MS have predominantly involved case reports and therefore only conventional MRI techniques. There have been a number of case reports involving patients with hypersomnolence and/or temperature dysregulation typically with bilateral hypothalamic lesions[110, 111, 156, 157]. In addition, Ueno et al reported a patient with childhood MS who developed right-sided hyperhidrosis with a contralateral hypothalamic plaque on MRI[152].

A quantitative MRI based study has been undertaken in patients with a CIS and at least two lesions on brain imaging, who are at high risk of developing MS[153]. In this study, Henry et al investigated 41 CIS patients and 49 controls for whole grey matter and focal grey matter atrophy. Although whole grey matter volumes were not different between patients and controls, there was a significant difference in volume for a number of grey matter areas when lesions were removed from the MRI analysis. These areas include the thalamus, basal ganglia and left hypothalamus.

In MS patients, further studies have investigated the hypothalamus from both an imaging and endocrine aspect. Kira et al assessed 27 MS patients and found that one third had a mild-to-moderately elevated prolactin with further studies suggesting this was due to hypothalamic dysfunction[155]. Overall the MS group had a significantly higher prolactin than healthy controls. Four of the patients with an elevated prolactin were found to have bilateral hypothalamic lesions on MRI compared to none of the patients with normal prolactin.

Further, neuro-endocrine studies in MS patients have predominantly concentrated on the HPA axis. These studies have typically been investigating the hypothesis that neuro-endocrine dysfunction may play a role in the pathogenesis of MS,

in particular the relationship between the HPA axis and immune system regulation. In the animal model of MS, experimental allergic encephalomyelitis, it has been proposed that susceptibility to developing the condition and disease activity may be related to an inability of the HPA system to release sufficient corticosteroids to suppress the immune system[158].

To this extent, Schumann and colleagues investigated 53 MS patients, including 35 with RRMS, 13 SPMS and 5 PPMS, with the corticotrophin releasing hormone (CRH) stimulation test[154]. In this test, IV CRH is administered followed by serial measurements of cortisol and adrenocorticotropic hormone (ACTH) levels with normally a rise of both. It is typically used to differentiate pituitary dependent Cushing's syndrome (Cushing's disease), in which there would be an exaggerated rise in cortisol and ACTH, from ectopic production (unaltered response). A subgroup of 45 patients also underwent MRI imaging to look for GAD-enhancing lesions as a marker of disease activity. The study found that patients with GAD-enhancing lesions had a significantly lower cortisol response to CRH than patients without. In addition there was a significant inverse correlation between the number of GAD-enhancing lesions and the cortisol response to the CRH stimulation. It was felt that these results provided support for the theory regarding the immuno-modulatory effect of the HPA axis in that high HPA activity suppresses disease activity.

Wei and Lightman performed a number of neuro-endocrine investigations as well as imaging in small groups of RRMS, SPMS and PPMS patients as well as healthy controls[43]. On the CRH stimulation test there was a lower cortisol response in SPMS patients as compared to controls. In addition, in patients with evidence of disease

activity on MRI, the ACTH response to CRH was significantly higher and the cortisol response to synthetic ACTH administration was significantly lower. Again therefore there was some evidence of HPA axis dysfunction, namely central upregulation as well as adrenal hyporesponsiveness, with some relation to markers of disease activity.

Fassbender at al have reported a comparable study to Schumann's in which they performed similar MRI and endocrine investigations on a group of 23 patients with RRMS who were having an acute relapse[159]. Additional investigations included serum and CSF markers of inflammation as well as a questionnaire-based assessment of depression and anxiety. They found that compared to healthy controls, MS patients had overall a significantly higher response to CRH stimulation. There was a significant correlation between depression and anxiety scores and the level of cortisol response to CRH. In contrast to the aforementioned study, patients with GAD enhancing lesions had a higher cortisol response to CRH. CSF white cell count, which could also be considered a marker of CNS inflammation, was also found to correlate with a higher cortisol response to CRH. These results suggested a role for dysfunction of the HPA axis in mood disorders in MS. Similarly there was an apparent relationship between HPA dysfunction and disease activity albeit the results are opposite to those of Schumann et al. The investigators took the alternative position that the abnormal neuro-endocrine results are as a consequence of immune activity.

Huitinga et al in a subsequent study to that described earlier, applied further investigations to the same cohort of post-mortem MS patients[160]. In this study, the potential effect of inflammatory mediators produced in active lesions in or near the hypothalamus on CRH and CRH/Vasopressin (VP) neurons was investigated. The

104

number of active lesions was found to inversely correlate with the number of CRH/VP neurons suggesting that there is relative impairment of the CRH system in those patients who have active lesions in and around the hypothalamus. It is of note that patients with a higher degree of immunologically active MS also had a shorter disease duration suggesting a worse disease course. This would appear to support the hypothesis that dysfunction of the HPA axis is related to disease activity, possibly due to impaired cortisol secretion leading to a lower ability to suppress immune responses.

3.4.2   MS-related fatigue – MRI correlations

Our study found no difference between T1 value of the hypothalamus in RRMS patients with and without fatigue and no correlation with fatigue severity. Therefore there was no evidence to support a role for the hypothalamus in MS-related fatigue. However, the potential for the hypothalamus to contribute to fatigue, especially in individual patients, cannot be fully discounted, particularly considering the likely multi-factorial pathophysiology of fatigue in MS and also the small numbers in this study.

To summarise, we have demonstrated abnormal T1 relaxation times in the hypothalamus of a cohort of RRMS patients. This confirms previous post-mortem studies of involvement of the hypothalamus in MS and demonstrates that this quantitative MRI technique may well provide a more comprehensive evaluation of the involvement of MS than can be demonstrated by conventional MRI techniques. We have also found no association between T1 values, and the subtle pathological abnormalities this represents, and MS-fatigue.

CHAPTER 4

Chapter 4.   CSF HYPOCRETIN-1 LEVELS, FATIGUE AND

HYPERSOMNOLENCE IN MS

4.1 Introduction

In chapter 3, we have demonstrated a significantly prolonged T1 relaxation time in the hypothalamus in MS patients. As discussed previously, the hypothalamus may be implicated in MS-related fatigue through a number of potential mechanisms, although notably we found no association between fatigue severity and T1 values of the hypothalamus in a small cohort of RRMS patients.

One potential mechanism for MS-fatigue, and detailed in chapter 1, is dysfunction of the hypocretin pathway. Hypocretin-containing neurons are located in the hypothalamus and project widely to receptors within the cerebral cortex, limbic system, brainstem and other deep grey matter structures such as the thalamus. Hypocretin-containing neurons are present in a significantly reduced number in narcoleptic patients[92]. Similarly, CSF hypocretin-1 levels have been found to be frequently low or absent in the CSF of patients with narcolepsy with cataplexy[96]. Narcolepsy is characterized by excessive daytime sleepiness but narcoleptics also report fatigue[81]. In MS, lesions within the hypothalamus have been shown, in case reports, to be associated with hypersomnia and low CSF hypocretin-1 levels[110, 111]. There is the potential for hypersomnia and fatigue to co-exist in MS patients and EDS may contribute to the patient's perception of fatigue[114].

4.1.1 Aims

To investigate this potential pathophysiological mechanism, we measured CSF hypocretin-1 levels in a cohort of patients with MS as well as other inflammatory and non-inflammatory neurological conditions. Specific aims were: (i) to assess any

relationship between hypocretin-1 levels and fatigue severity, (ii) to evaluate any relationship between hypocretin-1 levels and hypersomnolence, (iii) to compare hypocretin-1 levels in MS patients to patients with other neurological conditions.

4.2   Materials and methods

4.2.1   Subjects

One hundred patients who were undergoing CSF examination at a tertiary neurology centre were recruited. The group consisted of 58 females and 42 males with a median age of 42.5 years (IQR, 35 – 51). The patients were separated into three cohorts. The first group consisted of 34 MS patients, as defined by McDonald's criteria[143]. It comprised 22 females and 12 males. Twenty-six patients had RRMS, two had SPMS and six had PPMS[142].

The second group was composed of 24 patients with other inflammatory diseases. These included patients with neurosarcoidosis (n = 3), CIS (n = 8), infective/inflammatory encephalitis or meningitis (n = 3), inflammatory demyelinating polyradiculopathies or its variants (n = 10). The group comprised 7 females and 17 males.

The non-inflammatory diseases group comprised the remaining 42 patients made up of 29 females and 13 males. This group included patients with benign intracranial hypertension (n = 12), cerebrovascular disease (n = 6), primary headache syndromes (n = 4), axonal peripheral neuropathies (n = 3) as well as a wide variety of other conditions.

4.2.2   CSF collection and hypocretin measurement

CSF was obtained by lumbar puncture in all patients between 9am and 5pm. It was frozen immediately and stored at -80°C. The CSF hypocretin-1 levels were measured in crude, unextracted CSF using a custom-designed radioimmunoassay kit. Briefly, the hypocretin-1 antiserum was raised in a rabbit immunised with hypocretin-1 conjugated to bovine serum albumin by carbodiimide. The 125-I-labelled synthetic hypocretin-1 was prepared by the direct iodogen method and purified by reverse-phase high performance liquid chromatography. The antiserum did not cross react with any other hypothalamic peptides including hypocretin-2. The assay had a sensitivity of 0.2 fmol/tube with a 95% confidence interval. Intra- and interassay variation was less than 10%. CSF was also assessed for the presence of oligoclonal bands in 92 patients and the IgG index level in 90 patients in the hospital laboratory.

Approval was obtained from the Nottingham Research Ethics Committee. All subjects gave informed consent.

4.2.3   Fatigue and hypersomnolence assessment

Fatigue was quantified using the FSS and excessive daytime sleepiness by the ESS in 78 of the patients[23, 60]. The questionnaires were ascertained on the day of the lumbar puncture. The 78 patients consisted of 44 females and 34 males.

The MS group consisted of 14 females and 10 males, whilst the inflammatory group consisted of 4 females and 13 males and the non-inflammatory group consisted of 26 females and 11 males.

Upon evaluating the FSS scores, two groups of patients were identified within each cohort: fatigued patients (F) (FSS score $\geq$ 5.0) and non-fatigued patients (NF) (FSS score $\leq$ 4.0). The outstanding patients, with a FSS score of between 4.1 and 4.9, were not included in the F and NF group comparisons but their data was used in correlation analyses.

Similarly, applying the ESS led to two further groups of patients being identified within each cohort: patients with hypersomnolence (S) (ESS score > 10) and those without (NS) (ESS score < 8). Again outstanding patients with an ESS score of between 8 and 10, were not included in the S and NS group comparisons but were used in correlation testing.

4.2.4   Statistical analysis

The Kruskal-Wallis H-test and Mann-Whitney U-test were applied to compare the hypocretin-1 levels of the MS, I and NI patient groups. The Mann-Whitney U-test was also employed in comparisons between F/NF and S/NS cohorts. Spearman's rank correlation coefficient (rho) was determined to assess correlations between the hypocretin-1 levels as well as clinical and questionnaire-derived parameters. A p value of less than 0.05 was considered to be significant.

4.3   Results

4.3.1   Baseline characteristics

Characteristics including age, FSS and ESS scores are summarized in Tables 4.1 and 4.2 as well as disease duration and number of relapses in the preceding 2 years for the MS cohort. The median age of the patients with inflammatory diseases was significantly higher than both patients with MS and non-inflammatory diseases ($p = 0.024$ and $p = 0.041$ respectively). In addition there were a significantly higher proportion of male patients in the inflammatory cohort as compared to both the MS and non-inflammatory groups.

There was a significantly higher FSS score in MS patients as compared to those with non-inflammatory diseases ($p = 0.002$). In addition there were significantly higher FSS and ESS scores in patients with inflammatory diseases as compared to those with non-inflammatory disease ($p = 0.044$ and $p = 0.028$ respectively).

4.3.2   Hypocretin-1 levels and intergroup analysis

MS, inflammatory and non-inflammatory patients

The CSF hypocretin-1 levels for the MS, inflammatory and non-inflammatory groups are also reported in Tables 4.1, 4.2 and Fig 4.1, 4.2. There was a significantly lower hypocretin-1 level in the cohort of patients with inflammatory disease as compared to non-inflammatory diseases, ($p = 0.036$). This result was reproduced by assessment of the sub-group of 78 patients with fatigue and sleepiness data. Hypocretin-1 levels were also lower in patients with MS as compared to non-inflammatory disease however this only

111

approached significance when assessing the subgroup of patients with questionnaire data (p = 0.045). There was no significant difference in hypocretin-1 levels between the MS and other inflammatory disease cohorts.

Fatigued versus non-fatigued patients

Hypocretin-1 results for patients with and without fatigue are reported in Table 4.3. There were no significant differences in hypocretin-1 levels between F and NF groups.

Hypersomnolent versus non-hypersomnolent patients

Hypocretin-1 results are reported in Table 4.4. There were no significant differences in levels between S and NS groups.

4.3.3 Correlation analysis

Fatigue correlation and hypocretin-1

There was no significant correlation between CSF hypocretin-1 levels and fatigue severity as assessed by the FSS in patients with MS, inflammatory or non-inflammatory neurological diseases.

Hypersomnolence correlations and hypocretin-1

Similarly there was no significant correlation between tendency for excessive daytime sleepiness as assessed by the ESS and CSF hypocretin-1 levels in any of the disease cohorts.

Table 4.1   Characteristics of cohort of 100 patients

	MS	Inflammatory	Non-inflammatory	*p* Value
	n = 34	n = 24	n = 42	
Age (years)	41.5	48.5	41.5	0.024*
	(35 – 49.5)	(38 – 63)	(33 – 50)	0.041±
Disease-duration	3.5			
(years)	(2 – 7)			
Relapse rate	2			
	(1 – 2)			
Hypocretin-1	68.3	67.9	82.2	0.036±
(pmol/ml)	(56.4 – 91.4)	(60.5 – 80.1)	(68.3 – 102.2)	

Data are expressed as median (interquartile range)

Relapse rate is the number of relapses in the preceding 2 years

* Patients with inflammatory diseases compared to MS patients

± Patients with inflammatory diseases compared to those with non-inflammatory diseases
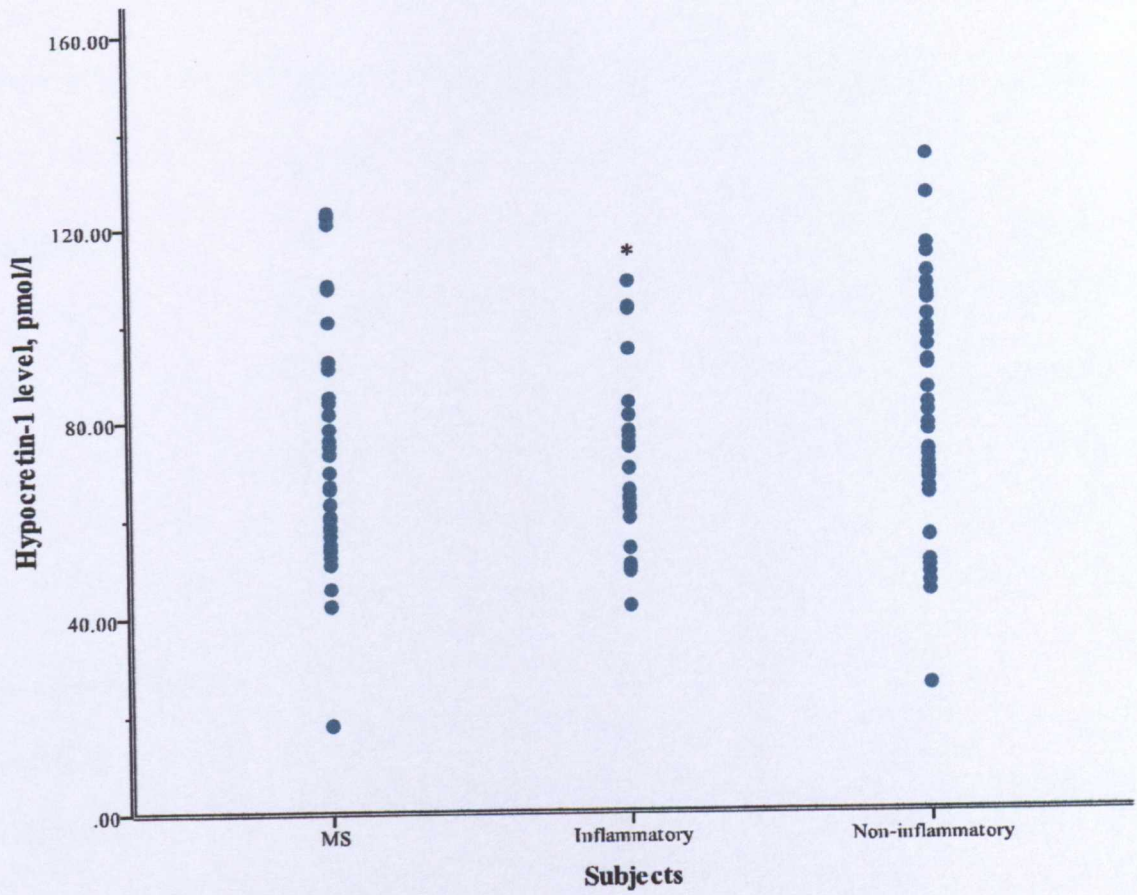
Fig 4.1   Scatterplot of hypocretin-1 levels in cohort of 100 patients

* Patients with inflammatory diseases compared to those with non-inflammatory diseases

(p = 0.036)

Table 4.2 Characteristics of subgroup of 78 patients with questionnaire data

	MS	Inflammatory	Non-inflammatory	p Value
	n = 24	n = 17	n = 37	
Age(years)	41.5	50	39	0.005*
	(36– 49)	(41 – 69)	(30 -50)	0.003±
FSS	5.4	5.8	3.7	0.002∞
	(4.4 – 6.1)	(3.5 – 6.3)	(2.6 – 5.1)	0.044 ±
ESS	10	10	6	0.028 ±
	(5.3 – 14.5)	(7.3 – 16.5)	(4 – 10.8)	
Disease-duration	3			
(years)	(2 – 17)			
Relapse rate	2			
	(1 – 2)			
Hypocretin-1	60.7	63.6	81.3	0.018±
(pmol/ml)	(54.3 – 80.7)	(52.3 – 75.9)	(67.2 – 98.1)	0.045∞

Data are expressed as median (interquartile range)

Relapse rate is the number of relapses in the preceding 2 years

FSS = Fatigue Severity Scale; ESS = Epworth Sleepiness Scale

* Patients with inflammatory diseases compared to MS patients

± Patients with inflammatory diseases compared to those with non-inflammatory diseases

∞ MS patients compared to those with non-inflammatory diseases
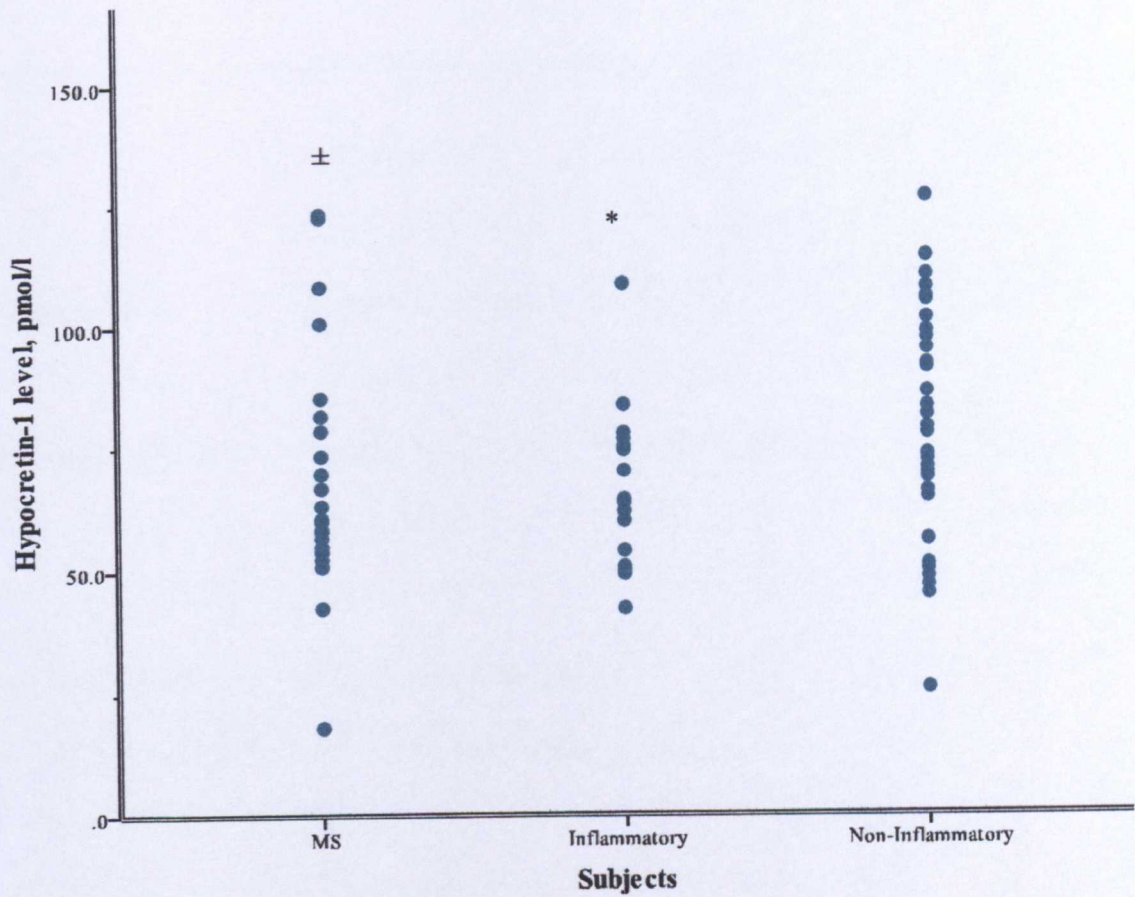
Fig 4.2 Scatterplot of hypocretin-1 levels in cohort of 78 patients with

questionnaire data

* Patients with inflammatory diseases compared to those with non-inflammatory diseases

(p = 0.018)

± MS patients compared to those with non-inflammatory diseases (p = 0.045)

116

Table 4.3   Hypocretin-1 levels (pmol/ml) in patients with and without fatigue

	F	NF	p Value
MS	60.6 (54 – 80)	68.3 (56 – 81.3)	0.65
	n = 17	n = 4	
Inflammatory	63.6 (49.9 – 76.6)	64.3 (57.9 – 91.6)	0.57
	n = 11	n = 5	
Non-inflammatory	94.3 (49.5 – 102.3)	73.2 (65.2 – 87.1)	0.22
	n = 10	n = 21	

Data are expressed as median (interquartile range)

F = patients with fatigue; NF = patients without fatigue

Table 4.4   Hypocretin-1 levels (pmol/ml) in patients with and without hypersomnolence

	S	NS	p Value
MS	60.6 (53.8 – 100.7)	69.7 (58.5 – 83.3)	0.38
	n = 11	n = 9	
Inflammatory	70.1 (61.9 – 77.7)	61 (52.9 – 83)	0.43
	n = 7	n = 6	
Non-inflammatory	91.3 (57.9 – 112.3)	80 (62.5 – 99.4)	0.43
	n = 9	n = 22	

Data are expressed as median (interquartile range)

S = patients with hypersomnolence; NS = patients without hypersomnolence

Clinical correlations and hypocretin-1

There was no significant correlation between hypocretin-1 levels and age, disease duration or relapse rate within any of the patient groups. These findings were reproduced with analysis of the subgroup of 78 patients with questionnaire data.

