The Impact of Obstructive Sleep Apnea-Hypopnea on Neurodegeneration in Patients with Multiple Sclerosis

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Abstract

Our recent study showed that obstructive sleep apnea-hypopnea (OSAH) was the most common sleep abnormality in the group of multiple sclerosis (MS) patients studied. Both MS patients and OSAH subjects from the general population show signs of neurodegeneration on magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS). However, the extent to which OSAH affects neurodegeneration in MS patients is unknown. The objective of this thesis was to examine the potential interaction effect between OSAH and MS on MRI and MRS markers of neurodegeneration. Our primary outcome measures were the metabolite ratios of N-acetyl groups/creatine (NA/Cr) in central and posterior brain, and normalized hippocampal volume. Reduced brain NA/Cr indicates neuronal injury or loss. Our secondary outcome measures were normalized brain volume, normalized cerebellar volume, normalized lateral ventricular volume, and cortical thickness. As previously reported, we found lower NA/Cr and reduced hippocampal volume in MS patients compared with subjects without MS, consistent with MS-associated neurodegeneration. We found a significant interaction effect of OSAH and MS treatment for posterior brain NA/Cr ($p = 0.0196$, 95% CI (0.023, 0.226)), suggesting an effect due to an interaction of immunomodulating treatment in MS and the pathology of OSAH. We did not find a significant interaction effect of MS and OSAH for central brain NA/Cr and normalized hippocampal volume, although we found that OSAH tended to be associated with reduced NA/Cr in subjects without MS ($p = 0.09$). In conclusion, we confirmed the presence of neurodegeneration in MS, but did not find a significant additional effect of OSAH on neurodegeneration in the group of MS patients studied. However, OSAH may be associated with central brain neurodegeneration in non-MS subjects. This potential finding would require confirmation in future studies.

Résumé

Notre étude récente démontre que le syndrome d'apnée-hypopnée obstructive du sommeil (SAHOS) est l'anomalie du sommeil la plus fréquente dans le groupe de patients atteints de la sclérose en plaques (SEP) étudié. Les patients atteints de la SEP et les populations souffrantes du SAHOS présentent des signes de neuro-dégénérescence détectable en imagerie et en spectroscopie par résonance magnétique (IRM et SRM). Cependant, l'influence du SAHOS sur la dégénérescence neuronale observée en SEP, s'il y en a une, n'est pas connue. L'objectif de cette thèse est d'examiner les effets de l'interaction potentielle entre le SAHOS et la SEP sur les marqueurs de neuro-dégénérescence IRM et SRM. Nos principales mesures de neuro-dégénérescence sont le ratio métabolique de N-acetylaspartate et de créatine (NA/Cr) dans les régions centrales et postérieures du cerveau ainsi que le volume de l'hippocampe normalisé. Un ratio NA/Cr diminué indique des pertes ou lésions neuronales. Nous avons choisi comme mesures exploratoires les volumes normalisés du cerveau, du cervelet et des ventricules, ainsi que l'épaisseur corticale. Tout comme des études précédentes l'ont démontré, nous avons également observé la diminution du ratio NA/Cr ainsi qu'une réduction du volume de l'hippocampe chez les patients SEP, ce qui correspond à une neuro-dégénérescence liée à la SEP. Nous avons observé un effet d'interaction significatif entre le SAHOS et le traitement contre la SEP, tel qu'indiqué par le ratio NA/Cr en région postérieure du cerveau, ($p = 0.0196$, 95% IC (0.023, 0.226)) et qui pourrait être dû à une interaction de traitement immunomodulateur dans la SEP et la pathologie du SAHOS. Nous n'avons pas trouvé un effet d'interaction significatif de la SEP et SOHAS pour le NA/Cr et volume de l'hippocampe dans la région centrale du cerveau, même si nous avons constaté que le SAHOS a tendance à être associé à une diminution NA/Cr chez des sujets sans SEP ($p = 0,09$). En conclusion, bien que nous ayons confirmé la présence de neuro-dégénérescence chez les patients atteints de SEP, nous n'avons pas trouvé un effet significatif supplémentaire du SAHOS sur la neuro-dégénérescence dans le groupe des patients atteints de SEP étudiés. Toutefois, le SAHOS pourrait être liée à la neuro-dégénérescence du cerveau central chez les sujets non atteints de la SEP. Cette hypothèse reste à être confirmée par de futures études.

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Abbreviations

AHI – Apnea-Hypopnea Index
BMI – Body-Mass Index
CHESS – Chemically Selective Saturation
CI – Confidence Interval
CNS – Central Nervous System
CPAP – Continuous Positive Airway Pressure
Cr – Creatine
EDSS – Expanded Disability Status Scale
FLAIR – Fluid Attenuated Inversion Recovery
GM – Grey Matter
mI – myo-inositol
MRI – Magnetic Resonance Imaging
MRS – Magnetic Resonance Spectroscopy
MRSI – Magnetic Resonance Spectroscopic Imaging
MS – Multiple Sclerosis
MSFC – Multiple Sclerosis Functional Composite Measure
NA – N-acetyl Groups
NAA – N-acetylaspartate
NAAG – N-acetylaspartyl-Glutamate
NMR – Nuclear Magnetic Resonance
OSAH – Obstructive Sleep Apnea–Hypopnea
PD – Proton Density
PDw – Proton Density-Weighted
PRESS – Point Resolved Spectroscopy
PSG – Polysomnography
RRMS – Relapsing–Remitting Multiple Sclerosis
SD – Standard Deviation
SNR – Signal To Noise Ratio
SPMS – Secondary–Progressive Multiple Sclerosis
PPMS – Primary–Progressive Multiple Sclerosis
PRMS – Progressive–Relapsing Multiple Sclerosis
RF – Radiofrequency
SIENAX – Structural Image Evaluation, Using Normalisation, Of Atrophy For Cross-Sectional Measurement
STEAM – Stimulated Echo Acquisition Mode
SVS – Single Voxel Spectroscopy
TE – Echo Time
TR – Repetition Time
T1 – Longitudinal Relaxation Time
T1w – T1-Weighted
T2 – Transverse Relaxation Time
T2w – T2-Weighted
VBM – Voxel-Based Morphometry
WM – White Matter

Introduction

In prior work from our group, obstructive sleep apnea-hypopnea (OSAH) is common in a group of patients with multiple sclerosis (MS). Both OSAH and MS can lead to neurodegeneration. The presence of both conditions may exacerbate the neurodegenerative process of MS. However, We do not know whether OSAH impacts neurodegeneration in MS. In this cross-sectional study, we used magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) to examine the potential interaction effect of MS and OSAH on markers of neurodegeneration. Our primary outcome measures were the metabolite ratios of N-acetyl groups/creatine (NA/Cr) in central and posterior brain, and normalized hippocampal volume. Reduced brain NA/Cr indicates neuronal injury or loss, and reduced regional and/or global volumes indicate neurodegeneration. Our secondary outcome measures were normalized brain volume, normalized cerebellar volume, normalized lateral ventricular volume, and cortical thickness.

Chapter 1 presents the background information on MS and OSAH, principles of MRI and MRS, the use of MR technology to measure neurodegeneration in MS, and a literature review of brain imaging studies in OSAH. Chapter 2 provides detailed experimental design, imaging protocol, MRI/MRS post processing, and statistical methods used. Chapter 3 summarizes primary and secondary results. Chapter 4 includes discussions of the results, limitations, future work, and conclusions of the study.

Chapter 1 Background and Literature Review

1.1 Introduction to MS

1.1.1 Epidemiology and clinical types of MS

In 1868, French neurologist Jean-Martin Charcot first identified and described a mysterious disease known today as multiple sclerosis (MS) (1). Almost 150 years later, the Multiple Sclerosis Trust estimates that there are 2.5 million people with MS in the world (2). The MS prevalence in Canada is among the highest in the world with an overall frequency of 240 per 100,000 people (3). MS is also one of the major disabling neurological diseases in young adults in North America, and is associated with an increased unemployment rate and depression (4, 5). Approximately 80-85% of patients are diagnosed with relapsing-remitting (RR) MS. RRMS onset occurs most typically in the second or third decade of life, and females are twice as likely to be diagnosed as males (6). Patients with RRMS experience recurrent but unpredictable attacks (relapses) with acute symptoms that usually recover partially or completely (remission) after each attack. Approximately 50% of patients with RRMS will progress into the secondary-progressive (SP) stage within 10 years. Patients with SPMS normally have less apparent remissions, and the disease produces a progressive clinical deterioration. There are also approximately 10% of patients diagnosed with primary-progressive (PP) MS. Patients with PPMS do not have attacks, but their disease course progresses with accumulation of disability from onset (7). In addition, an estimated 5% of patients are diagnosed with a very rare course of MS called progressive-relapsing (PR) MS. Patients with PRMS experience apparent attacks from onset, and the disease worsens steadily without remission (8).

1.1.2 Pathophysiology of MS

MS is an immune-mediated, chronic inflammatory disease of the central nervous system (CNS). The hallmark of MS is demyelinating plaques in the brain and spinal cord. Although the exact etiology of MS is unclear, researchers believe that MS is triggered by the combination of both genetic and environmental factors (9). In the initial stage of MS, auto-reactive T cells and demyelinating antibodies enter the central nervous system (CNS) by disrupting the blood-brain barrier. Consequently, the T cell-mediated inflammation induces acute swelling, and a cascade of signaling events that lead to immune-mediated attack on myelin and/or oligodendrocytes (6). In addition to demyelination, axonal injury also occurs due to the attack from a variety of reactive substances such as cytokines, proteolytic enzymes, and free radicals. Axonal injury eventually leads to axonal transection and degeneration, which provides an explanation for the irreversible neurological impairment in patients with progressive MS (10).

1.1.3 Clinical assessment of disabilities in MS

Neurologists commonly use the Kurtzke Expanded Disability Status Scale (EDSS) to quantify clinical disability in MS (11). Clinical disability in the EDSS is measured in eight functional systems, including pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, and other systems. The EDSS ranges from 0 to 10, with increments of 0.5 units. EDSS 0 indicates normal neurological function; 1.0 to 4.5 refers to patients with MS who are fully ambulatory while having minimal disability in some of the functional systems; 5.0 to 9.5 describes the level of impairment of ambulation, or the ability to transfer to/from a wheelchair or bed; 10 indicates death due to MS (11).

Although widely used, caution should also be taken when using the EDSS as a measure of neurological disability in MS. Beyond EDSS 4.5, the EDSS only assesses the level of ambulation, which does not reflect the wide variety of neurological impairments in patients with MS. The more recently developed MS functional composite measure (MSFC) may overcome some of the weaknesses of the EDSS (12, 13).

1.1.4 Treatment

Current clinically approved therapeutic strategies for treating RRMS patients are mainly disease-modifying immunomodulating treatments, including glatiramer acetate and interferon-β variants (14). Although immunomodulating treatments have been relatively non-effective in treating progressive MS (15), they have shown to reduce the frequency of acute, inflammatory lesions, and rate of accumulation of lesions compared with placebo controls in RRMS (16-19). In this study, we take into account immunomodulating treatments because they were used by a majority of patients with MS.

1.1.5 Sleep disorders in MS and OSAH

Patients with MS often experience more frequent sleep disturbances than the general population or patients with other chronic diseases (20). Patients with multiple sclerosis have significantly poorer sleep quality than normal controls, and poor sleep quality has been associated with fatigue in MS (21, 22). A recent study found a significant association of fatigue with sleep disorders in MS (23). Sleep disorders in MS include sleep disordered breathing, insomnia, narcolepsy, rapid eye movement (REM) sleep behaviour disorder, and restless legs syndrome (24, 25). Our group found that

obstructive sleep apnea-hypopnea (OSAH), the most prevalent form of sleep disordered breathing in the general population, is the most common sleep disorder in the population of MS patients studied (24).

1.2 Introduction to OSAH

1.2.1 OSAH

OSAH affects approximately 1-2% of women and 4% of men in the general middle-aged population in the US (26, 27). OSAH is characterized by repeated discrete episodes of upper airway narrowing (hypopnea) or complete collapse (apnea), which results clinically in repeated pauses in breathing or shallow breaths during sleep in the general population (28). The disrupted respiratory events are normally ended by a brief arousal, which increases the muscle tone of the upper airway to resume breathing. The consequences of OSAH include intermittent hypoxia (general lack of oxygen), hypoxemia (lack of oxygen in the arterial blood), and sleep fragmentation (28). OSAH is associated with obesity, excessive daytime sleepiness and may lead to chronic fatigue (28).

1.2.2 Pathogenesis and diagnosis of OSAH

The modulation of upper airway reflex is thought to be the key factor in the neurological pathogenesis of OSAH (29). During normal inspiration, the diaphragm creates a negative pressure in the upper airway. The pharyngeal dilator muscles (the genioglossus, the palatal muscles, and the pharyngeal constrictor muscles) are activated via respiratory centers to counter balance this force, in order to prevent collapse of the upper airway. This is called the upper airway reflex (30). When this mechanism fails, the upper airway

is at risk of collapsing. Both patients with OSAH and healthy controls have reduced upper airway dilator muscle activity at sleep onset (31). However, in patients with OSAH, the upper airway resistance drastically increases due to the reduced dilator muscle activity, which eventually leads to the complete collapse of the upper airway. In response, a combination of increased negative upper airway pressure, CO_2 pressure, and respiratory drive continue to rise until an arousal from sleep takes place. Consequently, all the dilator muscles are activated again and re-open the upper airway (32).

The exact mechanism of how the upper airway reflex becomes ineffective is complex. Three components of the upper airway reflex are considered important to the pathogenesis of OSAH (29). The first is the sensory component. Mechanoreceptors in the sensory component give feedback to the brain about sensory inputs such as muscle tone, airflow, pressure, and temperature. Dysfunction in mechanoreceptors result in decreased sensitivity to the input information from the upper airway and lungs. The impairments may preexist in patients with OSAH, or exist as a consequence of local damage to peripheral sensors, snoring, or OSAH itself (29). The second component is cortical arousability. As mentioned before, arousal during apnea is essential in restoring muscle tone and regaining breathing. The cortical arousability is reduced in OSAH, which means that in order to reach an arousal during apnea and/or hypopnea, one requires higher inspiratory input. Consequently it extends the duration and frequency of apneas and hypopneas. The exact reason for reduced arousability in OSAH is unclear, although it is thought to be associated with the sensitivities of chemoreceptors and mechanoreceptors (29). The third is the motor component. In OSAH, upper airway dilator muscles, particularly the genioglossus and the palatal muscles cannot provide

enough muscle tone to counter the negative pressure. The neural mechanisms of airway pressure regulation are complex. Some recent studies have proposed regulation pathways through nerve projections from the locus coeruleus, raphe nucleus, hypoglossal nucleus, nucleus tractus solitarius, and periobex (33, 34). Similar to the sensory components, impairment in the motor component could be due to a preexisting reduction in the muscle strength, or dysfunction due to snoring (29).

In-laboratory overnight polysomnography (PSG) is the gold standard to diagnose OSAH (35, 36). PSG allows measurement of the apnea-hypopnea index (AHI), calculated as the total number of apnea and hypopnea episodes per hour of sleep during PSG. An AHI of ≥ 15 is the standard threshold for the diagnosis of OSAH (37). Our group found a strong association specifically with OSAH and fatigue in MS (24).

1.2.3 Treatment

Continuous positive airway pressure (CPAP) is considered to be the most effective method in treating OSAH (38). The CPAP machine uses continuous air pressure to keep the airway open, therefore reducing respiratory disturbances during sleep (38). As a result, CPAP ultimately improves daytime sleepiness and performance associated with somnolence (39). However, patient compliance to the CPAP treatment remains a challenge for CPAP to reach its full therapeutic potential (40, 41).

1.2.4 Neural injury in OSAH

Patients with OSAH often exhibit neurobehavioural impairments, including fatigue, daytime sleepiness, depression, decreased vigilance, etc. (42). Oxidative stress as a

consequence of intermittent hypoxia provides a model for assessing the mechanism of neural injury in OSAH (43-45). Animal studies have shown that hypoxic insult to neurons induce modulations of ion channels (46), damage to the cerebellar cortex (47), brain stem and basal forebrain (48), and CA1 region of the hippocampus (49-51). However, it is rather difficult to demonstrate a direct association between the neurobehavioural impairments and OSAH in humans. Patients often have comorbidities such as cerebrovascular disease, hypertension, and diabetes that are also associated with neural injury (38).

Neuroimaging in humans provides a great potential to investigate non-invasively possible neuronal injury related to OSAH. The chapters below will introduce two neuroimaging techniques used in measuring neurodegeneration for this study. Following this, a review of published neuroimaging studies in MS and OSAH will be presented.

1.3 Introduction to MRI and MRS

1.3.1 Principles of MRI

Magnetic resonance imaging is a powerful non-invasive tool for examining the pathology of neurological diseases. It is based on the principles of nuclear magnetic resonance (NMR). Nuclei with an odd number of protons and/or neutrons have an angular momentum, which gives rise to a magnetic moment, or spin. A proton (^{1}H) in a water molecule is a good example of such nuclei. MRI primarily uses protons to generate images due to the abundance of water in the brain. When placed in a strong external magnetic field B_0, protons precess around the direction of B_0 at a specific frequency (Larmor Frequency), and exhibit a net magnetization in the direction of B_0. When we

apply a transient second magnetic field (a pulse) perpendicular to B_0 at specific radiofrequencies (RF), it interacts with the net magnetization and takes it out of its previous alignment with B_0. After the RF pulse is turned off, the net magnetization slowly returns to its original state. This process is called relaxation. The change in magnitude of the net magnetization vector can be measured by the signal detection coil in the MR scanner, and described in terms of current according to Faraday's law. The strength of the signal can then be represented as the intensity of that location in the image. Water in different chemical environments (tissues) has different physical properties in terms of proton density, T1 (longitudinal relaxation time), and T2 (transverse relaxation time). By carefully choosing the RF pulses, the repetition time (TR), and the echo time (TE), we can obtain images with optimized contrasts between specific tissues. This is important in MS because damaged tissues due to pathology may change the signal intensity and appear different from healthy tissues. Therefore, we can use MRI to assess MS disease activity and burden (52).

1.3.2 MR image modalities

As mentioned earlier, a combination of T1, proton density, and T2 parameters determines tissue intensities in MR images. Modalities are usually designed in a way that one of the three parameters is heavily weighted over the other two. A T1-weighted (T1w) image has maximized T1 difference between grey matter (GM), weight matter (WM), and cerebrospinal fluid (CSF). It gives good contrasts between tissues, and therefore is used as the input modality for a number of image-processing algorithms. A T1w image has bright WM, intermediate GM, and dark CSF. A T2-weighted (T2w) image emphasizes

on the T2 difference between tissues, which is sensitive to pathologies that affects T2 relaxation rates of tissues, e.g. inflammation, edema, etc. A T2w image has bright CSF and MS lesions, less bright GM, and dark WM. Conventionally, clinicians use total volume of lesions quantified on a T2w MR image to measure the accumulated MS disease burden (53). However, cross-sectional and longitudinal studies have not shown a strong correlation between T2w lesion volume and clinical disability (54-56). A proton density-weighted (PDw) image minimizes both the T1 and T2 effects, and the result is between T1w and T2w images. A PDw image has bright GM and lesions, less bright WM, and dark CSF. Fluid attenuated inversion recovery (FLAIR) is an inversion recovery pulse sequence designed to suppress the signal from liquid in the brain. By selecting an appropriate TI, FLAIR can produce images with better MS lesion contrast, particularly between CSF and periventricular MS lesions, than T2w and PDw images (57).

Figure 1-1 shows 4 types of MRI modalities of the same brain region of a patient with MS: T1-weighted (T1w), T2-weighted (T2w), proton density-weighted (PDw), and fluid attenuated inversion recovery (FLAIR) images. We can easily identify the hyperintense periventricular lesions using the FLAIR sequence.

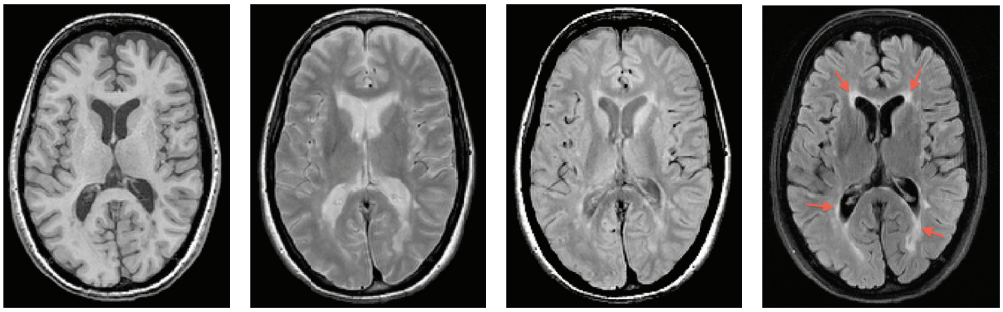


Figure 1-1. Axial MRI modalities of the same region of the brain. a) T1w; b) T2w; c) PDw; d) FLAIR (with arrows showing hyperintense periventricular lesions).

