UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

Zentrum für Molekulare Neurobiologie Hamburg,

Institut für Neuroimmunologie und Klinische Multiple Skleroseforschung

Prof. Dr. Roland Martin

Multiple Sclerosis related Fatigue and
the Hypothalamic-Pituitary-Adrenal Axis

Dissertation

zur Erlangung des Grades eines Doktors der Medizin

an der Medizinischen Fakultät der Universität Hamburg.

vorgelegt von:

Lars Nawrath,

aus Aachen

Hamburg 2009

Angenommen von der

Medizinischen Fakultät der Universität Hamburg am: 7.7.2010

Veröffentlicht mit Genehmigung der

Medizinischen Fakultät der Universität Hamburg.

Prüfungsausschuss, der Vorsitzende: Prof. Dr. C. Heesen

Prüfungsausschuss, zweiter Gutachter: PD Dr. C. Otte

Prüfungsausschuss, dritter Gutachter: Prof. Dr. C. Bamberger

Für Emma, Arthur und Jenny

Table of contents

Abbreviations

ACTH	Adrenocorticotropic Hormone
AUC	Area Under the Curve
CAMBS	Cambridge Multiple Sclerosis Basic Score
CK	Cytokines
CNS	Central Nervous System
CPMS	Chronic Progressive Multiple Sclerosis
CRH	Corticotropin-Releasing Hormone
Dex/CRH test	Dexamethasone/Corticotropin-Releasing Hormone Test
EAE	Experimental Allergic Encephalomyelitis
EDSS	Extended Disability Status Scale
ESS	Epworth Sleepiness Scale
FSS	Fatigue Severity Scale
GC	Glucocorticoid(s)
GM	Grey Matter
GR	Glucocorticoid Receptor(s)
HADS	Hospital Anxiety and Depression Scale
HAQUAMS	Hamburg Quality of Life Questionnaire in Multiple Sclerosis
HPA axis	Hypothalamic-Pituitary-Adrenal Axis
MFIS	Modified Fatigue Impact Scale
MS	Multiple Sclerosis
MSF	Multiple Sclerosis related Fatigue
MSNF	Multiple Sclerosis without related Fatigue
PPMS	Primary Progressive Multiple Sclerosis
PSQI	Pittsburgh Sleep Quality Index
RRMS	Relapsing Remitting Multiple Sclerosis
SDMT	Symbol Digit Modalities Test
SPMS	Secondary Progressive Multiple Sclerosis
WM	White Matter

1 Objective

Fatigue affects up to 60 percent of patients with multiple sclerosis (MS). MS related fatigue (MSF) is one of two major reasons for unemployment among people with MS in the USA. The pathophysiology of MSF appears to be complex. Neurohumoral, neuroimmune and structural pathology of the central nervous system (CNS) have been postulated as mechanisms for MSF development and persistence. However, a certain pathophysiology has not yet been definitely linked to MSF.

In numerous other diseases, fatigue is associated with hypothalamic, pituitary and diencephalic syndromes, which are often related to endocrine abnormalities. Diseases connected with fatigue (e.g. major depression, Cushing's syndrome or sleep disorders) have repeatedly displayed hypothalamic-pituitary-adrenal axis (HPA axis) hyperactivity.

Recent investigations have mainly shown HPA axis hyperactivity in MS patients. HPA axis hyperactivity thereby highly correlated with disease progression, cognitive impairment and brain atrophy. Inconsistent results were reported for HPA axis activity in relation to disability and depression.

The aim of this study was to investigate, whether MSF severity correlates with the extent of HPA axis hyperactivity. The combined dexamethasone/corticotropin-releasing hormone test (Dex/CRH test) was to be applied to 15 MSF patients and to 15 MS patients without related fatigue (MSNF) respectively, in order to assess central glucocorticoid receptor (GR) capacity.

Disturbed central GR feedback function was hypothesised to exist in MSF patients. For this reason, MSF was hypothesised to be partly explained by insensitivity of HPA axis to acute stimuli due to permanent HPA axis hyperactivity.

In addition, MSF severity and HPA axis function should independently be studied in relation to disability, cognitive impairment, sleep quality, daytime sleepiness and quality of life.

2 Introduction

2.1 Multiple sclerosis

Multiple Sclerosis (MS) is a complex immune-mediated inflammatory and neurodegenerative disease of the central nervous system (CNS) (Compston and Coles 2002), causing progressive substantial neurological disability in the majority of the patients affected (Confavreux et al. 2000).

MS might have been first mentioned in the 14th century in St. Thorlakr's island saga, in which the Viking Hala temporarily complains about blindness and speech disturbances. Cruveilhier, Carswell, Frerichs and Charcot were the first researchers describing MS pathology and symptoms in the 19th century (Schmidt 2002).

MS symptoms depend on the localisation of CNS lesions, which have a predilection for the optic nerves, periventricular white matter, brainstem, cerebellum and spinal cord. Therewith, frequent presenting symptoms are sensory disturbances (e.g. optic neuritis, paraesthesia) or long tract symptoms (e.g. limb weakness). Cerebellar (e.g. gait ataxia) and brainstem (e.g. bladder dysfunction) symptoms may develop later during the disease course. Psychosocial symptoms, including cognitive impairment, mood disturbance and MS related fatigue (MSF) also occur, whereas MSF can also be the presenting symptom in early stages of MS (Noseworthy et al. 2000, Thompson 2001).

According to clinical disease course, MS patients are divided into three groups (Lublin and Reingold 1996). About 15 per cent of MS patients present with a primary progressive MS (PPMS) from onset, partly with superimposed relapses and continuing progression between relapses. PPMS may be regarded as "MS amputated from its usually preceding relapsing-remitting phase" (Compston and Coles 2002, Vukusic and Confavreux 2007). The majority of patients present with an initial relapsing remitting MS (RRMS), which is defined as "clear disease relapses with full recovery or residual deficit upon recovery and lack of disease progression between relapses" (Lublin and Reingold 1996). RRMS subsequently transforms into secondary progressive MS (SPMS) in about 80 per cent of the cases (Kremenchutzky et al. 2006). SPMS is characterised by mainly continuous irreversible functional decline "with or without occasional relapses, plateaus and minor remissions" (Lublin and Reingold 1996). Disease process and speed are similar in most patients with chronic progressive MS (CPMS),

once a threshold of accumulated disability is passed, pointing to similar underlying pathological processes (Confavreux et al. 2003, Vukusic and Confavreux 2007).

MS prevalence is approximately 2.500.000 worldwide (Flachenecker and Zettl 2002). Germany belongs to the high-risk areas to develop MS with an estimated prevalence of 122.000 (Hein and Hopfenmüller 2000). MS aetiology appears to be multifactorial including genetic (Weinshenker 1996, Dyment at al. 1997, Steinman 2001), infectious and environmental (Noseworthy et al. 2000, Compston and Coles 2002, Marrie 2004) factors. In general, it is believed that MS is most likely to occur in a genetically susceptible host, who additionally encounters a certain or a number of environmental event(s) at a for the host critical time of exposure.

The exact way of MS initiation remains elusive at the time being. Two possible mechanisms were proposed (Prat and Antel 2005). First, after T cell activation outside the CNS via "molecular mimicry", bystander activation and/or superantigens, activated T cells may be able to migrate to the CNS and initiate proinflammatory reactions towards myelin epitopes (Kieseier et al. 1999, O'Connor et al. 2001, Steinman 2001, Prat and Antel 2005). A number of other immune active cells, e.g. macrophages, B cells or microglia, and immune active humoral substances, e.g. cytokines (CK), antibodies or proteins of the complement system, subsequently may become involved in the attack of neural tissue (Benveniste 1997, Kieseier et al. 1999, Noseworthy et al. 2000, O'Connor et al. 2001, Steinman 2001, Hemmer et al. 2002, Prat and Martin 2002). Second, "unidentified acquired insults" (viral infection) or "intrinsic abnormalities (neurodegenerative hypothesis)" may induce lethal changes in neurons or oligodendrocytes, which may trigger a T cell guided inflammatory sequence or a neurodegenerative cascade (Prat and Antel 2005).

During the entire course of the disease, four different stage and time dependent types of lesions in different areas of the CNS have been identified (Brück 2005, Lassmann et al. 2007, Pittock and Lucchinetti 2007). Classical focal active demyelinating lesions have been found in the white matter (WM) of patients presenting with acute relapse, mainly in RRMS (Lucchinetti et al. 2000). Slowly expanding chronic lesions in the WM, diffuse WM injury in normal appearing WM and grey matter (GM) lesions have rather been allocated to CPMS (Brück 2005, Kutzelnigg et al. 2005, Lassmann et al. 2007, Pittock and Lucchinetti 2007). However, inflammatory (Kuhlmann et al. 2002, Kutzelnigg et al. 2005) as well as

neurodegenerative (Kieseier and Hartung 2003, Brück 2005) elements have been identified in the above described four lesions, resulting in a new descriptive term of "simultaneous two component" disease (Zipp and Aktas 2006, Charil and Filippi 2007). Remyelination is supposed to occur at least during the early disease course, or even throughout the disease in about 20 per cent of patients (Lassmann et al. 2007). However, few is known about underlying mechanisms involving remyelination and neuron regeneration in MS (Lisak 2007).

Clinical symptoms may subside in consequence of resolution of inflammation, axonal remyelination and adaptive cortical reorganisation after loss of functional neural tissue (Reddy et al. 2000, Compston and Coles 2002, Rocca et al. 2005, Vollmer 2007). Inevitable disease progression may be triggered by events hitting the systemic immune system, altering the blood brain barrier function and modifying the function of CNS intrinsic immune cells (Prat and Antel 2005). Passing a threshold of irreversible axonal and neural loss secondary to the pathogenic cascade (Bermel and Bakshi 2006) as well as exhaustion of adaptive compensatory cortical reorganisation following CNS tissue loss (Bjartmar et al. 2003) may entail permanent disability.

2.2 MSF overview

MSF is a complex phenomenon. For MS patients affected, it is one of the most disabling symptoms. Definition and measurement proved to be difficult. The US National Multiple Sclerosis Society proposed the following, widely used definition of fatigue: "A subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities (Multiple Sclerosis Council for Clinical Practice Guidelines 1998).

MSF affects up to 58 per cent of patients (Colosimo et al. 1995, Bakshi et al. 2000, Tellez et al. 2005). Up to 40 per cent of MS patients regard MSF as their most disabling symptom (Krupp et al. 1988, Fisk et al 1994, Bergamaschi et al. 1997). 56 per cent of MS patients feel, that MSF limits their ability to perform physical and mental activities of daily living, the highest of any reported symptom (Freal et al. 1984, Krupp et al. 1989, Janardhan and Bakshi 2002). MSF is one of the two major reasons for unemployment among people with MS in the USA (Multiple Sclerosis Council for Clinical Practice Guidelines 1998). MSF is the initial symptom of MS in up to one third of the cases (Krupp et al. 1988), and may occur as the preceding symptom of a relapse or the main feature of a relapse (Freal et al. 1984). MSF is

present at all stages of the disease (Fisk et al. 1994). MSF is aggravated by heat, stress, depression and prolonged physical activity, whereas rest, sleep, positive experiences, moderate exercise, sex and decline in temperature alleviate MSF severity (Krupp et al. 1988, Bakshi 2003). Apart from heat, healthy people report similar exacerbating and ameliorating conditions for fatigue (Krupp et al. 1988).