Fatigue and hypersomnolence correlations

There were no significant correlations between FSS or ESS scores and clinical parameters recorded in the patient cohorts. There were, however, significant correlations between fatigue severity and daytime sleepiness in each of the patient cohorts; MS (rho = 0.473; $p = 0.019$), inflammatory (rho = 0.787; $p < 0.0001$) and non-inflammatory (rho = 0.377; $p = 0.023$).

4.3.4 Hypocretin-1 and CSF constituents

CSF was positive for oligoclonal bands in 34 patients and negative in 58 patients. There was no significant difference in hypocretin-1 levels between patients who were positive and those who were negative. Median IgG index was 0.55 (IQR, 0.47 – 0.83). There was no significant correlation between IgG index and hypocretin-1 levels.

4.4 Discussion

This study reports the CSF hypocretin-1 levels in a group of patients with a variety of neurological diseases under investigation at a tertiary neurology centre. The primary aim of this study was to prospectively investigate for an association between hypocretin-1 levels and fatigue severity, assessed by the FSS, and hypersomnolence, assessed by the

ESS, in a cohort of MS patients. It is, to the best of our knowledge, the first study to prospectively evaluate and report on this potential relationship in MS.

4.4.1 CSF hypocretin-1, fatigue and hypersomnolence

We found no association between FSS or ESS scores and hypocretin-1 levels in the MS, inflammatory or non-inflammatory cohorts. In addition there was no difference in median hypocretin-1 levels between patients with and without fatigue or hypersomnolence. This study, therefore, provides no support for the hypothesis that a potential pathophysiological factor in MS-related fatigue is hypocretin dysfunction or deficiency secondary to grey matter involvement in MS. This is despite finding supportive evidence for an association between self-reported perception of the severity of fatigue and EDS in all our patient cohorts.

There are a number of potential factors as to why no association was found in our study should such a relationship exist. Firstly, it is quite likely that within a population of MS patients, different pathophysiological mechanisms of fatigue exist between individuals. In addition, as discussed within the introduction, it is also quite likely that in a proportion of patients multifactorial mechanisms apply for generating fatigue. These would include the variety of primary fatigue mechanisms discussed such as immune-related, the neuroendocrine axis and autonomic nervous system dysfunction as well as the factors implicated in secondary fatigue such as pain or deconditioning. Within our study, potential confounding factors which may lead to secondary MS-related fatigue were not addressed prior to CSF examination. These include, for example, a sleep diary or withdrawal of potential medication which could lead to fatigue or

hypersomnolence. This study was performed on patients who were admitted for lumbar puncture as part of the management of their neurological symptoms or disease and as such these, potentially relevant, factors were unable to be addressed prior to the enrolment of subjects.

Further consideration must also be given to the patients who were enrolled. In addition to the fact that the cohorts of patients were neither age- nor sex-matched, there was a degree of selection bias in light of the fact that the patients were already being admitted for lumbar puncture. This would suggest that, for example, the MS patients may have active or early disease. Lumbar punctures are performed in patients to support a diagnosis of MS and therefore most often performed in patients with a short disease duration. In addition, the natural history of MS is that of a higher relapse rate initially with a gradual reduction in frequency with disease progression. MS relapses may be associated with destruction or dysfunction of the blood-brain barrier. Loss of integrity of this barrier has been shown to determine CSF protein levels and could potentially lead to alteration of levels of hypocretin-1[161]. It is of note, that our study revealed no association between hypocretin-1 levels and either disease duration or relapse rate within the preceding 2 years.

The techniques applied to assess fatigue and hypersomnolence also need to be considered as a potential confounding factor. Both the FSS and ESS, as discussed within the introduction, are employed frequently in studies and have a number of benefits including validity and reliability. Drawbacks however are rater bias as well as the potential ceiling effect of the FSS. This could lead to a lack of differentiation between degrees of fatigue severity within the cohort.

Regarding the laboratory techniques employed, during the collection of CSF, polystyrene containers were utilized. It has been recommended that, as a standard for studies of CSF biomarkers, polypropylene tubes are used as they have a low protein-binding potential[162]. It is therefore possible that the polystyrene tubes used during collection may have altered the levels of CSF hypocretin-1 in our study. However, whilst this effect has been demonstrated with other CSF biomarkers, there has been no such study performed to determine the effect on hypocretin-1[163]. In addition, this potential effect would be a constant within the study and should not have altered the inter-group analysis or comparisons to clinical deficits.

A further confounding factor is the fact that the HLA status was not assessed in the population of patients we studied. Both MS and narcolepsy are associated with HLA sub-types and furthermore there is evidence that hypocretin-1 deficiency in narcolepsy may also be associated with HLA subtype[96]. It is therefore possible that differentiating patients, within a study such as this, by HLA subtype would offer a clearer picture regarding any potential association.

Two previous studies have investigated patients with other neurological disorders and a potential association between EDS and hypocretin-1 levels. Martinez-Rodriguez et al reported a cohort of six patients with myotonic dystrophy type 1 who complained of hypersomnolence and evaluated them with the ESS and the MSLT[106]. There was no correlation between either tool and hypocretin-1 levels. A further study, evaluated the hypocretin-1 levels in ventricular CSF in 19 patients with advanced Parkinson's disease. The investigators employed the ESS but again no correlation was found[107].

Three studies have looked at hypocretin-1 levels in relation to measures of EDS in narcolepsy and other hypersomnias. One study evaluated 18 patients with narcolepsy plus cataplexy and found no difference in ESS scores between those with undetectable and detectable hypocretin-1 levels[97]. They did however find significantly shorter mean sleep latencies on MSLT in the cohort of patients with undetectable hypocretin-1. This supported an earlier study of 106 narcoleptic patients that reported more abnormal MSLTs in patients with low hypocretin-1 levels[96]. A further study of 24 patients with narcolepsy with or without cataplexy and primary hypersomnia found no correlation between hypocretin-1 levels and ESS score or sleep latency[164]. Considering these studies, it is possible that objective measures of alertness or EDS may be a more appropriate tool to investigate for any role of the hypocretin system in MS. We will apply objective measures in the study reported in the following chapter

4.4.2   CSF hypocretin-1 levels in MS

As discussed in the introduction, in addition to individual case reports of hypersomnia in MS patients with hypothalamic lesions, there have been four previous studies assessing groups of MS patients in terms of their hypocretin-1 levels. Kanbayashi et al reported eight MS patients whose CSF hypocretin-1 levels were not significantly different from patients without neuroimmunological disease[102]. Ripley et al studied 10 MS patients amongst a cohort of patients with a wide variety of neurological disorders and found levels within control range[165]. A study by Ebrahim et al found that low hypocretin-1 levels are present in some patients within a number of groups of neurological conditions, including a cohort of 44 MS patients, however, the mean hypocretin-1 level was within

normal range[105]. A study by Knudsen et al involved 23 RRMS patients and 25 patients with optic neuritis[113]. All but one patient had CSF hypocretin-1 levels measured and all were felt to be within normal range. In addition there was no difference in hypocretin levels between those undergoing an acute attack and those who were stable. Our study, in support of these previous reports, also found that although there is an apparent lower level of hypocretin-1 in patients with MS as compared to non-inflammatory conditions this does not reach significance

4.4.3 CSF hypocretin-1 levels in inflammatory disorders

Our study did find significantly lower hypocretin-1 levels in patients with inflammatory disorders, other than MS, as compared to non-inflammatory disorders. This again supports earlier studies discussed in the opening chapter. A number of investigators have reported significantly low levels in cohorts of patients with SLE, GBS and Miller-Fisher syndrome[102-105]. In addition, narcolepsy is felt to have an autoimmune origin with its HLA association and the recent report of a functional autoantibody[86]. The presence of CSF oligoclonal bands and/or an elevated IgG index may be evidence for a CNS immune-mediated disorder in individuals. Our study found no association between hypocretin-1 levels and either CSF marker.

One final point to note is our use of a custom-designed radioimmunoassay kit rather than the commercially available radioimmunoassay employed in the majority of referenced studies. We are confident that the measurements have internal consistency and validity, as intra-lab variation has consistently been low. As an analogy, the intra-lab coefficient of variation for CSF tau measurement is very low, whilst the inter-lab

variation can be greater than 50%[166]. In addition, inter-lab variation has been revealed in studies which have use the same commercially-available radioimmunoassay for hypocretin-1. For example, in one study comparatively very low levels were found in all patients with narcolepsy without cataplexy and also those with primary hypersomnia contrasting significantly with results from similar cohorts of patients in a study by different investigators[96, 164].

To conclude, our study found no association between CSF hypocretin-1 levels and fatigue severity as assessed by the FSS or propensity to excessive daytime sleepiness as determined by the ESS. We do report a significantly lower hypocretin-1 level in patients with inflammatory disorders as compared to non-inflammatory conditions.

CHAPTER 5

Chapter 5.   MODAFINIL FOR MS-FATIGUE: A PUPILLOGRAPHIC STUDY

5.1   Introduction

In chapter 1, a number of potential pharmacological and non-pharmacological treatments for MS fatigue were discussed. The drug that has most recently been evaluated is the wakefulness-promoting drug, modafinil. This drug has been shown to be effective in the treatment of hypersomnia, in particular in narcolepsy. It has been shown to activate neurons within deep grey matter structures such as the hypothalamus as well as the brainstem[118]. The results of studies evaluating the effect of modafinil on MS-fatigue, using questionnaire-based measures, are to-date mixed.

There are a number of physiological and laboratory techniques applied in studies, such as the Pupillographic Sleepiness Test (PST), which provide objective and reproducible measures of alertness and CNS vigilance. The PST has been shown to be an effective measure of alertness in a number of, predominantly hypersomnolent, conditions and can detect a treatment effect. However, it has been employed in only a limited number of MS studies.

5.1.1   Aims

Therefore to further explore the potential benefit of modafinil in MS-related fatigue, we applied a number of objective, as well as subjective, tools to a cohort of MS patients with and without fatigue and healthy controls. The specific aims of this study were: (i) to assess any effect of modafinil in participants as compared to placebo on measures of alertness in particular pupillography, (ii) to evaluate for any difference in baseline measures of alertness and vigilance in patients with and without fatigue, (iii) to compare

baseline measures of autonomic function in participants and any effect of modafinil on these measures.

5.2 Materials and methods

5.2.1 Subjects

Twenty-six patients with MS as defined by the McDonald's criteria were recruited[143]. The group consisted of 21 patients with RRMS, 3 with SPMS and 2 with PPMS[142]. There were 9 males and 17 females. All subjects were relapse and corticosteroid-free for at least one month prior to commencement of the study as well as for the duration of the study. No patients suffered from significant medical or psychiatric conditions that could confound the study. Prior ocular manifestations of MS or impaired visual function, as assessed by the Visual Function Questionnaire VFQ25, were exclusion criteria.

Subsequent to quantification of fatigue using the FSS, two groups of MS patients were identified: fatigued patients (F) (FSS score ≥ 5.0 [n = 16]) and non-fatigued patients (NF) (FSS score ≤ 4.0 [n = 9])[23]. The single remaining patient, with an FSS score of between 4.1 and 4.9, was not included in the F and NF group comparisons but their data was used in correlation analyses. No patients with fatigue were receiving medication that could potentially affect their fatigue. Three patients in each group were receiving glatiramer acetate treatment and one patient in the NF group was receiving intramuscular beta-interferon. One patient with SPMS was in the NF group whilst three patients in the F cohort had chronic progressive disease.

The normal control cohort consisted of 9 subjects matched in terms of gender and age. The controls had no history of neurological disease or other significant medical condition.

All participants completed the Beck Depression Inventory (BDI) upon recruitment to evaluate for any clinically significant depression (a cut-off of <19 was applied to indicate no or mild depression).

Approval was obtained from the Nottingham Research Ethics Committee. All subjects gave informed consent.

5.2.2   Pupillographic measures of alertness

5.2.2.1   Pupillographic sleepiness test

A monocular, infrared television pupillometer (PST setup version 1.20: AMTech, Weinheim, Germany) was used to measure pupil diameter in darkness. During each recording session subjects wore goggles with infrared filters to ensure no visible light, other than the pupillographic light source, entered the eye. The subject sat in a comfortable chair with their head positioned on a chin rest 70cm from the infrared camera. White-noise [60 dB(A)] was supplied via headphones to mask external noises. The subjects were instructed to fixate on the pupillographic light source, a red-light-emitting-diode positioned at the level of the camera. The pupil diameter was measured continuously for an 11 minute recording period at a frequency of 25Hz and provided the mean pupil diameter. Software driven artifact rejection was applied to the raw data to remove blinks and high-frequency noise. Oscillations of pupil diameter at frequencies of

≤0.8Hz were captured and subject to Fast Fourier Transform. This provided the total power of fluctuations. Absolute changes in pupil diameter were also recorded and subjected to low pass filtering. These were obtained as the cumulative differences between successive 0.64 seconds (16 data points at 25Hz) samples over 1 minute to yield the pupillary unrest index (PUI). Data were captured by dedicated software (AMTech, Weinheim, Germany) and stored for offline analysis.

5.2.3   Non-pupillary measures of alertness

5.2.3.1   Critical flicker fusion frequency

Critical Flicker Fusion Frequency (CFFF) is defined as the frequency at which a flickering light gives rise to the subjective sensation of a steady light. Subjects sat opposite a Flicker Fusion Monitor (model 1199, System 696 Ltd, London, UK) with four light emitting diodes arranged in a 1cm square on a black background. The mean of four measurements of the threshold at which the light changed from flickering to constant was obtained, two with increasing (flicker-to-fusion) and two with decreasing (fusion-to-flicker) frequencies. With increasing vigilance, a higher threshold is achieved.

5.2.3.2   Choice reaction time

Choice Reaction Time (CRT) provides an assessment of attention to a stimulus. It is as an indicator of psychomotor performance and has been shown to be sensitive to psychoactive compounds. Subjects were required to extinguish one of six equidistant red lights, illuminated at random, by pressing the associated response button as quickly as

possible. Two components were recorded; Recognition- (RRT) and Motor Reaction Time (MRT), which together are the Total Reaction Time (TRT). RRT was the time it took for the subject to notice the light, the measurement being the time between stimulus onset and the subject lifting their finger from the start button. MRT indexed the majority of the movement component of this task and was the time between the subject having lifted their finger from the start button and touching the response button. The mean reaction times of 50 trials were recorded.

5.2.3.3   Speech time

We used an example of automatic speech with subjects counting, taking their own time, from 1 to 10. Specific equipment, constructed within the department, was used to analyse this. The equipment involved a voice-operated timer which would begin at the first and end at the last phonation. Upon detecting phonation, the counts were directed to a 'phonation' timer and upon detecting silence the counts were directed to a 'pause' timer. Two values were obtained; phonation time and pause time. The phonation time was the summation of the ten individual digit phonation times whilst the pause time was the summation of the nine inter-phonation pause times. Both times are stable within normal individuals, however, a prolonged pause time has been shown to be an indicator of psychomotor retardation[167]. In addition, a decreasing pause time has been shown to indicate a response to treatment[168].

5.2.3.4 Visual analogue scale

Subjects rated their subjective state of mood using a computerized version of a visual analogue scale (VAS) developed by Norris[169]. There are 16 scales subdivided under three headings; alertness (nine items), anxiety (two) and contentedness (five), with rating of these groups based on a factor analysis carried out by Bond and Lader[170]. Higher scores in the 3 groups indicate increased alertness, calmness and contentedness respectively. We specifically analysed the alertness score in subjects.

5.2.3.5 Digit cancellation

In this investigation the digit 4 was randomly distributed in a matrix of 400 digits occurring 20 times in the whole sample. The subject had the task of crossing out the 4s as fast and accurately as they could. The time to complete the task was recorded. To minimize practice effects, four versions of these tests were prepared, and the subjects received the four versions in a quasi-random order. The test has been shown to be sensitive to psychotrophic compounds.

5.2.3.6 Hypersomnolence questionnaires

Patients completed the two most widely used questionnaires to assess sleepiness; the ESS and the SSS[60, 64]. Whilst the SSS routinely provides an indication of the immediate level of alertness of the patient, the ESS, on the other hand, is typically used to evaluate the subject's sleepiness over recent times. However, in this study as the ESS was administered both pre-and post-treatment, patients were asked to imagine how they

currently feel in terms of alertness in the context of the eight scenarios depicted in the ESS to also provide an immediate value of alertness.

5.2.4 Autonomic measures

5.2.4.1 Static pupillometry

Resting pupil diameter was measured with a binocular, infrared video pupillometer (Procyon Ltd, London, UK) under four luminance levels (darkness, 6, 91 and 360 cdm^{-2}) achieved with a calibrated internal light source within the pupillometer. The measurements were performed in a darkened room to prevent interference from external light sources. The pupil diameter was first recorded in darkness and then under each increasing luminance levels for two seconds at 4Hz and stored to disk for offline analysis.

5.2.4.2 Dynamic pupillometry

Pupil diameter during light and darkness reflex responses was measured with an infrared binocular television pupillometer (TVC 1015B, Applied Science Laboratories, Waltham, MA, USA) in a dark room. The sampling rate of the pupillometer was 60Hz and the detection accuracy better than 0.05mm.

The stimulus for the light reflex response were four light flashes (green, 565nm peak wavelength) of 200 millisecond duration and of incremental luminance (5.2, 41, 320 and 2050 cdm^{-2}) measured in the plane of the cornea. The light flashes were delivered at 25-second intervals, via a light-emitting-diode positioned 1cm from the subject's right eye and providing "full-face" light stimulation. Two parameters were

measured, firstly the amplitude of light reflex response i.e. the difference between the initial and minimal pupil diameters to a light flash, which is a measure of the parasympathetic response. Secondly, the 75% recovery time ($T_{3/4}$), that is the time taken to obtain 75% recovery from the peak of the pupil constriction and is a measure of sympathetic activity.

Darkness reflex responses were evoked by switching on an illuminated screen positioned 1m in front of the subject's eyes after a period of dark adaptation. The luminance of the screen was 1370 cdm^{-2} and was switched on for 10 seconds. This was followed by 20 seconds of darkness, a sufficient length of time for the dilatatory response to darkness to reach a plateau. The cycle was repeated with the mean of two parameters of darkness responses, measured. The parameters, both measures of sympathetic activity, were the initial velocity (the time to obtain 25% of maximum dilatation from the onset of the response, $T_{1/4}$) and the amplitude (the difference between dark- and light-adapted pupil diameters).

5.2.4.3   Non-pupillary autonomic measures

Blood pressure (BP) response to sustained handgrip is a non-invasive measure of autonomic function. BP was measured with the subject sat down using an electronic sphygmomanometer. They were then asked to achieve their maximum voluntary contraction (MVC) using a handgrip dynamometer. Whilst recording BP every minute, the subjects maintained handgrip at 20 per cent of MVC. This was maintained for as long as possible or for a maximum of 11 minutes. BP was recorded just prior to the release of handgrip. The measurement obtained was the difference between diastolic pressure just

prior to release and before starting. A normal response is an elevation in diastolic of $\geq 16mmHg$ with $\leq 10mmHg$ considered abnormal (11 – 15mmHg is borderline). This pressor response is considered a measure of sympathetic activity.

5.2.5 Drugs

Modafinil 200mg and placebo were prepared in identical capsules and administered orally. Modafinil has previously been studied in doses up to 400mg daily. In our study the dose of modafinil was selected on the basis of a beneficial response at this dose in a large multicentre study of patients with narcolepsy[117]. In previous studies of modafinil in patients with MS, there is evidence that, in those who respond, the majority do not need to go higher than 200mg daily[131]. In addition, one study revealed a benefit at a lower dose of 200mg but not the higher dose of 400mg daily[132].

5.2.6 Design

All subjects attended an acclimatization session at least one week prior to the first of two experimental sessions which were themselves separated by a two-week interval. Subjects were allocated to treatment according to a double-blind, balanced, crossover design.

5.2.7 Procedure

At the acclimatization session, as well as an introduction to the investigations, patients completed the fatigue and depression questionnaires and underwent a full neurological examination by an experienced observer. In the experimental sessions, pre-treatment tests comprised hypersomnolence questionnaires, digit cancellation, VAS, PST, darkness

reflex responses, CFFF, CRT, speech analysis and finally sustained hand-grip. The capsule was ingested immediately after completion of the pre-treatment tests. Post-treatment tests were carried out after two hours. The time course of the session was based on the pharmacokinetic profile of modafinil with t_{max} being approximately two hours following oral administration[171]. Post-treatment tests, in addition to the pre-treatment tests, included static pupillometry and light reflex responses.

5.2.8   Statistical analysis

The Kruskal-Wallis H-test and the Mann-Whitney U-test were applied to compare the autonomic and alertness measures between the F, NF and control groups. The Wilcoxon Signed Rank test was used to compare response to modafinil with response to placebo within each individual group. The Chi-Squared test was employed to assess the frequency of normal responses to the pressor test within each cohort. Spearman's rank correlation coefficient (rho) was determined to assess for any correlations between fatigue, sleepiness and pupillography results with other clinical and laboratory measures. A p value of less than 0.05 was considered to be significant.

5.3   Results

5.3.1   Baseline characteristics

Control and patient groups were matched in terms of gender and age (Table 5.1). There was no significant difference in terms of level of disability or duration of MS in patients with and without fatigue.

