FUNCTIONAL MAGNETIC RESONANCE IMAGING OF THE SPINAL CORD OF PATIENTS WITH MULTIPLE SCLEROSIS USING THERMAL STIMULATION

BY

AMANDA D. BERGMAN

A Thesis submitted to
the Faculty of Graduate Studies
In partial fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Physiology
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Functional Magnetic Resonance Imaging of the Spinal Cord of Patients with Multiple Sclerosis Using Thermal Stimulation

BY

Amanda D. Bergman

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

MASTER OF SCIENCE

Abstract

Functional magnetic resonance imaging of the spinal cord (Spinal fMRI) has been shown to detect activity in the spinal cord of healthy and spinal cord injured (SCI) subjects and is based on the SEEP (signal enhancement by extravascular protons) theory. The SEEP theory is a fMRI contrast mechanism based on water crossing the blood brain barrier (BBB) to increase extravascular water content in areas of neuronal activity. Here, the first study of spinal fMRI as applied to subjects with multiple sclerosis is presented. The aims of this study are: to test the SEEP theory in a model with increased permeability of the BBB; to investigate the neuronal activity that can be detected in the spinal cord of subjects with MS; and to further develop spinal fMRI as clinical tool. Spinal fMRI was carried out in 27 volunteers with MS and 4 healthy volunteers. Imaging was carried out in a 1.5 T clinical MR system, using established methods. Thermal stimulation at 15°C was applied to various dermatomes depending on the presence of lesions on T2 weighted scans and of functional deficits, and images were obtained in corresponding regions of the spinal cord. Patterns of neuronal activity were consistently observed in the spinal cord in response to stimulation which relied on the presence of a lesion in the region of the cord imaged and the severity of the deficits reported by the subjects. Signal intensity changes were significantly higher in MS patients than in healthy controls. Even with altered physiology of MS plaques, neuronal activity can be seen in recognizable patterns. Increased signal intensity changes in MS subjects support the theory that SEEP signal changes depend on the permeability of the BBB and they arise from a change in extravascular water content. As MS affects the lives of many people, any new insight into the disease has the potential to be extremely useful, both clinically and for research.

Acknowledgments

First, I would like to thank my supervisor, Dr. Patrick Stroman for his support and guidance and for his patience in helping me with my many computer problems. I would also like to thank Vicky Krause and Corinne LeBlanc for running the MR scanner to obtain the data.

Thanks to my committee, Drs. Kris Malisza, Jim Peeling, and Dave McCrea for their help and support throughout this project.

I would like to thank my family and friends for their support throughout the course of all of my schooling; for always being willing to offer a helping hand and for helping me through the rough spots.

Table of Contents

Abstract iii

Acknowledgements iv

List of Figures vii

List of Tables viii

Introduction 1
- Study Rationale 1
- Multiple Sclerosis 1
  - Diagnosis of MS 2
  - Differential Diagnosis 4
  - Natural History of MS 6
  - Pathophysiology of MS 9
    - Effects of Oligodendrocytes 11
    - Inflammation 13
    - Axonal Loss 16
    - Repair Mechanisms 17
  - Treatments of MS 19
  - MS in the Spinal Cord 22
- Spinal Cord and Sensory Transduction 23
  - Spinal Cord Circulation 26
  - Somatosensory Pathways 27
- Blood Brain Barrier 31
- Basic MRI 32
  - MRI Characteristics in Multiple Sclerosis 37
  - Spinal Cord MRI 41
- Functional MRI 42
  - BOLD Effect 42
  - SEEP Theory 45
  - Spinal Cord Functional MRI 47
  - Functional MRI and MS 49

Methods 52
- Subjects 52
- MR Data Acquisition 55
  - Functional Imaging 56
- Thermal Stimulation Paradigm 57
- Data Analysis 58

Results **62**
- Data Collected 62
- Patterns of Activity 63
    - MS Patients 63
    - Primary Progressive MS Patients 63
    - Relapsing Remitting MS Patients 67
    - Secondary Progressive MS Patients 67
- Changes in Signal Intensity 68

Discussion **72**
- Changes in Signal Intensity 72
    - Signal Intensity Changes in MS Subjects 74
    - Signal Intensity Changes by Subtype 75
- Patterns of Activity 78
    - Patterns observed 79
    - Primary Progressive MS Subjects 82
    - Relapsing Remitting MS Subjects 83
    - Secondary Progressive MS Subjects 84
    - Effects of Medications on Activity 85
- Future Work 91
- Conclusions 92

Appendix A **94**

Reference List **148**

List of Figures

		Page
Figure 1.	The paradigm followed for thermal stimulation. On the y axis, 0 represents baseline conditions while 1 represents stimulation.	60
Figure 2.	Fractional signal changes observed in spinal fMRI of healthy controls (blue) compared to MS patients (red) as they relate to the stimulation paradigm (black).	69
Figure 3.	Fractional signal changes observed in spinal fMRI of PPMS (purple), RRMS (blue), and SPMS (green) as they relate to the stimulation paradigm (black).	70
Figure 4.	Combined spinal fMRI results from the lumbar spinal cord showing the activity response to 10°C thermal stimulation of the L4 dermatome of healthy subjects. Images are in radiological orientation with the right side of the body towards the left side of the image, and dorsal is towards the bottom. From left to right the slices span approximately through the following spinal cord segments: fifth/fourth lumbar; third lumbar; second/first lumbar; 12^{th} thoracic; and 11^{th} thoracic. The colour scale shows red at points with the greatest consistency across subjects, through orange, yellow, and then green indicating that only three subjects showed activity. The schematic inlay (bottom right) shows the expected areas of activity in the spinal cord with a sensory or noxious stimulus. The schematic is magnified roughly by a factor of 5 compared to the MR images.	81

List of Tables

		Page
Table 1.	Examples of conditions that should be considered in the differential diagnosis of multiple sclerosis.	5
Table 2.	Details known about subjects and their condition; age, sex, subtype of multiple sclerosis, when the first symptoms were experienced, and when the subjects were diagnosed with multiple sclerosis.	53 - 54
Table 3.	The patterns of activity observed in the subjects with multiple sclerosis with the regions of the spine imaged and the presence or absence of lesions in the region imaged along with the symptoms experienced by the subjects at the time of the study.	64 - 66
Table 4.	P-values obtained when comparing the signal intensity changes of the subgroups of subjects with multiple sclerosis with each other and with healthy control subjects. (SP = secondary progressive MS; RR = relapsing remitting MS; PP = primary progressive MS).	71
Table 5.	Medications taken by subjects with MS at the time of the study.	86 - 87
Table 6.	Medications reported taken by the subjects with MS and their actions.	88 - 90

Introduction

Study Rationale

This study was performed to further develop functional magnetic resonance imaging of the spinal cord (spinal fMRI) as a clinical tool and to test two hypotheses. The first hypothesis involves the damage inflicted on the spinal cord of patients with multiple sclerosis (MS). We believe that patients with MS will have patterns of activity in spinal fMRI that are different than those of healthy controls. The second hypothesis involves a recently postulated fMRI contrast mechanism termed signal enhancement by extravascular protons (SEEP). This theory involves water crossing the blood brain barrier (BBB) into the central nervous system (CNS) upon activation of a region of neural tissue. As the BBB is not fully intact in many patients with MS, we propose that the percent signal changes observed with thermal stimulation on fMRI using the SEEP theory will be higher than those in healthy control subjects.

To test these hypotheses, knowledge of a variety of subjects is needed. These subjects include how the CNS normally functions, the changes which occur due to MS, how MRI can detect these changes, the theory behind functional MRI and spinal fMRI, and how fMRI has been used to learn more about MS previously.

Multiple Sclerosis

Multiple sclerosis is a disorder of the CNS that initially manifests as acute demyelination in focal lesions, and axonal loss with limited remyelination. As the disease progresses, chronic plaques of demyelination and astrocytosis with no functional remyelination are observed (1;2). MS is possibly the most common cause of neurological disability in young adults (1;3;4) with 50% of patients requiring help with walking within

fifteen years after the onset of disease (1). Throughout the world, approximately 2.5 million people have MS, predominantly affecting northern Europeans (1;4). The incidence and prevalence of MS are approximately 7 per 100,000 and 120 per 100,000 per year, respectively (1). In some areas, the lifetime risk may be as high as 1/400 (1).

MS plaques vary in size and may be located throughout the CNS (5). The hallmarks of MS pathology are myelin destruction, oligodendrocyte death, and axonal loss (6). The disease is hypothesized to be an organ-specific, T cell mediated autoimmune disease directed against oligodendrocytes and, for most, leading to impairments in both physical and psychological functions (7). These disabilities develop due to two mechanisms: as a result of incomplete recovery after relapse or disease progression (8).

Diagnosis of MS

The most widely used criteria for the diagnosis of MS are those put forth by Poser *et al* (9). These criteria divide patients into four subgroups: clinically probable MS, laboratory-supported clinical MS, clinically definite MS, and laboratory-supported definite MS. For clinically probable MS, dissemination in time or space must be shown. This would involve two exacerbations with clinical evidence of one lesion, one exacerbation with clinical evidence of two lesions, or one exacerbation with clinical evidence of one lesion and paraclinical evidence of one lesion. Laboratory-supported clinical MS involves the patient experiencing two exacerbations with cerebrospinal fluid (CSF) oligoclonal bands (OCB). Patients are diagnosed with clinically definite MS if both dissemination in time and in space can be shown. Therefore, the patient may have two attacks with clinical evidence of two separate lesions or two attacks with clinical

evidence of one lesion and paraclinical evidence of one lesion. Finally, to be diagnosed with laboratory-supported definite MS, the patient must experience a minimum of two exacerbations with clinical or paraclinical evidence of one lesions and CSF OCB; one exacerbation with clinical evidence of two separate lesions and CSF OCB; or one exacerbation, clinical evidence of one lesion and paraclinical evidence of a second lesion and CSF OCB.

These criteria have been revised by a panel of MS experts to include the involvement of MRI and to include progressive forms (10). A clinical diagnosis can still be made if dissemination in both time and space can be demonstrated, that is, two or more relapses with clinical evidence of two separate lesions. The diagnosis can also be made if one relapse is complemented by additional lesions shown by paraclinical evidence such as MRI abnormalities, CSF changes, and/or abnormal evoked potentials. The lesions still must be shown to be disseminated in time and space within the CNS (10).

MR abnormalities present in either the brain or spinal cord have been integrated into the new diagnostic criteria. As spinal cord lesions are more specific than brain lesions for MS and do not occur with aging, the presence of two discrete spinal cord lesions are considered positive MRI evidence even if the brain MRI is normal (11). In contrast, nine lesions on a brain MRI are considered as positive MRI evidence but if one spinal cord lesion is shown, only four lesions must be demonstrated on brain MRI (11).

CSF abnormalities can be found in the majority of patients at some point during the disease course (10). CSF protein electrophoresis shows oligoclonal IgG bands in more than 90% of patients. The presence of these oligoclonal bands strengthen the

evidence that the underlying pathology of MS is inflammatory at some point in its progression (1). Abnormalities may also include slightly elevated CSF white blood cell (WBC) count (typically ≤50 lymphocytes), elevated IgG within CSF compared to serum (IgG index), and identification of two or more unique oligoclonal bands by CSF protein electrophoresis (10). Oligoclonal bands do not always present early in the disease course but once present, they will always be found in that patient. They are found in 90 – 95% of MS patients later in the course of the disease. While OCBs are useful, they are also found in other conditions such as acute disseminated encephalomyelitis (ADEM), subacute sclerosing panencephalitis, Behçet's disease, systemic lupus erythemetosus, and sarcoidosis and therefore cannot be used as the only evidence for a diagnosis of MS (10).

In addition to MRI and CSF abnormalities, characteristic changes in evoked potentials can be used as paraclinical evidence for MS. Demyelination delays the latencies of visual, auditory, and somatosensory evoked potentials. It also delays central motor conduction times but the amplitude of the responses is not affected (1).

Besides meeting the aforementioned criteria, to diagnose a patient with MS, all other possible explanations must be ruled out for both the clinical signs and the paraclinical evidence (10). Therefore, the diagnosis of MS is one of exclusion.

Differential Diagnosis

There are many disorders one must consider in the differential diagnosis of multiple sclerosis. These include metabolic disorders, autoimmune diseases, infections, vascular disorders, genetic syndromes, diseases associated with lesions of the posterior fossa and spinal cord, psychiatric disorders, neoplastic diseases, and variants of multiple sclerosis (12-14). A more specific listing of these diseases can be found in Table 1.

Table 1: Examples of conditions that should be considered in the differential diagnosis of multiple sclerosis (12-14).

Type of Disorders	Specific Conditions
Metabolic Disorders	Disorders of B12 metabolism Leukodystrophies
Autoimmune Diseases	Sjogren's syndrome Systemic lupus erythematosus Behcet's disease Sarcoidosis Chronic inflammatory demyelinating polyradiculopathy Antiphospholipid-antibody syndrome
Infections	HIV-associated myelopathy HTLV-1 associated myelopathy Lyme disease Meningovascular syphilis Eales' disease
Vascular disorders	Spinal dural arteriovenous fistula Cavernous hemangiomata CNS vasculitis including retinocochlear cerebral vasculitis Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
Genetic syndromes	Hereditary ataxias Hereditary paraplegias Leber's optic atrophy Other mitochondrial cytopathies
Lesions of the posterior fossa and spinal cord	Arnold-Chiari malformation Nonhereditary ataxias Spondylotic and other myelopathies
Psychiatric disorders	Conversion reaction Malingering
Neoplastic diseases	Spinal cord tumours CNS lymphoma Paraneoplastic disorders
Variants of multiple sclerosis	Optic neuritis Isolated brain stem syndromes Transverse myelitis Acute disseminated encephalomyelitis Marburg disease Neuromyelitis optica

Natural History of MS

MS affects twice as many women as men (1;14). Disease onset usually occurs in the third or fourth decade but may occur earlier with 2% of patients acquiring the disease before age ten and 5% before age sixteen (1). In approximately one quarter of patients, the disease never affects their activities of daily living (1). As well, 10% of patients do well for over 20 years and are considered to have benign MS (14). Conversely, up to 15% become severely disabled within a short time (1). Life expectancy may be slightly shortened but most patients will live at least 25 years past diagnosis with the majority dying of unrelated causes (1;14). However, in rare cases, patients with severe forms of the disease may die within months or less after the onset of MS. Suicide is a risk even for young patients with mild symptoms (14).

Eighty percent of patients present with the relapsing remitting subtype of MS (RRMS) (1;7;14). This subtype presents as phases of relapse with full recovery between relapses. Episodes occur at random intervals but they initially average around one per year and decrease steadily thereafter (1). After 5 to 15 years, 70% of patients who originally presented with RRMS will advance to the secondary progressive phase of MS (SPMS) (7;14). During a relapse in RRMS, symptoms usually develop over several days and stabilize. They will then resolve, either spontaneously or in response to corticosteroids, within a few weeks (14). The relapses result from inflammation and demyelination with remission resulting from resolution of inflammation, redistribution of sodium channels of demyelinated axons, and remyelination (15). RRMS can present in many ways but typically starts with sensory disturbances, unilateral optic neuritis, diplopia (internuclear ophthalmoplegia), Lhermitte's sign (trunk and limb parethesias

evoked by neck flexion), limb weakness, clumsiness, gait ataxia, and neurogenic bladder and bowel symptoms (14). The main differences between RRMS and the other subgroups are that in RRMS there is little, if any, diffuse abnormality and wide spread atrophy is not observed in this subgroup.

SPMS is characterized by less than full recovery between relapses, a build up of persistent deficits, and disease progression between relapses (1;7;14;15). Progression between clinical relapses can be seen on MRI scans of the brain and spinal cord. This serves as evidence of greater white matter injury in patients with SPMS than in those with RRMS (16). SPMS can be differentiated from the other subgroups by a larger proportion of brain lesions appearing as hypointense on T1-weighted MRI and by atrophy of both the brain and spinal cord (17).

In 20% of patients, the disease is progressive from its onset, affecting the spinal cord and, less frequently, the optic nerve, cerebrum, or cerebellum (1;14). This form of MS, termed primary progressive (PPMS), affects an older, predominantly male population. This subtype has a less favourable prognosis than RRMS and is associated with fewer inflammatory lesions (1). The diagnosis of PPMS is usually more difficult than the other subgroups. In the various diagnostic criteria developed specifically for PPMS, an emphasis is placed on spinal cord imaging as lesions are often not found in the brain. Two spinal cord lesions may be sufficient to show dissemination in space when there is clinical evidence of MS. In some PPMS patients, only diffuse cord changes are found (18). Despite the severe disability experienced by patients in this subgroup, MR imaging usually shows few focal lesions in both the brain and spinal cord (17;19).

The prognosis is relatively good when sensory or visual symptoms predominate the course of MS in adults and there is complete recovery from individual episodes. This is most common in young women (1). Frequent relapses in the first two years, a progressive course from the onset, male sex, and early permanent motor or cerebellar findings, especially disturbances of coordination or balance, are predictive of a more severe clinical course (1;14). The level of disability experienced by the patient is usually determined by the onset of the progressive phase of the disease (1).

Susceptibility to MS is affected by both genetic and environmental factors. The recurrence risk is higher in children with both parents affected with MS (20%) than those with only one parent affected (2%). The risk does not increase for either those adopted into a family with someone affected with MS or the families of adoptees with MS (1). While believed to be polygenic, genetic factors cannot entirely explain susceptibility to the disease. This is most evident through twin studies. Monozygotic twins only have a 30% concordance rate of the disease (10). The environmental factors are also evident in the geographical distribution of the disease. Prevalence increases the further one lives from the equator with some exceptions. It is highest in northern Europe, southern Australia, and the middle part of North America (14). More evidence for environmental influence is evident in the fact that susceptibility changes for first generation immigrants from low susceptibility areas to areas of higher risk to approach that of the surrounding population.

Gray matter is not spared by MS (19). While cortical plaques are rarely seen on conventional MRI, they are frequently found at post-mortem (20). Grey matter changes may be significant and may contribute to both physical and cognitive impairments (16).

Recent quantitative MRS studies have shown the presence of normal appearing gray matter (NAGM) changes in MS. This study found that the normal appearing white matter (NAWM) and NAGM are abnormal in early RRMS, and that NAWM and NAGM abnormalities are not correlated with measures of lesion load (21). Abnormalities in the gray matter are often present, but are especially prevalent in patients with aggressive disease (12).

Pathophysiology of MS

There are three phases in the development of MS. The induction of the disease in the susceptible individual usually occurs early in life as a result of exposure to an extrinsic agent. The latent phase may last many years. Last, is the clinical expression of the disease (22). The pathologic hallmark of MS is the focal destruction of the myelin sheath (3;23). Evidence shows the pathogenic mechanisms leading to this damage is different in the various subgroups of MS patients (24). Once this stage has begun, MS is characterized by inflammatory mononuclear infiltrates, demyelination, a glial reaction, and axonal degeneration (25;26). All of these processes may occur simultaneously in the same individual. Over the course of the disease, inflammatory demyelinating lesions spread throughout the CNS, targeting primarily the optic nerves, brainstem, spinal cord, cerebellum, and periventricular white matter (3;13).

While lesions occurring within a single MS patient have been shown to have uniform patterns of inflammation, demyelination, axonal loss and remyelination (24), the pattern of disease between patients is highly variable (27). The course of the disease in an individual patient is very unpredictable even with the consistent pattern of damage.(14) Due to this variable pathology, the true age of a lesion and the time course

of injury cannot be determined (27). The pathological changes are found both in macroscopic lesions and normal appearing tissue (21).

The early symptoms of MS are believed to result from axonal demyelination which leads to the slowing or blockade of conduction (14). Myelinated axons conduct impulses faster than do nonmyelinated axons to and from the CNS. Myelin and myelin-forming cells provide extrinsic signals that may influence the physical distribution of axolemmal sodium channels, neurofilament phosphorylation, and axon caliber (28). Both axons and ion channels may also prove to be potential pathogenic targets of the disease in addition to the myelin sheath (23). MS may be a neurological syndrome with different immunopathological mechanisms, including cellular-mediated immune injury, complement- and antibody-mediated injury, or primary oligodendroglial dystrophy, triggering a final common pattern of CNS tissue injury rather than a single disease with a single cause (14;24).

As the lesion matures, many stages are involved including immune engagement, acute inflammatory injury of axons and glia, recovery of function and structural repair, and post-inflammatory gliosis and neurodegeneration (1). Changes also occur in the normal appearing white matter (NAWM). Pathological changes observed in NAWM include astrocyte hyperplasia, activation of microglia, small areas of perivascular inflammation and myelin breakdown products, and an increase in tissue water. A decrease in axonal density, alterations in neurofilament phosphorylation, and oligodendropathy have also been observed in the NAWM of patients with MS (29). While it has been generally accepted that the breakdown of the BBB is the initial event in lesion formation, a diffusion MRI study suggests the opposite, specifically that subtle

changes in NAWM may precede and possibly trigger the formation of demyelinated plaques. This is in agreement with the heterogeneous pathology of MS plaques observed in various patients (23). Further study in this area may elucidate this area more clearly.

Effects on the Oligodendrocytes

Oligodendrocytes synthesize and maintain the myelin sheath of up to 40 neighbouring nerve axons of the CNS. Myelin is needed for normal axonal conduction in which voltage-gated sodium channels cluster at the unmyelinated nodes of Ranvier, between myelin segments. From here, the action potential is propagated and spreads passively down the myelinated nerve segment to trigger another action potential at the next node (1).

Oligodendrocytes are a principal target of immune attack in MS (1). While the extent of survival of these cells varies from patient to patient, it is uniform within a given patient. This finding suggests that the focus of injury, such as myelin, mature oligodendrocytes, or progenitor cells, varies among patients (14). In any single lesion, oligodendrocyte destruction occurs via either apoptosis or necrosis, but not both (24). Oligodendrocytes are susceptible to damage via a number of immune mechanisms present within an inflammatory response. Activated macrophages or microglial cells could mediate oligodendrocyte injury via the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α or interferon (IFN)-γ (23). Other oligodendrogliotoxic factors include the generation of reactive oxygen and nitrogen species, the production of excitatory amino acids such as glutamate, the activation of complement components, the release of proteolytic and lipolytic enzymes, T cell-mediated injury via T cell products such as perforin and lymphotoxin, the interaction of

Fas antigen with Fas ligand, CD8+ class I MHC-mediated cytotoxicity, or persistent viral infection (23).

Oligodendrocytes can be found at higher densities in lesions relative to the periplaque white matter in patients with recent onset MS (30). In contrast, lesions in patients with a long history of MS demonstrate oligodendrocyte loss and limited remyelination localized to the edges of inactive plaques. These observations suggest that recurrent episodes of inflammation may cause the ability of oligodendrocytes to produce myelin to deteriorate (30). Although many oligodendrocyte precursor cells appear to survive the demyelination process in the chronic stage of MS, they appear to be in a fairly dormant state (31). Since lesion repair is more successful during the early course of MS, it suggests the proliferation and differentiation of oligodendrocyte precursor cells become gradually more impaired with progression of the disease (31;32). Therefore, the lesion environment changes, from one conducive to remyelination to one hampering endogenous repair processes. The causes of this loss of ability are unknown but may include the absence of growth factors implicated in remyelination, the presence of inhibitory molecules, or the presence of the scar tissue formed by astrocytes (31). Data suggest that the size of the oligodendrocyte precursor population gradually decreases with advancing age of the lesion. Complete destruction or severe depletion of the oligodendrocyte precursor population may occur in some cases of MS (31).

Axons are damaged in the process of demyelination in addition to oligodendrocytes (33). Removal of myelin may expose the unprotected axon to further injury by macrophages, lymphocytes or antibodies. This may explain why progressive neurologic deficits in patients with MS correlate with axonal injury and axonal loss (33).

Ultimately, irreversible neurological deficit in MS depends on loss of the ability of axons to conduct (34).

Demyelinated axons can discharge spontaneously and show increased mechanical sensitivity. Ephatic transmission, or cross-talk, can take place between neighbouring demyelinated axons, resulting in paroxysmal symptoms such as trigeminal neuralgia, ataxia, and dysarthria, or painful titanic posturing of the limbs, lasting one or two minutes and often triggered by touch or movement (1). Complete and incomplete conduction block can occur, with the axons losing the ability to transmit long trains of impulses accurately at physiological frequencies thus slowing conduction (22).

Inflammation

It is generally accepted that the immune system contributes to pathogenesis in MS (3). However, whether the tissue injury due to inflammatory mechanisms in MS is a primary or secondary event in lesion formation is still unclear (23). Inflammatory cells are typically found perivascularly (14), as cerebral venules are frequently at the centre of an MS lesion (13), but they may diffusely infiltrate into the parenchyma (14). Small lesions have a centrally placed vein which, when myelin breakdown products indicating recent activity of the lesion are present, is surrounded by an inflammatory cuff. Venules with perivascular lymphocytic cuffs are often seen extending from the demyelinated area into the apparently normal surrounding white matter (22). Histopathological and biochemical studies have reported astrocytosis, demyelination, perivascular inflammation, and edema in the NAWM of patients with MS along with an increase in tissue water (35). Abnormalities of neurofilament phosphorylation have also been reported in NAWM (35).

Most agree there is a heterogeneity in the demyelinating process of MS (3). Acute MS lesions display inflammation, shown by perivascular lymphocytes and macrophage infiltrates (20). They also show edema, active myelin breakdown, some axonal damage, as well as some remyelination and tissue repair (20;36). The composition of the inflammatory infiltrate varies depending on the stage of demyelinating activity (14). It is usually composed of a heterogeneous population of T cells, plasma cells, macrophages, and resident microglia with macrophages and microglia predominating in active lesions (14;37). Activated macrophages and microglial cells have been observed in association with demyelinating activity of lesions (3). Large numbers of macrophages and microglia are present in MS lesions, and the presence of myelin degradation products within the macrophage cytoplasm and macrophage activation markers are strong indicators for ongoing demyelinating activity (24). Macrophages may secrete various lymphokines and cytokines, which can disrupt the BBB and may increase neurologic deficits upon injury to myelin sheaths and oligodendrocytes (33).

The products of inflammation may also delay the development of the adaptive changes which follow demyelination (22), as inflammatory mechanisms contribute to axonal pathology as well as to demyelination (14;32). There is evidence supporting both the concept that the inflammatory reaction is a prerequisite for demyelination, and that it may represent an irrelevant secondary bystander effect triggered by the release of chemokines produced by activated macrophages, astrocytes, or endothelial cells (24). Apoptosis of lymphocytes appears to be the major mechanism for the resolution of inflammation in MS (37).

Inflammatory mediators, such as CD8+ T cells and antibodies, are implicated in axonal damage. Treatment with steroids or anti-inflammatory therapies decrease brain atrophy, pointing to involvement of the inflammatory response in the initiation of degeneration (32).

Chemokines have been shown to play a critical role in directing immune cell entry into the CNS. There is evidence for interplay between the cytokine tumor necrosis factor (TNF) and chemokine production by perivascular glial cells in the initiation of T cell entry into the CNS (32). This increase in expression of chemokines and their receptors in the CSF and serum correlate with the clinical activity of MS. Chemokines may be considered part of the innate CNS responses to inflammation or injury (32). Other innate mediators include the enzyme inducible nitric oxide synthase (iNOS) which is elevated in the CSF of MS patients. This enzyme has also been shown to be cytotoxic for oligodendrocytes *in vitro*. This is of interest as NO is an agent of demyelinating pathology (32).

Recent data suggest that inflammatory brain reactions lead to subtle axonal transport disturbances (28). A likely culprit in these reactions is nitric oxide (NO) which may act directly on normal or hypomyelinated axons, transiently blocking conduction and reversibly increasing deficits arising from already compromised pathways. However, when aggravated by high axonal firing frequency, NO causes irreversible structural changes to axons (1). Conduction block may also result from demyelination and may be restored by the reorganization of sodium channels along the internodal membrane (20).

Axonal Loss

The major, irreplaceable target of the disease process is the axon and the neuron that gives rise to it (26). Destruction of axons is a consistent feature of MS lesions which is considered likely to be a crucial factor in the persistent symptoms of progressive MS as it is the most disabling feature of the disease (10;20;25-28;37). This degeneration can lead to axonal loss, damage, or dysfunction (28). Confocal microscopic techniques have shown transection of axons within early demyelinating lesions (10). Axon loss has also been observed in NAWM (28). In fact, the destructive process underlying MS-related cerebral and spinal cord atrophy seems to be related more to diffuse and subtle changes in the NAWM rather than to focal macroscopic pathology (24;25). Studies relating MRI findings with histopathology have revealed substantial axonal loss in the spinal cords of patients with MS, whether focal lesions are present or not (18).

Chronic axonopathy may not be due directly to inflammation, but results from loss of trophic support normally provided to axons by myelin or glia, acting directly or through the maintenance of electrical activity, or both (1). Therefore, chronic axonal degeneration may slowly increase the clinical deficit, decaying a compromised but functioning pathway and leading to disease progression (1). The loss of axonal volume in MS is likely due to both the local injury of axons traversing demyelinating lesions and the Wallerian degeneration along the fiber pathway of lesioned axons (25). The most extensive axon loss seems to be associated with activated inflammation (14;27;37) and axonal damage occurs within both active and chronic MS plaques (23). Histopathological reports have found axonal damage in early inflammatory lesions

suggesting axonal loss may be present even in the early stages of the disease (38). The degenerative response is therefore an integral and early component of MS.

Inflammatory cells and mediators induce axonal loss as well as demyelination. Therefore, these two mechanism of damage occurring in MS may share a common effector mechanism (32). The extent of axonal damage in the primary demyelinating lesion of MS patients is associated with the number of activated microglia/macrophages and cytotoxic CD8+ lymphocytes (28). The highest incidence of acute axonal injury is found during active demyelination, associated with axonal damage in the periplaque and NAWM of actively demyelinating lesions (23;28). Also, low but significant ongoing axonal injury was observed in inactive demyelinated plaques, but not in completely remyelinated shadow plaques (23). Quantitative evidence has been found for a slow, chronic progression of axonal destruction in completely inactive demyelinated MS plaques, which may ultimately contribute to the clinical progression (23). Axonal damage and atrophy are now recognized as features of clinically early MS, regardless of the disease subtype (32).

Repair Mechanisms

Chronic plaques are usually completely demyelinated with marked astrocytic gliosis and a variable degree of axonal loss, some with inflammation at their edge (20). Most chronic lesions of MS are never remyelinated. Remyelination requires generation of new oligodendrocytes (15). Oligodendrocytic progenitor cells have been found in developing brain, normal adult human brain, and chronic lesions of MS. Data indicate the environment of many chronic lesions does not provide the appropriate signals for remyelination (15). It has been speculated that failure of remyelination by

premyelinating oligodendrocytes in chronic lesions of MS is due to an abnormal molecular composition of chronically demyelinated axons or an imbalance of growth factors that regulate myelination. Many persistently demyelinated CNS axons show decreased viability (26).

There are two fairly different mechanisms which may be utilized by the CNS after injury, regenerative repair and scar formation (3). As the acute inflammation resolves, the NO-induced physiological conduction block resolves (1). Symptoms also improve as surviving functional pathways are reorganized at the cellular and systems level. Together, these mechanisms account for remission early in the course of the disease (1).

Remyelination is a frequent feature in MS lesions and appears to be protective because axon destruction occurs much less frequently in remyelinated shadow plaques than in those with completely demyelinated axons (28). Myelin repair is believed to aid in the re-establishment of neurological function following acute attacks of inflammatory demyelination in MS (15;30). This may contribute to the slow recovery seen following some exacerbations of MS and is morphologically similar to repair observed in experimental models (30). Remyelination may be able to restore rapid nerve conduction and protect demyelinated axons from degeneration (15). In early MS lesions, significant myelin repair is mediated by both oligodendrocytes and Schwann cells (30). Remyelination by Schwann cells is mainly observed in the spinal cords of some patients, as they migrate in from the peripheral nervous system (PNS) to myelinate the demyelinated axons. The inflammatory response may also aid this process by releasing factors that could promote remyelination (24).

It has been suggested that reduced numbers of premyelinating oligodendrocytes and a lack of remyelination in chronic MS lesions may promote axonal degeneration (28). The extent of remyelination appears to be correlated with the degree of oligodendrocyte preservation in the lesions (23;24). Conversely, premyelinating oligodendrocytes are present in some chronic lesions of MS patients. Therefore remyelination is not necessarily limited by an absence of oligodendrocyte progenitors or their failure to generate oligodendrocytes (15). Failure of remyelination appears, in many cases, to be due to the failure of the precursor cells to proliferate and differentiate and not to the destruction of the local oligodendrocyte precursor population (31). Irreversible axonal injury and gliotic scarring may result as well. These consequences may result from repeated episodes of disease activity as it has been shown that remyelinated lesions can become targets of new demyelinating attacks (14;24). The development of gliosis is very prominent in the chronic lesion so it is likely to influence the degree of remyelination. Excessive production of glial cells may impede the migration of oligodendrocyte precursors to areas of demyelination. In addition, it appears that the potential for remyelination within a lesion is dependent on the degree of axonal loss or dysfunction that has also occurred (39).

Treatments of MS

While there is no cure for MS, there are many new treatments which have been shown to slow the progression of the disease for a time. The aims of treatment of MS are to decrease relapse rates, prevent fixed disability directly attributable to relapse, provide symptomatic management of fixed neurological deficits, prevent disability acquired through progression, and treat established progression (1). Most of the therapies

currently used for MS are based on modulating the immune response (3;40). These therapies work on reducing progression of the disease but have not been shown to induce myelin repair (3).

As myelin repair has yet to be achieved through available treatments, the reduction of relapse rates in MS is a very important goal in order to avoid damage to the myelin sheath (1). There are a variety of treatments that work towards this goal. These include Interferon beta (IFNβ)-1b (Betaseron) for both RRMS and SPMS; IFNβ-1a (Avonex), Glatiramer acetate (Copaxone), Immunoglobulin, and azathroprine for RRMS; and mitoxantrone hydrochloride for SPMS (1;14). As well, RRMS patients with more severe disabilities respond to high-dose IFNβ-1a (Rebif) (14). It is hypothesized that the immunopathology behind RRMS and SPMS stages of the disease are different so they require, with the exception of Betaseron, different treatments to obtain similar results (40). Along with reducing the number of relapses of the disease, in order to slow down or prevent the development of disability, the length of the relapses that do occur and the damage done in each relapse need to be reduced. Corticosteroids, such as methylprednisolone (given intravenously) or some oral steroids, have been shown to speed clinical recovery. MRI has shown they transiently restore the integrity of the BBB (1;14). As well as preventing disability due to relapse, deficits acquired through progression need to be prevented or at least reduced. Betaseron has been shown to be useful in this regard.