1.3.3 MRI and measuring brain atrophy in MS

MRI can be used to assess brain atrophy, a marker for neurodegeneration in neurological diseases (58). Brain atrophy indicates irreversible damage to the central nervous system, (52). A series of image processing techniques have been developed in the past two decades to measure total and regional volumes of the brain. Normalized total brain volume has become a reliable and sensitive marker for quantifying global neurodegeneration in MS (59, 60). To measure brain volume, one can use a tessellated mesh to model the brain surface, and extract the brain based on tissue intensity. Then the brain and skull are registered to a standard brain template, followed by tissue segmentation to generate the brain volume (59). The brain volume is multiplied by a volumetric scaling factor (normalization) based on the previous registration step, which corrects for normal variations in head size (59). In addition, patients with MS have regional brain atrophy including smaller hippocampi, smaller cerebellum, enlarged lateral ventricles, and thinner cortex (58, 61, 62). The initial steps of calculating these brain volumes usually involve intensity correction, registration to a standard pre-labeled brain

template, and estimation of the volume of the brain structure based on the volume in the standard brain template and the transformation matrix from registration (63). The raw volumes of hippocampi, cerebellum, and lateral ventricles are calculated first, and then normalized using the same volumetric scaling factor as in normalized total brain volume mentioned before.

1.3.4 Principles of MRS

Proton magnetic resonance spectroscopy is a technique for detecting the chemical profile in a given sample. When used on biological tissues, MRS reveals the metabolic products of biological reactions, which can be very useful in probing normal and dysfunctional metabolism. MRS is advantageous to conventional MRI in the sense that it can detect subtle changes of the chemical profile in normal appearing brain tissues, where pathological features are not detectable in conventional MRI. Based on the same principles as proton NMR, when we apply an external magnetic field, protons in different chemical environments experience different effective magnetic fields due to the chemical shielding effect from surrounding electrons. The strength of chemical shielding depends on the electron density around the proton, which can be affected by adjacent atoms. Therefore, protons with different neighbouring atoms resonate at slightly different frequencies. The difference of resonant frequencies between the proton of interest and the proton in a standard reference molecule (normally tetra-methylsilane, TMS) is called chemical shift. For convenience, chemical shift is expressed in parts-per-million (ppm), and is independent of the static magnetic field B_0. Using chemical shift, we can identify

the chemical environment of protons on an MR spectrum, and hence reveal the metabolic profile of the region of interest.

There are two basic types of spectroscopy commonly used in clinical applications, multi-voxel spectroscopy and single-voxel spectroscopy (SVS). Multi-voxel spectroscopy can provide single or multiple slices of regions in the brain based on one, two, and three-dimensional acquisition of multiple voxels. Multi-voxel spectroscopy is also known as MRS imaging (MRSI), for its ability to incorporate chemical information from each voxel with anatomical information in the brain from conventional MRI. MRSI can provide a wide coverage of the brain to illustrate the overall brain metabolic profile. In contrast, SVS usually provides higher signal to noise ratios for much more specific regions in the brain. Both localization techniques use three intersecting orthogonal excited slices to create a region of interest, a voxel.

Two commonly used localization techniques are STimulated Echo Acquisition Mode (STEAM) and Point RESolved Spectroscopy (PRESS) (64, 65). Both localization techniques use three intersecting orthogonal excited slices to create a region of interest, a voxel. STEAM offers voxels with sharper localization, whereas PRESS provides almost twice as much signal to noise ratio as STEAM, and is less sensitive to subject motion in the scanner than STEAM (64, 65). In order to measure concentrations of metabolites in the brain, one needs to suppress water signals first. Water concentration is normally in the 70 M range, whereas brain metabolites are usually in the range of 1 – 10 mM (66). A commonly used method to suppress water signal is called CHEmically Selective Saturation (CHESS) (67).

In an MRS spectrum of a human brain, we estimate the concentration of a metabolite by calculating the area under its resonance peak. In this project, the calculation has been done using the automated program LCModel for both MRSI and SVS (68). LCModel uses a combination of linear models and externally measured chemical references to estimate the concentration of each metabolite of interest (68). A spectrum of an MRSI voxel is shown in **Figure 1-2.**

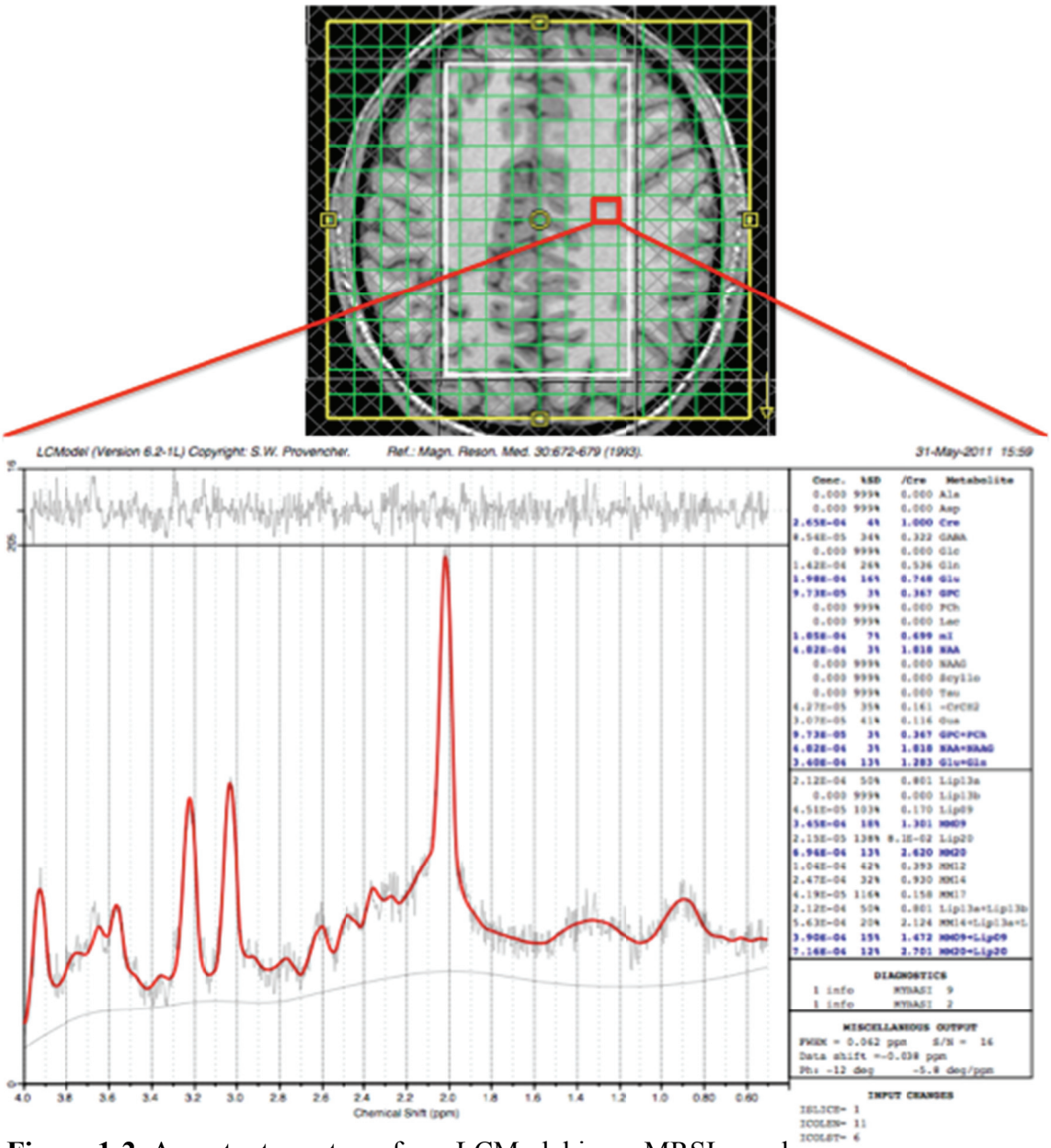


Figure 1-2. An output spectrum from LCModel in an MRSI voxel.

1.3.5 MRS and neuroaxonal injury in MS

MRSI allows the in-vivo monitoring of a number of brain metabolites. Of particular interest is the total signal from the resonance of N-acetyl groups (NA) at 2.0 ppm, which is composed primarily of the signal from N-acetylaspartate (NAA), with a small contribution from N-acetylaspartyl-glutamate (NAAG). NA can be used as an in-vivo marker of neuroaxonal integrity because both NAA and NAAG are synthesized in neuronal mitochondria, and are found primarily in neurons and axons in the adult human brain (69-71).

Another important brain metabolite assessed by MRS is creatine (Cr). Cr usually appears as a composite peak at 3.0 ppm, consisting of Cr and phosphocreatine (PCr). Cr and PCr are important reserves of high-energy biochemicals in neurons and glia (72). Since the total concentration of Cr and PCr is relatively stable and unaffected by MS, it is often used as an internal standard with which to normalize other metabolites (73). The ratio of NA/Cr is decreased in MS, generally interpreted as neuroaxonal injury and loss (74-76).

Other metabolites that can be assessed by MRS are choline (Cho), myo-inositol (mI), and glutamate. The Cho peak at 3.2 ppm consists of compounds that are associated with membrane phospholipids (72). In acute MS plaques, the ratio of Cho/Cr is increased, which is believed to indicate active demyelination and inflammation (74, 77). The mI peak appears at around 3.5 ppm. Elevated mI could possibly indicate glial cell proliferation or gliosis in MS (78-80). The glutamate peak is found at 2.35 ppm, and may indicate inflammation (81).

1.4 MRI/MRS studies on neuronal injury in OSAH

1.4.1 Introduction

There have been an increasing number of studies showing that untreated OSAH is associated with adverse health outcomes (82). The mechanisms of how OSAH increases medical morbidity remain unclear. However, neuroimaging studies in OSAH have significantly improved our understanding of brain structure and metabolism in patients with this condition (83). Researchers have applied techniques such as MRI and MRS to elucidate the central nervous system consequences of OSAH in the general population.

1.4.2 Brain atrophy and OSAH

MRI allows us to non-invasively examine the neuroanatomical abnormalities in patients with OSAH. In an early case-control study, Davies et al (84) quantitatively compared white matter hyperintensities in 45 patients with moderate to severe OSAH with 45 controls without OSAH. Although patients with OSAH had increased arterial blood pressure compared to controls, both groups had a similar prevalence of subclinical cerebrovascular disease on MRI. Another study conducted by Gale and Hopkins (85) examined memory performance and hippocampal volume in patients with severe OSAH. On comparison with a previously established normative sample of quantitative brain volumes, 36% of patients with OSAH had hippocampal atrophy (85). Hippocampal volumes were associated with oxygen saturation, and only with selected tests in memory performance in patients with OSAH (85).

Other than manual volumetric methods, a frequently used technique in brain MRI volumetric analysis is voxel-based morphometry (VBM) (86). VBM is an automated

method that compares the volume of grey matter between two groups of subjects on a voxel-by-voxel basis (86). Several VBM studies showed differences of grey matter volume in various brain regions between patients with OSAH and controls. However, results were often inconsistent. Researchers reported grey matter volume loss in the frontal, parietal, and temporal cortices, anterior cingulate, hippocampus, and cerebellum (87), and bilateral prefrontal cortex, bilateral inferior parietal gyri, right temporal cortex, occipital cortex, right thalamus, left putamen, caudate nucleus and pallidum, right hippocampus, parahippocampal gyrus, and cerebellum (right cerebellar hemisphere and vermis) (88). Both studies used a relatively liberal threshold for statistical significance without correcting for multiple comparisons (87, 88). Using the same technique, Morrell and colleagues found that patients with moderate OSAH had grey matter volume loss only in the left hippocampus (n=7) (89). They used a level of significance selected on the basis of an a priori hypothesis ($p<0.01$) (89). In a larger study, researchers found a reduction of grey matter volume in the right middle temporal gyrus, and the left cerebellum in patients with OSAH (90). Nonetheless, a more stringent statistical analysis ($p<0.05$, corrected for multiple comparisons using false discovery rate) found no difference in grey matter change in any region of the brain (91). In addition, reduced grey matter concentration was found in patients with moderate-severe OSAH in the left gyrus rectus, bilateral superior frontal gyri, left precentral gyrus, bilateral frontomarginal gyri, bilateral anterior cingulate gyri, right insular gyrus, bilateral caudate nuclei, bilateral thalami, bilateral amygdalo-hippocampi, bilateral inferior temporal gyri, and bilateral quadrangular and biventer lobules in the cerebellum (92). Interestingly, the same study also found no significant difference in grey matter volume between patients and controls,

suggesting that grey matter volume and concentration reflect different pathologies of OSAH (92). Torelli et al (93) showed decreased grey matter volume in left and right hippocampi and within more lateral temporal areas in patients with moderate-severe OSAH using VBM, and lower volumes of cortical grey matter, smaller right hippocampus, and right and left caudate in patients using Freesurfer (93-96). The factors that may account for the inconsistencies in these VBM studies are sample size and homogeneity, MRI scanner field strength, and statistical correction for both multiple comparisons and age (83).

In addition to grey matter, two studies also examined white matter integrity in OSAH. In an MRI population-based study, white matter disease in the brainstem was not associated with the AHI, with or without adjusting for potential confounders (97). In contrast, the arousal index (number of arousals per hour of sleep) was inversely associated with brainstem white matter disease, and the authors mentioned that its clinical significance should be further investigated (97). In a diffusion tensor imaging study, Macey and collegues (98) reported lower white matter integrity in the anterior corpus callosum, anterior and posterior cingulate cortex and cingulum bundle, right column of the fornix, portions of the frontal, ventral prefrontal, parietal and insular cortices, bilateral internal capsule, left cerebral peduncle, middle cerebellar peduncle and corticospinal tract, and deep cerebellar nuclei. This study suggested that axons linking major structures within the limbic system, pons, frontal, temporal and parietal cortices, and projections to and from the cerebellum were affected in patients with OSAH (98).

1.4.3 Neuronal injury and brain metabolites in OSAH

Compared with MRI, MRS can detect subtle metabolic impairment in the normal-appearing brain tissues in OSAH. Kamba et al. used MRSI to examine NA/Cr, Cho/Cr, and NA/Cho in mild and moderate to severe OSAH patients, as well as controls without neurological disease (99). They found that moderate to severe OSAH patients had significantly lower NA/Cho in the posterior periventricular white matter compared to both mild OSAH patients and controls. The authors concluded that these metabolite differences were due to cerebral damage caused by repeated apneic episodes (99). Although this study was the first to evaluate cerebral metabolism in OSAH using MRS, it was potentially confounded by the uncontrolled co-morbidities in patients with OSAH.

A later study by the same group was conducted to determine the relationship between NA/Cho in cerebral white matter and the severity of OSAH measured by AHI (100). 55 patients with severe OSAH were examined by MRSI and standard overnight PSG, after excluding the presence of hypertension, cardiac disease, diabetes mellitus, and hyperlipidemia (100). Results from this study showed that NA/Cho in the cerebral cortex was negatively associated with age, but not significantly associated with OSAH severity. However, NA/Cho in the posterior periventricular white matter was negatively associated with OSAH severity, after adjusting for age and cardiac disease (100). The authors' findings supported their previous study, and the hypothesis that cerebral hypoxia was responsible for the metabolic impairment in the deep white matter. In addition, the authors suggested an effect of cerebrovascular risk factors on cerebral metabolism in OSAH (100). Halbower et al. reported increased Cho/Cr in the left hippocampus, and decreased NA/Cho in both the left hippocampus and right frontal cortex in children with

severe OSAH, compared with age and sex-matched healthy children (101). The authors also found that children with severe OSAH had significant deficits in IQ and executive functions. However, there was no evaluation of the association between the cognitive performance and metabolic dysfunction. The authors speculated that untreated childhood OSAH could permanently limit the child's cognitive function (101). Although the results from the 3 studies were in general consistent, the interpretation of results was limited due to the non-specificity of NA/Cho.

Compared to NA/Cho, NA/Cr assesses neuroaxonal damage more specifically. Therefore, NA/Cr was examined in a few other studies. Bartlett et al. reported increased NA/Cr in the left hippocampus, and a significant association of NA/Cr and performance on a cognitive test in OSAH patients compared to age and occupation-matched controls (102). The authors also suggested that decreased Cr due to changes in brain bioenergetics caused by hypoxic damage was responsible for the increased NA/Cr (102).

In contrast, decreased NA/Cr was found in different areas in two studies. Achanatis et al. reported decreased NA/Cr in the frontal periventricular white matter in patients with untreated severe OSAH compared with age and sex-matched controls (103). They also found decreased Cho/Cr, decreased absolute NA and Cho in the frontal periventricular white matter. A subgroup analysis of patients without co-morbid medical conditions also agreed with the main results of the study (103). This study also suggested a link between neuroaxonal dysfunction in the frontal periventricular white matter and cognitive executive deficits in patients with OSAH (104, 105).

Sarchielli et al. also found lowered NA/Cr in frontal and temporal regions bilaterally in patients with moderate OSAH compared with controls (106). In addition, elevated

Cho/Cr in bilateral temporal regions, and elevated mI/Cr in both frontal and temporal regions were seen in the same group comparison. The authors suggested that repeated episodes of hypoxia were the possible origin of these metabolic dysfunctions in OSAH (106).

Tonon et al. conducted an interesting study to evaluate metabolic changes before and after continuous positive airway pressure (CPAP) treatment (107). They found that the absolute concentration of NA was lower in the parietal-occipital cortex in severe OSAH patients than in age and sex-matched controls at baseline. The reduction of NA persisted after 6 months of CPAP treatment, despite improved sleep quality and better cognitive performance (107). The results also showed significant correlations of minimum oxyhemoglobin saturation during sleep and the level of NA, as well as objective sleepiness measured by Multiple Sleep Latency Test and NA (107). This study supported the hypothesis that intermittent hypoxia, rather than arousals or sleep fragmentation, may be responsible for irreversible neuroaxonal damage (107).

In summary, the MRI/MRS results on OSAH were mostly inconsistent due to different experimental protocols and patient populations. Therefore, new studies with good experimental designs would be helpful to explain some of the inconsistent results of previous studies.

1.5 Hypothesis

We hypothesize that OSAH exacerbates neurodegeneration in patients with MS. The main outcome measures of neurodegeneration to be evaluated are NA/Cr and normalized hippocampal volumes. We expect a significant interaction effect of OSAH and MS to

reduce NA/Cr (indicating neuroaxonal injury) in the central brain and posterior cingulate medial cortex, as well as the size of hippocampus, after adjusting for potential confounders. Our secondary outcomes will be normalized brain volume, cerebellar volume, lateral ventricular volume and cortical thickness.

Chapter 2 Methodology

2.1 Ethical issues

The study was approved by the Research Ethics Board at the Montreal Neurological Institute and Hospital. All study subjects provided written informed consent for participation in the study.

2.2 Study design and subjects

The study design was cross-sectional. We used established clinical and laboratory criteria to diagnose MS (108). Based on a type 1 error rate of 5% and 80% power, we planned to recruit 40-60 patients with MS in order to be able to detect a clinically important difference of a 6% - 8% reduction in the NA/Cr ratio (109). Most MS patients for this study (n=42) were recruited from a population of patients who participated in a previous study on sleep abnormalities in MS (110), and had been recruited from the Montreal Neurological Hospital MS Clinic. These patients already underwent blood tests, pulmonary function testing, clinical evaluation, and polysomnography (PSG). If the PSG was performed > 6 months before the MRI/MRS examination, the subject was asked to undergo another PSG within 6 months of the MRI. Repeat PSG was mandatory if there had been a major change in clinical status (e. g. 10% change in body weight).

We also recruited 9 new MS patients from the Montreal Neurological Hospital MS Clinic. New patients with MS underwent the same evaluations as our previous patients, utilizing the same inclusion/exclusion criteria.

Controls without neurological disorders were recruited from a population of controls who participated in our previous study (n=16). Additional new controls without neurological

disease were recruited for this study as necessary (n=13). Controls were recruited from dermatology and preoperative clinics (patients and companions), local volunteers (friends of research team members), and from posted library advertisements. Overall, we recruited 49 patients with MS and 29 controls without neurological disorders for the study.

2.2.1 Inclusion criteria

For MS patients: 1) those recommended by the International Panel on the Diagnosis of MS (108), 2) relapse free for at least 30 days prior to study appointment.

2.2.2 Exclusion criteria

1) age <18 years, 2) pregnancy, 3) cognitive or psychiatric impairments which could preclude compliance with informed consent, study procedures, or study requirements, 4) presence of other significant neurological difficulties (other than MS), 5) presence of other significant pulmonary, otorhinological disorders, and medical disorders, 6) Expanded Disability Status Scale (EDSS) > 7.0, 7) forced vital capacity of < 60% on pulmonary function testing, 8) any value of > 1.5 times the upper limit or <0.75 times the lower limit (where applicable) of the reference range for any of the baseline/screening laboratory evaluations (with the exception of iron profile, vitamin B12 and folate levels) which is clinically significant, and 9) presence of metallic objects or other devices which could interfere with MRI. Exclusion criteria for normal controls were similar to those for MS subjects, except that controls did not undergo blood test or pulmonary function testing.

2.3 Study procedures

All subjects in this study underwent complete overnight in-laboratory PSG at the MUHC Sleep Laboratory. All subjects were evaluated by a physician researcher at the time of the MRI.