136

Scores on the BDI were found to be significantly higher in patients with fatigue than patients without fatigue and healthy controls. However, it was noted that only 3 subjects, who were all MS patients with fatigue, scored greater than 18 and no patients had severe depression.

5.3.2   Baseline alertness measures

We compared the pre-treatment results of the pupillographic and non-pupillary measures of alertness from the first experimental session undertaken by the subjects (Table 5.2). No significant difference was found in baseline PST results between the 3 cohorts of subjects. MS patients as a whole however, had a significantly lower CFFF as compared to healthy controls ($p = 0.017$). Interestingly, in patients with fatigue there was only a trend for a lower CFFF ($p = 0.057$) whilst it reached significance in patients without fatigue ($p = 0.019$).

On the CRT investigation, we found a significantly prolonged TRT in MS patients with fatigue as compared to controls ($p = 0.012$) which was due to a significantly prolonged MRT ($p = 0.024$). There was no difference in RRT and nor was there any significant difference between MS patients without fatigue and controls.

VAS alertness scores were significantly higher in controls as compared to MS-fatigue sufferers ($p = 0.004$). There was however no difference between those patients with and without fatigue. There were no other significant differences on the additional questionnaire based investigations although there was a trend for higher ESS scores in patients with fatigue as compared to those without but this did not reach significance ($p = 0.057$).

Table 5.1 Baseline characteristics

	F, n = 16	NF, n = 9	Controls, n = 9	p Value
Age (years)	40.5 (35 – 52)	43 (31 – 45)	36 (30.5 – 53)	
Sex, M/F	6/10	3/6	3/6	
EDSS	2.5 (2 – 4)	1.5 (1.5 – 2)		
Dis-Dur (years)	5 (3 – 11.5)	8 (5 – 12.5)		
FSS	5.6 (5.2 – 5.8)	2.3 (1.9 – 3.1)		<0.001
BDI	7 (3 – 12)	1 (0 – 3)	1 (0.5 – 4.5)	<0.001*

Data are expressed as median (interquartile range)

F = patients with fatigue; NF = patients without fatigue; EDSS = Expanded Disability Status Scale; Dis-Dur = disease duration; FSS = Fatigue Severity Scale; BDI = Beck Depression Inventory

* MS patients with fatigue compared to patients without fatigue and healthy controls

Table 5.2   Baseline alertness measures

	F, n = 16	NF, n = 9	Controls, n = 9	p Value
PUI (mm min^{-1})	4.44	4.07	3.48	
	(3.26 – 5.79)	(2.69 – 6.27)	(2.88 – 4.75)	
Power	813	892	652	
	(638 – 1333)	(487 – 1285)	(571 - 799)	
Mean Pupil	7.22	7.56	7.30	
Diameter (mm)	(6.20 – 8.40)	(5.46 – 8.00)	(6.70 – 8.47)	
CFFF (Hz)	29.4	29.8	32.5	0.019*
	(27.6 – 31.8)	(25.1 – 30.7)	(30.1 – 33.3)	
TRT (ms)	690	685	607	0.014±
	(633 – 795)	(646 – 716)	(577 – 651)	
RRT (ms)	386	369	378	
	(366 – 423)	(250 – 404)	(313 – 403)	
MRT (ms)	295	305	246	0.032±
	(268 – 329)	(290 – 329)	(225 – 298)	
Digit	60	49	47	
Cancellation (s)	(44 – 65.5)	(46 – 54)	(40.5 – 58.5)	
ESS	6	3	5	
	(5 – 11)	(0.5 – 8)	(2 – 5.5)	
SSS	2	1	1	
	(1 – 3)	(1 – 2)	(1 – 1)	

VAS	38.8	50.9	61.0	0.004±
	(29.8 – 53.7)	(39.0 – 65.7)	(51.6 – 67.4)	
Speech (s)	7.24	7.21	7.03	
	(5.82 – 9.52)	(5.63 – 7.83)	(5.84 – 10.77)	
Phonation (s)	3.55	3.44	3.51	
	(3.16 – 3.84)	(3.05 – 4.17)	(3.37 – 3.73)	
Pause (s)	3.75	3.26	3.71	
	(2.33 – 5.76)	(2.35 – 4.21)	(2.55 – 6.63)	

Data are expressed as median (interquartile range)

F = patients with fatigue; NF = patients without fatigue; PUI = Pupillary Unrest Index;

CFFF = Critical Flicker Fusion Frequency; TRT = Total Reaction Time; RRT = Recognition Reaction Time; MRT = Motor Reaction Time; ESS = Epworth Sleepiness Scale; SSS = Stanford Sleepiness Scale; VAS = Visual Analogue Scale

* MS patients without fatigue compared to healthy controls

± MS patients with fatigue compared to healthy controls

Table 5.3   Baseline sympathetic autonomic measures

	F, n = 16	NF, n = 9	Controls, n = 9	p Value
Sustained hand-grip (mmHg)§	11 (6 – 23)	19 (11 – 24)	16 (5 – 27)	
Normal response (%)∞	34.4	66.7	58.8	0.028*
Darkness reflex $T_{1/4}$ (mm s^{-1})	0.85 (0.73 – 0.92)	0.77 (0.72 – 0.90)	0.72 (0.63 – 1.02)	
Darkness reflex amplitude (mm)	3.85 (3.50 – 4.31)	3.92 (3.50 – 4.30)	3.86 (3.48 – 4.03)	
$T_{3/4}$ (s) 5.2 cdm^{-2}	1.43 (1.00 – 2.72)	1.54 (1.24 – 2.21)	1.41 (0.96 – 1.91)	
$T_{3/4}$ (s) 41 cdm^{-2}	1.85 (1.49 – 2.72)	1.74 (1.51 – 2.19)	3.09 (2.42 – 3.63)	0.004±
$T_{3/4}$ (s) 320 cdm^{-2}	2.82 (1.77 – 4.11)	2.88 (2.34 – 3.84)	3.13 (2.56 – 3.24)	
$T_{3/4}$ (s) 2050 cdm^{-2}	3.44 (2.06 – 5.01)	3.12 (2.29 – 3.50)	3.20 (2.65 – 3.85)	

Data are expressed as median (interquartile range)

F = patients with fatigue; NF = patients without fatigue; Normal response = percentage of

patients with a normal response on the sustained hand-grip test; $T_{1/4}$ = time to obtain 25%

of maximum dilatation from the onset of the response on darkness reflex testing; $T_{3/4}$ = time taken to obtain 75% recovery from the peak of the pupil constriction on light reflex response

§ Measure of the difference between the diastolic BP immediately prior to the commencement and release of sustained hand-grip

∞ Patients with a normal response to sustained hand grip

* MS patients with fatigue compared to those without fatigue

± MS patients without fatigue compared to healthy controls
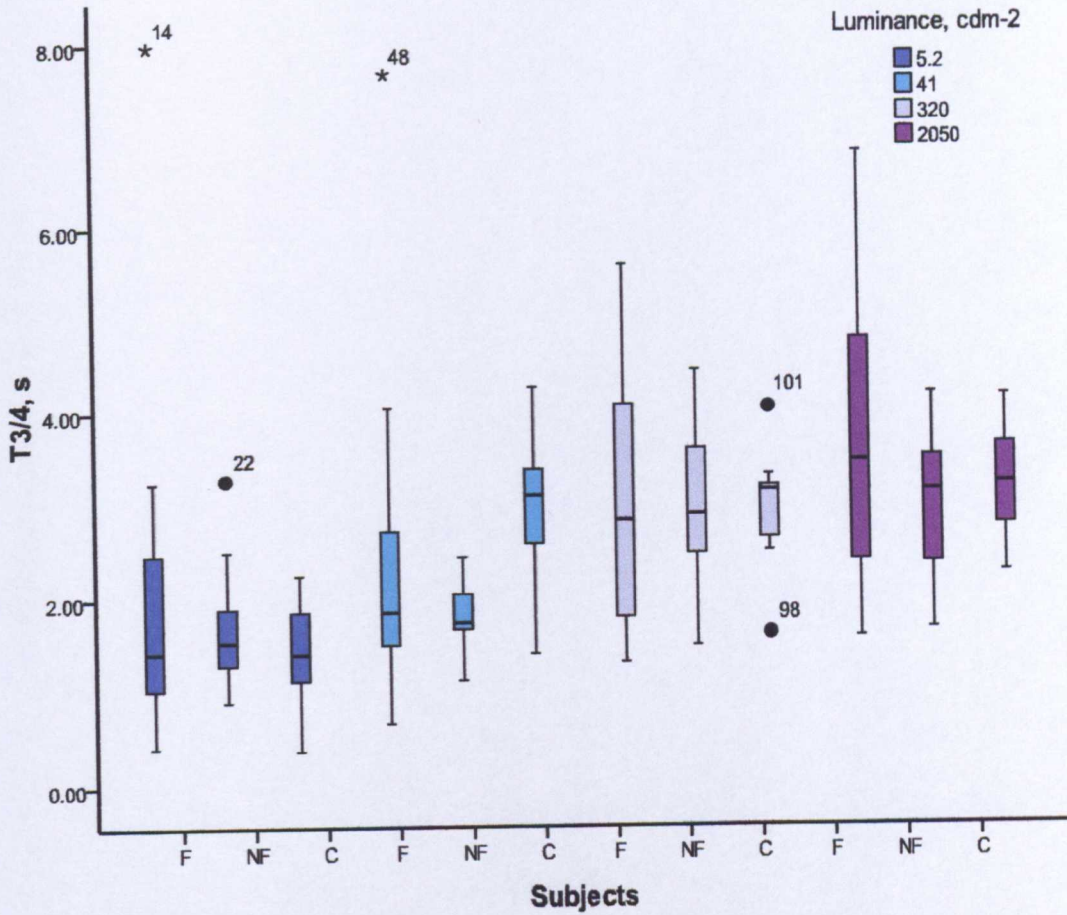
Figure 5.1   Light reflex response: baseline $T_{3/4}$ response of patients with and without fatigue and healthy controls, clustered in four incremental luminance units. $T_{3/4}$ is the time taken from peak of constriction to obtain 75% recovery. Columns represent median and interquartile range. Bars are extreme values.

F = patients with fatigue; NF = patients without fatigue; C = healthy controls
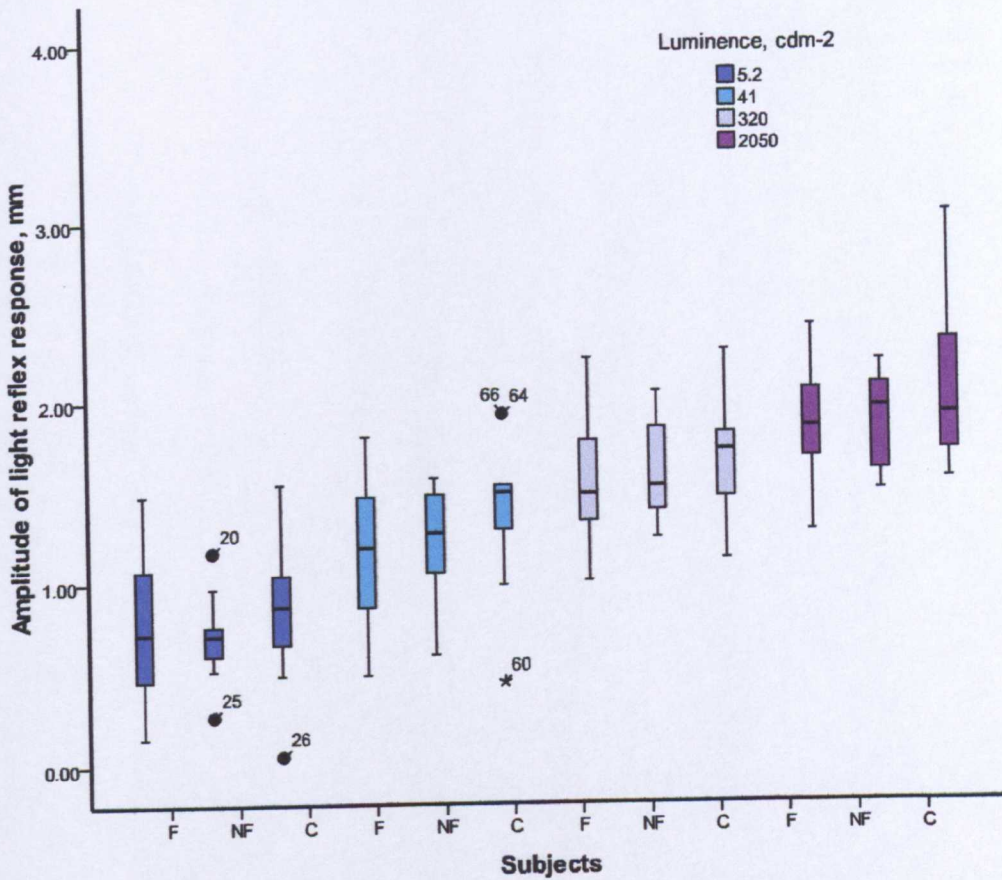
Figure 5.2 Light reflex response (parasympathetic measure): baseline amplitude of response of patients with and without fatigue and healthy controls, clustered in four incremental luminance units. Columns represent median and interquartile range. Bars are extreme values.

F = patients with fatigue; NF = patients without fatigue; C = healthy controls
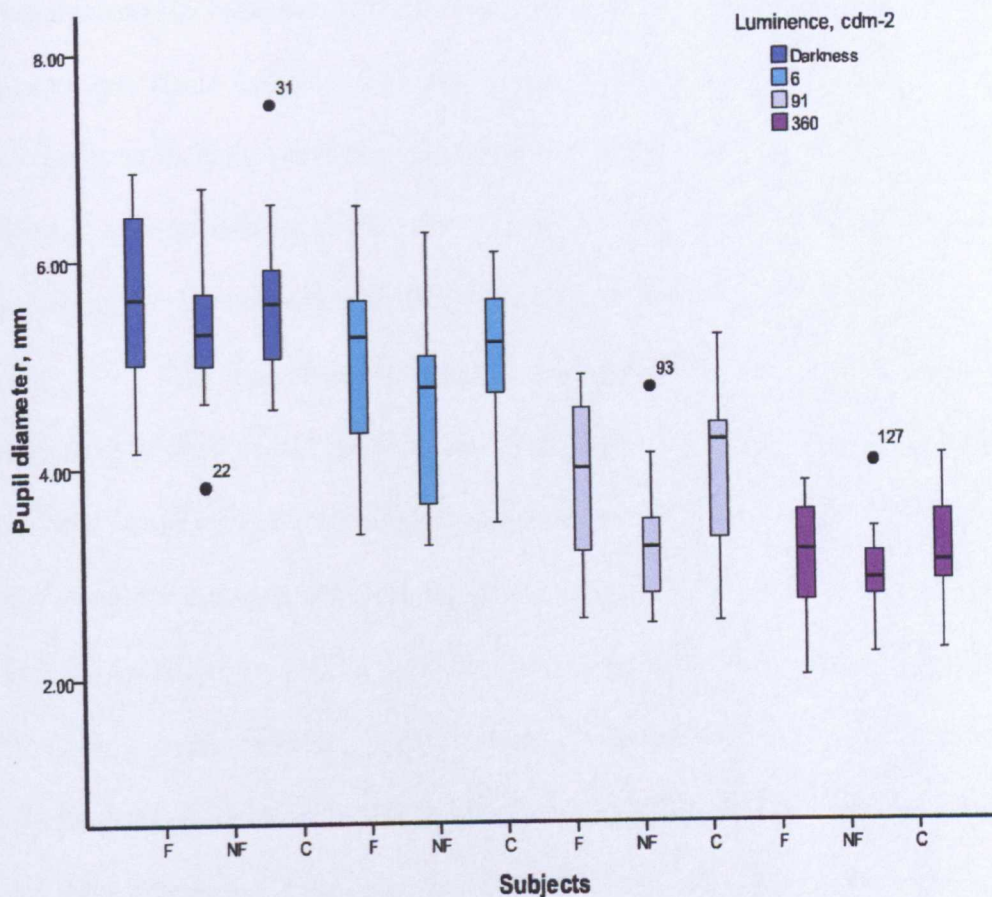
Figure 5.3 Resting pupil diameter: baseline pupil diameter of patients with and without fatigue and healthy controls during static pupillometry, clustered in four incremental luminance units. Columns represent median and interquartile range. Bars are extreme values.

F = patients with fatigue; NF = patients without fatigue; C = healthy controls

5.3.3 Baseline autonomic measures

We compared the baseline pupillary and non-pupillary measures of autonomic function between the three cohorts. For the static pupillometry and light reflex response investigations, which were not performed pre-treatment, we used the placebo post-treatment session data. For all other autonomic measures we used the pre-treatment results from the first experimental session (Table 5.3 and Fig 5.1 – 5.3).

The $T_{3/4}$ response during the light reflex at $41cdm^{-2}$ luminance was significantly slower in MS patients as compared to controls ($p = 0.01$). This remained significant when comparing non-fatigued patients to controls ($p = 0.004$) (Fig 5.1). There was a trend for a slower response in patients with fatigue as compared to controls ($p = 0.064$). As indicated on Figure 5.1 there was a single extreme outlier in the fatigue cohort with a very quick response. On removal of this outlier, this trend converted to a significantly slower response in the MS-fatigue sufferers as compared to healthy subjects ($p = 0.023$). There were, however, no significant differences in light reflex $T_{3/4}$ response at other luminance levels and nor were there any difference between patients with and without fatigue.

When comparing the pre-investigation and pre-release difference in diastolic BP during the sustained hand-grip investigation, we found no difference in the value between patient cohorts and healthy controls. However, it was noted that there was a significantly lower number of normal responses in patients with fatigue (34.4%) as compared to patients without fatigue (66.7%) [$p = 0.028$]. In addition there was a trend for a lower number of normal responses when comparing the MS-fatigue sufferers to

146

healthy controls (58.8%) [$p = 0.10$]. We found no significant differences in other baseline autonomic investigations.

5.3.4   Fatigue correlations in MS patients

We found a significant correlation between level of fatigue as measured by the FSS and mood as measured by the BDI (rho = 0.556; $p = 0.004$). There was a trend for higher levels of disability as measured by the EDSS to correlate with a higher level of fatigue but this did not reach significance (rho = 0.363; $p = 0.068$).

We also found a significant correlation between fatigue severity measured by the FSS and level of daytime sleepiness as measured by the ESS (rho = 0.407; $p = 0.039$) (Fig 5.4). There was also a significant inverse correlation between the measure of alertness as assessed by the VAS and the FSS scores (rho = -0.419; $p = 0.033$). No other objective or subjective measures of alertness correlated with level of fatigue.

We investigated for any association between the autonomic measures and fatigue severity but no significant correlation was found.

5.3.5   Hypersomnolence correlations in MS patients

On assessing baseline level of sleepiness as measured by the ESS, we found a significant inverse correlation with duration of MS symptoms (rho = -0.443, $p = 0.023$). On the alertness investigations we found a significant correlation between ESS score and RRT (rho = 0.482; $p = 0.013$) (Fig 5.5). Not unexpectedly, there were also significant correlations with the questionnaire based measures of alertness, the SSS (rho = 0.732; $p < 0.001$) and VAS (rho = -0.639; $p < 0.001$). With regard to the autonomic measures we
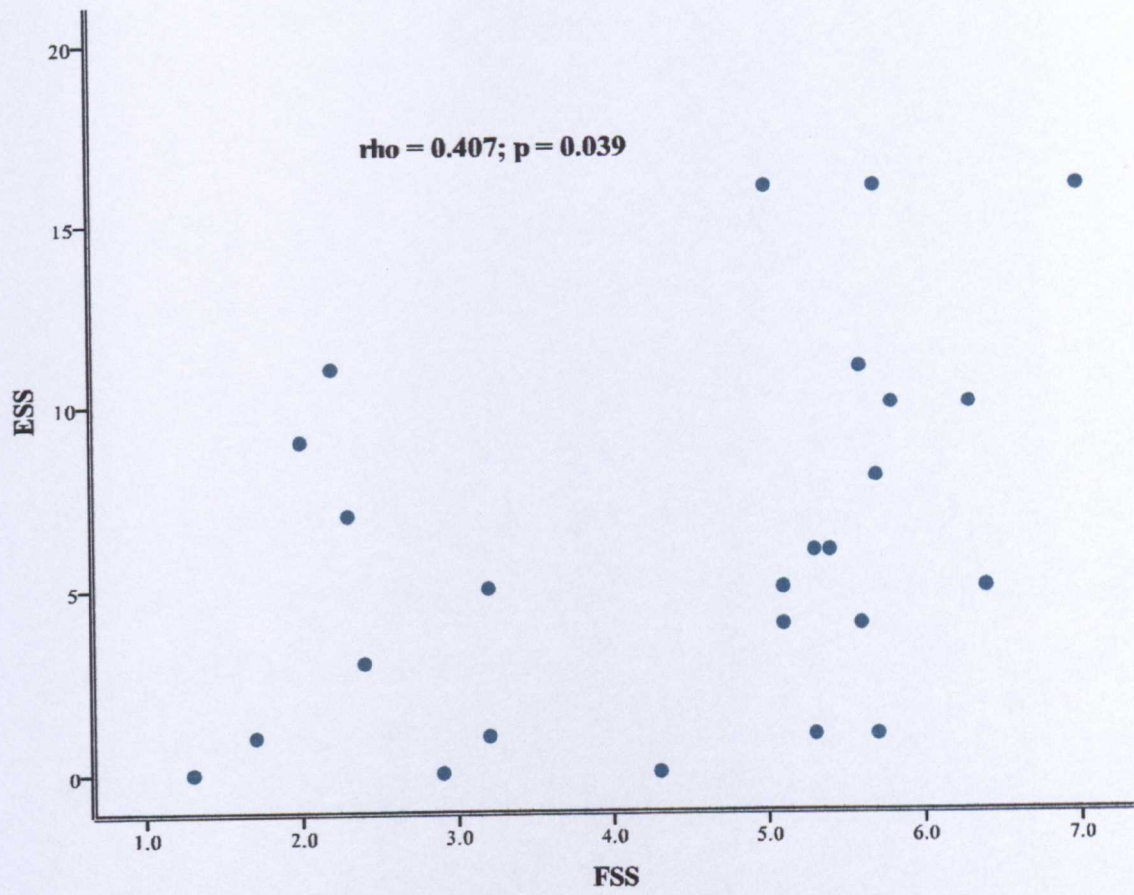
Figure 5.4   Scatterplot of fatigue severity and hypersomnolence scores on FSS and