No treatment has been able to completely stop the progression of the disease and eventually, in most patients, permanent neurological deficits will develop. Once they have developed, the patient is treated symptomatically, with the goals of therapy to

minimize pain and functional disability. Symptoms treated may include varied degrees of spasticity, bowel and bladder disturbances, erectile impotence, paroxysmal attacks, central pain, depression, sleep disturbances, and fatigue (1;14). For spasticity, muscle relaxants working at the level of the spinal cord such as Baclofen and Tizanidine are often used (1;14). Not all patients can be treated for spasticity as some may need a level of spasticity in order to walk if they have leg weakness. Therefore, treating their spasticity may lead to the greater functional deficit of being unable to walk (14). Bladder and bowel disturbances, and erectile impotence can be treated once their causes are determined (14). Often bladder symptoms are treated with self-catheterization and oxybutynin (1). Erectile impotence is treated with sildenafil citrate (1). Paroxysmal attacks usually respond well to carbamazepine and phenytoin (alone or together), acetazolamide, gabapentin, and pergolide (14). Fatigue has been successfully treated in many patients with amantadine and energy-conservation strategies. As well, depression and sleep disorders may contribute to the fatigue and once these are treated, the fatigue improves (14). More severe symptoms such as disabling, high-amplitude, cerebellar-outflow tremors, have not been shown to respond well to medication but may be treated through contralateral thalamic stimulation or ablative thalamotomy (14).

New treatments for MS are currently being investigated. Researchers are trying combining treatments, vaccination with MBP-specific T-cell receptor types, and blocking T cell entry into the brain. Thus far, none have been successful but research is still active in this area (1). As well, the search is still active to determine a therapy able to impact disease progression in the PPMS subtype. Thus far, none of the treatments developed for RRMS and SPMS have been useful in treating PPMS (7).

MS in the Spinal Cord

Spinal cord involvement is prominent in MS with lesions found in the cords of 75 – 90% of patients (8;12;18) even in the absence of spinal cord symptoms (12). Involvement of the spinal cord in MS is important in the development of disability (8) as spinal cord lesions cause symptoms more often than do brain lesions (17). As well, while spinal cord lesions cannot be consistently related to symptoms, they do relate weakly which is much more than can be shown with brain lesions (18). Disease activity in the spinal cord contributes to much of the disability developed in MS patients (41).

Spinal cord abnormalities due to MS range from single or multiple focal lesions to diffuse involvement of large regions of the cord. This said, most lesions do not extend more than two vertebral segments in length. The diffuse abnormalities are not seen as often, and usually occur in later stages of disease progression, but some patients will have only diffuse abnormalities from diagnosis on (18). Most spinal cord lesions are located in the cervical cord (5;8;18;41). In cross-section, most lesions are seen in the lateral and posterior white matter (5;18). While lesions do not spare the gray matter (18), they rarely involve the entire diameter of the cord (5).

Spinal cord lesions appear equally prevalent in both disabled and non-disabled patients (42). Atrophy has been more closely associated with disability than lesion load (8;41;42). Extensive axonal loss is seen in MS patients in comparison with controls (18;38;42) which is the main cause of atrophy in the spinal cord, with a small portion due to reduction in axonal diameter due to demyelination (41). Although this loss of axons may occur in focal lesions, it appears to be an independent process to that which results in demyelinating lesions as there is axonal loss apparent in normal appearing white

matter (12;18). While axonal damage within the brain may result in focal hypointense lesions on T1 weighted images due to the expanded extracellular spaces, in the spinal cord, the damage results in atrophy due to collapse of the tissue surrounding the area of loss (18).

Lesions in the spinal cord are important as they have greater potential to result in clinical symptoms and disability as the cord contains clinically important pathways such as the pyramidal tracts, spinothalamic tracts, and the posterior columns (43). Cord lesions are also important as they can be more specific for demyelination than lesions seen in the brain, especially in older adults, as vascular ischaemic lesions rarely occur in the spinal cord (43). Therefore, enhancing our knowledge of the process of MS in the spinal cord may lead to clinically relevant discoveries.

Spinal Cord and Sensory Transduction

The spinal cord can be divided into thirty-one segments grouped into eight cervical, twelve thoracic, five lumbar, five sacral segments, and one coccygeal nerve (44;45). Each segment gives off a pair of dorsal roots and a pair of ventral roots that join to become a spinal nerve. Cells making up the dorsal root axons are pseudounipolar sensory neurons and each dorsal root has a ganglion associated with it termed the dorsal root ganglion (DRG) in which the cell bodies of these neurons reside (44;45). Ventral root axons are made up of multipolar motor neurons, whose cell bodies lie in the spinal cord, thus the ventral roots do not have associated ganglia (44). The axons in the ventral roots arise mainly from cells in the ventral and lateral gray columns of the spinal cord (45). The dorsal root receives sensory afferent neurons while the ventral root contains motor efferent neurons.

As the spinal nerves contain both ventral and dorsal root fibres, they contain both motor and sensory information (44). The spinal nerves exit the vertebral column via intervertebral foramen within the column (44). Soon after exiting, the spinal nerves divide into anterior and posterior rami. The posterior rami innervate skin over the back of the neck and the trunk and the paraspinal musculature, while the anterior rami supply the anterolateral trunk and limbs (45).

A myotome is a group of muscles that have a common innervation from the same nerve root. Most muscles belong to more than one myotome as they are usually innervated by nerve roots from several adjacent spinal cord segments. In contrast, a dermatome is a cutaneous sensory area innervated by a single nerve root. Adjacent dermatomes overlap significantly, as do myotomes (45). This overlap can be explained by the fact that a single peripheral nerve may contain axons from several spinal nerves and multiple peripheral nerves may contain axons from the same single spinal nerve (44).

In adults, the spinal cord ends at a level near the first lumbar vertebra as the conus medullaris (45). There is an overall trend of a reduction in diameter as one moves caudally from the cervical region of the cord. This is due to the fact that all of the axons serving the entire spinal cord must pass through the cervical regions while only those serving the lumbosacral regions are present caudally (44). The exceptions to this observation are the cervical and lumbar enlargements. These regions of the cord innervate the limbs, thereby requiring more neurons to control these areas and receive the sensory input. The enlargements are due to an increase in gray matter in these segments. The cervical enlargement is still larger than the lumbar enlargement due to the increased

amount of white matter in the cervical cord but the lumbar enlargement has a larger diameter than the thoracic cord.

The organization of the spinal cord is opposite to that of the brain with white matter surrounding a central gray region (44). The gray matter mainly contains cell bodies while the white matter contains the long tracts of axons connecting the brain to the spinal cord. The white matter is white as most of its axons are myelinated. While the gray matter contains some axons, myelinated and unmyelinated, the white matter contains no cell bodies (44).

The gray matter has been organized into ten regions called the laminae of Rexed. The dorsal horn contains laminae I – VI. It receives the primary afferent axons from the dorsal roots and contains interneurons and projection neurons. Interneurons have short axons and synapse within a few hundred micrometres of their soma while projection neurons have long axons and are used to transmit information to more distant regions of the CNS (44). Lamina IX, in the ventral horns of gray matter, contains the cell bodies of the somatic motor neurons. This lamina is the main source of axons that leave the spinal cord. Autonomic motor neurons arise from lamina VII. Lamina VIII contains the interneurons associated with motor reflexes while lamina X surrounds the central canal. Lamina X includes the ependyma and a thin layer of gray matter, and is important in the perception of visceral pain (44).

The white matter is separated into three regions as determined by their relation to the central gray matter. The dorsal columns are dorsal and medial to the dorsal horns. At caudal to midthoracic levels, the fasciculus gracilis makes up the entire area of the dorsal columns but at cervical and upper thoracic levels, the fasciculus cuneatus occupies the

outer half, separated from the fasciculus gracilis by the dorsal intermediate sulcus (44). The fasciculus gracilis carries sensations of fine touch, proprioception and vibration from the legs, while the fasciculus cuneatus carries these sensations from the arms, thereby explaining their differing distribution. The lateral white columns are lateral to the gray matter between the ventral and dorsal horns and contain several tracts carrying sensory and motor information (44). The ventral white columns are medial and ventral to the ventral horns. Contained in these columns is the anterior lateral system consisting of important sensory tracts. This region also contains the ventral white commissure that connects the two halves of the spinal cord. It crosses the midline just ventral to the central canal (44).

Spinal Cord Circulation

The cervical region of the spinal cord receives its blood supply from branches of the vertebral arteries (44). The anterior artery is an anastomosis of branches of the right and left vertebral arteries, with each supplying one branch. It courses from the olivary nucleus to the conus medullaris in the anterior sulcus of the spinal cord, and supplies the ventral surface of the medulla and the anterior two-thirds of the spinal cord (46). It is continuous in the upper cervical region of the cord but is joined by anastomosing branches from the anterior radicular arteries in the segments caudal to the upper cervical region (46). The two posterior spinal arteries usually arise from the vertebral arteries but sometimes will arise from the posterior inferior cerebellar artery (PICA). They are connected to many posterior radicular arteries to form an anastomotic network on the posterior surface of the spinal cord (46). These arteries supply the posterior one-third of the spinal cord. The branches of the anterior and posterior spinal arteries form a vascular

plexus that surrounds the cord, and the arteries join in an anastomotic loop at the conus medullaris. The branches of the plexus surrounding the cord supply the majority of the white matter and the dorsal horns of the gray matter (46).

Each spinal nerve carries a spinal artery with it as it enters the intervertebral foramen (44). The spinal artery divides into dorsal and ventral radicular arteries which follow their respective nerve roots to the spinal cord. These arteries anastomose with the posterior or anterior spinal arteries as discussed above (44). The number and distribution of these radicular arteries can vary widely in an adult. Only around half of the dorsal radicular arteries actually develop fully and join the posterior spinal artery. As well, only about one third of the ventral radicular arteries join the anterior spinal arteries. Because of this, certain regions of the spinal cord, especially the thoracic region, are more susceptible to ischemic injury after the loss of a single spinal artery (44).

To supply the inner cord, sulcal arteries branch off the anterior spinal artery and its anastomoses and travel deep into the cord by way of the anterior median fissure. The midthoracic region is more susceptible to ischemia in this aspect as well, as it contains a smaller amount of sulcal arteries than other regions (46).

There is greater blood supply to the gray matter than to the white matter. The majority of gray matter, except the dorsal horns, is supplied by the sulcal arteries. The dorsal horns and funiculi are supplied by the posterior spinal arteries, posterior medullary feeders, and perforating pial branches (46).

Somatosensory Pathways

There are two somatosensory pathways: the dorsal column system and the anterior lateral system. The dorsal column system conveys fine tactile, vibratory, and

proprioceptive sensations (44). The primary afferents are large diameter myelinated Aβ fibres. These afferents have low thresholds and very small receptive fields, allowing for two-point discrimination (44).

The anterior lateral system (ALS) consists of the spinothalamic tract which terminates in the thalamus, the spinoreticular tract which terminates in the brainstem reticular formation, and the spinomesencephalic tract which terminates in the mesencephalon (44). The spinothalamic tract (STT) is the most important sensory pathway for sensation of pain and thermal sensation (47). Both the dorsal column and ALS pathways carry information from the periphery to the cerebral cortex with three neurons. With the exception of olfaction, all sensory pathways use this three neuron system going through the thalamus (44).

The first order neurons in this pathway are termed primary afferents. These axons have specialized peripheral receptors (44). The stimuli at the receptor are converted into receptor potentials, which are small, short-lived alterations in the membrane potential. In order to transmit the sensory information to the CNS, these receptor potentials must be converted into action potentials as receptor potentials cannot maintain the amplitude needed to transmit long distances (44). The initial amplitude of the receptor potential represents the intensity of the stimulus. As action potentials are all or none responses, intensity of the original stimulus is transmitted through the frequency at which they fire (44).

There are various types of sensory receptors. Photoreceptors respond to light while chemoreceptors respond to specific molecules which are perceived as taste and smell. Mechanoreceptors respond to mechanical force which may be perceived as touch,

proprioception, vibration, and sound (44). Nociceptors respond to chemicals released in response to tissue damage which is perceived as the various types of pain, and thermoreceptors respond to temperature (44;47).

There are both cold and warm thermoreceptors. Both are relatively slow adaptors to stimulation and they are among the few receptor types that discharge spontaneously under normal circumstances. Both types of thermoreceptors are active over a broad range of temperatures (47). At moderate skin temperatures, both types of thermoreceptors are active. As the temperature increases, the cold receptors begin to stop firing. The same is true for the warm receptors as the temperature decreases. Over temperatures of approximately 32 - 45°C, the increase in frequency of heat receptors firing is related linearly to the increase in temperature. As the temperature increases from 45 – 50°C, the firing rate of the heat receptors drop to zero with the nociceptors beginning to respond (44;47). Cold receptors are sensitive to skin temperatures around 24 – 34°C. Paradoxically, cold receptors also respond to temperatures between 45 – 55°C (44). As well as firing at higher temperatures, nociceptors respond to both cooling and cold pain. There are some nociceptors that fire at temperatures as high as 30°C while others only respond to colder temperatures (48).

In general, the first order neurons respond to a stimulus, transduce it, then convey the encoded information to the CNS (47). The soma of the primary afferent neurons are in the DRG, or cranial nerve ganglia and make connections in the ipsilateral gray matter of the spinal cord or medulla (44;47). Here, the primary afferents synapse with the second order neurons. These are projection neurons with their soma ipsilateral to the side of entry of the primary afferent. The second order axon crosses the midline and

terminates in the thalamus contralateral to the original stimulus (44;47). For the dorsal column system, this decussation is in the medulla while for the ALS, it occurs in the spinal cord within one or two segments of entry (44).

The cell bodies of the third order neurons are in the thalamus (44;47). The axon passes through the internal capsule and terminates in the cerebral cortex ipsilateral to its soma but contralateral to the original stimulus (44). Local circuits in the thalamus and intrinsic membrane properties of the third order neurons may modify the information received from the second order neurons before the signals are transmitted to the cerebral cortex (47).

The ALS conveys temperature and noxious information (44). This study involves thermal stimulation, thus a more detailed overview of the ALS is relevant. As discussed previously, the most prominent tract within the ALS is the spinothalamic tract. The primary afferents for this tract consist of small diameter myelinated Aδ and unmyelinated C fibres (44;46). These fibres travel to the CNS from nociceptors, thermoreceptors, and mechanoreceptors (47). The sensation of cold is mediated by Aδ and possibly by C fibres, while warm sensation is conveyed by warm-specific C fibres. Pain is conveyed by both Aδ and C nociceptors (46). Regardless of the sensation, all of the axons from these receptors enter the spinal cord in the lateral division of the dorsal root and bifurcate immediately, forming an ascending and a descending branch. Both branches travel for one to three segments in the dorsolateral tract (44). The branches then give off collateral fibres which leave the dorsolateral tract to enter the dorsal horn where they synapse with the second-order neurons. The Aδ fibres synapse primarily in laminae I and V of the

gray matter, while the C fibres synapse primarily in laminae II and I. Therefore, the majority of the primary afferents for this tract synapse in laminae I and V (44).

The cell bodies of the second order neurons send their axons to the contralateral thalamus. They cross the midline of the spinal cord in the ventral white commissure approximately at their level of origin and they ascend in the ventral part of the lateral funiculus (44;47). New axons join at the medial margin, maintaining a somatotopic organization as the tract travels rostrally (44). The tract maintains its position in the ventral lateral funiculus throughout the spinal cord and the medulla. In the rostral pons, it meets with the medial lemniscus and travels to the thalamus (44;47). The axons then terminate on the cell bodies of third order neurons in the ventral posterior lateral (VPL), ventral posterior inferior (VPI), centromedian (CM), and intralaminar nuclei of the thalamus (44). At the level of the thalamus, both the dorsal column/medial lemniscus system and the STT share the same third order neurons from the VPL and VPI to the cerebral cortex. The information from the dorsal columns dominates, allowing for localization of the STT sensations to be more precise (44).

Blood Brain Barrier

Of all of the organs in the body, the regulation of extracellular ionic and molecular composition within narrow limits is most important in the central nervous system (CNS) (45). The blood brain barrier (BBB) maintains the homeostatic microenvironment of the CNS tissue and acts as an interface between the vascular system and the CNS (44;46;49).

The BBB consists of the endothelial cells lining the capillaries within the CNS. These endothelial cells adhere strongly to one another with tight junctions which do not

allow any solutes or water to pass between the cells (44;45;47). Astrocytes on the CNS side of the BBB may also help to limit the movement of certain substances (47).

While the details of the structure and function of the blood spinal cord barrier (BSCB) in normal and pathological conditions are still not well known, the characteristics of the BSCB appear to be rather similar to those of the BBB. The physicochemical properties of the spinal cord endothelial cell membrane have been shown to be fairly analogous to those of the cerebral endothelium (50).

This barrier severely restricts the passage of large molecules and charged particles between the blood and CNS thereby regulating extracellular ion and neurotransmitter concentrations within the CNS (44;47;49). This protects the brain from the fluctuating composition of blood and minimizes the entry of compounds that may be toxic to the neural tissues (45). Passage across the BBB occurs via simple diffusion, facilitated diffusion, or active transport (45). Most molecules that cross the barrier have special transport mechanisms (44). Lipid-soluble compounds rapidly diffuse across the cell membranes while polar compounds require special carrier molecules driven either by concentration gradients or through the expenditure of energy in processes known as facilitated and active transport, respectively (45;46). Gas molecules such as oxygen and carbon dioxide diffuse freely and their concentrations equilibrate rapidly across the barrier. Pathological processes within the CNS may disrupt the barrier function of the BBB (47).

Basic MRI

Magnetic resonance is based on the interaction of an oscillating magnetic field with nuclear magnetic moments in a static magnetic field, B_o (51). MR imaging observes

the hydrogen (^1H) nuclei in water in the human body. When placed in a large magnetic field, these nuclei rotate (precess) about the field at a frequency called the Larmor frequency, which is proportional to B_o. There are two possible orientations for these nuclei, with the average direction either parallel or anti-parallel to B_o (51;52). More are aligned parallel as it is the lower energy state, so there is a net average magnetization parallel to B_o. This net magnetization is termed M_o (51). The magnitude of M_o depends on the number of ^1H nuclei in the tissue that contribute to the MR signal, and on the magnitude of B_o (51).

Within the bore of the magnet that produces the static field, there are coils to create additional fields parallel to B_o, but that vary linearly in intensity along the 3 directional axes. These fields are termed magnetic field gradients. Another coil is used to produce a weaker magnetic field, B_1, that is directed perpendicular to B_o, and oscillates rapidly with a frequency in the radio frequency (RF) range (52). This field is typically pulsed briefly, with a duration of a few milliseconds, and so is termed an "RF pulse". This pulse will have an effect on only those nuclei precessing at a frequency close to that of the RF field (52). The effect of B_1 is to move M_o from its orientation along B_o (conventionally defined as the z axis). When M_o has rotated 90° (into the xy plane), B_1 is called a 90° pulse. The net magnetization then rotates around B_o. This precession causes a magnetic field that changes in time which can produce a current in a coil. This current is the MR signal that is recorded (51;53). With different field gradients, the Larmor frequency, and therefore the MR signal frequency, is different in different regions of the body. This allows the regions of the body to be differentiated based on the specific Larmor frequencies.

After the RF pulse, the protons release the energy absorbed from the pulse in order to recover to their equilibrium state through two independent processes. Transverse relaxation is the exponential decay of the magnetization in the xy plane which occurs with the time constant T2. Longitudinal relaxation is the exponential recovery of the magnetization along the z axis which occurs with the time constant T1.

The dominant relaxation mechanism in biological tissues arises from the interaction between magnetic moments as they move randomly due to their thermal energy. Each magnetic moment will experience a field created by other magnetic moments, termed the "lattice", which fluctuates in time as other magnetic moments move nearby. This motion can have frequency components near multiples of the Larmor frequency and permit exchange of energy between the magnetic moment and the lattice (motion), or exchange of energy between two interacting magnetic moments. The transfer of energy to the lattice contributes to T1 relaxation. The transfer of energy between two magnetic moments does not result in a net change of energy, but dephases the magnetic moments and contributes to T2 relaxation. After the 90° RF pulse, M_o is precessing around B_o in the xy plane. As relaxation occurs, the longitudinal component of M_o grows towards the equilibrium value while the transverse component decays to zero. T2 is always less than or equal to T1 (51). In biological tissues at body temperature, T1 is commonly around 10 times longer than T2.

In practice, other factors can contribute to the decay of the xy component of the magnetization. These other factors include inhomogeneity of the large static field, sample-induced inhomogeneity, and the imaging gradients used. This factor is constant for a given imaging sequence. The sample-induced inhomogeneity is due to the fact that

different tissues within the body have varying magnetic properties so there is inhomogeneity at the interface between two different types of tissue. This is also constant throughout the imaging process for the same patient (51). The imaging gradients are used to determine slice thickness and encode spatial information into the MR signal in order to construct an image. This is transient throughout the imaging process but if the pulse sequence is designed properly, these effects should be eliminated as a source of decay of the transverse component of M_o (51). The result then is that the xy component of the magnetization decays with an exponential time constant T2*, which is shorter than T2.

By carrying out imaging in different ways, images can be produced in which contrast is based on T1, T2, T2*, or water density. A spin echo (SE) sequence starts with a 90° RF pulse which brings the axis of the precessing protons into the transverse (xy) plane. After a short time, these protons begin to dephase from one another. In this sequence, a 180° pulse is given after a time, t, and causes inversion of the magnetic moments. That is, the protons that had been precessing faster are now behind the ones precessing slower. As they are still precessing at the same speed as after the original RF pulse, those protons that were precessing faster than the others causing the loss of phase will "catch-up" to those precessing slower and they come back into phase again. This generates a signal in the receiver coil known as an echo (51). The 180° pulses can be applied again until, due to transverse relaxation, there is no longer a transverse component to the precession to generate an echo. As all of the protons experience the main field and sample-induced inhomogeneity both before and after the 180° pulse, these

static components contributing to T2* relaxation are cancelled out so all of the transverse relaxation in SE sequences is due to true T2 relaxation alone (51).

Another commonly used pulse sequence is gradient echo (GE) imaging. In GE imaging, the imaging gradients are a source of dephasing of the protons. A magnetic field gradient is applied to reverse this dephasing and thus refocus the magnetization in the xy plane in such a way that the main field and sample-induced inhomogeneity is not refocused. The result is an image in which the contrast is determined by differences in T2* (51).

Some important parameters in MR imaging are TR and TE. TR, or repetition time, is the time between two successive excitation RF pulses applied to the same slice of tissue (51;53). It is one of the factors that determines T1 weighting of an image in a spin echo sequence. If one compares two tissues, the tissue with a short TR will have relaxed before the tissue with a long TR. Therefore, when a short TR is used, M_o of the tissue with a short T1 will be larger when the 90° pulse is applied than that of the tissue with a long T1. This means that tissues with a short T1 will give a stronger signal and the image will be brighter (51;53). TE, or echo time, is the time between when the initial 90° RF pulse is applied and when the echo is acquired as the MR signal. For spin echo images, T2 weighting is determined by the TE (51). If a short TE is used, the net magnetization in the xy plain will have largely disappeared for tissues with short T2, so the MR signal will be small and the image will be dark. Conversely, for tissues with long T2, more of the net magnetization will be in the xy plane, so the MR signal will be larger and the image will be bright.

MR images are two-dimensional arrangements of pixels in a slice. These slices can be oriented in any direction. For clinical use, images are usually taken in three planes; coronal, sagittal, or transverse (52).

The relaxation times of the water protons in tissues are determined by the environment of the water in those tissues. Each voxel of an MR image has a signal which depends on the proton density, T1, and T2 or T2*. The weighting of the images depends on the imaging methods and parameters. Therefore, the signal intensity of each voxel, that is the contrast between voxels, relates directly to the physiological properties of the tissues imaged. In this study, the tissues of interest were white matter and gray matter in spinal cord. In the images for this study, gray matter is brighter than white matter. This is due to the T2-weighting of the images. Freely tumbling water has a large T2 value, resulting in it appearing bright on T2-weighted images (53). White matter contains highly oriented myelin and water can interact with the myelin, lowering T2 and causing white matter to appear darker than gray matter. The MS lesions also appear bright on T2-weighted images due to their high T2 and low T1 values, possibly due to the destruction of the highly oriented myelin allowing for an increase in freely tumbling water.

MRI Characteristics in Multiple Sclerosis

MRI, used for the diagnosis of MS, has become increasingly important in order to monitor disease activity, to establish prognostic parameters, and to find the radiological indices of key features of the MS lesion, such as inflammation, demyelination, remyelination, or axonal loss (23). It is the most sensitive technique for detecting MS lesions *in vivo* throughout the CNS (10;13). Current MRI technology allows MS lesions to be detected much earlier than in the past, aiding in diagnosis and providing useful

prognostic information (6). It can also be used to monitor therapies, both clinically and in research (6;13). MRI lesions are often "clinically silent" and MRI changes do not necessarily correlate well with clinical disability but may aid detection of disease activity before the development of clinical disability. This would be useful in determining courses of treatment (6;54). MS appears to be a more continuously active disease when monitored by MRI than previously believed, since new lesions appear to occur 7 to 10 times more frequently than clinical attacks in patients with RRMS and SPMS (10;13).

Normal appearing white matter (NAWM) surrounding MS lesions usually shows subtle abnormalities. The MRI findings correlate well with histopathological findings, which suggest focal high-signal intensity abnormalities represent typical demyelinated plaques, while less well-defined areas of mildly increased signal intensity represent partial demyelination histopathologically. The latter type of abnormality is associated with PPMS (12). The development of a variety of MRI techniques has improved our understanding of the pathologic evolution of MS. Newer MRI techniques are challenging the notion that all lesions evolve in this stereotyped pattern, in which focal BBB leakage and perivascular inflammation is the initiating event (29).

MRI can demonstrate macroscopic lesions associated with MS with high sensitivity, but lesions on standard T2 weighted MRI cannot be differentiated into their underlying pathology such as inflammation, demyelination, or axonal loss (23;55). While lesions on T2 weighted images are not specific for etiology, the T2 weighted lesion load is the most useful measure of disease burden (6;14;17) with over 95% of patients with MS demonstrating some degree of T2 weighted white matter abnormalities (1). There can be substantial T2 abnormalities even at the first clinical presentation of MS

symptoms (6). T2 lesion load and the number of changes over the first five years after diagnosis strongly correlate with changes in disability. Measurement of T2 lesion load has proven adequate in demonstrating therapeutic effects on macroscopic lesion bulk (20).

Both acute and chronic MS lesions appear bright on proton density (PD) and T2 weighted scans, as do other brain pathologies. MS lesions tend to appear round or ovoid in shape and usually ranging in size from a few millimeters to greater than a centimeter in diameter (13). Lesions greater than 2 cm in diameter are uncommon, and may be interpreted as neoplasms or abscesses on routine MRI (36). Rarely, disease foci can be very large, with a pseudotumour appearance and irregular areas of signal hyperintensity result from confluence of lesions. Slight signal alterations between or surrounding discrete lesions have also been observed on T2 weighted images of the brain and appear to represent a more diffuse component of the disease process. This phenomenon is seen mainly in the deep and periventricular white matter (13). On MRI, findings of multifocal lesions of various ages, especially those involving the periventricular white matter, brain stem, cerebellum, and spinal cord white matter, support the clinical impression of MS (14). When there is doubt as to the diagnosis of MS based on the first MRI, repeated MRI scans after several months may demonstrate that the lesions are "disseminated in time" (14).

T1 weighted lesions are suggestive of acute edema or tissue destruction (6). Chronic T1 hypointensities, termed chronic "black holes", are more specific for tissue destruction than T2 weighted abnormalities and reflect more severe tissue damage (6;10). Acute and actively demyelinating lesions enhance on T1 images after the administration

of intravenous gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA). Lesion enhancement reflects a breach of the normal BBB (6;10).

An MRI scan of the brain is suggested at least once in every patient with MS (13;18). Complementary MRI of the spinal cord may be considered for patients with unclear brain findings and if spinal cord symptoms predominate, as is often the case in PPMS. Spinal MRI is also recommended in patients with clinically definite MS who develop spinal cord symptoms first or have spinal cord symptoms that are progressive and possibly attributable to a different etiology (13).

Upon clinical suspicion of MS, for both brain and spinal cord MRI, sagittal images are performed with a T2 weighted sequence. If lesions are suspected from the sagittal scans, axial T2 weighted images help to determine if there is a lesion and outline anatomic lesion location clearly (13). Atrophy of the brain and spinal cord is common and is progressive as the disease advances. The degree of atrophy in the cerebrum, corpus callosum, and spinal cord, as well as third ventricle size, correlate directly with severity of disability (10).

Reparative mechanisms, including remyelination, may contribute to signal normalization (13). New lesions or the enlargement of preexisting lesions can be seen to occur simultaneously with the shrinkage of other previously active plaques demonstrating the continuous activity of the disease even when clinically silent. Diffusion tensor MRI has detected widespread cortical gray matter pathology further demonstrating the fact that gray matter is not spared (19).

Spinal Cord MRI

Many studies have shown the benefits of spinal cord imaging of patients with MS. It has a diagnostic role in patients with clinically isolated syndromes through visualizing spinal cord lesions in those with a normal brain MRI (18;43). Even patients with clinically definite MS may have a normal brain MRI but will have lesions in the spinal cord (43). For many patients with probable MS, spinal imaging will not change the diagnosis (56) but spinal cord MRI may rule out compressive spinal cord disease in those with symptoms suggestive of spinal cord lesions (18). Spinal MRI can increase the specificity of CNS examination of patients with probable MS and in some cases it may also increase sensitivity (18). One study found that in clinically definite MS, including spinal imaging in the MRI protocol increased the sensitivity of MRI to 100% (56). The main problem with MRI of the spinal cord is that spinal cord lesions are harder to detect than brain lesions (6;18).

The use of spinal MRI is not confined to those patients with spinal cord symptoms. In brain disorders, the finding of asymptomatic spinal cord lesions show spatial dissemination (18;56). As in the new MS diagnostic criteria, dissemination in space may be shown by MRI (18), the presence of asymptomatic spinal cord lesions could lead to the diagnosis of clinically definite MS. Many MS patients have spinal cord lesions, many of which are not associated with any clinical symptoms. In fact, approximately 50% of all MS patients have asymptomatic spinal cord lesions (56). These asymptomatic spinal cord lesions are quite rare in disorders other than MS which further aids in diagnosis (18). MRI studies have also shown a high frequency of asymptomatic

lesions in both the brain and spinal cord of patients presenting with a clinically isolated syndrome of the optic nerve, brain stem, or spinal cord (43).

In PPMS, it may aid in diagnosis as spinal cord lesions are much more common than brain lesions in this subtype. As well, diffuse spinal cord abnormalities may be the only finding in these patients. This follows the typically more severe disease course in these patients as diffuse abnormalities are typical of advanced disease. As such, these abnormalities are also found in later stages of SPMS (56).

To rule out possible vascular disease as the source of symptoms, spinal cord MRI is a useful tool. Lesions found in the brain due to vascular disease may be impossible to differentiate from MS lesions (56). In the spinal cord, this is not a problem as hypoxic/ischaemic lesions are extremely rare in the spinal cord of healthy subjects and as such, spinal cord lesions are more specific for demyelination than cerebral white matter lesions (6;43;56).

Functional MRI

BOLD Effect

Blood has magnetic properties which change with oxygenation state (52). Deoxyhemoglobin is paramagnetic and has a significant magnetic moment, while oxyhemoglobin is diamagnetic with a very small magnetic moment (51;52;57). Red blood cells containing deoxyhemoglobin exhibit magnetic susceptibility changes within the blood vessels relative to the surrounding tissue. This presence of deoxyhemoglobin leads to local magnetic field distortions or gradients within and around blood vessels (52). In the presence of these gradients, the nuclear spins of the hydrogen nuclei of water molecules various distances from the blood vessels experience different resonance

frequencies. The effects of this can lead to alterations in the transverse relaxation times (T2 and T2*). These effects can be used as a source of endogenous MR contrast in tissues that have been placed in these gradients (52). In T2 weighted images, signal intensity will decrease in voxels containing deoxyhemoglobin due to the decrease in T2 due to its paramagnetic properties. While the changes in T2* are 3.5 times larger than that of T2 (58), both the T2 and T2* changes are termed the BOLD (blood oxygenation-level dependent) effect (52). The BOLD effect can be detected with both spin-echo and gradient-echo sequences. However, as the signal changes are larger using gradient-echo and are easier to detect, gradient-echo is used most often.

The MR signal decays exponentially. This decay is described by the time, T2*, as demonstrated in Equation 1:

(Equation 1) $$S = S_o e^{(-TE/T2^*)}$$

Where S = signal intensity on activation and S_0 = baseline intensity. When there is more oxygen in the blood, the field is more uniform causing the signal to dephase more slowly. Therefore, T2* is longer with an increase in oxygen content (59). T2* is the characteristic time describing the apparent decay of the transverse component of the MR signal and includes both the effects of static field inhomogeneity and random interactions between magnetic moments. As described in the section on basic MRI, T2 describes only the true transverse relaxation arising from random interactions between magnetic moments.

The BOLD contrast relies on the macroscopic detection of changes in the microscopic magnetic fields surrounding red blood cells. When the hemoglobin in the red blood cell changes from the oxygenated to the deoxygenated state, there is a large

change in its magnetic properties as the red blood cell changes from being diamagnetic to paramagnetic (57). Therefore, the BOLD effect uses deoxyhemoglobin as an endogenous MRI contrast agent (57).

The physiological basis of functional neuroimaging is the fact that neuronal activity is associated with local changes in metabolism, particularly with glucose and oxygen consumption and via the neurovascular coupling in cerebral blood flow (CBF) and oxygenation (57). Positron emission tomography (PET) studies have documented that neuronal activation is accompanied by regional changes in blood flow and metabolism (52;60). While the blood flow is significantly increased by neuronal activity, oxygen consumption is not increased to the same degree (52). Therefore, oxygenation increases especially in the venular side of the capillaries and in the venous vessels (57). The concentration of deoxyhemoglobin decreases within the capillary bed and venous vessels. The decrease in paramagnetic molecules reduces the amount of dephasing induced. This increases the T2* for the stimulated tissue as compared to unstimulated tissue. The stimulated tissue has higher signal in T2* weighted images. This is the BOLD effect (51).

BOLD measures of neuronal activation are only indirectly related to synaptic activity. This is because the contrast is the result of a chain of physiologic events in response to neuronal activation. These events include changes in metabolism, oxygen consumption, hemodynamics, and blood oxygenation (57). As well, draining veins make the activity appear over a larger region than the neuronal activity actually occurs.

SEEP Theory

Recently, a new contrast mechanism has been suggested for functional MRI termed the signal enhancement by extravascular protons (SEEP) theory. The SEEP theory is based on an increase in the extravascular water content at sites of neuronal activation due to an increase in perfusion pressure. The signal change observed appears to develop mainly from an increase in proton density in the extravascular spaces of neuronal tissue (61). This effect has been shown to be larger by a factor of three in the spinal cord than in the brain which establishes its importance in spinal fMRI. Unlike the BOLD effect, the SEEP effect is detectable with SE imaging methods with very short echo times. This characteristic provides a much greater image quality (61). SEEP is not a change in relaxation time, thus the signal changes are bigger at lower echo time. This is because the signal has not had time to decay away due to relaxation.

It has been proven that there is an increase in blood flow to sites of increased neuronal activity with the BOLD effect (62). This means there is an increase in the perfusion pressure thereby increasing the hydrostatic pressure driving water out of blood vessels in areas of neuronal activation which is the basis for SEEP. Recent research has also pointed to astrocytes, glial cells within the CNS, as likely to play a significant role in neuronal activation as well (63;64). They may also contribute to the SEEP effect in that they take up ions and water in addition to neurotransmitter, namely glutamate, in the synaptic cleft. As SEEP is based on the movement of water and astrocytes are more numerous in the brain than are neurons, they will likely be shown to have a significant effect on the proton density signal changes with the SEEP effect.

