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TITOLO DELLA TESI DI DOTTORATO

"FATIGUE IN MULTIPLE SCLEROSIS: A CLINICAL AND MRI STUDY"

S.S.D. MED 26 (Neurologia)

Tutor: Dott.ssa Maria Donata Benedetti

Dottorando: Dott. Marco Turatti

Abitazione di Jean Martin Charcot

(Parigi,1825 - Nièvre, 1893)

217, Boulevard Saint Germain de Prés – Paris

[Per cortesia del Dott. A. Salviati]

Santa Ludwina da Schiedam

(Schiedam, 18 marzo 1380 – Schiedam 14 aprile 1433)

Santificata nel 1890 da Papa Leone XIII

INTRODUCTION

HISTORY OF MULTIPLE SCLEROSIS

The first multiple sclerosis (MS) case was described in 14th century in a 16 years-old girl with ambulation difficulties, headache and teethache. The young girl was known later as Santa Ludwina of Schiedam (130-1433) (Maeder R, 1979). At the age of 19 Ludwina suffered of eye blindness and was bed-bound. Ludwina's disease went on with progression even if with remission periods during which she was able to walk with assistance, until her death at 53 yers.

To find a second case of MS in history we need to wait the 19 century with the appearance on the scene of Sir August d'Este. He was the outlawed son of George the third, king of England. Sir August told about his disease in his diary. Retrospectively diagnosed as MS, the disease started at 26 year-old with several episode of optic neuritis. In 1822 he was sent to Driburg for thermal treatment where his symptoms went back. His diary contains enough informations to ensure he was affected by a relapsing-remitting form of MS, characterized by vision loss, diplopia, sensory impairment and bladder dysfunction.

The first description of MS lesions came from Robert Carswell (1793-1857) Atlas of Pathology, published in 1838. Carswell wrote: *"… a peculiar pathological status of spinal cord and pons, associated to atrophy"*. However, it was only with Jean-Martin Charcot (1825-1893), a french neurologist, that the correlation between MS symptoms and pathological changes on autoptic samples was established. Charcot named the condition *"sclerose en plaque"*. Moreover, Charcot was the father of the first diagnostic criteria for MS called Charcot triade: diplopia, ataxia and disartrya. Lastly, he provide a full histological description of lesions pointing out myelin loss and glial activation. At the beginning of 20th century Professor Denny-Brown observed that a damaged nerve, when stimulated, was nomore able to conduct stimulus to muscles. He concluded that demyelinated plaques were reason for conduction blocks in nerves and consequently in spine and brain.

Epidemiology of multiple sclerosis.

To date, we know that MS is an acquired immunomediated and inflammatory disease, characterized by inflammation, demyelination and primary and secondary axon degeneration (Pugliatti M, 2006). Data supporting the autoimmune theory are: (1) women are more affected as in other autoimmune diseases; (2) relatively steadiness of the disease during pregnancy; (3) frequent association with other autoimmune diseases and their presence in the parenthood; (4) association with type II HLA; (5) similarities with experimental allergic encephalomyelitis, used as a model for MS; (6) presence in serum and cerebro spinal fluid (CSF) of auto-antibodies against myelin's antigens (Marrie RA, 2004).

MS affects about 1.000.000 people between 17 and 65 years-old in the whole world. In 2000 MS prevalence rate in the white american population was around 191 cases per 100.000 inhabitants and the incidence rate was 7.3 per 100.000 years-person (Mayr WT, 2003). MS is twice as common in women as in men; men usually tend to have a later onset and a worse prognosis, probably depending on gender-associated factors on phenotypic and aetiological variability.

In Europe, mean prevalence rate is higher in northern countries, depending on better quality of diagnostic process, although great variability in the same area (Scotland, north Norway). Prevalence rates varies between 11 and 282 per 100.000 in women and between 10 and 123 in men, with a female:male rate between 1.1 and 3.4.

The highest prevalence rate has been noticed in age between 35 and 49 in all nations with the exception of Ireland, Great Britain and Norway, where the disease as a major prevalence in the 50 – 64 age group. Mean incidence rate in Europe is 4 cases per 100.000/year (Pugliatti M, 2006). Mortality rate in Europe stays in a range between 0.5 and 3.6 per 100.000.

In Italy prevalence rate for mainland is between 40 and 70 per 100.000 inhabitants (Malatesta G, 1991; Meucci G, 1992; Granieri E, 1996; Maddestra M, 1998; Totaro R, 2000), with the only exception of Salerno (35 per 100.000 in 1998) (Iuliano G, 1999) and Valle d'Aosta with 90 cases over 100.000

inhabitants (Sironi L, 1997). Female:Male rate varies between 1.2 and 2.3 and the higher prevalence rate has been found in age 35-49 years old, both in hinterland and Islands. The increase in prevalence and incidence across years can be ascribed to a better accuracy in diagnosis. (Granieri E, 1996).

Natural History of multiple sclerosis

MS is an autoimmune inflammatory disease affecting the CNS, which implies acute axonal damage caused by inflammation, mechanisms of functional and structural recovery and neurodegeneration. Primary target of inflammation is myelin, a protein involved in isolation of axons necessary for saltatory conduction. Consequences of demyelination may explain clinical and laboratory findings in MS. Partially demyelinated axons have a slower conduction giving a delay in evoked potentials. Demyelinated axons present spontaneous depolarisations and have a higher excitability, explaining symptoms as phosphenes and Lhermitte sign. Close demyelinated axons may cross-react and give rise to paroxysmal symptoms, such as trigeminal neuralgia (Compston A, 2002).

Symptoms and signs of MS poorly reflects the functional anatomy of demyelinated axons. Usually brain is involved, mostly when assessed by MRI. Lesions located in brainstem and cerebellum are more precisely related to specific symptoms. Spinal cord too is frequently affected by demyelinating lesions.

Since there are no single clinical or laboratory variables which can detect MS, diagnostic criteria were developed as combination of clinical and paraclinical data, since Schumacher Criteria were proposed (Schumacher FA, 1965). Afterwards, in 1982, diagnostic pathway has been modified introducing different degrees of certainty: clinically defined MS, defined MS with laboratoristic support, probable MS with laboratoristic support (Poser CM, 1983). In July 2000 the International Panel on the Diagnosis of MS has been instituted, with the aim of introducing diagnostic criteria for clinical practice and clinical trials (McDonald WI, 2001), which have been revised in 2005 (Polman CH, 2005).Moreover, MRI parameters and criteria for primary progressive MS (Thompson AJ, 2000)

(characterized by the absence of relapses and remission phases) were included in the diagnostic chart (Fig.1).

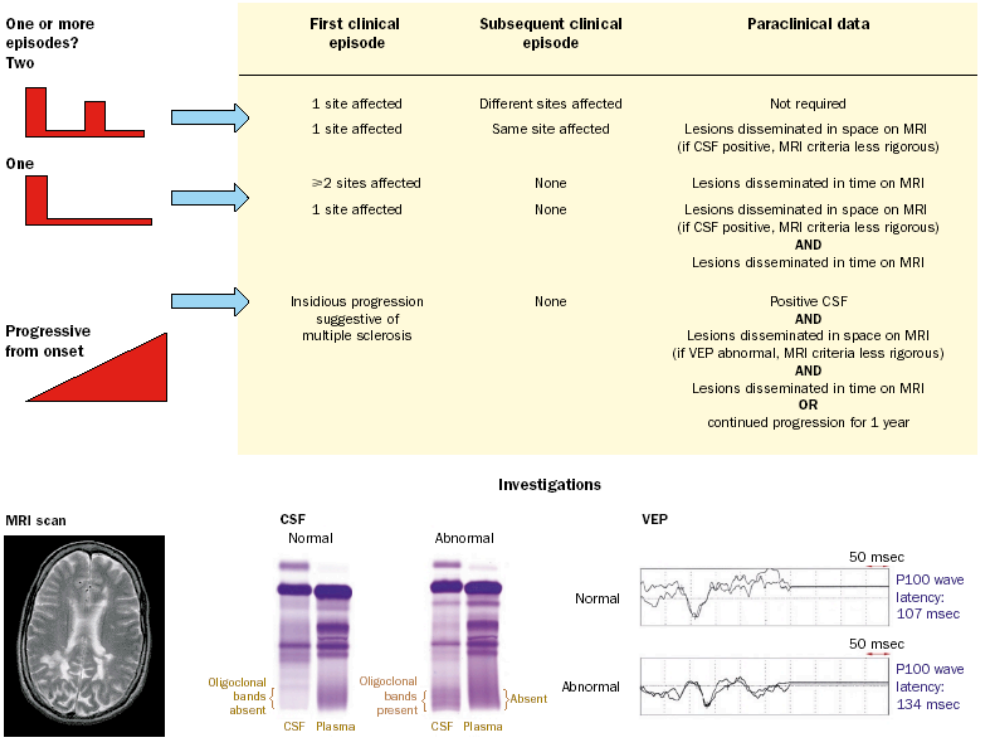


Figure 1. Diagnostic Criteria (McDonald 2001) with clinical and paraclinical variables. MRI= Magnetic Resonance Imaging; CSF= Cerebro-spinal Fluid; VEP= Visual Evoked Potentials.

The basis for the current diagnostic criteria (Tab. 1) is:

1. To have objective evidence for dissemination in time and space;
2. Clinical evidence with objective clinical sign. Diagnosis of MS based on clinical history may be possible if there is evidence of dissemination in time and space of lesions;
3. Laboratoristic and radiological tests, such as CSF analysis, visual evoked potentials (VEP) and mostly MRI, may support diagnosis if clinical evidence confirms at least one objective lesion;
4. A subject may be diagnosed as having MS or not having MS. A patients with a disease onset suggestive of MS but with unsatisfactory clinical and instrumental evaluation may be considered as affected by “possible MS”.

Clinical Presentation	Additional Data Needed for MS Diagnosis
Two or more attacks[a]; objective clinical evidence of two or more lesions	None[b]
Two or more attacks[a]; objective clinical evidence of one lesion	Dissemination in space, demonstrated by: ● MRI[c] *or* ● Two or more MRI-detected lesions consistent with MS plus positive CSF[d] *or* ● Await further clinical attack[a] implicating a different site
One attack[a]; objective clinical evidence of two or more lesions	Dissemination in time, demonstrated by: ● MRI[e] *or* ● Second clinical attack[a]
One attack[a]; objective clinical evidence of one lesion (monosymptomatic presentation; clinically isolated syndrome)	Dissemination in space, demonstrated by: ● MRI[c] *or* ● Two or more MRI-detected lesions consistent with MS plus positive CSF[d] *and* Dissemination in time, demonstrated by: ● MRI[e] *or* ● Second clinical attack[a]
Insidious neurological progression suggestive of MS	One year of disease progression (retrospectively or prospectively determined) *and* Two of the following: a. Positive brain MRI (nine T2 lesions or four or more T2 lesions with positive VEP)[f] b. Positive spinal cord MRI (two focal T2 lesions) c. Positive CSF[d]

If criteria indicated are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is MS; if suspicious, but the criteria are not completely met, the diagnosis is "possible MS"; if another diagnosis arises during the evaluation that better explains the entire clinical presentation, then the diagnosis is "not MS."

Table 1. The 2005 Revision to the McDonald Criteria for Multiple Sclerosis.