ESS respectively

FSS = Fatigue Severity Scale; ESS = Epworth Sleepiness Scale

rho = 0.482; p = 0.013
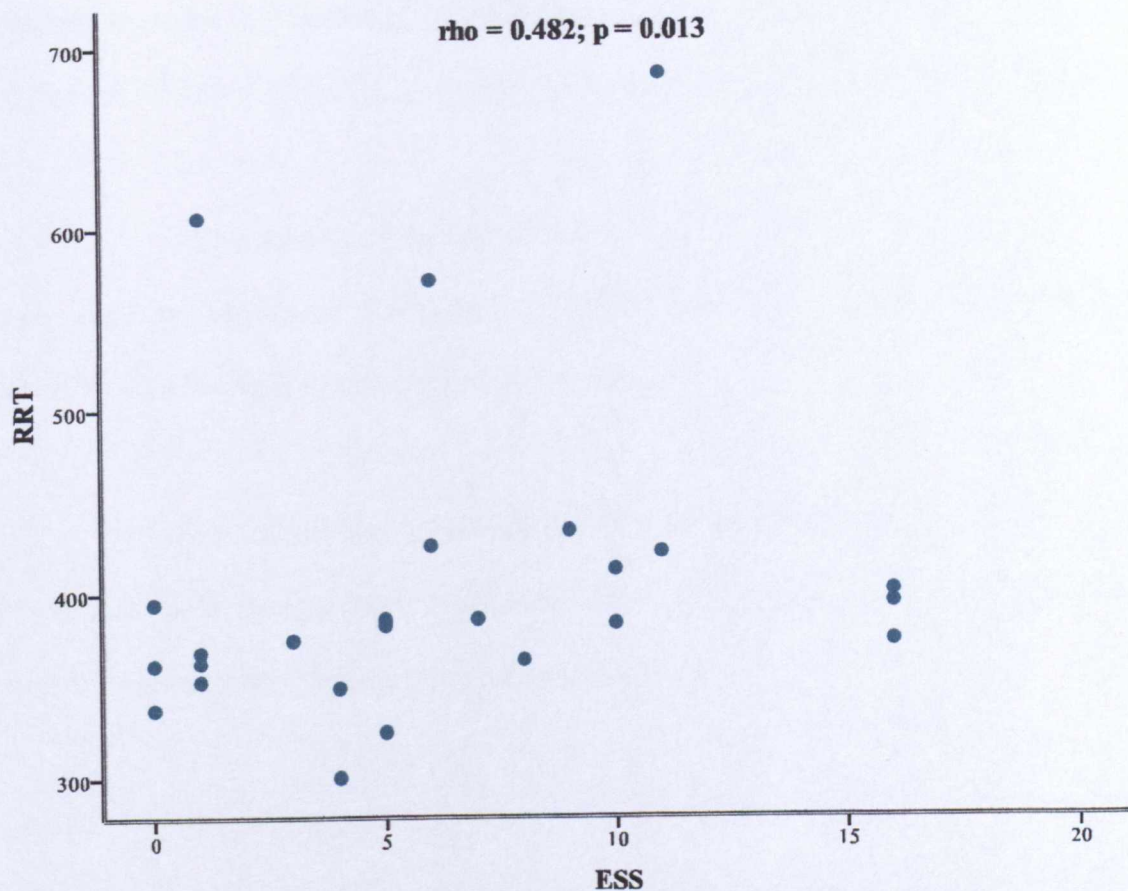
Figure 5.5   Scatterplot of the ESS scores and times of the RRT component of the

Choice Reaction Time test

ESS = Epworth Sleepiness Scale; RRT = Response Reaction Time

found significant correlations with the ESS and resting pupil diameter on static pupillometry at all four luminance levels [(darkness; rho = 0.500; p = 0.009) (6cdm^{-2}; rho = 0.424; p = 0.009) (91cdm^{-2}; rho = 0.428; p = 0.029) (360cdm^{-2}; rho = 0.479; p =0.013)].

5.3.6   PUI correlations in MS patients

There were no significant correlations of the PUI with any baseline demographic, autonomic or non-pupillographic alertness measures.

5.3.7   Response to modafinil and placebo within individual groups

Within each of the three cohorts, for both the alertness and autonomic investigations, we compared the response to placebo to that of modafinil.

5.3.7.1   Fatigue group

We found a significant reduction in two pupillographic measures of alertness; PUI (p = 0.02) and power of fluctuations (p = 0.03) after modafinil as compared to placebo (Fig 5.6 and 5.7). Figure 5.8 illustrates the significant increase in the CFFF threshold after modafinil (p = 0.006) as compared to placebo. The MRT of the CRT was also significantly quicker after modafinil in comparison to placebo (p = 0.015) (Fig 5.9). Finally in terms of alertness measures, there was a significant reduction in the pause time but not the speech time in MS-fatigue sufferers after modafinil as compared to placebo (p = 0.028) (Fig 5.10).

Within the fatigue cohort we found significant differences in the sympathetic, darkness reflex response post-modafinil as compared to post-placebo. There

150

was a larger change in the amplitude of the response (p =0.012) and a quicker initial velocity, $T_{1/4}$, (p = 0.006) post-placebo compared to post-modafinil (Fig 5.11 and 5.12). Static pupillometry was performed only post-drug treatment but we found a significant difference in terms of pupil size at 6 (p = 0.039) and 360 (p = 0.01) cdm^{-2} patients post-modafinil as compared to post-placebo but not at other luminance levels.

5.3.7.2   Non-fatigue group

There was no significant change in both pupillographic and non-pupillary measures of alertness after modafinil as compared to placebo in MS patients without fatigue. Similarly we only found a significant change in a single measure of autonomic function; resting pupil diameter at 91 cdm^{-2} (p = 0.038). There was evidence of a trend for a lower frequency of normal responses to sustained handgrip after modafinil (11.1%) compared to placebo (50.0%) [p = 0.079] but other autonomic investigations showed no significant change post-placebo as compared to post-modafinil.

5.3.7.3   Control group

Results were similar to those of the non-fatigue MS group. There were no significant changes found on alertness investigations. We again found a significant change on resting pupil diameter at 91 cdm^{-2} after modafinil as compared to placebo (p = 0.028) and also on a single measure in the light reflex response; the $T_{3/4}$ at the 41 cdm^{-2} luminance (p = 0.038).
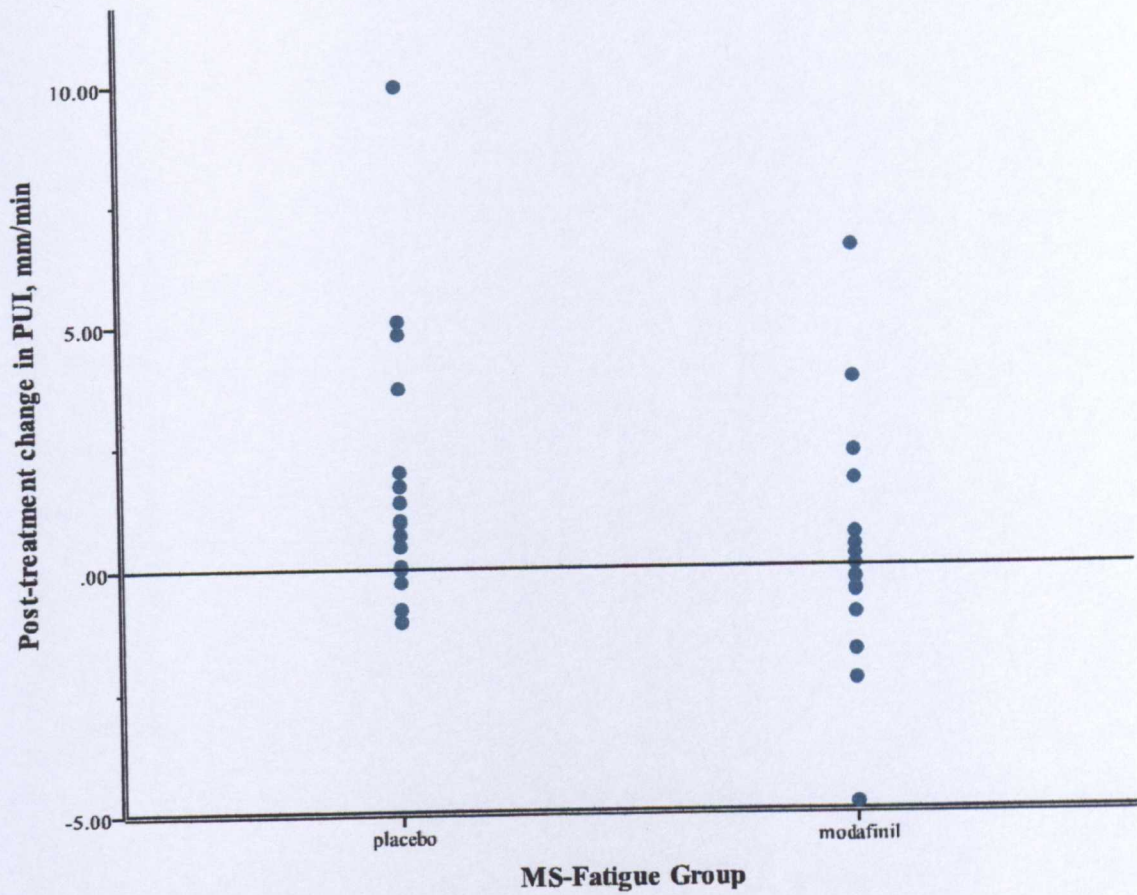
Figure 5.6   Scatterplot of change in PUI post-placebo and post-modafinil in the MS-fatigue group (p = 0.02)

PUI = Pupillary Unrest Index
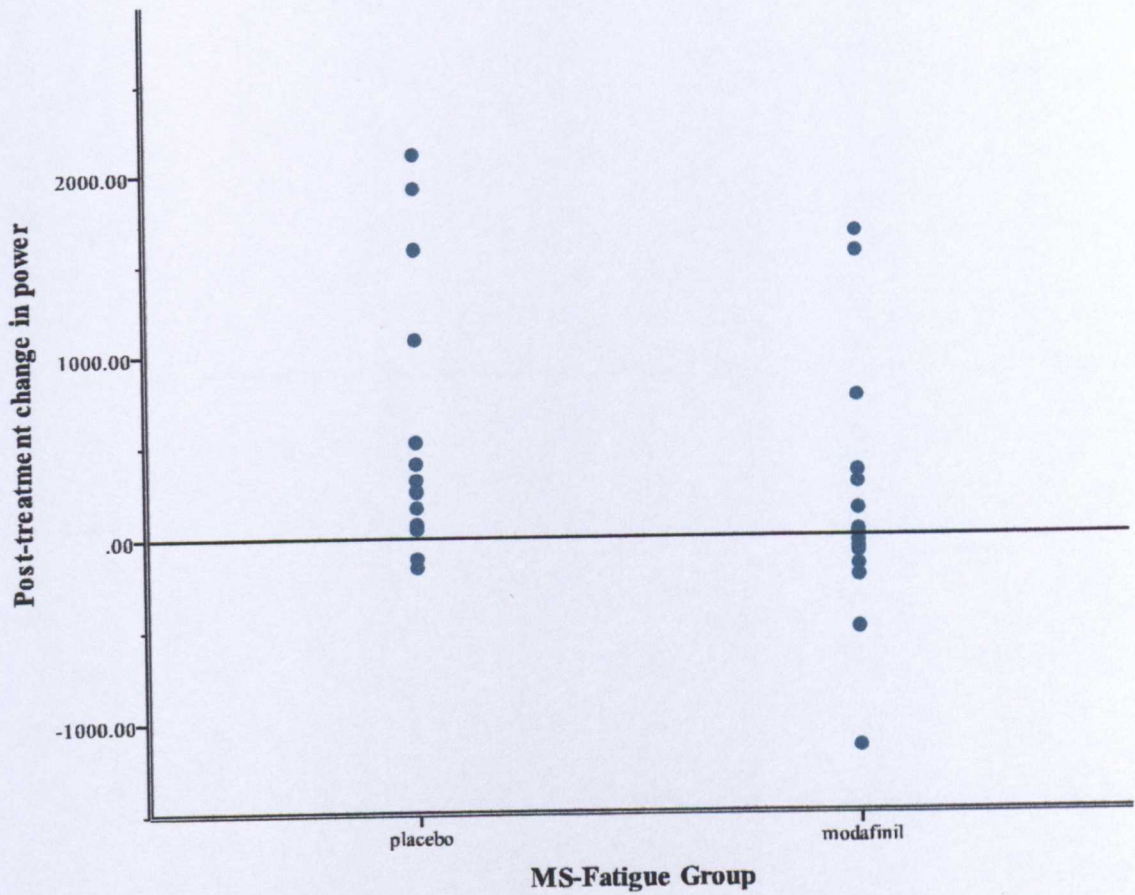
Figure 5.7   Scatterplot of change in total power of fluctuations post-modafinil and post-placebo in the MS-fatigue group ($p = 0.03$)
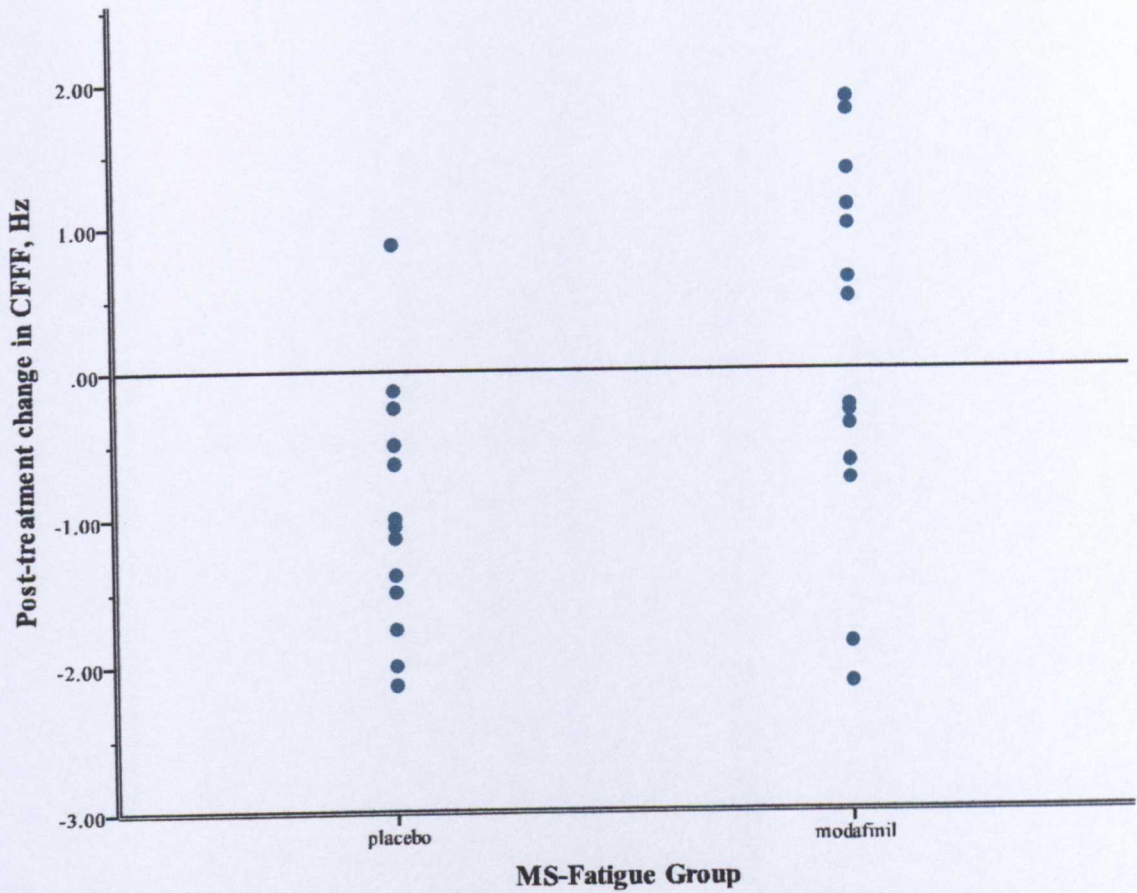
Figure 5.8   Scatterplot of change in CFFF threshold post-modafinil and post-placebo

in the MS-fatigue group ($p = 0.006$)

CFFF = Critical Flicker Fusion Frequency
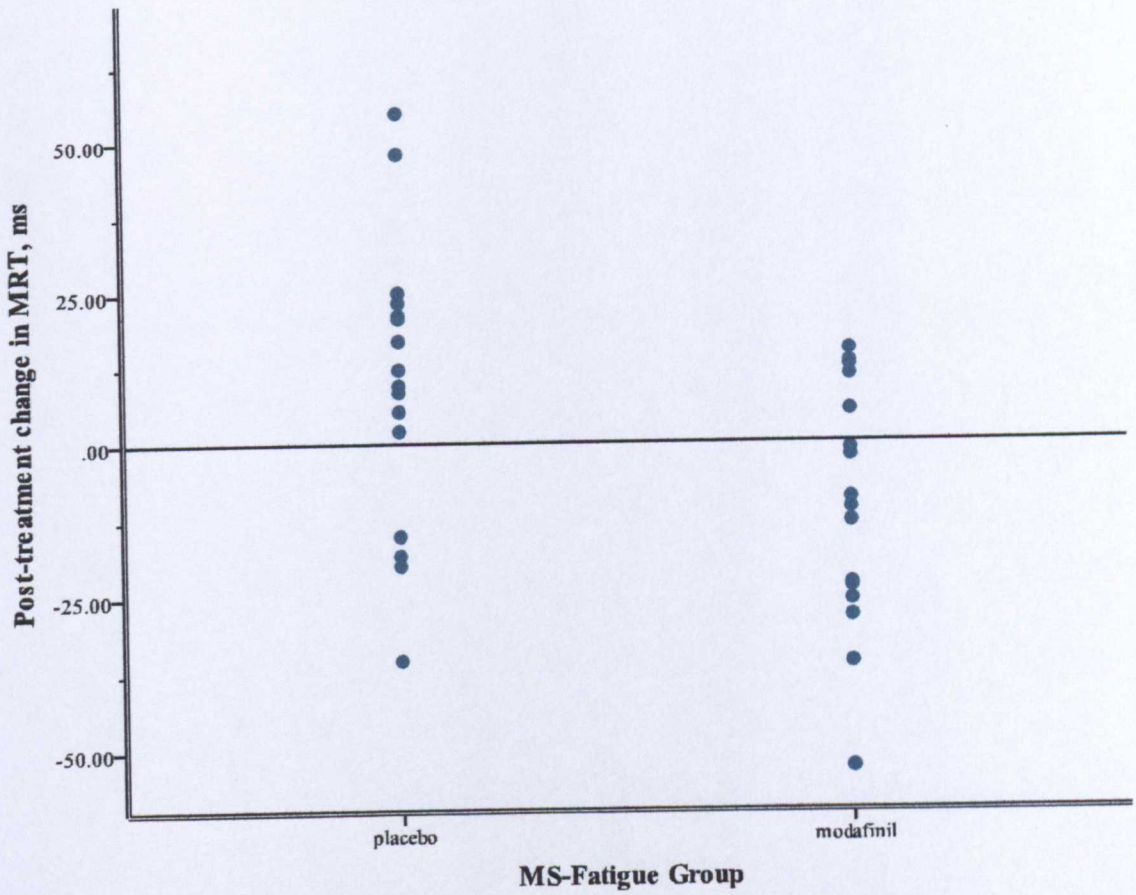
Figure 5.9 Scatterplot of change in the time of the MRT component of the Choice Reaction Time test post-modafinil and post-placebo in the MS-fatigue cohort ($p = 0.015$)