In a study performed by Stroman *et al.* fMRI data were obtained in the visual cortex at 1.5 T and 3T with both spin echo (SE) and gradient echo (GE) EPI (65). The imaging parameters were consistent with the currently accepted BOLD model and with published fMRI data. The slope and intercept at zero echo time were ascertained and compared to the standard BOLD model. BOLD changes are approximately 3.5 times higher with GE than with SE imaging at the same echo time. The data obtained did not show this as, consistent with previous observation in the spinal cord, the changes in signal intensity were the same in both GE and SE scans at the same echo time. The intercept of the linear relation between change in signal intensity and echo time were greater than zero every time. In fact, the intercept was consistently larger with SE than with GE imaging in contrast to that observed with the BOLD model.

A possible explanation for the positive intercept is an increase in the baseline signal intensity upon neuronal activation (65). Explanations of the change in baseline could include an increased blood volume or an increase in the extravascular water content in activated areas. If an increase in proton density occurs in the same fluid component where the BOLD effect occurs, then both SE and GE fMRI data should be sensitive to it (65). As SE is more sensitive, it suggests the signal occurs due to another contrast mechanism.

The baseline signal change appeared to occur only in areas where SE fMRI was more sensitive. To check this, the GE signal changes were considered in the areas where both SE and GE data showed signal changes for one subject (65). The signal changes were increased somewhat at each echo time within these selected areas but the changes did not affect the intercept extrapolated to an echo time of zero. This finding suggested

that the baseline signal intensity change does not occur in the same compartment where the BOLD effect is observed. If the baseline signal increase occurs in the water of the extravascular space with T2* shorter than that of arterial blood, GE data can be insensitive to the signal change (65).

In another study by Stroman *et al*, brain fMRI was performed at 0.2 T where the BOLD effect is negligible (66). They were able to obtain functional data using SEEP imaging parameters. They proposed a two-component model that explained how both BOLD and SEEP occurred with BOLD occurring on the venous side of neuronal activity and SEEP occurring on the arterial side. The magnitude of SEEP signal changes is of the same magnitude at 0.2 T, 1.5 T, and 3T. SEEP makes spinal fMRI possible.

Spinal Cord Functional MRI

Spinal fMRI can be used readily on clinical MR systems. It has been used to observe neuronal involvement in experiments involving motor information going to and sensory data coming from the skin. A strong correlation has been shown between spinal fMRI results and neuronal activity in the human spinal cord (61). Intensity changes related to neuronal activation have been observed in the cervical cord during a motor task and sensory stimulation at 1.5T. These signal changes correlated with expected neuronal activation from the stimuli; both in their spatial distribution and timing (67).

The time to reach maximal signal intensity change in the spinal cord appears to be longer (approximately 8 seconds) than BOLD changes in the brain. As well, if the stimulation was applied for longer periods of time, larger intensity changes were demonstrated (67). These observations are consistent with a combination of the BOLD

effect and baseline intensity changes, discussed previously with the SEEP theory, and indicate that spinal fMRI is feasible with both motor and sensory stimulation at 1.5 T.

In another study by Stroman *et al.*, it was shown that the signal changes detected did not follow the linear model of the BOLD effect and in fact, the signal changes were larger than predicted by the model (68). In this study, motor and sensory tasks were used to investigate signal changes in fMRI of the human spinal cord over a range of echo times. Plots of the fractional signal change as a function of echo time yielded linear functions with slopes corresponding to relaxation rate changes of -0.30 sec^{-1} with sensory stimulation and approximately -0.50 sec^{-1} with a motor task. However, when the data were extrapolated to an echo time of zero, the fractional signal change was approximately 2.5%, a value significantly greater than the intercept of zero expected (68). This strengthens the evidence that, in addition to the BOLD effect, there is a baseline signal change which occurs synchronized to neuronal activation in the spinal cord. Likely, it is due to an increase in the extravascular water content at sites of neuronal activity (23).

Another study by Stroman *et al.* involved cold stimulation applied at different times to different sensory dermatome regions overlying the right hand and forearm. These regions included the side of the palm (corresponding to C6), the little finger side of the palm (C8), and to the forearm below the elbow (C5) (69). The distribution of activity observed in this study followed expected patterns of neuronal activation with stimulation at the different dermatomal levels on the hand and forearm. As well, the neuronal activity was significantly different between the stimulation of the different dermatomes. The study demonstrates that intensity changes related to neuronal activation can be detected in the human cervical spinal cord (69).

As well as stimulating the cervical cord, the same group has measured fMRI signal intensity changes in the lumbar spinal cord with varying degrees of thermal stimulation applied to the L4 dermatome overlying the inner calf (61). The observed pattern of activity matches the neuronal anatomy of the spinal cord here as well. It shows primarily dorsal horn activity with expected components of motor activity associated with sensory stimulation. Another observation of this study was that much larger signal changes can be observed below a stimulation temperature of 15°C.

In this study, thermal stimulation was applied to the L4 dermatome of healthy subjects and to subjects with spinal cord injuries (SCI). This stimulation was caudal to the site of injury for all SCI subjects (61). Results of the study consistently demonstrated activity in the spinal cord even when the subjects could not feel the stimulus being applied. The signal intensity changes demonstrate the same stimulus-response pattern as that in noninjured subjects, but the areas of activity in the spinal gray matter are markedly altered. In subjects with complete injuries, there was enhanced ipsilateral and contralateral motor activity, while in subjects with incomplete injuries, the pattern of activity was similar to that of healthy subjects but with weaker activation. As well, the activity was seen slightly more rostral in the spinal cord than would be expected from observations of healthy subjects.

Functional MRI and MS

For most patients, the first sign of the disease is a clinically isolated syndrome involving the optic nerves, brainstem, or spinal cord with an acute and remitting course (19). While the clinical symptoms may disappear, irreversible tissue loss can occur at this stage of MS as well as in early RRMS. This has been shown in patients who have no

increase in neurological deficits and may even have a decrease in symptoms, yet they continue to accumulate lesions on T2 weighted MRI scans. Cortical reorganization likely contributes to the remissions and aids in preservation of normal function even at the early stages of the disease (19).

Functional MRI of patients with MS has shown that brain cortical reorganization is common, and is independent of disease duration and clinical phenotype (19). Demyelination alone is not likely to lead to these functional cortical changes. Our understanding of the factors associated with the accumulation of irreversible disability may be improved by the use of fMRI. This technique has the potential to provide important information about cortical reorganization that occurs due to MS related tissue damage (19).

It can be inferred that there is some cortical reorganization involved in multiple sclerosis as there is recovery of many functional deficits without the disappearance of the lesions in the CNS. Functional MRI studies have shown an increased recruitment of cerebral networks not usually involved in the performance of certain tasks. Cortical activation occurs over a diffuse sensorimotor network in RRMS patients with no detectable functional deficits (19;20). This increased recruitment of diverse areas of the cortex was also shown in a study while patients with RRMS and mild clinical disability were performing a simple cognitive task (19). Similar patterns have been observed in studies of patients with both SPMS and PPMS. For all subtypes of MS, the change in cortical activation pattern appears related to clinical disability and to disease burden as measured with conventional MRI and proton MRS. This reorganization allows many MS patients to maintain normal or near-normal functioning longer than they would without it.

Therefore, using a broader range of cortical areas through plasticity limits the damage associated with both macroscopic and microscopic changes in the CNS (19).

Recent fMRI data suggest that the progressive failure in the ability of the brain to compensate for damage due to disease progression may influence the rate of disability accumulation more than tissue loss alone (20). The failure of these mechanisms may result in the activation of previously silent "second order" compensatory areas which may serve to further extend the time without significant disability but eventually, these may fail as well (19).

Functional MRI has the potential to provide information about cortical reorganization following MS-related tissue damage. This may help us understand the factors involved with the accumulation of irreversible disability. The adaptations of the cortical synapses is suggested to contribute to functional recovery in MS and can be explored using fMRI (19). While fMRI of the brain has been performed on patients with MS, spinal fMRI of these patients has yet to be performed. In this study we propose to show spinal fMRI can visualize neuronal activity in the spinal cord of subjects with MS and that the patterns of activity seen will be affected by the disease.

Methods

Subjects

For this study, data were obtained from 27 multiple sclerosis patients of 29 attempted. Data could not be obtained from the two subjects due to spasms they experienced during data collection. For one, the leg spasms caused too much movement to obtain clear images. Meanwhile, the spasticity experienced by the second maintained her hips flexed to a 90° angle and she could not fit in the magnet. All but two of the subjects from whom data was obtained had chronic sensory or motor deficits in the trunk or limbs at the time of the study. Those with deficits had symptoms ranging from sensory disturbances in a small area of a limb to one patient who had no motor control below the neck. All patients provided written consent prior to entering the MR system. The year of first symptoms related to MS and the year of diagnosis were recorded as well as the subtype of progression of the disease (ie. Relapsing-remitting, primary progressive, secondary progressive). Duration from first symptoms ranged from approximately 4 to 35 years (mean = 18.7). Durations from diagnosis ranged from 3 to 32 years (mean = 15.0). The mean age was 51.4 years (range 21 to 70).

Six subjects had relapsing-remitting multiple sclerosis, 12 had secondary progressive, 8 had primary progressive, and for one subject, the subgroup of MS to which he belongs is unknown. The details for the individual subjects are in Table 2.

Healthy controls were obtained for this study. Data from four of five control subjects were obtained in the thoracic region of the spinal cord at the time of this study. Healthy controls for the cervical and lumbar regions of the spinal cord were obtained from previous studies using the same type of stimulus (61).

Table 2: Details known about subjects and their condition; age, sex, subtype of multiple sclerosis, when the first symptoms were experienced, and when the subjects were diagnosed with multiple sclerosis.

Subject #	Age	Sex	Type	First Symptoms	Diagnosed
1	46	Female	RR	May 1982	Sept 1982
2	53	Female	RR	1992	1994
3	21	Male	RR	Sept 1991	April 1994
4	48	Female	RR	Feb 1992	Feb 1992
5	48	Male	RR	3.5 days before diagnosis	1995
6	45	Female	RR	1985	Dec 2000
7	41	Female	SP	1992	1992
8	40	Male	SP	Sept 1999	Oct 1999
9	40	Female	SP	Aug 1983	Mar 1985
10	43	Female	SP	1978	1979
11	52	Female	SP	1983	1987
12	64	Female	SP	Mid 1980s	1999
13	52	Female	SP	1982	1983
14	57	Female	SP	1973	1973
15	56	Female	SP	1970	1971
16	57	Female	SP	1975	1983
17	49	Female	SP	1980	1988
18	53	Female	SP	Late 1960s	1978
19	54	Male	PP	Spring 1980	1994
20	70	Male	PP	Fall 1988	Spring 1997
21	62	Female	PP	1980	1988
22	58	Female	PP	1969	June 1999

Table 2: continued

Subject #	Age	Sex	Type	First Symptoms	Diagnosed
23	49	Female	PP	1979	1989
24	58	Female	PP	1979	1984/85
25	61	Female	PP with RR symptoms	1970	1971/72
26	53	Female	PP with RR symptoms	Late 1980s	Oct 1995
27	59	Male	Unknown	1983	1985

The experimental protocol was reviewed and approved by the Institute for Biodiagnostics' Human Research Ethics Board.

MR Data Acquisition

All studies were carried out on a 1.5 Tesla clinical MR system (General Electric, Signa Horizon LX) with subjects supine. All patients were positioned so as to enter the scanner head-first. A dedicated phase array spine coil was used for signal detection and a body coil was used for transmission of uniform radio frequency pulses.

Initially, localizer images were acquired in three planes (axial, sagittal and coronal) in the region of the cervical and upper thoracic region of the spine with a field of view (FOV) of 36cm, a slice thickness of 4 mm and a spacing of 0 mm with a 256 x 128 matrix. Sagittal T2-weighted anatomical images were acquired in the cervical region of the spine to identify demyelinated multiple sclerosis lesions using parameters normally used in clinical imaging for multiple sclerosis. These images were obtained using a fast spin-echo (FSE-XL) imaging sequence with a FOV = 26 cm, bandwidth = 15.63 kHz, echo time (TE) of 10 msec, a 256 x 160 matrix, 4 signal averages were employed (i.e. 4 NEX), and 3 mm thick slices with no gap between the slices. Spatial saturation pulses anterior to the spine were used to eliminate aliasing and motion artifacts, especially from the lungs. The repetition time (TR) was optimized to avoid T1-weighting while maintaining as short a scan time as possible. If lesions were suspected from observations of the sagittal scans, axial T2-weighted anatomical scans were performed in those regions for confirmation using the FSE-XL imaging sequence with a variable bandwidth, no phase wrap, surface coil intensity correction, bandwidth = 15.63 kHz, FOV = 18 cm, a 256 x 192 matrix, TE = 102, 4 mm slices, and spacing = 0.4. Spatial saturation pulses

were used anterior and to the left and right of the spine to eliminate aliasing. Again, the TR was selected for low T1-weighting but to maintain a minimal scan duration. A coronal localizer image was then performed on the lower thoracic and lumbar region of the spine again with a FSE-XL sequence with no phase wrap, FOV = 30 cm, slice thickness = 4 mm, spacing = 0 mm, a 256 x 160 matrix, 1 NEX, TE = 102 msec, echo train length = 16, and bandwidth = 15.63 KHz. Sagittal and axial T2-weighted anatomical scans were then performed using the parameters used in the cervical region of the cord.

Functional imaging

Functional time-course data were acquired using a single-shot fast spin-echo sequence (SSFSE). Sets of 8 slices spanning the area surrounding a lesion observed were imaged every 11 seconds. Slices were oriented transverse to the spinal cord with slices positioned at the intervertebral discs or the centres of the vertebral bodies according to methods established by Stroman *et al* (61;67-69). The slices were 7.5 mm thick with a field of view of 12 cm in a matrix of 128 x 128. Flow compensation gradients applied in the through-slice direction and spatial saturation pulses were applied to eliminate signal from surrounding regions anterior and to the right and left of the spine to eliminate aliasing and reduce motion artifacts from the surrounding visceral structures.

Each set of eight slices was also imaged using a fast spin-echo inversion-recovery (IR) sequence to obtain anatomical images on which to overlay the activation maps. These were obtained with a field of view of 12 cm and a matrix of 256 x 192 (TE = 17 msec, TR = 2000 msec, slice thickness 7.5 mm, 2 NEX, bandwidth = 31.25 KHz).

Thermal Stimulation Paradigm

Thermal stimulation was applied with a Medoc TSA-II thermal sensory analyzer controlled from a personal computer. The temperature probe was 3 x 3 cm and was placed on the dermatome corresponding to the level of the lesion in the spinal cord or on the area of functional (sensory or motor) deficit. Two regions of the spinal cord were imaged. For each region, the probe was placed on the left side of the body, then the right side.

The number of lesions found and functional deficits present determined the regions of the cord that were imaged. They included:

1. If two or more lesions were found in the spinal cord, regions corresponding to two of the lesions were imaged.
2. If only one lesion was found but there was a functional deficit corresponding to a region of the cord that did not appear to have a lesion, the region corresponding to the lesion was imaged followed by imaging the region of the cord corresponding to the dermatome(s) affected by the deficit. When imaging the spinal region corresponding to a deficit, the temperature probe was placed on a dermatome affected by the deficit.
3. If only one lesion was found and no functional deficits were present corresponding to a region of the cord not included in the lesion area, the region corresponding to the lesion was imaged. The second region imaged corresponded to the lumbar portion of the spinal cord with the temperature probe placed on the inner calf to stimulate the L4 dermatome.

4. If no lesions were found but functional deficits were present affecting different dermatomes, images were obtained from regions of the spinal cord corresponding to those dermatomes. The probe was placed on the respective dermatomes.
5. If no lesions were found and one functional deficit was present, images were obtained from regions of the spinal cord corresponding to that dermatome with the probe placed on the dermatome in which the deficit was present. The second region imaged corresponded to the lumbar portion of the spinal cord with the temperature probe placed on the inner calf to stimulate the L4 dermatome.
6. If no lesions could be found or deficits reported, the regions imaged corresponded to the lumbar portion of the spinal cord and to a region surrounding the 7^{th} cervical spinal cord segment. For imaging of the lumbar cord, the probe was placed as previously mentioned. For imaging of the cervical cord, the probe was placed on the palm of the hand to stimulate the C6 through C8 dermatomes.

For each experiment, sets of 8 slices were imaged repeatedly. For the first 6 sets of images, the temperature of the probe was held at 32°C. The temperature was then ramped down to 15°C over one set of images, held there for 4 sets, then ramped back up to 32°C over one set. This was repeated once then the experiment was finished with 8 sets of images with the temperature held at 32°C. Each set of images was acquired over 11 seconds.

Data Analysis

Data analysis was performed using custom-made software written in MatLab. The pattern of signal changes was modeled with the signal being higher during the

stimulation periods, while being lower for the baseline periods (Figure 1). Only data points obtained while the temperature was constant were included in the correlation.

Data analysis involved rigid-body registration. This entailed comparing all of the images in the time series to the first image, for each slice location. Rigid body translation and rotation were performed to best match all of the images by minimizing the sum of the squares of the differences between the reference image and that being aligned. The most any images were moved in any direction was 3 mm. Only the area spanning the spinal canal was included. The corrections were only made within the slices, not across slices in the superior/inferior direction. This registration procedure is not expected to completely correct small motion such as that which may arise due to CSF flow. The spinal cord does move slightly due to CSF flow but this movement is less than 1 mm per heart beat. As the pixel size in the study was 1 mm and the slices were imaged every 11 seconds, there should be no correspondence between the motion and the activity seen.

Images of the functional data were generated through signal intensity changes between the baseline and stimulation periods. Regions in which the signal intensity changes correlated with the pattern of stimulation with a correlation coefficient greater than 0.35 (corresponding to $P < 0.05$) were determined to be regions of neuronal activity related to the stimulation. The correlation value was shown in colour in order of descending degree of change from red through orange, yellow, then green on the images.

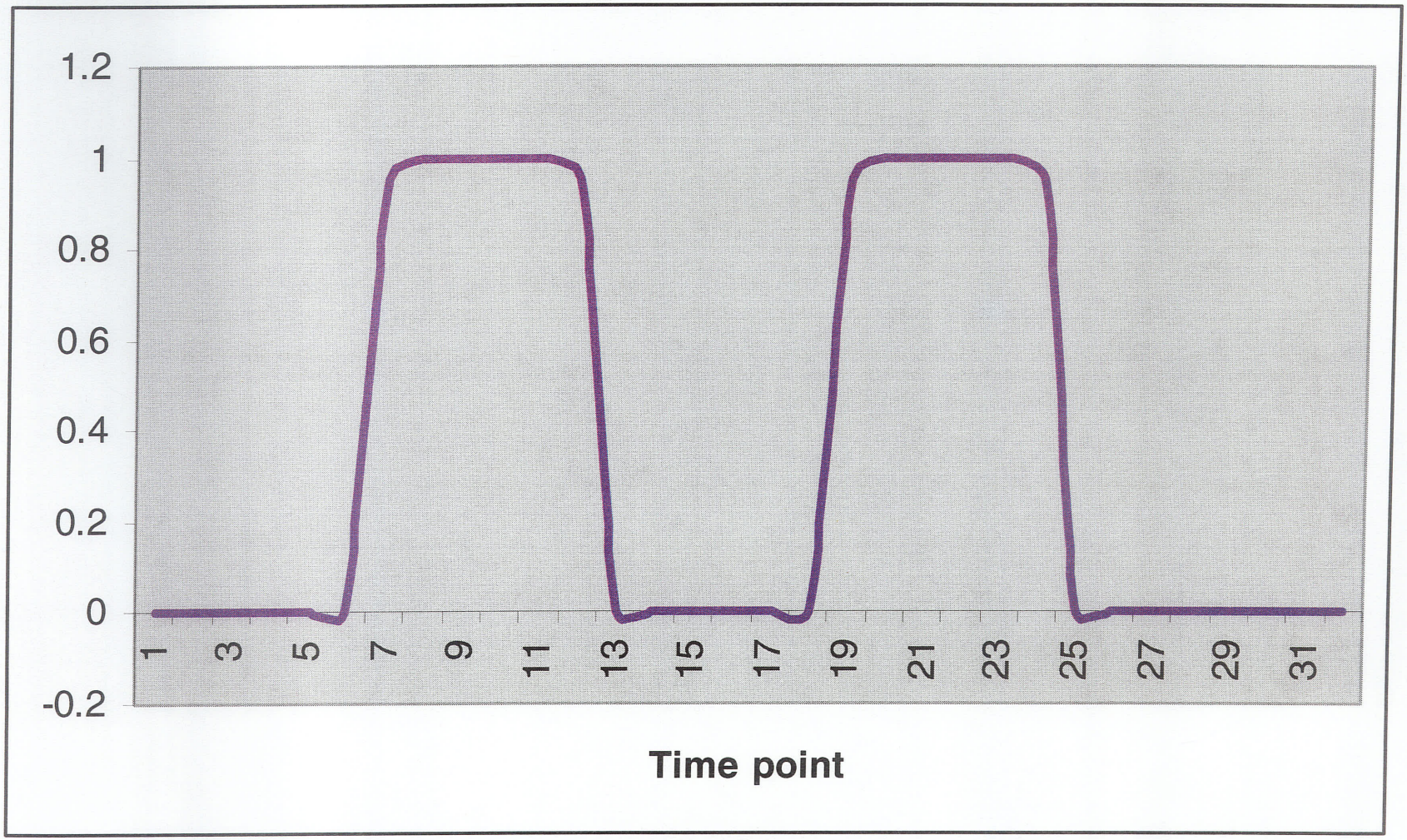

Figure 1. The paradigm followed for thermal stimulation. On the y axis, 0 represents baseline conditions while 1 represents stimulation.

Clustering was then performed due to the fact that of all of the active pixels seen, some showed true areas of activity while others may be false positives, arising most likely as a result of uncorrected motion. For clustering, all of the time courses within a series were compared using the sum of the squares of the differences. To split the active voxels into two clusters, the two time courses that were the most different were found. These were used as the starting point for two clusters. Each remaining active voxel was then assigned to the cluster which its time-course best matched, and the cluster average time course was updated each time another voxel was added. This procedure could be repeated to make more, smaller clusters. These clusters were then either included or excluded by their spatial location and their time courses. Clusters could be excluded by spatial location only if they were near the outer edge of spinal cord. Clustering gave a more accurate time course and map of activity present in the spinal cord.

Results

Data Collected

The plan for each experiment was to obtain four sets of data from each subject; one from each side of the body at two different dermatomal levels. This was achieved with three of the control subjects and seventeen of the MS subjects. With one subject with MS, we were able to obtain five sets of data, but only three sets of data were obtained for four of the MS subjects. Two sets of data were obtained for one control subject and three MS subjects, and only one set of data was obtained from one MS subject. For two of the subjects with MS and one of the control subjects, no data could be collected. The desired set of four experiments was not carried out in all subjects because of two problems. The primary problem was occasional technical difficulties with the thermal stimulator which limited the time available for data collection. This is because of the IBD/NRC imposed limit of only keeping volunteers in the MR magnet for a maximum of 90 minutes. The other source of problems was the inability of some subjects to lie still enough for all of the studies, usually due to leg spasms. For all subsequent tables, only subjects from whom data could be collected are included.

Anatomical images could only be used for a small proportion of subjects as there was too much movement by many of the subjects between the functional MRI experiments and the acquisition of anatomical images to overlay them. Even without anatomical images overlaid on the functional activity maps, it is quite evident where the areas of activity are located within the spinal cord.

Patterns of Activity

MS Patients

A summary of the symptoms, activation patterns, and areas imaged and stimulated of the subjects with multiple sclerosis can be found in Table 3 and the maps of active regions for all subjects can be found in Appendix A as Figures 1A through 31A. The figure numbers match the subject numbers in Table 3. As no patient has the same number or position of multiple sclerotic plaques, the functional data obtained could not be combined into one activity map. However, common patterns of activity were observed among the subjects with multiple sclerosis. In healthy controls, activity was observed in the dorsal horn at the level of the spinal cord corresponding to the dermatomes that were stimulated as well as in the central canal and ventral horn of more rostral levels of the spinal cord gray matter.

Primary Progressive MS Patients

For most of the subjects with primary progressive MS (PPMS), the pattern of activity appears to follow the severity of the symptoms reported by the subjects (Figures 19A – 26A). There is one exception in which the subject reported symptoms of no muscle control in the right leg and no fine motor skills in the right hand, yet the pattern of activity in this subject appears similar to that in healthy controls (Figure 19A). As all regions imaged in this group included MS plaque, effects of the presence/absence of a lesion cannot be commented on.

Table 3. The patterns of activity observed in the subjects with multiple sclerosis with the regions of the spine imaged and the presence or absence of lesions in the region imaged along with the symptoms experienced by the subjects at the time of the study.

Subtype	Subject #	Symptoms	Pattern of Activation*	Region of Spine Imaged**	Regions of Spinal Cord Stimulated	Presence of Lesion
RRMS	1	Tired, decreased sensation in fingertips and soles of feet especially when tired, sometimes a little double vision, trouble concentrating, numbness in hands and left leg and some motor, used to be right side, now mainly hands and left leg	Right: S: ---; M: --- Left: S: ---; M: ---	C4/5 - T1	palm of hand (C6 - C8)	Y
			Left: S: ---; M: ---	T8/9 - T12	calf (L4)	Y
	2	Most on left side, toe drags if tired (left), tingling in bottom of feet (sometimes going up to knee), balance, fatigue	Right: S: n; M: n Left: S: n; M: n	T10 - L1/2	sole of foot (L5/S1)	N
			Right: S: n; M: n Left: S: n; M: n	T8/9 - T12	calf (L4)	N
	3	Tremors (left arm), balance, fatigue	Right: S: n; M: n Left: S: -; M: ---	C2 - C5/6	upper arm (C4/5)	Y
			Right: S: n; M: n Left: S: 0; M: -	C4/5 - T1	palm of hand (C6 - C8)	Y
	4	None	Right: S: -; M: - Left: S: -; M: -	C4 - C7/T1	upper arm (C5)	Y
			Right: S: -; M: - Left: S: -; M: -	T9 - T12/L1	level with bellybutton (T10)	Y
	5	Lost flexibility in right hip area	Right: S: -; M: - Left: S: -; M: -	C4/5 - T1	palm of hand (C6 - C8)	Y
			Right: S: -; M: - Left - S: -; M: -	T8/9 - T12	calf (L4)	Y
	6	None	Right: S: -; M: - Left: S: -; M: -	C5 - T1/2	palm of hand (C6 - C8)	Y
			Right: S: -; M: - Left: S: -; M: -	C3 - C6/7	upper arm (C5)	Y
SPMS	7	Motor and sensory deficits in legs	Right: S: n; M: n Left: S: n; M: n	T8/9 - T12	calf (L4)	N
			Right: S: n; M: n Left: S: n; M: n	C4/5 - T1	palm of hand (C6 - C8)	N
	8	Unsteadiness, tingling in hands mainly, a little in legs and feet, very stiff from head to hip on right side	Right: S: n; M: n Left: S: n; M: n	C4 - C7/T1	palm of hand (C6 - C8)	Y
			Right: S: 0; M: --- Left: S: 0; M: 0	C4 - C7/T1	upper arm (C5)	Y
			Right: S: --; M: 0	T8/9 - T12	level with bellybutton (T10)	Y
	9	Legs weak, fingers numb, ringing in ear, spasms in legs, back pain when tired	Right: S: 0; M: 0 Left: S: 0; M: 0	C4/5 - T1	on fingers (C6 - C8)	Y
			Right: S: 0; M: 0 Left: S: n; M: n	C4/5 - T1	upper arm (C5)	Y
	10	Leg movement, problems with ambient heat or cold, tremors in hands, spasms in legs, sometimes sensory disturbances in legs	Right: S: -; M: --- Left: S: -; M: 0	C3 - C6/7	upper arm (C5)	Y
			Right: S: -; M: - Left: S: ---; M: 0	T9 - T12/L1	just above knee (L2/3)	Y

Table 3 continued

Subtype	Subject #	Symptoms	Pattern of Activation*	Region of Spine Imaged**	Regions of Spinal Cord Stimulated	Presence of Lesion
SPMS	11	No use of arms or legs (motor only)	Right: S: n; M: n Left: S: ---; M: n	C3/4 - C7	upper arm (C5)	Y
			Right: S: n; M: n Left: S: 0; M: n	T8/9 - T12	upper leg (L2/3)	Y
	12	Left leg motor, loss of balance	Right: S: --; M: --- Left: S: ---; M: n	T3/4 - T7	directly under armpit (T4/5)	Y
			Left: S: 0; M: -	C4/5 - T1	on palm (C6 - C8)	Y
	13	Motor in legs, tingling in hands, motor in hands	Right: S: 0; M: n Left: S: n; M: n	C5 - T1/2	on palm (C6 - C8)	Y
	14	Left hip dropped (can't lift leg/can't walk properly), eye problems, sensory in hands, voice problems, motor in arms	Right: S: n; M: n Left: S: n; M: n	C3/4 - C7	upper arm (C5)	Y
			Right: S: n; M: n Left: S: n; M: n	T8/9 - T12	calf (L4)	Y
	15	Motor in legs, fatigue, sensory in left foot	Right: S: n; M: n Left: S: n; M: n	C5/6 - T1	on palm (C6 - C8)	Y
			Right: S: n; M: n Left: S: -; M: -	T8/9 - T12	calf (L4)	Y
	16	Left leg weakness	Right: S: n; M: + Left: S: n; M: n	C4 - C7/T1	upper arm (C5)	Y
			Right: S: n; M: + Left: S: n; M: n	C5/6 - T2	on palm (C6 - C8)	Y
	17	Right leg little worse than left, both legs weakness, fluid retention, ring and little finger numbness	Right: S: n; M: n Left: S: 0: M: n	C3 - C6/7	upper arm (C5)	Y
			Right: S: 0; M: n Left: S: 0; M: n	T8/9 - T12	just above knee (L2/3)	Y
	18	Tired, trigeminal nerve pain (left), balance, slight motor in legs, bladder problems, leg spasms	Right: S: n; M: n Left: S: n; M: n	C6 - T2/3	on palm (C6 - C8)	Y
PPMS	19	No muscle control in right leg, no fine motor skills in right hand	Right: S: n; M: n Left: S: n; M: n	C2/3 - C6	upper arm (C5)	Y
	20	Left arm numb, left leg no sensory or motor	Left: S: 0; M: n Right: S: 0; M: 0	C6/7 - T2/3	on palm (C6 - C8)	Y
			Left: S: --; M: --	T6 - T9/10	bottom of ribs (T8)	Y
	21	Motor weakness in legs	Right: S: ---; M: + Left: S: ---; M: +	T8 - T11/12	level with bellybutton (T10)	Y
			Right: S: 0; M: + Left: S: 0; M: n	C4 - C7/T1	on palm (C6 - C8)	Y
	22	Balance, leg weakness, sleep disturbances	Right: S: --; M: + Left: S: n; M: n	C3/4 - C7	upper arm (C5)	Y
			Right: S: ---; M: + Left: S: ---; M: +	T7/8 - T11	bottom of ribs (T8)	Y
	23	Pins and needles both hands, both legs weakness, bladder/ bowel problems	Right: S: 0; M: n Left: S: 0; M: n	C4 - C7/T1	upper arm (C5)	Y
			Right: S: 0; M: n Left: S: 0; M: n	C6/7 - T3	just below elbow on outside of arm (T1/T2)	Y

Table 3 continued

Subtype	Subject #	Symptoms	Pattern of Activation*	Region of Spine Imaged**	Regions of Spinal Cord Stimulated	Presence of Lesion
PPMS	24	Fatigue, leg weakness	Right: S: 0; M: - Left: S: 0; M: n	C3/4 - C7	upper arm (C5)	Y
			Right: S: -; M: - Left: S: 0; M: -	T8/9 - T12	calf (L4)	Y
	25	Blind, leg weakness, poor balance, left arm weaker (buzz)	Right: S: 0; M: n Left: S: 0; M: n	C3 - C7/T1	just below elbow (C5/6)	Y
			Right: S: n; M: n Left: S: n; M: n	T9/10 - L1	calf (L4)	Y
	26	Drop foot, motor in arms, memory, incontinence	Left: S: -; M: n	C4/5 - T1	on palm (C6 - C8)	Y
Unknown Subgroup	27	Weaker in lower extremities, starting more in upper body	Left: S: n; M: + Right: S: -; M: -	C4 - C7/T1	just below elbow (C5/6)	Y
			Left: S: 0; M: n	T2/3 - T6	just under armpit (T4/5)	Y

* Note: S = sensory (dorsal horn) activity; M = motor (ventral horn) activity; n = same activity as controls; 0 = no activity seen; -/+ = slightly less/more activity than in controls; -- = much less activity than controls; --- = almost no activity seen

** Regions of the spine imaged = vertebral levels, not spinal cord levels

Relapsing Remitting MS Patients

The pattern of activity in patients with relapsing remitting MS (RRMS) appears to follow the deficits reported by the patients (Figures 1A – 6A). The two patients who reported no symptoms, while images were taken in the area in and surrounding lesions, had functional maps with slightly fewer active voxels but the same pattern as controls (Figures 4A & 6A). This is also true of the patient who reported only a slight loss of flexibility in his right hip area (Figure 5A). Of the three RRMS patients that reported experiencing multiple symptoms at the time of the study, one did not appear to have any lesions visible in the spinal cord. This patient had a functional map similar to controls (Figure 2A). Of the two remaining with symptoms, both had abnormal functional maps with the patient experiencing the most severe symptoms at the time of the study having the most abnormal functional map (Figure 1A).

Secondary Progressive MS Patients

As a group, the subjects with secondary progressive MS (SPMS) came into the study with the most severe reported deficits. As with the other MS patients, these subjects had patterns of activity that, for the most part, followed the symptoms (Figures 7A – 18A). However, there was more variation in this group than in the other groups. One patient in this group had motor and sensory deficits in her legs but no lesions were found in the spinal cord. She had a normal pattern of activity (Figure 7A). The subject that deviated most from the observed pattern in MS patients was a woman with no motor control of her body below the neck. For the scans in which the thermal stimulator was placed on her left side, she had motor activity only which would be expected in someone with her symptoms based on previous studies with fMRI of spinal cord injury (61;70).

The change came with stimulation of the right side. For these scans, her pattern of activity appeared similar to that of healthy controls with only slightly more motor activity (Figure 11A).

Changes in Signal Intensity

The change in percentage signal intensity for the subjects with MS was significantly greater than that for the control group with changes of 11.2% ± 0.5% and 9.0% ± 0.7%, respectively. The average signal intensity changes for these groups can be seen in Figure 2.

There was variation between subjects of different MS subgroups as well. The PPMS subgroup had signal changes significantly lower than that of the SPMS and RRMS subgroups. However, the SPMS and RRMS subgroups were not significantly different from one another. The average percent signal changes for the PP, SP, and RRMS subgroups were 8.6% ± 0.5%, 12.1% ± 0.9%, and 12.0% ± 1.0% respectively. The comparison of these subgroups can be found in Figure 3.