Many self-reporting questionnaires have been developed and serve as screening tools for MSF (Flachenecker et al. 2002, Schwid et al. 2002). The fatigue severity scale (FSS) has been the most widely used MSF scale in cross-sectional and longitudinal studies (Bakshi 2003). Because of the subjectivity in describing MSF, self-report instruments are probably the most useful tools to assess MSF practically (Tellez et al. 2005). MSF may be better evaluated combining a structured interview and a specific questionnaire, assessing MS and MSF impact on activities of daily life and on quality of life (Kotterba et al. 2003).

Mechanisms contributing to MSF or management strategies ameliorating MSF severity have not been deciphered unequivocally by MSF treatment studies. A multidisciplinary approach to treat MSF appears to be worthwhile (Kos et al. 2008). Practically, secondary causes of MSF should be excluded or treated first. Second, non-pharmacologic therapies including lifestyle changes (MS Council for Practical Guidelines 1998), exercise therapy (Rietberg et al. 2005) and psychological interventions (Thomas et al. 2006, van Kessel et al. 2008) should then be considered. Despite the weak study design and the ambiguous results of treatment studies for Amantadine (Murray 1985, The Canadian MS Research Group 1987, Cohen and Fisher 1989, Krupp et al. 1995, Pucci et al. 2007), Pemoline (Weinshenker et al. 1992, Krupp et al 1995) and Modafinil (Rammohan et al. 2002, Zifko et al. 2002, Stankof et al. 2005, Lange et al. 2009), pharmacologic management of MSF should be regarded as an option (Lapierre and Hum 2007).

2.3 Pathophysiology of MSF

The pathophysiology of MSF is complex. The majority of the existing literature points to a "central" origin of MSF (Comi et al. 2001). MSF may either be directly related to the disease process and/or may be caused secondarily, e.g. by disability, depression, sleep disturbance or immunotherapy (Schwid et al. 2002, Bakshi 2003).

A number of studies indicate, that proinflammatory mediators, which are thought to play a pivotal role in MS pathogenesis (Noseworthy et al. 2000, Steinman 2001, Lassmann et al. 2007), may participate in MSF development and persistence (Bertolone et al. 1993, Comi et al. 2001, Flachenecker et al. 2004). CK have been depicted to play a major role in other entities like sickness behaviour (Konsman et al. 2002, Dantzer 2006, Dantzer and Kelley 2007), chronic fatigue syndrome, obstructive sleep apnoea and postdialysis fatigue (Natelson 2001, Schwid et al. 2002). Sickness behaviour has been described to occur during infection or exacerbation of some autoimmune diseases. Clinical symptoms include malaise, lassitude, fatigue, numbness, coldness, muscle and joint aches as well as reduced appetite. Sickness behaviour has been pathophysiologically described as the bodies "highly organised strategy to fight infection". The orchestra of intrinsic reactions towards the invading organism is conducted by proinflammatory CK (Dantzer 2006). MSF might be the result of maladaptive, prolonged sickness behaviour without reconstitution of the body's neuroimmune allostasis after infection or inflammation (McEwen 1998, Dantzer and Kelley 2007).

Central autonomic dysfunction has been described in MSF (Flachenecker et al. 1999, Merkelbach et al. 2001, Flachenecker et al. 2003). MSF treatment studies have suggested, that MSF might be caused by disturbance of pathways of systems involved in general cerebral arousal, e.g. the ascending reticular activating system (Zimmermann and Hohlfeld 1999, Schapiro 2002, Stankof et al. 2005).

A number of investigators reported structural CNS abnormalities in MSF patients using a variety of imaging techniques, with the results being inconsistent and partly contradictory. A higher lesion load of the entire brain (Colombo et al. 2000, Tedeschi et al. 2007), GM atrophy (Tedeschi et al. 2007), subcortical GM pathology (Niepel et al. 2006, Pirko et al. 2007), GM pathology in specific areas (Colombo et al. 2000), WM pathology (Djaldetti et al. 1996, Tartaglia et al. 2004, Sanfilipo et al. 2006, Tedeschi et al. 2007), subcortical blood perfusion (Inglese et al. 2002) and metabolic (Roelcke et al. 1997) disturbances were demonstrated in MSF patients.

Disability (Colosimo et al. 1995, Tellez et al. 2005, Pittion-Vouyovitch et al. 2006), depression (Fisk et al. 1994, Ford et al. 1998, Bakshi et al. 2000, Siegert and Abernethy 2005) or sleep disturbances (Attarian et al. 2004, Lobentanz et al. 2004) have been found to be

associated with MSF and may be involved in MSF development, persistence and/or aggravation.

2.4 MS and the hypothalamic-pituitary-adrenal axis

Data collected in animal models of acute and chronic MS provided first insights into the role of the hypothalamic-pituitary-adrenal axis (HPA axis). Impaired HPA axis function was demonstrated in Lewis rats, in which susceptibility to experimental autoimmune encephalomyelitis (EAE), a monophasic animal model of acute MS, is subsequently increased (Mason et al. 1990). Corticosterone levels started increasing shortly prior to the development of signs of paralysis and peaked at the time of maximum clinical disease. Spontaneous recovery began, when corticosterone plasma levels were maximal. Adrenalectomy shortly after the onset of the first attack of paralysis resulted in death of the animals. Adequate replacement of corticosterone plasma levels in adrenalectomised animals resulted in a similar clinical disease course as in the original model and recovery was complete (MacPhee et al. 1989). Blockage of central glucocorticoid receptors (GR) in Lewis rats after EAE induction resulted in disease exacerbation (Bolton and Flower 1989). Further information was gained after introducing a new animal model of chronic relapsing EAE, which more closely resembles MS than the other monophasic EAE animal models (Stefferl et al. 2001). HPA axis response during first relapse was adequate and suppressed clinical disease activity. HPA axis activity dropped by 50 per cent during a second relapse compared to the first bout. External corticosterone supplementation after remission of the first relapse resulted in prolongation of relapse free interval, but eventually animals relapsed. After entering the progressive disease phase, HPA axis products returned to baseline values and an antibody-mediated pathogenesis was observed (Stefferl et al. 2001).

Findings of these animal studies led to the assumption, that patients with MS may exhibit a hyporesponsive HPA axis, making them more susceptible to the disease. However, a variety of post-mortem, basal and dynamic testing of the HPA axis revealed the opposite: MS patients largely exhibited a hyperactive HPA axis. Post-mortem studies in MS patients revealed, that adrenal size of MS patients was significantly enlarged (Reder et al. 1994). Besides, significantly elevated Cortisol levels in the cerebrospinal fluid were observed (Erkut et al. 2002). Number of corticotropin-releasing hormone (CRH) secreting neurons in the periventricular nucleus of the hypothalamus was significantly increased (Erkut et al. 1995, Purba et al. 1995). In addition, CRH expressing neurons were colocalised by Vasopressin,

which is an indicator of chronic HPA axis activation (Erkut et al. 1995). CRH secreting neurons also exhibited an enhanced activity (Huitinga et al. 2003). In vivo studies of HPA axis activity demonstrated, that basal plasma concentration of adrenocorticotropic hormone (ACTH) and Cortisol in clinically stable MS patients were elevated (Michelson et al. 1994). Dexamethasone suppression was significantly reduced in about 50 per cent of MS patients with active disease compared to about 15 per cent in healthy controls (Reder et al. 1987). Application of the combined Dexamethasone-suppression/CRH-stimulation test (Dex/CRH test) exhibited a heterogeneous HPA axis response in MS patients with active disease, with the majority of the patients revealing a hyperactive HPA axis (Grasser et al. 1996). HPA axis hyperactivity was confirmed in a number of studies in MS patients with active (Fassbender et al. 1998, Then Bergh et al. 1999b, Ysrraelit et al. 2008) and clinically stable disease (Heesen et al. 2002, Schumann et al. 2002, Gold et al. 2005b, Ysrraellit et al. 2008). In detail, HPA axis hyperactivity significantly increased with disease progression (Then Bergh et al. 1999b, Heesen et al. 2002) and significantly correlated with cognitive dysfunction (Heesen et al. 2002, Gold et al. 2005b) as well as depression (Fassbender et al. 1998). HPA axis hyperactivity was also significantly associated with brain atrophy (Schumann et al. 2002).

Conclusively, animal data of models resembling human MS point to a defective HPA axis, which increases susceptibility of acute EAE but not progression in chronic relapsing EAE. In contrast, human data in general suggests HPA axis hyperactivity, which appears to be related to the MS intrinsic disease process.

2.5 Fatigue and HPA axis in other diseases

Central fatigue is generally associated with hypothalamic, pituitary and diencephalic syndromes, which in turn are related to endocrine abnormalities (Chaudhuri and Behan 2004). Fatigue is a symptom of a number of other diseases, which show altered HPA axis function. On the one hand, there are fatigue related diseases with hypoactive HPA axis like chronic fatigue syndrome, posttraumatic stress syndrome or fibromyalgia (Elenkov et al. 1999, Chaudhuri and Behan 2004, Cleare 2003). On the other hand, fatigue connected diseases like chronic stress, Cushing's syndrome, sleep disorders or major depression are associated with a hyperactive HPA axis (Holsboer et al. 1994, Elenkov et al. 1999, Vgontzas and Chrousos 2002).

2.6 Study objective

The aim of this study has been to investigate, whether MSF severity correlates with the extent of HPA axis hyperactivity. The combined Dex/CRH test has been applied to MS patients with and without MSF, so that central GR feedback capacity could be assessed. Disturbed central GR feedback function was hypothesised to exist in MSF patients. Thus, MSF was hypothesised to be partly explained by insensitivity of the HPA axis to acute stimuli due to permanent HPA axis hyperactivity.

3 Patients and methods

3.1 Recruitment of participants

This study had been approved by the local ethics committee of the chamber of doctors in Hamburg (Ärztekammer Hamburg).

First, the patient database of the MS outpatient clinic of the Department of Neurology, University Hospital Hamburg-Eppendorf, was screened for potential participants. MS patients, who refused participating in any study during a former visit to the outpatient clinic a priori, were excluded. In addition, the following exclusion criteria were applied: an incomplete address in the database, a missing fatigue screening score (see next paragraph), an incomplete medical record, a current pregnancy, a current MS relapse, a relapse during the last three months, a steroid treatment within the last three months, a known endocrine abnormality or clinical evident psychiatric disease. Only patients definitely fulfilling MS-diagnostic criteria (Poser 1983, McDonald et al. 2001) were included into this study.