MRT = Motor Reaction Time
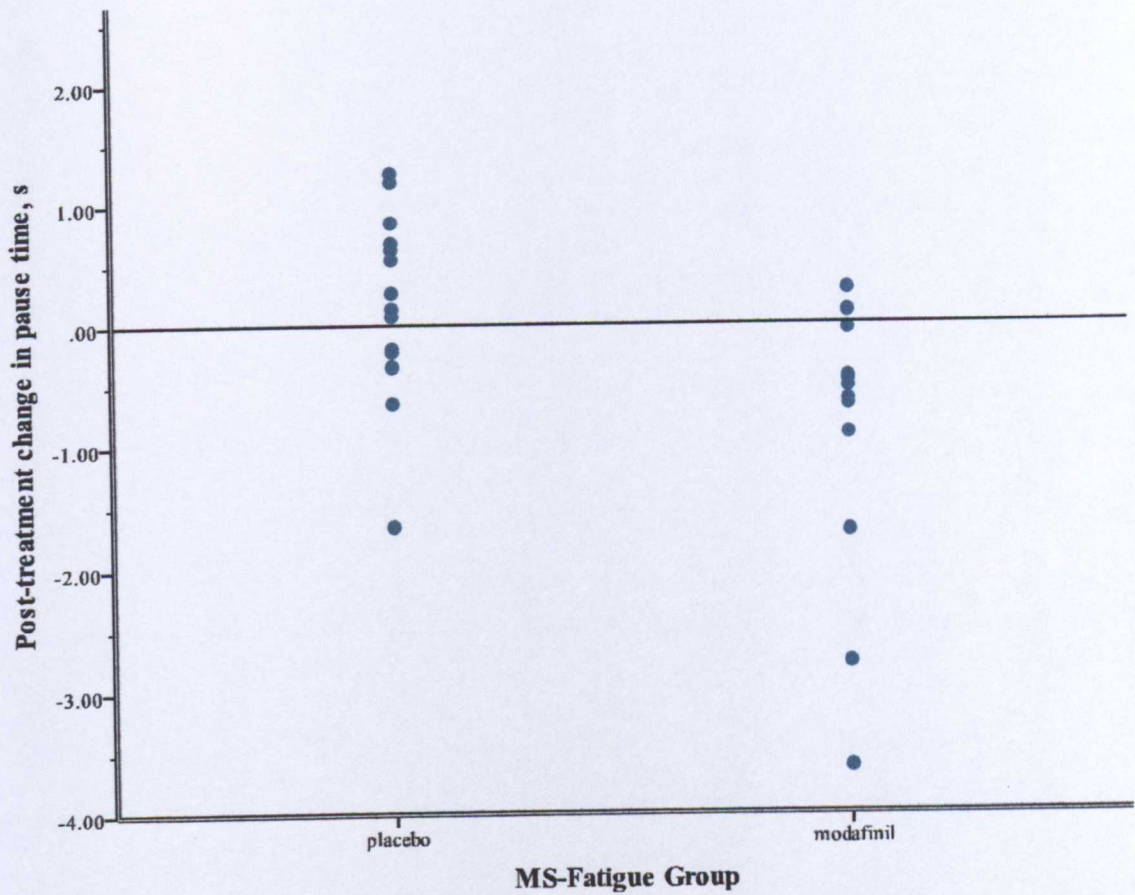
Figure 5.10   Scatterplot of change in pause time on speech analysis post-modafinil and post-placebo in the MS-fatigue cohort ($p = 0.028$)
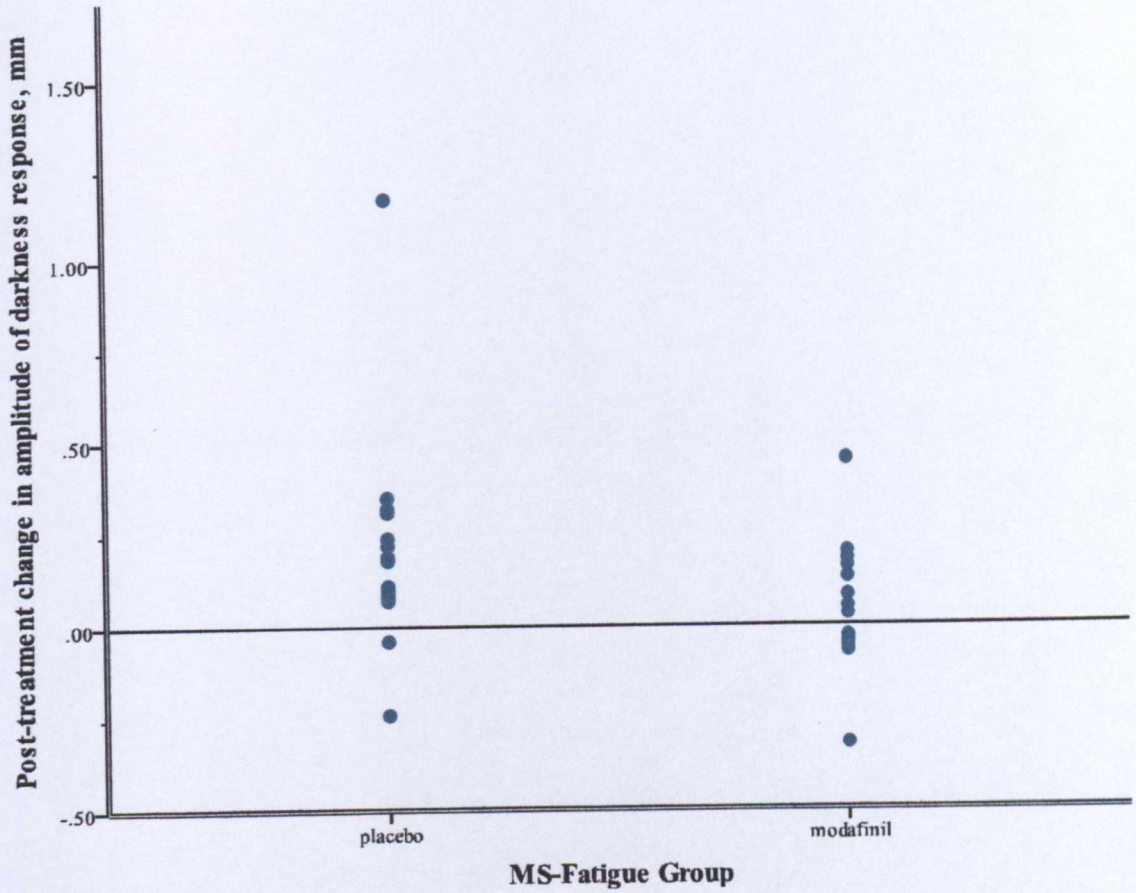
Figure 5.11 Scatterplot of the change in the amplitude of the darkness reflex response

post-modafinil and post-placebo ($p = 0.012$)
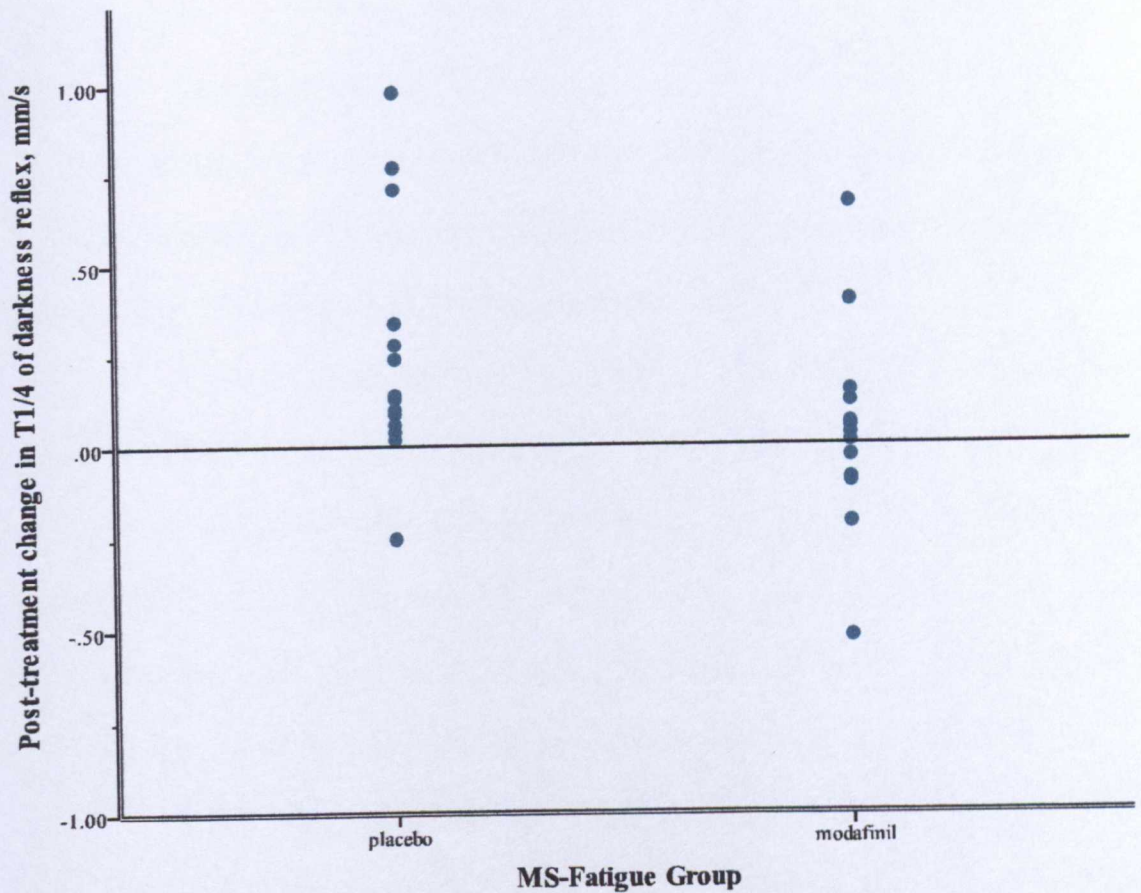
Figure 5.12   Scatterplot of change in $T_{1/4}$ of the darkness reflex response post-modafinil

and post-placebo ($p = 0.006$)

$T_{1/4}$ = time to obtain 25% of maximum dilatation from the onset of the response on

darkness reflex testing

5.4   Discussion

5.4.1   Baseline alertness

We found evidence of a significantly lower CFFF in MS patients as compared to controls. CFFF as a measure is most frequently used in psychopharmacology to investigate for the potential effects of medication on CNS vigilance/activation[119, 172].

It has also been studied as an objective tool to identify minor cognitive difficulties in patients with hepatic dysfunction. Minimal hepatic encephalopathy (MHE) is defined as, otherwise unexplained, cognitive abnormalities only detectable on psychometric or neuropsychological testing i.e. without evidence of overt encephalopathy. A study by Sharma et al of 156 patients with cirrhosis found a lower CFFF in those with MHE as compared to those without[173].

CFFF has also been employed in studies involving patients with hypersomnia. Saletu et al found a significantly lower CFFF in 17 treatment-free narcoleptics as compared to healthy controls[174]. Similarly, Schneider at al found significantly lower CFFF in small groups of 10 patients with narcolepsy and untreated OSAHS as compared to controls[175].

This investigative tool has been applied in MS studies but primarily to evaluate visual function as opposed to vigilance. In a study of 122 MS patients, 48% had an abnormally low CFFF threshold, as defined as greater than three standard deviations from the mean CFFF of a group of 28 healthy individuals[176]. This included 41% of a subgroup of 27 MS patients who denied any history of visual involvement whilst 51% of

patients with current or previous visual involvement had abnormal CFFF thresholds. In addition the frequency of abnormal CFFF results increased with increasing disability.

The question from this study arises as to whether in the MS group, especially those without a history of visual involvement, the abnormal CFFF was due to subclinical optic neuropathy or impaired CNS vigilance.

In another study of 23 MS patients the mean CFFF threshold was significantly lower than that of healthy controls with 39% felt to have an abnormally low value[177]. This however compared to 78% of the MS patients having abnormal visual evoked potentials (VEPs). This may suggest that VEPs are more sensitive at detecting optic nerve demyelination. Alternatively, the two investigations may measure either different pathologies or evaluate different systems, such as visual and cognitive. It is of note that in the same study, of seven patients with optic neuritis, five had abnormal CFFF thresholds but two of these had normal VEPs which suggests that conversely VEPs are not necessarily more sensitive at detecting demyelination.

In patients with definite MS, a study has shown a significant reduction in CFFF threshold with increasing body temperature[178]. This was the opposite response to that seen in healthy controls. Whilst, in view of this correlation with Uhthoff's phenomenon, this study may support the CFFF as a tool for assessing optic nerve dysfunction, vigilance and fatigue may also be exacerbated by heat in MS.

As mentioned in the introduction, aminopyridines are potassium channel blockers that prolong the duration of the action potential and potentiate synaptic transmission. They have been evaluated as a symptomatic treatment potentially effective against intermittent conduction block. In a placebo-controlled study involving 20 patients

with temperature-sensitive MS, the effect of 4-Aminopyridine on 'visual function', which comprised visual acuity and CFFF analysis, was evaluated[179]. Although the CFFF aspect was not reported separately for all patients, there was a significant improvement in visual function with treatment. This could be explained by improvement in vision, as there was also a significant decrease in P100 latency with treatment, but it is of note that aminopyridines have provided a subjective benefit in MS-fatigue.

In our study, there was a significantly lower CFFF threshold in MS patients as compared to controls. These results would support lower levels of vigilance and attention in MS although the contribution of visual dysfunction cannot be completely discounted. Indeed, patients with fatigue had a lower CFFF threshold than controls but this did not reach significance. The potential impact of fatigue on CNS vigilance as measured by the CFFF could be reassessed by a further study with a larger cohort of patients.

Our study also found a significantly prolonged MRT in MS patients with fatigue as compared to controls. There was no difference however between patients with and without fatigue nor was there any difference between non-fatigued patients and controls. The MRT is the time from the subject's finger being lifted off its original position to when it reaches the button associated with the light that has come on. This time, as its name suggests, is primarily a measure of motor speed rather than information processing. It would be influenced by disability affecting the upper limb, for example weakness or ataxia, as well as visual function. It is of note that the EDSS was not significantly different between the MS groups however individual motor and cerebellar Functional System scores, which may be more relevant, were not evaluated.

A number of studies by different groups have been undertaken to evaluate choice reaction time in MS patients primarily to assess information processing speed. A wide variety of methodologies and equipment have been used thus making comparisons difficult. An example of one such study was that undertaken by Reicker at al which employed simple, choice and semantic Reaction Time (RT) tests in a cohort of 60 MS patients and 60 healthy individuals[180]. The simple RT test involved pressing the space bar when the letter 'X' appeared on a screen. The choice RT test involved two different stimuli (the words 'duck' and 'kite') with the subject having to press one of two buttons depending on which word was presented. Finally, the semantic RT test required patients to allocate a variety of different words presented individually into a number of different categories represented by different buttons. Overall, MS patients had prolonged RTs compared to controls with increased divergence of RTs with increasing complexity of the task. Whilst RTs were not separated into their cognitive and motor components and it was felt that motor dysfunction could account for some of the difference between cohorts, there was also evidence of cognitive dysfunction particularly by the increasing difference in groups with complexity. This study supports the idea that CRT can be employed to assess information processing in MS but again direct comparison between our study and previous studies remains difficult.

5.4.2   Baseline autonomic

Autonomic dysfunction is common in MS with one study showing 90% of patients complaining of at least one autonomic symptom with, as expected, the most common symptoms relating to sphincter control[181]. Cardiovascular symptoms are less common,

with between 24 – 54% complaining of orthostatic dizziness[181, 182]. Our study found some evidence of cardiovascular autonomic dysfunction with an abnormally low number of normal responses to sustained hand-grip in MS-fatigue patients. This supports a number of previous studies which have also found some evidence of autonomic dysfunction on cardiovascular reflex function tests. Typically tests include heart rate response to deep breathing, valsalva and standing as well as BP response to standing and isometric hand-grip.

Gunal et al reported that in a cohort of 22 RRMS patients, 10 patients (45%) had at least one abnormal cardiovascular test compared to no abnormal tests in the control group[181]. Three patients (14%) were classified as having autonomic dysfunction on the basis of two or more abnormal tests. The most common abnormality was found on heart rate response to breathing. In this study however, only a single patient had an abnormal response to sustained hand-grip. In a similar study, four MS patients (25%) from a cohort of 16, had abnormal cardiovascular reflexes although only one patient (6%) had autonomic dysfunction.

A further study utilizing the same cardiovascular investigations was performed on 40 MS patients and found similar results with four patients (10%) classified as having autonomic dysfunction[182]. The most frequently abnormal test in this study, however, was isometric hand-grip with a normal response occurring in only half the patients. This study also reported a correlation between the presence of autonomic dysfunction and MRI lesions in the brainstem.

Sterman and colleagues employed a slightly different battery of cardiovascular reflex tests, including heart rate and BP response to cold but not hand-

grip, and detected autonomic dysfunction in 9 of 22 patients (41%)[183]. Again the most frequently abnormal test was that of heart rate response to deep breathing. In addition they found a correlation with an increasing number of abnormal autonomic tests and sphincter disturbance on the Kurtzke Functional Score.

A study performed by Pepin and colleagues assessed isometric hand-grip in 104 MS patients[184]. Eighteen patients (17%) failed to achieve a normal response compared to none of the normal controls (N = 25). In our study, we found a much higher number of patients who failed to achieve a normal response compared to this earlier study. There are a number of methodological differences between the studies. In our study, the exercise was performed at 20% as opposed to 30% of MVC. In addition, the duration of the exercise was capped at 11 minutes if the subject had not fatigued prior to that. In the Pepin study the mean time to fatigue was approximately four and a half minutes. They also employed equipment that performed beat-by-beat BP monitoring whilst we performed serial electronic measurements including one just prior to release.

If patients failed to reach fatigue by 11 minutes in view of the lower work rate required, then this potentially could lead to false positive results, however, the majority of MS patients reached fatigue prior to this cut-off. Similarly, the fact that the controls more commonly achieved a normal response with the same protocol suggests that the work rate was adequate to induce a pressor response. Both studies may be subject to patients perceiving fatigue earlier and therefore shortening the duration of the test. However, this argument is counterbalanced by results from the Pepin study which identified a lower BP rise in MS patients, compared to controls, at all stages of the test and not just at the fatigue point.

This study found a significantly lower number of normal BP responses to isometric hand-grip in patients with fatigue as compared to those without. This does raise the possibility of autonomic dysfunction contributing to MS-fatigue. We do note that there was little evidence of autonomic disturbance with pupillary autonomic testing, in MS as compared to healthy controls, and no significant differences in these pupillary measures between patients with and without fatigue. Two previous studies have investigated the potential relationship between autonomic function and fatigue in MS and have provided conflicting results.

Flachenecker et al investigated 60 MS patients and 30 controls with cardiovascular reflex testing and fatigue questionnaires[48]. They found a significantly lower heart rate response to standing in patients with fatigue as compared to those without as well as healthy controls. Both the orthostatic heart rate response and the isometric handgrip results correlated significantly with fatigue scores. It was felt that this study provided evidence for a relationship between sympathetic dysfunction and fatigue.

In a study involving 84 MS patients, Merkelbach et al evaluated the results of cardiovascular reflex tests and a number of questionnaire-based fatigue scores[46]. They found that 27% of patients had evidence of autonomic dysfunction, with the isometric hand-grip test most frequently abnormal. Patients with SP- or PPMS were more frequently affected than patients with RRMS. There were no significant correlations between autonomic tests and any of three fatigue scores. Weak correlations were only noted when patients were divided according to type of MS with different fatigue scores correlating with different autonomic tests in SPMS. However, incongruent results were also found including less impairment of orthostatic BP response in RRMS patients with

fatigue. Overall it was felt from this study that the relationship between fatigue and autonomic dysfunction appears weak.

The results of our study do add some support to the suggestion that cardiovascular autonomic dysfunction may contribute to MS-fatigue. We do acknowledge that our study involved a small number of patients compared to the two reports described above and only a single cardiovascular reflex test as compared to a number of other studies described earlier. The complaint of fatigue is likely to have a multifactorial basis in a heterogenous population of MS sufferers and as such autonomic dysfunction may well play a significant role in a number of individual patients although it is difficult to conclude as to whether it is a frequent significant contributor to fatigue in the MS population as a whole.

5.4.3 Fatigue and hypersomnolence correlations

We found no correlation between subjective measures of fatigue and sleepiness and our primary objective measure of alertness, pupillography. We did find a significant correlation between EDS severity as assessed by the ESS and the RRT component of the CRT. As discussed earlier, various tools assessing CRTs have been employed in MS and these have provided some evidence of dysfunction of information processing. We found no significant baseline difference in RRT between patients with and without fatigue nor any association between fatigue severity and RRT. Although our findings suggest that patients with higher levels of EDS have slower information processing, it is important to recognise that there still remains a motor component to the RRT score. The RRT comprises a motor aspect with the patient having to lift their finger from a button as well

as the cognitive aspect. Our study found no correlation between autonomic measures and fatigue, sleepiness or pupillography.

5.4.4 Response to modafinil

In this study, to the best of our knowledge, we are the first to report the effect of modafinil on MS-fatigue using objective measures of alertness and vigilance, as well as the subjective questionnaire-based measures which are commonly used in such studies. The main methodological flaw is the small number of subjects in each cohort however as a pilot study it provides a basis for a further larger study to determine further the role of modafinil. We have however demonstrated that in patients with fatigue there is a response to modafinil in terms of pupillographic measures of alertness, CFFF threshold, CRT measures and the pause time of automatic speech.

As discussed in chapter 1, only a small number of studies have previously reported the use of the PST in MS patients. A number of methodological differences apply between our investigation and previous studies. Firstly, in the study by Egg and colleagues, a larger number of patients were recruited but the group was more heterogenous with a significant number of patients with a single attack, undergoing an acute relapse or on treatment with interferon-beta[78]. Only a single measure from the PST investigation, the PUI, was determined. Although the FSS was used, patients were not separated into those with and without fatigue and simply correlation analysis was performed. In addition, they were primarily utilizing pupillography as a measure of autonomic imbalance rather than of alertness. Their study reported no significant difference in terms of PUI between patients and controls. The baseline results of our

study support this finding. They did however find an unexpected, significant inverse correlation between PUI and fatigue severity in the MS group. This would suggest a rather contradictory, higher degree of fatigue according to the FSS was associated with a higher degree of alertness as determined by the lower PUI. Their conclusion was that autonomic instability was not associated with MS fatigue severity.

A further report by the same group, also used a similar larger but more heterogenous MS cohort[113]. Hypersomnolence was evaluated but not fatigue. Medication that could potentially contribute to EDS were only excluded on the day of the investigation. They found no difference in PUI between patients and controls and no correlation between PUI and self-reported EDS. Again the baseline results of our study support their findings.

Although there was no correlation between any PST measure and fatigue or sleepiness severity, we have found a significant reduction in the PUI and the power of fluctuations with modafinil, as compared to placebo, in patients with fatigue. This indicates that patients with MS related fatigue show an increased alertness with modafinil. This response was not seen within healthy subjects or MS patients without fatigue.

Speech time, whilst infrequently employed as a tool in studies, has been shown, predominantly in neuro-psychiatric disorders, to be a measure of psychomotor retardation. This is to the best of our knowledge the first study to employ speech time analysis in MS.

Hoffman et al noted that 12 patients with a unipolar affective disorder had a significantly prolonged pause time as compared to controls and this correlated with a

clinically-evaluated scale of psychomotor retardation[167]. In a 1976 report, Szabadi et al described four patients with moderate severity depression in whom they assessed automatic speech[168]. Phonation time remained constant but was variable between individuals. Pause time, however, significantly decreased with recovery from depression. There was no change in pause time in four healthy subjects and therefore the improvement could not be explained simply by practice effect.

A study involving 13 schizophrenic patients undergoing an acute exacerbation detected a longer mean pause time in a rote- (automatic) speech task as compared to controls[185]. They did however feel that tasks with higher cognitive and linguistic demands (speech analysis of a description of a cartoon) would more readily detect differences between controls and schizophrenic patients. However this, so-called, free-speech task would not lend itself as well to repetitive assessment such as that required for investigating a treatment effect and explains our use of automatic speech in measuring a response to treatment in this study.

It is noted that BDI scores were higher in patients with fatigue than those without or healthy controls. Therefore, it might be suggested that the response to modafinil in this cohort is due to the effect on psychomotor retardation which might occur in patients with depression. There have been limited studies into the potential use of modafinil either as primary or adjunctive treatment in depression and, to-date, the evidence does not support the use of modafinil in the treatment of depression[186]. In addition, although there was a significant difference in BDI scores between the cohorts, neither the frequency nor severity of depression were great in the fatigue cohort.

It was noted that there was a significant improvement with modafinil in the MRT component of the CRT in patients with fatigue. There was no significant improvement in patients without fatigue and healthy controls. Although the RRT was not significantly different compared to placebo, it is possible that this result is also indicative of an improvement in psychomotor function.