When compared with the control subjects, the PPMS subgroup was not significantly different from the control subjects but the SP and RRMS subgroups had significantly different signal intensity changes. Table 4 illustrates the p-values obtained through the student's t-test comparing the various groups.

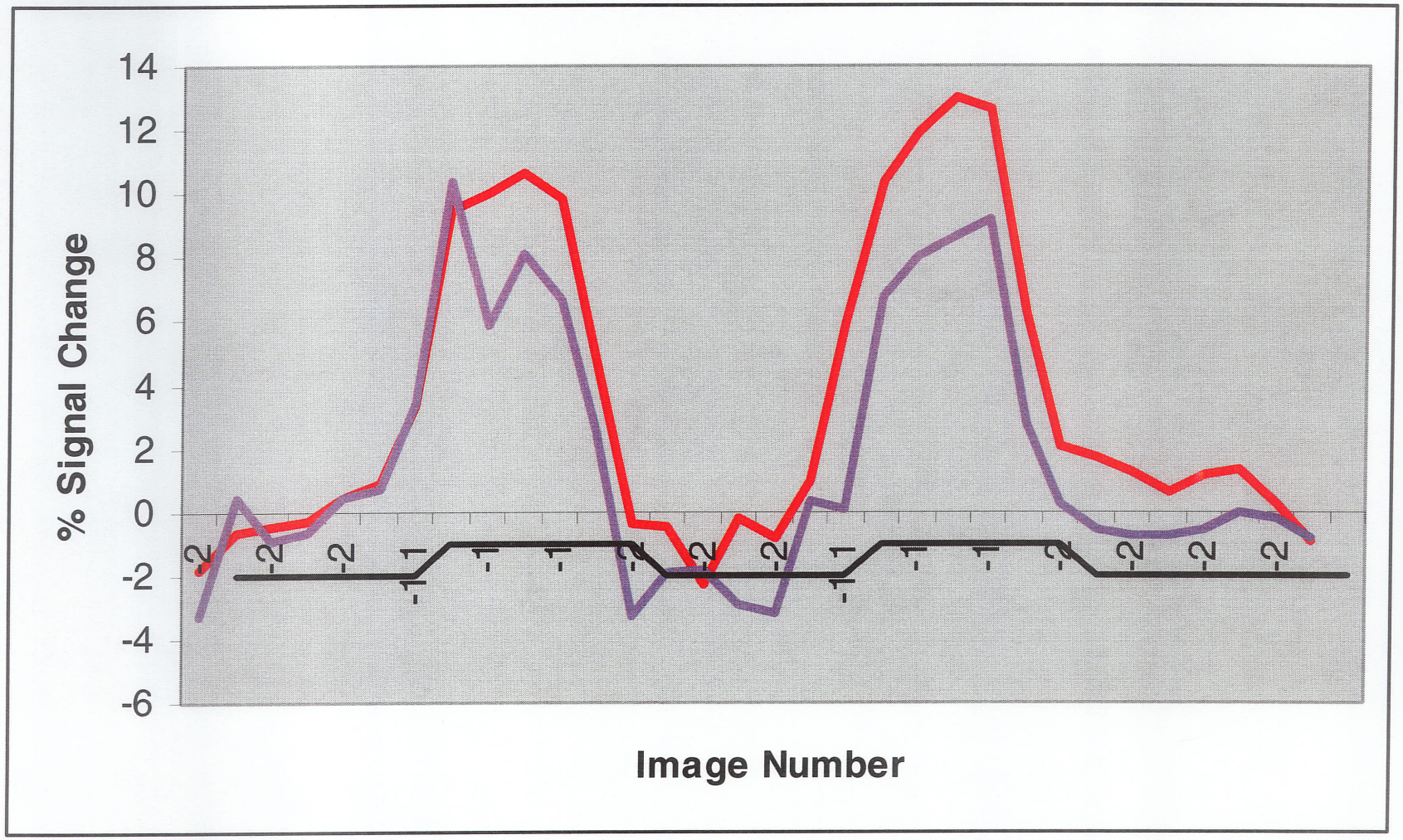

Figure 2. Fractional signal changes observed in spinal fMRI of healthy controls (blue) compared to MS patients (red) as they relate to the stimulation paradigm (black).

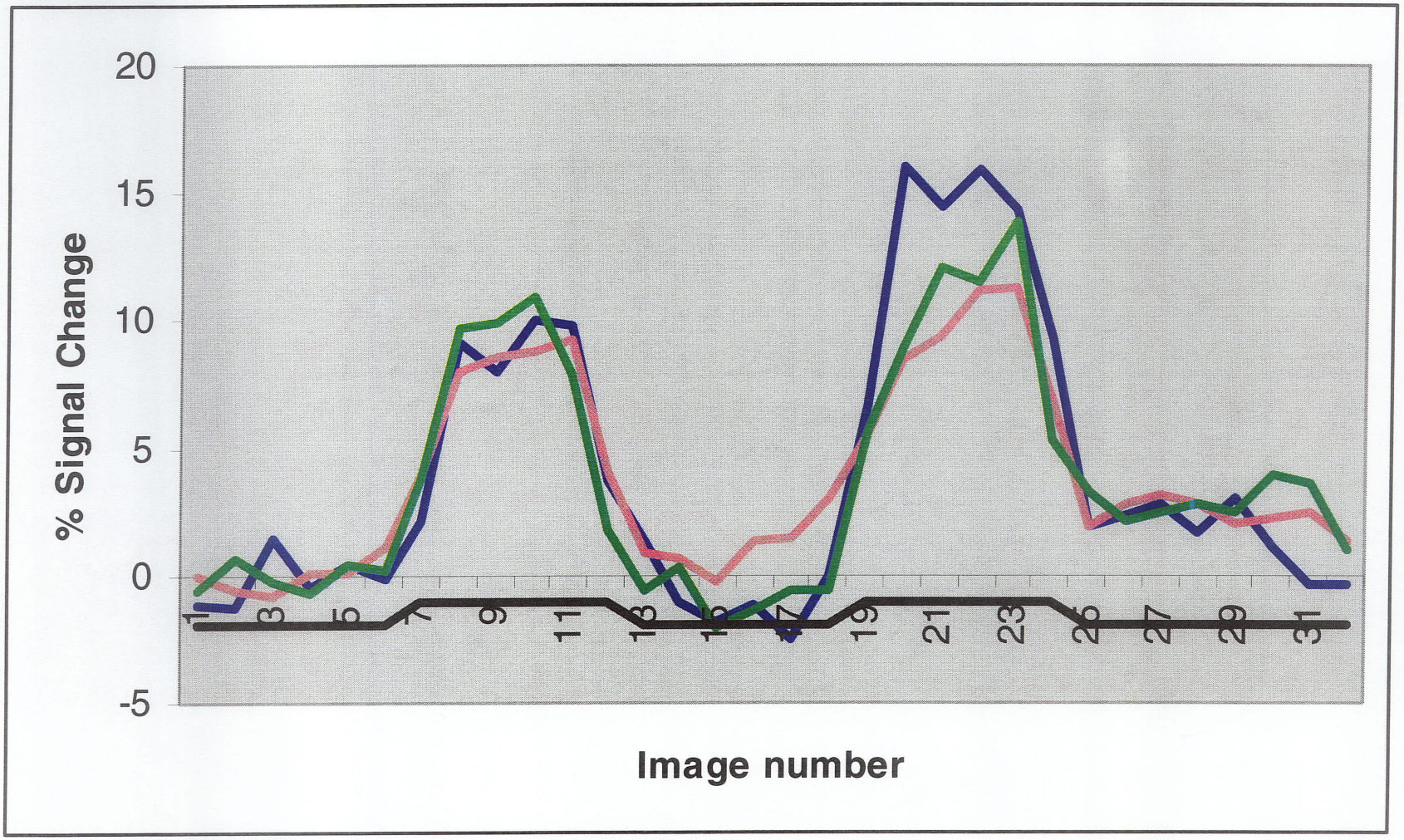

Figure 3. Fractional signal changes observed in spinal fMRI of PPMS (purple), RRMS (blue), and SPMS (green) as they relate to the stimulation paradigm (black).

Table 4. P-values obtained when comparing the signal intensity changes of the subgroups of subjects with multiple sclerosis with each other and with healthy control subjects. (SP = secondary progressive MS; RR = relapsing remitting MS; PP = primary progressive MS)

Groups Compared	p-values
SP vs. PP	0.0053
SP vs. RR	0.87
SP vs. Controls	0.061
PP vs. RR	0.043
PP vs. Controls	0.62
RR vs. Controls	0.17

Discussion

Changes in Signal Intensity

With respect to signal intensity changes, the purpose of this study was to show that predictable changes in signal intensity could be observed in the spinal cord of subjects with MS in response to thermal stimulation using the SEEP theory. These changes were seen throughout the study with the signal intensity increasing above baseline in the regions imaged when the thermal stimulation was applied.

The SEEP theory is based on water crossing the blood-CNS barrier (BCNSB). As the blood brain barrier (BBB) and the blood spinal cord barrier (BSCB) have similar characteristics (50), any reference to the BBB in this discussion will refer to both the BBB and the BSCB unless otherwise stated. Water can diffuse across the plasma membranes that compose the BBB but too slowly to be physiologically useful. However, this is not the only way in which water can cross the BBB.

A family of water channels, aquaporins, is found throughout the body. While in the body, eleven subtypes of aquaporins have been found in mammals, six subtypes have been found in the brain. These six are AQP1, AQP3, AQP4, AQP5, AQP8, and AQP9 (71). Of these, AQP4 is the most abundant in the brain and the brain is the main site of AQP4 involvement in the body (72).

Three of the subtypes in the brain have only recently been found in astrocyte cultures; AQP3, AQP5, and AQP8. Their role in the brain has yet to be determined (71). Meanwhile, AQP1 is involved in the formation of CSF. This subtype is found in capillary beds throughout the body. The exception is in the brain, where it is only found in the choroid plexus (72).

In the brain, AQP4 is found in plasma membranes of astrocytes, endothelial cells, and ependymocytes but is most abundant in astrocytic end feet that are against vascular and pial membranes. This subtype is believed to be the most important for water transfer during physiological processes, most importantly, neuronal activity. AQP4 is a bi-directional channel for water and is driven purely by osmotic gradients. AQP9 is also found on astrocytes and endothelial cells but AQP 4 is the most important for water transport. AQP 9 may also have a role in energy metabolism as, along with water, it serves as transport for glycerol (72).

Astrocytes have been found to have important roles in maintaining a homeostatic extracellular environment for neurons. They appear to fill any gap between the other cerebral elements such as blood vessels, pial surfaces, nodes of Ranvier, and neuronal cell bodies. Their end-feet lie alongside microvessels and often surround synapses completely (73). Astrocytes are likely the source for metabolic substrates for neurons and can take up neurotransmitters, water, and other ions in the synaptic cleft both during basal and increased neuronal activity and serve as connections between the neural elements and the blood (73).

Many of the channels found on brain endothelial cells for other substances cotransport water. These include the glucose transporter Glut1, monocarboxylate transporter Mct1, and the $NaKCl_2$ cotransporter. It has been suggested these may also be involved in the transendothelial transport of water. Mct1 and Glut1 are also expressed in perivascular membranes (72).

Positron emission tomography studies are based on the fact that water crosses the BBB. In fact, water is used as a tracer to measure cerebral blood flow in many studies as

it traverses the BBB quickly (60). While the barrier is not freely permeable to water, it crosses quickly enough to offer a close estimate that is further modified with various models (60). These findings along with the molecular channels and cotransporters discussed above show that, contrary to the belief for many years, water does cross the BBB and does so at a rapid enough rate to be physiologically significant.

Signal Intensity Changes in MS Subjects

The fact that the increase in signal intensity was significantly greater in subjects with MS than in healthy subjects was not expected. A possible explanation for this finding is the widespread abnormalities believed to be present throughout the BBB. There is increasing evidence that there are chronic deficits in the BBB both before and after macroscopic enhancing MS lesions can be seen by means of T1-weighted MRI. This includes changes in the BBB throughout normal appearing white matter (NAWM) in MR images. As the SEEP theory is based on water crossing the BBB with increased permeability, more water could cross, causing an increase in the signal intensity greater than that of subjects without these abnormalities.

While these changes have been seen using magnetic resonance spectroscopy (MRS), their pathophysiology is not yet known (23). Two possible explanations have been suggested. The first suggests that Wallerian (retrograde) degeneration may occur in the axons that have been damaged within the macroscopic lesions (23). The second suggestion is that changes in the structure in the NAWM may precede actual macroscopic changes and may indeed trigger the formation of lesions (23;35). This hypothesis that the microscopic damage precedes, and possibly causes, the macroscopic damage comes from evidence that there is a low-grade defect of the BBB throughout the nervous system of

patients with MS. One group working with MS patients found a significant signal increase on Gd-DTPA scans that were originally thought to be nonenhancing (ie. no large BBB defects due to inflammation) (11). Another group also found a low-grade leakage through the BBB of chronic lesions (22). As well, increased signal intensity was seen with Gd-DTPA in NAWM which points to diffuse BBB damage in patients with MS (11). This low-grade leakage may continue to occur throughout the BBB until it hits a threshold beyond which irreversible damage occurs and a macroscopic lesion develops (35). Alternatively, the two processes of microscopic and macroscopic damage may not be linked but occur through similar mechanisms that vary in severity between patients (35).

The fact that increased signal intensity is observed in spinal fMRI of patients with MS, compared to healthy control subjects, gives evidence that we are indeed observing an effect that depends on the permeability of the BBB, consistent with an increase in extravascular water content. This increase was seen in regions of the spinal cord both with and without macroscopic lesions visible on T2-weighted anatomical images. This conclusion is also supported by previous studies of traumatic spinal cord injury with thermal stimulation below the site of injury, which demonstrated identical signal changes in injured subjects as in a healthy control group (70).

Signal Intensity Changes by Subtype

The difference between the PPMS subgroup and the RR and SPMS subgroups was not surprising, but the lack of difference between the PPMS subjects and the controls was not expected. PPMS lesions tend to manifest differently than do RR and SPMS. Inflammation is not a prominent feature of PPMS (8;11) rather the disability seen with

this subtype is believed to occur due to axonal loss (8). In contrast, inflammation is a principal feature of both RR and SPMS. The lesion patterns are also different with the patterns in patients with RR or SPMS being rather similar while patients with PPMS tend to have fewer and smaller lesions (13).

Commonly in RRMS, the relation between conventional MRI abnormalities and clinical condition is poor (11). The T2 weighted and Gd enhanced T1 weighted brain MRI show approximately ten active lesions for every clinical relapse. Slightly lower levels of activity are found in SPMS and much less activity is found in PPMS (20). RRMS is characterized by the presence of active lesions, seen as a high number of Gd-DTPA enhancing lesions (17).

SPMS is associated with the presence of large confluent hyperintense lesions on T2 weighted MRI in contrast to the multiple focal lesions characteristic of RRMS (17;18). These lesions may partly appear as hypointense on T1 weighted scans (17). Patients with benign MS have been reported to have smaller T2 lesion loads compared to SPMS patients matched for disease duration, and to display much less Gd enhancement compared to those with early RR disease (54).

The conventional MRI characteristics of PPMS include a tendency to reduce lesion loads and to reduce the rate of new lesion formation (11). Patients with PPMS develop fewer T2 lesions in the brain (ie. have lower T2 lesion loads), have less T1 hypointensity, and have a lower frequency of inflammatory lesions than patients with SPMS, as determined by little enhancement observed with the administration of intravenous Gd-DTPA in T1-weighted images. This is all despite comparable levels of disability (11). Patients with PPMS have more widespread abnormalities in the spinal

cord than in the brain with some patients having diffuse increase in signal intensity throughout the spinal cord on MR imaging (18). As well, with advancing disease, such as in SP and PPMS, atrophy of the brain and spinal cord often becomes apparent with the largest degree of atrophy seen in PPMS (13;18). In the PPMS subgroup, spinal cord MRI parameters correlate well with spinal symptoms which emphasizes the importance of spinal cord involvement in PPMS (17). Brain abnormalities seem to explain most of the symptoms and disability in RR and SPMS but in PPMS, spinal cord abnormalities seem to be more important clinically than brain abnormalities (17). PPMS patients display few new T2 lesions on monthly brain scanning and most new lesions do not enhance with conventional doses of Gd-DTPA (54). In PPMS patients, conventional MRI scans of the brain typically show a relatively low amount of macroscopic white matter abnormalities which is in contrast with their severe and irreversible disability (74).

Disease activity as shown by the number of new and enhancing lesions is also much lower in patients with PPMS than in the other two subgroups (13). However, mild disruption to the BBB is believed to occur throughout the CNS in PPMS (11). While this likely occurs to a lesser extent in the RR and SPMS subtypes, these latter two MS subtypes also have the large disruptions brought on by inflammatory lesions. This corresponds well with our observation that the percent signal changes in the PPMS subgroup did not increase to that of the RR and SPMS subgroups. However, it does not explain the fact that the PPMS subgroup showed signal intensity changes that were not significantly different from the healthy subjects.

Patterns of Activity

Changes in the patterns of activity observed upon stimulation were expected to occur depending on where in the nervous system the damage had occurred. The possible sites of damage in any subject are at the level of the receptors, peripheral nerves, spinal nerves, sensory neurons, motor neurons, white matter tracts, and regions of the brain. If the receptors were damaged, the change in pattern of activity would depend of the types of receptors and the size of the region affected. There would likely be no change in any activity in the ventral horns as they contain motor efferents. If there was a large area of receptors affected within the dermatome affected, there would be a decrease in sensory stimulation to the dorsal horns. The decrease in neuronal activity would be expected to be proportional to the number of receptors affected. If peripheral nerves were affected, the change in activity would likely be similar to that observed if the receptors were damaged.

Damage to spinal nerves would affect the pattern of activity observed with stimulation. There would be no sensory input from the dermatomes innervated by those nerves. As well, as the activity observed in the ventral horn is postulated to be inhibition of the motor reflex to withdraw, the pattern of activity in the ventral horn would likely decrease. This pattern would also be observed if specifically sensory neurons were affected. If motor neurons were exclusively affected, there would probably not be a change in neuronal activation as the damage would be to efferents running from the cord.

The effect of injury to white matter tracts would depend on the tracts damaged. Damage to ascending tracts would not affect neuronal activation in the dorsal horns from sensory input but it may affect patterns of activity observed in the ventral horns as the

inhibition of the motor reflex to withdraw is stimulated by sensory input to higher centres in the brain. This input would not reach the brain without the ascending white matter tracts in the spinal cord. While damage to the descending white matter tracts also would not affect neuronal activation in the dorsal horns, the patterns of activity in the ventral horn would be affected. How it would be affected is uncertain. There would be no inhibition of the motor reflex but without inhibition, there may actually be a motor reflex to withdraw from the stimulus.

Damage to the brain could affect the patterns of activity observed with thermal stimulation in various ways, depending on the regions affected. In general, if motor areas were affected, the inhibition of motor reflex may either be increased or decreased with no changes to the patterns observed in the dorsal horns. Damage to sensory areas will make no change to dorsal horns, but may decrease the motor reflex.

Patterns Observed

We had initially believed that the subjects with MS would have different patterns of activity than control subjects based on their T2 lesion load in the spinal cord. However, the patterns followed the severity and type of deficit the patient was experiencing at the time of the study more than the presence of a lesion in the region of the spinal cord stimulated and imaged. Conversely, if there were no lesions present in the regions imaged, the patterns of activity appeared similar to those of control subjects.

In the 2 subjects we studied for which no lesions were observed in the spinal cord, there was no difference in the pattern of activity compared to that typically observed in control subjects. However, response patterns similar to that in control subjects were also seen in subjects with observable lesions. As this project is part of a larger study of spinal

fMRI, comparisons can be made to previous studies of healthy controls and patient groups with spinal cord injuries (SCI) (61;70). Most patterns of neuronal activity appeared to follow patterns observed in either healthy controls or subjects with incomplete or complete SCI, with the patterns progressing from healthy controls through incomplete SCI to complete SCI as the severity of symptoms experienced by the subject increased.

In healthy controls, the pattern of activity observed was primarily a sensory response in the ipsilateral dorsal region, with motor activity observed bilaterally as seen in Figure 4. In previous studies of subjects with complete spinal cord injury, the sensory response was diminished, but the motor response was enhanced bilaterally and around the central canal (70). Subjects with incomplete injuries had a diminished sensory response, and did not have an augmented motor response.

As MS plaques are regions of demyelination and axonal damage, impulse conduction through regions surrounding these plaques should be altered, either through a decrease in conduction speed or a complete blockage of conduction through the area. As well, there is damage throughout the NAWM in the CNS of patients with MS (55). Therefore, a change in the pattern of activity would be expected even in regions imaged in which a macroscopic T2 visible lesion was not observed. However, this phenomenon was not observed in this study as the 2 patients for whom a spinal cord lesion could not be visualized appeared to have a pattern of neuronal activity similar to that of healthy controls. Possible reasons for these observations may include the fact that these subjects had not yet developed the extent of microscopic damage that could affect impulse conduction. Another possibility is, due to the fact that there were only 2 subjects for

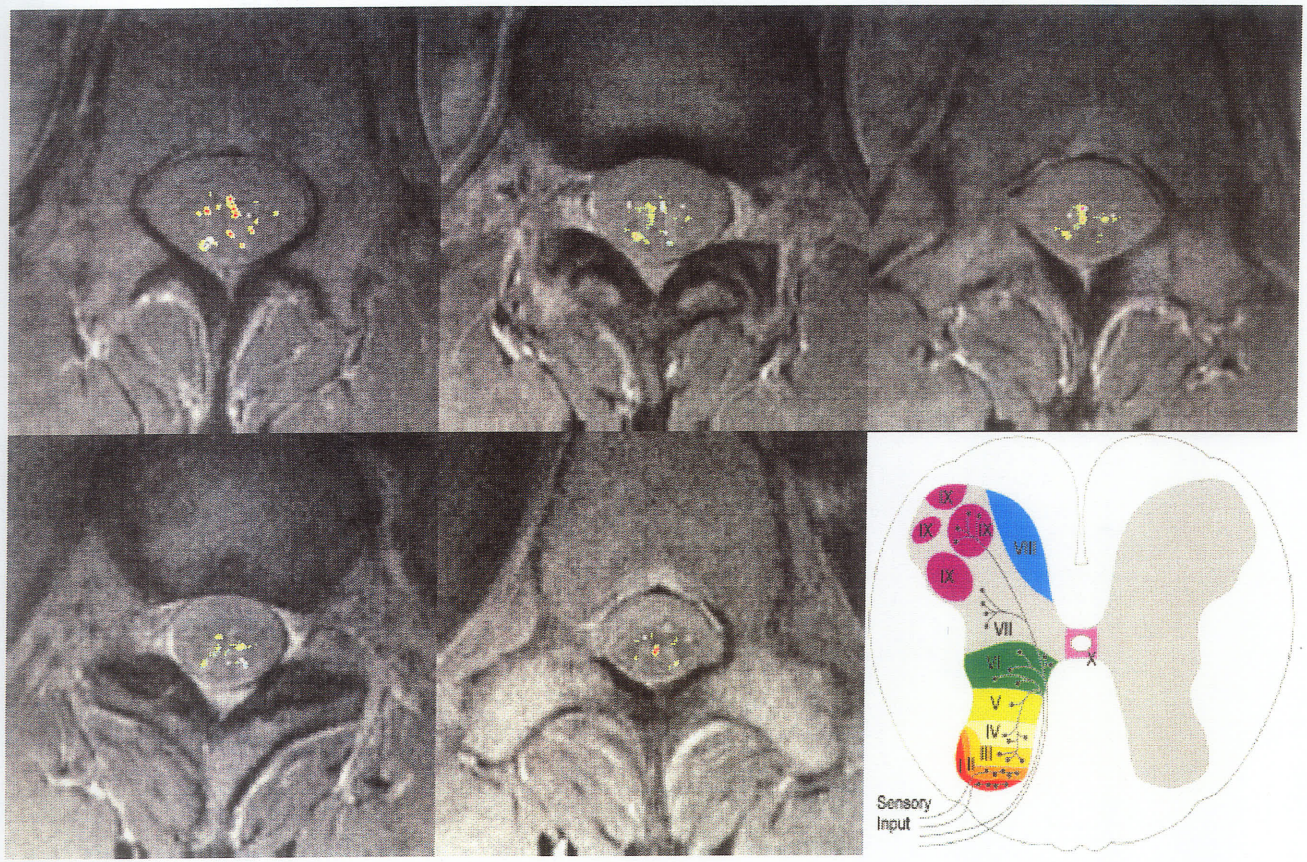

Figure 4. Combined spinal fMRI results from the lumbar spinal cord showing the activity response to 10°C thermal stimulation of the L4 dermatome of healthy subjects. Images are in radiological orientation with the right side of the body towards the left side of the image, and dorsal is towards the bottom. From left to right the slices span approximately through the following spinal cord segments: fifth/fourth lumbar; third lumbar; second/first lumbar; 12th thoracic; and 11th thoracic. The colour scale shows red at points with the greatest consistency across subjects, through orange, yellow, and then green indicating that only three subjects showed activity. The schematic inlay (bottom right) shows the expected areas of activity in the spinal cord with a sensory or noxious stimulus. The schematic is magnified roughly by a factor of 5 compared to the MR images (61). (Used with permission of P.W. Stroman)

whom data was obtained in regions without macroscopic lesions, there were too few subjects to draw a conclusion as they may be exceptions to the group. If more patients were imaged in areas without a lesion the trend may continue or the pattern of activity observed may follow the severity of functional deficit as it does with regions surrounding a lesion.

The observation that the patterns of activity in MS subjects followed the functional deficits experienced more so than the T2 lesion load could be an effect of plasticity of the CNS. New connections may be made to compensate for the damaged areas. This plasticity has been observed throughout the CNS in response to injury (75).

Primary Progressive MS Subjects

As a primary progressive course of MS is associated with higher severity of symptoms (18), it would be expected that the subjects in this subgroup would show patterns of activity similar to patients with SCI. Most of these subjects did, with the change in pattern from healthy controls depending on the severity of their symptoms. However, Subject 19 did not follow this pattern (Figure 19A), showing a pattern of activity like that of healthy controls despite reporting symptoms of no muscle control in her right leg and no fine motor skills in her right hand. One possible explanation of this observation is that this subject was not imaged with stimulation of the legs. Images were taken with stimulation of the hand and at the bottom of the ribs, which corresponded to regions of the spinal cord in which a lesion was present. Some change was expected in the pattern of activity when the right hand was stimulated but the observed pattern with stimulation at the bottom of ribs was expected as no deficit was reported in this region. A possible explanation of no change in the pattern of activity when the right hand was

stimulated is that the symptoms she reported were motor and she still had some control of the hand. However, other patients in this subgroup reported only motor symptoms yet their patterns of activity were not those of healthy controls.

Relapsing Remitting MS Subjects

Of all of the MS subtypes, the group of patients with RRMS had a pattern of activity closest to that observed for healthy controls. This was likely due to the fact that the majority of the subjects in this group had no symptoms or symptoms less severe than the subjects in the other subtypes at the time of the study. To demonstrate the differences within this subgroup, patients with different severity of symptoms will be discussed.

Subject 4 was experiencing no symptoms related to MS at the time of the study. At all sites stimulated, she showed a pattern of activity similar to that of healthy controls. The only difference was that slightly less activity was observed in both dorsal and ventral horns. This level and pattern of activity was also observed in Subjects 5 and 6. Subject 5 was experiencing a slight loss of flexibility in the right hip area at the time of the city while Subject 6 was not experiencing any MS related symptoms. The reason for this slight decrease in activity while maintaining similar patterns of activity is not known. A possibility is that it may be due to changes in neuronal function in the spinal cord from previous lesions not resulting in functional deficits.

The subject with the most severe symptoms in this subgroup, Subject 1, also had a pattern of activity that deviated the most from healthy controls. There was almost no activity observed in the regions of the spinal cord corresponding to the dermatomes stimulated. This observation coincides with observations in other subgroups of subjects with symptoms of similar severity. As the subject reported numbness in the hands and

left leg, the lack of activity is not surprising. Subjects with complete spinal cord injury from previous studies who could not feel the stimulus due to an injury well above the site being stimulated had a pattern of decreased sensory activity but increased motor activity. The difference in the pattern of activity observed in Subject 1 may be due to the fact that the damaged neurons in this subject were likely running through, and possibly originating from, the lesion in the region of the spinal cord imaged, in contrast to the damage being present above with the region imaged remaining relatively intact.

Secondary Progressive MS Subjects

The subjects with SPMS had the most varied patterns of activity, most likely due to the wide range of symptoms experienced within this group at the time of the study. Two subjects are discussed that followed patterns similar to that of other subgroups. As well, one subject that followed patterns similar to other subgroups with stimulation on one side of the body but did not with stimulation of the opposite side.

The symptoms relating to the limbs reported by Subject 18 were mild weakness in the legs and leg spasms with no symptoms in the arms. Imaging was performed with the thermal stimulator placed on the palm of the left, then the right hands. The pattern of activity observed was akin to that of healthy controls (Figure 18A). This would be expected as no symptoms were reported in the arms. In contrast, Subject 10 reported involuntary leg movement, problems with ambient heat or cold, tremors in hands, spasms in legs, and sometimes sensory disturbances in legs. The patterns of activity observed were very abnormal on thermal stimulation of the upper arm and just above the knee. They did not follow a pattern similar to either healthy controls or SCI subjects. On the upper arms, slightly less activity was seen compared to that of healthy controls but very

little motor activity was seen on the right and no motor activity was seen on the left. The right and left legs varied more from one another. The pattern with stimulation of the right leg was weaker but in the same pattern as healthy controls. Meanwhile, there was little sensory and no motor activity observed on the activation maps with stimulation of the left leg.

Another pattern of interest is that of Subject 11. She is the subject with no motor control below her neck. For scans in which the thermal stimulator was placed on her left side, she had motor activity only, similar to a pattern observed in complete spinal cord injured patients, which would be expected with her symptoms. Conversely, when the thermal stimulator was placed on the right side of her body, her pattern of activity appeared similar to that of healthy controls with only slightly more motor activity. This was true for both sites on which the thermal stimulator was placed, the upper arm and the upper leg. The reason for this is unknown. The subject was not asked if the stimuli were felt and if they felt different so it may or may not have been due to the ability to feel the stimulus. Another possibility is that the neuronal activity shown on the activation map was due to plasticity of the spinal cord. In an attempt to maintain neural integrity, plasticity has been well documented in neurodegenerative diseases, including MS (75).

Effects of Medications on Activity

We had originally thought that some of the medications taken by the MS subjects may have affected the pattern of activity seen on the activation maps for these subjects. Many of the subjects were on medications that affect neurotransmitters in the CNS. The medications patients were on at the time of the study can be found in Table 5, and their main actions are presented in Table 6. Those of most interest include GABA agonists,

Table 5. Medications taken by subjects with MS at the time of the study.

Subject #	Medications
1	Interferon beta-1b (Betaseron) Rofecoxib (Vioxx)
2	Indapamide (Lozide) Lisinopril Rofecoxib (Vioxx) Amantadine
3	Interferon beta-1b (Betaseron) Fluoxetine (Prozac)
4	Fludrocortisone (Florinef) Hydrocortisone (Cortef)
5	Mirtazapine (Remeron) Alprazolam
6	None
7	Baclofen (Lioresal) Insulin
8	Interferon beta-1a (Rebif)
9	Prednisone Oxybutynin (Ditropan) ASA Citalopram (Celexa) Baclofen (Lioresal)
10	None
11	Baclofen (Lioresal) Tizanidine (Zanaflex) Citalopram (Celexa) Tolterodine (Detrol) Lorazepam
12	Betahistine (Serc) Amantadine Levothyroxine Tolterodine (Detrol) Fluoxetine (Prozac) Premarin Amlodipine (Norvasc)
13	Amantadine Propranolol (Inderal) Baclofen (Lioresal)
14	Diazepam Progesterone Estrodiol (Estrogel) Tolterodine (Detrol) Ibuprofen Amitryptoline (Zorco)

Table 5 continued

Subject #	Medications
15	Felodipine Amantadine Lisinopril Levodopa Zopiclone Citalopram (Celexa)
16	ASA (Bufferin)
17	Baclofen (Lioresal) Methylphenidate (Ritalin) Triphasil
18	Amiloride (Midamor) Citalopram (Celexa) Baclofen (Lioresal) Nifedipine Clonazepam Estradiol Progesterone Mitoxantrone (Novantrone)
19	Hydrochlorothiazide (Avalide) Modafinil (Alertec)
20	Carbamazepene (Tegretol) Tizanidine (Zanaflex)
21	Raloxifene (Evista) Tizanidine (Zanaflex) Oxybutnin (Ditropan) Lorazipam
22	Raloxifene (Evista)
23	Amantadine Oxybutinin (Ditropan)
24	Simvastatin Amiltryptoline (Zorco) Citalopram (Celexa)
25	Interferon beta-1b (Betaseron) Gabapentin (Neurontin) Citalopram (Celexa) 222s
26	Glatiramer acetate (Copaxone) Carbamazepene (Tegretol) Levothyroxine (Eltroxin) Rofecoxib (Vioxx) Lorazipam
27	Fenofibrate (Lipidil) Tizanidine (Zanaflex) Gabapentin (Neurontin)

Table 6. Medications reported taken by the subjects with MS and their actions (46;49;76-83).