In the diagnostic panel is a terminology deserving a precise definition in order to understand its importance for a correct diagnosis.

a. *Definition of "attack":* synonymous for relapse, it is the acute onset of neurological symptoms consistent with MS, in case of clinical evidence that causative lesions have an inflammatory and demyelinating nature. An attack has to last at least 24 hours (Poser CM, 1983). Although medical history may suggest an attack, diagnosis need to be supported by noticing clinical objectivation of at least one lesion. Moreover, a second event has to occur at least 30 days after a first event.

b. *CSF analysis:* CSF analysis gives evidence of the inflammatory nature of lesions, useful when MRI criteria are not fully satisfied. CSF analysis is considered abnormal if IgG oligoclonal bands are detected in the absence of corresponding bands in serum(Andersson M, 1994), and/or Link index is elevated (Link H, 1977). CSF analysis does not provide informations on time and space dissemination of lesions and attacks.

c. *Visual Evoked Potentials:* VEP are considered suggestive of demyelination in case of increased latency with normal amplitude and morphology (Halliday

AM, 1993); they may support the diagnosis to give objectivation of a clinical apparent or asymptomatic lesion of the visual system (Gronseth GS, 2000).

d. *MRI:* brain and spinal lesion give evidence of dissemination in time and space. The international study group has adopted Barkhof and Tintorè diagnostic criteria (Barkhof F, 1997; Tintorè M, 2000), because of higher specificity and accuracy compared to previous studies by Fazekas et al (Fazekas F, 1988) and Paty et al (Paty DW, 1988). Barkhof's Criteria required the presence of at least 3 of subsequent 4 items: 1) one lesion with enhancement after gadolinium or 9 T2-hyperintense lesions if no enhancement noticed; 2) at least 1 infra-tentorial lesion; 3) at least 1 juxtacortical lesion (U fibers involved); 4) at least 3 periventricular lesion (Table 2). Lesions are considered if larger than 3 mm; moreover, a spinal cord lesion may replace a brain lesion (Mc Donald WI, 2001). In the 2005 revision of diagnostic criteria, the International Panel reached a consensus on spinal cord lesions importance:

    1. Spinal cord MRI is a useful tool in differential diagnosis. Whether lesions have been detected in healthy subjects brain, nor have been in healthy spine (Kidd D, 1993; Bot JCJ, 2002; Lycklama G, 2003).
    2. Lesions of spinal cord suggestive of MS (no oedema; T2-hyperintense areas in spinal cord; at least 3 mm in diameter, but shorter than 2 segments; less than half transversal spinal cord involvement) are useful for diagnosis if space dissemination lacks (Dalton CM, 2003; Bot JCJ, 2004; Brex P, 1999).
    3. For dissemination in space, one spinal lesion is equal to a brain lesion; a spinal lesion enhancing after gadolinium administration is equal to a brain enhancing lesion and can be considered twice (a single spine enhancing lesion is equivalent to a brain enhancing lesion plus an infratentorial brain lesion). Moreover, a spine lesion may contribute with brain lesions to reach the 9 T2-depending lesions required by Barkhof Criteria (Table 2).
    4. Spinal lesion should be focal and well defined in T2 sequences to be considered in MS diagnosis.

5. Serial spine MRI when there is no evidence of symptomatic myelitis is not useful for dissemination in time (Dalton CM, 2003). This tool is only a support for MS diagnosis when spine involvement is clinically suspected.

Table 2. Magnetic Resonance Imaging Criteria to Demonstrate Brain Abnormality and Demonstration of Dissemination in Space

Original McDonald Criteria	2005 Revisions
Three of the following: 1. At least one gadolinium-enhancing lesion or nine T2 hyperintense lesions if there is no gadolinium-enhancing lesion 2. At least one infratentorial lesion 3. At least one juxtacortical lesion 4. At least three periventricular lesions	Three of the following: 1. At least one gadolinium-enhancing lesion or nine T2 hyperintense lesions if there is no gadolinium enhancing lesion 2. At least one infratentorial lesion 3. At least one juxtacortical lesion 4. At least three periventricular lesions
NOTE: One spinal cord lesion can substitute for one brain lesion/	NOTE: A spinal cord lesion can be considered equivalent to a brain infratentorial lesion: an enhancing spinal cord lesion is considered to be equivalent to an enhancing brain lesion, and individual spinal cord lesions can contribute together with individual brain lesions to reach the required number of T2 lesions.

Based on data from Barkhof and colleagues[20] and Tintoré and coworkers.[21]

McDonald Criteria published in 2001 consider both enhancing lesions and new T2 lesions for time dissemination. In 2005, a revision of diagnostic criteria confirmed the utility of T2 lesions for time dissemination also if appearing earlier than 3 months after a previous scan as required in 2001 Criteris (Tab. 3). However, new T2 lesions in the first weeks after onset can not be considered a new and independent event. New MRI T2 lesions have to occur at least after 1 month from disease onset. Therefore any new T2 lesion detected in whatever follow-up scan compared to a reference one done at least 1 month from onset, can satisfy MR criteria for dissemination in time (Table 3).

Original McDonald Criterion	2005 Revisions
1. If a first scan occurs 3 months or more after the onset of the clinical event, the presence of a gadolinium-enhancing lesion is sufficient to demonstrate dissemination in time, provided that it is not at the site implicated in the original clinical event. If there is no enhancing lesion at this time, a follow-up scan is required. The timing of this follow-up scan is not crucial, but 3 months is recommended. A new T2- or gadolinium-enhancing lesion at this time then fulfills the criterion for dissemination in time. 2. If the first scan is performed less than 3 months after the onset of the clinical event, a second scan done 3 months or longer after the clinical event showing a new gadolinium-enhancing lesion provides sufficient evidence for dissemination in time. However, if no enhancing lesion is seen at this second scan, a further scan not less than 3 months after the first scan that shows a new T2 lesion or an enhancing lesion will suffice.	1. There are two ways to show dissemination in time using imaging: a. Detection of gadolinium enhancement at least 3 months after the onset of the initial clinical event, if not at the site corresponding to the initial event b. Detection of a *new* T2 lesion if it appears at any time compared with a reference scan done at least 30 days after the onset of the initial clinical event

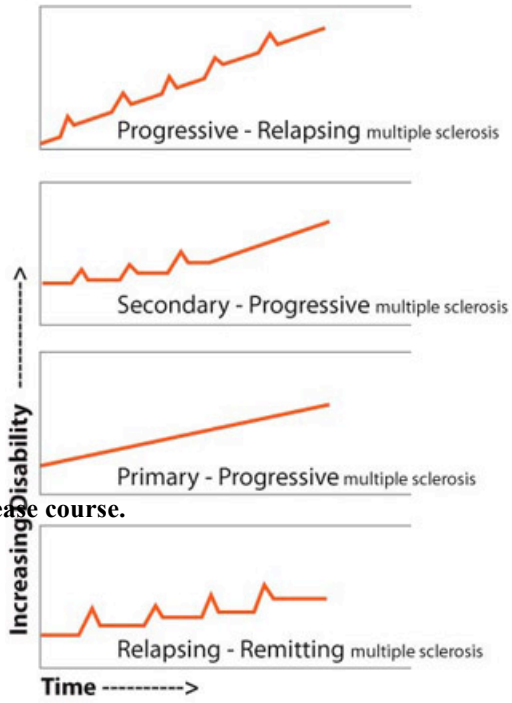
Tabella 3. MRI diagnostic criteria for Time dissemination.

In 2010 a new revision of diagnostic criteria (Polman et al, 2010) introduced the opportunity to demonstrate time dissemination with a single MRI scan (1 gadolinium enhancing lesion plus 1 or more non-enhancing lesions). In the new Criteria to demonstrate dissemination in space at least 1 lesion in two different areas (periventricular, juxtacortical, spinal cord, infratentorial) are required.

In MS differential diagnosis many medical conditions have to be considered. In young adults one has to consider vascular diseases, such as antifosfolipides syndrome, systemic ehritematousus lupus (SEL), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Takayasu disease. Some infectious diseases as Human T-lymphotropic virus Type I infection and Lyme disease may present t onset like MS. Monophasic demyelinating disease, as acute demyelinated encephalomyelitis, Devic syndrome (Wingerchuk DM, 1999) and transverse myelitis represent a diagnostic challenge.

MS may present in different forms with great variability in disability accrual. MS course is expression of two clinical phenomena: recurrence of neurological symptoms (acute onset of neurological impairment with complete or partial recovery) and progression (irreversible symptoms worsening lasting more than 3 months). Relapses are clinical manifestation of focal and recurrent inflammation in CNS (Youl BD, 1991). Several pathological (Evangelou N, 2000) and imaging (Losseff NA, 1996; Arnold DL, 1999; Ciccarelli O, 2001; Filippi M, 2003) studies have demonstrated that progression and disability are related to early and progressive axonal loss, meaning degenerative processes. In about 85% of MS patients, relapses are the only expression of disease in the first years, defining the relapsing-remitting course of MS. A percentage of these patients, increasing proportionally with disease duration, the course changes toward secondary progression. Fifteen percent of patients have a primary progressive onset. Relapses affected 40% of patients in primary and secondary progressive phases (Confavreux C, 2000, 2006).

Currently, four different disease course are recognised, according to Lublin e Reingold, 1996 (Fig.2). The most common course is the one defined as



relapsing-remitting *(RR)*, with recurrence of neurological symptoms and signs. The secondary progressive *(SP)* course follow a RR disease, evolving in chronic progression with or without superimposed relapses. Primary progressive *(PP)* MS is defined when progression of symptoms is present t onset without relapses during time. Progressive relapsing (PR) MS replicate PP MS, but shows relapses in time. In addition, few author have introduced the definition of transitional MS to indicate MS patients with an isolated relapse before or after progression (Filippi M, 2005). From population study Lyon Multiple Sclerosis Cohort we have learned that in RR MS there is an higher proportion of female patients (F=68%; M=61%) compared to secondary progressive MS. However, RR and SP MS share age at onset of RR phase, symptoms at onset, recovery from onset and remission duration between first and second attack. Otherwise, disease duration resulted almost double in the secondary progressive group (Confavreaux C, 2006). This is in line with the well known phenomenon, that sees a growing proportion of patients with an initial RR disease course will linearly convert to secondary progression over time (McAlpine D and Compston ND, 1952; Confavreux C, 1977; Wukusic S and Confavreux C, 2003). Conversion rate to secondary progressive course is about 2-3% per year (Wukusic S and Confavreaux C, 2003), with a median time to progression of 19 years (Amato MP, 2000).

Observational data collected in MS natural history studies suggest that disease phenotype and course are age-depending.

Positive prognostic factors are visual and sensory symptoms at onset and complete recovery of relapses. On the contrary, motor involvement has a

negative prognostic value. Quoad Vitam prognosis is worse if MS affects aged male. Even a high relapse rate with poor recovery and a short time between onset and second attack are considered negative prognostic factors (Weinshenker BG, 1989). However, the factor that influence disability the most is the beginning of progressive phase.

MS patients accumulate disability through 2 different mechanisms: partial recovery after relapses and disease progression. RRMS patients accumulate disability slower compared to PP patients, from the onset. Anyway, after a disability limiting the patients to walk less than 500 meters (equal to EDSS 4.0), the following disability does not correlate anymore with disease presentation (Confavreux C, 2000).

DEFINITION OF FATIGUE

Fatigue has both central and peripheral components, whose relative contribution to fatigue appears to be task dependent (Enoka RM, 1992). Central fatigue refers to an activity-induced inability to fully activate a muscle voluntarily. Peripheral fatigue implies that the ability of the muscle to produce force is reduced.

During sustained muscle actions, central and peripheral fatigue have been described to develop during maximal (Bigland-Ritchie B, 1983), as well as submaximal (Losher WN, 1996), voluntary efforts. During intermittent muscle actions, both central and peripheral fatigue develop when the effort is maximal (Taylor JL, 2000), whereas, when the effort is submaximal fatigue has been shown to be caused mainly by peripheral mechanisms (Bigland-Ritchie B, 1986). Commonly discussed factors that affect peripheral fatigue include energy supply (Sahlin K, 1998), muscle fiber-type distribution (Tesch P, 1978; Thorstensson A, 1976), muscle strength before fatigue (Hunter SK, 2003; Kanehisa H, 1997), and the length of the muscle (Fitch S, 1985). Possible mechanisms of central fatigue are suboptimal facilitation from the motor cortex (Taylor JL, 2000), decreased facilitation from muscle spindles (Macefield G, 1991), increased inhibition from group III and IV afferents (Garland SJ, 1991-1995), and desensitization of the motorneurons (Kernell D, 1969). Moreover, the ability of the central nervous system to fully activate a muscle during maximal efforts has been described to vary substantially between muscle groups, muscle action types, and individuals (Behm DG, 2002; Loscher WN, 2002; Westing SH, 1991).