2.4 Polysomnography

PSG was performed using the Harmonie System (Stellate, Montreal, QC), with recording of standard electroencephalographic leads (C4/C3/F3/F4/O1/O2/M1/M2), bilateral electrooculogram, chin, anterior tibialis & extensor digitorum electromyograms, airflow via nasal pressure cannula, thoracoabdominal movements via inductive plethysmography (Respitrace Systems, Ardsley, NY), single lead EKG, pulse oximetry, snoring, digital video recording, and body position.

A single certified PSG technologist with expert physician review scored all polysomnographic records, according to American Academy of Sleep Medicine Task Force (AASM) criteria (111) except that respiratory analysis was based on standard AASM research criteria (112), where an apnea was defined as airflow cessation for ≥ 10 sec and a hypopnea as airflow reduction for ≥ 10 sec by ≥ 50%, or associated with arousal or desaturation ≥ 4%. The number of apneas and hypopneas per hour of sleep were used to calculate the AHI.

2.5 MRI/MRS data acquisition

A combination of ^{1}H MR imaging and spectroscopic examinations of the brain were conducted for each subject on a SIEMENS TIM TRIO scanner at 3.0 T (Siemens AG,

Erlangen, Germany) at the McConnell Brain Imaging Centre of the Montreal Neurological Institute.

2.5.1 MRI data acquisition

For each subject, the magnet isocentre was placed at thalamic eminentia media. 192 transverse contiguous 1-mm-thick T1-weighted anatomical images were acquired parallel to the callossal line, using a 3D gradient-recalled echo technique (FLASH, TR 18 ms, TE 5ms, 256 x 256 matrix, 256-mm field of view, 27-degree flip angle) (**Figure 2-1a**). Following that, 60 contiguous slices of 3-mm-thick proton density (PD) (**Figure 2-1b**) and T2-weighted images (TR 2100ms, TE 16ms (PD), 80ms (T2), 256 x 256 matrix, 256-mm field of view, 120 degree flip angle) were acquired using a dual-echo spin-echo sequence (**Figure 2-1c**). In addition, 60 contiguous slices of 3-mm-thick Fluid Attenuated Inversion Recovery (FLAIR)-weighted images (TR 10000ms, TE 83ms, TI 2500ms, 256 x 256 matrix, 256-mm field of view, 120-degree flip angle) were also acquired (**Figure 2-1d**).

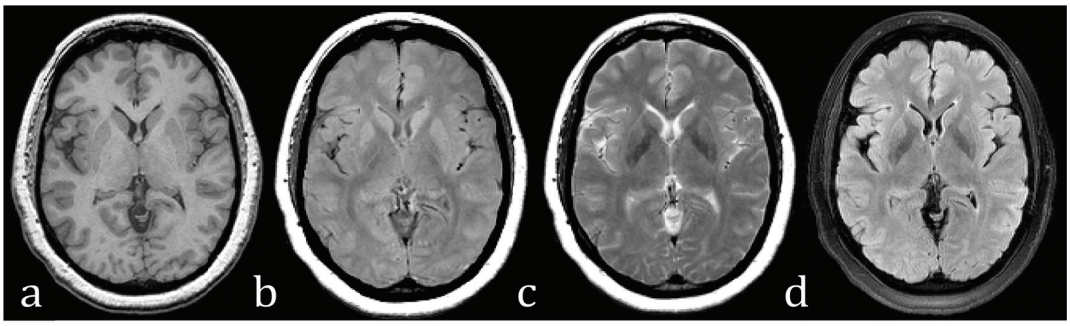
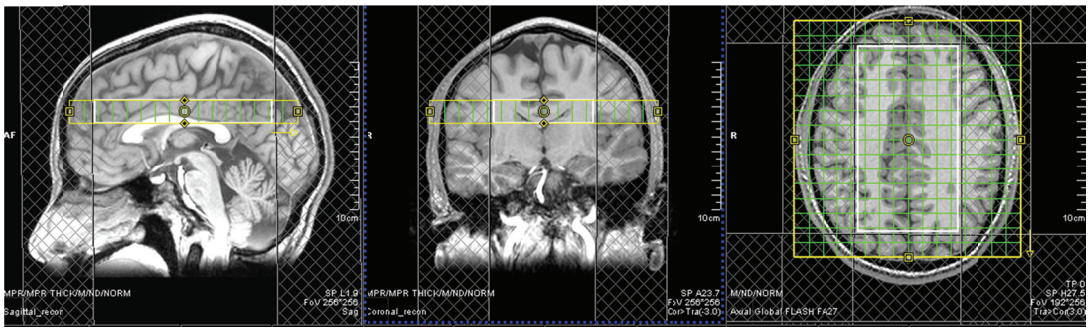


Figure 2-1. Acquired MR images. a) T1-weighted image, b) PD-weighted image, c) T2-weighted image, and d) FLAIR-weighted image.

2.5.2 MRS data acquisition

The MRSI image was acquired using a PRESS sequence (TR 3000 ms, TE 30 ms, 16 x 16 phase encodes, 160 x 160 field of view, 2 signal averages with concentric k-space weighting). A water-suppression pulse (CHESS) was placed at the beginning of the MRSI sequence. The PRESS excitation volume was LR (Left-Right): 70mm, AP (Anterior-Posterior): 125mm, FH (Feet-Head): 15mm, and included mainly the deep white matter of the superior and medium frontal gyrus, and of the pre-central gyrus (**Figure 2-2a**). Voxels in this area could provide a reliable estimate of overall NA/Cr in the central brain. Within the region of interest (ROI) of 70 mm (LR) x 125 mm (AP) x 15 mm (FH), 6 x 12 voxels were chosen for analysis.

Single voxel spectroscopy (SVS) image was acquired using a PRESS sequence (TR 2000 ms, TE 30 ms, voxel size of left-right 30 mm, anterior-posterior 30 mm, and foot-head 20 mm, 196 signal averages). A water-suppression pulse (CHESS) was placed at the beginning of the SVS sequence. The voxel was placed in the posterior-cingulate area (RL30mm, AP30mm, FH20mm) to cover the medial parietal-occipital grey matter (**Figure 2-2b**).



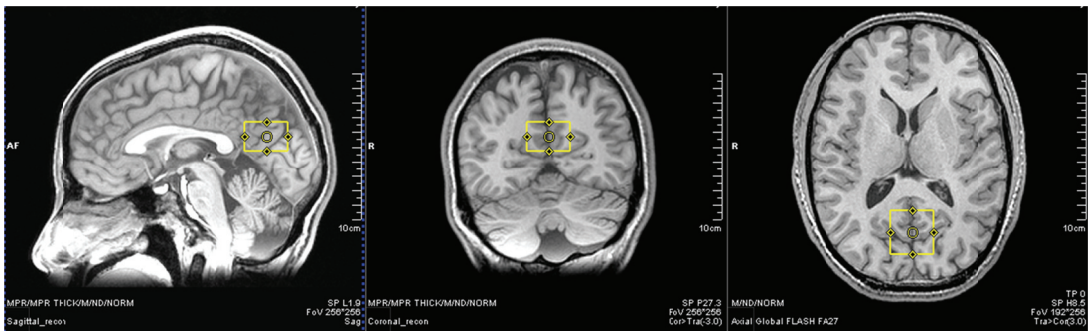


Figure 2-2. First row: a) MRSI volume of interest; second row: b) SVS volume of interest.

2.6 Clinical Assessment

All study subjects underwent a clinical evaluation at the time of the MRI/MRS with a physician-researcher. This included a medical history, comprehensive sleep assessment (sleep questionnaires), vital signs, height, weight, and completion of standardized questionnaires to assess fatigue (Fatigue Severity Scale (113)) and Multidimensional Fatigue Inventory (114), subjective sleepiness (Epworth Sleepiness Scale (114)), sleep quality (Pittsburgh Sleep Quality Index (114)), pain (using a 10 cms visual analog scale), depression (Centre for Epidemiologic Studies – Depression Scale (115)), stress (Perceived Stress Scale (116), and health-related quality of life (Medical Outcomes Study Short Form-36 (SF-36) (117). MS subjects also underwent a physical exam including neurological exam with calculation of the EDSS (118). As noted above, new MS subjects also underwent pulmonary function testing and blood test.

2.7 Outcome measures

The main outcome measures of neurodegeneration were NA/Cr in central and posterior brain, and normalized hippocampal volumes. Our secondary exploratory outcomes were

normalized brain, cerebellar, and lateral ventricular volumes, as well as cortical thickness.

2.8 MRI post-processing

Six processing pipelines were applied to each subject to generate T2 lesion masks and all MRI outcome measures. The automated T2 lesion masks were first generated by the Classify Quick Pipeline (Simon Francis, Magnetic Resonance Spectroscopy Unit, MNI) (119). The lesion masks were then manually corrected by myself (I received specialized training to do so) after visual quality control of the results. An example of a lesion mask before and after correction is shown in **Figure 2-3**. For the MRI outcome measures, normalized hippocampal, lateral ventricular and cerebellar volumes were calculated from an automated segmentation tool (Prof. Louis Collins, Image Processing Lab, MNI) (120). Segmentation images for quality control are shown in **Figure 2-4** (hippocampi), **Figure 2-5** (lateral ventricles), and **Figure 2-6** (cerebellum). Normalized brain volumes (NBV) were generated using SienaX (FSL, Oxford) (59). Brain segmentation images for quality control are shown in **Figure 2-7**. Cortical thickness was calculated using Freesurfer (Freesurfer, Harvard) (121). The cortical thickness segmentation image for quality control is shown in **Figure 2-8**. I also visually checked all automated results.

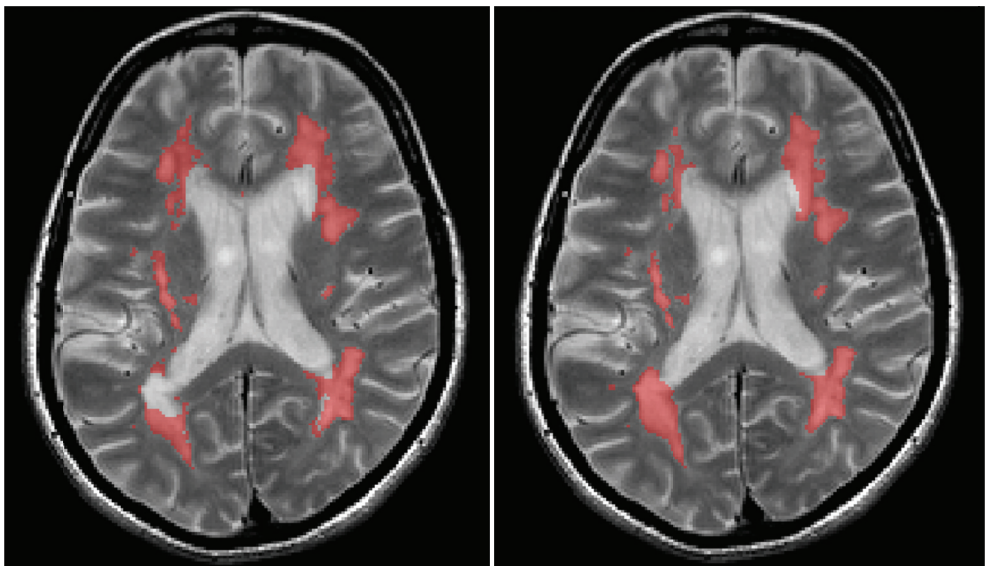


Figure 2-3. Left: automated T2 lesion mask (in red) before manual correction superimposed on a T2-weighted image; Right: T2 lesion mask after manual correction (in red) superimposed on a T2-weighted image.

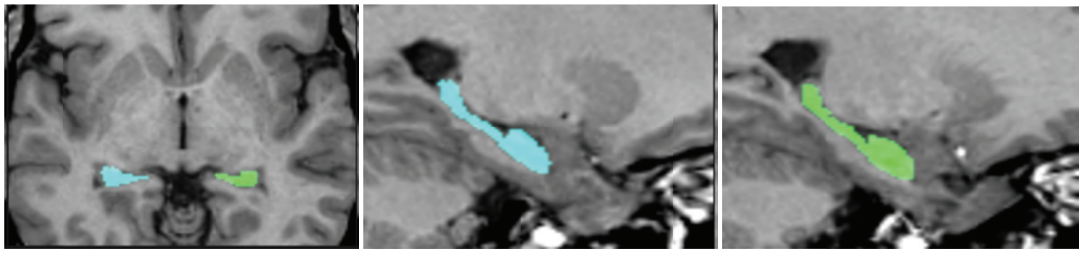


Figure 2-4. Automated segmentations of the left and right hippocampi in T1-weighted images. Left: both hippocampi labeled in colours in the axial view of a T1-weighted image; Middle: the left hippocampus labeled in turquoise in the sagittal view; Right: the right hippocampus labeled in green in the sagittal view.

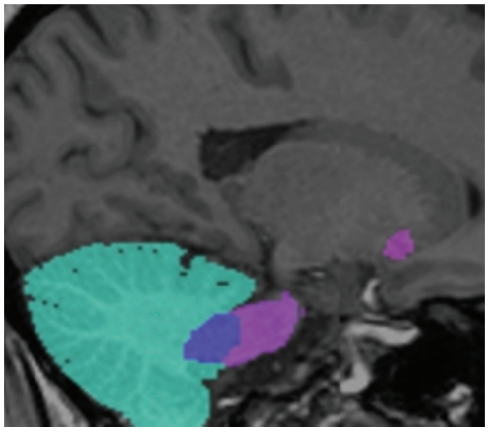


Figure 2-5. An automated segmentation of the cerebellum labeled in turquoise in a T1-weighted image.

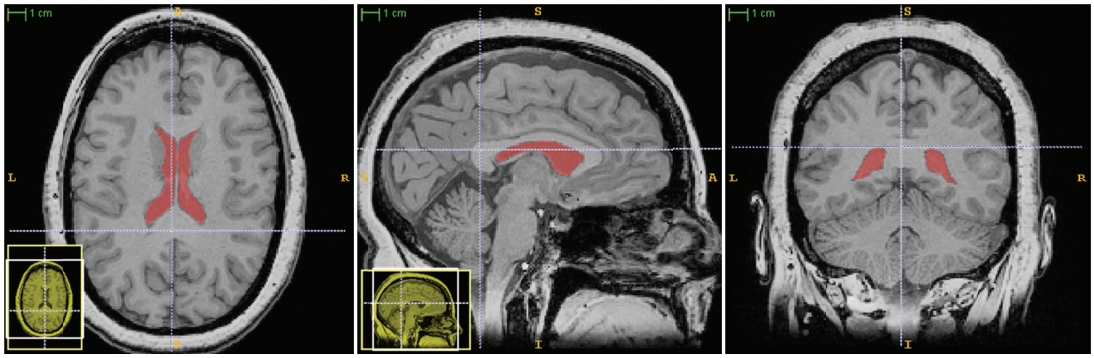


Figure 2-6. An automated segmentation of the lateral ventricles labeled in red in T1-weighted images.

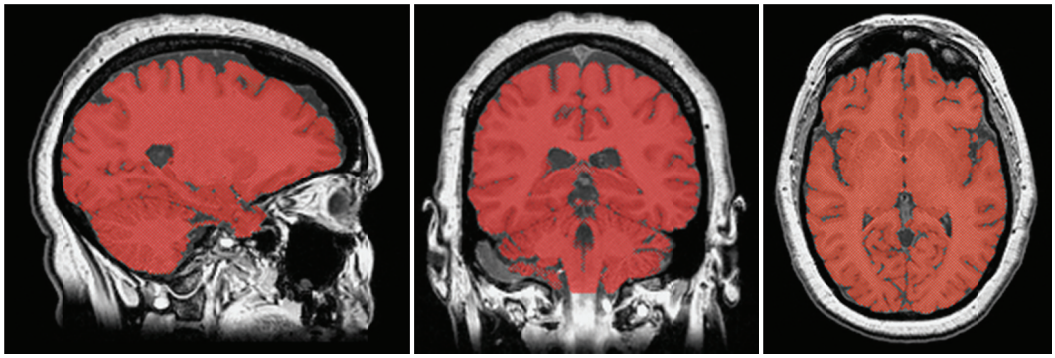


Figure 2-7. An automated segmentation of the brain labeled in red in T1-weighted images.

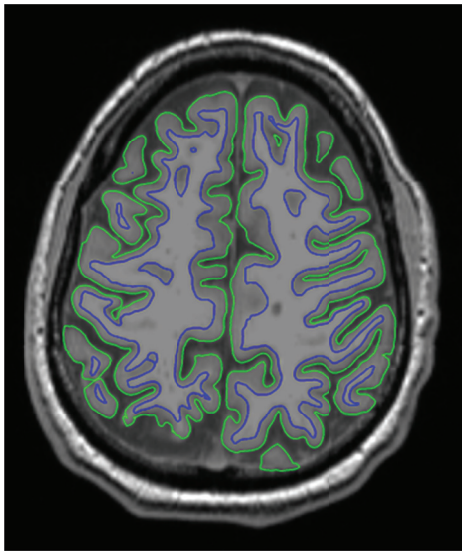


Figure 2-8. An automated segmentation of the neo cortex in a T1-weighted image. The blue and green surfaces indicate the pial and inner surfaces of the cortex.

2.9 MRS post-processing

Metabolite resonance intensity ratios of NA/Cr were determined using a combination of a locally developed software (AVIS; Samson Antel PhD, Magnetic Resonance Spectroscopy Unit, MNI) and an automated metabolite quantification software, LC Model (122). Concentration ratios of NA relative to intra-voxel creatine were computed in both MRSI and SVS. All spectra in MRSI and SVS were visually checked by a specially trained individual to ensure high data quality. In addition, the following criteria were used to determine the inclusion of all spectra: 1) full width half maximum (FWHM) $\leq$ 0.08 ppm, 2) signal to noise ratio (SNR) > 10, 3) NA/Cr % standard deviation (SD) < 20, 4) a relatively stable baseline. Spectra selection was also double-checked by a computer script to ensure data quality. An example of spectra selection in AVIS is shown in **Figure 2-9**. The mean values of NA/Cr in MRSI were then calculated from included

spectra for every subject. All MRI/MRS post-processing was completed by myself who was blinded to the OSAH status of the study subjects.

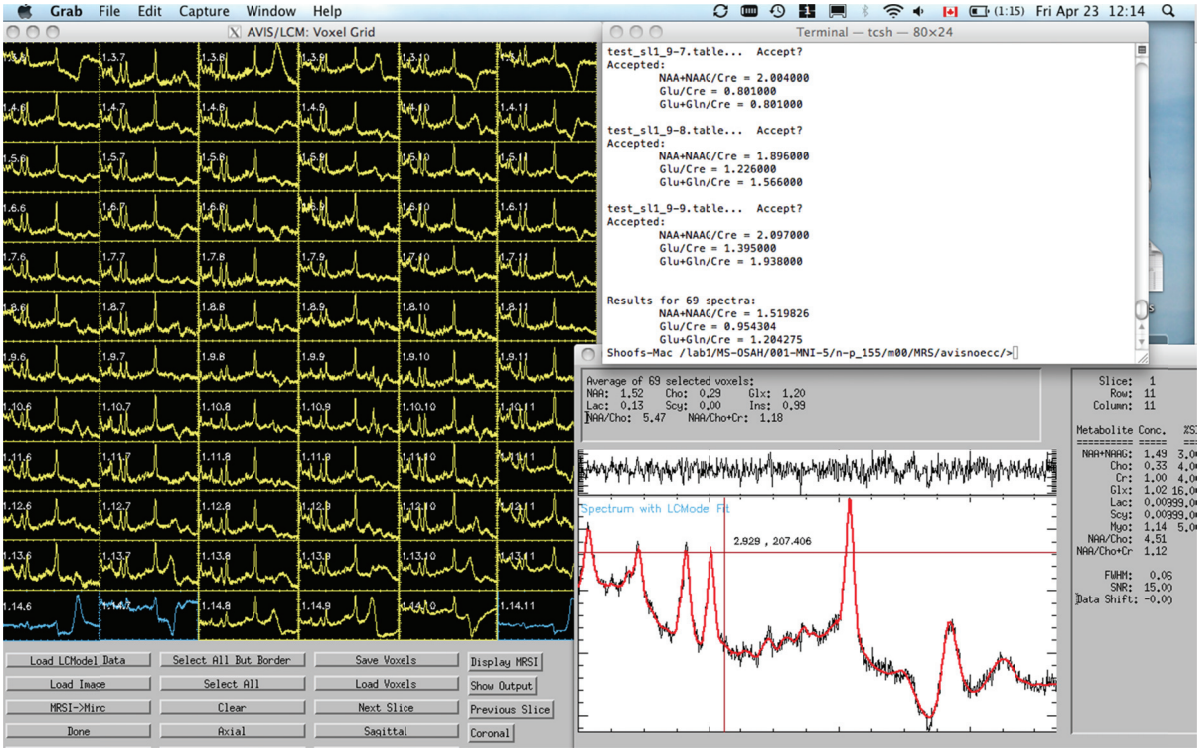


Figure 2-9. Spectrum selection in MRSI using AVIS. The yellow spectra were the ones that met the quality criteria.

2.10 Reliability of MRI measures

Prior to initiation of the research study, we evaluated the test/retest reliability of our MRI protocol. Five healthy subjects (mean age = 23.4 ± 2.2) underwent MRI/MRS on two separate occasions (range from 1 hour to 30 days).