Medication	Actions
Alprazolam	Benzodiazepine Treat anxitey disorders, agoraphobia
Amantadine HCl	Antiviral agent for prophlaxis and treatment of influenza A Antiparkinsonian actions Treat drug-induced extrapyramidal disorder
Amiloride HCl (Midamor)	Potassium-sparing diuretic (inhibitor of renal epithelial sodium channels)
Amitriptyline HCl (Zorco)	Tricyclic antidepressant Muscarinic receptor antagonist Sedative
Amlodipine (Norvasc)	Calcium channel antagonist Treat hypertension, angina
ASA (Aspirin)	Analgesic, antipyretic, antiinflammatory effects
Avalide (Hydrochloro-thiazide)	Thiazide diuretic Inhibitor of sodium-chloride symport Treat hypertension, CHF, nephrolithiasis due to idiopathic hypercalciuria, nephrogenic diabetes insipidus
Baclofen (Lioresal)	$GABA_B$ agonist Antispastic
Betahistine (Serc)	Antivertigo Histamine H1 receptor agonist; H3 receptor antagonist
Bufferin (ASA)	Analgesic, Antipyretic, Antiinflammatory, Antirheumatic activity Long-term palliative treatment of mild to moderate pain and inflammation of arthritic and other inflammatory conditions
Carbamazepene (Tegretol)	Tricyclic compound Treat bipolar depression, epilepsy
Citalopram (Celexa)	Antidepressant Selective serotonin reuptake inhibitor (SSRI)
Clonazepam	Benzodiazepine Potent antiseizure medication, antianxiety
Conjugated estrogens (Premarin)	Conjugated equine estrogen Hormone replacement therapy
Diazepam	Benzodiazepine Treat seizure activity (generalized tonic-clonic, status epilepticus) GABA agonist (antispastic activity), antianxiety
Estradiol	Hormone replacement therapy
Estradiol (Estrogel)	Hormone replacement therapy; topical
Felodipine	Calcium channel antagonist (decrease bp) Treat hypertension, CHF, Raynaud's phenomenon
Fenofibrate (Lipidil)	Potential antiatherothrombotic effects Treat hyperlipidemia

Table 6 continued

Medication	Actions
Fludrocortisone acetate (Florinef)	Adrenocorticosteroid Treat Addison's disease Increases blood pressure
Fluoxetine HCl (Prozac)	SSRI, Antidepressant, Psychotropic Treat depression, obsessive-compulsive disorder, panic disorder, social phobia, posttraumatic stress disorder, etc
Gabapentin (Neurontin)	Antiseizure drug Spasmolytic agent in patients with MS
Glatiramer acetate (Copaxone)	Immunomodulatory effects Decrease the frequency of relapses in RRMS
Hydrocortisone (Cortef)	Glucocorticoid Treat acute exacerbations of MS and many more
Ibuprofen	Antiinflammatory, analgesic, antipyretic activity
Indapamide hemihydrate (Lozide)	Thiazide diuretic Increase sodium and chloride excretion Treat hypertention, CHF
Insulin	Anabolic actions
Interferon beta-1a (Rebif)	Antiviral activity Immunomodulatory activity Decrease frequency of clinical exacerbations in RRMS
Interferon beta-1b (Betaseron)	Antiviral properties Immunomodulatory properties Decrease frequency of exacerbations in RRMS
Levodopa/ Carbidopa (Apo levocarb)	Dopamine precursor Treat Parkinsonism
Levothyroxine (L-thyroxine) (Eltroxin)	Thyroid agent, hormone/hormone modifier Treat thyroid carcinoma, goiter, hypothyroidism, myxedema coma, hypothyroidism, pituitary TSH suppression
Lisinopril	Angiotensin converting enzyme (ACE) inhibitor Treatment of hypertention and CHF, decreases bp
Lorazepam	Benzodiazepine Used in treatment of status epilepticus, antianxiety
Methylphenidate (Ritalin)	Amphetamine Treat narcolepsy and ADHD
Mirtazapine (Remeron)	Antidepressant
Mitoxantrone (Novantrone)	Chemotherapy (inhibits DNA and RNA synthesis) Treatment of refractory acute leukemia, pediatric and adult acute myelogenous leukemia, non-Hodgkin's lymphomas, and breast cancer
Modafinil (Alertec)	CNS stimulants Narcolepsy
Nifedipine	Calcium channel antagonist Antiarrhythmic, antianginal effects Antihypertensive effects, decreases bp

Table 6 continued

Medication	Actions
Oxybutynin chloride (Ditropan)	Muscarinic receptor antagonist Antispasmodic properties Indicated for overactive bladder
Prednisone	Glucocorticoid Acute exacerbations of MS
Progesterone	Hormone therapy
Propranolol (Inderol)	Beta-adrenergic receptor antagonist Treat hypertension
Raloxifene HCl (Evista)	Selective estrogen receptor modulator Hormone replacement therapy Prevent and treat postmenopausal osteoporosis
Rofecoxib (Vioxx)	NSAID, COX-2 inhibitor Antiinflammatory, antipyretic, analgesic activity Treat dysmenorrhea, osteoarthritis
Simvastatin	Competitive inhibitor of HMG-CoA reductase Treat disorders involving elevated plasma levels of LDL
Tizanidine (Zaniflex)	Alpha-adrenoceptor agonist Treat spasticity in MS or after stroke
Tolterodine L-tartrate (Detrol)	Muscarinic receptor antagonist Treat urinary incontinance
Triphasil	Oral contraceptive
Zopiclone (Imovane)	Sleeping pill (hypnotic) Treat insomnia

acetylcholine muscarinic receptor antagonists, and analgesics. Others that could possibly affect neuronal activity observed on stimulation include benzodiazepines, dopamine precursors, selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, amphetamines, hypnotics, analeptics, β-blockers, and α-adrenergic agonists. These medications all have effects on neurotransmitters within the central and peripheral nervous systems but their actions on spinal cord activity, if present, are likely indirect.

Despite the fact that these medications affect neurotransmitters in the spinal cord, they did not appear to affect the activation maps produced from the thermal stimulation. This is evident in the fact that even within the same patient, the pattern of activity observed followed the deficits experienced by the subjects more than any other factors. In patients, the pattern of activity at one level of the spinal cord or from stimulation of one side of the body may appear to have activation maps similar to healthy controls. Meanwhile, at another level of the spinal cord, or from stimulation of the other side of the body, the pattern of activity may appear more similar to spinal cord injured subjects from previous studies.

Future Work

For future studies, it would be useful to image more subjects in regions of the spinal cord without lesions to determine if our observations were accurate or an aberration. As well, if the same subject was imaged both in regions surrounding or within a lesion and in regions with no lesions, it may shed more light on these findings. The subjects should also be asked specifically about their perceptions of the stimulus. That is, did it feel the same on both sides and at all sites stimulated, and did it feel like

heat or not. Some patients reported that they felt the stimulation but that it did not feel like heat.

In order to help develop Spinal fMRI as a clinical tool, a study in which a cohort of subjects is followed with Spinal fMRI scans at set intervals could prove useful. The results obtained could then be compared to their clinical progression to determine if Spinal fMRI is clinically useful in tracking treatment or progression of MS.

Conclusions

The difference observed in spinal fMRI signal changes between healthy volunteers, and those with chronic MS, supports the theory that the SEEP signal changes depend on the permeability of the BBB, and that they arise from a change in extravascular water content. The findings of the present study also suggest that the SEEP contrast mechanism may provide a means of assessing patients with multiple sclerosis to supplement conventional MRI and other clinical assessment methods. The fact that there are differences seen between the PPMS subgroup and the RR and SPMS subgroups further supports the conclusion that the differences seen in the percent signal changes with thermal stimulation reflect the differences in pathology within MS patients.

Overall, using spinal fMRI, subjects with MS had a pattern of activity that followed the severity and distribution of their deficits if the images were taken in the presence of a lesion. The data show that even with an altered physiology within and outside the areas of macroscopic demyelinated multiple sclerotic plaques, neuronal activity can be seen in recognizable patterns.

As MS is a currently incurable disease that affects the lives of many people, any new insight into the disease has the potential to be extremely useful, both clinically and for research.

A long term goal of this and previous studies is to develop spinal fMRI as a clinical tool to help clinicians to better understand the injury sustained by their individual patient. Previously, spinal fMRI has been shown to demonstrate a pattern of activity in subjects with spinal cord injury that correlates well to their site and quality of deficit. In the present study, the patterns of activity observed in subjects with MS correlated to their deficits. Therefore, this study shows the great potential of spinal fMRI as a useful clinical tool in the research regarding MS and the management of the patients with the disease.

Appendix A

Maps of Active Regions for All Subjects

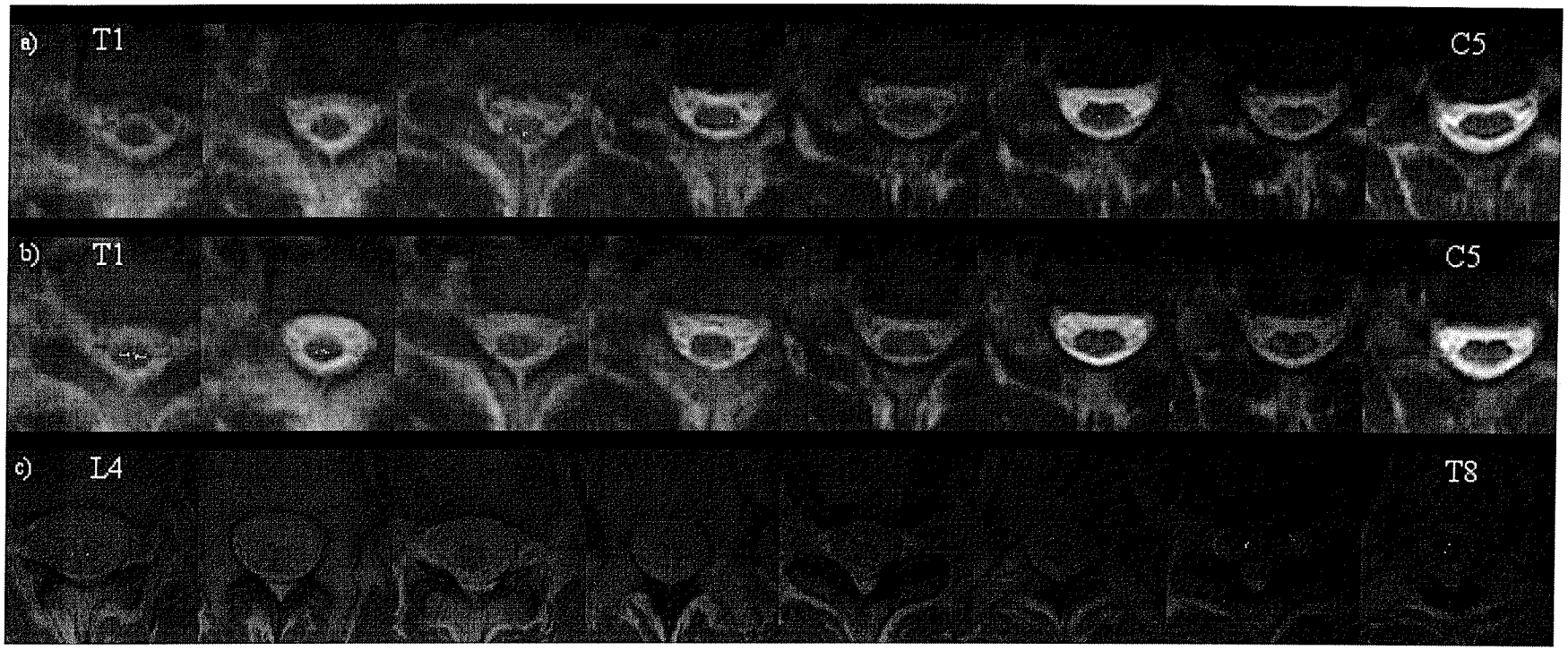

Figure 1A. The pattern of activity for a 46 year old female with RRMS. The subject began experiencing symptoms in May 1982 and was diagnosed with MS in September 1982. Her symptoms at the time of the study were fatigue, decreased sensation in fingertips and soles of feet especially when tired, sometime double vision, trouble concentrating, numbness in hands and left leg, some motor ability mainly in the hands and left leg. Her medications were Interferon beta-1b (Betaseron) and Rofecoxib (Vioxx). For (a) and (b), thermal stimulation was applied to the C6 – C8 dermatomes on the palm of the hand, on the left and right respectively. The slices were taken from C4/5 – T1 vertebral levels, from right to left. For (c), thermal stimulation was applied to the L4 dermatome on the left calf. The slices were taken from T8/9 – T12 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

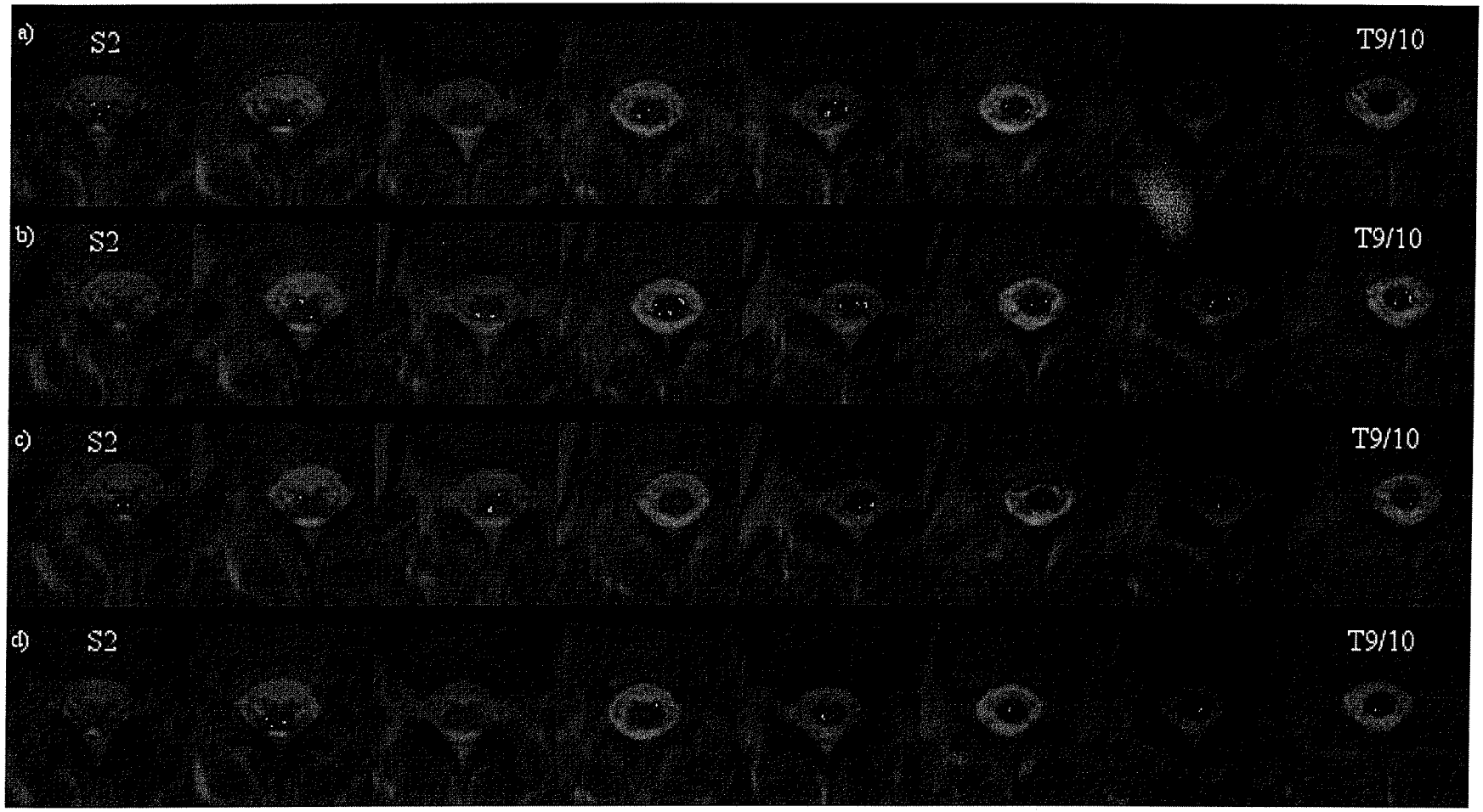

Figure 2A (continued on next page)

Figure 2A. The pattern of activity for a 53 year old female with RRMS. The subject began experiencing symptoms in 1992 and was diagnosed with MS in 1994. Her symptoms at the time of the study were left drop foot if tired, tingling on the bottom of the feet which sometimes goes up to the knee, balance problems, and fatigue. Her medications were Indapamide (Lozide), Lisinopril, Rofecoxib (Vioxx), and Amantadine. For (a) and (b), thermal stimulation was applied to the L5 dermatome on the sole of the foot, on the left and right respectively. The slices were taken from T10 – L1/2 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the L4 dermatome on the calf, on the left and right respectively. The slices were taken from T10 – L1/2 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

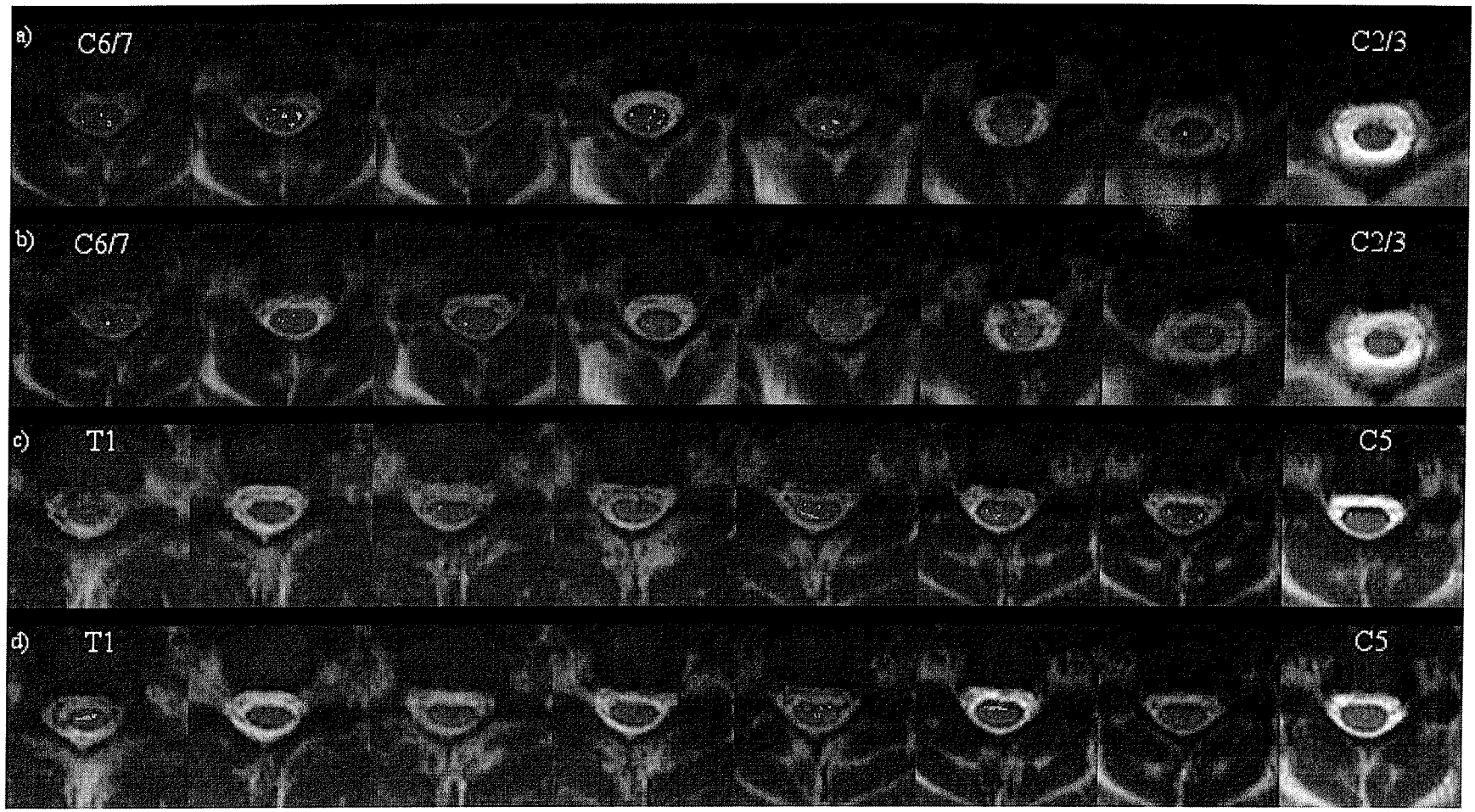

Figure 3A (continued on next page)

Figure 3A. The pattern of activity for a 21 year old male with RRMS. The subject began experiencing symptoms in 1991 and was diagnosed with MS in 1994. His symptoms at the time of the study were tremore I the left arm, balance problems, and fatigue. His medications were Interferon beta-1b (Betaseron) and Fluoxetine (Prozac). For (a) and (b), thermal stimulation was applied to the C4/5 dermatome on the upper arm near the shoulder, on the right and left respectively. The slices were taken from C2 – C5/6 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the C6 – C8 dermatome on the palm of the hand, on the right and left respectively. The slices were taken from C4/5 – T1 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

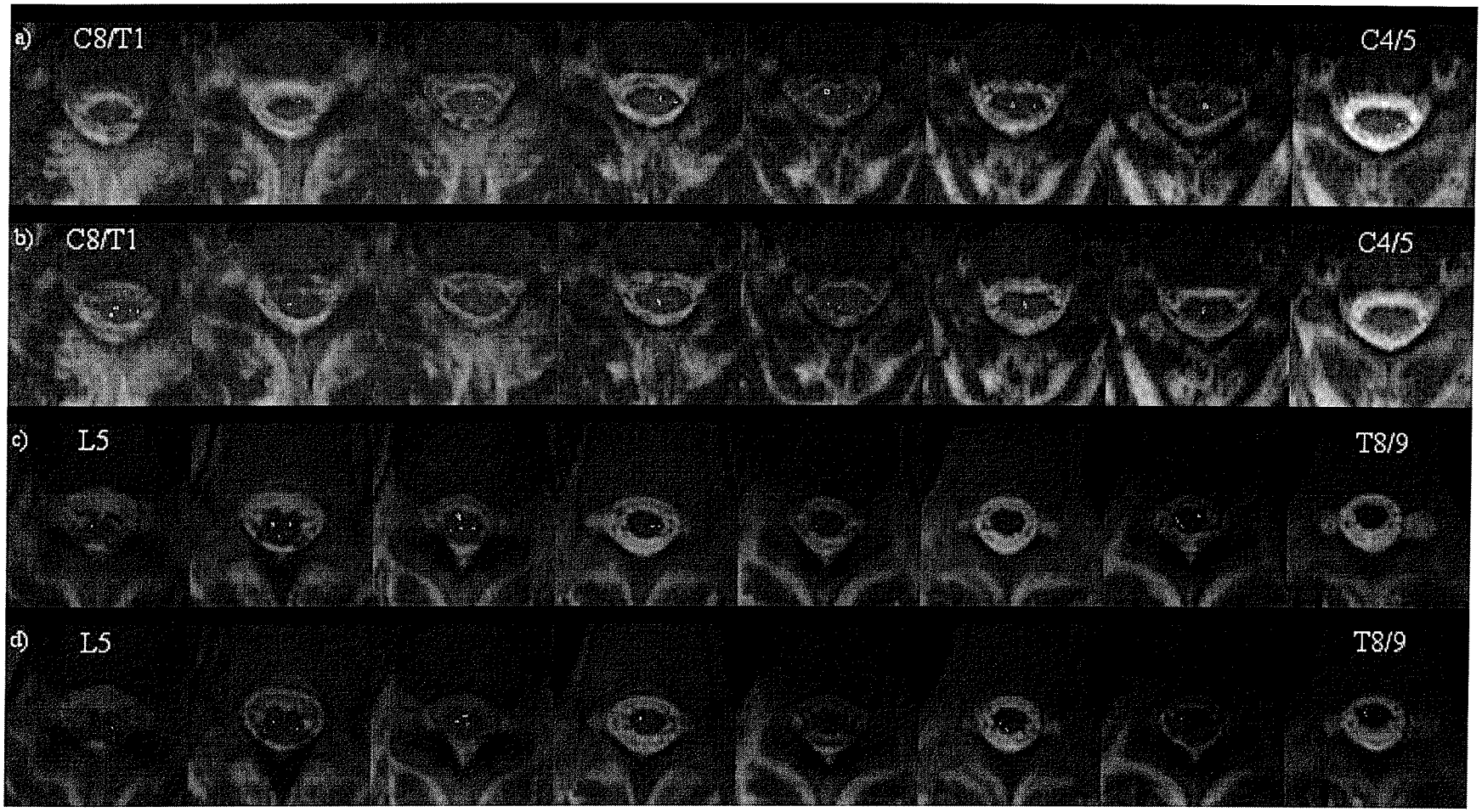

Figure 4A (continued on next page)

Figure 4A. The pattern of activity for a 48 year old female with RRMS. The subject began experiencing symptoms and was diagnosed with MS in February of 1992. She was not experiencing any MS related symptoms at the time of the study. Her medications were Fludrocortisone (Florinef) and Hydrocortisone (Cortef). For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C4 – C7/T1 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the T10 dermatome on the side of the body level with the bellybutton, on the left and right respectively. The slices were taken from T9 – T12/L1 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

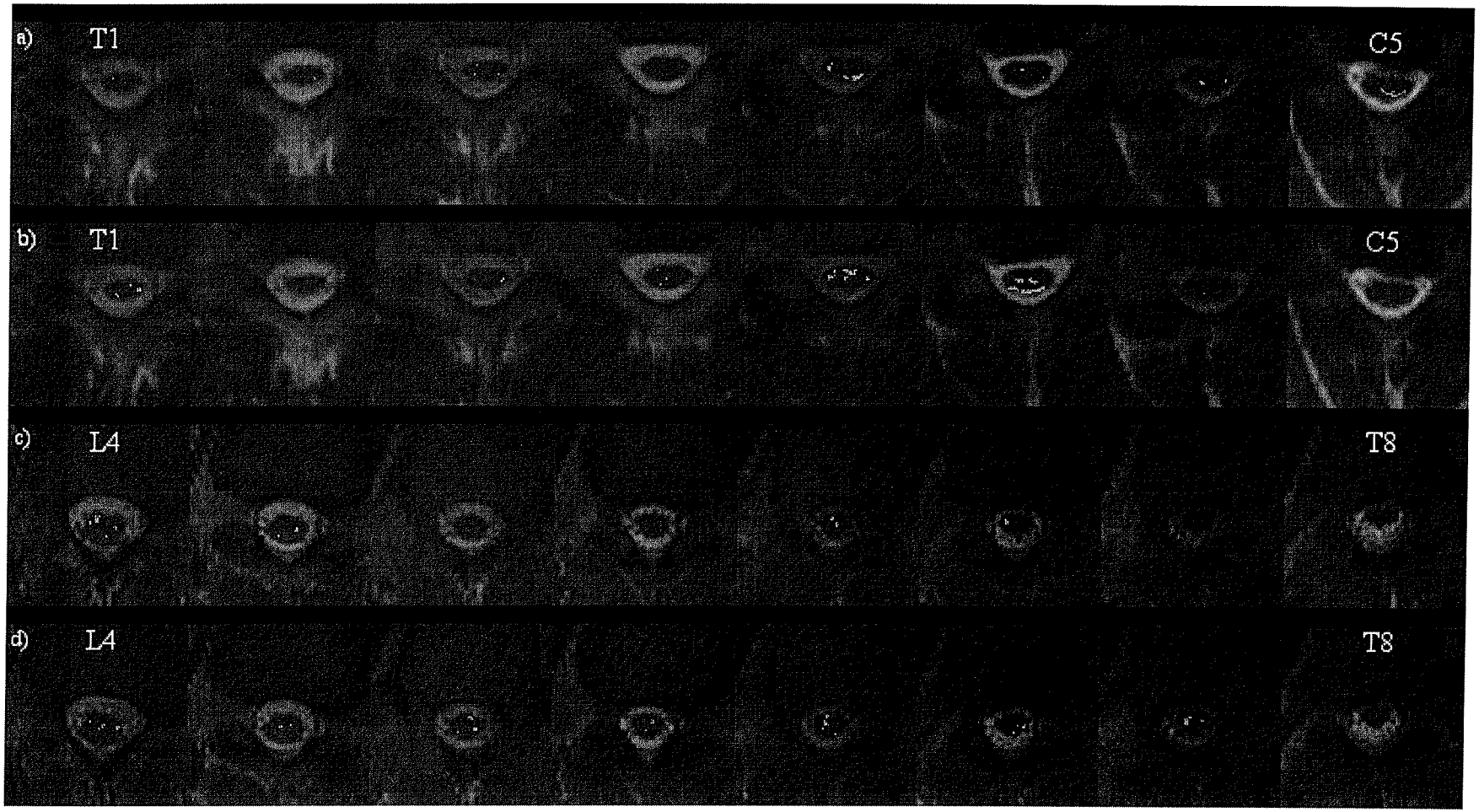

Figure 5A (continued on next page)

Figure 5A. The pattern of activity for a 48 year old male with RRMS. The subject began experiencing symptoms and was diagnosed with MS in 1995. His symptom at the time of the study was a loss of flexibility in the right hip area. His medications were Mirtazapine (Remeron) and Alpazalum. For (a) and (b), thermal stimulation was applied to the C6 - 8 dermatomes on the palm of the hand, on the left and right respectively. The slices were taken from C4/5 – T1 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the L4 dermatome on the calf, on the right and left respectively. The slices were taken from T8/9 – T12 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

Figure 6A (continued on next page)

Figure 6A. The pattern of activity for a 45 year old female with RRMS. The subject began experiencing symptoms in 1985 and was diagnosed with MS in December 2000. She had no symptoms and was taking no medications at the time of the study. For (a) and (b), thermal stimulation was applied to the C6 – 8 dermatomes on the palm of the hand, on the left and right respectively. The slices were taken from C5 – T1/2 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C3 – C6/7 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

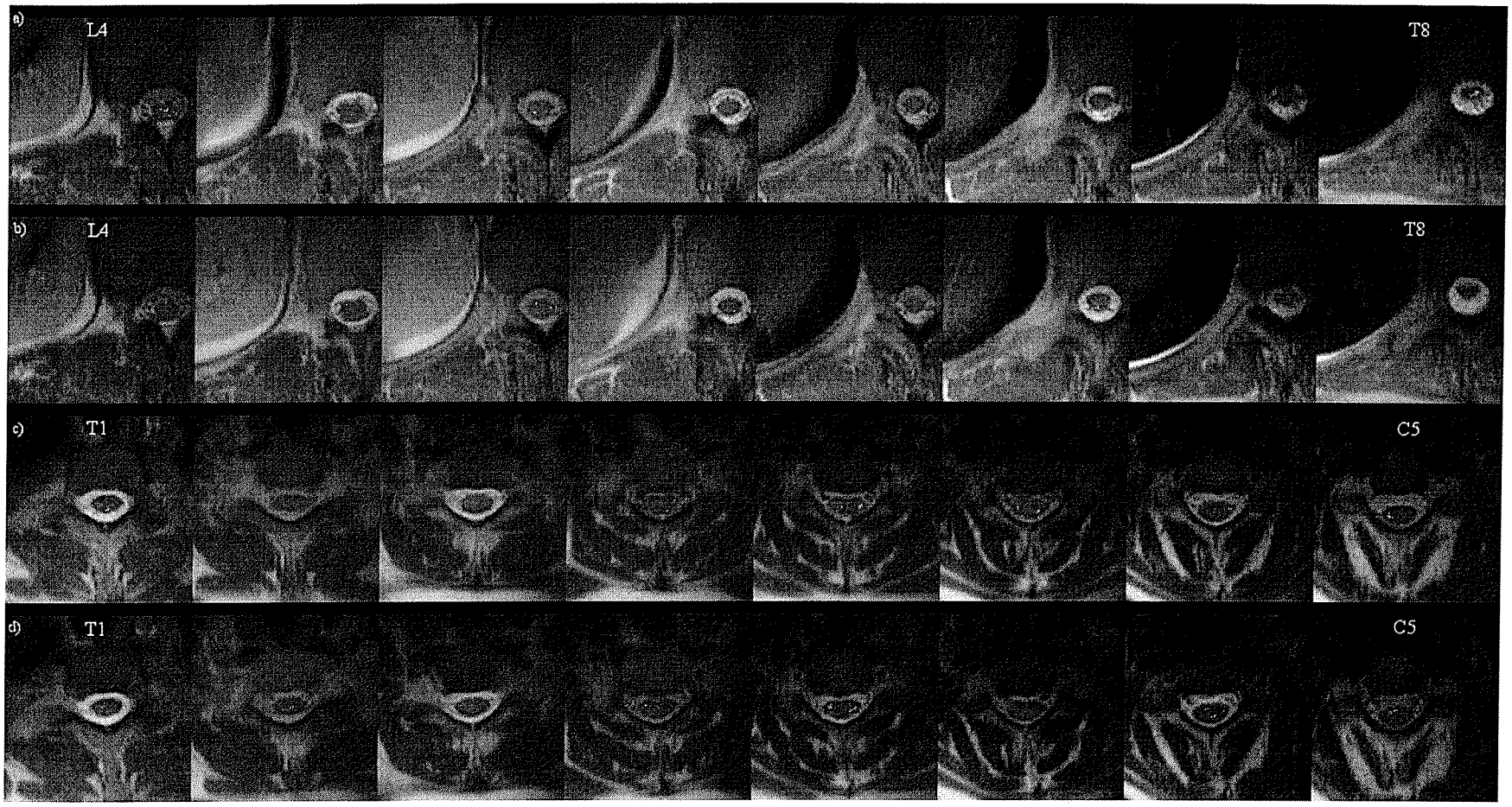

Figure 7A (continued on next page)

Figure 7A. The pattern of activity for a 41 year old female with SPMS. The subject began experiencing symptoms and was diagnosed with MS in 1992. Her symptoms at the time of the study were motor and sensory deficits in the legs. Her medications were Baclofen (Lioresal), and Insulin. For (a) and (b), thermal stimulation was applied to the L4 dermatome on the calf, on the right and left respectively. The slices were taken from T8/9 – T12 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the C6 – C8 dermatomes on the palm of the hand, on the left and right respectively. The slices were taken from C4/5 – T1 vertebral levels. The levels indicated on the images are the approximate levels of the spinal cord imaged.