FATIGUE IN MULTIPLE SCLEROSIS

Fatigue is an overwhelming sense of tiredness, lack of energy and feeling of exhaustion, which affects up to 80% of patients with MS and impacts significantly their quality of life (Fisk et al., 1994). It interferes with work, family life and social activities (Kos et al., 2008). Fatigue, as a subjective and non-specific symptom, can be often confused with weakness and/or depressed mood. Usually fatigue is present even at rest and it is worse in the second part of the day. Fatigue has not to be mistaken with fatigability, which is a generalised sense of exhaustion affecting patients after few minutes of physical activity, not present at or after rest. Fatigue may occur at any stage of MS, and affects even patients with mild disease course (Krupp et al.,1989). In some occasions, fatigue may be the first symptom of the disease, preceding of weeks or months the first disease episode. Fatigue is often present in patients as a chronic condition. However, some MS patients may experience transient fatigue because of external factors (i.e. exercise, disease-modifying therapy, temperature increase, etc). Fatigue in MS has not been clearly associated with disability (Schwartz et al., 1996).

Fatigue can be divided in peripheral, central and mental fatigue. Peripheral fatigue is generated by loss of force-generating energy within the muscles. Central fatigue is due to inability to sustain the central drive to spinal neurons. Mental fatigue is a reduction in cognitive performance affecting mostly memory and abstract thought. In multiple sclerosis, fatigue is supposed to be of central (supraspinal) origin, as demonstrated by interpolated transcranial magnetic stimuli during voluntary effort (Lee et al., 2008) and by the reduction of intracortical inhibition, pre- and post-exercise, in fatigued patients (Liepert et al., 2005). Fatigue in MS is likely to be due, at least in part, to presence of conduction block in demyelinated axons (Steve at al., 2010). This hypothesis was supported by an electroencephalographic study showing an increase in cortical activation and reduced cortical inhibition during a simple motor task in fatigued MS patients (Leocani et al., 2001).

Some 40 to 70 percent of individuals with MS who experience fatigue do so on a daily basis, typically up to 6 hours, usually in the afternoon (Freal et al., 1984;

Fisk et al., 1994). Longitudinal studies suggested that people with MS who have severe fatigue do not experience significant spontaneous improvement of fatigue over intervals as long as 2 years (Cookfair et al., 1997). This is in contrast to individuals who do not have MS, whose fatigue fluctuates significantly over intervals as short as 2 weeks (Vercoulen et al., 1996a). Krupp et al. (Krupp et al., 1988) systematically defined the following characteristics that distinguished fatigue in MS from normal fatigue:

- It comes on easily.
- It prevents sustained physical functioning.
- It is worsened by heat.
- It interferes with responsibilities.
- It interferes with physical functioning.
- It causes frequent problems.

Features that did not distinguish fatigue in individuals with MS from those without MS are worsening of fatigue associated with exercise, stress, depression, prolonged physical activity, and time of day (afternoons), and improvement of fatigue with rest, sleep, positive experiences, and sex.

At any point in time, the percentage of people with chronic fatigue in general practices ranges from 24 to 37 percent; almost 70% of these cases presumably are due to medical and psychiatric conditions (Bates et al., 1993; Kroenke et al., 1988; Buchwald et al., 1987). Although the prevalence of comorbid medical conditions associated with fatigue has not been evaluated in MS populations, MS patients are subject to all the common conditions associated with fatigue and require thorough evaluation before clinicians assume that fatigue is caused primarily by MS. Common comorbid medical conditions associated with fatigue include infectious diseases, anaemia, hypo- or hyperthyroidism, cardiovascular disease, pulmonary disease, renal disease, and hepatic disease.

Empirical literature on the relationship between stress, coping, and anxiety and fatigue is poor. Few studies address the relationship between depression and fatigue. Prevalence estimated of the rate of depression among persons with MS range from 14 to 57 percent (Joffe et al., 1987; Whitlock and Siskind, 1980),

while estimated of lifetime prevalence range from 37 to 54 percent (Minden et al., 1987; Schiffer et al., 1983).

Clark et al. reported disrupted sleep in 25 to 35 percent of patients with MS (Clark et al., 1992). Common causes were neurogenic bladder dysfunction, spasticity, spasms, anxiety, depression and pain (Clark et al., 1992). Less commonly, the aetiology is a primary sleep disorder such as sleep apnoea or periodic leg movements, but limited evidence suggests that the frequency of these disorders in MS is still much greater than in control populations (Ferini-Strambi et al., 1994). Though, it is reasonable to imagine a relationship between disrupted sleep disorders and fatigue, but studies have had mixed results. A challenge to distinguish daytime complaints of fatigue from excessive daytime sleepiness related to primary or secondary sleep disorders. Tiredness, fatigue, and sleepiness are considered similar symptoms by many patients. However, no study has directly evaluated the benefits of improved sleep on daytime fatigue.

PRIMARY MS FATIGUE

Studies that tried to identify the component of fatigue directly related to the MS disease process (primary fatigue) have investigated characteristics of MS associated with fatigue severity. A clinical trial of interferon-beta 1a in mild relapsing remitting MS showed a correlation between FSS scores and EDSS scores at baseline and at week 104 (Cookfair et al., 1997). Correlations between EDSS and FSS remained after adjusting for Beck Depression Inventory scale. The majority of low disability patients with MS (EDSS range of 1–3.5) reported severe fatigue; almost 60 percent had FSS scores of 5.0 at baseline (FSS range of 1–7). Only persons with baseline FSS scores < 5.0 showed a worsening in FSS score by week 104 (37 percent of study participants). The majority of participants showed stable and persistent levels of fatigue over the 104 weeks of the study. This ceiling effect of self-reported fatigue severity in individuals even mildly affected may be responsible for the observation in previous small cross-sectional studies reporting little or no correlation between fatigue severity and disability. The results of the Cookfair

study (1997) confirm the high prevalence of severe fatigue in even mildly disabled individuals with MS. This finding is in agreement with previous studies in which one-third to one-half of patients with MS reported fatigue precedes the onset of other symptoms of MS, even by years (Krupp et al., 1988; Fisk et al, 1994). The appearance of fatigue prior to other symptoms of MS may be directly related to MS disease process, since approximately one-third to one-half of patients presenting with their first identifiable symptoms of MS (i.e., optic neuritis, transverse myelitis) have disseminated white matter lesions at MRI that must have been present for some time prior to diagnosis (Morissey et al., 1993; Beck et al., 1993). The finding that FSS scores did not correlate with T2 lesion burden in the Cookfair et al. study does not take away attention from this hypothesis, because a study on the association of frontal lobe and basal ganglia hypometabolism with fatigue in MS also did not find any correlation between MRI abnormalities and fatigue (Roelcke et al., 1997). Despite the known association between MS disease process and fatigue, the pathophysiologic mechanisms resulting in the subjective sense of fatigue are still unclear. Four hypothetical sources of this fatigue has been identified. First, evidence from event-related potential recordings during auditory memory tasks suggests an impairment between stimulus evaluation and activation of motor programs (Sandroni et al., 1992). This is consistent with the observation of frontal lobe hypometabolism in fatigued individuals with MS (Roelcke et al., 1997) and could account for cortical component of fatigue. The mechanism of this cortical impairment is unknown, but could involve conduction block involving intracortical circuits. Second, fatigue could be a manifestation of intermittent conduction block in partially demyelinated axons of central motor pathways (Fig. 3). Current evidence supports a cortical component to the subjective sense of fatigue, since some studies were unable to substantiate this hypothesis (Sheean et al., 1997).

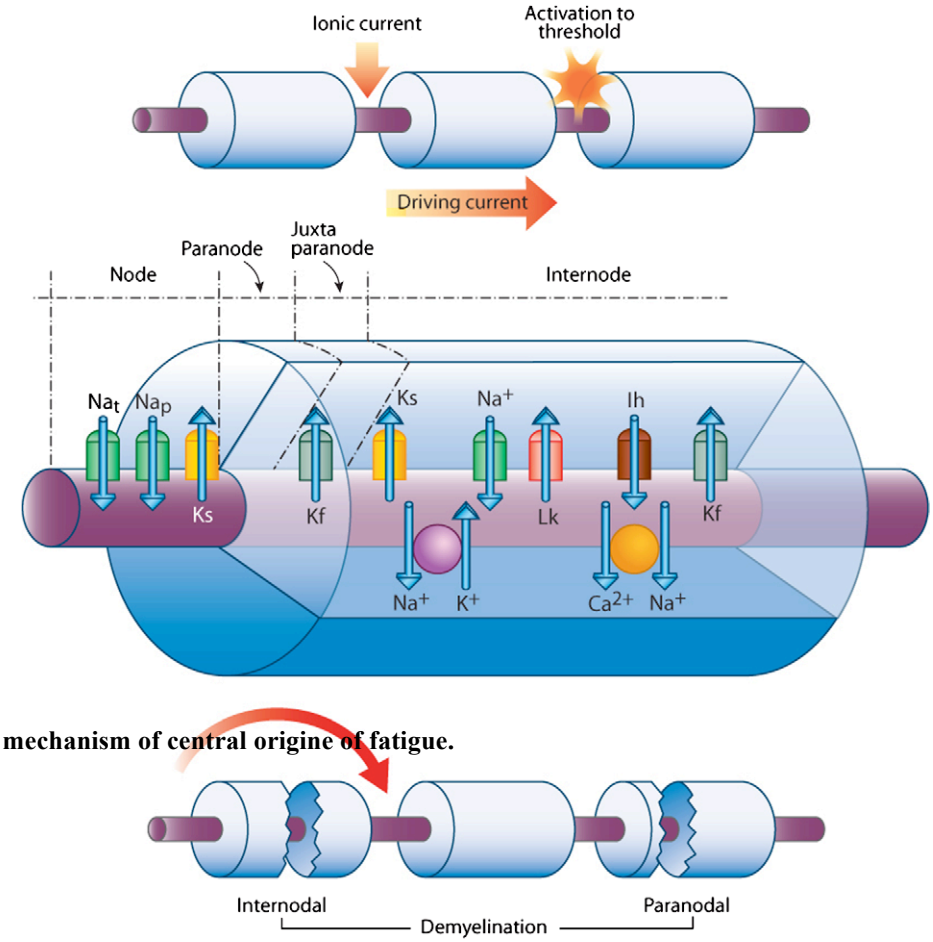


Figure 3. Possible mechanism of central origine of fatigue.

In a myelinated axon, action potentials are generated at the node of Ranvier by transient ‘‘opening” of voltage-gated sodium channels, thereby resulting in an influx of sodium ions, termed ionic current, and membrane depolarization. This influx of sodium ions sets up a driving current that excites the subsequent node to threshold, resulting in opening of more voltage-gated sodium channels and ultimately axonal conduction. The process by which an action potential is propagated or ‘‘jumps” from one node of Ranvier to the next is referred to as saltatory conduction. Different channels are distributed unevenly along the axonal membrane: two classes of sodium channels are found in high concentrations at the node, including voltage-gated sodium channels as well as persistent Na+ channels. Slow potassium channels are also present at the node as well as in the internode. Fast potassium channels are almost exclusively located in the juxta-paranode and are a redistributed to the node in demyelinated axons thereby interfering with impulse conduction and contributing to conduction failure. Inward rectifier channels are permeable to both potassium and sodium ions and act to limit the extent of axonal hyperpolarization, whereas the electrogenic sodium/potassium pump serves to reverse ionic fluxes that may be generated through activity. In demyelinated axons, the safety factor of transmission is reduced thereby resulting in either hyperpolarizing or depolarizing conduction block. Dysfunction of sodium/ potassium pump, recently reported in multiple sclerosis, results in increased intra-axonal sodium levels, which in turn causes reverse operation of the sodium–calcium exchanger, an increase in intraxonal calcium concentration and ultimately axonal degeneration.