Averages of absolute percent differences (percent differences were calculated by dividing the difference between the two scans by the average of the two scans for each subject) for NA/Cr ratios are presented in **Table 2-1**. Average of absolute percent differences for

normalized brain, hippocampal, cerebellar, and lateral ventricular volumes are listed in **Table 2-2**.

The average NA/Cr ratio percent differences were 1.25% for MRSI, and 5.01% for SVS. The average percent differences ranged from 1.20 % to 3.75 % for brain volumes in all brain regions of interest. These results indicated good reliability of our MRI/MRS protocol.

Table 2-1. Reliability Assessment of Brain Metabolites: Average Percent Differences

	MRSI (%)	SVS (%)
NA/Cr	1.25	5.01

Legend: MRSI = Magnetic Resonance Spectroscopic Imaging; SVS = Single Voxel Spectroscopy; NA/Cr = concentration ratio of N-acetyl groups/ creatine.

Table 2-2. Reliability Assessment of Brain Volumes: Average Percent Differences

Outcomes	%
Normalized Brain Volume	1.52
Normalized Hippocampal Volume	3.75
Normalized Lateral Ventricular Volume	3.07
Normalized Cerebellar Volume	2.65
Cortical Thickness	1.20

2.11 Statistical analysis

All statistical analyses were done in the statistical package R 2.13.0 (www.r-project.org). We calculated a multiple linear regression model for each outcome measure using a step-wise selection procedure. We started the procedure with a full model including all independent (exposure) variables.

$$Y = \beta_0 + \beta_1 MS + \beta_2 OSAH + \beta_3 MS \times OSAH + \beta_4 MS\ treated + \beta_5 OSAH\ treated + \beta_6 BMI + \beta_7 age + \beta_8 sex$$

Each outcome measure Y was the dependent (outcome) variable for every model tested. $ß_0$ was the intercept, which stood for the predicted value of Y when all the independent variables were set to zero. Each ß in front of an independent variable was the partial regression coefficient for the corresponding variable, which could be interpreted as the effect size of the corresponding variable on the outcome while holding all other independent variables constant. OSAH and MS status, and the interaction between OSAH and MS were all included in the model as independent categorical variables. Other independent variables were chosen based on the results of univariate analyses, previous empirical findings, and clinical experience. They included age, sex, BMI, OSAH treatment (CPAP), and MS treatment (treated with immunomodulating treatment). OSAH status, MS status, OSAH treated, MS treated, and sex were all categorical variables, where no OSAH, no MS, no treatment, and male were used as reference levels. All other variables were continuous variables.

After the full model was established, we started removing independent variables one by one, starting from the interaction variable of MS and OSAH. We removed the interaction variable when 1) it was not significant, and 2) removing it did not decrease the overall model fit, indicated by the adjusted R^2 value. In other words, if the interaction variable was significant, or removing an insignificant interaction variable decreased the overall model fit, we chose not to remove it from the model. Following the interaction variable, we then moved on to the least significant variable from the model. If removing the variable decreased overall model fit, we then put the removed variable back into the model, and continued to remove the second least significant variable instead. We

repeated this process until the adjusted R^2 value reached its maximum. MS and OSAH status were included in all models regardless of their significance.

For primary outcomes, a Bonferroni correction was applied on each regression model's overall p value. For the secondary outcomes, no correction was performed due to the exploratory nature of these analyses. Sub-group exploratory analyses were also performed in MS and Non-MS patients using OSAH as the independent variable, accounting for confounders such as age and sex. All levels of significance was set at $p < 0.05$.

Chapter 3 Results

3.1 Clinical and demographic characteristics

The clinical and demographic characteristics of 29 controls and 49 patients with MS are shown in **Table 3-1**. Age, sex, BMI, immunomodulatory treatment, and CPAP treatment were all included in multiple regression models as independent variables. We have three categories of AHI. The AHI Now indicates most recent AHI from each subject. The AHI 1st variable indicates the first AHI measured from all subjects. We generated the AHI combined variable to estimate the clinical severity of OSAH in all subjects. The reason for doing so is that the AHI of patients who were treated by CPAP was calculated while on treatment, which essentially reduced the natural episodes of apnea and hypopnea. Therefore, we combined the first AHI of subjects who are currently treated by CPAP, and the most recent AHI of subjects who are not currently treated by CPAP to generate the AHI combined variable. It is based on the assumption that AHI does not change significantly over time when the BMI of patients does not change. MS and control subjects were of similar mean age, but subjects with OSAH were older than those without OSAH. There were more females in the MS group. Mean BMI was somewhat higher in MS patients with OSAH, but mean values for all groups were in the non-obese range. Most MS patients had the RR form of the disease, and most (approximately 60%) were on immunomodulating treatment. Mean AHI indices were similar in MS and control subjects.

Table 3-1. Clinical and demographic characteristics of study subjects.

		Control (n = 29)		MS (n = 49)	
		Without OSAH	With OSAH	Without OSAH	With OSAH
n		16	13	17	32
Age (years)[1]		43.17 ± 12.27	52.48 ± 15.21	47.36 ± 10.10	53.04 ± 9.83
sex (% of female)		56.25%	38.46%	64.71%	59.38%
BMI[1]		24.3 ± 3.0	25.3 ± 6.1	23.9 ± 5.4	28.6 ± 5.3
# of subjects treated by CPAP		-	3 (23%)	-	12 (38%)
EDSS (range, median)		-	-	1 - 6.5 (2.5)	1 - 6.5 (3.75)
MS disease duration (years)[1]		-	-	15.1 ± 9.1	17.6 ± 8.1
MS disease type n (%)	RR	-	-	15 (88.2%)	22 (68.8%)
	SP	-	-	2 (11.8%)	9 (28.1%)
	PR	-	-	0	1 (3.1%)
T2 lesion volume (cc)[1]		-	-	13.85 ± 12.26	15.00 ± 14.8
Immunomodulatory treatment or chemotherapy at evaluation n (%)		-	-	10 (58.8%)	22 (68.8%)
Apnea-Hypopnea Index Now[1, a]		8.9 ± 4.1	25.1 ± 14.9	9.8 ± 3.8	22.4 ± 17.4
Apnea-Hypopnea Index 1st[1, b]		9.1 ± 4.5	32.1 ± 10.5	10.0 ± 4.1	28.3 ± 13.6
Apnea-Hypopnea Index combined[1, c]		8.9 ± 4.1	31.0 ± 10.8	9.8 ± 3.8	30.3 ± 14.1

[1] mean ± SD, [a] most recent apnea-hypopnea index, [b] first apnea-hypopnea index, [c] if the patient was treated by CPAP at the time of PSG, the first apnea-hypopnea index was used. MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea; BMI = Body Mass Index; CPAP = Continuous Positive Airway Pressure; EDSS = Kurtzke Expanded Disability Status Scale; RR = Relapsing-Remitting; SP = Secondary-Progressive; PR = Progressive-Relapsing

The summary of MRI and MRS outcome measures are shown in **Table 3-2**. All multiple regression models are shown and analyzed in chapters 3.2 and 3.3.

Table 3-2. Summary of MRI and MRS outcome measures.

		Control (n = 29)		MS (n = 49)	
		Without OSAH	With OSAH	Without OSAH	With OSAH
n		16	13	17	32
Primary Outcome Measures	**Central Brain NA/Cr**	**1.715 ± 0.093**	**1.634 ± 0.074**	**1.526 ± 0.107**	**1.524 ± 0.127**
	Posterior Brain NA/Cr	**1.440 ± 0.092**	**1.387 ± 0.107**	**1.304 ± 0.077**	**1.399 ± 0.230**
	Normalized Hippocampal Volume (cc)	**7.27 ± 0.60**	**7.39 ± 0.71**	**6.79 ± 0.86**	**6.81 ± 0.78**
Secondary Outcome Measures	Normalized Brain Volume (cc)	1578.21 ± 94.84	1552.46 ± 71.08	1473.24 ± 149.61	1462.72 ± 137.44
	Normalized Cerebellar Volume (cc)	161.18 ± 11.31	155.32 ± 5.72	151.57 ± 15.31	148.47 ± 16.70
	Normalized Lateral Ventricular Volume (cc)	18.97 ± 7.77	29.81 ± 16.01	42.99 ± 21.49	39.08 ± 19.15
	Cortical Thickness (mm)	2.58 ± 0.06	2.54 ± 0.08	2.50 ± 0.13	2.50 ± 0.11

Values shown are mean ± SD. MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea; NA/Cr = the ratio of N-acetylaspartate/creatine

3.2 Primary outcome measures

3.2.1 NA/Cr in central brain

The parameters of the model for central brain NA/Cr are shown in **Table 3-3**. The model was highly significant with a Bonferroni-corrected overall p = 1.11E-08. The final multiple linear regression model was:

Central Brain NA/Cr = 1.879 - 0.172 x MS - 0.046 x OSAH + 0.0631 x MS x OSAH - 0.0038 x Age

After controlling for age (p = 3.65E-04), the MS effect remained significant (p = 6.64E-06, 95% CI (-0.242, -0.101)) reducing central brain NA/Cr by -10.03%, whereas OSAH did not have a significant effect (p = 0.2405). We did not observe a significant interaction effect between OSAH and MS on central brain NA/Cr (p = 0.196). The magnitude of the OSAH effect was much smaller than the MS effect (2.68% vs 10.03%).

Table 3-3. The multiple regression model parameters for central brain NA/Cr.

Central Brain NA/Cr (n=78)			
Adjusted R^2 of the model	0.4272		
p of the model*[1]	**1.11E-08**		
Intercept	1.879		
Variables	ß (%)[a]	95% CI of ß	p
MS*	-0.172 (-10.03)	**(-0.242, -0.101)**	**6.64E-06**
OSAH	-0.046 (-2.68)	(-0.123, 0.0313)	0.2405
Age*	-0.0038 (-0.22)	**(-0.0058, -0.0018)**	**3.65E-04**
MS x OSAH	0.0631 (3.68)	(-0.0333,0.160)	0.196

[1]Bonferroni corrected; * $p < 0.05$; [a] The % change of ß was calculated by dividing β by the mean central brain NA/Cr in normal controls. e.g. MS: -0.172/1.715 = -10.03%

MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea; NA/Cr = the ratio of total N-acetylaspartate groups/creatine.

3.2.2 NA/Cr in posterior brain

The parameters of the model for posterior brain NA/Cr are shown in **Table 3-4**. The overall model was significant after Bonferroni correction (p = 0.0139). The final multiple linear regression model was:

Posterior Brain NA/Cr = 1.440 - 0.166 x MS - 0.042 x OSAH + 0.116 x MS x OSAH + 0.0514 x MS treatment - 0.0449 x OSAH treatment

After controlling for treatment effects, MS remained significant (p = 1.30E-04, 95% CI (-0.248, -0.0843)) and reduced posterior brain NA/Cr by 11.53% on average. We did not observe a significant OSAH effect (p = 0.296). However, there was a significant positive interaction effect (p = 0.0252, 95% CI (0.0148, 0.217)), which increased posterior brain NA/Cr by 8.06%.

Table 3-4. The multiple regression model parameters for posterior brain NA/Cr.

Posterior Brain NA/Cr (n=78)			
Adjusted R^2 of the model	0.1526		
p of the model*[1]	**0.0139**		
Intercept	1.44		
Variables	ß (%)[a]	95% CI of ß	p
MS*	-0.166 (-11.53)	**(-0.248, -0.0843)**	**1.30E-04**
OSAH	-0.042 (-2.92)	(-0.122, 0.0376)	0.296
MS treatment	0.0514 (3.57)	(-0.0120, 0.115)	0.111
OSAH treatment	-0.0449 (-3.12)	(-0.112, 0.0224)	0.188
MS x OSAH*	0.116 (8.06)	**(0.0148, 0.217)**	**0.0252**

[1]Bonferroni corrected, * $p < 0.05$, [a] The % effect size of ß on the mean posterior brain NA/Cr in normal controls. e.g. MS: -0.166/1.440 = -11.53%

MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea; NA/Cr = the ratio of N-acetylaspartate and creatine

After examining the clinical and demographic information in Table 3-1, we decided to replace the interaction of MS and OSAH in the original regression model by the interaction of MS treatment status and OSAH. The reason was that the MS with OSAH group had many more subjects being treated with immunomodulating treatment than the MS without OSAH group. The treatment effect may be a more appropriate explanation for the interaction effect between MS and OSAH on NA/Cr. Therefore, the revised multiple regression model (in **Table 3-5**) was:

Posterior Brain NA/Cr = 1.429 - 0.166 x MS - 0.0198 x OSAH + 0.124 x MS treatment x OSAH - 0.0246 x MS treatment - 0.0416 x OSAH treatment

After replacing the old interaction variable, the new model overall fit was improved with a Bonferroni-corrected p = 0.0103. The MS effect remained significant (p = 2.47E-03, 95% CI (-0.166, -0.037)) and the new interaction of MS treatment and OSAH became more significant (p = 0.0169, 95% CI (0.023, 0.226)), and had a larger effect size (8.61%). The effect size of interaction (8.61%) was even higher than the negative MS effect (-7.01%). OSAH remained insignificant in this model (p = 0.545).

Table 3-5. The revised multiple regression model parameters for posterior brain NA/Cr.

Posterior Brain NA/Cr (n=77)			
Adjusted R^2 of the model	0.161		
p of the model*[1]	**0.0103**		
Intercept	1.429		
Variables	ß (%)[a]	95% CI of ß	p
MS*	-0.101 (-7.01)	**(-0.166, -0.037)**	**2.47E-03**
OSAH	-0.0198 (-1.38)	(-0.085, 0.045)	0.545
MS treatment	-0.0246 (-1.71)	(-0.115, 0.066)	0.591
OSAH treatment	-0.0416 (-2.89)	(-0.108, 0.025)	0.218
MS treatment x OSAH*	0.124 (8.61)	**(0.023, 0.226)**	**0.0169**

[1]Bonferroni corrected, ***** $p < 0.05$, [a] The % effect size of ß on the mean posterior brain NA/Cr in normal controls. e.g. MS: -0.101/1.440 = -7.01%

MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea; NA/Cr = the ratio of N-acetylaspartate and creatine

3.2.3 Normalized hippocampal volume

The parameters of the model for normalized total hippocampal volume are shown in **Table 3-6**. The overall model was significant after Bonferroni correction ($p = 0.025$). The final multiple linear regression model was:

Normalized Total Hippocampal Volume = 7150.5 - 573.5 x MS + 47.3 x OSAH + 316.8 x Sex (female)

MS had a significant effect of -7.89% ($p = 1.90E-03$, 95% CI (-928.3, -218.8)) on normalized hippocampal volume after correcting for sex as a confounder. We did not

observe a significant interaction effect. In addition, we did not observe a significant OSAH effect on normalized hippocampal volume (p = 0.786). The OSAH effect remained much smaller than the MS effect (0.65% vs -7.89%).

Table 3-6. The multiple regression model parameters for total normalized hippocampal volume

Normalized Total Hippocampal Volume (mm^3) (n=78)			
Adjusted R^2 of the model	0.111		
p of the model*[1]	**0.025**		
Intercept	7150.5		
Variables	ß (%)[a]	95% CI of ß	p
MS*	-573.5 (-7.89)	**(-928.3, -218.8)**	**1.90E-03**
OSAH	47.3 (0.65)	(-297.9, 392.5)	0.786
Sex (female)	316.8 (4.26)	(-22.9, 656.4)	0.067

[1]Bonferroni corrected, * $p < 0.05$, [a] The % effect size of ß on the mean normalized total hippocampal volume in normal controls. e.g. MS: -573.5/7270 = -7.89%

MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea

3.3 Secondary outcome measures

3.3.1 Normalized brain volume

The parameters of the model are shown in **Table 3-7**. The overall model was significant (p = 9.96E-06). The final multiple linear regression model was:

Normalized Brain Volume = 1778.3 – 44.3 x MS + 24.3 x OSAH – 5.2 x Age

+44.9 x Sex (female) – 72.8 x MS Treatment

After correcting for sex and age (p = 2.92E-05), we did not observe a significant effect of MS (p = 0.205), OSAH (p = 0.377), or the interaction of MS and OSAH (not included in the final model) on normalized brain volume. However, the treatment of MS had a significant effect of -4.61% (p = 0.0352, 95% CI (-140.5, -5.2)), indicating a reduced brain volume in those on MS treatment.

Table 3-7. The revised multiple regression model parameters for normalized brain volume.

Normalized Brain Volume (cc) (n=78)			
Adjusted R^2 of the model	0.298		
p of the model*	**9.96E-06**		
Intercept	1778.3		
Variables	ß (%)[a]	95% CI of ß	p
MS	-44.3 (-2.81)	(-113.2, 24.6)	0.205
OSAH	24.3 (1.54)	(-30.2, 78.7)	0.377
Age*	-5.2 (0.33)	**(-7.5, -2.9)**	**2.92E-05**
Sex (female)	44.9 (2.84)	(-7.1, 96.8)	0.0894
MS treatment*	-72.8 (-4.61)	**(-140.5, -5.2)**	**0.0352**

***** $p < 0.05$, [a] The % effect size of ß on the mean normalized brain volume in normal controls. e.g. MS: -44.3/1578.2 = -2.81%

MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea

3.3.2 Normalized cerebellar volume

The parameters of the model for normalized cerebellar volume are shown in **Table 3-8**. The overall model was significant (p = 3.713E-04). The final multiple linear regression model was:

Normalized Cerebellar Volume = 155.03 – 6.02 x MS – 2.9 x OSAH + 44.9 x Sex (female)

After correcting for the significant sex effect (p = 0.0017), MS remained significant (p = 0.003, 95% CI (-16.0, -3.4)) in the model with an effect of -6.72%. We did not observe any significant effect of OSAH (p = 0.377), or the interaction of MS and OSAH (not included in the final model) on normalized cerebellar volume.

Table 3-8. The multiple regression model parameters for normalized cerebellar volume.

Normalized Cerebellar Volume (cc) (n=78)			
Adjusted R^2 of the model	0.1867		
p of the model*	**3.713E-04**		
Intercept	155.0		
Variables	ß (%)[a]	95% CI of ß	p
MS*	-9.7 (-6.02)	**(-16.0, -3.4)**	**0.003**
OSAH	-2.9 (-1.80)	(-9.1, 3.3)	0.377
Sex (female)*	10 (6.20)	(3.9, 16.1)	**0.0017**

***** $p < 0.05$, [a] The % effect size of ß on the mean normalized cerebellar volume in normal controls. e.g. MS: -9.7/161.2 = -6.02%

MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea

3.3.3 Normalized lateral ventricular volume

The parameters of the model for normalized lateral ventricular volume are shown in **Table 3-9**. The overall model was significant (p = 7.204E-04). The final multiple linear regression model was:

Normalized Lateral Ventricular Volume = 7580.4 + 21204.2 x MS +8379.0 x OSAH +263.9 x Age – 11181.0 x MS x OSAH

After correcting for age (p = 0.139), MS remained significant (p = 9.16E-04, 95% CI (8977.7, 33430.6)) in the model with an effect of 111.8%, indicating increased lateral ventricular size with MS. We did not observe a significant effect of OSAH (p = 0.216), or the interaction of MS and OSAH (0.187) on normalized lateral ventricular volume.

Table 3-9. The multiple regression model parameters for normalized lateral ventricular volume.

Normalized Lateral Ventricular Volume (mm^3) (n=78)			
Adjusted R^2 of the model	0.1863		
p of the model*	**7.204E-04**		
Intercept	7580.4		
Variables	ß (%)[a]	95% CI of ß	p
MS*	21204.2 (111.8)	**(8977.7, 33430.6)**	**9.16E-04**
OSAH	8379.0 (44.2)	(-5002.6, 21760.5)	0.216
Age	263.9 (13.9)	(-87.3, 615.1)	0.139
MS x OSAH	-11181.0 (-58.9)	(27911.2, 5549.2)	0.187

* $p < 0.05$, [a] The % effect size of ß on the mean normalized lateral ventricular volume in normal controls. e.g. MS: 21.20/18.97 = 111.8%

MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea

3.3.4 Cortical thickness

The parameters of the model for cortical thickness are shown in **Table 3-10**. The overall model was significant (p = 2.910E-03). The final multiple linear regression model was:

Cortical Thickness = 2.686 – 0.053 x MS – 0.0080 x OSAH – 0.003 x Age

After correcting for the age effect (p = 0.0121), MS remained significant (p = 0.0296, 95% CI (-0.100, -0.005)) in the model with an effect of -2.05%, indicating reduced cortical thickness in MS subjects. We did not observe any significant effect of OSAH (p = 0.748), or the interaction of MS and OSAH (not included in the model) on cortical thickness.

Table 3-10. The multiple regression model parameters for cortical thickness.