While consulting the outpatient clinic, every patient usually completes a modified FSS amongst a battery of questionnaires and tests. For screening purposes, the FSS is reduced to seven questions. Every item is rated from 1 (strong disagreement) to 5 (strong agreement), so that a sum score can be generated ranging from 7 to 35. According to the last sum score of the modified FSS, patients were assigned to the MSNF group with a score lower than 14, whereas patients with a score above 28 were allocated to the MSF group. An afore-described cut-off (Bakshi et al. 1999, Flachenecker et al. 2002) was adjusted to the modified FSS for group allocation.

Detailed information about the purpose and the aim of the study were outlined in a letter, which was sent to the potential participants. Side effects of Dexamethasone (Fortecortin®, 1.5 mg, Merck, Germany) were described. Potential participants were informed to be called within the next 14 days. Of the 88 patients written to, 35 patients could not be contacted. When talking on the phone, emerged questions were discussed first. 22 patients denied to participate or met one of the above-mentioned exclusion criteria. An appointment was scheduled with 31 MS patients, after agreeing on participating in the study.

A couple of days before the scheduled test day, a second letter was sent to the participants, comprising information about the course of the study day. One tablet Dexamethasone (Fortecortin®, 1.5 mg, Merck, Germany) was enclosed. As explained orally during the first phone call and written meticulously in the letter, the Dexamethasone ought to be taken at 11:00 pm the night before the scheduled appointment. Participants were asked neither to drink coffee nor to smoke the two hours preceding the Dex/CRH test. Within two days before the actual test day, participants were called again to reassure, that the second letter had arrived and participants were reminded to take the Dexamethasone.

3.2 Test day

Patients were asked to arrive in the outpatient clinic at 1:30 pm. Evolved questions of the patient were answered and exclusion criteria were gone through again. Prior to the beginning of the study, every patient had to consent in written form. Following the examination of quality of life and cognitive impairment, the neurological history was taken and a thorough neurological examination (with the first five patients being examined under supervision) was carried out, in order to classify the individual disability. Around 2:20 pm, an i.v. canula was inserted and the first blood sample was taken at 2:30 pm. The following questionnaires were always completed in the below-listed sequence. 29 of the patients sat during the procedure and two participants preferred to lie down. Participants were not allowed to eat or to leave the room, except for using the bathroom, which one person asked to do so. Table water was offered.

3.3 Questionnaires and clinical scores

3.3.1 Hamburg Quality of Life Questionnaire in Multiple Sclerosis (HAQUAMS)

The HAQUAMS is a disease specific quality of life instrument and consists of 36 items, which can be rated from 1 to 5. The higher the computed sum score, the worse the quality of life. Internal consistency was high. The HAQUAMS showed to be a reliable and valid instrument for quality of life assessment in MS (Gold et al. 2001).

3.3.2 Symbol digit modalities test (SDMT)

SDMT requires examinees to match numbers to a series of different symbols presented randomly on a single test page, using a key found at the top. 120 matches can be made in total, and each subject has to match as many as possible, while working in a consistent order. The score is the number of correct matches obtained within 90 seconds. It is possible to

convert the raw score into a standardised score, after correcting for age and school education. The scoring range is from –3 to 3. -3 indicates cognitive impairment and -2.5 to 0 is considered a borderline status (Smith 1968).

3.3.3 Extended disability status scale (EDSS)

The EDSS is seen as the gold standard to describe the neurological disability and overall function in MS patients (Schmidt and Hoffmann 2002). Based upon taking a neurological history and performing a standardised neurological examination, eight functional systems are graded from 0 (no disability) to 5 or 6 (maximal impairment) except for miscellaneous, which is rated dichotomously (0=none, 1=any other neurological finding attributed to MS). After combining the systems, a score between 0 (no disability detected) and 10 (death) on a 20-point ordinal scale is given. Scores up to 3.5 are largely dependent on the functional systems, whereas ambulation mainly determines highly graded disability (Kurtzke 1983). Interrater reliability was described to be fair to substantial. Intrarater reliability was moderate to almost perfect in mildly disabled patients (Sharrack and Hughes 1996, Sharrack et al. 1999), but varied in highly disabled patients (Hobart et al. 2000). Validity is higher in less disabled patients (Sharrack et al. 1999).

3.3.4 Cambridge Multiple Sclerosis Basic Score (CAMBS)

The CAMBS aims at recording the current status of a patient in the context of the clinical course. Four indices (disability, relapse, progression and handicap) are scored from 1 (no impairment) and 5 (most severe impairment) (Mumford and Compston 1993). The CAMBS is a reliable and responsive outcome measure, but has a limited validity (handicap domain) (Sharrack and Hughes 1996, Sharrack et al. 1999). Interrater reliability is considered moderate (Mumford and Compston 1993).

3.3.5 Fatigue Severity Scale (FSS)

The FSS was introduced to assess perceived fatigue in patients with MS and systemic Lupus erythematodes as well as to differentiate fatigue from clinical depression. Subjects are asked to rate nine statements on a scale from 1 (strong disagreement) to 7 (strong agreement) according to the level of fatigue felt over the preceding week. The individual score is the mean of the numerical response to the nine statements. There is a scoring range from 1 to 7, with 4 to 5 indicating a borderline status (Krupp et al. 1989). The FSS demonstrated internal consistency and reliability (Krupp et al. 1989, Flachenecker et al. 2002), but has limited face validity as a measure of fatigue severity (Schwid et al. 2002).

3.3.6 Modified Fatigue Impact Scale (MFIS)

The Fatigue Impact Scale (FIS) was developed to evaluate the effects of fatigue on the quality of life in MS patients (Fisk et al. 1994). 21 of 40 items of the original version were selected in 1998 to propose the multidimensional MFIS. According to the perceived influence of fatigue, subjects rate the statements from 0 (never) to 4 (almost always). The higher the computed score, the greater the impact of fatigue on a patient's life. It is further possible to form a cognitive, a physical and a psychosocial subscale (Multiple Sclerosis Council for Clinical Practice Guidelines 1998). The MFIS showed a satisfactory reliability and at best moderate validity (Flachenecker et al. 2002).

3.3.7 Epworth Sleepiness Scale (ESS)

The ESS was devised as a screening instrument for excessive daytime sleepiness in patients with nocturnal sleeping problems. Subjects are asked to estimate the probability of falling asleep during the performance of eight typical activities of daily life. Every statement is rated from 0 (never) to 3 (high), thus giving a scoring range from 0 to 24. A sum score above 10 is understood as a pathologically increased daytime sleepiness (Johns 1991). Internal consistency, reliability, and validity were demonstrated (Johns 1991, Johns 1992).

3.3.8 Pittsburgh Sleep Quality Index (PSQI)

The PSQI is used to assess the sleep quality of patients. The 19 self-rated questions assess a wide variety of factors related to sleep quality, e.g. sleep duration, use of sleeping medication and specific sleep-related problems. These 19 items are grouped into seven component scores, each weighted equally on a 0 to 3 scale. The seven component scores are then summed to yield a global score, ranging from 0 to 21. The empirical cut-off is 5. A satisfactory internal consistency, reliability and validity were shown (Buysse et al. 1989).

3.3.9 Hospital Anxiety and Depression Scale (HADS)

The HADS was implemented as a screening tool for depression and anxiety in patients. The questionnaire consists of 14 items, seven attributed to depression and anxiety respectively. Patients are asked to grade the statements from never (0) to almost always (3). Two sum scores (depression and anxiety) are calculated. The Cut-off is 11, with 8 to 10 representing a borderline status (Zigmond and Snaith 1983). The HADS showed satisfactory internal consistency, reliability and validity (Herrmann 1997).

3.4 The technical procedure

3.4.1 The course of the Dex/CRH test

The Dex/CRH test was first implemented in a sample of depressive patients (von Bardeleben and Holsboer 1989). The original version was altered and applied to MS patients for the first time in 1996 (Grasser et al. 1996). Patients are treated with 1.5 mg Dexamethasone perorally at 11:00 pm the night before the test day. An intravenous canula is inserted at 2:20 pm the next day. 10 ml blood samples are taken at 2:30 pm, 3:00 pm, 3:30 pm, 4:00 pm and 4:30 pm. At 3:02 pm, 100 micrograms synthetic CRH (CRH-Ferring, Kiel, Germany) reconstituted in 1 ml 0.09 % saline is injected within 30 seconds. A total of 10 syringes is drawn into prechilled tubes at the corresponding time and stored in iced water at about 4°C. The S-Monovette® 7.5ml Z (Sarstedt, Germany) was used for Cortisol quantification, the S-Monovette® 2.7ml Z (Sarstedt, Germany) was used for ACTH measurement. If a canula congested, a bolus of 10 ml 0.9% saline was given. Afterwards, 10 ml blood were aspirated and dismissed before proceeding. After winning the last sample at 4:30 pm, 150 µg Trasylol (Aprotinin) and then 250 µl Tetriplex (EDTA) are added immediately to every specimen, which is subsequently carefully swivelled. Following the centrifugation with fourthousandfold gravitational force for ten minutes at room temperature, the plasma is removed and put in pipettes Eppendorf (Eppendorf Nelkehr-Hinz GmbH, Germany). The acquired specimen is frozen and stored at −80°C. The Department of Clinical Chemistry of the University Hospital Eppendorf performed the following assays (see below), in order to measure ACTH and Cortisol plasma levels.

3.4.2 ACTH Assay

An immunoluminometric 2-step assay (Nichols Advantage, San Juan Capistrano, CA) was used for the determination of ACTH in plasma. Two monoclonal antibodies, one luminescent labelled and the other immobilised on the inner surface of the tube, recognize different binding sites on ACTH to form a sandwich-type complex bound to the tube. The luminescence signal is directly proportional to the ACTH concentration. The detection limit was 1 pg/ml. The intra- and interassay coefficients of variation were 2.7 % and 6.4 %, respectively.

3.4.3 Cortisol Assay

Cortisol was measured by a dual antibody chemoluminiscence assay using the Elecsys System 2010 kit (Roche, Grenzach-Whylen, Germany). The detection limit was 3.6 ng/ml. Intra- and interassay coefficients of variation were 1.3 % and 1.5 %, respectively.

3.5 Data analysis

First, the demographic data of the two groups (MSNF, MSF) were compared. Qualitative scales were studied (gender, disease form, immunotherapy) using the chi-square test. Subsequent to the calculation of the mean values and the standard deviations of the quantitative scales (FSS, MFIS, ESS, PSQI, EDSS, CAMBS subscales, HADS, HAQUAMS), variance analysis was performed applying the student t-test for independent samples, in order to compare characteristics of MSNF and MSF patients.

The following variables were calculated for the Dex/CRH test:
- Baseline values for ACTH and Cortisol by forming a mean value of the parameters won at 14:30 and 15:00 ("ACTH base" and "Cortisol base")
- Maximum of ACTH and Cortisol by picking the highest parameter for each substance after CRH-stimulation ("ACTH max" and "Cortisol max")
- Delta (δ) max in levels of ACTH and Cortisol by subtracting baseline values from maximum levels ("ACTH δmax" and "Cortisol δmax")
- Area under the curve (AUC) from 15:30 to 16:30 by using the trapezoidal rule ("ACTH AUC" and "Cortisol AUC").