Third, recruitment of alpha motor neurons is impaired because of corticospinal tract involvement and could result in increased energy demands for muscle activation (Rice et al., 1992). Lastly, abnormal co-activation of agonists and antagonists associated with spasticity could increase energy demands (Olgiati et al., 1988).

SECONDARY MS FATIGUE

Fatigue in MS may also be secondary to disease mechanism. Three elements are involved in secondary MS fatigue: deconditioning, respiratory muscles weakness and pain.

Deconditioning. Although MS is strictly a disease of the CNS, peripheral sources of fatigue could involve a number of mechanisms. Many studies suggested an intramuscular component to fatigue in MS (Miller et al., 1990; Kent-Braun et al., 1994; Sharma et al., 1995). Authors founded a relationship between the impaired muscle fatigue and the Ashworth score of spasticity and foot tapping rates, suggesting changes in muscle secondary to upper motor neuron impairment. However, the researchers were not able to correlate muscle fatigue with an individual's subjective sense of fatigue. These results suggest that aerobic exercise and strengthening may be important to prevent secondary changes in muscle due, in part, to deconditioning.

Respiratory Muscle Weakness. Another potential peripheral source of fatigue is respiratory muscle weakness. Even patients with low disabiity may demonstrate a reduced exercise capacity at least partially due to inspiratory or expiratory muscle fatigue (Foglio et al., 1994). As the disease progresses and patients become wheelchair-bound, respiratory muscle weakness may become an important factor of peripheral fatigue and may also result in significant sleep disruption (Smeltzer et al., 1996). The contribution of deconditioning to respiratory muscle weakness and the potential improvements in respiratory muscle function with exercise and expiratory training need to be confirmed in further studies. No studies have attempted to link subjective fatigue with respiratory muscle weakness in MS patients.

Pain. The relationship between pain and MS fatigue has not been clarified. About 40 to 53 percent of people with MS experience chronic pain, often ill-defined in aetiology (Moulin et al., 1988; Warnell, 1991; Archibald et al., 1994). Of interest is the common association of chronic widespread or regional pain, sleep disturbance, and fatigue in MS (Warnell, 1991; Archibald et al., 1994).

Fatigue is influenced by physical, social, cultural, and institutional environment. However, the evidence for these relationships is almost nonexistent. One

exception is the effect of elevations in core and ambient temperature on neurological function and fatigue, a subject reviewed by Syndulko et al. (1996). Many authors published observations and controlled studies of heating reactions since Uhthoff's initial report in 1890 estimated the incidence of heat sensitivity in individuals with MS to be between 60 percent and 80 percent. A similar incidence of heat-related worsening of MS fatigue was documented in two studies (Freal et al., 1984; Krupp et al., 1988). Moreover, this phenomenon can be observed with core temperature elevations as little as 0.5°F (i.e., normal diurnal temperature fluctuations). Therefore, heat sensitivity must be considered as a dimension of fatigue during everyday activities of daily living as well as during the heat waves of summer. The mechanisms by which heat may worsen neurological signs and symptoms in MS remain still unclear. Presumably, the enhanced susceptibility of demyelinated axons to conduction block with elevations in temperature plays an important role in this phenomenon (Davis and Jacobson, 1971; Rasminsky, 1973).

MAGNETIC RESONACE IN MS WITH FATIGUE

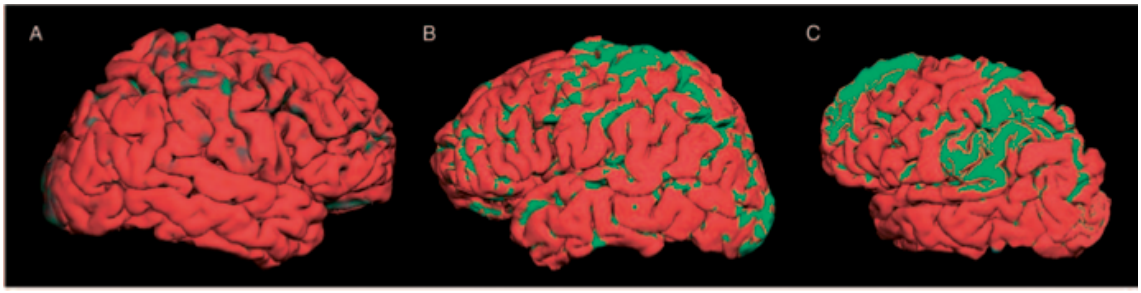
Conventional MRI studies, based on T1 and T2 lesion load, have revealed discordant findings (van der Werf et al., 1998; Mainero et al., 1999). Sepulcre et al., found a correlation between fatigue in MS and white matter (WM) lesion load (Sepulcre et al., 2009). In this study, fatigue correlates with the presence of MS lesions in the right parieto-temporal WM, a key region for attentional mechanisms. This region is particularly critical for the so-called alerting network, but it is also a part of the orienting network. Spatial inattention has been linked in humans to disruption of the right superior occipitofrontal fasciculus, lesions of which correlated with the presence of fatigue in Sepulcre's study. That also showed an association between fatigue and Other deep WM of the left frontal lobe, including the anterior corpus callosum.

More recently, morphometric MRI has shown a correlation between fatigue and white and grey matter volume (Tedeschi et al., 2007), partially supporting other findings which suggested a link between fatigue and dysfunction of deep grey matter regions (Inglese et al., 2007). The results of Tedeschi et al. suggest that

fatigue is correlated with diffuse CNS involvement even in MS patients with low disability, as patients with high-fatigue showed significantly higher lesion load, and WM, grey matter (GM) atrophy than patients with low-fatigue. Although patients with higher FSS had higher lesion load, the univariate and multivariate statistical analysis did not identify lesion load as a risk factor of fatigue. Conversely, data suggest that fatigue, independent of disability, is significantly related to a destructive pathological process involving both WM and GM (as measured by WM and GW atrophy). Previous MRI studies on smaller patients' samples have unsuccessfully attempted to correlate fatigue and MRI measures such as T2 lesion load, brain atrophy, monthly gadolinium enhancing lesions and magnetization transfer ratios (Van den Werf et al., 1998; Bakshi et al., 1999; Mainero et al., 1999; Codella et al., 2002). Recently, in a longitudinal study performed in a cohort of 134 patients with MS (Marrie et al., 2005) no correlation was found between fatigue (measured by the Sickness Impact Profile's Sleep and Rest Scale) and brain atrophy at baseline. However, there was a significant association between worsening fatigue during the initial 2 years and progressive brain atrophy over the next 6 years, when comparing patients who showed an increased fatigue with those who showed a decreased fatigue.

Other imaging studies have hypothesized that fatigue is related to damage of specific white matter pathways and cortical areas in the frontal and parietal regions (Sepulcre et al., 2009). In GM analysis, the authors found that atrophy of areas of the left superior frontal gyrus and both middle frontal gyri correlated with the presence of fatigue. As the areas in the left hemisphere are closely related to the WM regions where more lesions are located, their atrophy could be construed as indicating retrograde degeneration of neurons projecting through the affected WM region or transynaptic degeneration after axon transection in the WM lesions. Moreover these results suggest the presence of an atrophy threshold for the generation of fatigue, because of founding an increasing association between fatigue score and cortical volume going from the healthy group to non-fatigue MS patients and from these to fatigued MS patients.

Roelcke et al. suggested a possible role of the striatum–thalamus–frontal cortex pathway in the genesis of fatigue, suggested by the finding of a reduced metabolic activity of the putamen, the lateral and medial prefrontal cortex, the premotor cortex and the right supplementary motor area in severely fatigued MS patients (Roelcke et al. 1997). Calabrese et al. confirmed and extended these observations, and further indicated that a pathological process affecting the striatum–thalamus–frontal cortex pathway occurs in fatigued RR MS. Indeed, while a diffuse frontal-temporal cortical thinning characterizes patients with RR MS compared to healthy subjects, a more pronounced thinning of the cortical areas involved in the striatum–thalamus–frontal cortex pathway distinguishes fatigued RR MS patients (Calabrese et al., 2010) (FIG.4) from non-fatigue MS patients.



The main finding of Andreasen et al. was that primarily fatigued MS patients have regional atrophy involving functionally related grey matter structures and nearby white matter. Principal areas were the frontal and parietal lobes and the basal ganglia. The two largest atrophy clusters involved grey and white matter in/nearby the dorsolateral prefrontal cortex and posterior parts of the parietal lobe (FIG.5).

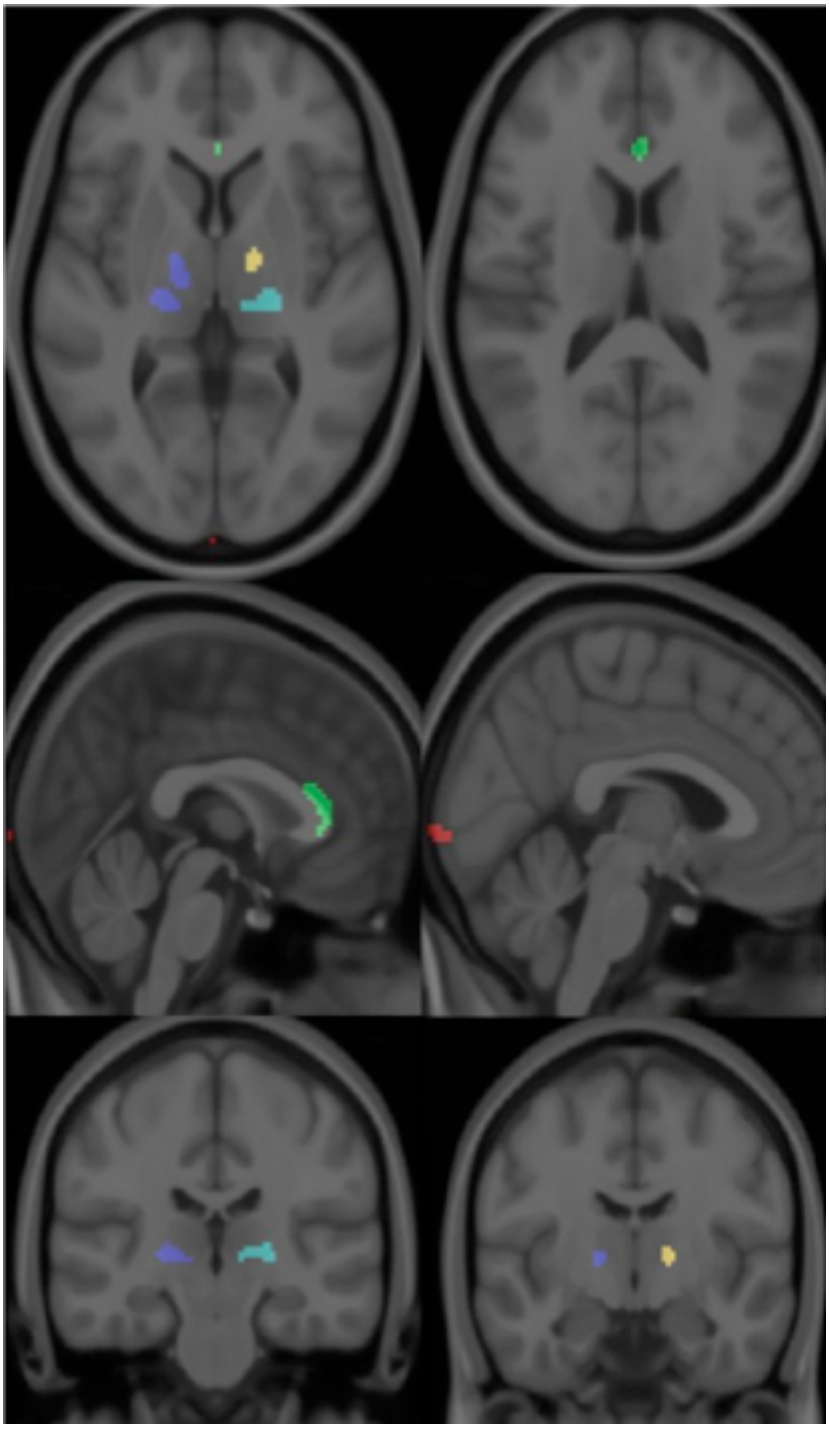


Figure 5. Brain atrophy in deep grey matter structures.