Cortical Thickness (mm) (n=78)			
Adjusted R^2 of the model	0.1377		
p of the model*	**2.910E-03**		
Intercept	2.686		
Variables	ß (%)[a]	95% CI of ß	p
MS*	-0.053 (-2.05)	**(-0.100, -0.005)**	**0.0296**
OSAH	-0.0080 (-0.31)	(-0.055, 0.040)	0.748
Age*	-0.003 (-0.12)	(-0.005, -0.001)	**0.0121**

* $p < 0.05$, [a] The % effect size of ß on the mean cortical thickness in normal controls. e.g. MS: -0.053/2.58 = -2.05%

MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea

3.3.5 Subgroup exploratory analyses

We performed exploratory analyses on MS and non-MS control subjects separately. The goal was to investigate the potential effect of OSAH in patients with and without MS.

MS subgroup analysis

Results in the MS group revealed a significant effect of age on central brain NA/Cr (p = 0.0075, 95% CI (-0.0080, -0.0012)). OSAH had a trend effect of increasing NA/Cr in posterior brain (p = 0.073, 95% CI (-0.006, 0.128)), for the same reason of MS treatment explained in **Chapter 3.2.2**. We did not observe a significant OSAH effect on central brain NA/Cr (p = 0.489) after correcting for the age effect. In addition, we did not find a significant OSAH effect on normalized hippocampal volume (p = 0.961).

Table 3-11. The multiple regression model parameters for the three primary outcomes in the subgroup of MS subjects.

	Adjusted R^2 of the model	Variables	ß	95% CI of ß	p[1]
Central Brain NA/Cr (n=49)	0.3491	OSAH	0.024	(-0.046, 0.095)	0.489
		Age*	**-0.0046**	**(-0.0080, -0.0012)**	**0.0075**
Posterior Brain NA/Cr (n=49)	0.035	OSAH	0.061	(-0.006, 0.128)	0.073
Normalized Hippocampal Volume (mm^3) (n=49)	-0.03	OSAH	11.98	(-473.7, 497.6)	0.961

[1]uncorrected, *p < 0.05 MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea; NA/Cr = the ratio of N-acetylaspartate and creatine

Non-MS subgroup analysis

Results in the non-MS group showed a trend of reduced NA/Cr in central brain NA/Cr (p = 0.09, 95% CI (-0.113, 0.009)), after correcting for the significant age effect (p =

0.0069, 95% CI (-0.005, -0.001)). We did not find a significant OSAH effect on either posterior brain NA/Cr (p = 0.166) or normalized hippocampal volume (p = 0.637).

Table 3-12. The multiple regression model parameters for the three primary outcomes in the subgroup of non-MS subjects.

	Adjusted R^2 of the model	Variables	ß	95% CI of ß	p[1]
Central Brain NA/Cr (n=29)	0.3491	OSAH	-0.052	(-0.113, 0.009)	0.090
		Age*	**-0.003**	**(-0.005, -0.001)**	**0.0069**
Posterior Brain NA/Cr (n=29)	0.035	OSAH	-0.0524	(-0.128, 0.023)	0.166
Normalized Hippocampal Volume (mm^3) (n=29)	-0.03	OSAH	116.6	(-384.29, 617.53)	0.637

[1]uncorrected, * $p < 0.05$, MS = Multiple Sclerosis; OSAH = Obstructive Sleep Apnea-Hypopnea; NA/Cr = the ratio of N-acetylaspartate and creatine

Chapter 4 Discussion and Conclusions

4.1 Discussion of results

This is the first study to examine the potential impact of OSAH on neurodegeneration in patients with MS. In our study population, we confirmed previous reports of degeneration in MS. Even though we did not find a significant additive effect of OSAH on neurodegeneration in MS, we found a suggestion of an OSAH effect on central brain neurodegeneration in non-MS subjects. In this Discussion we will summarize our main findings, followed by our study limitations, future work, and study conclusions.

In our study, MS has a significant effect on all three primary outcome measures. After correcting for the significant age effect, MS reduced NA/Cr in the central brain by 10.03%, and in the posterior brain by 11.53% compared with normal controls. MS also decreased the size of bilateral hippocampi by 7.89%. Our results are consistent with previously reported findings that MS is associated with neuroaxonal damage.

In the original posterior brain NA/Cr regression model, the positive significant interaction of MS and OSAH with an effect size of 6.76% is unexpected. From all the MRS studies presented earlier, one would expect a cumulative effect of MS and OSAH on neuroaxonal integrity, leading to more severe neuroaxonal injury than the effect of each disease alone, and perhaps even more than the simple addition of the two effects. When looking at the boxplot in the appendix **Figure 3-5**, one notes the unusually high NA/Cr in the group with MS and OSAH, in contrast to the value of central brain NA/Cr of the same group. In addition, the clinical and demographic characteristics of subjects in **Table 3-1** show that the MS with OSAH group has a few clinical features that are different from other groups. For example, compared with the MS without OSAH group, the MS with OSAH

group has a higher percentage of SPMS patients (28% vs 11%), and a higher percentage of patients receiving immunomodulatory treatment or chemotherapy (69% vs 59%). In addition, MS patients with OSAH had higher percentage of subjects treated by CPAP, compared to controls with OSAH (38% vs 23%).

Compared with MS disease types and CPAP treatment in OSAH, there appears to be more evidence to support the association between immunomodulatory treatment in MS and increased NA/Cr. There has been one study on the effect of CPAP on cortical NAA over a period of 6 months. However, the authors did not find a significant change after a 6-month period of CPAP treatment on patients with OSAH (107). SPMS is a more severe form of MS and usually exhibits more severe neuroaxonal loss in terms of decreased NA/Cr (123). Therefore, the treatment information may be able to provide a more sensible explanation to the elevated NA/Cr. Indeed, Narayanan et al. found that when treated with interferon ß-1b, patients with MS had a mean increase of NA/Cr of 5.5% over a 12-month period in central brain, whereas the untreated group had a decrease in NA/Cr (124). Another study found that NA/Cr increased by 10.7% after two years of treatment with glatiramer acetate, in contrast to a 8.9% decrease in the untreated group (125). Since these two medications are used in the MS patients in our study, we decided to test the interaction term again by replacing the MS status in the original interaction with MS treatment. This change slightly improved the overall model fit and significance, as well as the significance of the interaction variable. Nonetheless, the MS treatment main effect is not significant itself in both the new and old model. Considering the significant interaction effect of MS treatment and OSAH, and the insignificant MS treatment effect alone, we suspect a potential neuro-protective effect from the MS

disease-modifying therapies that alter the complex mechanism of neural injury in OSAH. Inflammation and reactive oxygen species resulted from chronic intermittent hypoxia may be suppressed by the anti-inflammatory treatments, which enhance the natural recovery mechanism in neuronal mitochondria. One may speculate where this enhancement takes place. By visually examining the acquisition image of MRSI and SVS in **Figure 2-2**, we also note that posterior and central brain regions could have different grey/white matter ratios. Since we only observe a significant interaction effect in posterior brain NA/Cr, it may suggest that the enhanced recovery mechanism occurs more likely in grey matter than white matter. Although we have not quantitatively compared the GM/WM ratios in these two regions, this hypothesis is worth being tested, because grey matter contains a greater number of neuronal cell bodies, where NA is produced.

We did not find a significant interaction effect of MS and OSAH on central brain NA/Cr or on normalized hippocampal volume. Nonetheless, this observation does not rule out the possibility that such an effect may still exist in patients not on immunomodulatory treatments. We did not find a significant OSAH main effect on any primary outcome either. However, we can see that in both central and posterior brain, OSAH has a mean negative effect on NA/Cr, ranging from 1.38% to 2.92%. The 95% confidence intervals also suggest that OSAH could decrease NA/Cr in central brain by up to 7.1%, and in posterior brain by up to 5%. This is consistent with findings from Tonon et al. that OSAH decreased NA/Cr in the posterior brain by a mean of 7.8% (107). The different results could be due to different OSAH severity in the two studies. Tonon et al. studied severe OSAH patients with a mean AHI of 58 events/hr, in contrast to approximately 30

events/hr in our study population with OSAH. The insignificant effect of OSAH on hippocampal volume does not agree with previous studies. This could also be due to the fact that subjects in our cohort did not have severe OSAH. However, this is the first study that examined hippocampal volumes in OSAH using a specific segmentation tool for the hippocampus. Previous findings regarding decreased hippocampal volumes were reported using the non-hypothesis driven VBM technique. Furthermore, a proportion of our OSAH subjects in both groups were treated with CPAP. The effect of CPAP was not examined on hippocampal volume. In summary, the discrepancies between our findings and the literature could be due to a combination of factors, such as disease severity, CPAP/MS treatment, and different image processing techniques used.

The regression models for all three primary outcomes measures of neurodegeneration are significant after Bonferroni correction. This indicates that each model selection is valid and includes exposure variables that can account for the variance in the outcome measure. The adjusted R^2 values show how well each regression model fits the data. The regression model for central brain NA/Cr explains as much as 42.7% of the variance of the NA/Cr data, whereas the regression model for normalized hippocampal volume only explains 11.1% of the variance of the data. There are clearly unknown factors that contribute to the variance in the data. Moreover, in contrast to using continuous variables, using categorical variables in linear models usually provide a poorer fit of the model. We did not use AHI as a continuous measure of disease severity because the use of CPAP treatment decreased the actual AHI in patients, making it an unreliable marker of disease severity. We should be careful with our interpretation on the effects of independent variables from these models, even though the models are all significant.

In our secondary analyses, all regression models are significant. The MS effect is significant on normalized cerebellar and lateral ventricular volumes, and cortical thickness, indicating MS-associated neurodegeneration. While these findings agree with the literature, we did not find the expected MS effect on normalized brain volume in our study population. In fact, MS treatment had a significant and negative effect on normalized brain volume, with a mean decrease of 4.6%. However, one should be careful with the interpretation of such results in a cross sectional study. Our patients with MS who were treated with immunomodulating agents were more likely to have a more severe disease course, where continuous and irreversible neurodegeneration can occur. The treatment effect on brain size in this case should be interpreted to be a consequence of group bias instead of the actual effect of the treatment. The non-significant OSAH effect is generally smaller than the MS effect on our secondary outcome measures. This also makes it more difficult to detect significant changes in an inhomogeneous population.

Our subgroup exploratory analyses suggest a potential trend for an OSAH effect on central brain NA/Cr in non-MS subjects after correcting for the age effect, indicating that OSAH may be associated with diffuse, central brain neuronal injury in the general population. This finding is novel, although it requires further investigation with more untreated OSAH subjects in an *a priori* study design.

4.2 Limitations

There are several limiting factors in this study. Its cross sectional design does not allow us to fully investigate the evolution of neurodegeneration with respect to the progression

of MS and OSAH over time. Even though MS and control subjects were similar in age, the unmatched MS and OSAH treatment groups add extra difficulty in elucidating the impact of OSAH on neurodegeneration in MS. Because a proportion of subjects with OSAH were treated, we were unable to use AHI as a continuous variable in the linear regression models. The MS treatment effect likely also has an impact on our primary outcome measures, adding complexity to our interpretation of the results.

4.3 Future work

We plan to classify different tissues in the MRSI and SVS volumes of interest in our study subjects. Using this information, we plan to evaluate the potential grey matter/white matter effect on NA/Cr in central and posterior brain, using multiple regression analysis. We also plan to perform a multi-voxel analysis in MRSI. Currently we are using the mean values of all voxels, which is likely to reduce the statistical power and miss the regional changes in NA/Cr.

4.4 Conclusions

We used MRI and MRS to measure the potential impact of OSAH on neurodegeneration in patients with MS, compared to controls subjects without MS. We found reduced regional brain size and lower NA/Cr in MS patients compared to subjects without MS, consistent with MS-associated neurodegeneration. We also found a novel significant interaction effect of MS treatment and OSAH on posterior brain NA/Cr which may indicate that MS immunomodulating treatment has a protective effect on posterior brain neuronal injury in MS subjects with OSAH. We did not find an additional significant

impact of obstructive sleep apnea-hypopnea on neurodegeneration in MS patients in our study, although obstructive sleep apnea-hypopnea tended to be associated with reduced central brain NA/Cr in subjects without MS. This finding suggests that obstructive sleep apnea-hypopnea may have a diffuse effect on neuroaxonal integrity in the brain in subjects without MS, however future studies would need to confirm this finding.

Study Consent Forms

A. Research Ethics Board approved consent form for controls

McGill University Health Center, Montreal Neurological Hospital

Sleep Abnormalities in Multiple Sclerosis: Potential Benefits of Treatment and Effect on Neurodegeneration: Brain Imaging

Informed Consent - Controls
(Revised September, 2009)

Principal Investigator: D. A. Trojan, MD
Co-Investigators: J. Kimoff, MD, D. Arnold, MD, S. Narayanan, PhD, A. Bar-Or, MD, Y. Lapierre, MD, D. Da Costa, PhD, A. Benedetti, PhD, K. Schwartzman, MD

1. Purpose of study:

In a previous study, we found a high frequency of sleep abnormalities in multiple sclerosis (MS) patients. The purpose of this study is to examine the effect of sleep abnormalities detected in MS patients on the brain, as well as possible causes of sleep abnormalities in MS patients. To do this, we will study the brain of both multiple sclerosis patients and normal controls who do and do not have sleep abnormalities with special radiological techniques. You are being recruited for the study as a healthy control who does not have multiple sclerosis, and other neurological and inflammatory disorders.

2. Procedures:

To participate in this part of the study, you will be asked to come to the Montreal Neurological Hospital for one visit, requiring approximately three hours. You may also be requested to undergo an overnight sleep study at the Sleep Laboratory of the Royal Victoria Hospital if you have not undergone such a study in the last six months. During the visit at the Montreal Neurological Hospital, a medical history and sleep history, and vital signs will be obtained (30 minutes). You will be asked to complete eight questionnaires to assess fatigue, sleepiness, sleep quality, pain, depression, stress, and quality of life (one hour). You will have blood samples drawn to measure immunologic and hormonal factors, and molecules implicated in neurodegeneration. You will then undergo a magnetic resonance scan of your brain at the McConnell Brain Imaging Centre at the Montreal Neurological Institute and Hospital, which will take approximately one hour. Magnetic resonance makes it possible to visualize the brain using a magnetic field and radiowaves. For the magnetic resonance examination, you have to lie still on a bed inside a ventilated magnet. This is a confined space. If you feel uncomfortable you can talk to the technician and come out of the magnet at any time. You will hear knocking sounds while the scanner takes a picture of your brain. Because of the strong magnetic fields it is necessary to take certain precautions. You will be asked to fill in and sign a questionnaire before undergoing the magnetic resonance examination to make sure that

there are no contraindications to the exam, such as a heart pacemaker. If you require a sleep study, the test will be conducted in the same way that it is done for routine clinical sleep tests. You will be asked come to the Sleep Laboratory in the evening (approximately 9 PM), sleep through the night, and be free to leave in the morning. The test records your heart beat, breathing movements, brain waves, oxygen levels, and movements of your eyes, chin, and legs. This is done with tiny wires attached to your skin with a special cream and tape, a little plastic tube close to your nose openings, elastic bands around your waist and chest, and a oxygen sensor on your finger. You will be monitored by an infra-red (invisible light) video camera. The recording will last the entire night.

It is possible that study data will be used for future research for projects directly related to the disease process of multiple sclerosis and other related disorders, and sleep abnormalities, and will be reviewed by a duly constituted Research Ethics Board.

3. Advantages of proposed study:

There are no direct advantages for participation in this part of the study for you. However, if you are found to have a sleep disorder, you will be offered a consultation with a sleep specialist at the end of the study. This study may provide information on the possible effects and causes of sleep abnormalities on the brain of multiple sclerosis patients.

4. Disadvantages of the proposed study:

The only known disadvantage of the study is the time commitment required to undergo the evaluations. Magnetic resonance examination is a safe procedure. If you have any contraindications to having a magnetic resonance scan, such as a heart pacemaker, you will not be asked to participate in the study. The main side effect with the examination is the intermittent noise during the scan. You will be provided with earplugs for comfort. To date no harmful effects have been demonstrated. The sleep testing is not painful or dangerous. There may be some minor inconvenience associated with sleeping with wires attached. A technologist will be present throughout the night to monitor your sleep and to assist you in any way, such as to help you to the washroom, or deal with any wires or sensors which become detached. Blood tests require needle insertion. Possible side effects from needle insertion are minimal, and include pain at the site of insertion of the needle during and possibly for several days after the test. There is also a small risk of bleeding or infection at the site.

5. Withdrawal from the study:

Your participation in this part of the study is voluntary, and you may withdraw at any time, including during the study procedures themselves, without prejudice to yourself or your treatment. The data obtained prior to your withdrawal will be treated in the same way as for subjects who complete the entire study. To the extent possible, the available data will be analyzed for any reports or communications resulting from the study.

6. Other information:

Research scans are not routinely examined for abnormalities. However, any incidental findings found during the study will be communicated to you, and upon your request, to your physicians.

You will be reimbursed for parking expenses or travel expenses, and meals for the study visits (up to $100 for the entire study).

Confidentiality: Your confidentiality will be preserved during the study. Data will not be released to anyone other than the study investigators and research personnel. Identifiable data will be kept in a locked filing cabinet in a locked office. Research information will be identified with a confidential code. Study data will be entered into a computer file without including patient names. The computers used are password protected, and 128 bit encryption will be used. Your name and identifiable information will not be revealed in any communications or reports resulting from the study. Identifiable data will be kept for five years.

Please note that the Research Ethics Board or Quality Assurance Officers duly authorized by it may access study data for quality assurance purposes.

CONTACT INFORMATION
You may address any questions regarding the study now and in the future, to the Principal Investigator, Dr. Daria Trojan (Room 138 at the Montreal Neurological Hospital, tel. 514-398-8911). If you have any questions regarding your rights as a research subject and you wish to discuss them with someone not conducting the study, you may wish to contact the Montreal Neurological Hospital, Patient Ombudsman at 514-934-1934, ext. 48306. If you have any other kind of comments or concerns, or need assistance regarding your participation as a research subject in this project, please contact the MNH Patient's Committee, room 354, tel. 514-398-5358).

Declaration of Consent:

I agree to be to be contacted by a member of the Research Ethics Board, at the discretion of the Research Ethics Board.

I, (name of participant) _____, have read the above description of the protocol entitled "Sleep abnormalities in multiple sclerosis: potential benefits of treatment and effect on neurodegeneration: brain imaging" with one of the investigators (name of investigator) _____. The investigator has explained all aspects of the protocol to me. I fully understand the procedures, advantages, disadvantages of the study, which have been explained to me. I freely and voluntarily consent to participate in this study.

Further, I understand that I may seek information about each test either before or after it is given, that I am free to withdraw from the testing at any time if I desire, and that any personal information will be kept confidential.

Signature: _____

Subject Date Contact Number

Signature: _____

Investigator Date Contact Number

B. Research Ethics Board approved consent form for patients with MS

McGill University Health Center, Montreal Neurological Hospital

Sleep Abnormalities in Multiple Sclerosis: Potential Benefits of Treatment and Effect on Neurodegeneration: Brain Imaging

Informed Consent - Patients
(Revised October, 2010)

Principal Investigator: D. A. Trojan, MD
Co-Investigators: J. Kimoff, MD, D. Arnold, MD, S. Narayanan, PhD, A. Bar-Or, MD, Y. Lapierre, MD, D. Da Costa, PhD, A. Benedetti, PhD, K. Schwartzman, MD

1. Purpose of study:

In a previous study, we found a high frequency of sleep abnormalities in multiple sclerosis (MS) patients. We are now conducting a study to examine the effect of sleep abnormalities detected in MS patients on the brain, as well as possible causes of sleep abnormalities in MS patients. To do this, we will study the brain of multiple sclerosis patients and normal control subjects who do and do not have sleep abnormalities with special radiological techniques.

2. Procedures:

To participate in this study, you will be asked to come to the Montreal Neurological Hospital for one visit, requiring approximately three hours. You may be requested to undergo an overnight sleep study at the Sleep Laboratory of the Royal Victoria Hospital if you have not undergone such a study in the last six months (if your were not part of our previous research projects, the sleep study will be necessary). During the visit at the Montreal Neurological Hospital, a medical history or interval medical history will be obtained (10 minutes), a history of sleep difficulties will be obtained (10 minutes), and a neurological exam will be performed (20 minutes). You will be asked to complete questionnaires to assess fatigue, sleepiness, sleep quality, pain, depression, stress, and quality of life (one hour). You will have blood samples drawn to measure immunologic and hormonal factors, and molecules implicated in neurodegeneration. If you have not participated in our previous research projects, blood tests will also be done on these samples to ensure that you do not have medical difficulties that can cause fatigue or disturb your sleep. Pulmonary function tests will be done at the Royal Victoria Hospital (20 minutes) if not done previously as part of our previous research studies, or if there has been a significant change in your respiratory symptoms. You will be accompanied for this. If you meet the study inclusion criteria, you will then undergo a magnetic resonance scan of your brain at the McConnell Brain Imaging Centre at the Montreal Neurological Institute and Hospital, which will take approximately one hour. Magnetic resonance makes it possible to visualize the brain using a magnetic field and radiowaves. For the magnetic resonance examination, you have to lie still on a bed inside a ventilated magnet.

This is a confined space. If you feel uncomfortable you can talk to the technician and come out of the magnet at any time. You will hear knocking sounds while the scanner takes a picture of your brain. Because of the strong magnetic fields it is necessary to take certain precautions. You will be asked to fill in and sign a questionnaire before undergoing the magnetic resonance examination to make sure that there are no contraindications to the exam, such as a heart pacemaker.