After determining mean values and standard deviations of endocrine variables (base, max, δmax and AUC) for each substance, group (MSF, MSNF) differences for endocrine variables were computed by means of student t-test for independent samples. In addition, clinical scores (FSS, MFIS, ESS, PSQI, SDMT, EDSS and HADS) were correlated with the endocrine parameters enumerated above, computing Pearson's correlation coefficient.

As the nominal level of significance, $p < 0.05$ was set, allowing $0.05 < p < 0,1$ in order to detect a trend. This analysis was conducted with statistical software (SPSS 12.0).

3.6 Appliances/ Equipment

Large for ACTH:

- Dual antibody chemoluminiscence assay using the Elecsys System 2010 (Roche Grenzach-Whylen, Germany) with:
  - Amerlite (HRP and reinforcer)
  - Byk Sanguec (microperoxidasis)
  - Immulite- DPC (alcalic phosphatasis)
  - Acridinester

Large for Cortisol:

- Immunoluminetric 2-step assay (Nichols Advantage, San Juan Capistrano, CA) with:
  - Cortisol-antibody-conjugates (R1 and R2)
  - mIMP (monoclonal Immuno Magnetic Particle)
  - Calibrators 1-6 (for different Cortisol concentrations)

Others:

- Centrifuge Labofuge A (Heraeus Christ GmbH, Germany)
- Refridgerator Kryotec −80°C (Hans-S Schröder GmbH, Germany)

Small for ACTH and Cortisol:

- S-Monovette® 7.5ml Z, incl. additive carrier and clot activator (Sarstedt, Germany) for Cortisol
- S-Monovette® 2.7ml Z, incl. 1.6mg EDTA per ml blood (Sarstedt, Germany) for ACTH
- Multi-Adapter for S-Monovette® (Sarstedt, Germany)
- I.V. indwelling canula 18G/ 1 3/4" LUER, Vasofix®, Braunüle® (Braun, Germany)
- Transparent dressing, Tegaderm™ I.V. (3M Health Care, Germany)
- Closure stopper with injection port (IN-Stopper) (Braun, Germany)
- Disposable hypodermic needle 21G/ 1 1/2" LUER, Sterican® Size 2 (Braun, Germany)
- Single-use syringes, 2-piece, LUER, Inject 2ml (Braun, Germany)
- Single-use syringes, 2-piece, LUER, Inject 10ml (Braun, Germany)
- Capillary tubes with stopper 12 mm (Hamburg, Germany)
- Canulas 32 mm and 45 ml (BOC Ohmeda AB, Sweden)
- Connecta Plus 3 (Helsingburg, Sweden)
- Scale Ken Albstadt (Ebingen, Deutschland)
- Pipette Eppendorf (Eppendorf Nelkehr- Hinz GmbH, Germany)
- Pipette tips: blue and yellow (Sarstedt, Germany)

- Microtubes 1.5ml (Sarstedt, Germany)
- Winged infusion set Luer Lock 21G, 0.8x20mm (Braun, Germany)
- Powder-Free Latex Exam Gloves, SAFE SKIN™ SATIN PLUS™ (Kimberley- Clark, Thailand)

Pharmacy:

- Cutasept F Skin desinfectant (Bode Chemie Hamburg, Germany)
- Fortecortin® (Dexamethason) 1.5 mg pills (Merck, Germany)
- CRH-Ferring® (100 micrograms), ampoules with dry substance and solvent (Ferring, Kiel, Germany)
- Saline 0.9 % 10ml (Braun, Germany)
- Trasylol (Schering, Germany)
- Titriplex (Schering, Germany)

4 Results

4.1 Demographic data

31 patients participated in this study (15 MSNF patients, 16 MSF patients). One MSF patient did not fill in ESS, PSQI and HADS. Also, the same patient's Cortisol specimen was processed incorrectly, so that those Cortisol plasma levels could not be analysed. Hence, this patient was excluded from statistical analysis. The 30 remaining patients were included into the study (demographic data see table 1, next page). Both groups did not significantly differ in terms of age and gender. The MSF patients group exhibited significantly higher scores in fatigue (FSS, MFIS). There was a trend toward longer disease duration and more CPMS disease courses in MSF patients group. MSF patients group was significantly more disabled (EDSS, CAMBS Disability) and reported significantly more recent disease progression (CAMBS Progression). Both groups denied symptoms of clinical relapse (CAMBS Relapse) and did not significantly differ in terms of their cognitive impairment (SDMT). Quality of life (HAQUAMS) was significantly reduced in MSF patients group. Patients of both groups received Interferon-beta, Glatiramer Acetate, Methotrexat, Mitoxantron and Mycophenolate Mofetil. Immunotherapy was carried out in nine patients of each group, so that there was no significant group difference.

A minority of participants took CNS active medication. Three MSF patients received antidepressive drugs (one a combination of Amitryptilin and Sertralin, one Trimipramin and one Citalopram). Two patients received Modafinil therapy, one in combination with Carbamazepin.

Scores for daytime sleepiness (ESS), sleep quality (PSQI) and depression (HADS) were significantly higher in MSF patients group. However, the three scores last mentioned revealed a floor effect, reducing statistical power for this part of the analysis.

Table 1: Demographic data of the participants (MSNF, MSF)

	MSNF (n=15)	MSF (n=15)	p
Gender (m/f)	6/9	6/9	>0.99*
Age	42.87 ± 10.18	46.31 ± 11.35	0.38
Disease duration	8.6 ± 5.57	13.80 ± 9.68	*0.09*
Disease course (RR/SP/PP)	11/2/2	6/8/1	*0.07**
FSS	13.67 ± 6.26	54.88 ± 5.99	**<0.001**
MFIS	7.53 ± 8.09	59.38 ± 11.47	**<0.001**
ESS	3.27 ±2.37	10.53 ± 4.72	**<0.001**
PSQI	3 ± 1.6	7.2 ± 4.14	**0.002**
EDSS	2.3 ± 1.84	4.44 ± 1.47	**0.001**
CAMBS $_{disability}$	1.93 ± 0.8	2.94 ± 0.25	**<0.001**
CAMBS $_{relapse}$	1.6 ± 0.63	1.38 ± 0.5	0.279
CAMBS $_{progression}$	1.53 ± 0.64	2.44 ± 0.89	**0.003**
CAMBS $_{handicap}$	2 ± 1.07	3.63 ± 0.81	**<0.001**
SDMT	0.13 ± 1.3	-0.47 ± 1.69	0.278
HADS $_{depression}$	3.13 ± 3.5	7.93 ± 3.31	**0.001**
HAQUAMS $_{total}$	1.48 ± 0.37	2.86 ± 0.55	**<0.001**
Immunotherapy	9	9	>0.99*

Data presented as mean values ± standard deviation,

p according to student t-test for independent samples or χ^2-test*,

significant results (p < 0.05) in bold print, *trends (0.05 < p < 0.1) in italic writing*

4.2 Variance analysis for MSF and HPA axis

Both groups (MSNF, MSF) revealed a similar HPA axis response pattern after Dexamethasone suppression and CRH stimulation. Figures 1 and 2 demonstrate the similar distribution and development of the endocrine parameters of both groups during the application of the Dex/CRH test.
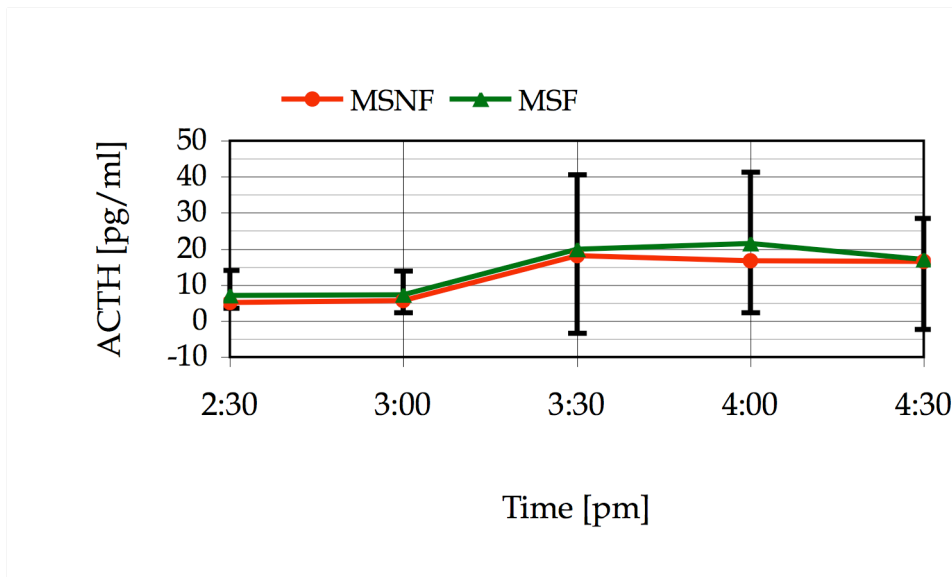

Figure 1: ACTH plasma concentrations (mean values ± standard deviation) are plotted against the time course.
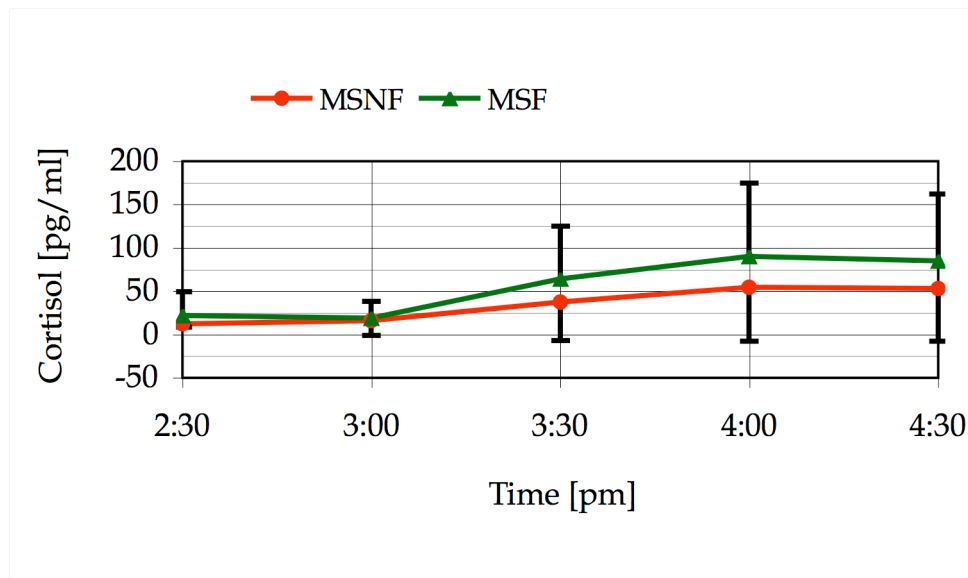

Figure 2: Cortisol plasma concentrations (mean values ± standard deviation) are plotted against the time course.

When comparing endocrine parameters of MSNF and MSF patients (base, max, δmax and AUC of each, ACTH and Cortisol), significant differences were not found (see table 2).