CFFF as a tool has been used in healthy controls to compare psychoactive drugs and has been shown to have the potential to detect the effect of treatment. Hou et al demonstrated, with a reduction in CFFF threshold, the sedative effect of the anti-histamine diphenhydramine, as compared to placebo, in a cohort of healthy subjects[172].

As discussed earlier, we found evidence of a significantly lower CFFF in MS patients as compared to control and this supports the findings of a number of previous trials employing CFFF in MS. The question is raised as to whether this provides evidence, as suggested by previous studies, of visual impairment or whether it is indicative of impaired vigilance. It is of note that we found that a significantly higher CFFF threshold post-modafinil as compared to placebo in patients with fatigue. It is not readily conceivable how modafinil might affect visual function. In addition we found no significant difference in MS patients without fatigue and it would have been likely that if the higher threshold was due to improved visual function, then this result would have been reproduced in this cohort. We feel this finding supports the concept that CFFF is at least in part a measure of vigilance. We found no significant difference in CFFF with modafinil as compared to placebo in healthy individuals. This supports the previous findings of two separate studies in which modafinil was shown to have no significant effects on CFFF threshold in healthy subjects[119, 172].

In terms of autonomic measures, we did find significant changes in the darkness reflex responses in terms of the amplitude and the initial speed of response (both indicative of altered sympathetic activity) with modafinil, as compared to placebo, in the MS-fatigue group. There has previously been rather mixed results with modafinil in regards to the darkness reflex in earlier studies involving healthy individuals. One study showed no effect whilst an earlier study showed an increase in initial velocity with modafinil but no change in amplitude[119, 172]. Although we only found limited evidence of autonomic disturbance both at baseline and with treatment it is conceivable that modafinil could affect autonomic measures. As discussed previously, modafinil has been shown to activate a number of cell groups which are involved in an arousal network[118]. This network includes noradrenergic neurons which project from the LC to the cerebral cortex as well as influencing other nuclei such as the TMN. The question, however, of whether modafinil has a direct or indirect effect on sympathetic activity is as yet unanswered.

Overall, our study provides evidence, with a number of objective measures, of a benefit with modafinil in terms of alertness and vigilance in patients with MS-fatigue. Again as discussed in the introduction, a number of studies have evaluated the potential use of modafinil in MS-fatigue but with varied results. Notably, the two earliest reported studies both had methodological flaws. The first by Zifko et al found a significant improvement in both ESS and FSS after three months treatment however this was an open-label, non-placebo-controlled study[131]. A study by Rammohan at al found a similar improvement in ESS and FSS as well as the MFIS after two weeks treatment with modafinil 200mg[132]. It is of note, however, that this was a single-blind study

171

albeit with a crossover, placebo arm. The response did not persist with an increased dose of modafinil 400mg possibly suggesting a placebo effect that waned with time. In addition, ESS was not measured after placebo but rather post-modafinil scores were compared to a baseline measurement.

Stankoff et al reported in 2005 a large double-blind, placebo-controlled study in which they found no significant difference between treatments after five weeks in either MFIS or ESS[133]. They did however comment on unpublished post-hoc analysis which suggested a differential effect modafinil may have on the physical aspect of the MFIS score in patients with, as compared to those without, excessive daytime sleepiness as determined by the ESS.

We also note the lack of response to modafinil in the non-fatigued and control cohorts on PST, CFFF and speech measures. This supports previous studies which have shown a limited effect of modafinil on cognitive tests as well as fatigue and sleepiness questionnaires in healthy subjects[187]. It is noted however that there are mixed results on previous studies assessing the response of PST measures to modafinil in healthy individuals. One study demonstrated no significant difference in PUI with modafinil as compared to placebo although in a second study by the same group a significant reduction in PUI was found[119, 172]. Overall, however, we feel that this lack of response in other cohorts, supports the concept, suggested by Stankoff et al in their post-hoc analysis, that the response to modafinil is not based on a class effect but rather there is a differential effect. In their study it was determined both by type of MS and ESS score or rather the presence of hypersomnolence, whilst in our study it was the presence of fatigue.

A question that arises from these results is to what these neurophysiological and laboratory tools are specifically measuring and, although objective, whether these provide any quantitative assessment of MS fatigue. MS fatigue is almost certainly multifactorial within groups of patients and may even be so in individual patients. It could be argued that questionnaire based assessment and detection of MS-fatigue is a non-specific measure and does not distinguish between aetiology of fatigue. In addition, rater bias and ceiling effects contribute to the problems of fatigue questionnaires.

It is likely that in some MS patients with fatigue, that one or more of alertness, sleepiness, CNS vigilance and psychomotor retardation are contributing to the symptom of fatigue. For example, from previous studies it is evident that some MS patients will perceive that a liability to drop off to sleep in the day is a component of fatigue and vice versa[114]. Therefore, we feel it is likely that the finding of a benefit with modafinil in measures of these symptoms is relevant to some patients and may indicate a role for modafinil in some patients with MS-fatigue. Isolating those patients in clinical practice, in whom the benefit is most likely to occur, is the difficult factor.

To conclude, this study demonstrates the first objective, laboratory and neurophysiological evidence of improvement in measures of alertness, vigilance and psychomotor retardation with modafinil in patients with MS-fatigue. It supports a role for modafinil in the treatment of MS-fatigue although further studies may be important to identify if certain sub-groups of fatigue sufferers would respond best to treatment.

CHAPTER 6

Chapter 6. SUMMARY AND CONCLUSIONS

6.1   Summary

This thesis began by presenting a brief general overview of MS. Although traditionally considered a white matter CNS disease, the evidence of involvement of grey matter and in particular deep grey matter structures in MS was described. Fatigue, a frequent and major symptom in MS, was then discussed in detail. A number of potential mechanisms have been proposed as to the pathophysiology of MS-fatigue and several of these processes imply a key role for deep grey matter structures. Disruption of cortico-subcortical circuits that involve cortical grey matter and the basal ganglia and thalamus has been suggested by studies involving advanced imaging techniques[17, 58]. Autonomic dysfunction has been demonstrated, including with cardiovascular reflex function tests, to be present in MS patients and may contribute to MS-fatigue[48]. This suggests a role for the hypothalamus, from which descending autonomic neurons originate. Neuroendocrine disturbance has also been suggested as contributing to MS-fatigue. This further implicates the hypothalamus through its neuroendocrine function with neurons projecting towards and stimulating secretion of hormones from the pituitary.

The introduction also discussed a component of the arousal network, the hypothalamic hypocretin-containing neurons. These have been demonstrated to be reduced in number, potentially through an autoimmune mechanism, in narcolepsy[92]. Dysfunction or deficiency of the hypothalamic hypocretin pathway could also contribute to MS-fatigue. Whether a disorder of a pathway which primarily leads to EDS may also contribute to fatigue is somewhat contentious. It is, however, reasonable to consider that impaired alertness in subjects may contribute to a sense of fatigue. There is evidence of

an association between self-reported fatigue and hypersomnolence in a variety of conditions including MS[114].

The work presented in this thesis explored the role of the deep-grey matter in MS-fatigue. Conventional MRI techniques, whilst essential for the diagnosis of MS, have been relatively less effective at providing a correlation with symptoms and disability. In addition, conventional MRI is limited in its ability to demonstrate lesions and abnormalities in the grey matter of MS patients. It was illustrated in the introductory chapter the value of non-conventional MRI techniques, including quantitative MRI modalities, both in terms of their closer association with clinical features and their application to small structures.

In the first study we applied a quantitative MRI technique, T1 relaxation time measurement, to three deep grey matter structures in a cohort of RRMS patients. Using the FSS questionnaire, patients were evaluated in terms of their fatigue. Significantly higher median T1 values were demonstrated in the thalamus and putamen, as compared to healthy controls. This has not been demonstrated on previous T1 relaxation time studies of RRMS patients. In addition, we found significantly higher median T1 values of the thalamus in patients with fatigue as compared to those without. A significant correlation between the T1 value of the thalamus and fatigue severity was also shown. The abnormal T1 values of these deep grey matter structures, although indicative of subtle pathological abnormalities, and potentially a consequence of diseases processes, such as Wallerian degeneration, were not associated with the most overt MRI evidence of disease burden, the T2-lesion load. It was noted that T2 lesion volume, in support of previous studies, was not associated with fatigue severity. This study

176

supported the value of quantitative MRI techniques in providing a more comprehensive picture of disease burden. In addition, it endorsed the assertion of previous studies that non-conventional MRI modalities may provide a better correlation with symptomatology. Similarly it provided supportive evidence for a role of the thalamus in MS-fatigue possibly through disruption of cortico-subcortical circuits.

The second study to be presented in this thesis expanded on this finding by applying T1 mapping to another deep grey matter structure, the hypothalamus, in a cohort of RRMS. The hypothalamus, although involved in MS, has not been previously assessed by quantitative MRI techniques. The same method of T1 relaxation time measurement was applied whereby the largest volume of the structure was included whilst avoiding contamination from neighbouring white matter or CSF. With this approach, the study demonstrated a significantly higher T1 value in MS patients as compared to healthy controls. This finding endorsed the conclusions derived from the first study and suggested that a diffuse process may be affecting these interconnected deep grey matter structures. To this extent, and in contrast to the first study, T2-lesion volume was associated with the hypothalamic T1 value. Also in this study, and in light of the potential involvement of the hypothalamus in a number of fatigue mechanisms, fatigue severity was measured in the cohort. No significant association with FSS scores was shown.

Having provided evidence for the presence of subtle abnormalities within the hypothalamus in MS, its potential relationship to fatigue was further explored in the third and fourth studies presented in this thesis. In the third study, although we had previously found no evidence of an association between an MRI-derived measure of the hypothalamus and fatigue, we employed a laboratory technique to further assess the

hypothalamus and more specifically the hypocretin pathway. As detailed in the introduction, the measurement of CSF hypocretin-1 levels has been clearly shown to demonstrate involvement of the hypocretin system in conditions such as narcolepsy[96]. In addition, levels have been shown to be associated with the severity of hypersomnolence[97]. Low CSF hypocretin-1 levels have been demonstrated in a number of other conditions including cases of secondary hypersomnia and inflammatory disorders[110, 111].

In this third study, as well as CSF hypocretin-1 levels, we prospectively measured both fatigue severity and propensity to EDS with questionnaires in cohorts of patients with MS, inflammatory and non-inflammatory neurological disorders. Although CSF hypocretin-1 levels have been previously measured in cohorts of MS patients, its potential relationship to clinical features has not been studied. The addition of a measurement of hypersomnolence in this study was important to allow patients to be differentiated according to EDS as well as fatigue although it was noted that FSS scores correlated significantly with ESS scores in all cohorts. The study demonstrated significantly lower CSF hypocretin-1 levels in patients with inflammatory diseases other than MS as compared to the non-inflammatory cohort. There was, however, no significant difference between the MS group and the other patient cohorts. In addition, we found no association between fatigue or EDS severity and hypocretin-1 levels and no difference in hypocretin-1 levels between patients with and without fatigue or hypersomnolence. This suggested that hypocretin dysfunction or deficiency secondary to grey matter involvement in MS is not a significant or frequent factor role in MS-fatigue.

A further potential hypothalamic role in MS-fatigue, that of autonomic dysfunction, was addressed within an arm of the fourth and final study presented in this thesis. Baseline investigations included a number of autonomic investigations, principally pupillary reflex studies but also a single sympathetic cardiovascular reflex function test. Although there were no significant differences in the pupillary measures, there were a significantly lower number of normal BP responses to isometric hand-grip in patients with fatigue as compared to those without. This does raise the possibility of autonomic dysfunction contributing to MS-fatigue.

The primary aim of the final study again derived from the results of the first and second studies, that of pathology within deep grey matter structures in MS. It is generally agreed that multidisciplinary approaches to MS-fatigue management are most appropriate and also that convincing evidence for specific pharmacological agents does not exist. Studies in MS-fatigue are complicated by the reliance on subjective measures of fatigue and certainly these tools have, so far, provided conflicting evidence in limited studies of modafinil. Although its mechanism is yet to be clarified, evidence supports an interaction with the arousal network involving a number of deep grey matter and brainstem nuclei. In this study neurophysiological and laboratory measures of alertness, principally pupillography as well as non-pupillary measures of vigilance, were utilised to explore more objectively the role of modafinil. It was demonstrated that in MS patients with fatigue, there was a significant improvement with modafinil, as compared to placebo, in a number of these measures including the PUI, power of pupil fluctuations, CFFF threshold and speech-pause time. Furthermore, this was not simply a class effect

but rather specific to fatigue sufferers. This supports a role for modafinil in MS-fatigue management potentially through its effect on the arousal network.

6.2   Conclusions

The principal aim of this thesis was to investigate the potential role of deep grey matter structures in MS-fatigue. It was a broad aim and for that it may be open to criticism. Similarly, the use of a number of investigational tools within the thesis may also provoke comment. However, fatigue is a rather heterogenous, poorly-described symptom in clinical practice and, by common consensus, has a multifactorial basis both between and often also within MS patients. It therefore represents a challenging area to study for the MS research community and a heterogenous approach may be considered a strength and necessity.

This thesis also represents a body of work which evolved as the studies were completed. Initially quantitative MRI-based studies were employed and, in light of its ability to detect subtle abnormalities, were expanded to other deep grey matter areas. Subsequent to abnormalities being determined in the hypothalamus, alternative laboratory tools were utilised to explore additional pathophysiological mechanisms. Finally, considering the expanding knowledge base of its mode of action and its current favour, it was appropriate to investigate the role of modafinil in fatigue. However, rather than repeat subjective questionnaire-based studies, a study incorporating objective measures was devised to attempt to reduce the well established criticisms of fatigue studies.

In the first two studies, through applying a method of T1 relaxation time measurement that had been shown in previous studies to be effective in demonstrating

pathology in pyramidal tracts and the cervical spinal cord, we revealed abnormalities in three deep grey structures, the thalamus, putamen and hypothalamus[139, 140]. This supported the use of this technique in expanding the knowledge of disease burden in MS as abnormal T1 relaxation time measurements in the deep grey matter had not been previously demonstrated in RRMS. In addition, non-conventional MRI techniques had never previously been used to investigate the hypothalamus in MS. The presence of abnormalities in these structures that are well interconnected raises the possibility of a common diffuse process accounting for this pathology. Wallerian degeneration, thought to account for pathology in NAWM, would be most readily considered although only limited evidence existed in these studies for this theory.

In chapter 2, it was also shown that the T1 value of the thalamus correlated significantly with fatigue severity with higher thalamic T1 values occurring in patient with fatigue as compared to those without. The conventional measure of disease burden, T2-lesion volume, was not associated with fatigue and nor were the T1 relaxation times of other deep grey matter structures. This would support the concept raised by previous studies in MS that suggest the spatial localization of pathology is more important than the overall disease burden in accounting for clinical features. Similarly, it also supports the theory that non-conventional MRI modalities may be more relevant in terms of addressing the clinical-radiological paradox.

In chapter 3, we reported no association between the pathology detected in the hypothalamus and fatigue severity. However, in light of the number of mechanisms that exist by which the hypothalamus could affect fatigue, further studies specifically to address individual mechanism were performed. In the study detailed in chapter 4, we

found no relationship between CSF hypocretin-1 levels and fatigue or hypersomnolence in MS. This was notably a prospective study but with a number of methodological flaws. Patients were neither age- nor sex-matched and potential conditions or treatments which could contribute to secondary fatigue were not addressed. In addition, there was a degree of selection bias as to the patients enrolled who were undergoing an LP for management of their condition rather than primarily as part of the study.

In the study detailed in chapter 5 we demonstrated a significantly lower number of normal responses on a sympathetic cardiovascular reflex test in patients with fatigue as compared to those without. It is possible that the abnormalities in the hypothalamus detected in chapter 3 may account for this autonomic disturbance.

In the final study we demonstrated a significant response to modafinil, as compared to placebo, in MS fatigue sufferers in two measures of alertness on pupillography. We also detected significant improvement in other, non-pupillary, measures of alertness including; CFFF threshold, a measure of CNS vigilance and the pause time on speech analysis, a sensitive measure of psychomotor retardation. These responses were not a class effect in this study and these measures were not significantly different at baseline in this cohort as compared to patients without fatigue or healthy controls. This study therefore provides support for the use of modafinil in MS-fatigue.

Overall a number of conclusions may be drawn from this thesis. Firstly, deep grey matter structures, whilst frequently appearing normal on conventional MRI, are involved in MS. Furthermore, this pathology may contribute to MS-fatigue possibly through abnormalities within nuclei or their connections that are involved in cortico-subcortical circuits. It appears likely that non-conventional MRI techniques are required

to both demonstrate pathology and highlight the specific localisation of pathology relevant to clinical features.

There is also evidence of abnormal sympathetic autonomic tests in MS patients with fatigue and this is potentially due to more frequent hypothalamic involvement in MS than that appreciated by conventional MRI. Only a single cardiovascular reflex test was performed and as such we are unable to determine whether there is true autonomic dysfunction. Although, in the same cohort, further pupillary autonomic investigations were not different between fatigue and non-fatigue sufferers, it would certainly be more likely that cardiovascular, as opposed to alternative autonomic system dysfunction would contribute to fatigue. Pathology within the hypothalamus, as demonstrated by abnormal T1 values, could certainly account for autonomic abnormalities. This pathology could potentially also lead to disturbance of other hypothalamic functions. However, it appears that, other than rare acute cases, the hypocretin pathway is not frequently impaired in MS and nor does it appear to be a significant or frequent contributor to MS-fatigue.

We have demonstrated robust evidence of the effectiveness of modafinil in MS-fatigue sufferers. Modafinil is classed as a wakefulness-promoting drug and as such is principally used in hypersomnolent conditions. Its role in MS-fatigue management has, therefore, never been clear. However, modafinil does appear to act on areas of the brain which are involved in MS. In addition, whilst severe, acute hypersomnolence is a rare syndrome in MS, it does appear that fatigue and at the very least a perception of EDS may coexist in MS. It may be that this perception is in fact the lack of alertness and

vigilance with fatigue that we have demonstrated to respond to modafinil on neurophysiological and laboratory measures.

As I have discussed throughout this thesis, MS-fatigue almost certainly has a heterogenous basis. For this reason, it certainly cannot be stated that modafinil will be effective for all fatigue sufferers. Nevertheless, modafinil on the strength of our study will contribute positively towards fatigue management in MS patients. However, in light of its multifactorial nature, it might be necessary to try and clarify if there are any specific characteristics these fatigue sufferers possess which leads to this modafinil response.

6.2.1   Recent MRI literature of grey matter involvement in MS

There have been a number of more recent studies that have evaluated T1 relaxation time measurements in MS since the studies I have detailed in chapters 2 and 3. In addition an increasing body of literature has developed evaluating the impact of grey matter involvement in MS. I intend to discuss this in more detail in order that the MRI studies described in this thesis are seen in a more up-to-date context and prior to discussing future potential developments and applications.

Longitudinal studies have been reported of T1 relaxation time measurements in NAWM and normal-appearing grey matter (NAGM) in both early RR- and PPMS cohorts[138, 188]. Baseline results in these studies have shown significantly higher T1 values in both grey and white matter fractions in MS patients as compared to healthy controls. This MRI technique has also demonstrated a significant change in NAWM and NAGM over even a relatively short period of time in the early PPMS cohort although this was not found in RRMS patients. In PPMS, this increase in pathology

demonstrated by T1 measurements correlated significantly with the degree of change in clinical deficit, in particular walking ability. This was despite relatively small changes in the clinical measures[138]. Further assessment of the clinical relevance of cortical and deep grey matter involvement in MS has been studied principally with non-conventional MRI techniques other than T1 relaxation time measurements.

Studies have reported abnormal grey matter MTR measurements through employing either global or regional NAGM analysis in cohorts of RR- and SPMS patients[189, 190]. In addition, significant associations were found between grey matter MTR measures and locomotor ability and, furthermore, it was shown that this grey matter pathology was a better predictor of clinical outcome than that found in NAWM[189]. Grey matter pathology has also been demonstated, using MTR analysis, in PPMS patients including those with only a short disease duration[191, 192]. The clinical relevance of the damage detected in NAGM in PPMS was eloquently demonstrated in a study by Khaleeli et al in which an association was found between abnormal MTR measurements in the motor cortex and performance in the motor components of the Multiple Sclerosis Functional Composite as well as EDSS scores[191].

Cortical and deep grey matter volume measurements have also provided evidence of the clinical impact of grey matter involvement in MS. Grey matter atrophy has been demonstrated in both RR- and SPMS patients and was associated with degree of disability[193, 194]. In addition, as compared to white matter, the rate of grey matter atrophy increased significantly with advancing disease[193]. A number of studies have assessed the role of grey matter involvement in cognitive dysfunction. Sanfilipo et al demonstrated impaired performance in a number of neuropsychological tests in MS

patients[194]. They also found a differential affect of grey and white matter atrophy on performance with white matter volume predicting working memory and processing speed and grey matter volume associated with verbal memory. Specific regional grey matter atrophy and its role in cognitive function was investigated in a further study on RR- and SPMS patients[195]. Deep grey matter, in particular thalamic, atrophy, was associated with impairment in processing speed whilst atrophy of the amygdala correlated with verbal memory performance.

Regional grey matter abnormalities have also been eloquently demonstrated with advanced MRI techniques in the cervical spinal cord and cerebellum. Agosta et al found magnetization transfer MRI-detectable damage in the grey matter of macroscopically-normal, cervical spinal cord in a cohort of RRMS patients[196]. Furthermore, this pathology was associated with disability as measured by the EDSS. Cerebellar grey matter atrophy has been demonstrated by Anderson et al in SPMS with lower volumes in patients with cerebellar dysfunction and an association between atrophy and upper limb function as measured by the nine-hole peg test[197].

Finally, there are a number of more recent studies employing non-conventional MRI techniques to further explore the potential role of grey matter in MS-fatigue. Tedeschi et al demonstrated that in a large cohort of RRMS patients with low disability scores, patients with fatigue had significantly greater global grey and white matter atrophy as compared to those without fatigue[198]. Investigations of focal grey matter atrophy have however had mixed results in studies involving smaller numbers of MS patients. Benedict et al reported no association between FSS scores and volume measurements in deep (thalamus and caudate) and cortical (amygdala and hippocampus)

grey matter regions[195]. On the otherhand, Sepulcre at al found a significant association between fatigue severity and cortical, predominantly frontal region, atrophy[199].