If you require a sleep study, the test will be conducted in the same way that it is done for routine clinical sleep tests. You will be asked come to the Sleep Laboratory in the evening (approximately 9 PM), sleep through the night, and be free to leave in the morning. The test records your heart beat, breathing movements, brain waves, oxygen levels, and movements of your eyes, chin, and legs. This is done with tiny wires attached to your skin with a special cream and tape, a little plastic tube close to your nose openings, elastic bands around your waist and chest, and an oxygen sensor on your finger. You will be monitored by an infra-red (invisible light) video camera. The recording will last the entire night.

It is possible that study data will be used for future research for projects directly related to the disease process of multiple sclerosis and other related disorders, and sleep abnormalities, and will be reviewed by a duly constituted Research Ethics Board.

3. Advantages of proposed study:

There are no direct advantages for participation in this part of the study for you. However, if you are found to have a sleep disorder, you will be offered a consultation with a sleep specialist at the end of the study. This study may provide information on the possible effects and causes of sleep abnormalities on the brain of multiple sclerosis patients.

4. Disadvantages of the proposed study:

The only known disadvantage of the study is the time commitment required to undergo the evaluations. Magnetic resonance examination is a safe procedure. If you have any contraindications to having a magnetic resonance scan, such as a heart pacemaker, you will not be asked to participate in the study. The main side effect with the examination is the intermittent noise during the scan. You will be provided with earplugs for comfort. To date no harmful effects have been demonstrated. The sleep testing is not painful or dangerous. There may be some minor inconvenience associated with sleeping with wires attached. A technologist will be present throughout the night to monitor your sleep and to assist you in any way, such as to help you to the washroom, or deal with any wires or sensors which become detached. Blood tests require needle insertion. Possible side effects from needle insertion are minimal, and include pain at the site of insertion of the needle during and possibly for several days after the test. There is also a small risk of bleeding or infection at the site.

5. Withdrawal from the study:

Your participation in this part of the study is voluntary, and you may withdraw at any time, including during the study procedures themselves, without prejudice to yourself or your treatment. The data obtained prior to your withdrawal will be treated in the same way as for subjects who complete the entire study. To the extent possible, the available data will be analyzed for any reports or communications resulting from the study.

6. Other information:

Research scans are not routinely examined for abnormalities. However, any incidental findings found during the study will be communicated to you, and upon your request, to your physicians.

You will be reimbursed for parking expenses or travel expenses, and meals for the study visits (up to $100 for the entire study).

Confidentiality: Your confidentiality will be preserved during the study. Data will not be released to anyone other than the study investigators and research personnel. Identifiable data will be kept in a locked filing cabinet in a locked office. Research information will be identified with a confidential code. Study data will be entered into a computer file without including patient names. The computers used are password protected, and 128 bit encryption will be used. Your name and identifiable information will not be revealed in any communications or reports resulting from the study. Identifiable data will be kept for five years.

Please note that the Research Ethics Board or Quality Assurance Officers duly authorized by it may access study data for quality assurance purposes.

CONTACT INFORMATION
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Declaration of Consent:
I agree to be to be contacted by a member of the Research Ethics Board, at the discretion of the Research Ethics Board.

I, (name of participant) _____, have read the above description of the protocol entitled "Sleep abnormalities in multiple sclerosis: potential

benefits of treatment and effect on neurodegeneration: brain imaging" with one of the investigators (name of investigator) _____. The investigator has explained all aspects of the protocol to me. I fully understand the procedures, advantages, disadvantages of the study, which have been explained to me. I freely and voluntarily consent to participate in this study.

Further, I understand that I may seek information about each test either before or after it is given, that I am free to withdraw from the testing at any time if I desire, and that any personal information will be kept confidential.

Signature:_____

Subject Date Contact Number

Signature:_____

Investigator Date Contact Number

Appendix

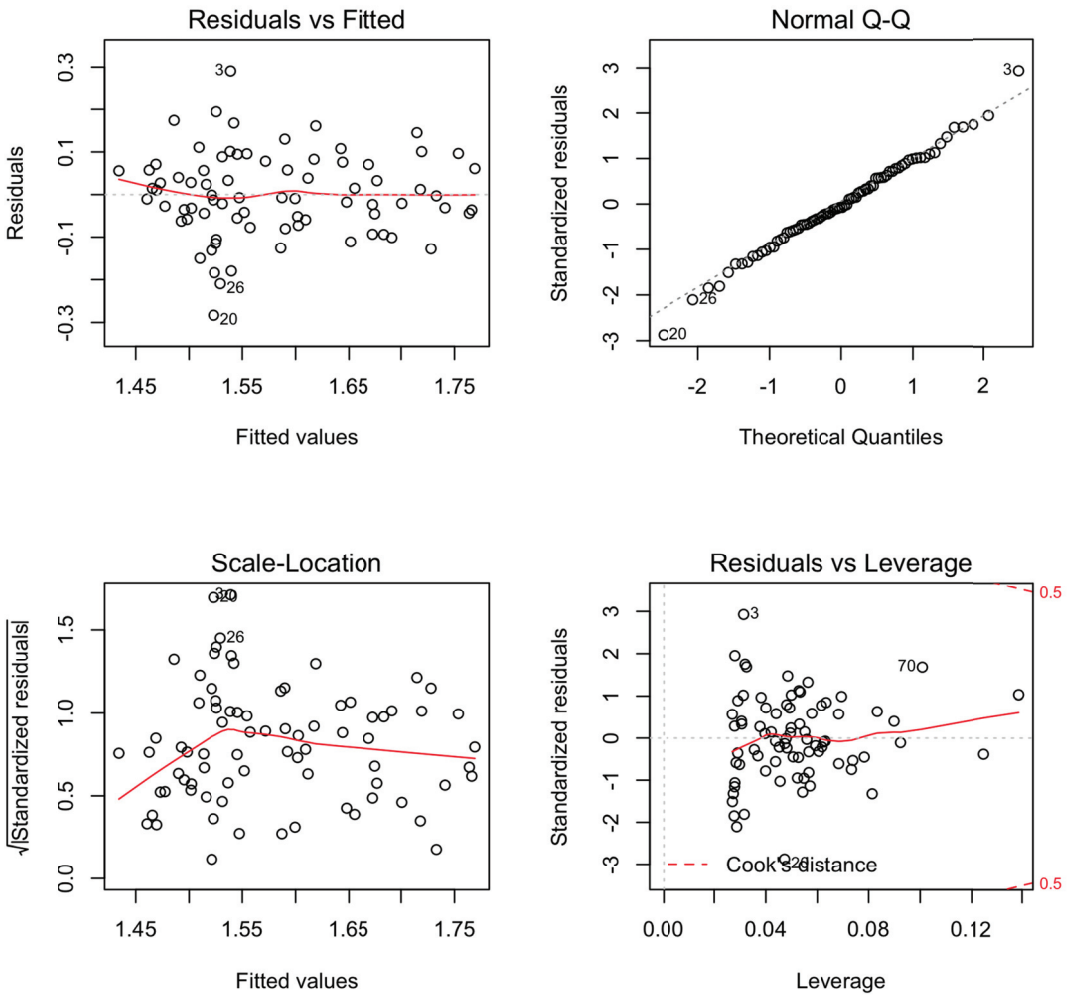

Figure 3-1. Diagnostic plots of central brain NA/Cr data (n = 78).

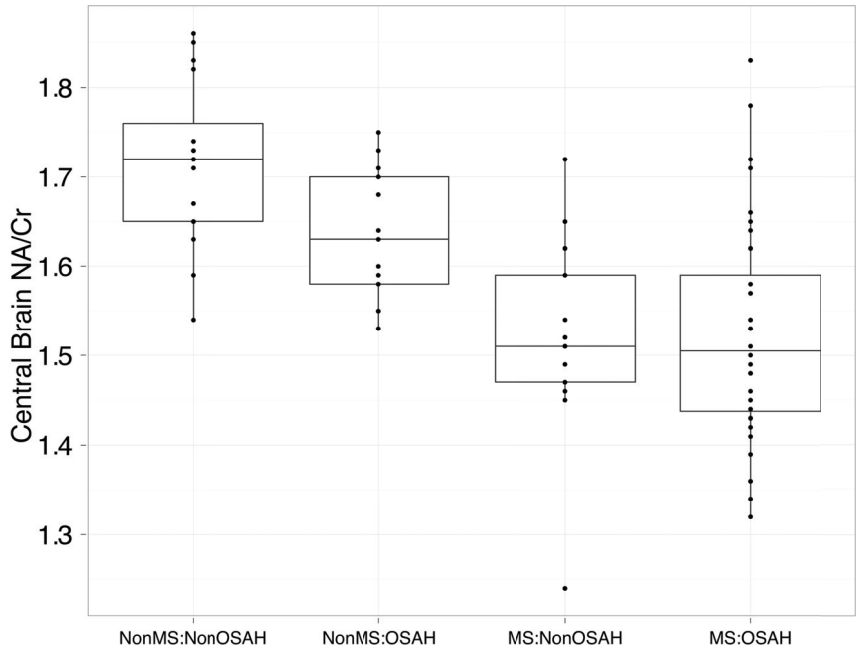

Figure 3-2. Box plots of central brain NA/Cr data (n = 78).

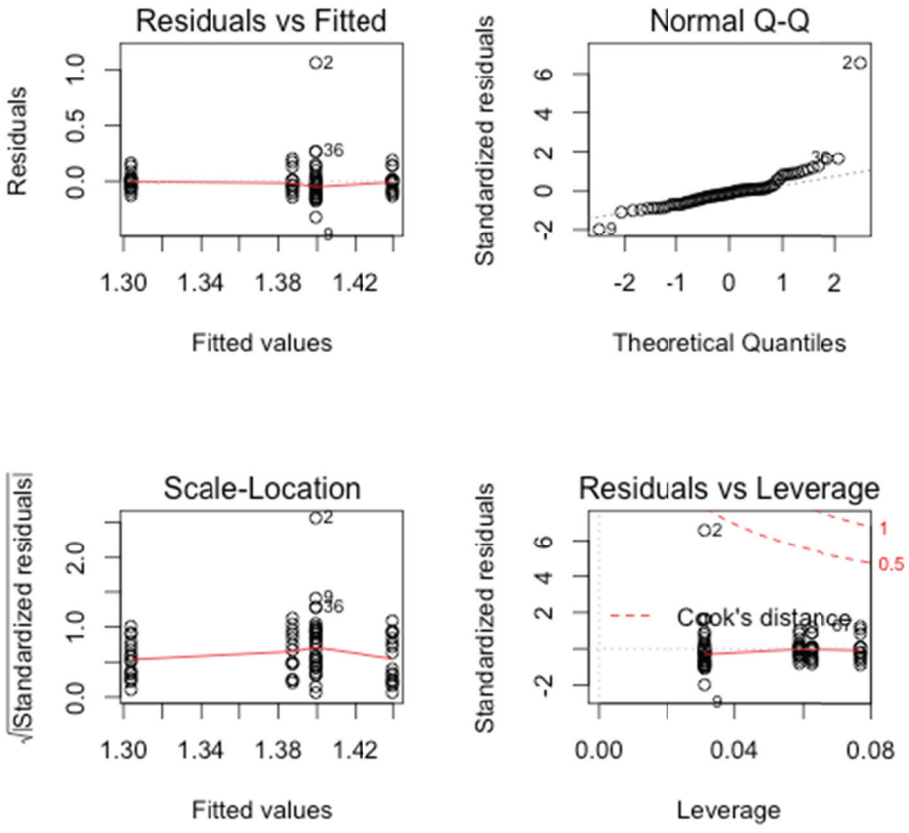

Figure 3-3. Diagnostic plots of NA/Cr data in posterior brain before removing the outlier (n = 78).

Figure 3-3 indicated an outlier with a value of 2.465, compared to a group (MS with OSAH) mean ± SD of = 1.399 ± 0.230. Since this value was more than 4 standard deviations from the group mean, we opted to eliminate the outlier in order to maintain normality of the data and apply a linear regression model. **Figure 3-4** confirmed normal distribution after removing the outlier. **Figure 3-5** showed data distribution in each group of subjects.

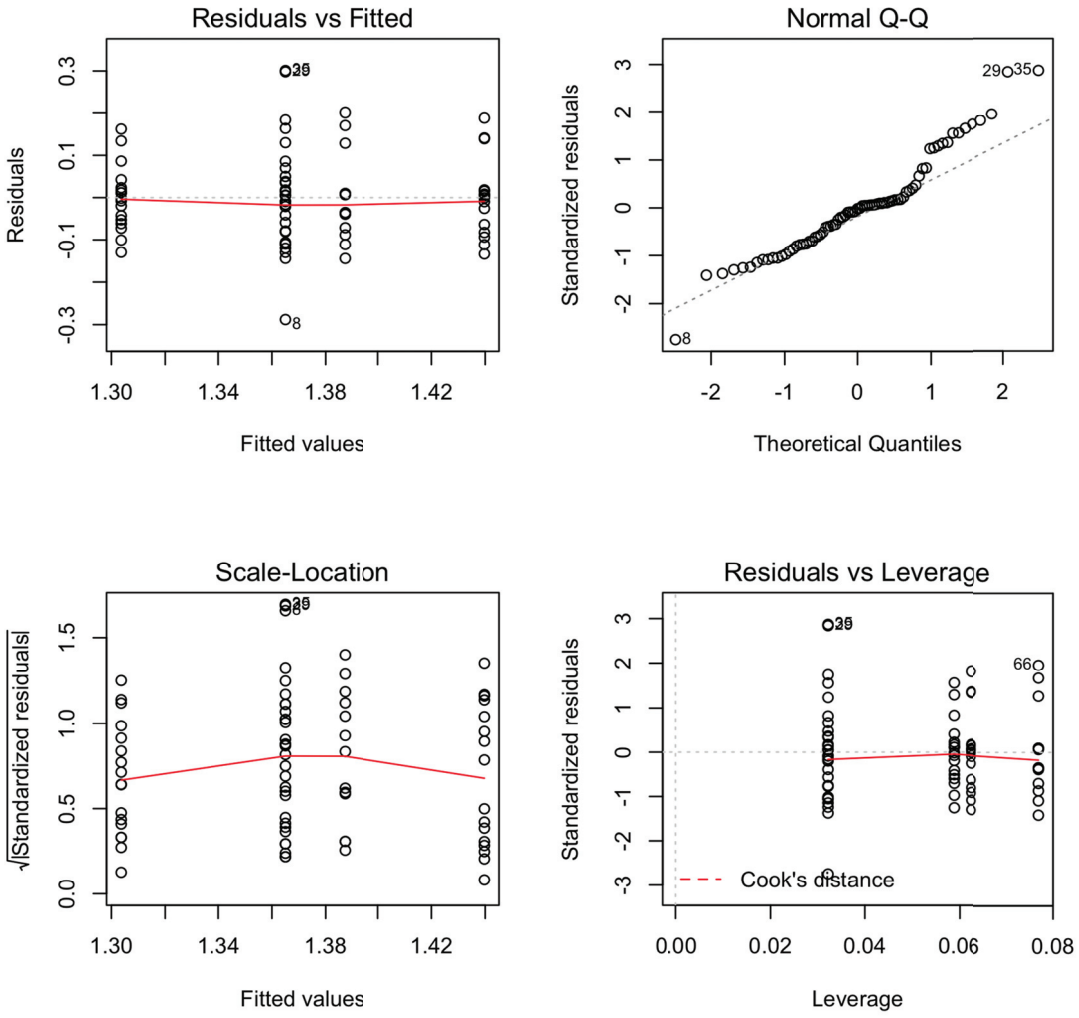

Figure 3-4. Diagnostic plots of NA/Cr data in posterior brain after removing the outlier (n = 77).

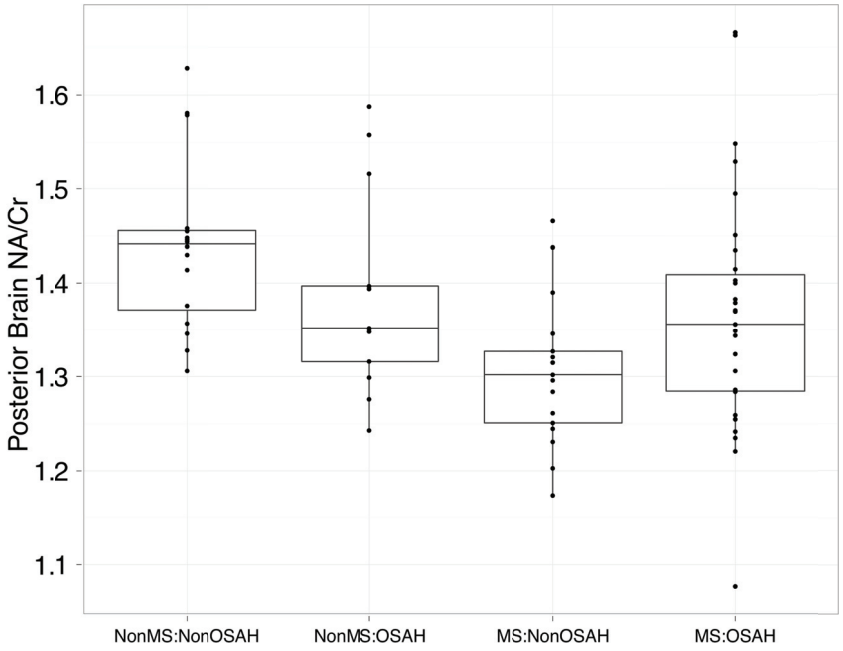

Figure 3-5. Box plots of posterior brain NA/Cr data after removing the outlier (n = 77).

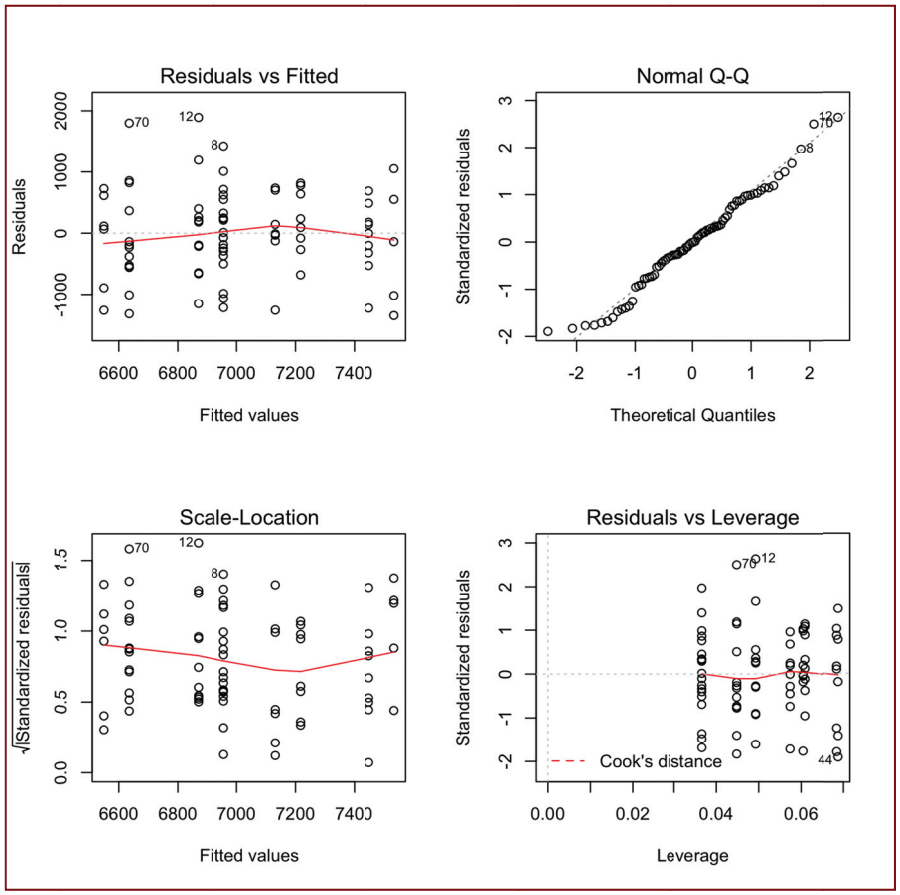

Figure 3-6. Diagnostic plots of normalized hippocampal volume data (n = 78).

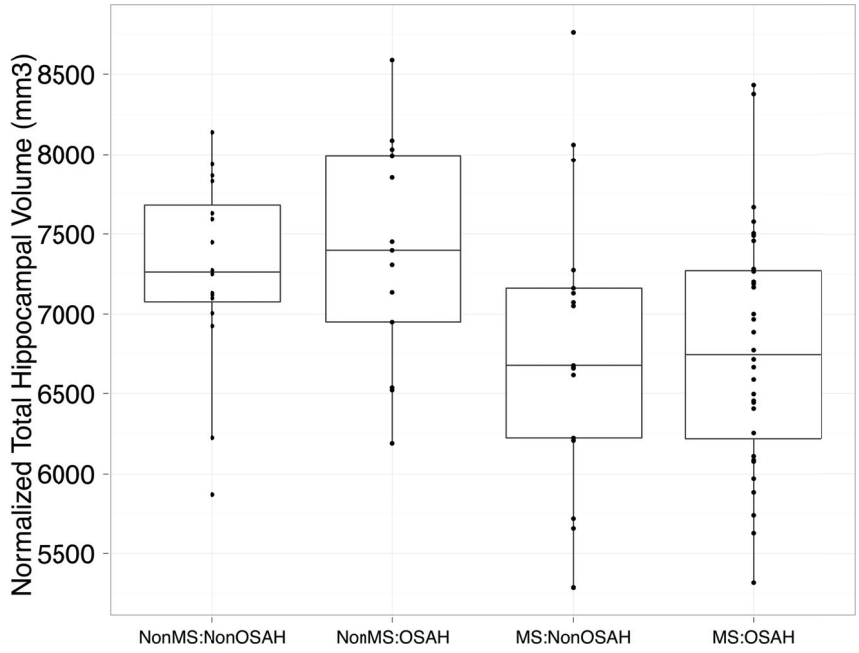

Figure 3-7. Box plots of normalized hippocampal volume data (n = 78).