Also other clusters involved the limbic lobe, the anterior cingulate and mesial aspect of the superior frontal gyrus (Andreasen et al., 2010). These findings demonstrated a concordance between central muscle activation impairment and atrophy within areas of the motor circuit among primary fatigued patients, involving the primary motor cortex and premotor area. This may suggest that

the consequences of regional atrophy, with respect to physical disability, are reduced because of functionally compensatory activity, and that the same mechanism induces increased neuronal work and thereby triggers the perception of motor fatigue. Also, regional atrophy in primary fatigued patients compared to healthy controls involved the thalamic ventral anterior and ventral lateral nuclei which are involved in motor planning and action execution (Draganski et al., 2008). In non-fatigued patients regional atrophy was found within the thalamus compared to healthy controls. In all cases, nine clusters of atrophy were identified, and several appeared within the motor network from cerebellum and rostral to that. The involvement of the anterior cingulate, medial frontal cortex and striatum may imply that motivational disturbances play a role. Lesions within these areas have been correlated to lack of spontaneity, apathy and akinetic mutism. The recent finding of regional atrophy affecting cortex and adjacent white matter and the involvement of the basal ganglia strongly support the cortico-subcortical-circuit interruption as a pathological substrate of primary fatigue in MS. This is emphasized by the convergence of central motor activation impairment and atrophy within areas of the motor circuit in primary fatigued patients including premotor cortex and primary motor cortex.

Studies of functional MRI have shown widespread cortical activation during a simple motor task in RR, primary progressive (PP) and secondary progressive (SP) MS (Filippi et al., 2002). MS patients show greater pre-fatigue activation of brain regions involved in motor responses (precentral gyrus, cerebellum, insula and cingulated gyrus), compared to healthy controls. Increase in cortical activation has been interpreted as an adaptive response to dysfunction of motor pathways (FIG.6).

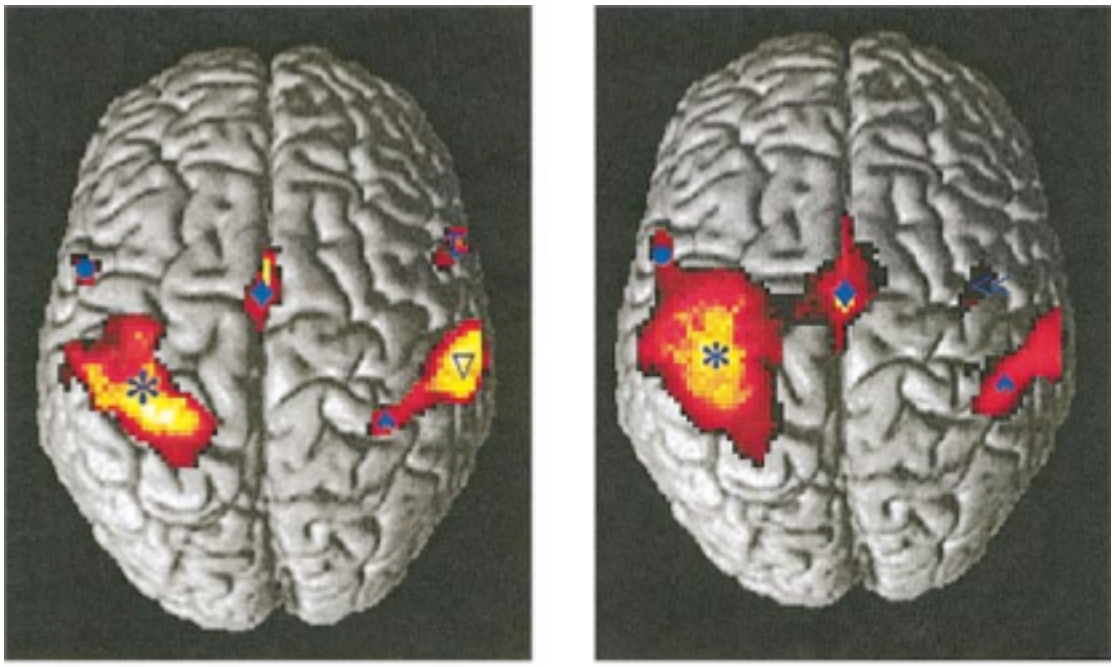


Figure 6. Activated areas in non fatigue MS patients (on the left) and fatigue MS patient (on the right). From Filippi et al., 2022

Fatigue in MS may be a result of cortico-subcortical (thalamus and basal ganglia) interactions during motor tasks planning and execution. Moreover, an increase in activation of anterior cingulated cortex, involved in attentional task and executive functions, has been reported in MS patients affected by fatigue, probably as a compensatory mechanism (Filippi et al., 2002). In addition, the some group with Rocca et al., investigated temporary fatigue in MS patients under weekly treatment with IFNβ-1a (Rocca et al., 2007). The authors showed a progressive reduction of the activation of the primary Sensory Motor Cortex during follow-up, independently of the presence/absence of fatigue, in the two groups of patients. The within-group analysis of fMRI changes in this study also showed different patterns of brain recruitment in MS patients with and without reversible fatigue. In particular, during tasks, MS patients with reversible fatigue after IFNβ-1a injection showed increased activations of the basal ganglia (thalami and lenticular nuclei). Conversely, in MS patients without reversible fatigue, before IFNβ-1a injection, the thalamus was more activated, as well as the cerebellum. Compared to MS patients with reversible fatigue, those with permanent fatigue tended to have an increased recruitment of the SII, cerebellum, and several regions in the parietal lobes. Conversely, MS patients

with reversible fatigue showed increased activations of the primary SMC, basal ganglia, thalami, SMA, CMA, and several regions located in the frontal lobes (FIG.7).

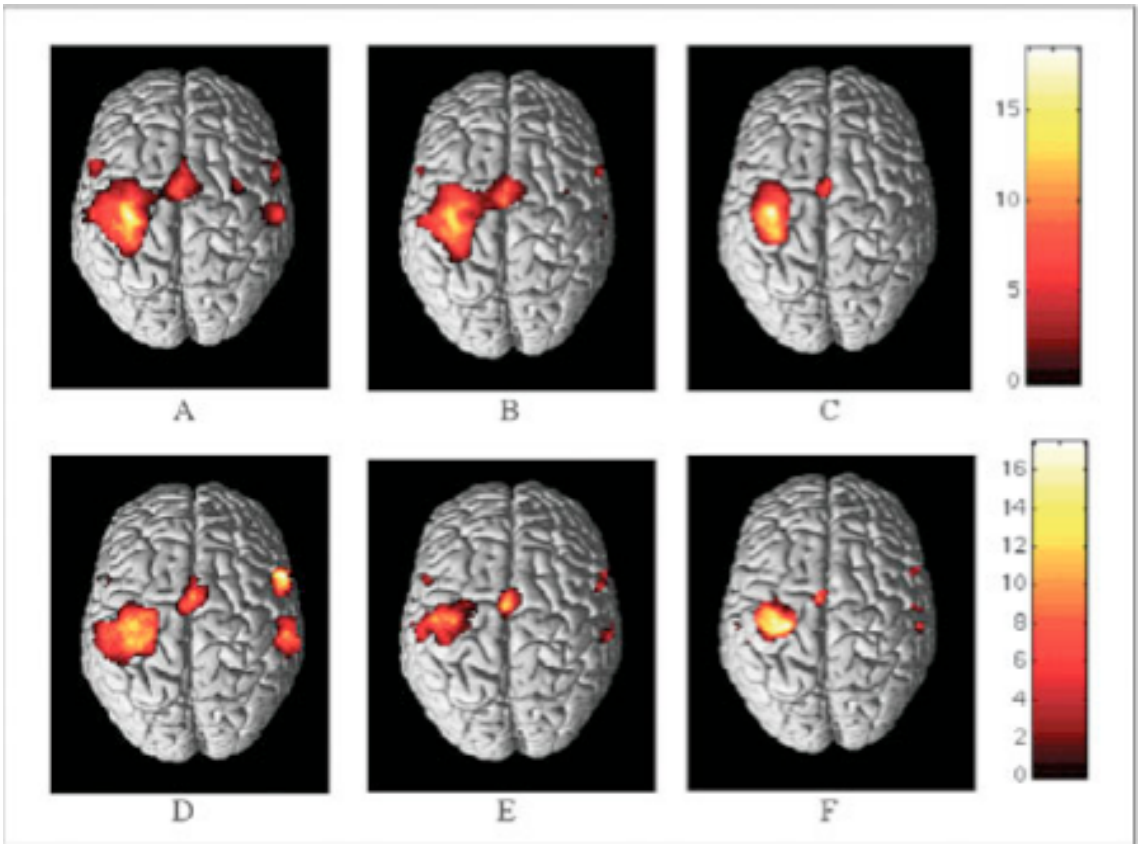


Figure 7. Activeted areas in MS patients affected by fatigue secondary to IFNB-1° injection. From Rocca et al., 2007.

All these studies suggest that some MS patients might have an increased susceptibility to fatigue, which is related to a different sensorimotor circuitry recruitment during the performance of motor activity. In particular, it seems likely that potentially fatigable MS patients tend to have a baseline "overactivation" of the basal ganglia, frontal lobes, and cingulum. With time, this "overactivation" might result in a depletion of the functional properties of these regions and in their inability to undergo the dynamic changes observed in MS patients without reversible fatigue and, as a consequence, lead to the onset of "chronic" fatigue.

Lastly, cerebral metabolism studies support this findings, having demonstrated a reduced glucose metabolism in cortical motor and basal ganglia regions which correlates with fatigue severity (Roelcke et al., 1997). The authors compared

two MS groups, showing predominant cerebral glucose metabolism reductions bilaterally in a prefrontal area involving the lateral and medial prefrontal cortex and adjacent white matter, in the premotor cortex, putamen, and in the right supplementary motor area of MS fatigued patients. In addition, there was cerebral glucose metabolism reduction in the white matter extending from the rostral putamen toward the lateral head of the caudate nucleus. Fatigue severity scale values were inversely related to cerebral glucose metabolism in the right prefrontal cortex. Cerebral glucose metabolism in the cerebellar vermis and anterior cingulate was relatively higher in fatigued MS patients than in non-fatigued patients. These data strengthen the hypothesis that fatigue in MS is associated with frontal cortex and basal ganglia dysfunction that could result from demyelination of the frontal white matter.

AIM OF THE STUDY

Fatigue in MS is likely of central origin. MS patients with fatigue are supposed to have demyelinating lesions within specific brain networks involved in maintaining a central drive to motor neurons. As reported by previous functional MRI studies, fatigued MS patients show greater activation of cerebral areas and a greater number of brain activated areas, compared to healthy subjects and MS patients without fatigue. These findings are believed to reflect adaptive changes and brain plasticity. On the other hand, correlations between specific cerebral networks activation and brain atrophy support a neurodegenerative origin of fatigue.

In the present study a group of mildly disabled RRMS patients with and without fatigue and a group of healthy subjects underwent conventional, morphometric and functional MRI, with the following objectives: 1) Defining the pattern of activated brain areas in MS patients with fatigue during the performance of a simple motor task at rest (primary outcome) and after a maximal effort hand-grip exercise

3) Comparing the characteristics of activated areas across groups (controls, non-fatigued and fatigued MS subjects)

4) Correlating brain activation patterns and brain volumes with clinical variables (neurological disability, fatigue severity, etc).