Therefore, through advanced MRI techniques, there is an ever-increasing body of evidence of grey matter involvement in MS and its clinical impact both in terms of locomotor disability, cognitive dysfunction and fatigue.

6.2.2   Potential developments and future applications from this thesis

Larger patient cohorts will be necessary to further evaluate the techniques, results and conclusions of this thesis. Additional development of techniques may also be appropriate. This process is, in part, already underway. Our group has already submitted a study for publication in which the MRI analysis of the hypothalamus has been adapted. ROIs were delineated on high-resolution MP-RAGE images, prior to co-registering these ROIs on the T1 map images. The MP-RAGE sequences have the advantage, over the method described in this thesis, of providing a somewhat better anatomical view of structures such as the hypothalamus. Upon applying this to a slightly larger cohort of RRMS patients, significantly higher T1 values were again found in the hypothalamus, as compared to healthy individuals. In addition a further quantitative MRI measure, hypothalamic volume, was determined although no significant difference was found. This study also detected a significant correlation between fatigue severity and T1 relaxation time. These results may also require further evaluation and clarification with larger cohorts. In addition, possibly using techniques described in this thesis, it would be appropriate to attempt to determine by which mechanism pathology in the hypothalamus contributes to fatigue.

The application of neurophysiological and laboratory measures of alertness more often in fatigue studies has to be considered. We utilised a number of measures, however, it would seem appropriate to be more selective as the overriding advantage of questionnaires is that they are a quick, low-technology solution. Pupillography is well established and is not time consuming. It requires a small amount of equipment and a separate room but, for the researcher, performing the investigation is very straightforward. Performing speech analysis is quicker than completing the FSS and it is also relatively low-tech. It is a sensitive measure although as speech is highly individualistic, analysis has to be performed intra- rather than inter-cohort. Determining CFFF threshold is also quick and low-tech but in MS is confounded by its possible dual detection of visual dysfunction.

Whilst these measures are robust and provide objective evidence, it remains important to be aware that these measures may not completely correlate with patient's symptoms and in particular quality of life (QoL). In view of its significant impact, QoL tools should run alongside objective assessments when determining the effectiveness of treatments for fatigue. A future study of modafinil would be appropriate with a larger cohort, possibly a more limited number of objective investigations and a sensitive QoL measure. A larger patient cohort study of modafinil would have the potential to identify factors which may influence response to treatment. It is apparent that the way forward in the management of MS-fatigue will be to identify specific features or characteristics, whether clinical, MRI, CSF or laboratory, which can lead to a tailored fatigue treatment program for the individual.

References

1. Lassmann H. Pathology of multiple sclerosis. In: Compston A, Ebers G, Lassman H, McDonald I, Matthews B, Wekerle H, editors. McAlpine's Multiple Sclerosis. 3rd ed. London: Churchill Livingstone; 1998. p. 323-58.

2. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. N Engl J Med 1998;338 (5):278-85.

3. Brownell B, Hughes JT. The distribution of plaques in the cerebrum in multiple sclerosis. J Neurol Neurosurg Psychiatry 1962;25:315-20.

4. Kidd D, Barkhof F, McConnell R, Algra PR, Allen IV, Revesz T. Cortical lesions in multiple sclerosis. Brain 1999;122 (Pt 1):17-26.

5. Peterson JW, Bo L, Mork S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann Neurol 2001;50 (3):389-400.

6. Huitinga I, De Groot CJ, Van der Valk P, Kamphorst W, Tilders FJ, Swaab DF. Hypothalamic lesions in multiple sclerosis. J Neuropathol Exp Neurol 2001;60 (12):1208-18.

7. Cifelli A, Arridge M, Jezzard P, Esiri MM, Palace J, Matthews PM. Thalamic neurodegeneration in multiple sclerosis. Ann Neurol 2002;52 (5):650-3.

8. Bakshi R, Ariyaratana S, Benedict RH, Jacobs L. Fluid-attenuated inversion recovery magnetic resonance imaging detects cortical and juxtacortical multiple sclerosis lesions. Arch Neurol 2001;58 (5):742-8.

9.   Chard DT, Griffin CM, McLean MA, Kapeller P, Kapoor R, Thompson AJ, et al. Brain metabolite changes in cortical grey and normal-appearing white matter in clinically early relapsing-remitting multiple sclerosis. Brain 2002;125 (Pt 10):2342-52.

10.   Narayana PA, Doyle TJ, Lai D, Wolinsky JS. Serial proton magnetic resonance spectroscopic imaging, contrast-enhanced magnetic resonance imaging, and quantitative lesion volumetry in multiple sclerosis. Ann Neurol 1998;43 (1):56-71.

11.   Sailer M, Fischl B, Salat D, Tempelmann C, Schonfeld MA, Busa E, et al. Focal thinning of the cerebral cortex in multiple sclerosis. Brain 2003;126 (Pt 8):1734-44.

12.   De Stefano N, Matthews PM, Filippi M, Agosta F, De Luca M, Bartolozzi ML, et al. Evidence of early cortical atrophy in MS: relevance to white matter changes and disability. Neurology 2003;60 (7):1157-62.

13.   Codella M, Rocca MA, Colombo B, Martinelli-Boneschi F, Comi G, Filippi M. Cerebral grey matter pathology and fatigue in patients with multiple sclerosis: a preliminary study. J Neurol Sci 2002;194 (1):71-4.

14.   Griffin CM, Chard DT, Parker GJ, Barker GJ, Thompson AJ, Miller DH. The relationship between lesion and normal appearing brain tissue abnormalities in early relapsing remitting multiple sclerosis. J Neurol 2002;249 (2):193-9.

15.   Ciccarelli O, Werring DJ, Wheeler-Kingshott CA, Barker GJ, Parker GJ, Thompson AJ, et al. Investigation of MS normal-appearing brain using diffusion tensor MRI with clinical correlations. Neurology 2001;56 (7):926-33.

16. Wylezinska M, Cifelli A, Jezzard P, Palace J, Alecci M, Matthews PM. Thalamic neurodegeneration in relapsing-remitting multiple sclerosis. Neurology 2003;60 (12):1949-54.

17. Roelcke U, Kappos L, Lechner-Scott J, Brunnschweiler H, Huber S, Ammann W, et al. Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue: a 18F-fluorodeoxyglucose positron emission tomography study. Neurology 1997;48 (6):1566-71.

18. Katon WJ, Buchwald DS, Simon GE, Russo JE, Mease PJ. Psychiatric illness in patients with chronic fatigue and those with rheumatoid arthritis. J Gen Intern Med 1991;6 (4):277-85.

19. Multiple Sclerosis Council for Clinical Practice Guidelines. Fatigue and multiple sclerosis: evidence-based management strategies for fatigue in multiple sclerosis. Washington, DC: Paralyzed Veterans of America; 1998.

20. Freal JE, Kraft GH, Coryell JK. Symptomatic fatigue in multiple sclerosis. Arch Phys Med Rehabil 1984;65 (3):135-8.

21. Krupp LB, Alvarez LA, LaRocca NG, Scheinberg LC. Fatigue in multiple sclerosis. Arch Neurol 1988;45 (4):435-7.

22. Scheinberg L, Holland N, Larocca N, Laitin P, Bennett A, Hall H. Multiple sclerosis; earning a living. N Y State J Med 1980;80 (9):1395-400.

23. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989;46 (10):1121-3.

24. Janardhan V, Bakshi R. Quality of life in patients with multiple sclerosis: the impact of fatigue and depression. J Neurol Sci 2002;205 (1):51-8.

25. Djaldetti R, Ziv I, Achiron A, Melamed E. Fatigue in multiple sclerosis compared with chronic fatigue syndrome: A quantitative assessment. Neurology 1996;46 (3):632-5.

26. Sheean GL, Murray NM, Rothwell JC, Miller DH, Thompson AJ. An electrophysiological study of the mechanism of fatigue in multiple sclerosis. Brain 1997;120 (Pt 2):299-315.

27. Sandroni P, Walker C, Starr A. 'Fatigue' in patients with multiple sclerosis. Motor pathway conduction and event-related potentials. Arch Neurol 1992;49 (5):517-24.

28. Krupp LB, Elkins LE. Fatigue and declines in cognitive functioning in multiple sclerosis. Neurology 2000;55 (7):934-9.

29. Foglio K, Clini E, Facchetti D, Vitacca M, Marangoni S, Bonomelli M, et al. Respiratory muscle function and exercise capacity in multiple sclerosis. Eur Respir J 1994;7 (1):23-8.

30. Smeltzer SC, Lavietes MH, Cook SD. Expiratory training in multiple sclerosis. Arch Phys Med Rehabil 1996;77 (9):909-12.

31. Krupp LB, LaRocca NG, Muir J, Steinberg AD. A study of fatigue in systemic lupus erythematosus. J Rheumatol 1990;17 (11):1450-2.

32. Heesen C, Nawrath L, Reich C, Bauer N, Schulz KH, Gold SM. Fatigue in multiple sclerosis: an example of cytokine mediated sickness behaviour? J Neurol Neurosurg Psychiatry 2006;77 (1):34-9.

33. Flachenecker P, Bihler I, Weber F, Gottschalk M, Toyka KV, Rieckmann P. Cytokine mRNA expression in patients with multiple sclerosis and fatigue. Mult Scler 2004;10 (2):165-9.

34. Rieckmann P, Albrecht M, Kitze B, Weber T, Tumani H, Broocks A, et al. Cytokine mRNA levels in mononuclear blood cells from patients with multiple sclerosis. Neurology 1994;44 (8):1523-6.

35. Giovannoni G, Thompson AJ, Miller DH, Thompson EJ. Fatigue is not associated with raised inflammatory markers in multiple sclerosis. Neurology 2001;57 (4):676-81.

36. Bertolone K, Coyle PK, Krupp LB, Doscher CA. Cytokine correlates of fatigue in multiple sclerosis [abstract]. Neurology 1993;43:A356.

37. Coyle PK, Krupp LB, Mohr K, Doscher C, Mehta PD. Immune activation markers in neurological patients with symptomatic chronic fatigue [abstract]. Ann Neurol 1991;30 (2):P279.

38. Rudick RA, Barna BP. Serum interleukin 2 and soluble interleukin 2 receptor in patients with multiple sclerosis who are experiencing severe fatigue. Arch Neurol 1990;47 (3):254-5.

39. Wingerchuk DM, Benarroch EE, O'Brien PC, Keegan BM, Lucchinetti CF, Noseworthy JH, et al. A randomized controlled crossover trial of aspirin for fatigue in multiple sclerosis. Neurology 2005;64 (7):1267-9.

40. Demitrack MA. Neuroendocrine correlates of chronic fatigue syndrome: a brief review. J Psychiatr Res 1997;31 (1):69-82.

41. Neeck G, Federlin K, Graef V, Rusch D, Schmidt KL. Adrenal secretion of cortisol in patients with rheumatoid arthritis. J Rheumatol 1990;17 (1):24-9.

42. Chikanza IC, Petrou P, Kingsley G, Chrousos G, Panayi GS. Defective hypothalamic response to immune and inflammatory stimuli in patients with rheumatoid arthritis. Arthritis Rheum 1992;35 (11):1281-8.

43. Wei T, Lightman SL. The neuroendocrine axis in patients with multiple sclerosis. Brain 1997;120 (Pt 6):1067-76.

44. Gottschalk M, Kumpfel T, Flachenecker P, Uhr M, Trenkwalder C, Holsboer F, et al. Fatigue and regulation of the hypothalamo-pituitary-adrenal axis in multiple sclerosis. Arch Neurol 2005;62 (2):277-80.

45. McDougall AJ, McLeod JG. Autonomic nervous system function in multiple sclerosis. J Neurol Sci 2003;215 (1-2):79-85.

46. Merkelbach S, Dillmann U, Kolmel C, Holz I, Muller M. Cardiovascular autonomic dysregulation and fatigue in multiple sclerosis. Mult Scler 2001;7 (5):320-6.

47. Rowe PC, Calkins H. Neurally mediated hypotension and chronic fatigue syndrome. Am J Med 1998;105 (3A):15S-21S.

48. Flachenecker P, Rufer A, Bihler I, Hippel C, Reiners K, Toyka KV, et al. Fatigue in MS is related to sympathetic vasomotor dysfunction. Neurology 2003;61 (6):851-3.

49. Leocani L, Colombo B, Magnani G, Martinelli-Boneschi F, Cursi M, Rossi P, et al. Fatigue in multiple sclerosis is associated with abnormal cortical activation to voluntary movement--EEG evidence. Neuroimage 2001;13 (6 Pt 1):1186-92.

50.   Perretti A, Balbi P, Orefice G, Trojano L, Marcantonio L, Brescia-Morra V, et al. Post-exercise facilitation and depression of motor evoked potentials to transcranial magnetic stimulation: a study in multiple sclerosis. Clin Neurophysiol 2004;115 (9):2128-33.

51.   Colombo B, Martinelli Boneschi F, Rossi P, Rovaris M, Maderna L, Filippi M, et al. MRI and motor evoked potential findings in nondisabled multiple sclerosis patients with and without symptoms of fatigue. J Neurol 2000;247 (7):506-9.

52.   Bakshi R, Miletich RS, Henschel K, Shaikh ZA, Janardhan V, Wasay M, et al. Fatigue in multiple sclerosis: cross-sectional correlation with brain MRI findings in 71 patients. Neurology 1999;53 (5):1151-3.

53.   Mainero C, Faroni J, Gasperini C, Filippi M, Giugni E, Ciccarelli O, et al. Fatigue and magnetic resonance imaging activity in multiple sclerosis. J Neurol 1999;246 (6):454-8.

54.   Bruno RL, Creange SJ, Frick NM. Parallels between post-polio fatigue and chronic fatigue syndrome: a common pathophysiology? Am J Med 1998;105 (3A):66S-73S.

55.   Schuurman PR, Bosch DA, Bossuyt PM, Bonsel GJ, van Someren EJ, de Bie RM, et al. A comparison of continuous thalamic stimulation and thalamotomy for suppression of severe tremor. N Engl J Med 2000;342 (7):461-8.

56.   Hua Z, Guodong G, Qinchuan L, Yaqun Z, Qinfen W, Xuelian W. Analysis of complications of radiofrequency pallidotomy. Neurosurgery 2003;52 (1):89-99; discussion 99-101.

57.   Bhatia KP, Marsden CD. The behavioural and motor consequences of focal lesions of the basal ganglia in man. Brain 1994;117 (Pt 4):859-76.

58.   Filippi M, Rocca MA, Colombo B, Falini A, Codella M, Scotti G, et al. Functional magnetic resonance imaging correlates of fatigue in multiple sclerosis. Neuroimage 2002;15 (3):559-67.

59.   The International Classification of Sleep Disorders. 2nd ed. Westchester, IL: American Academy of Sleep Medicine; 2005.

60.   Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14 (6):540-5.

61.   Johns MW. Reliability and factor analysis of the Epworth Sleepiness Scale. Sleep 1992;15 (4):376-81.

62.   Chervin RD, Aldrich MS. The Epworth Sleepiness Scale may not reflect objective measures of sleepiness or sleep apnea. Neurology 1999;52 (1):125-31.

63.   Chervin RD, Aldrich MS, Pickett R, Guilleminault C. Comparison of the results of the Epworth Sleepiness Scale and the Multiple Sleep Latency Test. J Psychosom Res 1997;42 (2):145-55.

64.   Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC. Quantification of sleepiness: a new approach. Psychophysiology 1973;10 (4):431-6.

65.   Carskadon MA, Dement WC, Mitler MM, Roth T, Westbrook PR, Keenan S. Guidelines for the multiple sleep latency test (MSLT): a standard measure of sleepiness. Sleep 1986;9 (4):519-24.

66.   Mitler MM, Gujavarty KS, Browman CP. Maintenance of wakefulness test: a polysomnographic technique for evaluation treatment efficacy in patients with

excessive somnolence. Electroencephalogr Clin Neurophysiol 1982;53 (6):658-61.

67. Smith JM, Misiak H. Critical flicker frequency (CFF) and psychotropic drugs in normal human subjects-a review. Psychopharmacologia 1976;47 (2):175-82.

68. Wilhelm H, Wilhelm B. Clinical applications of pupillography. Journal of Neuro-ophthalmology 2003;23 (1):42-9.

69. Yoss RE, Moyer NJ, Hollenhorst RW. Pupil size and spontaneous pupillary waves associated with alertness, drowsiness, and sleep. Neurology 1970;20 (6):545-54.

70. Danker-Hopfe H, Kraemer S, Dorn H, Schmidt A, Ehlert I, Herrmann W. Time-of-day variations in different measures of sleepiness (MSLT, pupillography, and SSS) and their interrelations. Psychophysiology 2001;38 (5):828-35.

71. Wilhelm B, Wilhelm H, Lüdtke H, Streicher P, Adler M. Pupillographic assessment of sleepiness in sleep-deprived healthy subjects. Sleep 1998;21 (3):258-65.

72. Wilhelm B, Giedke H, Lüdtke H, Bittner E, Hofmann A, Wilhelm H. Daytime variations in central nervous system activation measured by a pupillographic sleepiness test. J Sleep Res 2001;10 (1):1-7.

73. Regen F. Association of pui and waking EEG during sleep deprivation. Journal of Sleep Research 2004;13 (1):1.

74. Merritt S, Schnyders H, Patel M, Basner R, O'Neill W. Pupil staging and EEG measurement of sleepiness. Int J Psychophysiol 2004;52 (1):97-112.

75.   McLaren J, Hauri P, Lin S, Harris C. Pupillometry in clinically sleepy patients. Sleep Med 2002;3 (4):347-52.

76.   Wilhelm H, Lüdtke H, Wilhelm B. Pupillographic sleepiness testing in hypersomniacs and normals. Graefes Arch Clin Exp Ophthalmol 1998;236 (10):725-9.

77.   Wilhelm B, Luedtke H, Widmaier D, Wilhelm H, Ruehle K. Therapy control in sleep apnoea patients by means of a pupillographic sleepiness test. Sleep 1998;21 (Suppl.):266.

78.   Egg R, Högl B, Glatzl S, Beer R, Berger T. Autonomic instability, as measured by pupillary unrest, is not associated with multiple sclerosis fatigue severity. Mult Scler 2002;8 (3):256-60.

79.   Frauscher B, Egg R, Brandauer E, Ulmer H, Berger T, Poewe W, et al. Daytime sleepiness is not increased in mild to moderate multiple sclerosis: a pupillographic study. Sleep Med 2005;6 (6):543-7.

80.   Chervin RD. Sleepiness, fatigue, tiredness, and lack of energy in obstructive sleep apnea. Chest 2000;118 (2):372-9.

81.   Bailes S, Libman E, Baltzan M, Amsel R, Schondorf R, Fichten CS. Brief and distinct empirical sleepiness and fatigue scales. J Psychosom Res 2006;60 (6):605-13.

82.   Daniels E, King MA, Smith IE, Shneerson JM. Health-related quality of life in narcolepsy. J Sleep Res 2001;10 (1):75-81.

83. Beusterien KM, Rogers AE, Walsleben JA, Emsellem HA, Reblando JA, Wang L, et al. Health-related quality of life effects of modafinil for treatment of narcolepsy. Sleep 1999;22 (6):757-65.

84. Becker PM, Schwartz JR, Feldman NT, Hughes RJ. Effect of modafinil on fatigue, mood, and health-related quality of life in patients with narcolepsy. Psychopharmacology (Berl) 2004;171 (2):133-9.

85. Fredrikson S, Carlander B, Billiard M, Link H. CSF immune variables in patients with narcolepsy. Acta Neurol Scand 1990;81 (3):253-4.

86. Smith AJ, Jackson MW, Neufing P, McEvoy RD, Gordon TP. A functional autoantibody in narcolepsy. Lancet 2004;364 (9451):2122-4.

87. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998;92 (5):1 page following 696.

88. de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998;95 (1):322-7.

89. Overeem S, Mignot E, van Dijk JG, Lammers GJ. Narcolepsy: clinical features, new pathophysiologic insights, and future perspectives. J Clin Neurophysiol 2001;18 (2):78-105.

90. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000;355 (9197):39-40.

91. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000;6 (9):991-7.

92. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000;27 (3):469-74.

93. Lodi R, Tonon C, Vignatelli L, Iotti S, Montagna P, Barbiroli B, et al. In vivo evidence of neuronal loss in the hypothalamus of narcoleptic patients. Neurology 2004;63 (8):1513-5.

94. Ellis CM, Simmons A, Lemmens G, Williams SC, Parkes JD. Proton spectroscopy in the narcoleptic syndrome. Is there evidence of a brainstem lesion? Neurology 1998;50 (2 Suppl 1):S23-6.

95. Kaufmann C, Schuld A, Pollmacher T, Auer DP. Reduced cortical gray matter in narcolepsy: preliminary findings with voxel-based morphometry. Neurology 2002;58 (12):1852-5.

96. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002;59 (10):1553-62.

97. Baumann CR, Khatami R, Werth E, Bassetti CL. Hypocretin (orexin) deficiency predicts severe objective excessive daytime sleepiness in narcolepsy with cataplexy. J Neurol Neurosurg Psychiatry 2006;77 (3):402-4.

98. Kanbayashi T, Inoue Y, Chiba S, Aizawa R, Saito Y, Tsukamoto H, et al. CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. J Sleep Res 2002;11 (1):91-3.

99. Scammell TE, Nishino S, Mignot E, Saper CB. Narcolepsy and low CSF orexin (hypocretin) concentration after a diencephalic stroke. Neurology 2001;56 (12):1751-3.

100. Nokura K, Kanbayashi T, Ozeki T, Koga H, Zettsu T, Yamamoto H, et al. Hypersomnia, asterixis and cataplexy in association with orexin A-reduced hypothalamic tumor. J Neurol 2004;251 (12):1534-5.

101. Nevsimalova S, Vankova J, Stepanova I, Seemanova E, Mignot E, Nishino S. Hypocretin deficiency in Prader-Willi syndrome. Eur J Neurol 2005;12 (1):70-2.

102. Kanbayashi T, Ishiguro H, Aizawa R, Saito Y, Ogawa Y, Abe M, et al. Hypocretin-1 (orexin-A) concentrations in cerebrospinal fluid are low in patients with Guillain-Barre syndrome. Psychiatry Clin Neurosci 2002;56 (3):273-4.

103. Nishino S, Kanbayashi T, Fujiki N, Uchino M, Ripley B, Watanabe M, et al. CSF hypocretin levels in Guillain-Barre syndrome and other inflammatory neuropathies. Neurology 2003;61 (6):823-5.