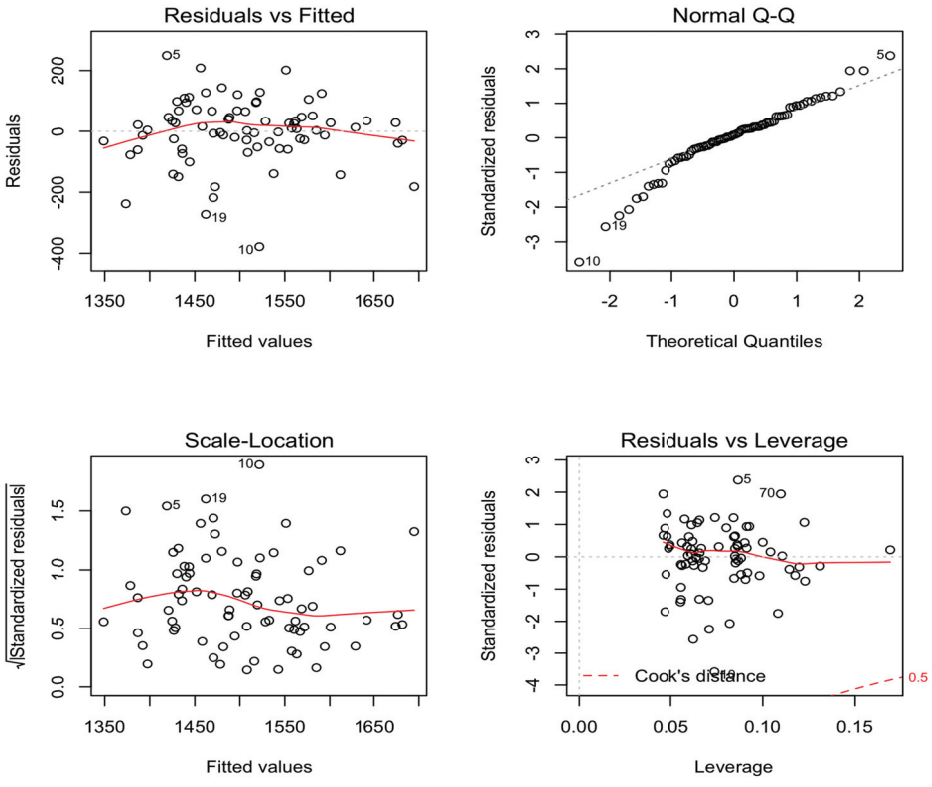

Figure 3-8. Diagnostic plots of normalized brain volume data (n = 78).

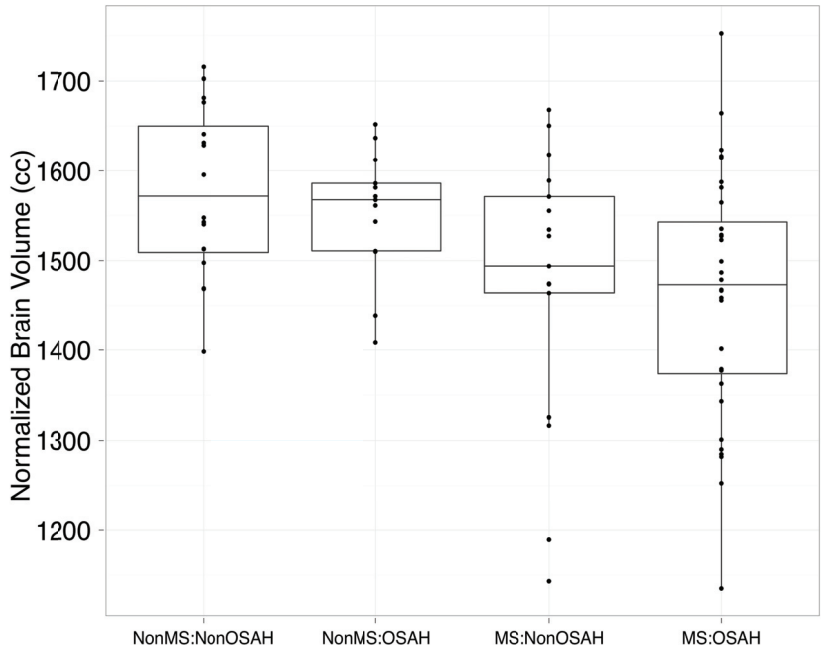

Figure 3-9. Box plots of normalized brain volume data (n = 78).

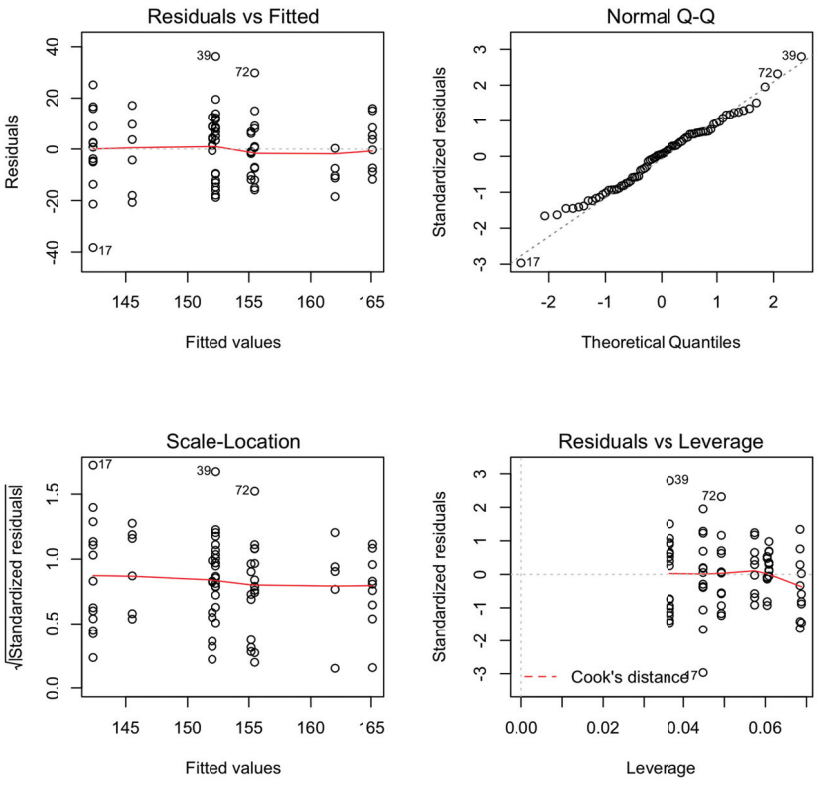

Figure 3-10. Diagnostic plots of normalized cerebellar volume data.

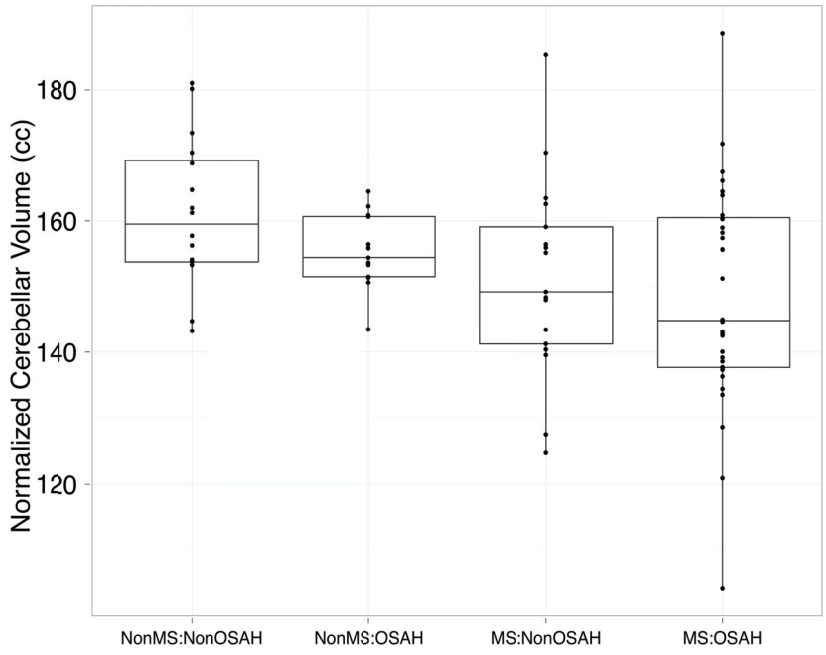

Figure 3-11. Box plots of normalized cerebellar volume data (n = 78).

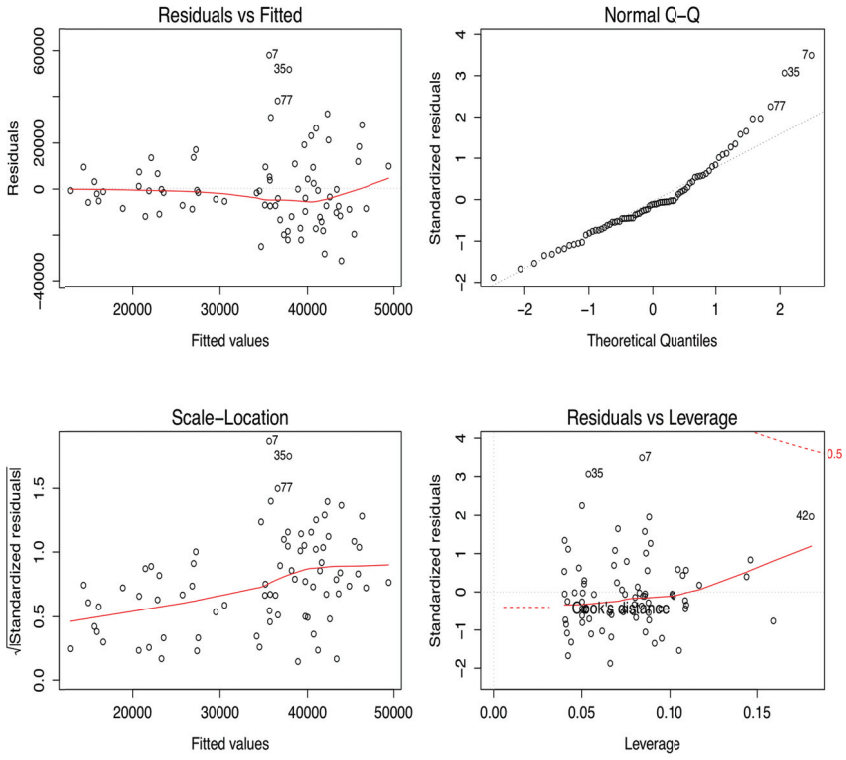

Figure 3-12. Diagnostic plots of normalized lateral ventricular volume data (n = 78).

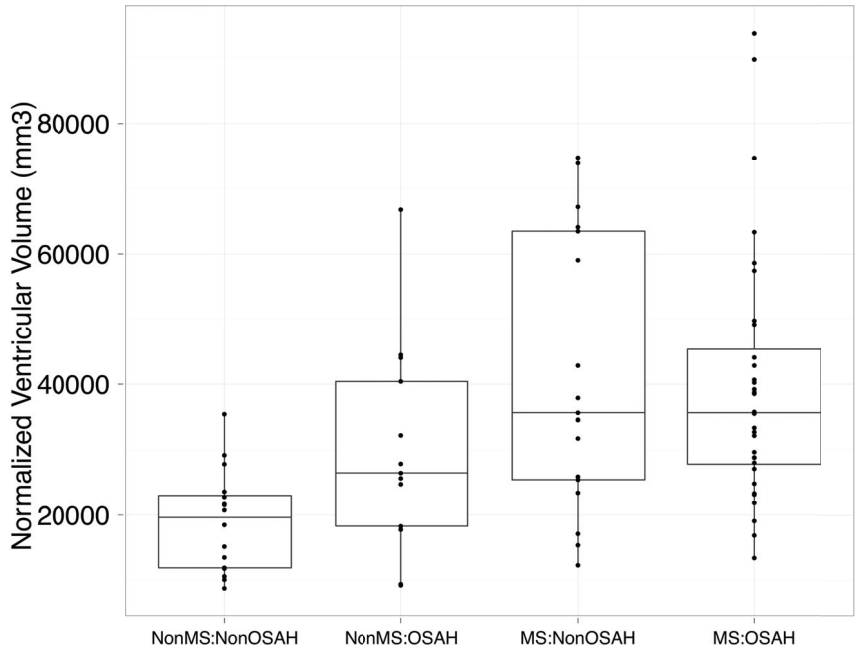

Figure 3-13. Box plots of normalized lateral ventricular volume data (n = 78).

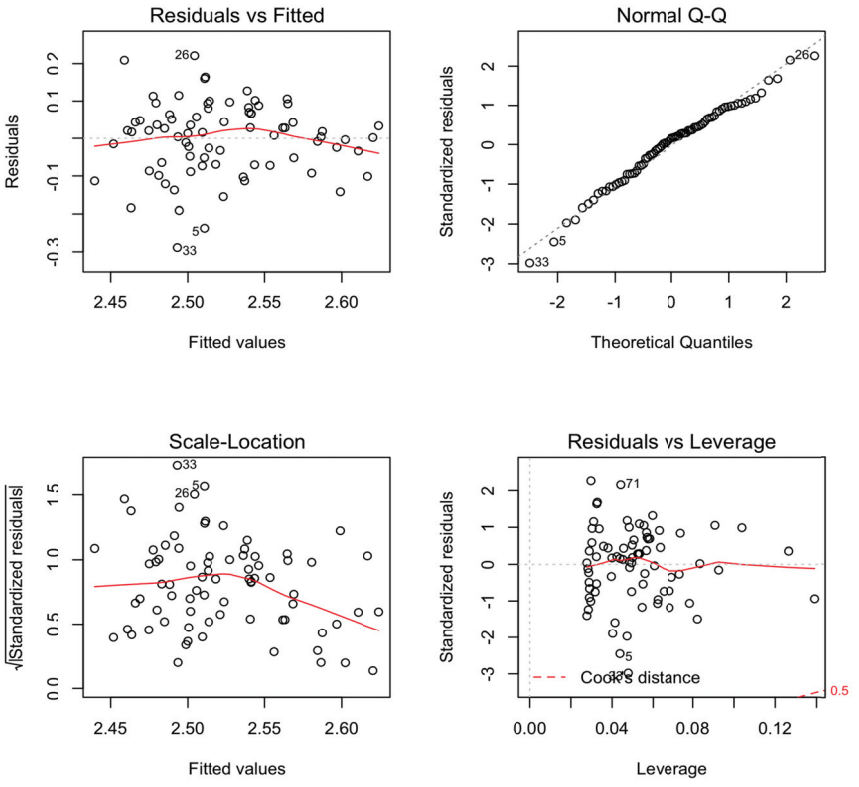

Figure 3-14. Diagnostic plots of cortical thickness data (n = 78).

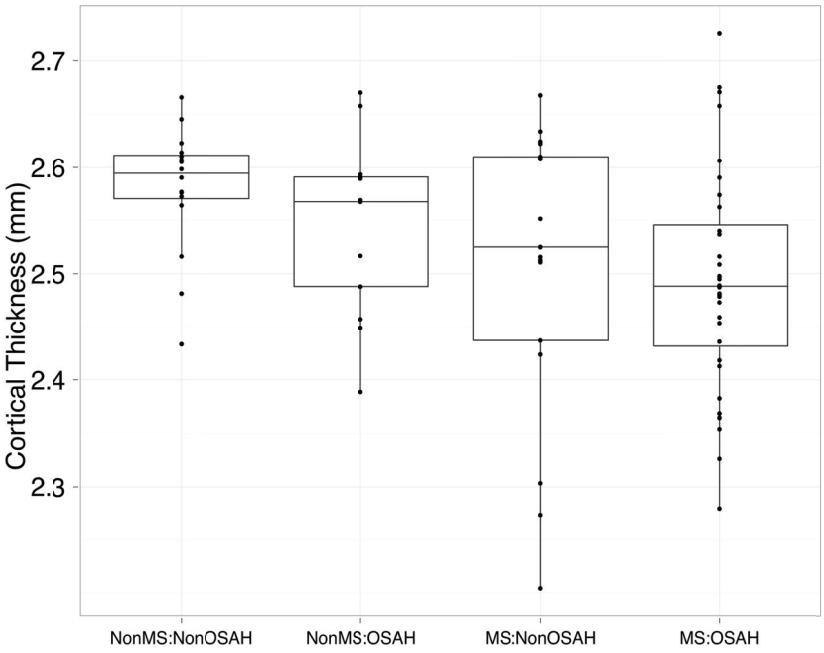

Figure 3-15. Box plots of cortical thickness data (n = 78).

References:

1. Clanet M. Jean-Martin Charcot. 1825 to 1893. Int MS J. 2008 Jun;15(2):59-61.
2. Prevalence and incidence of multiple sclerosis. [cited 2012 April 2nd]; Available from: http://www.mstrust.org.uk/atoz/prevalence_incidence.jsp.
3. Beck CA, Metz LM, Svenson LW, Patten SB. Regional variation of multiple sclerosis prevalence in Canada. Mult Scler. 2005 Oct;11(5):516-9.
4. Wallin MT, Wilken JA, Turner AP, Williams RM, Kane R. Depression and multiple sclerosis: Review of a lethal combination. J Rehabil Res Dev. 2006 Jan-Feb;43(1):45-62.
5. Guidelines MSCfCP. Fatigue and multiple sclerosis: evidence-based management strategies for fatigue in multiple sclerosis: Multiple Sclerosis Council for Clinical Practice Guidelines.; 1998.
6. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med. 2000 Sep 28;343(13):938-52.
7. Thompson AJ, Montalban X, Barkhof F, Brochet B, Filippi M, Miller DH, et al. Diagnostic criteria for primary progressive multiple sclerosis: a position paper. Ann Neurol. 2000 Jun;47(6):831-5.
8. 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 Apr;46(4):907-11.
9. Hohlfeld R. Biotechnological agents for the immunotherapy of multiple sclerosis. Principles, problems and perspectives. Brain. 1997 May;120 (Pt 5):865-916.
10. Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci. 2008;31:247-69.
11. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983 Nov;33(11):1444-52.
12. Fischer JS, Rudick RA, Cutter GR, Reingold SC. The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment. National MS Society Clinical Outcomes Assessment Task Force. Mult Scler. 1999 Aug;5(4):244-50.
13. Kalkers NF, Bergers L, de Groot V, Lazeron RH, van Walderveen MA, Uitdehaag BM, et al. Concurrent validity of the MS Functional Composite using MRI as a biological disease marker. Neurology. 2001 Jan 23;56(2):215-9.
14. Aktas O, Kieseier B, Hartung HP. Neuroprotection, regeneration and immunomodulation: broadening the therapeutic repertoire in multiple sclerosis. Trends Neurosci. 2010 Mar;33(3):140-52.
15. Hartung HP, Aktas O. Bleak prospects for primary progressive multiple sclerosis therapy: downs and downs, but a glimmer of hope. Ann Neurol. 2009 Oct;66(4):429-32.
16. Li DK, Paty DW. Magnetic resonance imaging results of the PRISMS trial: a randomized, double-blind, placebo-controlled study of interferon-beta1a in relapsing-remitting multiple sclerosis. Prevention of Relapses and Disability by Interferon-beta1a Subcutaneously in Multiple Sclerosis. Ann Neurol. 1999 Aug;46(2):197-206.
17. Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. The IFNB Multiple Sclerosis Study Group and The

University of British Columbia MS/MRI Analysis Group. Neurology. 1995 Jul;45(7):1277-85.
18. Comi G, Filippi M, Wolinsky JS. European/Canadian multicenter, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imaging--measured disease activity and burden in patients with relapsing multiple sclerosis. European/Canadian Glatiramer Acetate Study Group. Ann Neurol. 2001 Mar;49(3):290-7.
19. Zhao GJ, Koopmans RA, Li DK, Bedell L, Paty DW. Effect of interferon beta-1b in MS: assessment of annual accumulation of PD/T2 activity on MRI. UBC MS/MRI Analysis Group and the MS Study Group. Neurology. 2000 Jan 11;54(1):200-6.
20. Bamer AM, Johnson KL, Amtmann D, Kraft GH. Prevalence of sleep problems in individuals with multiple sclerosis. Mult Scler. 2008 Sep;14(8):1127-30.
21. Lobentanz IS, Asenbaum S, Vass K, Sauter C, Klosch G, Kollegger H, et al. Factors influencing quality of life in multiple sclerosis patients: disability, depressive mood, fatigue and sleep quality. Acta Neurol Scand. 2004 Jul;110(1):6-13.
22. Trojan DA, Arnold D, Collet JP, Shapiro S, Bar-Or A, Robinson A, et al. Fatigue in multiple sclerosis: association with disease-related, behavioural and psychosocial factors. Multiple Sclerosis. 2007;13(8):985 -95.
23. Veauthier C, Radbruch H, Gaede G, Pfueller CF, Dörr J, Bellmann-Strobl J, et al. Fatigue in multiple sclerosis is closely related to sleep disorders: a polysomnographic cross-sectional study. Multiple Sclerosis Journal. 2011;17(5):613 -22.
24. Kaminska M, Kimoff R, Benedetti A, Robinson A, Bar-Or A, Lapierre Y, et al. Obstructive sleep apnea is associated with fatigue in multiple sclerosis. Multiple Sclerosis Journal. 2011 Dec 19.
25. Brass SD, Duquette P, Proulx-Therrien J, Auerbach S. Sleep disorders in patients with multiple sclerosis. Sleep Med Rev. 2010 Apr;14(2):121-9.
26. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med. 1993 Apr 29;328(17):1230-5.
27. Bixler EO, Vgontzas AN, Lin HM, Ten Have T, Rein J, Vela-Bueno A, et al. Prevalence of sleep-disordered breathing in women: effects of gender. Am J Respir Crit Care Med. 2001 Mar;163(3 Pt 1):608-13.
28. Collop NA. Obstructive sleep apnea syndromes. Semin Respir Crit Care Med. 2005 Feb;26(1):13-24.
29. Broderick M, Guilleminault C. Neurological aspects of obstructive sleep apnea. Ann N Y Acad Sci. 2008 Oct;1142:44-57.
30. Horner RL, Innes JA, Murphy K, Guz A. Evidence for reflex upper airway dilator muscle activation by sudden negative airway pressure in man. J Physiol. 1991 May;436:15-29.
31. Fogel RB, Trinder J, White DP, Malhotra A, Raneri J, Schory K, et al. The effect of sleep onset on upper airway muscle activity in patients with sleep apnoea versus controls. J Physiol. 2005 Apr 15;564(Pt 2):549-62.
32. Jordan AS, White DP. Pharyngeal motor control and the pathogenesis of obstructive sleep apnea. Respir Physiol Neurobiol. 2008 Jan 1;160(1):1-7.