METHODS

STUDY SUBJECTS' SELECTION:

Patients will be selected from the MS Outpatient Clinic at G. Rossi Hospital in Verona (Italy) according to the following inclusion criteria:

1. diagnosis of MS according to Polman's criteria;
2. relapsing-remitting disease course;
3. age 18-60;
4. EDSS=0-4.5;
5. right-hand dominance (Edinburg Handedness Inventory score >=0.90);
6. complaint of fatigue for at least 50% of days for more than 6 weeks;
7. mean Fatigue Severity Scale score equal to or higher than 5.0. An additional group of RRMS patients without fatigue and with mean FSS score <5.0 – matched by age, gender, and EDSS score to patients with fatigue - will be selected from the same source.

Exclusion criteria will be:

1. motor/sensory impairment of the right upper limb which could interfere with f-MRI evaluation;
2. one or more relapses in the previous year;
3. steroids in the previous year;
4. treatment with amantadine and/or 4-aminopyridine within the previous month;
5. Montgomery and Asberg Depression Rating Scale score >=17.

Disease-modifying therapies are permitted. A group of right-handed healthy subjects, age- and gender-matched to MS patients, will be used as normal controls.

Table 2. Study Inclusion Criteria.

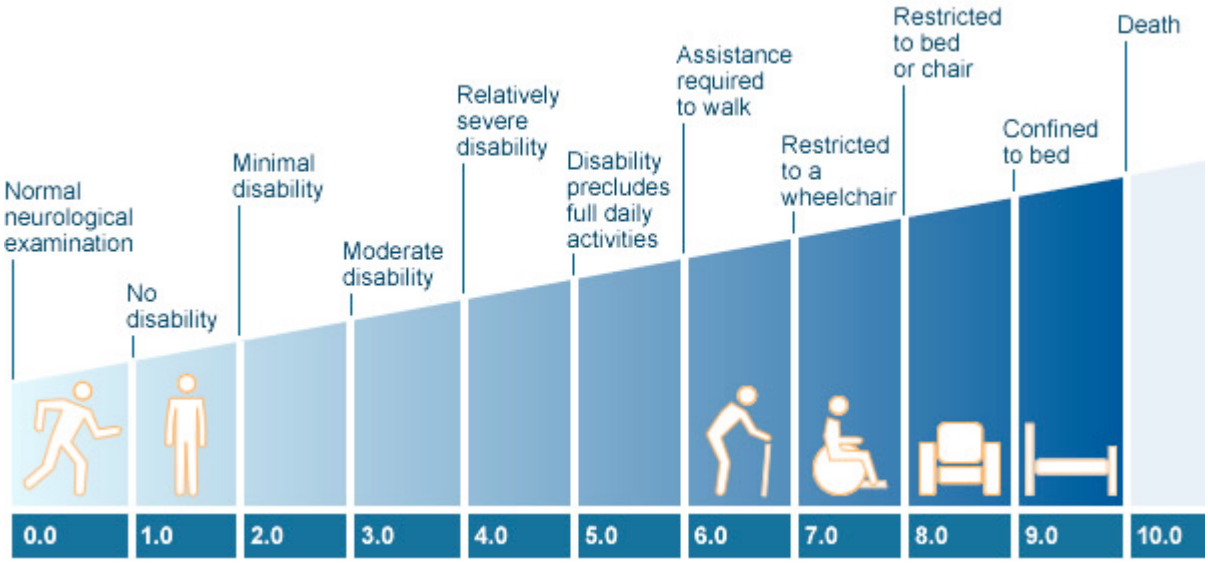
Fatigue	n-Fatigue	Controls
Right-handed dominance Age 18 - 55		
Diagnosis of MS according to Polman's Criteria		Age and Sex Matched
Relapsing-remitting disease course		
0 < EDSS ≤ 4.5		
Fatigue ≥ 50% of day in the last 6 weeks	Fatigue < 50% of day in the last 6 wks	
FSS ≥ 5	FSS < 5	

CLINICAL VARIABLES AND MEASURES:

Demographic and clinical variables will include: age; gender; education; age at disease onset; disease duration.

The following scales will be administered to patients:

Expanded Disability Status scale (EDSS): The EDSS quantifies disability in eight Functional Systems (FS) and allows neurologists to assign a Functional System Score (FSS) in each of these. The Functional Systems are: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, mental, other. EDSS steps 1.0 to 4.5 refer to people with MS who are fully ambulatory. EDSS steps 5.0 to 9.5 are mostly defined by the impairment of ambulation. EDSS step 10 means death due to MS (FIG.8).



Multiple Sclerosis Functional Composite (MSFC): The MSFC is a multidimensional clinical outcome measure that includes quantitative tests of leg function/ambulation (Timed 25-Foot Walk), arm function (9-Hole Peg Test), and cognitive function (Paced Auditory Serial Addition Test, PASAT). The MSFC is based on the concept that scores for these three dimensions - arm, leg, and cognitive function - are combined to create a single score that can be used to detect change over time in MS patients. This is done by creating Z- scores for each component of the MSFC and averaging them to create an overall composite score (the MSFC score). The components of the MSFC are:

1) *Time 25-Foot Walk*: The Timed 25-Foot Walk is a quantitative measure of lower extremity function. It is the first component of the MSFC administered at each visit. The patient is directed to one end of a clearly marked 25-foot course and is instructed to walk 25 feet as quickly as possible, but safely. The task is immediately administered again by having the patient walk back the same distance. Patients may use assistive devices when doing this task.
2) *9-Hole Peg Test*: The 9-HPT is a quantitative measure of upper extremity (arm and hand) function. It is the second component of the MSFC to be administered. Both the dominant and non-dominant hands are tested twice (two consecutive trials of the dominant hand, followed immediately by two consecutive trials of the non-dominant hand).
3) *PASAT* is a measure of cognitive function that specifically assesses auditory information processing speed and flexibility, as well as calculation ability. The PASAT is presented on audiocassette tape or compact disc (CD) to control the rate of stimulus presentation. Single digits are presented every 3'' and the patient must add each new digit to the one immediately prior to it. The test score is the number of correct sums given (out of 60 possible) in each trial. To minimize familiarity with stimulus items in clinical trials and other serial studies, two alternate forms have been developed; the order of these should be counterbalanced across testing sessions. The PASAT is the last measure of the MSFC that is administered at each visit.

Edinburgh Handedness Inventory: it is a measurement scale used to assess the dominance of a person's right or left hand in everyday activities. The inventory can be used by an observer assessing the person, or by a person self-reporting hand use. The latter method tends to be less reliable due to a person over- attributing tasks to the dominant hand. Patients scoring equal to or higher than 0,90 are considered having right-hand dominance.

Montgomery and Asberg Depression Rating Scale (MADRS): it is a ten-item diagnostic questionnaire which psychiatrists use to measure the severity of depressive episodes in patients with mood disorders. Patients

with a score equal to or higher than 17 were considered affected by depression and were not included in the study.

Fatigue Severity Scale (FSS): it is a method of evaluating fatigue in MS and other conditions including Chronic Fatigue Immune Dysfunction Syndrome (CFIDS) and Systemic Lupus Erythmatosus (SLE). The FSS is designed to differentiate fatigue from clinical depression, since both share some symptoms. The FSS is a questionnaire containing nine statements that attempt to explore severity of fatigue symptoms. The subject is asked to read each statement and circle a number from 1 to 7, depending on how appropriate he/she feels the statement applied to him/her over the preceding week. A low value indicates that the statement is not very appropriate whereas a high value indicates agreement. Fatigue cut-off is FSS score equal or higher than 5.0.

Just before MRI scans patients will be evaluated with finger tapping rate. The subjects are asked to put their hands on the table in the prone position, with fingers approximately 1 cm apart from each other. They then lift their fingers (except thumbs) and then tap the table in the following order: little, ring, middle, and index finger. They are asked to perform this movement as fast as they can for one minute. Rate will be obtained dividing the numbers of tapping by 60 seconds (n°/s).

IMAGING EVALUATIONS:

Functional MRI Acquisition:

Functional images will be acquired on a 1.5 T MR scanner (Symphony, Siemens, Erlangen, Germany) equipped with EPI capability and a standard transient/receive (TR) head coil and foam cushions to minimize head movement. The subject's head will be stabilized with adjustable padded restraints on both sides. Study subjects will be instructed to remain as still as possible throughout the experiment. Functional MRI scanning will be conducted

with a T2-weighted echo planar imaging (EPI) sequence (36 slices, slice thickness 3 mm); In the protocol, 110 volumes will be acquired, alternating five activation and five control cycles (rest), resulting in a 5 min EPI recording.
Two different conditions will be used:
1) Resting State – low-frequency BOLD fluctuations (LFBFs) fMRI
2) Simple task: finger-tapping stimulation
The fMRI paradigm used will be a block design sequence (BABAB), for the motor task, with periods of a 30 seconds of rest (B) and periods of 30-seconds visually cued hand movement (A). The sequence (BABAB) was repeated before and after the fatigue protocol. Fatigue protocol consists of a maximal effort hand-grip exercise sustained for 3 minutes. Patients will be scanned while performing movements of flexion-extension of the last four fingers of the right hand. Patients will be instructed to avoid mirror-movements during MRI acquisitions and will be asked to keep their eyes closed during MRI.
Body temperature will be assessed before f-MRI evaluation

Structural MRI acquisition:

Conventional images will be acquired using turbo spin-echo proton density- and T2-weighted sequences on the axial plane and spin-echo T1-weighted images (sagittal slice orientation). Brain tissues volumes will be calculated with SIENAX, tool in FSL, obtaining a total brain volume (TBV), grey matter (GM) volume and white matter (WM) volume.

Clinical and structural MRI measures analysis:

Demographic, clinical and radiological variables differences between fatigued and non-fatigued MS patients will be analyzed with t test and Kruskal-wallis test. Correlations between clinical variables, radiological measures and functional scale scores will be analyzed with Spearman's correlation coefficient.

Functional MRI analysis:

Image post-processing will be performed on an independent computer workstation. f-MRI data will be analyzed using the Brainvoyager software and FSL software. First, images will be realigned to the first one to correct for subject motion, spatially normalized into the stereotaxic space of Talairach and Tournoux (1988), and smoothed wit a 10-mm, 3D Gaussian filter. Variations in BOLD contrast associated with motor task will be assessed on a pixel-by-pixel basis with general linear model and the theory of Gaussian fields. Significant hemodynamic changes for each contrast will be assessed using t statistical parametric maps. A random-effect analysis will be performed, for comparison between groups; the paired t-test will be used to assess activation differences within individual groups, while ANOVA and t test will be used for comparison across groups. The correlation between BOLD changes and FSS scores and brain volumes will be also evaluated with a linear regression analysis.

Resting state analysis was performed with MELODIC, tool of FSL. MELODIC 3.0 uses Independent Component Analysis to decompose a single or multiple 4D data sets into different spatial and temporal components. For ICA group analysis, MELODIC uses either Tensorial Independent Component Analysis (TICA, where data is decomposed into spatial maps, time courses and subject/session modes) or a simpler temporal concatenation approach. MELODIC can pick out different activation and artefactual components without any explicit time series model being specified.

Structural MRI analysis:

Brain tissue volume, normalised for subject head size, was estimated with SIENAX (Smith 2001, Smith 2002), part of FSL (Smith 2004). SIENAX starts by extracting brain and skull images from the single whole-head input data (Smith 2002). The brain image is then affine-registered to MNI152 space (Jenkinson 2001, Jenkinson 2002) (using the skull image to determine the registration scaling); this is primarily in order to obtain the volumetric scaling factor, to be used as a normalisation for head size. Next, tissue-type segmentation with partial volume estimation is carried out (Zhang 2001) in order to calculate total

volume of brain tissue (including separate estimates of volumes of grey matter, white matter, peripheral grey matter and ventricular CSF).