Table 2: Variance analysis for MSNF versus MSF

		MSNF group	MSF group	p*
ACTH	Base	5.4 ± 2.36	7.16 ± 6.69	0.345
	Max	20.87 ± 22.59	22.63 ± 20.81	0.823
	δmax	15.47 ± 20.59	15.47 ± 14.77	1
	AUC	103 ± 92	121.38 ± 110.12	0.619
Cortisol	Base	14.33 ± 8.86	20.67 ± 23.1	0.33
	Max	57.53 ± 62.59	93.33 ± 84.73	0.199
	δmax	43.2 ± 55.52	72.67 ± 72.03	0.22
	AUC	282.4 ± 301.48	454.13 ± 409.98	0.202

Data presented as mean values ± standard deviation,

*p according to student t-test for independent samples

Cortisol levels of six patients were below 10 [pg/ml] at all times and three more participants displayed a maximum Cortisol concentration of 16 [pg/ml] after CRH stimulation, pointing to a hyporesponsive HPA axis. Four out of nine participants belonged to the MSF patients group. The majority (three MSNF patients, four MSF patients) exhibited EDSS scores greater than four. The nine subjects mentioned did not have any further clinical feature in common.

4.3 Correlations for clinical scores and endocrine parameters

First, FSS, MFIS, ESS and PSQI were correlated with endocrine parameters. None of the results were significant (see table 3). Correlations were performed for EDSS, SDMT and HADS, each being correlated with the parameters of the HPA axis (see table 4, next page). Correlations for EDSS and HADS depression with endocrine parameters did not reveal any significant results. Significant results were obtained for the correlation between SDMT and ACTH base. The more impaired the patients were, the less suppressed the patient's ACTH base after pretreatment with Dexamethasone ($r=-0.36$, $p=0.046$), which is displayed in figure 3 (next page). A trend could be demonstrated for the correlation between SDMT and ACTH AUC ($r=-0.31$, $p=0.087$) as shown in figure 3 (next page).

Table 3: Correlation analysis for fatigue associated scales and endocrine parameters

		FSS		MFIS		ESS		PSQI	
		r*	p*	r*	p*	r*	p*	r*	p*
ACTH	Base	0.17	0.361	0.15	0.43	0.09	0.626	-0.25	0.186
	Max	0.08	0.667	0.06	0.738	-0.01	0.959	-0.16	0.389
	δmax	0.05	0.795	0.03	0.857	-0.04	0.838	-0.13	0.503
	AUC	0.12	0.51	0.11	0.574	0.02	0.912	-0.18	0.349
Cortisol	Base	0.20	0.283	0.1	0.612	-0.01	0.962	-0.21	0.28
	Max	0.25	0.177	0.18	0.335	0.11	0.566	-0.03	0.873
	δmax	0.24	0.202	0.19	0.326	0.13	0.497	0.02	0.917
	AUC	0.26	0.169	0.17	0.366	0.1	0.601	-0.05	0.81

*Values as r and p according to two-tailed Pearson's correlation coefficient

Table 4: Correlation analysis of SDMT, EDSS and HADS with endocrine parameters

		SDMT		EDSS		HADS	
		r*	p*	r*	p*	r*	p*
ACTH	Base	**-0.36**	**0.046**	-0.07	0.706	-0.11	0.559
	Max	-0.27	0.14	-0.11	0.542	-0.03	0.877
	δmax	-0.23	0.22	-0.12	0.527	-0.004	0.984
	AUC	*-0.31*	*0.087*	-0.1	0.595	-0.04	0.844
Cortisol	Base	-0.25	0.182	-0.21	0.273	-0.08	0.695
	Max	-0.12	0.52	-0.071	0.709	0.04	0.831
	δmax	-0.08	0.695	-0.03	0.889	0.07	0.724
	AUC	-0.14	0.46	-0.09	0.655	0.03	0.864

r* and p* according to two-tailed Pearson's correlation coefficient,

significant results (p < 0.05) in bold print, *trends (0.05 < p < 0.1) in italic writing*
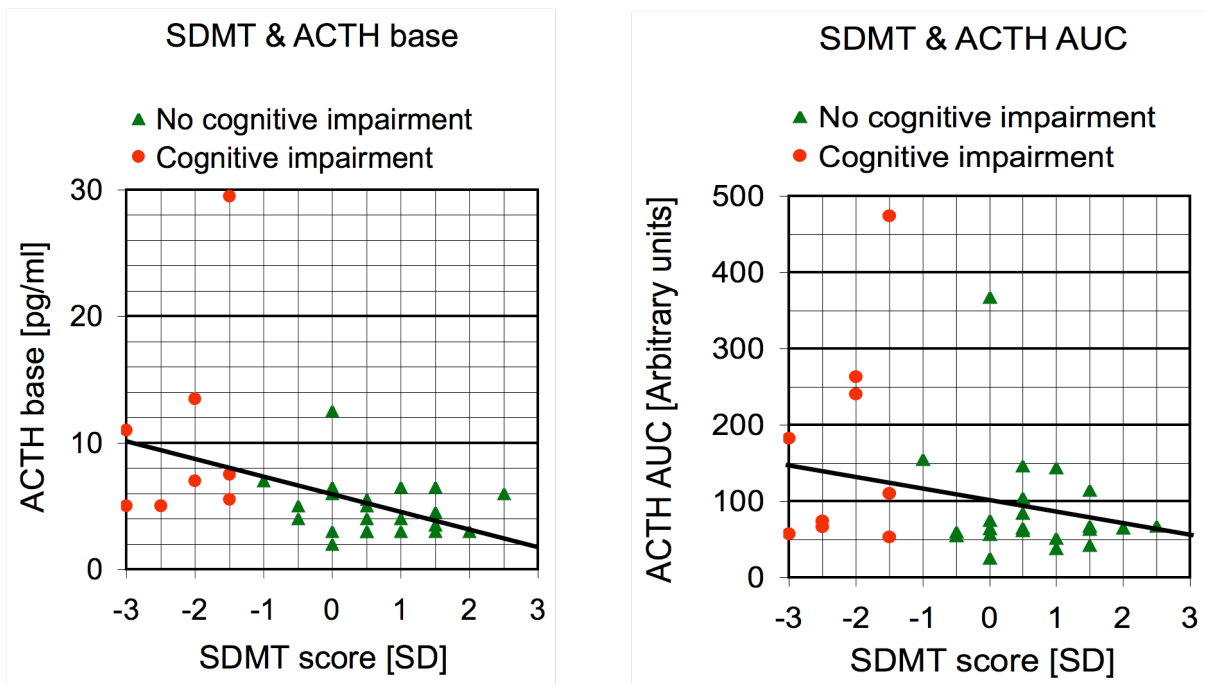

Figure 3: Endocrine parameters are plotted against the grade of cognitive impairment, whereas the raw score has been converted to the standard score. The straight line represents the correlation in both scatterplots.

5 Discussion

5.1 MSF and HPA axis activity

Fatigue is a common and disabling symptom of MS patients. Despite the proposal of a number of possible mechanisms for MSF development and persistence, a certain pathophysiology has not yet been definitely linked to MSF. This study investigated, whether MSF is associated with HPA axis hyperactivity due to central GR dysfunction. The combined Dex/CRH test was applied to thirty participants. Endocrine plasma levels acquired by means of the standardised Dex/CRH test did not differ significantly between groups of MSF and MSNF patients.

Several studies have been conducted, in order to investigate the HPA axis activity in MS (Heesen et al. 2007). After applying basal, dynamic and post-mortem testing, the majority of the research findings have pointed to HPA axis hyperactivity in MS patients. However, some of the studies have displayed results of normal or hypoactive HPA axis response pattern (Grasser et al. 1996, Wei and Lightman 1997). Four studies examined the relation of MSF severity and HPA axis regulation (Wei and Lightman 1997, Heesen et al. 2002, Gottschalk et al. 2005, Ysrraelit et al. 2008). Three of these studies have not detected any significant correlation between MSF severity and endocrine parameters, which have been quantified by basal (Wei and Lightman 1997, Ysrraelit et al. 2008) as well as dynamic (Heesen et al. 2002, Ysrraelit et al. 2008) testing. However, these reports comprised some methodical shortcomings. All three reports have not primarily investigated the relation between MSF and HPA axis regulation. MSF scores have revealed a floor effect in patients of all diesease courses (Heesen et al. 2002) or only of those with RRMS (Ysrraelit et al. 2008). MSF scores have not been published (Wei and Lightman 1997). Besides, most patients have been investigated within four weeks of an acute relapse (Wei and Lightman 1997). By contrast, Gottschalk et al. (2005) have specifically matched clinically stable MSF and MSNF patients with RRMS disease course. Their MSF patient sample has exhibited a centrally caused HPA axis hyperactivity. ACTH plasma levels of MSF patients have been significantly elevated after CRH stimulation, indicating disturbed central GR feedback function.

Gottschalk et al. (2005) have postulated that there is an association of proinflammatory CK and both, MSF and HPA axis upregulation, in RRMS patients. Concerning HPA axis hyperactivity, this may be supported by findings, that antidepressive treatment has

"normalised" HPA axis activity in RRMS patients with relapse (Then Bergh et al. 2001) and has reduced severity of MSF as well as depression in RRMS patients (Mohr et al. 2003). Concerning MSF, a number of results support the idea of CK induced sickness behaviour. Proinflammatory CK following whole blood stimulation of peripheral blood mononuclear cells have been significantly higher in MSF patients of this sample (Heesen et al. 2006). Increased proinflammatory CK mRNA levels in whole blood samples of MSF patients have also been reported (Flachenecker et al. 2004). MSF therapy with amantadine and pemoline has resulted in reduction of proinflammatory CK (serum) and of MSF severity (Bertolone et al. 1993). Contradictory findings by Giovannoni et al. (2001) may be explained by their choice of other markers for sickness behaviour and the heterogeneity of MS. CK have been described to exert direct action on the endocrine system and CNS (Turnbull and Rivier 1999, Harbuz 2002). A number of CK have been shown to activate the HPA axis directly (Besedovsky and del Rey 1996, Turnbull and Rivier 1999, Dunn 2006). CK have been postulated to be causative for sickness behaviour, which is experienced during infection (Konsman et al. 2002, Dantzer and Kelley 2007). Thereby, neuroimmune communication may follow the fast neural pathway or the slow humoral pathway with direct immune to brain transmission (Konsman et al. 2002, Dantzer 2006). The latter has been thought to occur at areas without blood brain barrier, less restrictive blood brain barrier or in areas of breakdown of blood brain barrier as in relapse (Konsman et al. 2002, Schulz and Engelhardt 2005, Dantzer 2006, Kulmatycki and Jamali 2006). CK have been demonstrated to be produced by structures of the brain (e.g. hypothalamus) and to bind on intracerebral structures like microglia, astrocytes and neurons (Kulmatycki and Jamali 2006). Increased levels of proinflammatory CK, CK production on longer duration and in higher quantities, faulty downregulation of CK and sensitisation to CK might induce and hold up sickness behaviour (Dantzer 2006). MSF might be the result of maladaptive, prolonged sickness behaviour without reconstitution of the body's neuroimmune allostasis after infection or inflammation (McEwen 1998). Hence, it is conceivable to explain MSF partly by processes similar of those involved in sickness behaviour.