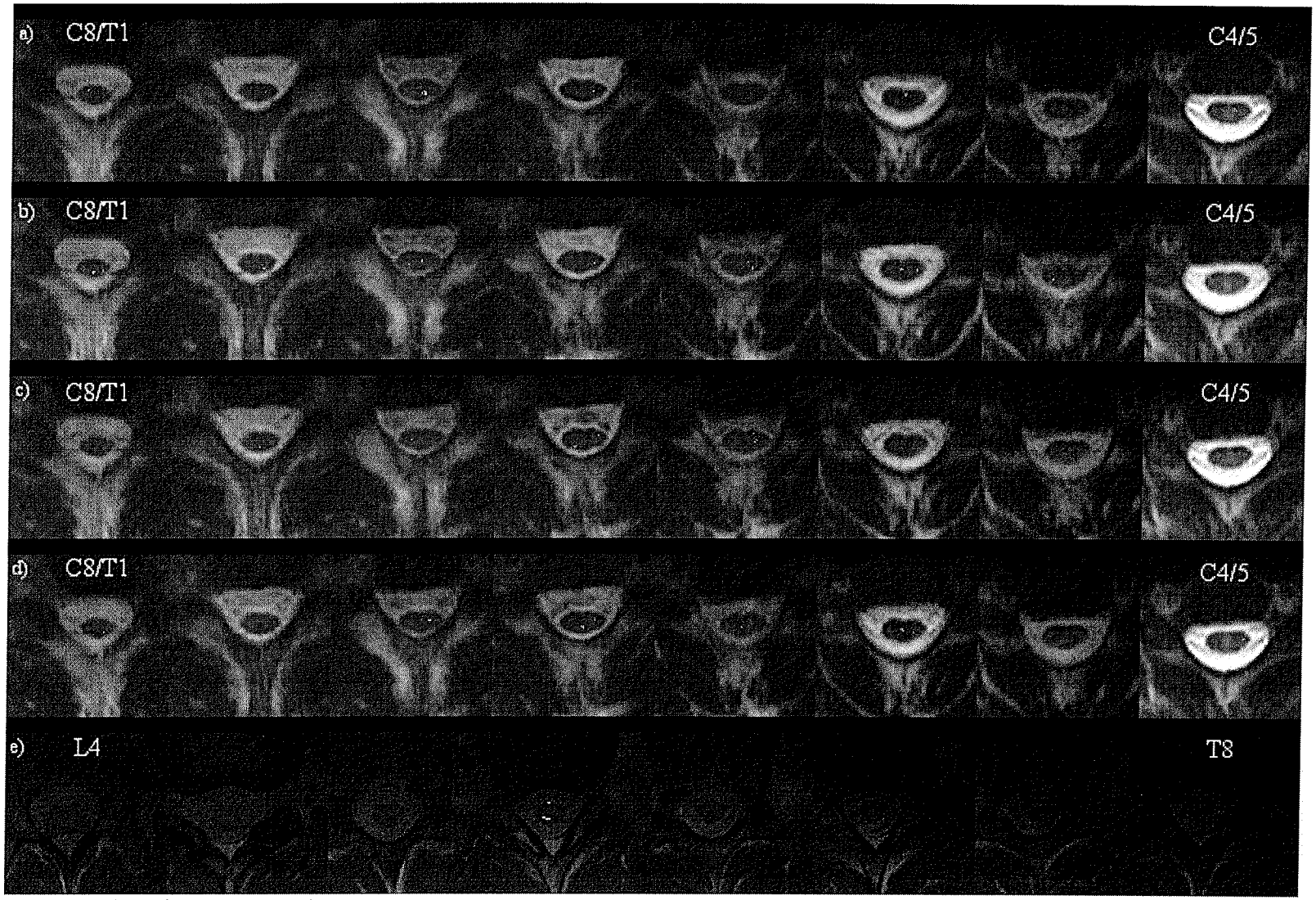

Figure 8A (continued on next page)

Figure 8A. The pattern of activity for a 40 year old male with SPMS. The subject began experiencing symptoms in September 1999 and was diagnosed with MS in October 1999. His symptoms at the time of the study were unsteadiness, tingling mainly in the hands with a little in legs and feet, and very stiff from head to hip on the right side. His medication was Interferon beta-1a (Rebif). For (a) and (b), thermal stimulation was applied to the C6 – C8 dermatomes on the palm of the hand, on the left and right respectively. The slices were taken from C4 – C7/T1 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C4 – C7/T1 vertebrae, right to left. For (e), thermal stimulation was applied to the T10 dermatome on the right side of the body level with the bellybutton. The slices were taken from T8/9 – T12 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

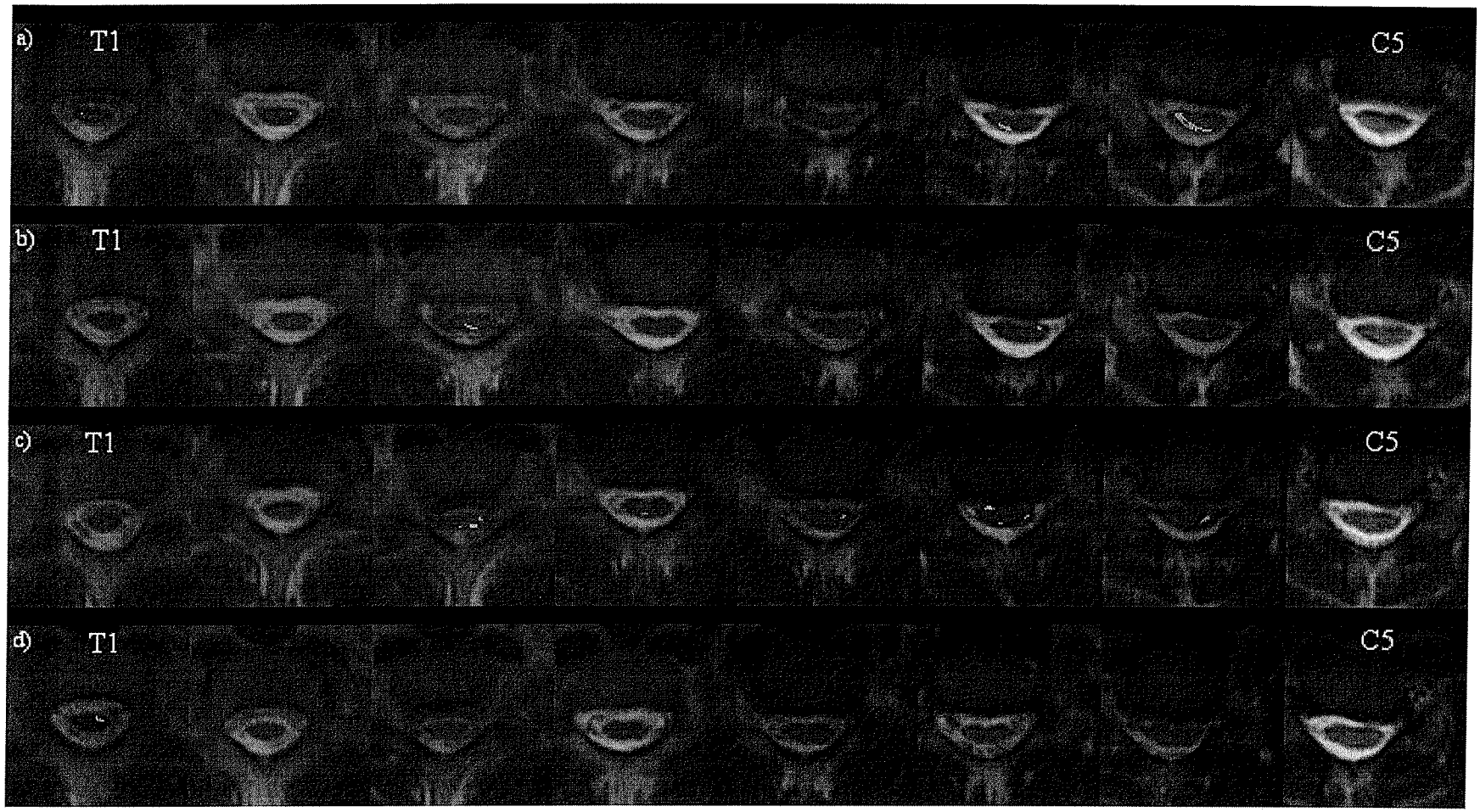

Figure 9A (continued on next page)

Figure 9A. The pattern of activity for a 40 year old female with SPMS. The subject began experiencing symptoms in 1983 and was diagnosed with MS in 1985. Her symptoms at the time of the study were weakness in the legs, numbness in the fingers, ringing in the ears, leg spasms, and back pain when she's tired. Her medications were Prednisone, Oxybutynin (Ditropan), ASA, Citalopram (Celexa), and Baclofen (Lioresal). For (a) and (b), thermal stimulation was applied to the C6 – C8 dermatomes on the fingers, on the left and right respectively. The slices were taken from C4/5 – T1 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the C5 dermatome on the upper arm, on the right and left respectively. The slices were taken from C4/5 – T1 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

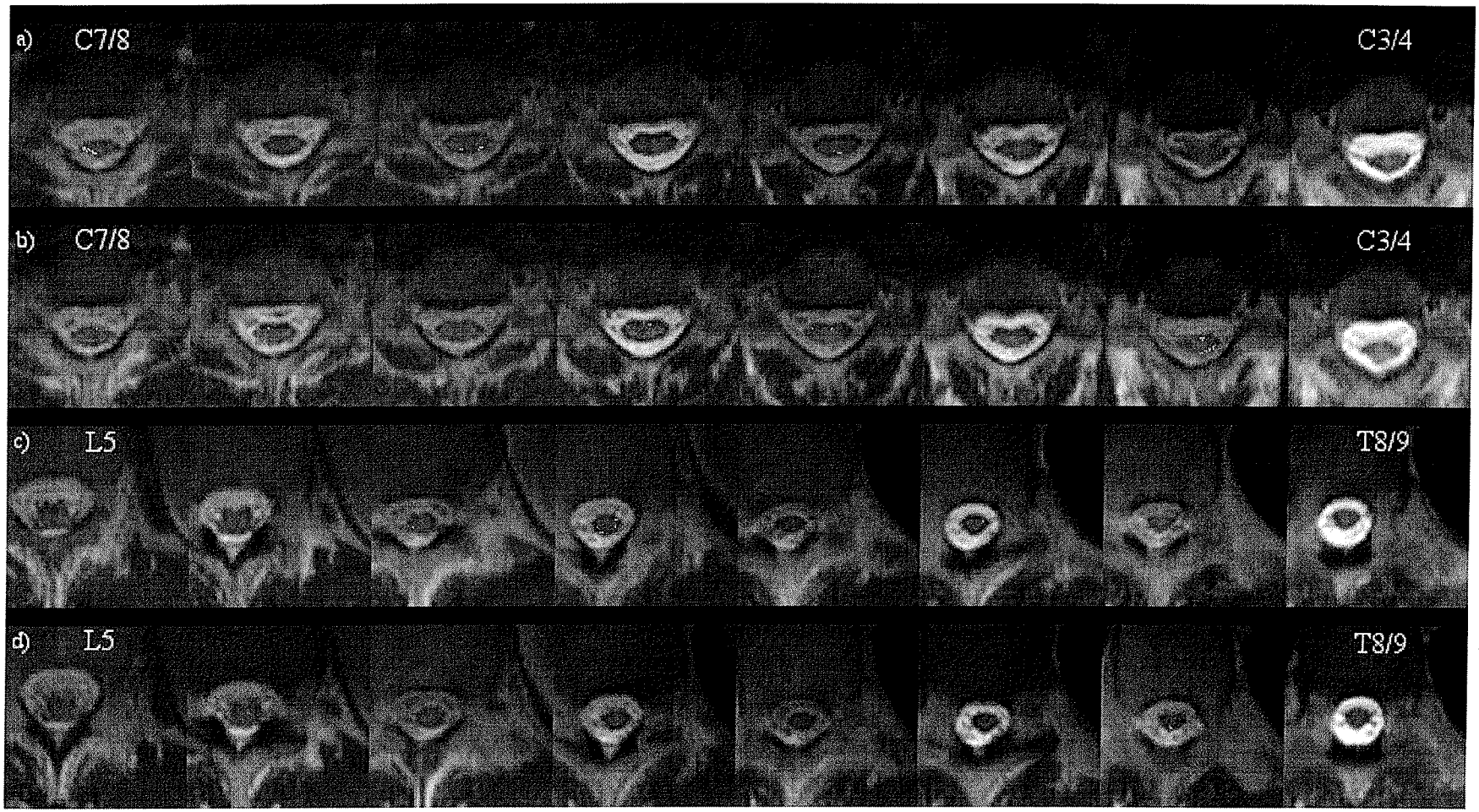

Figure 10A (continued on next page)

Figure 10A. The pattern of activity for a 43 year old female with SPMS. The subject began experiencing symptoms in 1978 and was diagnosed with MS in 1979. Her symptoms at the time of the study were motor problems in her legs, problems with ambient heat or cold, tremors in hands, spasms in legs, and at times, sensory disturbances in legs. She was on no medications. For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C3 – C6/7 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the L3 dermatome on the upper leg, on the right and left respectively. The slices were taken from T9 – T12/L1 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

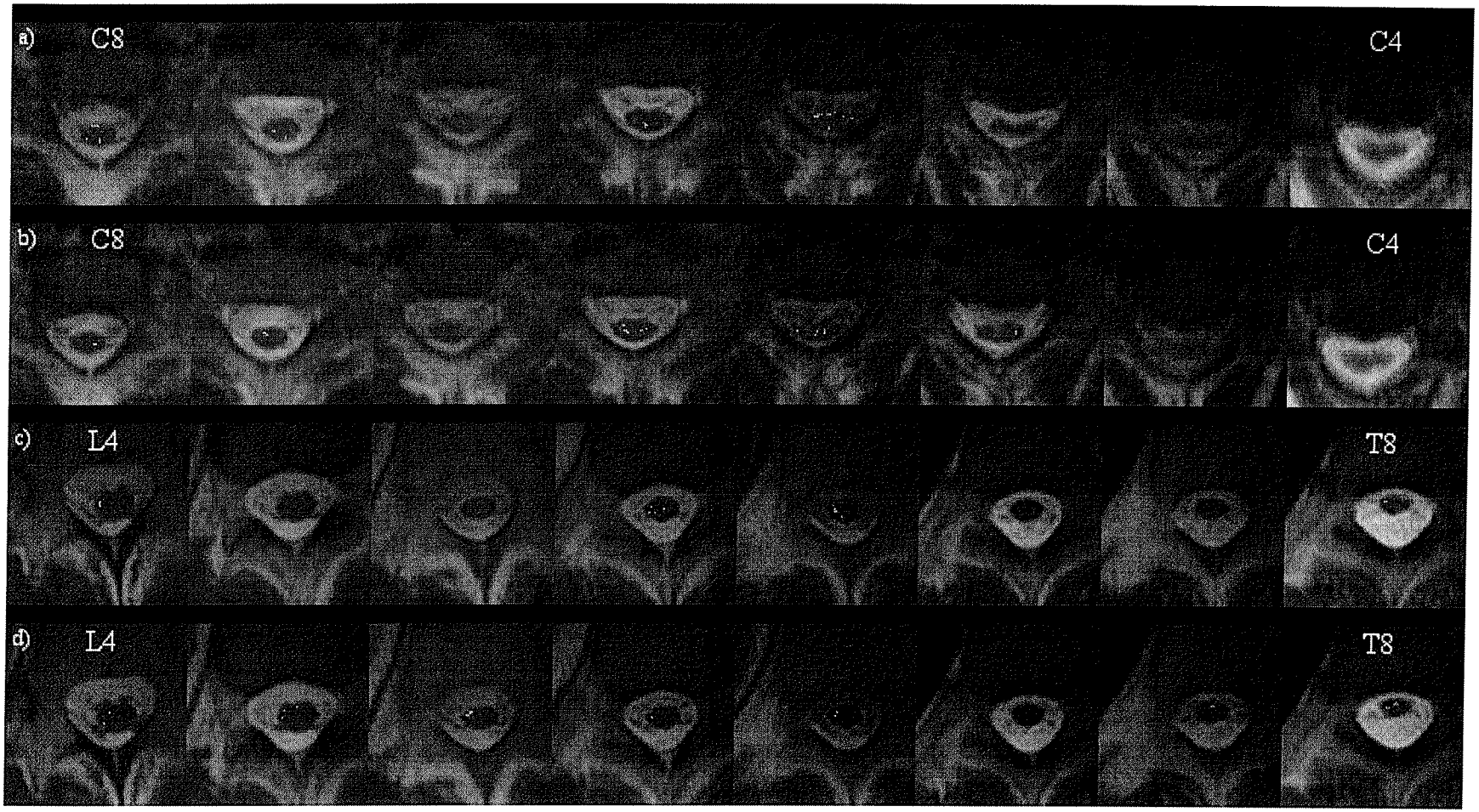

Figure 11A (continued on next page)

Figure 11A. The pattern of activity for a 52 year old female with SPMS. The subject began experiencing symptoms in 1983 and was diagnosed with MS in 1987. Her symptoms at the time of the study were no motor control below her neck. Her medications were Baclofen (Lioresal), Tizanidine (Zanaflex), Citalopram (Celexa), Tolterodine (Detrol), and Lorazipam. For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C3/4 – C7 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the L3 dermatome on the upper leg, just above the knee, on the right and left respectively. The slices were taken from T8/9 – T12 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

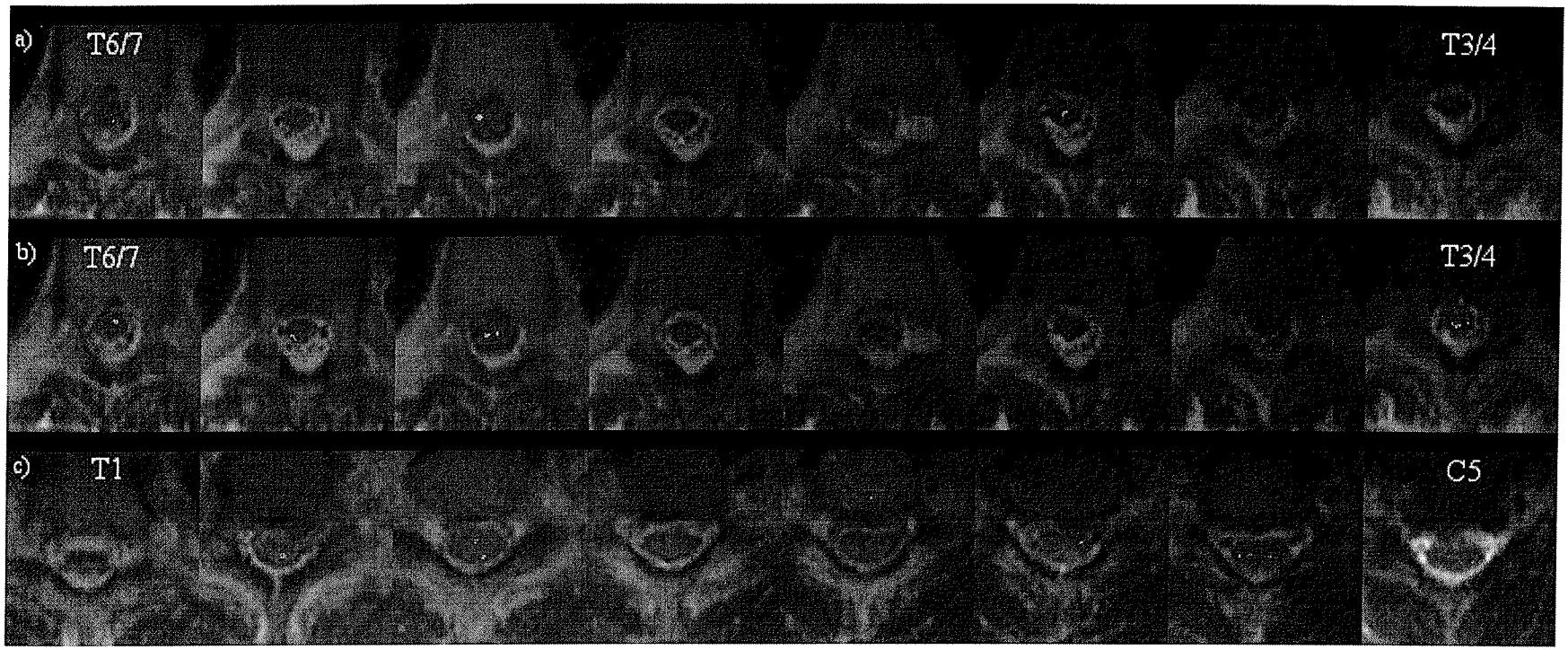

Figure 12A. The pattern of activity for a 64 year old female with SPMS. The subject began experiencing symptoms in the mid 1980s and was diagnosed with MS in 1999. Her symptoms at the time of the study consisted of motor problems in the left leg and a loss of balance. Her medications were Betahistine (Serc), Amantadine, L Thyroxine, Tolterodine (Detrol), Fluoxetine (Prozac), Premrin, and Amlodipine (Norvasc). For (a) and (b), thermal stimulation was applied to the T5 dermatome on the side of the chest under the armpit, on the right and left respectively. The slices were taken from T3/4 – T7 vertebral levels, from right to left. For (c), thermal stimulation was applied to the C6 – 8 dermatomes on the palm of the left hand. The slices were taken from C4/5 – T1 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

Figure 13A. The pattern of activity for a 52 year old female with SPMS. The subject began experiencing symptoms in 1982 and was diagnosed with MS in 1983. Her symptoms at the time of the study consisted of motor problems in the legs, and tingling and motor problems in the hands. Her medications were Amantadine, Propranolol (Inderal), and Baclofen (Lioresal). For both (a) and (b), thermal stimulation was applied to the C6-8 dermatomes on the palm of the hand, on the left and right respectively. The slices were taken from C5 – T1/2 vertebral levels, from right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

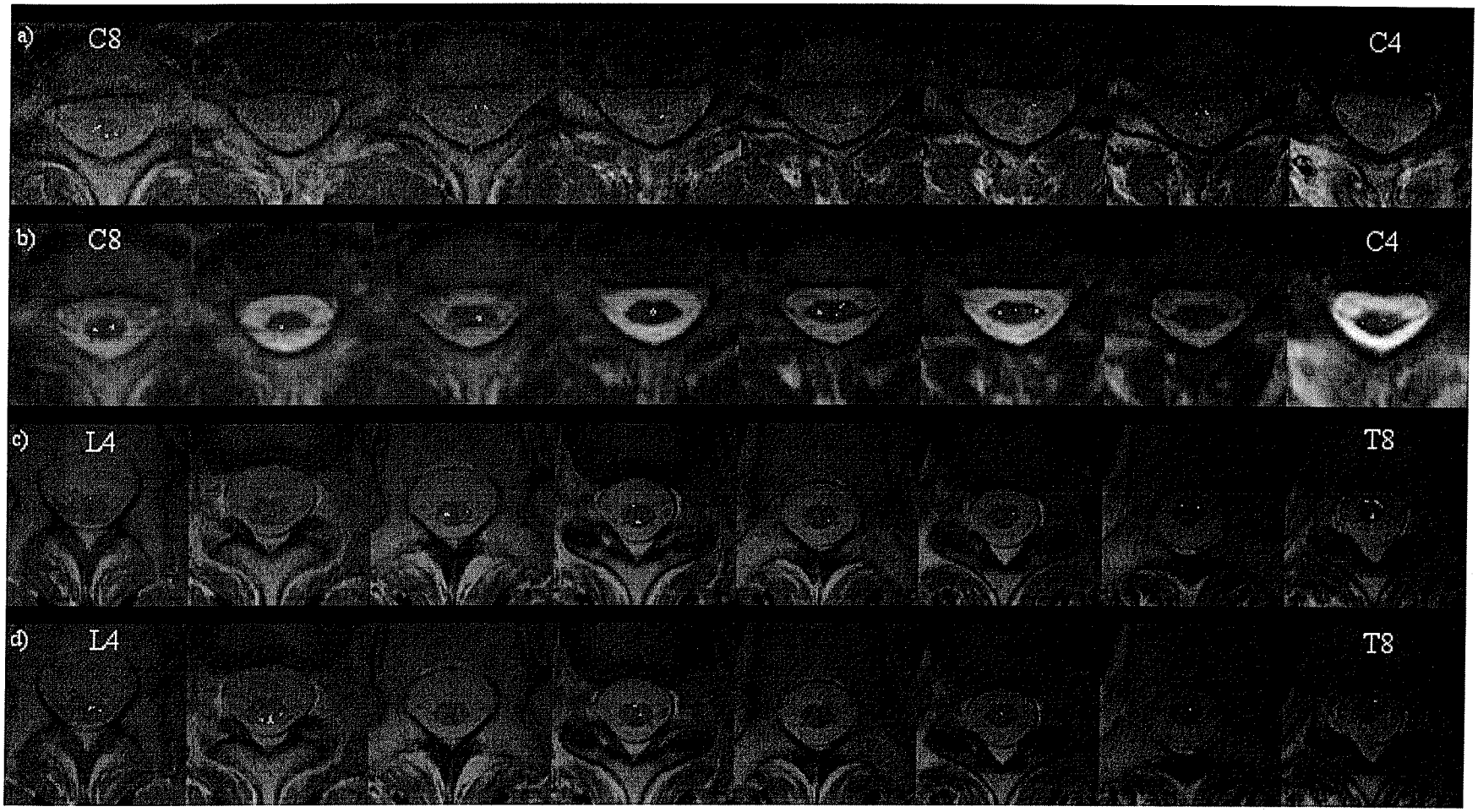

Figure 14A (continued on next page)

Figure 14A. The pattern of activity for a 57 year old female with SPMS. The subject began experiencing symptoms and was diagnosed with MS in 1973. Her symptoms at the time of the study consisted of a left dropped hip, eye problems, sensory deficits in hands, voice problems, and motor problems in her arms. Her medications were Diazepam, Progesterone, Estrogel, Tolterodine (Detrol), Ibuprofen, and Amiltryptoline (Zorco). For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C3/4 – C7 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the L4 dermatome on the calf, on the right and left respectively. The slices were taken from T8/9 – T12 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

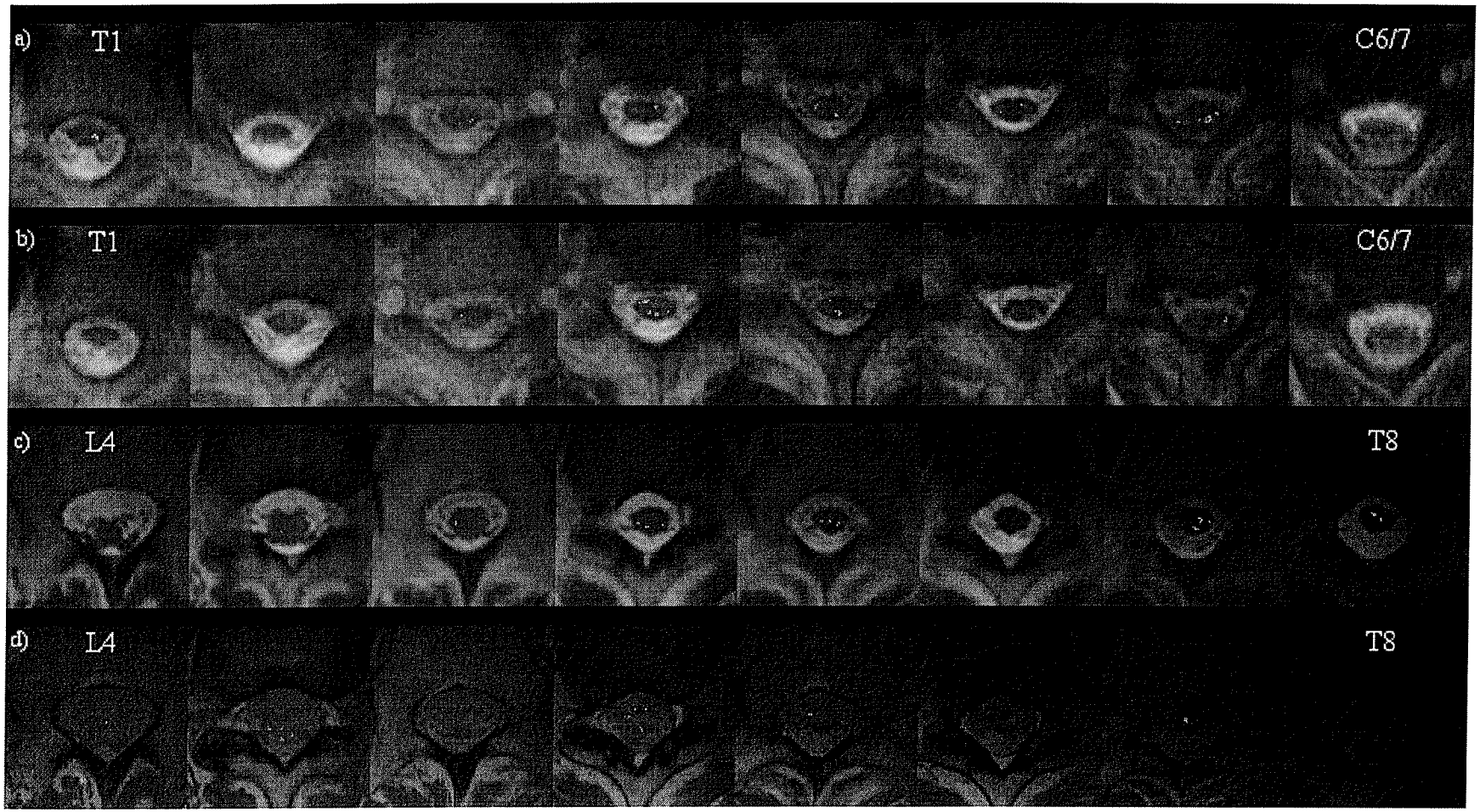

Figure 15A (continued on next page)

Figure 15A. The pattern of activity for a 56 year old male with SPMS. The subject began experiencing symptoms in 1970 and was diagnosed with MS in 1971. His symptoms at the time of the study consisted of motor problems in both legs, fatigue, and sensory loss in the left foot. His medications were Felodipine, Amantadine, Lisinopril, Levodopa (Apo Levocarb), Zopiclone (Apo-Zopiclone), and Citalopram (Celexa). For (a) and (b), thermal stimulation was applied to the C6-8 dermatome on the palm of the hand, on the left and right respectively. The slices were taken from C5/6 – T1 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the L4 dermatome on the calf, on the right and left respectively. The slices were taken from T8/9 – T12 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

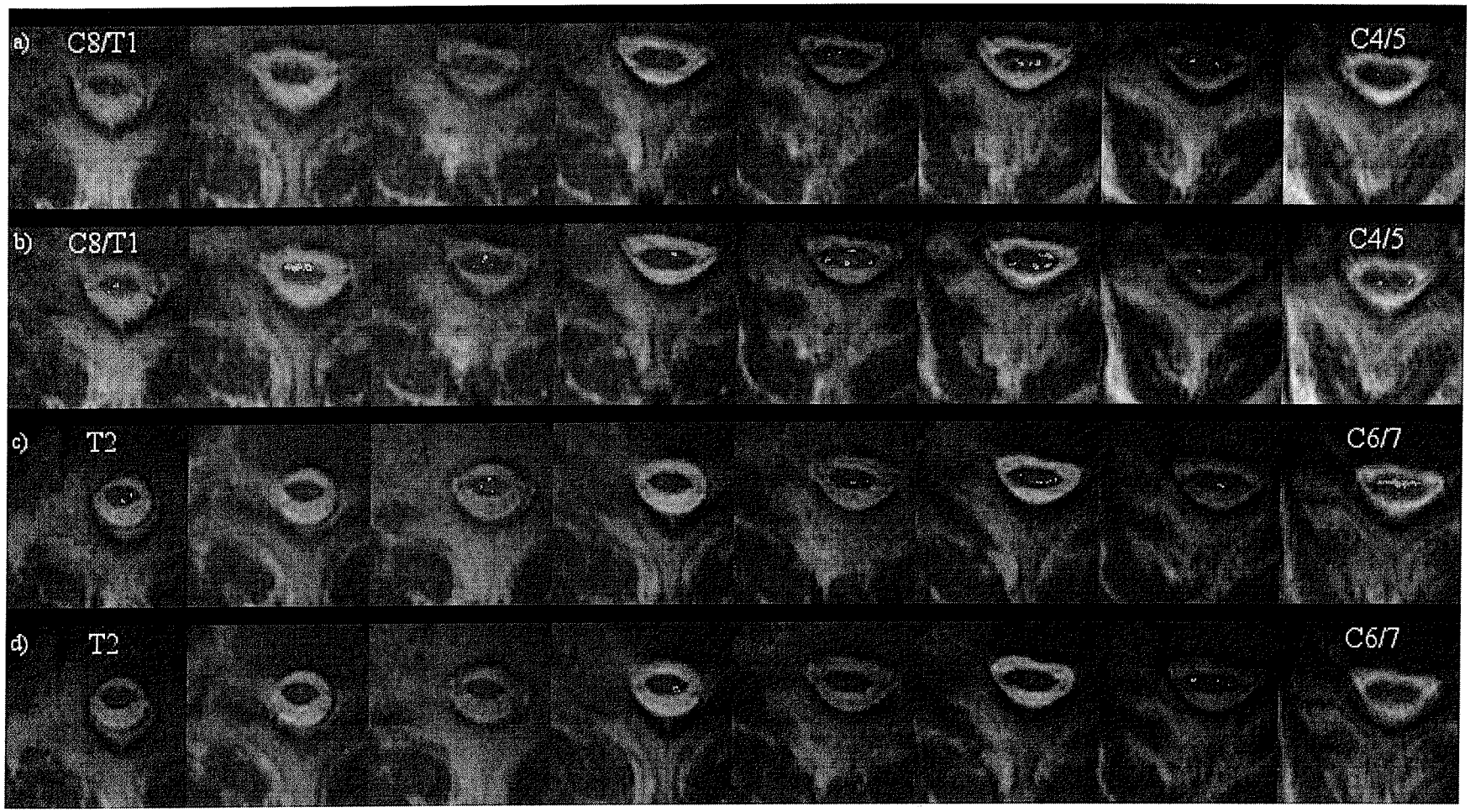

Figure 16A (continued on next page)

Figure 16A. The pattern of activity for a 57 year old male with SPMS. The subject began experiencing symptoms in 1975 and was diagnosed with MS in 1983. His symptom at the time of the study was left leg weakness. His medication was Bufferin. For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C4 – C7/T1 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the C6 – C8 dermatome on the palm of the hand, on the right and left respectively. The slices were taken from C5/6 – T2 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

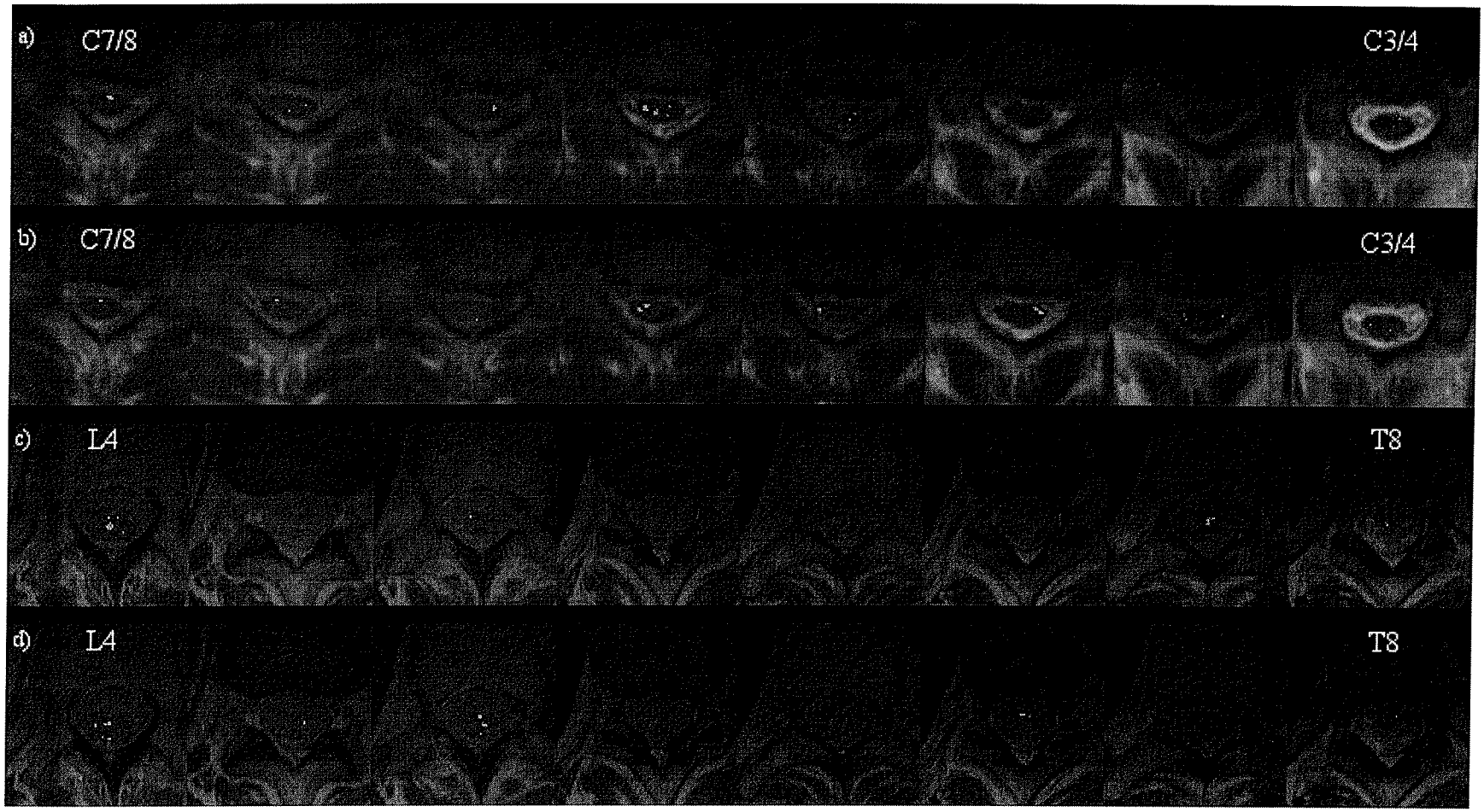

Figure 17A (continued on next page)