SAMPLE SIZE DETERMINATION:

Very few studies have focused on sample size calculation in the f-MRI research field.

We calculated sample size according to the functional MRI parameters considered as the primary outcome of the study (i.e. pattern of activated brain areas in MS patients with fatigue during the performance of a simple motor task). We used the same assumptions of a previous study which focused on power analysis for within-group f-MRI experiments in a single population, using a verbal working memory task to obtain estimates of percent signal change and of intra-subject and inter-subjects variability in order to generate power curves. These estimates were used in simulation experiments (somato-sensory stimulation) showing a good correspondence with the results of the cognitive task experiment (JE Desmond et al., 2002).

In order to detect a signal change before and after motor task performance of approximately 0.5% and a spatial smoothing at FWHM of 5 mm, at least 12 patients are needed to insure a 80% power with alfa=0.05 at the single voxel level.

STATISTICAL ANALYSIS

Brain f-MRI was analysed with Brainvoyager QX 2.0 AND FSL. Statistical analysis was conducted with SPSS 16.0.

Analysis between pre and post application of “fatigue protocol” were conducted with paired t-Test and Wilcoxon Rank Test. Analysis between groups were conducted with unpaired t-test, Mann-Whitney Test and ANOVA.

RESULTS

Sample description.

Twelve MS patients (6 with fatigue and 6 without fatigue) were selected from the database of Outpatients Clinic in Policlinico Hospital in Verona. Tree healthy subjects were enrolled in the study. In table are shown demographic and clinical data (TAB.3).

	SEX	AGE§	EDSS§	MSFC (z) §
n-Fatigue	3 F 3 M	39 (28-49)	2 (0-4)	-0,6506 (-2,1919-0,1018)
Fatigue	5 F 1 M	48 (40-60)	2 (1-2,5)	-0,3914 (-2,2828-0,1462)

	9HPT§	25FWT§	PASAT§
n-Fatigue	-0,6782 (-2,0594-1,2623)	0,3121 (-0,7421-0,4502)	-1,2859 (-3,4802-0,7426)
Fatigue	-0,1980 (-1,2970-0,4356)	0,3165 (0,1609-0,4195)	-0,4579 (-3,0662 - -0,1681)

§: Median and range

Table 2. Summary of demographical and clinical variables of study groups.

The two groups of patients, MS with fatigue and MS without fatigue, did not differ fin terms of demographical variables and disability, expressed both with EDSS score and MSFC and its sub-items (25FWT, 9HPT and PASAT) (TAB.4).

Patient #	Sex	Age	EDSS	MSFC	BV§	GMV§	WMV§
1	F	60	2,5	-0,339	1418907,70	707317,21	711590,49
2	M	40	2,0	-2,2828	1592113,46	798597,81	793515,65
3	F	53	2,0	0,0049	1515526,79	735764,89	779761,90
4	F	49	2,0	-0,8091	1408599,68	705374,31	703225,37
5	F	43	1,0	-0,4490	1545471,57	748944,56	796527,01
6	F	47	2,5	0,1462	1490771,78	773567,70	717204,08
7	M	28	0,0	-2,1919	1501808,87	720204,63	781604,24
8	F	49	3,0	-1,2692	1442068,74	731388,18	710680,56
9	M	40	4,0	-0,7120	1417498,30	798363,83	709134,47
10	F	47	1,0	-0,5892	1442394,12	715878,13	726515,99
11	F	33	1,0	0,1018	1565159,42	781054,43	784104,99
12	M	38	3,5	-0,0906	1595570,87	766891,69	828679,18

Demographical, clinical and structural data of MS patients.
F: Female; M: Male; EDSS: Expanded Disabilituy Status Scale; MSFC: Multiple Sclerosis Functional Composite; BV: Brain volume; GMV: Grey Matter volume; WMV: White Matter volume.
§ mm^3

S PATIENTS VS
EALTHY
JBJECTS

MRI EVALUATION

MORPHOLOGICAL EVALUATION

Analysis of total brain, grey matter and white matter volume were conducted running Sienax on T1 3D images. This analysis was conducted on MS patients, with and without fatigue, and on healthy subjects.

The results of the analysis are shown in TAB.5, while FIG.9 shows segmentation process on patients.

	SEX	Brain Vol§	GM Vol§	WM Vol§
n-Fatigue	3 F 3 M	1472100 (1417498,30-1595570,87)	725796,40 (708363,83-781054,43)	754060,11 (709134,47-828679,18)
Fatigue	5 F 1 M	1503100 (1408599,68-1592113,46)	742354,72 (705374,31-798597,81)	748482,99 (703225,37-796527,01)

§: Median and range

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	Sig. (2-tailed)
Brain Volume	Equal variances assumed	,046	,834	,027	10	,979
	Equal variances not assumed			,027	9,996	,979
Grey Matter Volume	Equal variances assumed	,213	,655	,395	10	,701
	Equal variances not assumed			,395	9,572	,701
White Matter Volume	Equal variances assumed	,048	,832	-,242	10	,814
	Equal variances not assumed			-,242	9,900	,814

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	Sig. (2-tailed)
Brain Volume	Equal variances assumed	1,420	,255	-2,386	13	,033
	Equal variances not assumed			-3,071	4,648	,031
Grey Matter Volume	Equal variances assumed	1,545	,236	-2,542	13	,025
	Equal variances not assumed			-3,306	4,759	,023
White Matter Volume	Equal variances assumed	6,937	,021	-1,889	13	,081
	Equal variances not assumed			-2,638	5,659	,041

Table 3. Structural variables and statistical analysis of study groups.

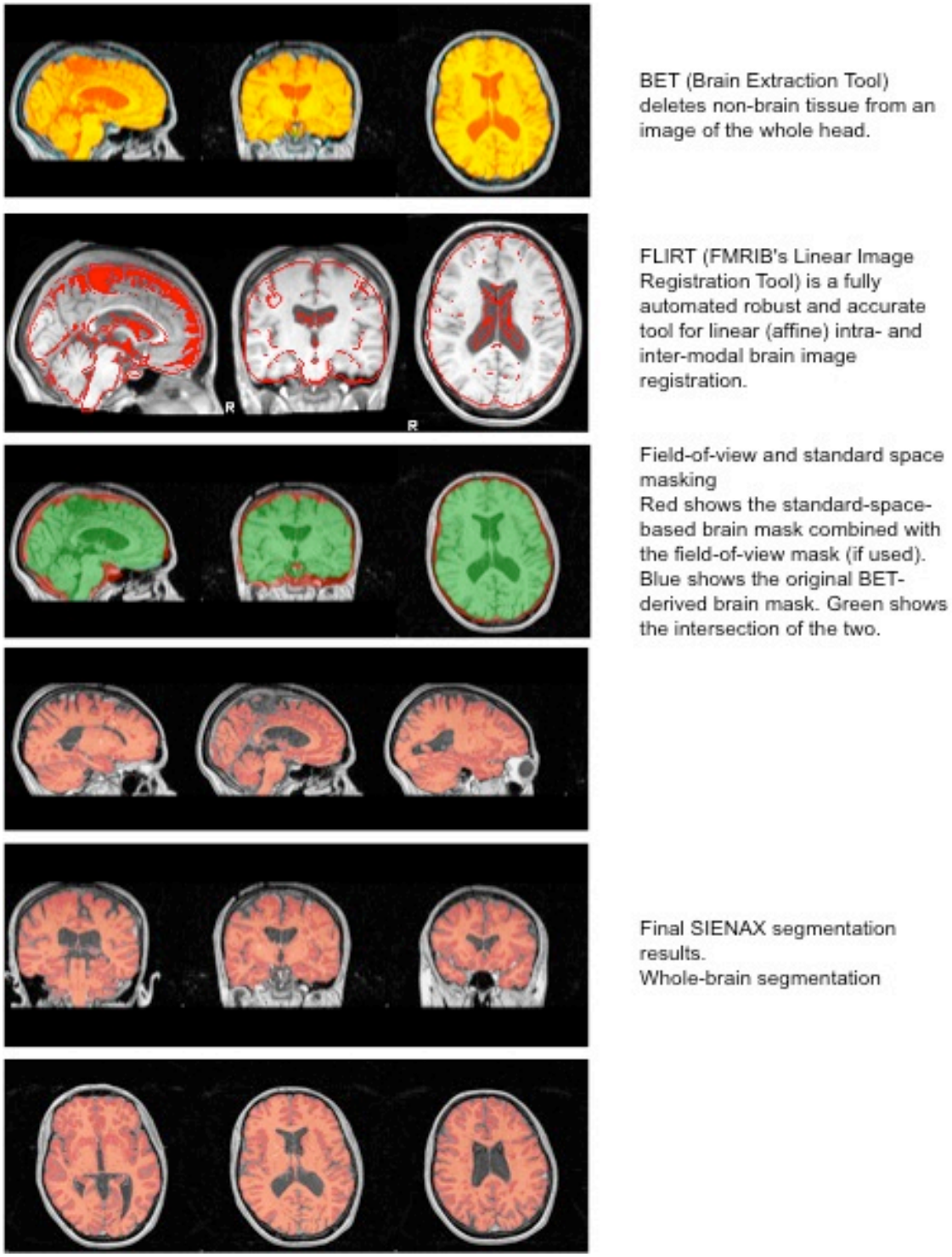


Figura 9. Segmentation processes in SIENAX analysis.

The between-group analysis on fatigued versus not-fatigued MS patients did not reveal any differences in volumes (TAB.). The between-group analysis on MS patients versus healthy subjects showed a significantly higher total brain

volume, grey matter volume and white matter volume in controls compared to MS patients (TAB.).

There was no correlation between FSS score and brain volumes.

FUNCTIONAL EVALUATION

In order to reduce artefact and noise-related signal components, a series of mathematical operations is typically performed prior to statistical data analysis. The most essential steps of these preprocessing operations are:

1) Removal of global signal fluctuations
2) Slice scan timing correction
3) Head motion detection and correction (FIG.10)
4) Spatial and temporal smoothing of the data
5) Removal of linear and non-linear trends in voxel time courses

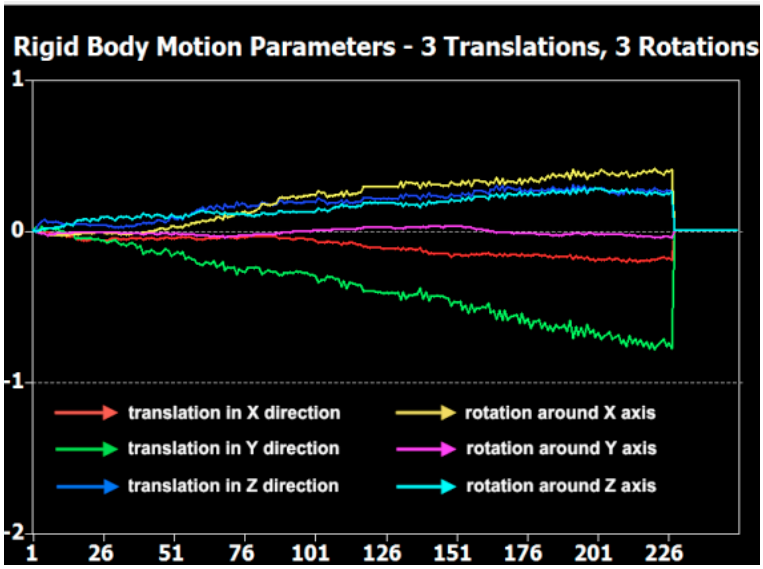


Figure 10. Head motion detection and correction.

To guarantee a good alignment result in any situation, the coregistration (FIG.11) process has been split into two main stages:

1) Initial alignment (IA)
2) Fine-tuning alignment (FA)

The goal of the initial alignment step is to bring the functional and anatomical data sets in close proximity from a potentially very different starting point. If, for example, the functional data is recorded in the axial plane while the anatomical

data is recorded in the sagittal plane, the initial alignment should bring them at least in the same global orientation, i.e. by using large rotations and translation values. The fine-tuning alignment step on the other hand assumes that the two data sets are already pretty close to each other, which may allow iterative minimization techniques to do an automatic fine adjustment of the alignment in a similar way as during 3D motion correction.