Immunotherapy may account for the normal HPA axis response pattern despite the presence of significantly increased levels of CK in MSF patients of this sample. In contrast to Gottschalk et al.'s (2005) study population, patients of both groups in this sample received immunotherapy. Glatiramer Acetate has not yet been investigated in terms of its influence on HPA axis activity. However, it has been repeatedly shown, that Glatiramer Acetate modulates

T cell and CK profile of treated patients (Hartung et al. 2004). In contrast to cross-sectional studies of Interferon therapy (Reder and Lowy 1992, Limone et al. 2002), longitudinal studies have reported an influence of Interferon therapy on HPA axis function. Immediately after starting Interferon therapy, the HPA axis has displayed a marked hyperactivity in healthy volunteers and RRMS patients (Goebel et al. 2002, Then Bergh et al. 2007), which has levelled off after one year of Interferon therapy in RRMS patients (Goebel et al. 2005, Then Bergh et al. 2007). In addition, Interferon therapy has been found to modulate CK profile in MS patients (Weber et al. 1998, Dunn 2000). Modulation of CK profile may account for similar results concerning endocrine parameters despite the presence of proinflammatory CK as a potential activator of the HPA axis in MSF patients of this sample, whereas the exact mechanism remains unclear.

Differing results concerning fatigue as side effect of MS immunotherapy have been obtained. On the one hand, fatigue has not been reported as a side effect of Glatiramer Acetate (Johnson et al. 1998, Cohen et al. 2007). On the other hand, some authors have reported fatigue as a side effect of Interferon therapy (Neilley et al. 1996, Walther and Hohlfeld 1999), whereas fatigue has been subordinated to "flu like symptoms" (Walther and Hohlfeld 1999), a collection of symptoms similar to the symptoms of sickness behaviour. Furthermore, fatigue has been shown to increase after initiation of Interferon therapy (Simone et al. 2006). In contrast, longitudinal studies have been able to show a beneficial effect of immunotherapy on MSF severity, whereas Glatiramer Acetate compared to Interferon has been superior in improving MSF symptoms (Metz et al. 2004, Hadjimichael et al. 2008). In line with these findings, MSF and Interferon therapy have not significantly correlated (Flachenecker et al. 2002, Tellez et al. 2005). In conjunction with the latter observations and with no significant group difference concerning immunotherapy in this investigation, one might conclude, that the probability of a recruiting bias with acute MSF as a side effect of immunotherapy in this sample appears to be low.

Another possible explanation for discrepancy of findings may be ascribed to the differing characteristics of MS study population. Gottschalk et al. have recruited mildly disabled RRMS patients. MSNF patients of this sample displayed similar characteristics. On the contrary, MSF patients of this study presented with a significantly higher degree of disability, exhibited a trend towards CPMS disease course and reported significant recent disease progression.

First, the observed change of pathological pattern over time in the MS animal model may give reasons for differing findings concerning HPA axis activity in study populations. Acute EAE has been accompanied by profound HPA axis activation due to inflammation (MacPhee et al. 1989, Mason et al. 1990). A similar HPA axis reaction has been described during the first bout of chronic relapsing EAE. However, a change of pathogenesis to a complement- and antibody-mediated process has been observed during disease progression in chronic relapsing EAE. During this process, the HPA axis has still reacted normoresponsively to novelty stress, but hyporesponsively to inflammatory stress. Disease progression seemed to be associated with a selective desensitisation of the HPA axis to inflammation, by what animals subsequently have ended up dying (Stefferl et al. 2001). Human data appear to be in line with the hypothesis of a change of HPA axis activity over time due to differences in pathological observations depending on the disease course (Kuhlmann et al. 2002, Kutzelnigg et al. 2005). MSF patients in this analysis were mainly in their SPMS disease phase. Reduction of T cell mediated inflammation and intact blood brain barrier has been described in CPMS (Lassmann et al. 2007), so that neurodegenerative pathogenesis may outweigh inflammatory pathogenesis (Zipp and Aktas 2006, Charil and Filippi 2007). Findings of diffuse inflammation in CPMS instead of former waves of focal inflammatory infiltrates in RRMS (Kuhlmann et al. 2002, Kutzelnigg et al. 2005) may be interpreted as being no longer able to cross the threshold necessary for HPA axis activation (Tsigos and Chrousos 1994). However, studies investigating HPA axis function in MS have demonstrated, that increasing disability and disease progression have been accompanied by upregulation of HPA axis activity in cross-sectional (Then Bergh et al. 1999b, Heesen et al. 2002) and longitudinal studies (Gold et al. 2005b), so that HPA axis activity conversion during disease progression appears to be unlikely.

Second, GC insensitivity of immune cells in RRMS patients due to decreased GR affinity and sensitivity (DeRijk et al. 2004, van Winsen et al. 2005, Ysrraelit et al. 2008) may account for HPA axis hyperactivity. In line, another study of altered GC signal transmission has demonstrated that binding capacity of GR has not been diminished on blood lymphocytes but function of GR has been disturbed systemically (Then Bergh et al. 1999a). In depression, proinflammatory CK have been shown to interfere with GR function, which has caused HPA axis activation (Pace et al. 2007). GC insensitivity may be the result of chronic HPA axis activation in RRMS patients, resembling the pathomechanism of GC resistance syndrome (Pariante 2004, Charmandari et al. 2008). As a consequence, immune cells, which are less

sensitive to GC, might be responsible for deteriorating clinical disease status due to less controlled inflammation (DeRijk et al. 2004). GC insensitivity may also explain, why MS patients with HPA axis hyperactivity do not show any signs of hypercortisolism. Interestingly, HPA axis hyperactivity in RRMS patients with relapse has reversed to "normal" HPA axis activity following Moclobemide treatment (Then Bergh et al. 2001). This may be interpreted as an existing adjustable capability of HPA axis during early disease course. Later in the disease course, GC insensitivity of immune cells has diminished in CPMS patients in two studies (DeRijk et al. 2004, van Winsen et al. 2005), but not in another study (Ysrraelit et al. 2008). Findings may have differed due to unequal methods or general MS heterogeneity. Decreasing GC insensitivity in at least some CPMS patients may suggest, that there may be an additional mechanism for HPA deregulation in late stages of MS.

Third, other studies investigating HPA axis activity provide support for a heterogeneous HPA axis response to dynamic testing. Grasser et al. (1996) applied the Dex/CRH test to RRMS and SPMS patients with acute exacerbation. The majority of their sample exhibited a hyperactive HPA axis. However, some of their patients have shown a physiological response pattern and a third subgroup have exhibited a blunted HPA axis response to the Dex/CRH challenge. Hyporesponsiveness has also been observed in this study sample, whereas seven of the nine participants with blunted HPA axis response shared high disability ratings. A similar description has been provided by two other studies (Grasser et al. 1996, Wei and Lightman 1997), with hyporesponsiveness being more likely to occur in MS patients with a progressive disease form, high disability scores and long disease duration. HPA axis heterogeneity in CPMS patients with higher disability ratings is further supported by two postmortem studies. A higher active lesion load in the hypothalamus inhibited HPA axis activity. (Huitinga et al. 2003, Huitinga et al. 2004). The closer the lesions to the hormone secreting neurons, the stronger the neuron specific inhibition appeared to be (Huitinga et al. 2004). Such inhibition resulted in a worse and more fatal disease course in septic MS patients. Their ability to secrete an adequate amount of endogenous Cortisol to the acute septic inflammatory stimulus was reduced (Huitinga et al. 2003), which may be comparable to septic patients with adrenal insufficiency (Marik and Zaloga 2002). Hypothalamic lesions were present in more than 90 % of brains of these MS patients at autopsy. More than 50 per cent of these lesions appeared to be active. Hypothalamic lesion load was reflected in a general high lesion load throughout the whole brain (Huitinga et al. 2001). Patients at autopsy had previously all been in their progressive disease phase with high disability scores after long disease duration, mainly

having suffered from SPMS (Huitinga et al. 2003, Huitinga et al. 2004). In contrast to this study, increasing HPA axis activation has also been observed in MS patients with higher disability scores and parallel to disease progression (Then Bergh et al. 1999b, Heesen et al. 2002, Gold et al. 2005b). Differences may be explained by the observation, that seven (of 15) highly disabled MS patients of this sample exhibited HPA axis hypoactivity. Gold et al. (2005a) have postulated, that the transition from inflammatory to degenerative disease phase, i.e. from RRMS to SPMS, might be marked by a considerable HPA axis hyperactivity secondary to axonal or neuronal lesions in areas, which usually inhibit HPA axis activity. The HPA axis hyperactivity may eventually decrease CNS inflammation, while neurodegeneration remains unchanged. This idea is supported by results of a significant correlation between the magnitude of HPA axis hyperactivity and the speed of disease progression as well as worsening of disability scores (Gold et al. 2005b). Besides, their patient sample exhibited a positive correlation between the degree of cognitive impairment and the extent of HPA axis hyperactivity (Heesen et al. 2002, Gold et al. 2005b). Furthermore, cognitive impairment (Bermel and Bakshi 2006, Sanfilipo et al. 2006) and HPA axis hyperactivity (Schumann et al. 2002) have been both found to be associated with brain atrophy. As a result of findings of HPA axis hypo- and hyperactivity in highly disabled CPMS patients with long disease duration, Gold et al. (2005a) have speculated, that the Dex/CRH test for investigating the HPA axis function of MS patients might be a prognostic tool, in order to predict future disease course severity.

Conclusively, MSF and HPA axis response pattern are postulated to be secondary phenomena caused by the individual MS intrinsic pathology and disease course. Humoral substances (e.g. CK, immunotherapy), humoral mechanisms (e.g. GC insensitivity) and neurodegeneration (e.g. hypothalamic MS lesions) may cause MSF and may change HPA axis function. The longer the disease duration, the more influential factors have been identified, causing a heterogeneous HPA axis response in CPMS patients. Besides, hyporesponsive as well as hyperresponsive HPA axis pattern in early stages of MS may have a prognostic value. The degree of HPA axis deregulation in early stages of MS may be a reflection of the extent of disease activity, i.e. total lesion load, and might therefore predict speed of disease progression and development of permanent disability.

5.2 MSF and disability

MSF scores moderately correlated with disability scales, which is in line with the results of the vast majority of studies correlating MSF and disability (Colosimo et al. 1995, Bakshi et al. 2000, Flachenecker et al. 2002, Iriarte et al. 2000, Kroencke et al. 2000, Lobentanz et al. 2004, Tellez et al. 2005, Pittion-Vouyovitch et al. 2006). Controlling for depression, age and disease duration has resulted in a lesser but still significant correlation between MSF and disability (Bakshi et al. 2000, Flachenecker et al. 2002, Tellez et al. 2005). MSF frequency and severity have been shown to increase with disease progression (Colosimo et al. 1995, Flachenecker et al. 2002, Tellez et al. 2005), which has mainly been attributed to the accumulating disability in CPMS disease course (Colosimo et al. 1995, Kroencke et al. 2000, Pittion-Vouyovitch et al. 2006).