Figure 17A. The pattern of activity for a 49 year old female with SPMS. The subject began experiencing symptoms in 1980 and was diagnosed with MS in 1988. Her symptoms at the time of the study consisted of weakness in both legs that is worse in the right leg than in the left, fluid retention, and numbness in the right ring and little finger. Her medications were Baclofen (Lioresal), Methylphenidate (Ritalin), and Triphasil. For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the right and left respectively. The slices were taken from C3 – C6/7 vertebral levels, right to left. For (c) and (d), thermal stimulation was applied to the L3 dermatome on the leg, just above the knee, on the left and right respectively. The slices were taken from T8/9 – T12 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

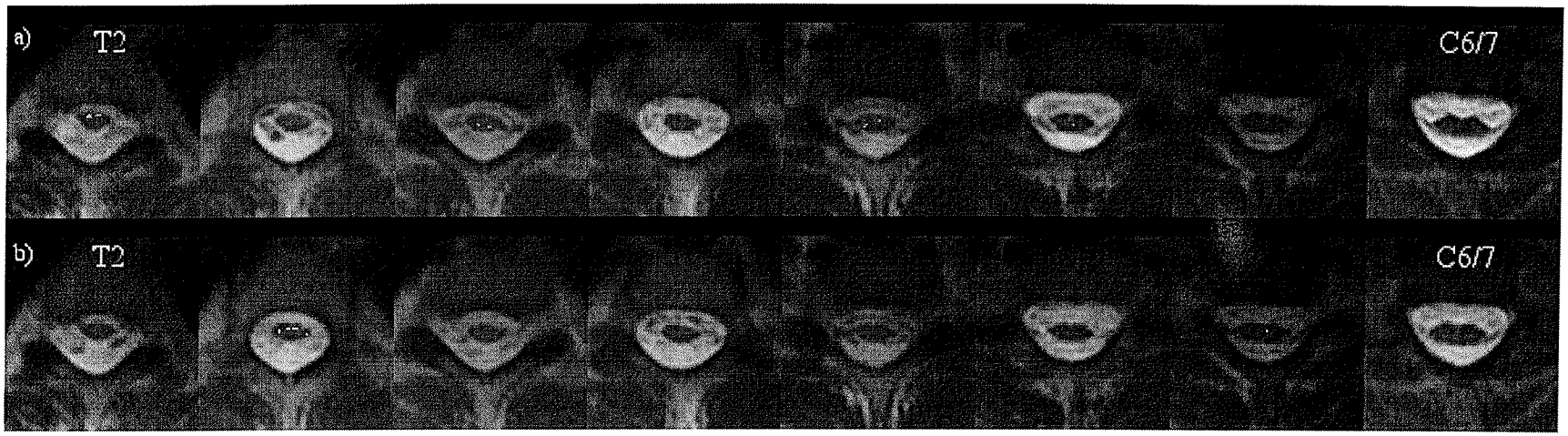

Figure 18A. The pattern of activity for a 53 year old female with SPMS. The subject began experiencing symptoms in the late 1960s and was diagnosed with MS in 1978. Her symptoms at the time of the study consisted of fatigue, left trigeminal nerve pain, poor balance, some motor problems in legs, bladder problems, and leg spasms. Her medications were Amiloride (Midamor), Citalopram (Celexa), Baclofen (Lioresal), Nifedipine (Apo-Nifid), Clonazepam, Estradiol, Progesterone, and Mitoxantrone HCl (Novantrone). For both (a) and (b), thermal stimulation was applied to the C6 - 8 dermatomes on the palm of the hand, on the left and right respectively. The slices were taken from C5/6 – T2 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

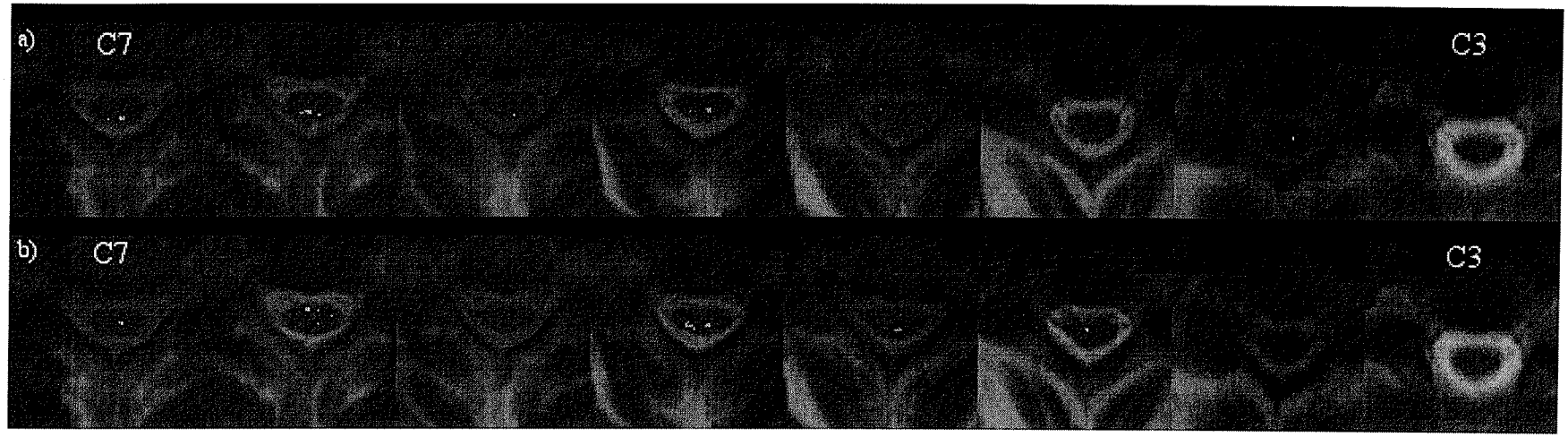

Figure 19A. The pattern of activity for a 54 year old male with PPMS. The subject began experiencing symptoms in 1980 and was diagnosed with MS in 1994. His symptoms at the time of the study were no motor control in the right leg and no fine motor skills in the right hand. His medications were Hydrochlorothiazide (Avalide) and Modafinil (Altertec). For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the right and left respectively. The slices were taken from C2/3 – C6 vertebral levels, from right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

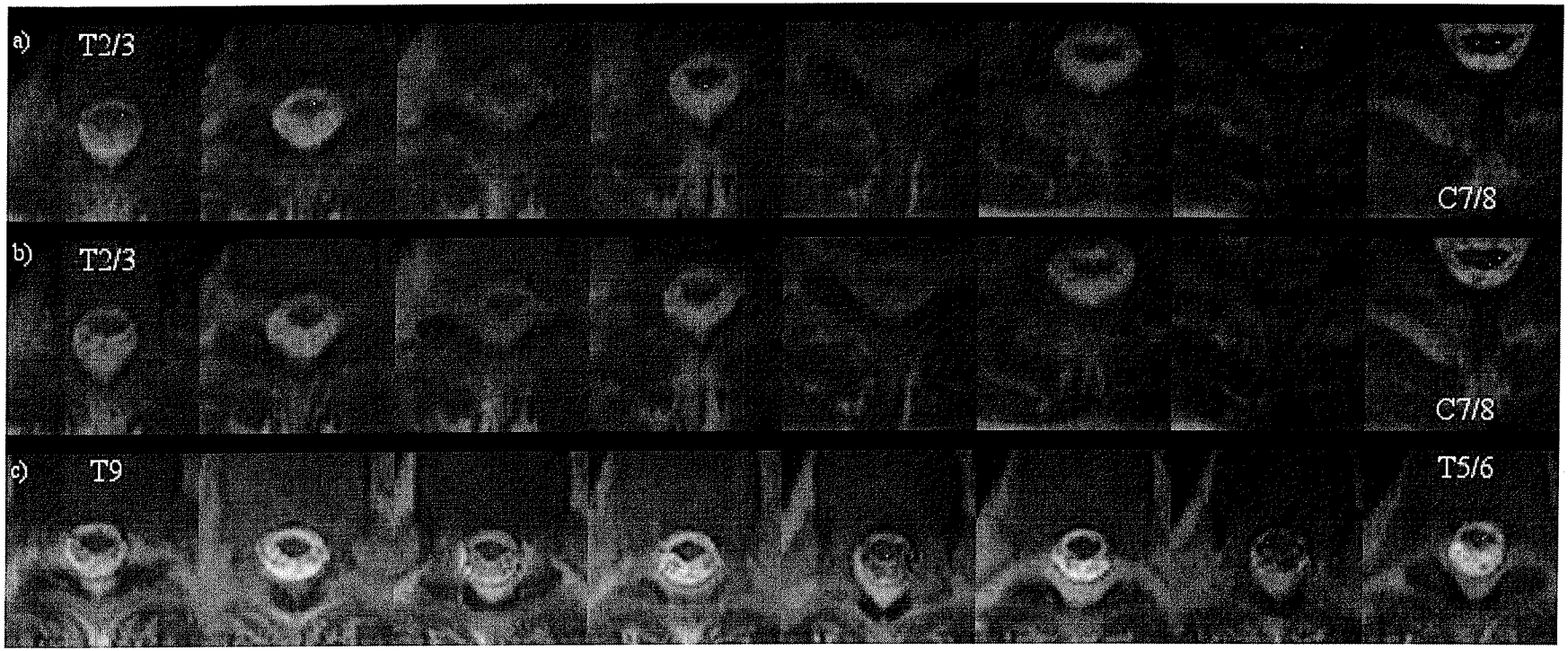

Figure 20A. The pattern of activity for a 70 year old male with PPMS. The subject began experiencing symptoms in 1988 and was diagnosed with MS in 1997. His symptoms at the time of the study were numbness in the left arm and no sensory or motor ability in the left leg. His medications were Carbamazepene (Tegretol) and Tizanidine (Zanaflex). For (a) and (b), thermal stimulation was applied to the C6 – C8 dermatome on the palm of the hand, on the left and right respectively. The slices were taken from C6/7 – T2/3 vertebral levels, from right to left. For (c), thermal stimulation was applied to the T8 dermatome on the side of the chest level with the bottom of the ribs on the left. The slices were taken from T6 – T9/10 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

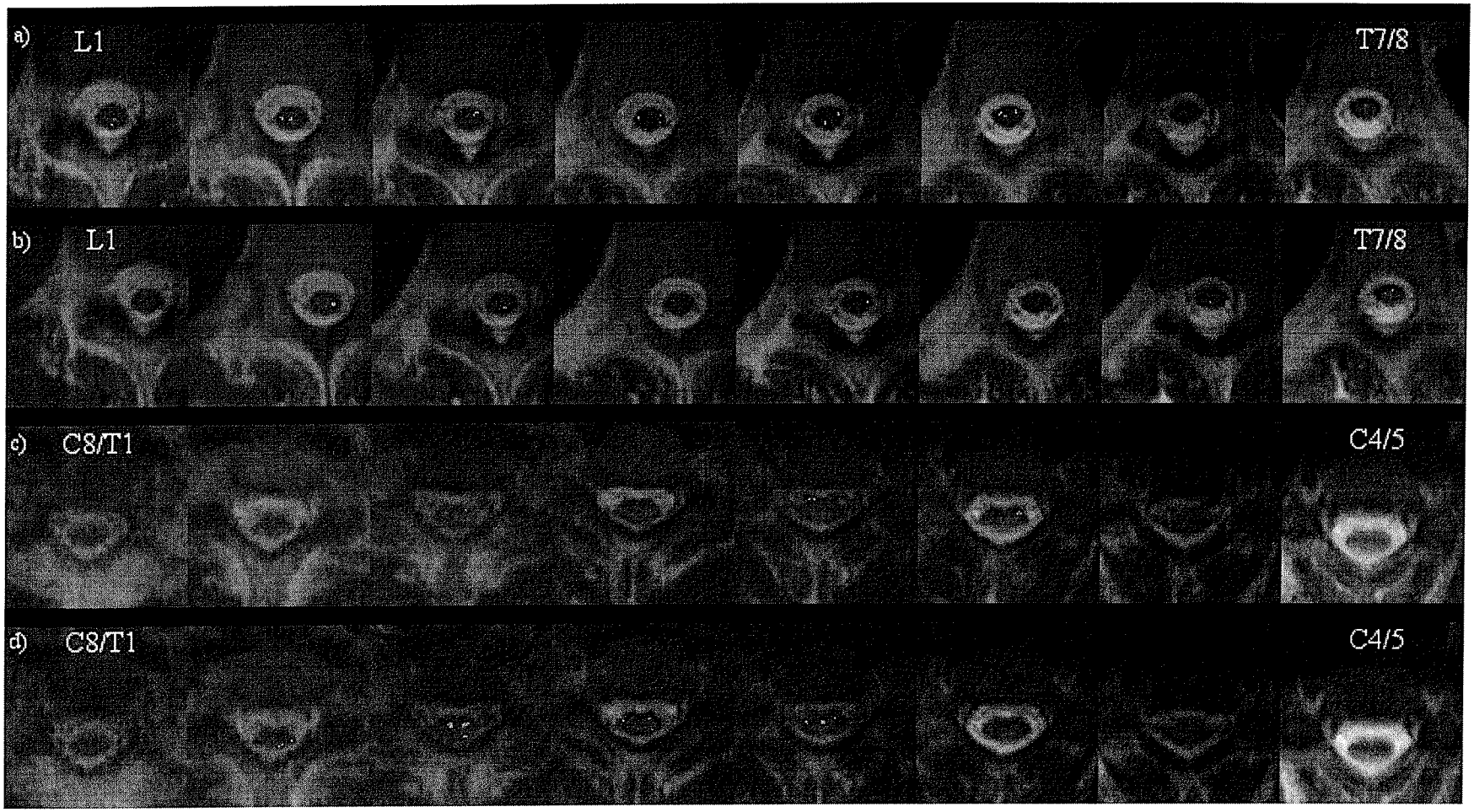

Figure 21A (continued on next page)

Figure 21A. The pattern of activity for a 62 year old female with PPMS. The subject began experiencing symptoms in 1980 and was diagnosed with MS in 1988. Her symptom at the time of the study was motor weakness in her legs. Her medication were Raloxifene (Evista), Tizanidne (Zanaflex), Oxybutinin (Ditropan), and Lorazipam. For (a) and (b), thermal stimulation was applied to the T10 dermatome on the side of the body at the level of the bellybutton, on the left and right respectively. The slices were taken from T8 – T11/12 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the C6 – C8 dermatomes on the palm of the hand, on the left and right respectively. The slices were taken from C4 – C7/T1 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

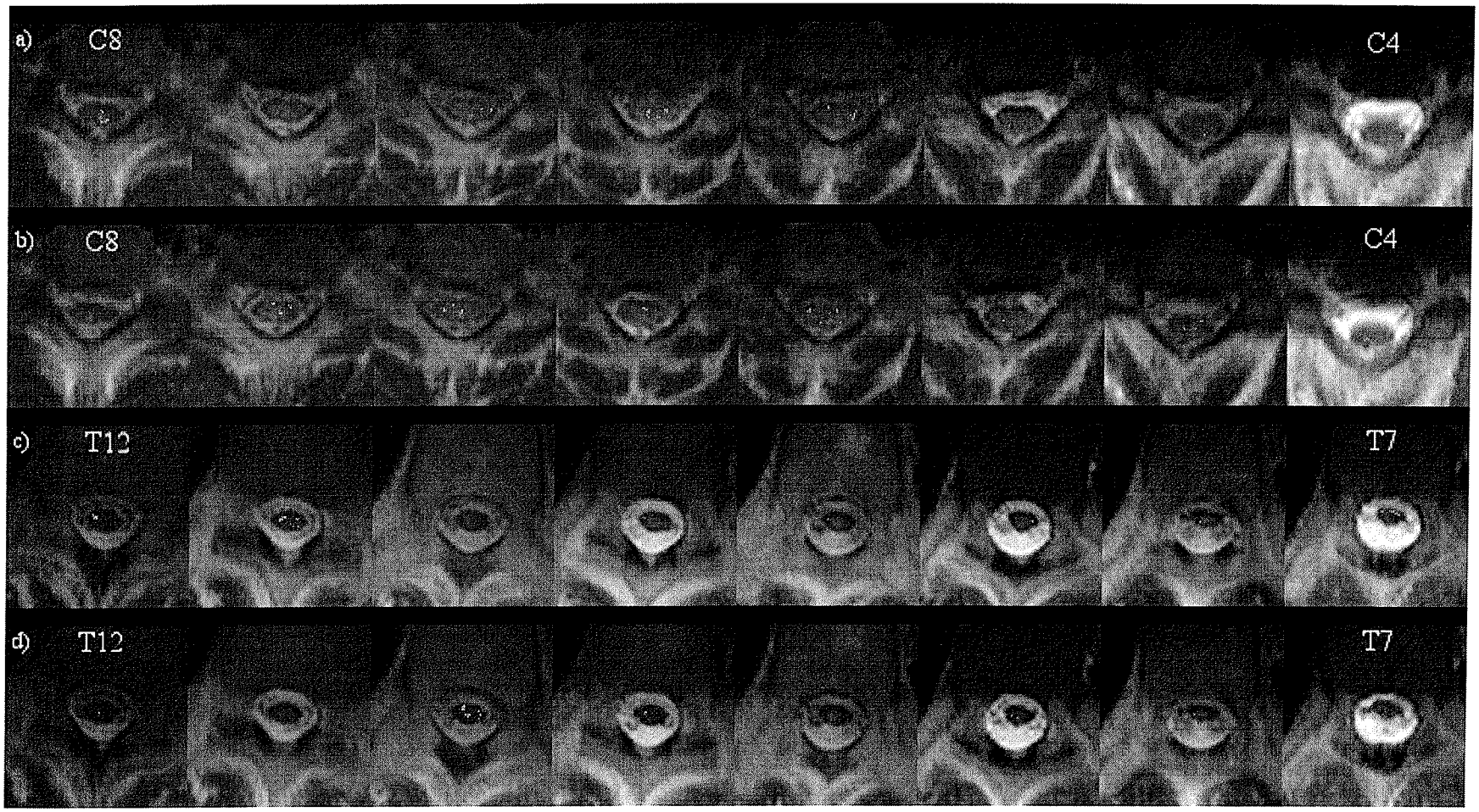

Figure 22A (continued on next page)

Figure 22A. The pattern of activity for a 58 year old female with PPMS. The subject began experiencing symptoms in 1969 and was diagnosed with MS in 1999. Her symptoms at the time of the study were loss of balance, leg weakness, and sleep disturbances. Her medication was Raloxifene (Evista). For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C3/4 – C7 vertebral levels, from right to left. For (c) and (d), thermal stimulation was applied to the T8 dermatome on the side of the chest level with the bottom of the ribs, on the right and left respectively. The slices were taken from T7/8 – T11 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

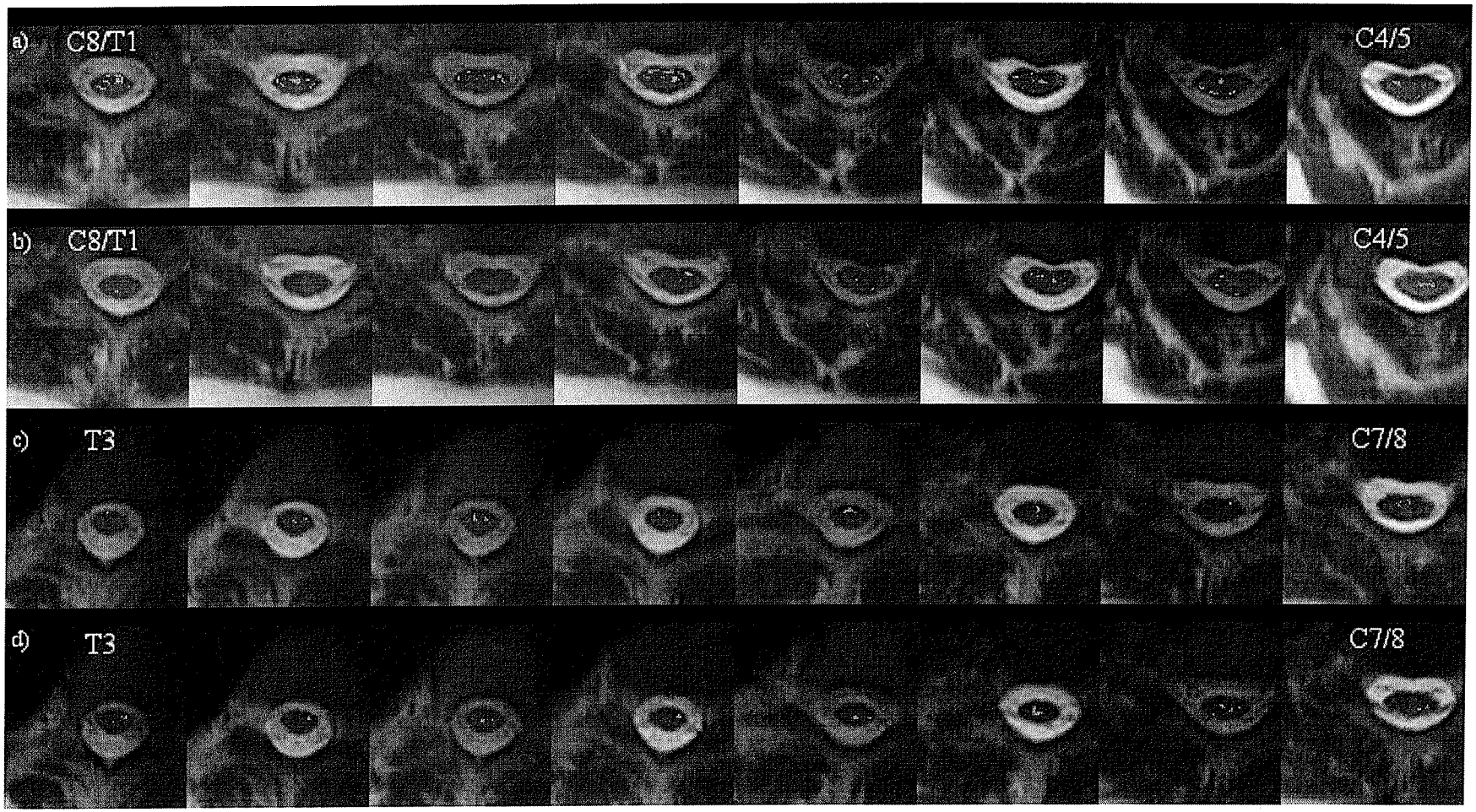

Figure 23A (continued on next page)

Figure 23A. The pattern of activity for a 49 year old female with PPMS. The subject began experiencing symptoms in 1979 and was diagnosed with MS in 1989. Her symptoms at the time of the study consisted of weakness in both legs, "pins and needles" in both hands, and bladder and bowel problems. Her medications were Amantadine and Oxybutinin (Ditropan). For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C4 – C7/T1 vertebral levels, right to left. For (c) and (d), thermal stimulation was applied to the T1 dermatome on the outside of the arm just below the elbow, on the right and left respectively. The slices were taken from C6/7 – T3 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

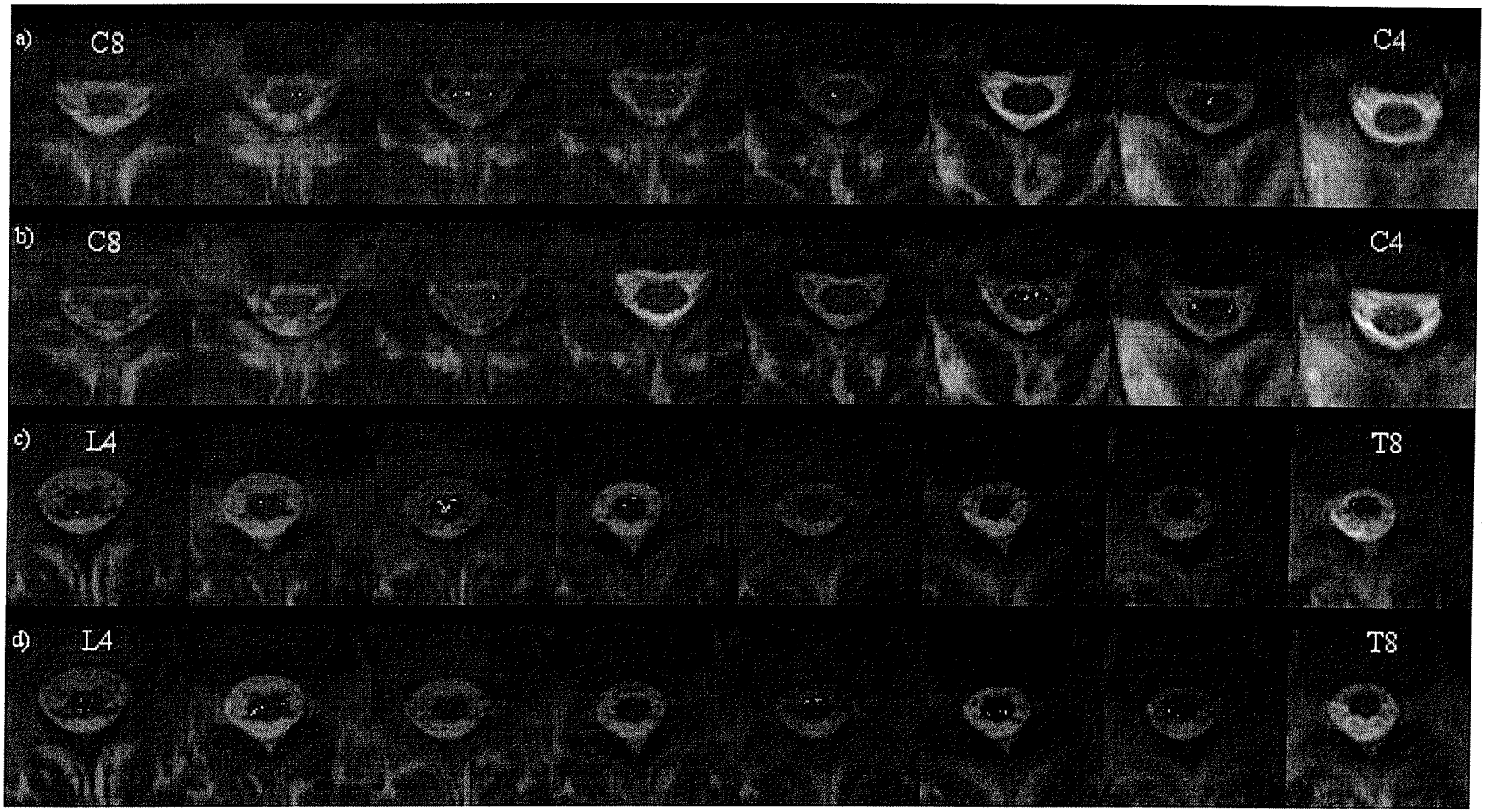

Figure 24A (continued on next page)

Figure 24A. The pattern of activity for a 58 year old female with PPMS. The subject began experiencing symptoms in 1979 and was diagnosed with MS in 1984/85. Her symptoms at the time of the study consisted of fatigue and leg weakness. Her medications were Simvastatin, Amiltryptomline (Zorco), and Citalopram (Celexa). For (a) and (b), thermal stimulation was applied to the C5 dermatome on the upper arm, on the left and right respectively. The slices were taken from C3/4 – C7 vertebral levels, right to left. For (c) and (d), thermal stimulation was applied to the L4 dermatome on the calf, on the left and right respectively. The slices were taken from T8/9 – T12 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

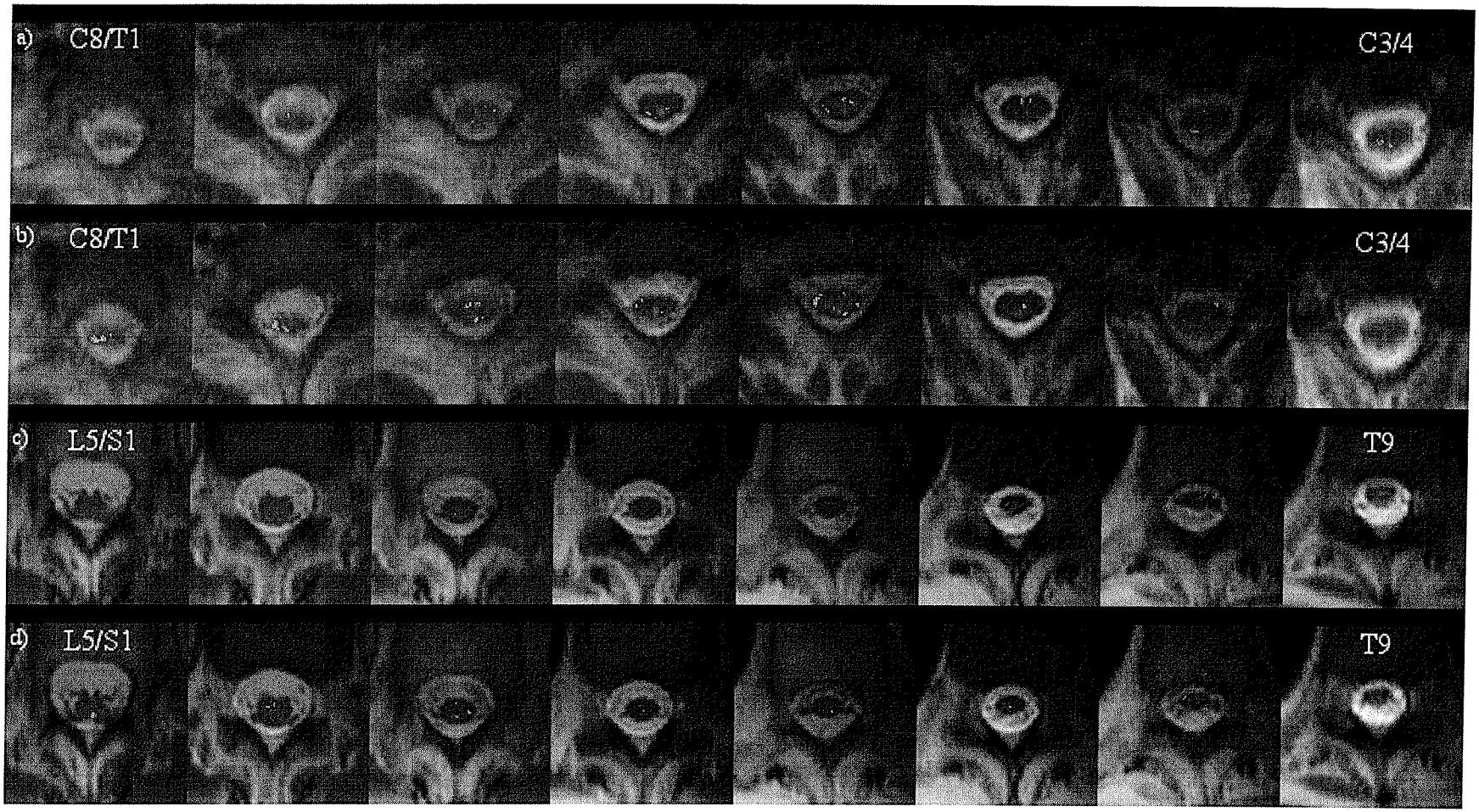

Figure 25A (continued on next page)

Figure 25A. The pattern of activity for a 61 year old female with PPMS with relapsing remitting symptoms. The subject began experiencing symptoms in 1970 and was diagnosed with MS in 1971/72. Her symptoms at the time of the study consisted of leg weakness, poor balance, weakness in the left arm, and she was blind. Her medications were Interferon beta-1b (Betaseron), Gabapentin, Citalopram (Celexa), and 222s. For (a) and (b), thermal stimulation was applied to the C6 dermatome on the inner arm, just below the elbow, on the left and right respectively. The slices were taken from C3 – C7/T1 vertebral levels, right to left. For (c) and (d), thermal stimulation was applied to the L4 dermatome on the calf, on the right and left respectively. The slices were taken from T9/10 – L1 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

Figure 26A. The pattern of activity for a 53 year old female with PPMS with relapsing remitting symptoms. Thermal stimulation was applied to the C6-8 dermatomes on the left hand with the slices taken from C4/5 – T1 vertebral levels, right to left. The subject began experiencing symptoms in the later 1980s and was diagnosed with MS in October 1995. Her symptoms at the time of the study consisted of drop foot, motor problems in her arms, memory difficulties, and incontinence. Her medications were glatiramer acetate (Copaxone), Carbamazepene (Tegretol), Eltroxen, Rofecoxib (Vioxx), and Lorazipam. The levels indicated on the images are the approximate levels of the spinal cord imaged.