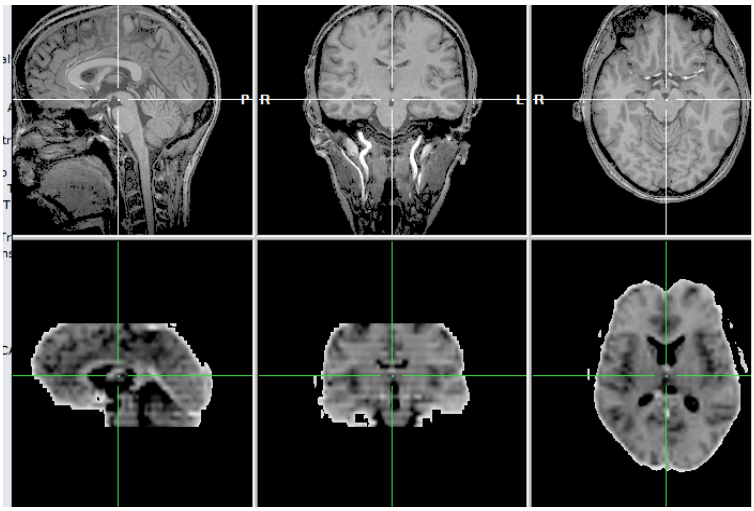


Figure 11. Coregistration of functional scanning on anatomical images.

In the original definition (Talairach & Tournaux, 1988), the midpoint of the anterior commissure (AC) is located first, serving as the origin of Talairach space. The brain is then rotated around the new origin (AC) so that the posterior commissure (PC) appears in the same axial plane as the anterior commissure. The connection of AC and PC in the middle of the brain forms the y-axis of the Talairach coordinate system. The x-axis runs from the left to the right hemisphere through AC. The z-axis runs from the inferior part of the brain to the superior part through AC. In order to further specify the x and z-axes, a y-z plane is rotated around the y (AC-PC) axis until it separates the left and right hemisphere (mid-sagittal plane). This completes the first part of Talairach transformation, often called AC-PC transformation. The obtained AC-PC space is attractive for individual clinical applications, especially presurgical mapping and neuronaviagation since it keeps the original size of the brain intact while providing a common orientation for each brain.

For a full Talairach transformation, a cuboid in AC-PC space is defined that runs parallel to the three axes defined in the first step enclosing precisely the cortex. This cuboid or bounding box requires specification of additional landmarks specifying the borders of the cerebrum. The bounding box is sub-divided by several sub-planes. The midsagittal y-z plane separates two sub-cuboids containing the left and right hemisphere, respectively. An axial (x-y) plane through the origin separates two sub-cuboids containing the space below and above the AC-PC plane. Two coronal (x-z) planes, one running through AC and one running through PC, separate three sub-cuboids; the first contains the anterior portion of the brain anterior to the AC, the second contains the space between AC and PC and the third contains the space posterior to PC. These planes separate 12 sub-cuboids. In a final Talairach transformation step, each of the 12 sub-cuboids is expanded or shrunken linearly to match the size of the corresponding sub-cuboid of the standard Talairach brain. To reference any point in the brain, x, y, z coordinates are specified in millimeters of Talairach space. Talairach & Tournoux (1988) also defined the proportional grid to reference points within the defined cuboids (Fig.12).

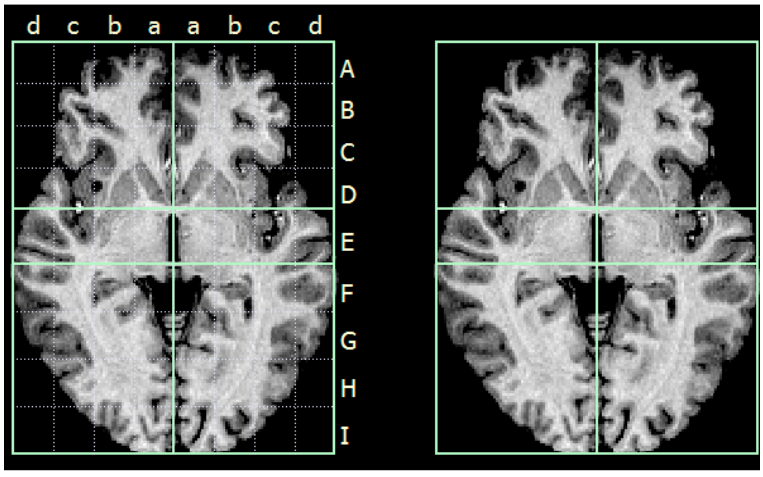


Figure 12. Talairach space coregistration.

For each activated area number of voxel was calculated and used for statistical purposes.

Analysis of functional data before application of fatigue protocol showed activation of left sensorimotor cortex (L-SMC), bilateral supplementary motor area (BIL-SMA), left and right cerebellum in both fatigue and non fatigue MS

patients. Non fatigue patients activated also right sensorimotor area (R-SMA) and left inferior frontal gyrus (L-IFG). After fatigue protocol the same pattern of activation was observed, with the appearance of activation of R-SMA in fatigued patients.

Areas activated during simple motor task are shown in Tab. 5

Pre Fatigue

	L-SMC	R-SMC	BIL-SMA	L-IFG	L-Cerebellum	R-Cerebellum
F-MS	*		*		*	*
NF-MS	*	*	*	*	*	*

Post Fatigue

	L-SMC	R-SMC	BIL-SMA	L-IFG	L-Cerebellum	R-Cerebellum
F-MS	*	*	*		*	*
NF-MS	*	*	*	*	*	*

L-SMC: left sensorimotor cortex
R-SMC: right sensorimotor cortex
BIL-SMA: bilateral supplementary motor area
L-IGF: left inferior frontal gyrus

F-MS: Fatigue MS patients
NF-MS: Non-fatigue MS patients

Table 4. Activated area in patients groups before and after fatigue protocol.

Within-group analysis in fatigued MS patients before and after fatigue protocol did not shown any statistical difference. Within-group analysis in non fatigue MS patients showed a significant difference in activation in left somatosensory motor area (p=0,028) (TAB.6).

Table 5. Within-group analysis of functional data.

PRE-POST FATIGUE IN N-FATIGUE-MS GROUP						
	L-SMC Pre-Fatigue vs Post-Fatigue	R-SMC Pre-Fatigue vs Post-Fatigue	BIL-SMA Pre-Fatigue vs Post-Fatigue	L-IFG Pre-Fatigue vs Post-Fatigue	L-Cerebellum Pre-Fatigue vs Post-Fatigue	R-Cerebellum Pre-Fatigue vs Post-Fatigue
p	,917	,715	,753	,753	,917	,753

PRE-POST FATIGUE IN FATIGUE-MS GROUP						
	L-SMC Pre-Fatigue vs Post-Fatigue	R-SMC Pre-Fatigue vs Post-Fatigue	BIL-SMA Pre-Fatigue vs Post-Fatigue	L-IFG Pre-Fatigue vs Post-Fatigue	L-Cerebellum Pre-Fatigue vs Post-Fatigue	R-Cerebellum Pre-Fatigue vs Post-Fatigue
p	**,028**	,249	,463	,116	,345	,249

Wilcoxon Signed Rank test. L-SMC: left sensory-motor cortex. R-SMC: right sensory-motor cortex. BIL-SMA: bilateral supplementary motor area. L-IGF: left inferior frontal gyrus.

Between-group analysis (fatigue MS versus non fatigue MS) showed no difference before and after fatigue protocol (Tab.7).

Table 6. Between-group analysis of functional data.

PRE-FATIGUE IN F-MS GROUP vs NF-MS GROUP						
	L-SMC	R-SMC	BIL-SMA	L-IFG	L-Cerebellum	R-Cerebellum
p	,180	,818	,819	,699	,589	,818

POST-FATIGUE IN F-MS GROUP vs NF-MS GROUP						
	L-SMC	R-SMC	BIL-SMA	L-IFG	L-Cerebellum	R-Cerebellum
p	,132	,699	1,000	,310	,310	,937

Mann-Whitney Test. L-SMC: left sensory-motor cortex. R-SMC: right sensory-motor cortex. BIL-SMA: bilateral supplementary motor area. L-IGF: left inferior frontal gyrus.

No correlation between FSS score and activated areas before and after fatigue was observed.

Resting state analysis showed no differences in baseline activated network between the two groups and before/after application of fatigue protocol.

DISCUSSION

The present study has not been able to detect any correlation between MRI and clinical variable expressed as EDSS and MSFC score. These findings agree with some study in the literature (van der Werf et al., 1998; Mainero et al., 1999) where no correlation was found between fatigue and both standard MRI variables (lesion load) and structural variables (brain tissue volume). However, Tedeschi and coworkers have shown a correlation between fatigue and white and grey matter volume in a large sample of MS patients (222 patients), although there were few subjects in non fatigue group (n=25) compared to fatigue MS (n=197) (Tedeschi et al., 2007). Our inability to reach the same finding may be due to a lack in power of the present study because of the low number of patients in each group (fatigue MS=6; non fatigue MS=6). Tedeschi et al. suggested that fatigue is correlated to diffuse CNS involvement even in MS patients with low disability, as patients with high-fatigue showed significantly higher WM and grey matter (GM) atrophy than patients with low-fatigue. Furthermore, they suggest that in MS, independent of disability, WM and GM atrophy is a risk factor to have fatigue.

Regarding functional data analysis, our MS patients showed greater activation during a single motor task in bilateral sensory-motor cortex, bilateral supplementary motor area, bilateral cerebellum and left inferior frontal gyrus. After application of fatigue protocol the same areas were detected with the only exception of fatigue group, in which the contralateral motor cortex was silent before fatigue protocol, while it was recruited after fatigue protocol. This results confirm data collected from Filippi et al. in 2002 using a similar study protocol (Filippi et al., 2002). To date this pattern of brain activation was still waiting for confirmational data. Notwithstanding, our between-group analysis, both before and after fatigue, did not reply Filippi et al. data, which showed significant differences of activation in these areas. Probably also in this case our sample is to small to detect differences in activated and deactivated neurons. However, we can confirm that in fatigue origin process numerous structures are involved. Compared to healthy subjects, MS patients, even disregarding the presence of fatigue, need the cooperation of more network to reach the same task. Indeed,

MS patients required a greater activation in controlateral sensorymotor area and cerebellum. This can be probably explained by an alteration of cortical and subcortical network involved in motor task and perception functions, requiring activation of contralateral areas in both hemispheres.

Within-group analysis in fatigue patients did not find any change before and after application of the “fatigue protocol”. However, in non fatigue-MS group we found a significant decrease in activation of left sensory-motor area after the “fatigue protocol” ($p= 0.028$), which has no clear explanation and needs to be confirmed in a larger sample. As pure speculation, we may interpret this results as reflection of loss of the central drive in L-SMC neurons. In the hypothesis that fatigue in MS has a central and supraspinal origin, decrease in activation of the L-SMC during a right-handed task explain reduction in central ability to sustain prolonged activation in fatigued conditions.

CONCLUSIONI

Fatigue in MS patients is not related to demographic and clinical variables expression of disability (EDSS, MSFC and related sub-items).

MS patients during a single motor task showed a predominant activation of bilateral sensory-motor cortex, bilateral supplementary motor area, bilateral cerebellum and left inferior frontal gyrus.

Our analysis showed no differences between patients groups in terms of activation volumes.

In the fatigue-MS group we did not find any change before and after application of the “fatigue protocol”. In the non fatigue-MS group we found a significant decrease in activation of left sensory-motor area after the “fatigue protocol” ($p= 0.028$), which has no clear explanation and needs to be confirmed in a larger sample.

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