Further support for a relation between MSF and disability has been gained by results of imaging studies. GM atrophy may be considered the best marker of disability (Agosta et al. 2006, Pirko et al. 2007) and has been shown to significantly correlate with disability ratings (Tedeschi et al. 2005). Disability ratings have correlated better with brain atrophy in CPMS than in RRMS patients (Ge 2000), possibly due to predominantly existing GM pathology in CPMS compared to RRMS patients (Pirko et al. 2007). Cortical reorganisation in early stages of MS may compensate CNS tissue loss, hence avoiding development of early disability (Reddy et al. 2000, Pantano et al. 2002, Bjartmar et al. 2003). A number of investigators have reported similar structural CNS abnormalities in MSF patients. MSF patients have presented with a significant higher lesion load of the entire brain (Colombo et al. 2000, Tedeschi et al. 2007) and significant GM atrophy (Tedeschi et al. 2007). Following a longitudinal study, Marrie et al. (2005) have postulated, that MSF in RRMS patients may be a prognostic marker for future brain atrophy, which was independent of disability. This independency is hypothesised to be explained by the aforementioned effective compensatory mechanisms concerning cortical reorganisation (Reddy et al. 2000, Pantano et al. 2002, Bjartmar et al. 2003), although this has not yet been investigated in relation to MSF. Early lesion load has been postulated to predict subsequent brain atrophy (Chard et al. 2003). Thus, rate of GM atrophy was hypothesised to predict the speed of disease progression and hence the amount of accumulating disability (Agosta et al. 2006). Hence, MSF is postulated to be partly explained by mechanisms causing permanent neurological disability as axonal loss and neurodegeneration. Mild correlation of MSF and disability in early stages of MS may be explained with successful compensatory cortical reorganisation and successful remission after

relapse (Inglese et al. 2002, Vollmer 2007), thus avoiding early disability. However, extensive cortical reorganisation may be the cause for MSF at this stage of MS (Kos et al. 2008). Correlation between MSF and disability increases in CPMS, possibly due to exhaustion of cortical reorganisation as well as increasing lesion load and brain atrophy (Tedeschi et al. 2005). These mechanisms are hypothesised to result in parallel deterioration of MSF and disability (Inglese et al. 2002, Bjartmar et al. 2003). Severity of MSF is postulated to predict the extent of upcoming brain atrophy, which might predict future severity of disability.

5.3 MSF and quality of life

MSF persistence may also have a psychosocial component. MSF has been demonstrated to be one of the two most disabling factors in MS (Fisk et al 1994). MSF has been more frequent and more severe the longer the disease has lasted and the more disabled the patient has been (Tellez et al. 2006). MSF has been shown to contribute to the morbidity associated with MS by limiting energy and endurance and by adversely affecting mood, outlook and ability to cope with accompanying symptoms (Bakshi 2003). Therefore, it is not surprising, that MSF was significantly associated with a reduced quality of life in this study confirming results from other reports (Fisk et al. 1994, Amato et al. 2001, Gold et al. 2001, Janardhan and Bakshi 2002, Merkelbach et al. 2002, Lobentanz et al. 2004, Pittion-Vouyovitch et al. 2006). MSF has remained significantly associated with impaired quality of life even after adjusting for depression and disability (Janardhan and Bakshi 2002, Merkelbach et al. 2002). As depression and disability, MSF has been an important, possibly independent determinant of quality of life (Nortvedt et al. 1999, Janardhan and Bakshi 2000, Amato et al. 2001, Janardhan and Bakshi 2002).

Vice versa, subjective fatigue has been found to increase by psychosocial factors, namely low sense of control and focussing on bodily sensation (Vercoulen et al. 1996), both given in a state of permanent neurological dysfunction when relying on helping hands to perform acts of daily living. MSF patients, who have been able to create environments appropriate of their physical and psychological needs, have reported lower fatigue scores (Krupp and Rivzi 2002). Moderate fitness training and yoga, which are supposed to improve sense of control, have been able to reduce fatigue severity (Oken et al. 2004) and have improved quality of life (Schulz et al. 2004). Cognitive behaviour therapy has been able to reduce MSF severity (van Kessel et al. 2008) and education has been found to be protective in terms of MSF severity (Tedeschi et al. 2007).

Hence, psychosocial factors and low quality of life are hypothesized to contribute partially to MSF severity. Conversely, MSF seems to be an independent primary predictor of quality of life.

5.4 MSF, HPA axis activity and depression

A significant correlation between scores of MSF and depression was detected despite the exclusion of clinically obvious depression. HADS scores revealed a floor effect , reducing the statistical power for this correlation. Reviewing the literature in MS, there has been some controversy about the relationship between MSF and depression in MS. A minority of studies have found no or a weak correlation between MSF and depression (Krupp et al. 1988, Vercoulen et al. 1996, Iriarte et al. 2000). In contrast, others have reported a significant correlation between MSF and depression, even after adjusting for possible influential factors, e.g. disability (Fisk et al. 1994, Ford et al. 1998, Bakshi et al. 2000, Kroencke et al. 2000, Flachenecker et al. 2002, Tellez et al. 2005, Pittion-Vouyovitch 2006). Differences in these findings may be attributed to small sample size (Krupp et al. 1988), CNS active medication (Krupp et al. 1988, Vercoulen et al. 1996), rather low MSF scores (Iriarte et al. 2000) or use of other rating scales for MSF and/or depression (Krupp et al. 1988, Vercoulen et al. 1996, Iriarte et al. 2000). MSF has been found to independently predict depression (Lobentanz et al. 2004) and vice versa (Flachenecker et al. 2002). Depressed MS patients have demonstrated a greater likelihood of being fatigued (Bakshi et al. 2000), with depressive scores also predicting changes on MSF scales (Tellez et al. 2006). Persistent MSF has been more likely to occur in patients with severe disability and severe depression (Tellez et al. 2006) Conclusively, the majority of published results supports the view of an association between MSF and depression.

HADS scores and endocrine parameters as obtained through the combined Dex/CRH test did not significantly correlate, which is in line with findings of other studies (Then Bergh et al. 1999b, Heesen et al. 2002, Ysrraelit et al. 2008). In contrast, severity of depression has correlated with the extent of HPA axis hyperactivity and inflammatory disease activity in RRMS patients with active disease (Fassbender et al. 1998). Differences in findings might be explained by the state of disease activity (inflammation) and the clear-cut RRMS patient sample (Fassbender et al. 1998). In general, proinflammatory CK (Flachenecker et al. 2004, Heesen et al. 2006, Pace et al. 2007) and HPA axis hyperactivity (Holsboer et al. 1994, Gottschalk et al. 2005) might be involved in the pathogenesis of both, MSF and major

depression. In addition, MS, MSF and major depression seem to share a number of epidemiological, aetiological and clinical features (Joffe 2005, Siegert and Abernethy 2005, Kos et al. 2008, Gold and Irwin 2009). However, both entities may exist independent of each other, suggesting additional mechanisms for MSF development and persistence. The latter may be the case in this study sample. Furthermore, MSF, depression and poor quality of life are hypothesised to be intertwined in a vicious cycle, possible causing continuous deterioration among each other if not disrupted. Perpetuation of the cycle may cause aggravation of the three features involved, if not recognised and therapeutically interrupted.

5.5 MSF, HPA axis activity and sleep-wake regulation

MSF scales and diminished sleep quality as well as MSF scales and increased daytime sleepiness exhibited a moderate correlation in this study sample. However, only a minority of participants exceeded the previously described cut-offs of the scales used (PSQI, ESS) (Buysse et al. 1989, Johns 1991). Besides, a floor effect of both scales was detected. In line with observations of this study, diminished sleep quality of MSF patients has been described before, either by using sleep logs and actigraphy (Attarian et al. 2004, Stanton et al. 2006) or by using PSQI (Lobentanz et al. 2004). Sleep disturbance has been found to be a contributor to MSF (Strober and Arnett 2005). MS associated sleep distraction has been described to be caused either by MS intrinsic mechanisms, e.g. disease severity, or by sleep disorders, e.g. restless leg syndrome or insomnia (Attarian et al. 2004, Fleming and Pollak 2005, Stanton et al. 2006). A significant positive correlation between MSF and increased daytime sleepiness has been described before (Rammohan et al. 2002, Kotterba et al. 2003, Attarian et al. 2004). On the contrary, no correlation has been found between MSF scales and daytime sleepiness (ESS, pupillography) in RRMS patients (Frauscher et al. 2005). The latter result may be explained by the mild disability of that distinct MS patient sample, so that contributing factors to daytime sleepiness (Fleming and Pollak 2005) in those patients might not have been developed yet. In addition, the ESS scores of those participants have revealed a floor effect.

The HPA axis is a central element for sleep regulation. HPA axis activity follows a circadian rhythm with a nadir during the night for a regenerating sleep (Siegel 2004, Buckley and Schatzberg 2005, Saper et al. 2005). However, the correlation between endocrine parameters and PSQI nor the correlation between endocrine parameters and ESS revealed significant results in this patient sample. In contrast, endocrine abnormalities (ACTH, Cortisol) and raised inflammatory markers have been observed in chronic insomnia (Vgontzas et al. 2001).

Activation of the HPA axis or administration of GC have induced arousal and sleeplessness (Vgontzas and Chrousos 2002). Downregulation of the activated HPA axis has reduced symptoms of chronic insomnia (Basta et al. 2007). Several reasons may account for differing findings. Most important of all, scoring in both scales (ESS, PSQI) revealed a floor effect. Second, we applied a dynamic test to these participants, which measures GR feedback function at the pituitary level. Thus, slightly altered basal concentrations of ACTH and Cortisol at night or disturbance of circadian rhythm would not have been detected, even if some of the patients had been diagnosed with diminished sleep quality and/or subsequent excessive daytime sleepiness.

MSF seems to be associated with sleep disorders and subsequent excessive daytime sleepiness in a certain fraction of MS patients, with HPA axis hyperactivity not playing a causative role in this sample. Long disease duration, progressive disease course and subsequent moderate to high disability ratings are postulated to be contributing to sleep disturbance in this sample.

5.6 MSF, HPA axis activity and cognitive impairment

FSS scores and cognitive impairment did not significantly correlate in this study, whereas a trend was detected between MFIS and SDMT scores. This may be explained by the description, that the FSS has been described to rather measure the physical component of MSF. In contrast, MFIS had rather a cognitive emphasis (Tellez et al. 2005). Findings of this study are in line with results published before, showing that cognitive fatigue was found to be a contributor to the development of cognitive impairment (Krupp and Rivzi 2002). The frequency of cognitive impairment in MS patients ranges from 30 to 70 per cent (Krupp and Rivzi 2002, Chiaravalloti and DeLuca 2008), pointing to an overlap of MSF and cognitive impairment in some MS patients.