104. Baumann CR, Bassetti CL. CSF hypocretin levels in Guillain-Barre syndrome and other inflammatory neuropathies. Neurology 2004;62 (12):2337; author reply 2337.

105. Ebrahim IO, Semra YK, De Lacy S, Howard RS, Kopelman MD, Williams A, et al. CSF hypocretin (Orexin) in neurological and psychiatric conditions. J Sleep Res 2003;12 (1):83-4.

106. Martinez-Rodriguez JE, Lin L, Iranzo A, Genis D, Marti MJ, Santamaria J, et al. Decreased hypocretin-1 (Orexin-A) levels in the cerebrospinal fluid of patients with myotonic dystrophy and excessive daytime sleepiness. Sleep 2003;26 (3):287-90.

107. Drouot X, Moutereau S, Nguyen JP, Lefaucheur JP, Creange A, Remy P, et al. Low levels of ventricular CSF orexin/hypocretin in advanced PD. Neurology 2003;61 (4):540-3.

108. Overeem S, van Hilten JJ, Ripley B, Mignot E, Nishino S, Lammers GJ. Normal hypocretin-1 levels in Parkinson's disease patients with excessive daytime sleepiness. Neurology 2002;58 (3):498-9.

109. Baumann CR, Stocker R, Imhof HG, Trentz O, Hersberger M, Mignot E, et al. Hypocretin-1 (orexin A) deficiency in acute traumatic brain injury. Neurology 2005;65 (1):147-9.

110. Iseki K, Mezaki T, Oka Y, Terada K, Tomimoto H, Miki Y, et al. Hypersomnia in MS. Neurology 2002;59 (12):2006-7.

111. Kato T, Kanbayashi T, Yamamoto K, Nakano T, Shimizu T, Hashimoto T, et al. Hypersomnia and low CSF hypocretin-1 (orexin-A) concentration in a patient with multiple sclerosis showing bilateral hypothalamic lesions. Intern Med 2003;42 (8):743-5.

112. Gledhill RF, Bartel PR, Yoshida Y, Nishino S, Scammell TE. Narcolepsy caused by acute disseminated encephalomyelitis. Arch Neurol 2004;61 (5):758-60.

113. Knudsen S, Jennum P, Korsholm K, Sheikh S, Gammeltoft S, Frederiksen J. Normal levels of cerebrospinal fluid hypocretin-1 and daytime sleepiness during

attacks of relapsing-remitting multiple sclerosis and monosymptomatic optic neuritis. Mult Scler 2008;14 (6):734-8.

114. Attarian HP, Brown KM, Duntley SP, Carter JD, Cross AH. The relationship of sleep disturbances and fatigue in multiple sclerosis. Arch Neurol 2004;61 (4):525-8.

115. Taphoorn MJ, van Someren E, Snoek FJ, Strijers RL, Swaab DF, Visscher F, et al. Fatigue, sleep disturbances and circadian rhythm in multiple sclerosis. J Neurol 1993;240 (7):446-8.

116. Vetrugno R, Stecchi S, Scandellari C, Pierangeli G, Sabattini L, D'Angelo R, et al. Sleep--wake and body core temperature rhythms in multiple sclerosis with fatigue. Clin Neurophysiol 2007;118 (1):228-34.

117. Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy: US Modafinil in Narcolepsy Multicenter Study Group. Neurology 2000;54 (5):1166-75.

118. Scammell TE, Estabrooke IV, McCarthy MT, Chemelli RM, Yanagisawa M, Miller MS, et al. Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci 2000;20 (22):8620-8.

119. Hou R, Freeman C, Langley R, Szabadi E, Bradshaw C. Does modafinil activate the locus coeruleus in man? Comparison of modafinil and clonidine on arousal and autonomic functions in human volunteers. Psychopharmacology (Berl) 2005;181 (3):537-49.

120. Mathiowetz V, Matuska KM, Murphy ME. Efficacy of an energy conservation course for persons with multiple sclerosis. Arch Phys Med Rehabil 2001;82 (4):449-56.

121. Oken BS, Kishiyama S, Zajdel D, Bourdette D, Carlsen J, Haas M, et al. Randomized controlled trial of yoga and exercise in multiple sclerosis. Neurology 2004;62 (11):2058-64.

122. Patti F, Ciancio MR, Reggio E, Lopes R, Palermo F, Cacopardo M, et al. The impact of outpatient rehabilitation on quality of life in multiple sclerosis. J Neurol 2002;249 (8):1027-33.

123. Rosenberg GA, Appenzeller O. Amantadine, fatigue, and multiple sclerosis. Arch Neurol 1988;45 (10):1104-6.

124. Cohen RA, Fisher M. Amantadine treatment of fatigue associated with multiple sclerosis. Arch Neurol 1989;46 (6):676-80.

125. Krupp LB, Coyle PK, Doscher C, Miller A, Cross AH, Jandorf L, et al. Fatigue therapy in multiple sclerosis: results of a double-blind, randomized, parallel trial of amantadine, pemoline, and placebo. Neurology 1995;45 (11):1956-61.

126. Management of multiple sclerosis in primary and secondary care. Clinical Guideline 8. National Institute for Clinical Excellence; 2003 Nov.

127. Pucci E, Branas P, D'Amico R, Giuliani G, Solari A, Taus C. Amantadine for fatigue in multiple sclerosis. Cochrane Database Syst Rev 2007 (1):CD002818.

128. Weinshenker BG, Penman M, Bass B, Ebers GC, Rice GP. A double-blind, randomized, crossover trial of pemoline in fatigue associated with multiple sclerosis. Neurology 1992;42 (8):1468-71.

129. Mainero C, Inghilleri M, Pantano P, Conte A, Lenzi D, Frasca V, et al. Enhanced brain motor activity in patients with MS after a single dose of 3,4-diaminopyridine. Neurology 2004;62 (11):2044-50.

130. Sheean GL, Murray NM, Rothwell JC, Miller DH, Thompson AJ. An open-labelled clinical and electrophysiological study of 3,4 diaminopyridine in the treatment of fatigue in multiple sclerosis. Brain 1998;121 (Pt 5):967-75.

131. Zifko UA, Rupp M, Schwarz S, Zipko HT, Maida EM. Modafinil in treatment of fatigue in multiple sclerosis. Results of an open-label study. J Neurol 2002;249 (8):983-7.

132. Rammohan KW, Rosenberg JH, Lynn DJ, Blumenfeld AM, Pollak CP, Nagaraja HN. Efficacy and safety of modafinil (Provigil) for the treatment of fatigue in multiple sclerosis: a two centre phase 2 study. J Neurol Neurosurg Psychiatry 2002;72 (2):179-83.

133. Stankoff B, Waubant E, Confavreux C, Edan G, Debouverie M, Rumbach L, et al. Modafinil for fatigue in MS: a randomized placebo-controlled double-blind study. Neurology 2005;64 (7):1139-43.

134. van Waesberghe JH, Castelijns JA, Scheltens P, Truyen L, Lycklana ANGJ, Hoogenraad FG, et al. Comparison of four potential MR parameters for severe tissue destruction in multiple sclerosis lesions. Magn Reson Imaging 1997;15 (2):155-62.

135. van Walderveen MA, Barkhof F, Pouwels PJ, van Schijndel RA, Polman CH, Castelijns JA. Neuronal damage in T1-hypointense multiple sclerosis lesions

demonstrated in vivo using proton magnetic resonance spectroscopy. Ann Neurol 1999;46 (1):79-87.

136.   Parry A, Clare S, Jenkinson M, Smith S, Palace J, Matthews PM. White matter and lesion T1 relaxation times increase in parallel and correlate with disability in multiple sclerosis. J Neurol 2002;249 (9):1279-86.

137.   Fu L, Matthews PM, De Stefano N, Worsley KJ, Narayanan S, Francis GS, et al. Imaging axonal damage of normal-appearing white matter in multiple sclerosis. Brain 1998;121 (Pt 1):103-13.

138.   Manfredonia F, Ciccarelli O, Khaleeli Z, Tozer DJ, Sastre-Garriga J, Miller DH, et al. Normal-appearing brain t1 relaxation time predicts disability in early primary progressive multiple sclerosis. Arch Neurol 2007;64 (3):411-5.

139.   Vaithianathar L, Tench CR, Morgan PS, Constantinescu CS. Magnetic resonance imaging of the cervical spinal cord in multiple sclerosis--a quantitative T1 relaxation time mapping approach. J Neurol 2003;250 (3):307-15.

140.   Vaithianathar L, Tench CR, Morgan PS, Wilson M, Blumhardt LD. T1 relaxation time mapping of white matter tracts in multiple sclerosis defined by diffusion tensor imaging. J Neurol 2002;249 (9):1272-8.

141.   van Walderveen MA, van Schijndel RA, Pouwels PJ, Polman CH, Barkhof F. Multislice T1 relaxation time measurements in the brain using IR-EPI: reproducibility, normal values, and histogram analysis in patients with multiple sclerosis. J Magn Reson Imaging 2003;18 (6):656-64.

142.   Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory

Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology 1996;46 (4):907-11.

143.   McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50 (1):121-7.

144.   Kurtzke J. Disability rating scales in multiple sclerosis. Ann NY Acad Sci 1984;436:347-360.

145.   Sonka M, Hlavac V, Boyle R, Image Processing, Analysis and Machine Vision. 2nd ed. California: Brooks and Cole Publishing; 1998.

146.   Barnes D, McDonald WI, Landon DN, Johnson G. The characterization of experimental gliosis by quantitative nuclear magnetic resonance imaging. Brain 1988;111 (Pt 1):83-94.

147.   Barnes D, McDonald WI, Johnson G, Tofts PS, Landon DN. Quantitative nuclear magnetic resonance imaging: characterisation of experimental cerebral oedema. J Neurol Neurosurg Psychiatry 1987;50 (2):125-33.

148.   van Waesberghe JH, Kamphorst W, De Groot CJ, van Walderveen MA, Castelijns JA, Ravid R, et al. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Ann Neurol 1999;46 (5):747-54.

149.   Sawlani V, Gupta RK, Singh MK, Kohli A. MRI demonstration of Wallerian degeneration in various intracranial lesions and its clinical implications. J Neurol Sci 1997;146 (2):103-8.

150. Simon JH, Kinkel RP, Jacobs L, Bub L, Simonian N. A Wallerian degeneration pattern in patients at risk for MS. Neurology 2000;54 (5):1155-60.

151. Cookfair DL, Fischer J, Rudick R, Jacobs L, Herndon R, Richert J, et al. Fatigue severity in low disability MS patients participating in a phase III trial of Avonex (IFNb-1a) for relapsing multiple sclerosis [abstract]. Neurology 1997;48 (suppl 2):A173.

152. Ueno M, Tokunaga Y, Terachi S, Gondo K, Hara T. Asymmetric sweating in a child with multiple sclerosis. Pediatr Neurol 2000;23 (1):74-6.

153. Henry R, Shieh M, Okuda D, Evangelista A, Gorno-Tempini M, Pelletier D. Regional grey matter atrophy in clinically isolated syndromes at presentation. J Neurol Neurosurg Psychiatry 2008;79 (11):1236-44.

154. Schumann EM, Kumpfel T, Then Bergh F, Trenkwalder C, Holsboer F, Auer DP. Activity of the hypothalamic-pituitary-adrenal axis in multiple sclerosis: correlations with gadolinium-enhancing lesions and ventricular volume. Ann Neurol 2002;51 (6):763-7.

155. Kira J, Harada M, Yamaguchi Y, Shida N, Goto I. Hyperprolactinemia in multiple sclerosis. J Neurol Sci 1991;102 (1):61-6.

156. Edwards S, Lennox G, Robson K, Whiteley A. Hypothermia due to hypothalamic involvement in multiple sclerosis. J Neurol Neurosurg Psychiatry 1996;61 (4):419-20.

157. Martinez-Rodriguez J, Munteis E, Roquer J. Periodic hyperthermia and abnormal circadian temperature rhythm in a patient with multiple sclerosis. Mult Scler 2006;12 (4):515-7.

158. Mason D, MacPhee I, Antoni F. The role of the neuroendocrine system in determining genetic susceptibility to experimental allergic encephalomyelitis in the rat. Immunology 1990;70 (1):1-5.

159. Fassbender K, Schmidt R, Mössner R, Kischka U, Kühnen J, Schwartz A, et al. Mood disorders and dysfunction of the hypothalamic-pituitary-adrenal axis in multiple sclerosis: association with cerebral inflammation. Arch Neurol 1998;55 (1):66-72.

160. Huitinga I, Erkut Z, van Beurden D, Swaab D. Impaired hypothalamus-pituitary-adrenal axis activity and more severe multiple sclerosis with hypothalamic lesions. Ann Neurol 2004;55 (1):37-45.

161. Reiber H. Flow rate of cerebrospinal fluid (CSF) - a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases. J Neurol Sci 1994;122:189-203.

162. Teunissen CE, Petzold A, Bennett JL, Berven FS, Brundin L, Comabella M, et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology 2009;73 (22):1914-22.

163. Lewczuk P, Beck G, Esselmann H, Bruckmoser R, Zimmermann R, Fiszer M, et al. Effect of sample collection tubes on cerebrospinal fluid concentration of tau proteins and amyloid beta peptides. Clin Chem 2006;52:332-4.

164. Ebrahim IO, Sharief MK, de Lacy S, Semra YK, Howard RS, Kopelman MD, et al. Hypocretin (orexin) deficiency in narcolepsy and primary hypersomnia. J Neurol Neurosurg Psychiatry 2003;74 (1):127-30.

165. Ripley B, Overeem S, Fujiki N, Nevsimalova S, Uchino M, Yesavage J, et al. CSF hypocretin/orexin levels in narcolepsy and other neurological conditions. Neurology 2001;57 (12):2253-8.

166. Lewczuk P, Beck G, Ganslandt O, Esselmann H, Deisenhammer F, Regeniter A, et al. International quality control survey of neurochemical dementia diagnostics. Neurosci Lett 2006;409 (1):1-4.

167. Hoffmann G, Gonze J, Mendlewicz J. Speech pause time as a method for the evaluation of psychomotor retardation in depressive illness. Br J Psychiatry 1985;146:535-8.

168. Szabadi E, Bradshaw C, Besson J. Elongation of pause-time in speech: a simple, objective measure of motor retardation in depression. Br J Psychiatry 1976;129:592-7.

169. Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971;10 (21):181-91.

170. Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974;47:211-18.

171. Robertson PJ, Hellriegel E. Clinical pharmacokinetic profile of modafinil. Clin Pharmacokinet 2003;42 (2):123-37.

172. Hou R, Langley R, Szabadi E, Bradshaw C. Comparison of diphenhydramine and modafinil on arousal and autonomic functions in healthy volunteers. J Psychopharmacol 2007;21 (6):567-78.

173. Sharma P, Sharma B, Puri V, Sarin S. Critical flicker frequency: diagnostic tool for minimal hepatic encephalopathy. J Hepatol 2007;47 (1):67-73.

174.   Saletu M, Anderer P, Saletu-Zyhlarz G, Mandl M, Zeitlhofer J, Saletu B. Event-related-potential low-resolution brain electromagnetic tomography (ERP-LORETA) suggests decreased energetic resources for cognitive processing in narcolepsy. Clin Neurophysiol 2008;119 (8):1782-94.

175.   Schneider C, Fulda S, Schulz H. Daytime variation in performance and tiredness/sleepiness ratings in patients with insomnia, narcolepsy, sleep apnea and normal controls. J Sleep Res 2004;13 (4):373-83.

176.   Daley M, Swank R, Ellison C. Flicker fusion thresholds in multiple sclerosis. A functional measure of neurological damage. Arch Neurol 1979;36 (5):292-5.

177.   Salmi T. Critical flicker frequencies in MS patients with normal or abnormal pattern VEP. Acta Neurol Scand 1985;71 (5):354-8.

178.   Accornero N, De Vito G, Rotunno A, Perugino U, Manfredi M. Critical fusion frequency in MS during mild induced hyperthermia. Acta Neurol Scand 1989;79 (6):510-4.

179.   Davis F, Stefoski D, Rush J. Orally administered 4-aminopyridine improves clinical signs in multiple sclerosis. Ann Neurol 1990;27 (2):186-92.

180.   Reicker L, Tombaugh T, Walker L, Freedman M. Reaction time: An alternative method for assessing the effects of multiple sclerosis on information processing speed. Arch Clin Neuropsychol 2007;22 (5):655-64.

181.   Gunal D, Afsar N, Tanridag T, Aktan S. Autonomic dysfunction in multiple sclerosis: correlation with disease-related parameters. Eur Neurol 2002;48 (1):1-5.

182. Vita G, Fazio M, Milone S, Blandino A, Salvi L, Messina C. Cardiovascular autonomic dysfunction in multiple sclerosis is likely related to brainstem lesions. J Neurol Sci 1993;120 (1):82-6.

183. Sterman A, Coyle P, Panasci D, Grimson R. Disseminated abnormalities of cardiovascular autonomic functions in multiple sclerosis. Neurology 1985;35 (11):1665-8.

184. Pepin E, Hicks R, Spencer M, Tran Z, Jackson C. Pressor response to isometric exercise in patients with multiple sclerosis. Med Sci Sports Exerc 1996;28 (6):656-60.

185. Cannizzaro M, Cohen H, Rappard F, Snyder P. Bradyphrenia and bradykinesia both contribute to altered speech in schizophrenia: a quantitative acoustic study. Cogn Behav Neurol 2005;18 (4):206-10.

186. Candy M, Jones L, Williams R, Tookman A, King M. Psychostimulants for depression. Cochrane Database Syst Rev 2008 (2):CD006722.

187. Randall D, Fleck N, Shneerson J, File S. The cognitive-enhancing properties of modafinil are limited in non-sleep-deprived middle-aged volunteers. Pharmacol Biochem Behav 2004;77 (3):547-55.

188. Davies GR, Hadjiprocopis A, Altmann DR, Chard DT, Griffin CM, Rashid W, et al. Normal-appearing grey and white matter T1 abnormality in early relapsing-remitting multiple sclerosis: a longitudinal study. Mult Scler 2007;13 (2):169-77.

189. Fisniku LK, Altmann DR, Cercignani M, Tozer DJ, Chard DT, Jackson JS, et al. Magnetization transfer ratio abnormalities reflect clinically relevant grey matter damage in multiple sclerosis. Mult Scler 2009;15 (6):668-77.

190. Vrenken H, Pouwels PJ, Ropele S, Knol DL, Geurts JJ, Polman CH, et al. Magnetization transfer ratio measurement in multiple sclerosis normal-appearing brain tissue: limited differences with controls but relationships with clinical and MR measures of disease. Mult Scler 2007;13 (6):708-16.

191. Khaleeli Z, Cercignani M, Audoin B, Ciccarelli O, Miller DH, Thompson AJ. Localized grey matter damage in early primary progressive multiple sclerosis contributes to disability. Neuroimage 2007;37 (1):253-61.

192. Rovaris M, Judica E, Sastre-Garriga J, Rovira A, Sormani MP, Benedetti B, et al. Large-scale, multicentre, quantitative MRI study of brain and cord damage in primary progressive multiple sclerosis. Mult Scler 2008;14 (4):455-64.

193. Fisher E, Lee JC, Nakamura K, Rudick RA. Gray matter atrophy in multiple sclerosis: a longitudinal study. Ann Neurol 2008;64 (3):255-65.

194. Sanfilipo MP, Benedict RH, Weinstock-Guttman B, Bakshi R. Gray and white matter brain atrophy and neuropsychological impairment in multiple sclerosis. Neurology 2006;66 (5):685-92.

195. Benedict RH, Ramasamy D, Munschauer F, Weinstock-Guttman B, Zivadinov R. Memory impairment in multiple sclerosis: correlation with deep grey matter and mesial temporal atrophy. J Neurol Neurosurg Psychiatry 2009;80 (2):201-6.

196. Agosta F, Pagani E, Caputo D, Filippi M. Associations between cervical cord gray matter damage and disability in patients with multiple sclerosis. Arch Neurol 2007;64 (9):1302-5.

197. Anderson V, Fisniku L, Altmann D, Thompson A, Miller D. MRI measures show significant cerebellar gray matter volume loss in multiple sclerosis and are associated with cerebellar dysfunction. Mult Scler 2009;15 (7):811-7.

198. Tedeschi G, Dinacci D, Lavorgna L, Prinster A, Savettieri G, Quattrone A, et al. Correlation between fatigue and brain atrophy and lesion load in multiple sclerosis patients independent of disability. J Neurol Sci 2007;263 (1-2):15-9.

199. Sepulcre J, Masdeu JC, Goni J, Arrondo G, Velez de Mendizabal N, Bejarano B, et al. Fatigue in multiple sclerosis is associated with the disruption of frontal and parietal pathways. Mult Scler 2009;15 (3):337-44.

APPENDICES

Appendix 1. Fatigue Severity Scale

Read each of the following 9 statements and circle a number from 1 to 7 based on how accurately it reflects your condition and the extent to which you agree or disagree that the statement applies to you. A low value (e.g. 1) indicates strong disagreement and a high value (e.g. 7) strong agreement with the statement.

- My motivation is lower when I am fatigued

- Exercise brings on my fatigue

- I am easily fatigued

- Fatigue interferes with my physical functioning

- Fatigue causes frequent problems for me

- My fatigue prevents sustained physical functioning

- Fatigue interferes with carrying out certain duties and responsibilities

- Fatigue is amongst my three most disabling symptoms

- Fatigue interferes with my work, family, or social life

Appendix 2. Epworth Sleepiness Scale

Consider the following situations, how likely are you to doze off or fall asleep, in contrast to feeling just tired? Even if you have not done some of these things recently try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation:

0 = would never doze

1 = slight chance of dozing

2 = moderate chance of dozing

3 = high chance of dozing

- Sitting and reading

- Watching TV

- Sitting inactive in a public place (e.g. a theatre or a meeting)

- As a passenger in a car for an hour without a break

- Lying down to rest in the afternoon when circumstances permit

- Sitting and talking to someone

- Sitting quietly after a lunch without alcohol

- In a car, whilst stopped for a few minutes in the traffic

Appendix 3. Ethics approval

The studies described in chapters' 2 through 5 were performed on healthy volunteers and patients following the approval of the Nottingham Research Ethics Committee. Reference numbers: NS100201 and NS090102. In addition, the study in chapter 5 was approved by the Medicines Control Agency. Reference number: MF 8000/12346.