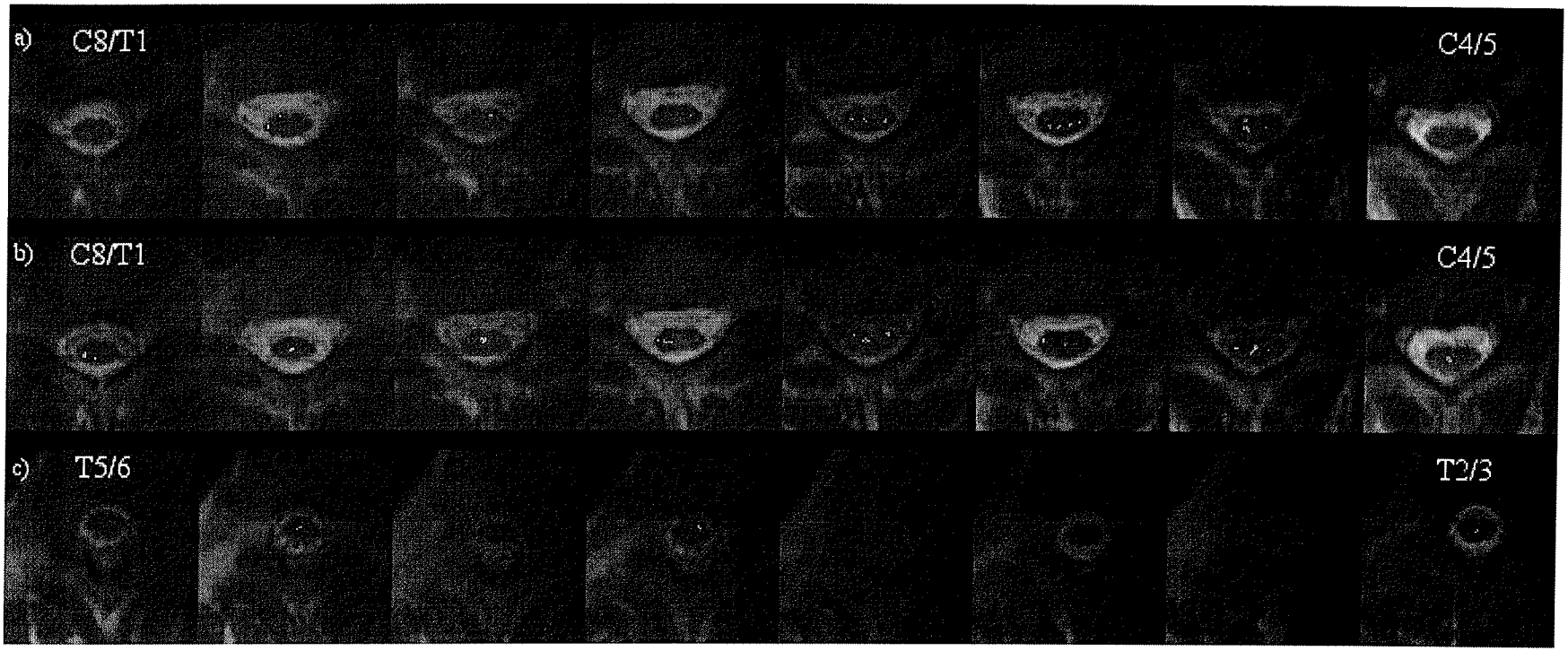

Figure 27A. The pattern of activity for a 59 year old male MS of unknown subtype. The subject began experiencing symptoms in 1983 and was diagnosed with MS in 1985. His symptoms at the time of the study was weakness in the lower extremities which was starting more in the upper body. His medications were Fenofibrate (Lipidil), Tizanidine (Zanaflex), and Gabapentin (Neurontin). For (a) and (b), thermal stimulation was applied to the C6 dermatome on the lower arm, on the left and right respectively. The slices were taken from C4 – C7/T1 vertebral levels, from right to left. For (c), thermal stimulation was applied to the T4/5 dermatome on the side of the chest directly under the armpit on the left. The slices were taken from T2/3 – T6 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

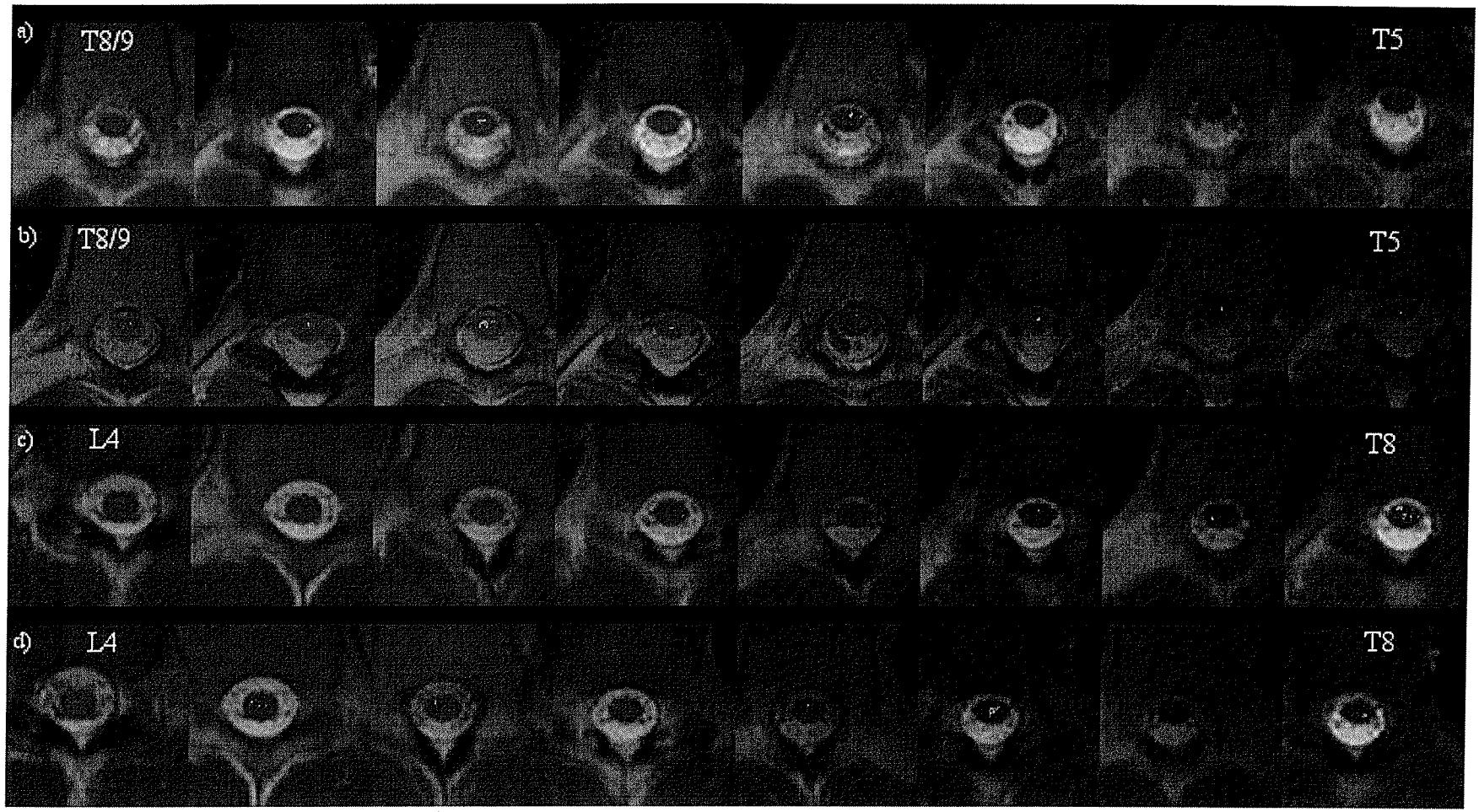

Figure 28A (continued on next page)

Figure 28A. The pattern of activity for a 28 year old male control subject. For (a) and (b), the thermal stimulator was placed on the T5 dermatome, on the side of the body underneath the armpit on the left and right respectively. The slices taken were from T5/6 to T9 vertebral levels, from right to left. For (c) and (d), the thermal stimulator was placed on the T8 dermatome, on the side of the body at the level of the bottom of the ribcage, on the right and left respectively. The slices were from T8/9 to T12 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

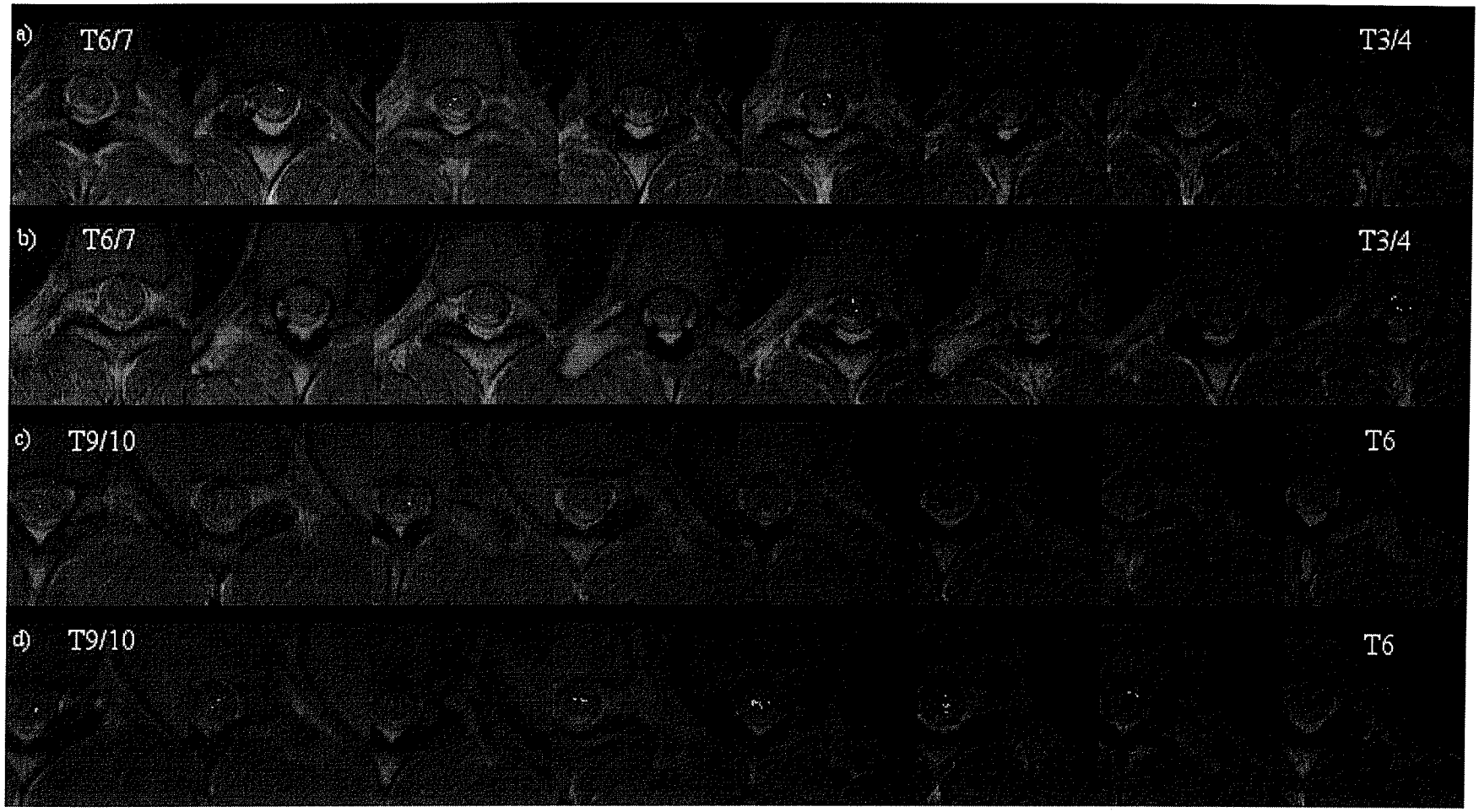

Figure 29A (continued on next page)

Figure 29A. The pattern of activity for a female control subject. For (a) and (b), the thermal stimulator was placed on the T5 dermatome, on the side of the body underneath the armpit, on the left and right respectively. The slices taken were from T3/4 to T7 vertebral levels, from right to left. For (c) and (d), the thermal stimulator was placed on the T8 dermatome, on the side of the body at the level of the bottom of the ribcage, on the right and left respectively. The slices were from T6/7 to T10 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

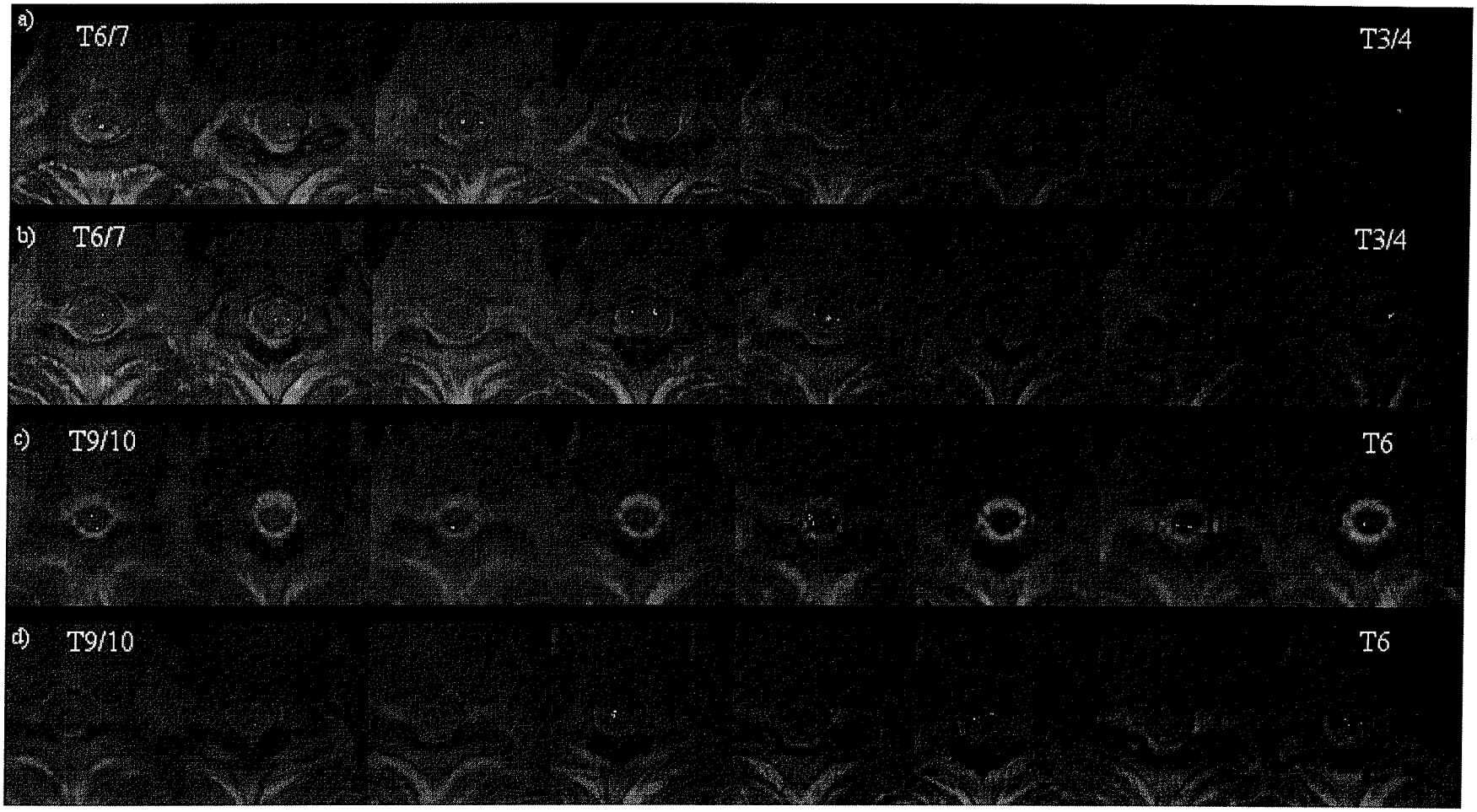

Figure 30A (continued on next page)

Figure 30A. The pattern of activity for a male control subject. For (a) and (b), the thermal stimulator was placed on the T5 dermatome, on the side of the body underneath the armpit on the left and right respectively. The slices taken were from T3/4 to T7 vertebral levels, from right to left. For (c) and (d), the thermal stimulator was placed on the T8 dermatome, on the side of the body at the level of the bottom of the ribcage, on the right and left respectively. The slices were from T6/7 to T10 vertebral levels, right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

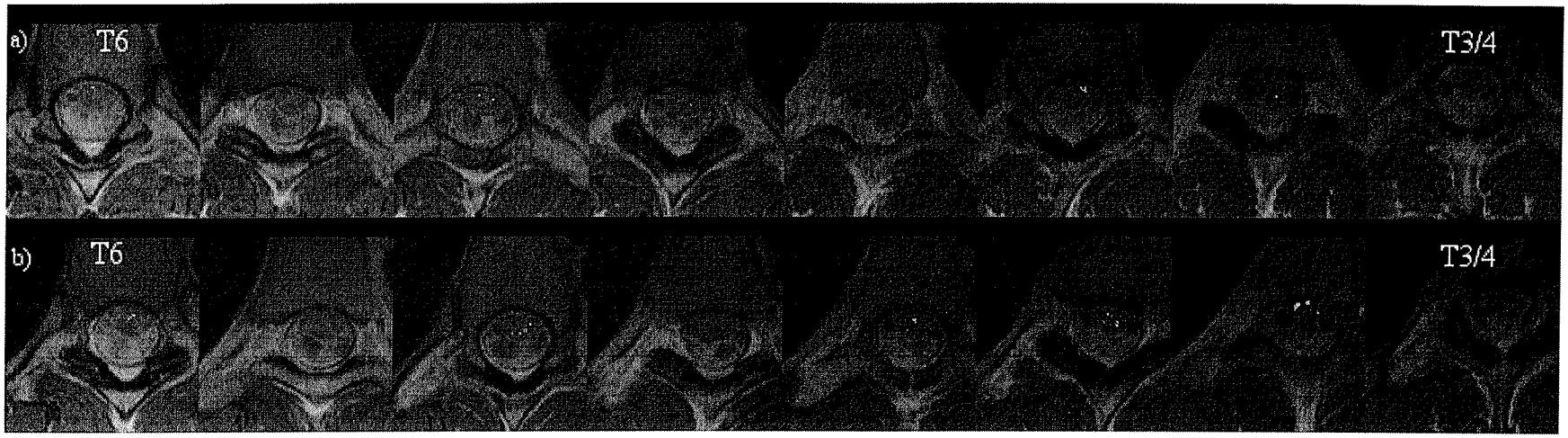

Figure 31A. The pattern of activity for a 27 year old female control subject. For (a) and (b), the thermal stimulator was placed on the T5 dermatome, on the side of the body underneath the armpit, on the left and right respectively. The slices taken were from T3/4 – T6/7 vertebral levels, from right to left. The levels indicated on the images are the approximate levels of the spinal cord imaged.

Reference List

1. Compston A, Coles A. Multiple sclerosis. (2002) The Lancet; 359:1221-31.

2. Tartaglia MC, Narayanan S, De Stefano N, Arnaoutelis R, Antel SB, Francis SJ Santos AC, Lapierre Y, Arnold DL. (2002) Choline is increased in pre-lesional normal appearing white matter in multiple sclerosis. Journal of Neurology; 249:1382-90.

3. Soldan MMP, Rodriguez M. (2002) Heterogeneity of pathogenesis of multiple sclerosis: implications for promotion of remyelination. The Journal of Infectious Diseases; 186(Suppl 2):S248-S253.

4. Gonen O, Moriarty DM, Li BSY, Babb JS, He J, Listerud J, Jacobs D, Markowitz CE, Grossman RI. (2002) Relapsing-remitting multiple sclerosis and whole-brain N-acetylaspartate measurement: evidence for different clinical cohorts - initial observations. Radiology; 225:261-8.

5. Thielen KR, Miller GM. (1996) Multiple sclerosis of the spinal cord: magnetic resonance appearance. Computer Assisted Tomography; 20(3):434-8.

6. Arnold DL, Matthews PM. (2002) MRI in the diagnosis and management of multiple sclerosis. Neurology; 58(Suppl 4):523-31.

7. Fassas A, Passweg JR, Anagnostopoulos A, Kazis A, Kozak T, Havrdova E, Carreras E, Graus F, Kashyap A, Openshaw H, Schipperus M, Deconinck E, Mancardi G, Marmont A, Hansz J, Rabusin M, Zuazu Nagore FJ, Besalduch J, Dentamaro T, Fouillard L, Hertenstein B, La Nasa G, Musso M, Papineschi F, Rowe JM, Saccardi R, Steck A, Kappos L, Gratwohl A, Tyndall A, Samijn J; Autoimmune Disease Working Party of the EBMT (European Group for Blood and Marrow Transplantation). (2002) Hematopoietic stem cell transplantation for multiple sclerosis: a retrospective multicenter study. Journal of Neurology; 249(8):1088-97.

8. Stevenson VL, Leary SM, Losseff NA, Parker GJM, Barker GJ, Husmani Y, Miller DH, Thompson AJ. (1998) Spinal cord atrophy and disability in MS: a longitudinal study. Neurology; 51:234-8.

9. Poser CM, Patty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Tourtellotte WW. (1983) New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Annals of Neurology; 13(3):227-31.

10. Keegan BM, Noseworthy JH. (2002) Multiple sclerosis. Annual Reviews Medicine; 53:285-302.

11. Ingle GT, Thompson AJ, Miller DH. (2002) Magnetic resonance imaging in primary progressive multiple sclerosis. Journal of Rehabilitation Research and Development; 39(2):261-72.

12. Lycklama à Nijeholt GJ, Bergers E, Kamphorst W, But J, Nicolay K, Castelijns JA, van Waesberghe JHTM, Ravid R, Polman CH, Barkhof F. (2001) Post-mortem high-resolution MRI of the spinal cord in multiple sclerosis: a correlative study with conventional MRI, histopathology, and clinical phenotype. Brain; 124:154-66.

13. Fazekas F, Barkhof F, Filippi M, Grossman RI, Li DKB, McDonald WI, McFarland HF, Paty DW, Simon JH, Wolinsky JS, Miller DH. (1999) The contribution of magnetic resonance imaging to the diagnosis of multiple sclerosis. Neurology; 53:448-56.

14. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. (2000) Multiple Sclerosis. The New England Journal of Medicine; 343(13):938-52.

15. Chang A, Tourtellotte WW, Rudick R, Trapp B. (2002) Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. New England Journal of Medicine; 346(3):165-73.

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

17. Lycklama à Nijeholt GJ, van Walderveen MAA, Castelijns JA, van Waesberghe JHTM, Polman C, Scheltens P, Rosier PFWM, Jongen PJH, Barkhof F. (1998) Brain and spinal cord abnormalities in multiple sclerosis: correlation between MRI parameters, clinical subtypes and symptoms. Brain; 121:687-97.

18. Lycklama G, Thompson A, Filippi M, Miller D, Polman C, Fazekas F, Barkhof F. (2003) Spinal-cord MRI in multiple sclerosis. The Lancet Neurology; 2:555-62.

19. Filippi M, Rocca MA. (2003) Disturbed function and plasticity in multiple sclerosis as gleaned from functional megnetic resonance imaging. Current Opinion in Neurology; 16:275-82.

20. Miller DH. (2002) MRI monitoring of MS in clinical trials. Clinical Neurology and Neurosurgery; 104:236-43.

21. Griffen CM, Chard DT, Parker GJM, Barker GJ, Thompson AJ, Miller DH. (2002) The relationship between lesion and normal appearing brain tissue in early relapsing remitting multiple sclerosis. Journal of Neurology; 249:193-9.

22. McDonald WI, Miller DH, Barnes D. (1992) The pathological evolution of multiple sclerosis. Neuropathology and Applied Neurobiology; 18:319-34.

23. Lucchinetti C, Brück W, Noseworthy J. (2001) Multiple sclerosis: recent developments in neuropathology, pathogenesis, magnetic resonance imaging studies and treatment. Current Opinion in Neurology; 14:259-69.

24. Lucchinetti CF, Brueck W, Rodriguez M, Lassmann H. (1998) Multiple sclerosis: lessons from neuropathology. Seminars in Neurology; 18(3):337-49.

25. De Stefano N, Iannucci G, Sormani MP, Guidi L, Bartolozzi ML, Comi G, Federico A, Filippi M. (2002) MR correlates of cerebral atrophy in patients with multiple sclerosis. Journal of Neurology; 249:1072-7.

26. Ransohoff RM, Howe CL, Rodriguez M. (2002) Growth factor treatment of demyelinating disease: at last, a leap into the light. TRENDS in Immunology; 23(11):512-6.

27. Ure DR, Rodriguez M. (2002) Preservation of neurologic function during inflammatory demyelination correlates with axon sparing in a mouse model of multiple sclerosis. Neuroscience; 111(2):399-411.

28. Neumann H. (2003) Molecular mechanisms of axonal damage in inflammatory central nervous system diseases. Current Opinion in Neurology; 16:267-73.

29. Werring DJ, Brassat D, Droogan AG, Clark CA, Symms MR, Barker GJ, MacManus DG, Thompson AJ, Miller DH. (2000) The pathogenesis of lesions and normal-appearing white matter changes in multiple sclerosis: a serial diffusion MRI study. Brain; 123(8):1667-76.

30. Murray PD, McGavern DB, Sathornsumetee S, Rodriguez M. (2001) Spontaneous remyelination following extensive demyelination in associated with improved neurological function in a viral model of multiple sclerosis. Brain; 124:1403-16.

31. Wolswijk G. (2002) Oligodendrocyte precursor cells in the demyelinated multiple sclerosis spinal cord. Brain; 125(2):338-49.

32. Owens T. (2003) The enigma of multiple sclerosis: inflammation and neurodegeneration cause heterogeneous dysfunction and damage. Current Opinion in Neurology; 16:259-65.

33. Rodriguez M, Scheithauer B. (1994) Ultrastructure of multiple sclerosis. Ultrastructural Pathology; 18:3-13.

34. Davie CA, Barker GJ, Webb S, Tofts PS, Thompson AJ, Harding AE, McDonald WI, Miller DH. (1995) Persistent functional deficit in multiple sclerosis and autosomal dominant cerebellar ataxia is associated with axons loss. Brain; 118:1583-92.

35. Werring DJ, Clark CA, Droogan AG, Barker GJ, Miller DH, Thompson AJ. (2001) Water diffusion is elevated in widespread regions of normal-appearing

white matter in multiple sclerosis and correlates with diffusion in focal lesions. Multiple Sclerosis; 7:83-9.

36. Castriota-Scanderbeg A, Sabatini U, Fasano F, Floris R, Fraracci L, Di Mario M, Nocentini U, Caltagirone C. (2002) Diffusion of water in large demyelinating lesions: a follow-up study. Neuroradiology; 44:764-7.

37. Brück W, Lucchinetti C, Lassmann H. (2002) The pathology of primary progressive multiple sclerosis. Multiple Sclerosis; 8:93-7.

38. Brex PA, Leary SM, O'Riordan JI, Miszkiel KA, Plant GT, Thompson AJ, Miller DH. (2001) Measurement of spinal cord area in clinically isolated syndromes suggestive of multiple sclerosis. Journal of Neurology, Neurosurgery, and Psychiatry; 70:544-7.

39. Davie CA, Silver NC, Barker GJ, Tofts PS, Thompson AJ, McDonald WI, Miller DH. (1999) Does the extent of axonal loss and demyelination from chronic lesions in multiple sclerosis correlate witht he clinical subgroup? Journal of Neurology, Neurosurgery, and Psychiatry; 67:710-5.

40. Sicotte NL, Liva SM, Klutch R, Pfieffer P, Bouvier S, Odesa S, Wu TCJ, Voskuhl RR. (2002) Treatment of multiple sclerosis with the pregnancy hormone estriol. Annals of Neurology; 52:421-8.

41. Losseff NA, Webb SL, O'Riordan JI, Page R, Wang L, Barker GJ, Tofts PS, McDonald WI, Miller DH, Thompson AJ. (1996) Spinal cord atrophy and disability in multiple sclerosis: a new reproducible and sensitive MRI method with potential to monitor disease progression. Brain; 119:701-8.

42. Kidd D, Thorpe JW, Kendall BE, Barker GJ, Miller DH, McDonald WI, Thompson AJ. (1996) MRI dynamics of brain and spinal cord in progressive multiple sclerosis. Journal of Neurology, Neurosurgery, and Psychiatry; 60:15-9.

43. O'Riordan JI, Losseff NA, Phatouros C, Thompson AJ, Moseley IF, MacManus DG, McDonald WI, Miller DH. (1998) Asymptomatic spinal cord lesions in clinically isolated optic nerve, brainstem, and spinal cord syndromes suggestive of demyelination. Journal of Neurology, Neurosurgery, and Psychiatry; 64:353-7.

44. Kingsley RE. Concise Text of Neuroscience. Seconded. Philadelphia: Lippincott Williams & Wilkins; 2000. Pg 22,24,25,27,34,46,145,146,150,154,155,165, 166,178,179,187,188.

45. Goldman L. Cecil Textbook of Medicine. 21st ed. Philadelphia: W.B. Saunders Company; 2000. Pg 2185,2098.

46. Goetz CG. Textbook of Clinical Neurology. 2nd ed. Philadelphia: Elsevier; 2003. Pg 352,390,398, 401,402,820.

47. Berne RM, Levy MN (eds). Physiology. 4th ed. London: Mosby; 1998. Pg 85, 109,110,119

48. Green BG. (2004) Temperature Perception and Nociception. Journal of Neurobiology; 61(1):13-29.

49. Marx J. Rosen's Emergency Medicine: Concepts and Clinical Practice. 5th ed. Mosby, Inc; 2002. Pg 287,1501.

50. Sharma HS, Westman J (eds). Blood-Spinal Cord and Brain Barriers in Health and Disease. London: Elsevier Academic Press; 2004. Pg 437.

51. Brown MA, Semelka RC. MRI Basic Principles and Applications. 3rd ed. New Jersey: John Wiley & Sons, Inc; 2003. Pg 1,4,8,13-15,21,25,27,29-31,69,73,94, 173.

52. Gillies RJ (ed). NMR in Physiology and Biomedicine. Toronto: Academic Press; 1994. Pg 16,25,26,139,140.

53. Weishaupt D, Kochli VD, Marineck B. How Does MRI Work? An Introduction to the Physics and Function of Magnetic Resonance Imaging. New York: Springer-Verlag; 2003. Pg 6,12.

54. Miller DH, Albert PS, Barkhof F, Francis G, Frank JA, Hodgkinson S, Lublin FD, Paty DW, Reingold SC, Simon J. (1996) Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. Annals of Neurology; 39:6-16.

55. Fu L, Matthews PM, De Stefano N, Worsley KJ, Narayanan S, Francis GS, Antel JP, Wolfson C, Arnold DL. (1998) Imaging axonal damage of normal-appearingwhite matter in multiple sclerosis. Brain; 121:103-13.

56. Lycklama à Nijeholt GJ, Uitdehaag BMJ, Bergers E, Castelijns JA, Polman CH, Barkhof F. (2000) Spinal cord magnetic resonance imaging in suspected multiple sclerosis. European Radiology; 10:368-76.

57. Moonen CTW, Bandettini PA (eds). Functional MRI. New York: Springer-Verlag; 1999. Pg 3,10,79,80,103,115.

58. Bandettini PA, Wong EC, Jesmanowicz A, Hicks RS, Hyde JS. (1994) Spin-echo and gradient-echo EPI of human brain activation using BOLD contrast: a comparative study at 1.5 T. NMR in Biomedicine; 7(1-2):12-20.

59. Buxton RB. Introduction to Functional Magnetic Resonance Imaging: Principles & Techniques. Cambridge: Cambridge University Press; 2002. Pg 399.

60. Ohta S, Meyer E, Fujita H, Reutens DC, Evans A, Gjedde A. (1996) Cerebral [^{15}O]water clearance in humans determined by PET: I. Theory and normal values. Journal of Cerebral Blood Flow & Metabolism; 16:765-80.

61. Stroman PW, Tomanek B, Krause V, Frankenstein UN, Malisza KL. (2002) Mapping of neuronal function in the healthy and injured human spinal cord with spinal fMRI. NeuroImage; 17:1854-60.

62. Menon RS, Ogawa S, Kim SG, Ellermann JM, Merkle H, Tank DW, Ugurbil K. (1992) Functional brain mapping using magnetic resonance imaging. Signal changes accompanying visual stimulation. Investigative Radiology; 27(Suppl 2):S47-S53.

63. Abbott NJ. (2002) Astrocyte - endothelial interactions and blood-brain barrier permeability. Journal of Anatomy; 200:629-38.

64. Piet R, Vargova L, Sykova E, Poulain DA, Oliet SHR. (2004) Physiological contribution of the astrocytic environment of neurons to intersynaptic crosstalk. Proceedings of the National Academy of Sciences; 101(7):2151-5.

65. Stroman PW, Krause V, Frankenstein UN, Malisza KL, Tomanek B. (2001) Spin-echo versus gradient-echo of MRI with short echo times. Magnetic Resonance Imaging; 19:827-31.

66. Stroman PW, Malisza KL, Onu M. (2003) Functional magnetic resonance imaging at 0.2 Tesla. NeuroImage; 20(2):1210-4.

67. Stroman PW, Ryner LN. (2001) Functional MRI of motor and sensory activation in the human spinal cord. Magnetic Resonance Imaging; 19:27-32.

68. Stroman PW, Krause V, Malisza KL, Frankenstein UN, Tomanek B. (2001) Characterization of contrast changes in functional MRI of the human spinal cord at 1.5 T. Magnetic Resonance Imaging; 19:833-8.

69. Stroman PW, Krause V, Malisza KL, Frankenstein UN, Tomanek B. (2002) Functional magnetic resonance imaging of the human cervical spinal cord with stimulation of different sensory dermatomes. Magnetic Resonance Imaging; 20:1-6.

70. Stroman PW, Kornelsen J, Bergman A, Krause V, Ethans K, Malisza K, Tomanek B. (2004) Noninvasive assessment of the injured human spinal cord by means of functional magnetic resonance imaging. Spinal Cord; 42:59-66.

71. Badaut J, Lasbennes F, Magistretti PJ, Regli L. (2002) Aquaporins in brain: distribution, physiology, and pathophysiology. Journal of Cerebral Blood Flow & Metabolism; 22:367-78.

72. Amiry-Moghaddam M, Ottersen OP. (2003) The molecular basis of water transport in the brain. Nature Reviews Neuroscience; 4:991-1001.

73. Bouzier-Sore AK, Merle M, Magistretti PJ, Pellerin L. (2002) Feeding active neurons: (re)emergence of a nursing role for astrocytes. Journal of Physiology - Paris; 96:273-82.

74. Rovaris M, Bozzali M, Santuccio G, Ghezzi A, Caputo D, Montanari E, Bertolotto A, Bergamaschi R, Capra R, Mancardi G, Martinelli V, Comi G, Filippi M. (2001) *In vivo* assessment of the brain and cervical cord pathology of patients with primary progressive multiple sclerosis. Brain; 124(12):2540-9.

75. Martino G. (2004) How the brain repairs itself: new therapeutic strategies in inflammatory and degenerative CNS disorders. Lancet Neurology; 3(6):372-8.

76. Katzung BG (ed). Basic & Clinical Pharmacology. 8th ed. New York: Lange Medical Books/McGraw-Hill; 2001. Pg 89,114,166,172,193,214,254-256,400, 406,412,457-459,464,465,470,499,501,503,537,591,601,607,650,664,672,698, 943,972.

77. Hardman JG, Limbird LE (eds). Goodman & Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York: McGraw-Hill; 2001. Pg 145,162,167, 169,235,237,240,252,254,279,357,401,403,406,410,452,459,513,522,537,538, 556,560,565,692,693,696,710,711,715,739,773,776,822,853,860,864,872,978, 993,1429,1477,1478,1572,1608,1611-1615,1669,1684,1739.

78. Moore DP, Jefferson JW. Handbook of Medical Psychiatry. 2nded. St. Louis: Mosby; 2004. Pg 491.

79. Archer DF, EstroGel Study Group. (2003) Percutaneous 17beta-estradiol gel for the treatment of vasomotor symptoms in postmenopausal women. Menopause; 10(6):516-21.

80. Noble J (ed). Textbook of Primary Care Medicine. 3rd ed. St. Louis: Mosby, Inc; 2001. Pg 331,1626.

81. Leger D, Metlaine A, Choudat D. (2005) Insomnia and Sleep Disruption: Relevance for Athletic Performance. Clinics in Sports Medicine; 24(2):269-85.

82. da Costa SS, de Sousa LC, Piza MR. (2002) Meniere's disease: overview, epidemiology, and natural history. Otolaryngologic Clinics of North America; 35(3):455-95.

83. Siebert DJ, Bochner F, Imhoff DM, Watts S, Lloyd JV, Field J, Gabb BW. (1983) Aspirin kinetics and platelet aggregation in man. Clinical Pharmacology and Therapeutics; 33(3):367-74.