33. Chamberlin NL, Eikermann M, Fassbender P, White DP, Malhotra A. Genioglossus premotoneurons and the negative pressure reflex in rats. J Physiol. 2007 Mar 1;579(Pt 2):515-26.
34. Douse MA, White DP. Serotonergic effects on hypoglossal neural activity and reflex responses. Brain Res. 1996 Jul 8;726(1-2):213-22.
35. Chesson AL, Jr., Berry RB, Pack A. Practice parameters for the use of portable monitoring devices in the investigation of suspected obstructive sleep apnea in adults. Sleep. 2003 Nov 1;26(7):907-13.
36. Kushida CA, Littner MR, Morgenthaler T, Alessi CA, Bailey D, Coleman J, Jr., et al. Practice parameters for the indications for polysomnography and related procedures: an update for 2005. Sleep. 2005 Apr 1;28(4):499-521.
37. Medicine AAoS. International Classification of Sleep Disorders: Diagnostic and Coding Manual. 2nd ed. Westchester, IL2005.
38. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. Physiol Rev. 2010 Jan;90(1):47-112.
39. Engleman HM, Martin SE, Deary IJ, Douglas NJ. Effect of continuous positive airway pressure treatment on daytime function in sleep apnoea/hypopnoea syndrome. Lancet. 1994 Mar 5;343(8897):572-5.
40. Sin DD, Mayers I, Man GC, Pawluk L. Long-term compliance rates to continuous positive airway pressure in obstructive sleep apnea: a population-based study. Chest. 2002 Feb;121(2):430-5.
41. Zozula R, Rosen R. Compliance with continuous positive airway pressure therapy: assessing and improving treatment outcomes. Curr Opin Pulm Med. 2001 Nov;7(6):391-8.
42. Nowak M, Kornhuber J, Meyrer R. Daytime impairment and neurodegeneration in OSAS. Sleep. 2006 Dec;29(12):1521-30.
43. Becker HF, Jerrentrup A, Ploch T, Grote L, Penzel T, Sullivan CE, et al. Effect of nasal continuous positive airway pressure treatment on blood pressure in patients with obstructive sleep apnea. Circulation. 2003 Jan 7;107(1):68-73.
44. Prabhakar NR, Kumar GK. Oxidative stress in the systemic and cellular responses to intermittent hypoxia. Biol Chem. 2004 Mar-Apr;385(3-4):217-21.
45. Prabhakar NR. Sleep apneas: an oxidative stress? Am J Respir Crit Care Med. 2002 Apr 1;165(7):859-60.
46. Haddad GG, Jiang C. Mechanisms of anoxia-induced depolarization in brainstem neurons: in vitro current and voltage clamp studies in the adult rat. Brain Res. 1993 Oct 22;625(2):261-8.
47. Pae EK, Chien P, Harper RM. Intermittent hypoxia damages cerebellar cortex and deep nuclei. Neurosci Lett. 2005 Feb 28;375(2):123-8.
48. Veasey SC, Davis CW, Fenik P, Zhan G, Hsu YJ, Pratico D, et al. Long-term intermittent hypoxia in mice: protracted hypersomnolence with oxidative injury to sleep-wake brain regions. Sleep. 2004 Mar 15;27(2):194-201.
49. Gozal D, Daniel JM, Dohanich GP. Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat. J Neurosci. 2001 Apr 1;21(7):2442-50.
50. Gu Z, Jiang Q, Zhang G. Extracellular signal-regulated kinase 1/2 activation in hippocampus after cerebral ischemia may not interfere with postischemic cell death. Brain Res. 2001 May 18;901(1-2):79-84.

51. Row BW, Kheirandish L, Neville JJ, Gozal D. Impaired spatial learning and hyperactivity in developing rats exposed to intermittent hypoxia. Pediatr Res. 2002 Sep;52(3):449-53.
52. Arnold DL, Matthews PM. MRI in the diagnosis and management of multiple sclerosis. Neurology. 2002 Apr 23;58(8 Suppl 4):S23-31.
53. Paty DW. Magnetic resonance in multiple sclerosis. Curr Opin Neurol Neurosurg. 1993 Apr;6(2):202-8.
54. Khoury SJ, Guttmann CR, Orav EJ, Hohol MJ, Ahn SS, Hsu L, et al. Longitudinal MRI in multiple sclerosis: correlation between disability and lesion burden. Neurology. 1994 Nov;44(11):2120-4.
55. Miller DH. Magnetic resonance in monitoring the treatment of multiple sclerosis. Ann Neurol. 1994;36 Suppl:S91-4.
56. Filippi M, Horsfield MA, Morrissey SP, MacManus DG, Rudge P, McDonald WI, et al. Quantitative brain MRI lesion load predicts the course of clinically isolated syndromes suggestive of multiple sclerosis. Neurology. 1994 Apr;44(4):635-41.
57. De Coene B, Hajnal JV, Gatehouse P, Longmore DB, White SJ, Oatridge A, et al. MR of the brain using fluid-attenuated inversion recovery (FLAIR) pulse sequences. AJNR Am J Neuroradiol. 1992 Nov-Dec;13(6):1555-64.
58. Anderson VM, Fox NC, Miller DH. Magnetic resonance imaging measures of brain atrophy in multiple sclerosis. J Magn Reson Imaging. 2006 May;23(5):605-18.
59. Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, Federico A, et al. Accurate, Robust, and Automated Longitudinal and Cross-Sectional Brain Change Analysis. Neuroimage. 2002;17(1):479-89.
60. Zivadinov R, Reder AT, Filippi M, Minagar A, Stuve O, Lassmann H, et al. Mechanisms of action of disease-modifying agents and brain volume changes in multiple sclerosis. Neurology. 2008 Jul 8;71(2):136-44.
61. Sicotte NL, Kern KC, Giesser BS, Arshanapalli A, Schultz A, Montag M, et al. Regional hippocampal atrophy in multiple sclerosis. Brain. 2008;131(4):1134 -41.
62. Brex PA, Jenkins R, Fox NC, Crum WR, O'Riordan JI, Plant GT, et al. Detection of ventricular enlargement in patients at the earliest clinical stage of MS. Neurology. 2000 Apr 25;54(8):1689-91.
63. Collins DL, Holmes CJ, Peters TM, Evans AC. Automatic 3-D model-based neuroanatomical segmentation. Human Brain Mapping. 1995;3(3):190-208.
64. Frahm J, Bruhn H, Gyngell ML, Merboldt KD, Hanicke W, Sauter R. Localized high-resolution proton NMR spectroscopy using stimulated echoes: initial applications to human brain in vivo. Magn Reson Med. 1989 Jan;9(1):79-93.
65. Bottomley PA. Spatial localization in NMR spectroscopy in vivo. Ann N Y Acad Sci. 1987;508:333-48.
66. Kreis R, Ernst T, Ross BD. Development of the human brain: in vivo quantification of metabolite and water content with proton magnetic resonance spectroscopy. Magn Reson Med. 1993 Oct;30(4):424-37.
67. Haase A, Frahm J, Hanicke W, Matthaei D. 1H NMR chemical shift selective (CHESS) imaging. Phys Med Biol. 1985 Apr;30(4):341-4.
68. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med. 1993 Dec;30(6):672-9.

69. Birken DL, Oldendorf WH. N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. Neurosci Biobehav Rev. 1989 Spring;13(1):23-31.
70. Bjartmar C, Battistuta J, Terada N, Dupree E, Trapp BD. N-acetylaspartate is an axon-specific marker of mature white matter in vivo: a biochemical and immunohistochemical study on the rat optic nerve. Ann Neurol. 2002 Jan;51(1):51-8.
71. Simmons ML, Frondoza CG, Coyle JT. Immunocytochemical localization of N-acetyl-aspartate with monoclonal antibodies. Neuroscience. 1991;45(1):37-45.
72. Miller BL. A review of chemical issues in 1H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. NMR Biomed. 1991 Apr;4(2):47-52.
73. De Stefano N, Narayanan S, Mortilla M, Guidi L, Bartolozzi ML, Federico A, et al. Imaging axonal damage in multiple sclerosis by means of MR spectroscopy. Neurol Sci. 2000;21(4 Suppl 2):S883-7.
74. Arnold DL, Matthews PM, Francis G, Antel J. Proton magnetic resonance spectroscopy of human brain in vivo in the evaluation of multiple sclerosis: assessment of the load of disease. Magn Reson Med. 1990 Apr;14(1):154-9.
75. Matthews PM, De Stefano N, Narayanan S, Francis GS, Wolinsky JS, Antel JP, et al. Putting magnetic resonance spectroscopy studies in context: axonal damage and disability in multiple sclerosis. Semin Neurol. 1998;18(3):327-36.
76. Caramanos Z, Narayanan S, Arnold DL. 1H-MRS quantification of tNA and tCr in patients with multiple sclerosis: a meta-analytic review. Brain. 2005 Nov;128(Pt 11):2483-506.
77. Arnold DL, Matthews PM, Francis GS, O'Connor J, Antel JP. Proton magnetic resonance spectroscopic imaging for metabolic characterization of demyelinating plaques. Ann Neurol. 1992 Mar;31(3):235-41.
78. Kapeller P, Brex PA, Chard D, Dalton C, Griffin CM, McLean MA, et al. Quantitative 1H MRS imaging 14 years after presenting with a clinically isolated syndrome suggestive of multiple sclerosis. Mult Scler. 2002 May;8(3):207-10.
79. Fernando KT, McLean MA, Chard DT, MacManus DG, Dalton CM, Miszkiel KA, et al. Elevated white matter myo-inositol in clinically isolated syndromes suggestive of multiple sclerosis. Brain. 2004 Jun;127(Pt 6):1361-9.
80. Vrenken H, Barkhof F, Uitdehaag BM, Castelijns JA, Polman CH, Pouwels PJ. MR spectroscopic evidence for glial increase but not for neuro-axonal damage in MS normal-appearing white matter. Magn Reson Med. 2005 Feb;53(2):256-66.
81. Srinivasan R, Sailasuta N, Hurd R, Nelson S, Pelletier D. Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T. Brain. 2005;128(5):1016 -25.
82. Punjabi NM. The Epidemiology of Adult Obstructive Sleep Apnea. Proc Am Thorac Soc. 2008;5(2):136-43.
83. Zimmerman ME, Aloia MS. A review of neuroimaging in obstructive sleep apnea. J Clin Sleep Med. 2006 Oct 15;2(4):461-71.
84. Davies CW, Crosby JH, Mullins RL, Traill ZC, Anslow P, Davies RJ, et al. Case control study of cerebrovascular damage defined by magnetic resonance imaging in patients with OSA and normal matched control subjects. Sleep. 2001 Sep 15;24(6):715-20.

85. Gale SD, Hopkins RO. Effects of hypoxia on the brain: neuroimaging and neuropsychological findings following carbon monoxide poisoning and obstructive sleep apnea. J Int Neuropsychol Soc. 2004 Jan;10(1):60-71.
86. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage. 2000 Jun;11(6 Pt 1):805-21.
87. Macey PM, Henderson LA, Macey KE, Alger JR, Frysinger RC, Woo MA, et al. Brain morphology associated with obstructive sleep apnea. Am J Respir Crit Care Med. 2002 Nov 15;166(10):1382-7.
88. Yaouhi K, Bertran F, Clochon P, Mezenge F, Denise P, Foret J, et al. A combined neuropsychological and brain imaging study of obstructive sleep apnea. J Sleep Res. 2009 Mar;18(1):36-48.
89. Morrell MJ, McRobbie DW, Quest RA, Cummin AR, Ghiassi R, Corfield DR. Changes in brain morphology associated with obstructive sleep apnea. Sleep Med. 2003 Sep;4(5):451-4.
90. Morrell MJ, Jackson ML, Twigg GL, Ghiassi R, McRobbie DW, Quest RA, et al. Changes in brain morphology in patients with obstructive sleep apnoea. Thorax. 2010 Oct;65(10):908-14.
91. O'Donoghue FJ, Briellmann RS, Rochford PD, Abbott DF, Pell GS, Chan CH, et al. Cerebral structural changes in severe obstructive sleep apnea. Am J Respir Crit Care Med. 2005 May 15;171(10):1185-90.
92. Joo EY, Tae WS, Lee MJ, Kang JW, Park HS, Lee JY, et al. Reduced brain gray matter concentration in patients with obstructive sleep apnea syndrome. Sleep. 2010 Feb;33(2):235-41.
93. Torelli F, Moscufo N, Garreffa G, Placidi F, Romigi A, Zannino S, et al. Cognitive profile and brain morphological changes in obstructive sleep apnea. Neuroimage. 2011 Jan 15;54(2):787-93.
94. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron. 2002 Jan 31;33(3):341-55.
95. Fischl B, Salat DH, van der Kouwe AJ, Makris N, Segonne F, Quinn BT, et al. Sequence-independent segmentation of magnetic resonance images. Neuroimage. 2004;23 Suppl 1:S69-84.
96. Segonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, et al. A hybrid approach to the skull stripping problem in MRI. Neuroimage. 2004 Jul;22(3):1060-75.
97. Ding J, Nieto FJ, Beauchamp NJ, Jr., Harris TB, Robbins JA, Hetmanski JB, et al. Sleep-disordered breathing and white matter disease in the brainstem in older adults. Sleep. 2004 May 1;27(3):474-9.
98. Macey PM, Kumar R, Woo MA, Valladares EM, Yan-Go FL, Harper RM. Brain structural changes in obstructive sleep apnea. Sleep. 2008 Jul;31(7):967-77.
99. Kamba M, Suto Y, Ohta Y, Inoue Y, Matsuda E. Cerebral metabolism in sleep apnea. Evaluation by magnetic resonance spectroscopy. Am J Respir Crit Care Med. 1997 Jul;156(1):296-8.
100. Kamba M, Inoue Y, Higami S, Suto Y, Ogawa T, Chen W. Cerebral metabolic impairment in patients with obstructive sleep apnoea: an independent association of obstructive sleep apnoea with white matter change. J Neurol Neurosurg Psychiatry. 2001 Sep;71(3):334-9.

101. Halbower AC, Degaonkar M, Barker PB, Earley CJ, Marcus CL, Smith PL, et al. Childhood obstructive sleep apnea associates with neuropsychological deficits and neuronal brain injury. PLoS Med. 2006 Aug;3(8):e301.
102. Bartlett DJ, Rae C, Thompson CH, Byth K, Joffe DA, Enright T, et al. Hippocampal area metabolites relate to severity and cognitive function in obstructive sleep apnea. Sleep Med. 2004 Nov;5(6):593-6.
103. Alchanatis M, Deligiorgis N, Zias N, Amfilochiou A, Gotsis E, Karakatsani A, et al. Frontal brain lobe impairment in obstructive sleep apnoea: a proton MR spectroscopy study. Eur Respir J. 2004 Dec;24(6):980-6.
104. Engleman HM, Kingshott RN, Martin SE, Douglas NJ. Cognitive function in the sleep apnea/hypopnea syndrome (SAHS). Sleep. 2000 Jun 15;23 Suppl 4:S102-8.
105. Naegele B, Pepin JL, Levy P, Bonnet C, Pellat J, Feuerstein C. Cognitive executive dysfunction in patients with obstructive sleep apnea syndrome (OSAS) after CPAP treatment. Sleep. 1998 Jun 15;21(4):392-7.
106. Sarchielli P, Presciutti O, Alberti A, Tarducci R, Gobbi G, Galletti F, et al. A 1H magnetic resonance spectroscopy study in patients with obstructive sleep apnea. Eur J Neurol. 2008 Oct;15(10):1058-64.
107. Tonon C, Vetrugno R, Lodi R, Gallassi R, Provini F, Iotti S, et al. Proton magnetic resonance spectroscopy study of brain metabolism in obstructive sleep apnoea syndrome before and after continuous positive airway pressure treatment. Sleep. 2007 Mar;30(3):305-11.
108. 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 Jul;50(1):121-7.
109. De Stefano N, Narayanan S, Francis GS, Arnaoutelis R, Tartaglia MC, Antel JP, et al. Evidence of axonal damage in the early stages of multiple sclerosis and its relevance to disability. Arch Neurol. 2001 Jan;58(1):65-70.
110. Kaminska M, Kimoff R, Benedetti A, Robinson A, Bar-Or A, Lapierre Y, et al. Obstructive sleep apnea is associated with fatigue in multiple sclerosis. Mult Scler. 2011 Dec 19.
111. Iber C, Medicine AAoS. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications: American Academy of Sleep Medicine; 2007.
112. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. Sleep. 1999 Aug 1;22(5):667-89.
113. Krupp LB, Larocca NG, Muirnash J, Steinberg AD. The Fatigue Severity Scale - Application to Patients with Multiple-Sclerosis and Systemic Lupus-Erythematosus. Archives of Neurology. 1989 Oct;46(10):1121-3.
114. Smets EMA, Garssen B, Cull A, deHaes JCJM. Application of the multidimensional fatigue inventory (MFI-20) in cancer patients receiving radiotherapy. Brit J Cancer. 1996 Jan;73(2):241-5.
115. Radloff LS. The CES-D Scale. Applied Psychological Measurement. 1977 June 1, 1977;1(3):385-401.
116. Cohen S, Williamson GM. Perceived Stress in a Probability Sample of the United-States. Clar Symp. 1988:31-67.

117. Ware JE, Kosinski M, Dewey JE, Gandek B. SF-36 health survey: manual and interpretation guide. Boston: Nimrod Press; 1993.
118. Kurtzke JF. Rating Neurologic Impairment in Multiple-Sclerosis - an Expanded Disability Status Scale (Edss). Neurology. 1983;33(11):1444-52.
119. Francis S. Automatic Lesion Identification in MRI of Multiple Sclerosis Patients. Montreal, Quebec: McGill Univeristy; 2004.
120. Collins DL, Pruessner JC. Towards accurate, automatic segmentation of the hippocampus and amygdala from MRI by augmenting ANIMAL with a template library and label fusion. Neuroimage. 2010;52(4):1355-66.
121. Dale AM, Fischl B, Sereno MI. Cortical Surface-Based Analysis: I. Segmentation and Surface Reconstruction. Neuroimage. 1999;9(2):179-94.
122. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magnetic Resonance in Medicine. 1993;30(6):672-9.
123. Filippi M, Agosta F. Imaging biomarkers in multiple sclerosis. J Magn Reson Imaging. 2010 Apr;31(4):770-88.
124. Narayanan S, De Stefano N, Francis GS, Arnaoutelis R, Caramanos Z, Collins DL, et al. Axonal metabolic recovery in multiple sclerosis patients treated with interferon beta-1b. J Neurol. 2001 Nov;248(11):979-86.
125. Khan O, Shen Y, Caon C, Bao F, Ching W, Reznar M, et al. Axonal metabolic recovery and potential neuroprotective effect of glatiramer acetate in relapsing-remitting multiple sclerosis. Mult Scler. 2005 Dec;11(6):646-51.