This study could anew confirm results, when cognitive impairment has significantly correlated with the extent of HPA axis hyperactivity (Heesen et al. 2002). Gold et al. (2005b) have speculated that accumulating lesions in areas involved in memory, attention and information processing, e.g. prefrontal cortex, thalamus and hippocampus (Nolte 2002), may disturb the usually sent inhibitory signals. As a consequence, HPA axis hyperactivity may evolve, which is postulated to further damage GC sensitive brain areas (prefrontal cortex, thalamus, hippocampus). GC have been shown to reduce number and length of hippocampal dendrites and to inhibit neurogenesis as seen through sustained hippocampal atrophy years

after a depressive episode (Höschl and Hajek 2001). As a support of this hypothesis, imaging studies have found a significant association between cognitive impairment and regional CNS atrophy, namely hippocampal volume loss (Mungas et al. 2005), 3rd ventricle width (Benedict et al. 2004) and atrophy of the temporal lobe (Benedict et al. 2005). As a consequence, cerebral reorganisation with increased levels of activation as seen in further imaging studies (Chiaravalloti and DeLuca 2008) may explain cognitive impairment but also the moderate association with cognitive fatigue. HPA axis hyperactivity may be regarded as a secondary phenomenon in this context.

5.7 Study weakness

Heterogeneity of recruited participants in this study may be considered a study weakness.. As already described in the introduction of this thesis, epidemiology, aetiology, pathogenesis, histopathology, clinical course and the wide range of clinical presentation of MS and MSF make it difficult to recruit a homogenous patient sample. Especially in CPMS, more and more MS features overlap and complicate unequivocal conclusions. In this study, immunotherapy, esp. Interferon therapy, and presence of depression may have resulted in a type II error. Besides, the low number and the diverse characteristics of participants reduce statistical power of findings.

The combined Dex/CRH test has been implemented in a sample of depressed patients (von Bardeleben and Holsboer 1989). Sensitivity of detecting HPA axis deregulation has been estimated to be 80 per cent (Pace et al. 2007). However, the Dex/CRH test has been described to demonstrate GR feedback dysfunction at the pituitary level only and has limited value concerning conclusions on other elements of HPA axis function (Schulz et al. 2005). Disturbance of the circadian rhythm or of the basal concentrations (Vgontzas et al. 2001, Buckley and Schatzberg 2005, Basta et al. 2007) cannot be discovered. Therefore, dynamic HPA axis testing may be insufficient in inquiries of HPA axis pathology.

Also, the HPA axis as part of the stress system does not follow the concept of homoeostasis. No clear-cut references of parameters (ACTH, Cortisol) can be defined, since the HPA axis responds to acute and chronic stress with different activation, which is at the time being necessary (and physiological) to maintain the body's integrity (McEwen 1998, Schulz et al. 2005). Hence, it depends on the temporal framework, when parameters of the HPA axis are quantified, in order to conclude appropriately. Few is known about the HPA axis behaviour

over time (Mohr and Pelletier 2006), because the majority of the studies conducted so far have followed a cross-sectional design. Hence, the cross-sectional concept of this study might be another reason why conclusions on results may be limited due to the snapshot on the sample.

Another weakness of this study is the absence of a control group. Theoretically, both study groups may have presented with HPA axis hyperactivity, which was then not detected due to the lack of healthy controls.

5.8 Conclusion and Perspective

The results of this study in the context of other investigations underline the complex nature of MSF. A number of MS features (e.g. disease course, disability, quality of life) caused by MS-intrinsic pathogenesis (inflammation, neurodegeneration, compensatory neuronal reorganisation) intermingle, thus complicating inquiry of MSF pathology. MSF is hypothesized to be caused by every single factor but also by every conceivable combination of factors. MSF prevalence seems to depend on the number of factors involved, since e.g. disabled MS patients with progressive disease course are more likely to present with MSF. The great variety of clinical presentation and underlying pathology emphasize the importance of a multidimensional approach to every MSF patient, in order to detect the individual MSF characteristics and to begin appropriate treatment.

In summary, two theories of MSF development and persistence are proposed. First, the humoral fatigue hypothesis comprises changes in endocrine and immune active systems, e.g. HPA axis regulation and CK. Second, the structural hypothesis includes the pathological processes of WM and GM as well as the cortical reorganisation following CNS tissue loss. Both models are amended by psychosocial factors, which may reflect humoral and structural pathology. Both hypotheses are supposed to complement each other.

Conflicting results concerning the association of MSF severity and HPA axis activity have been obtained. A significant correlation of MSF severity and HPA axis hyperactivity could only be shown in RRMS patients without relapse. Three other studies including this one could not demonstrate this relationship. Differences of observations may be attributed to disease course (RRMS, CPMS), disease activity (without exacerbation, relapse, progression), GR affinity and sensitivity, prevailing pathological pattern (inflammation, neurodegeneration),

CNS lesion localisation (prefrontal cortex, hippocampus hypothalamus) and immunotherapy. A humoral and a structural hypothesis is generated (see also Gold et al. 2005a).

First, inflammatory lesions and mediators (e.g. CK) in RRMS may cause HPA axis activation. Depending on GR function of immune cells, inflammation may either be controlled (GC sensitive immune cells) or may result in uncontrolled inflammation (GC insensitive immune cells), possibly increasing HPA axis activation. Increasing inflammation, i.e. higher number of CNS lesions, may culminate in exhaustion of cortical reorganisation, possibly inducing MSF. Furthermore, a larger number of CNS lesions may increase vulnerability to subsequent permanent damage. GC insensitivity may be the reason for faster disease progression due to uncontrolled disease. The latter may result in higher disability scores and greater brain atrophy. On the one hand, the combination of MSNF and "normoactive" HPA axis in RRMS patients might stand for a good prognosis due to a controlled disease and adequate cortical reorganisation. On the other hand, MSF and HPA axis hyperactivity in RRMS patients might indicate a worse prognosis concerning upcoming disease progression. MSF severity may predict upcoming brain atrophy and HPA axis hyperactivity may predict disability progression. Both features (MSF, HPA axis hyperactivity) may be a reflection of total lesion load.

Second, accumulated lesion load, i.e. total brain atrophy, and CNS lesions in specific areas may account for HPA axis hypo- and hyperactivity in late stages of MS. Neurodegeneration in areas involved in HPA axis feedback control, e.g. prefrontal cortex or hippocampus may cause further disinhibition of HPA axis. This might in turn decrease inflammation and at the same time mark the transition from RRMS to SPMS. Direct inhibitory lesions in the hypothalamus might be responsible for hypoactive HPA axis. Hypoactive HPA axis may even result in worse disease course. Insufficient HPA axis response to inflammation might accelerate disease progression, possibly allowing unrestricted disease dissemination. MSF may further be aggravated by total lesion load and GM atrophy.

Hence, MSF and HPA axis activity may be secondary phenomena to the body's reaction to the attacking disease (MS), due to which allostasis is kept or not (McEwen 1998, Schulz et al. 2005). However, pathological findings, i.e. MSF or HPA axis deregulation, may indicate loss of allostasis in neural, endocrine and/or immune function, which might subsequently worsen

prognosis. The longer the disease duration, the more influential factors concerning allostasis in MS have been identified, complicating the interpretation of study findings.

Future investigations of MSF and HPA axis function should have a longitudinal design. Moreover, homogenous patient samples should alleviate interpretation of study findings. Instead of restricting investigations to one entity, a wider screening incorporating clinical, laboratory and imaging markers should help overcoming previous methodical shortcomings.

6 Summary

Introduction: Multiple sclerosis (MS) is a complex immune-mediated inflammatory and neurodegenerative disease of the central nervous system (CNS). Recent studies were repeatedly able to demonstrate hypothalamic-pituitary-adrenal axis (HPA axis) hyperactivity in MS patients. MS related fatigue (MSF) is one of its most disabling symptoms. Postulated mechanisms for MSF development include neuroendocrine, neuroimmune and structural abnormalities of the central nervous system. Other diseases with fatigue (e.g. chronic fatigue syndrome, major depression) have been found to be associated with HPA axis deregulation. **Objective:** This study investigated, whether MSF severity correlates with the extent of HPA axis hyperactivity. **Patients and methods:** In a prospective, cross-sectional study, 31 clinically stable MS patients definitely fulfilling MS-diagnostic criteria were recruited from the database of the MS outpatient clinic of the Department of Neurology, University Hospital Eppendorf. Exclusion criteria were applied (incomplete address in the database, a missing fatigue screening score (see next paragraph), an incomplete medical record, current pregnancy, current MS relapse, relapse during the last three months, steroid treatment within the last three months, known endocrine abnormality or clinical evident psychiatric disease). 16 Patients with MSF and 15 patients without MSF were enrolled into the study, using empirical cut-offs for the scores determined by the fatigue severity scale (FSS). Fatigue severity was correlated with endocrine parameters (ACTH, Cortisol) won by the combined dexamethasone/corticotropin-releasing hormone test (Dex/CRH test). Clinical characteristics (sleep quality, daytime sleepiness, disability, cognitive impairment, depression, quality of life) of the participants were examined. **Results:** MSF severity and endocrine parameters were not significantly associated. MSF severity significantly correlated with disease disability, diminished sleep quality, excessive daytime sleepiness and reduced quality of life. No correlation was found between MSF severity and cognitive impairment. The degree of cognitive impairment significantly correlated with the extent of HPA axis hyperactivity. **Discussion:** MSF severity did not significantly correlate with HPA axis hyperactivity. MSF and HPA axis activity may be secondary phenomena to the body's reaction to MS, possibly having a prognostic value in early stages of MS. HPA axis hyperactivity in cognitively impaired MS patients might be the result of neural loss of inhibiting cortico-subcortical pathways. Future studies should involve larger and more homogeneous patient samples.

7 References

Agosta et al. 2006: Agosta F, Rovaris M, Pagani E, Sormani MP, Comi G, Filippi M. (2006) Magnetization transfer MRI metrics predict the accumulation of disability 8 years later in patients with multiple sclerosis. Brain. Oct;129(10):2620-7.

Amato et al. 2001: Amato MP, Ponziani G, Rossi F, Liedl CL, Stefanile C, Rossi L. (2001) Quality of life in multiple sclerosis: the impact of depression, fatigue and disability. Mult Scler. Oct;7(5):340-4.

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

Bakshi et al. 2000: Bakshi R, Shaikh ZA, Miletich RS, Czarnecki D, Dmochowski J, Henschel K, Janardhan V, Dubey N, Kinkel PR. (2000) Fatigue in multiple sclerosis and its relationship to depression and neurologic disability. Mult Scler. Jun;6(3):181-5.

Bakshi 2003: Bakshi R. (2003) Fatigue associated with multiple sclerosis: diagnosis, impact and management. Mult Scler. Jun;9(3):219-27.

Basta et al. 2007: Basta M, Chrousos GP, Vela-Bueno A, Vgontzas AN. (2007) Chronic insomnia and stress system. Sleep Med Clin. Jun;2(2):279-291.

Benedict et al. 2004: Benedict RH, Weinstock-Guttman B, Fishman I, Sharma J, Tjoa CW, Bakshi R. (2004) Prediction of neuropsychological impairment in multiple sclerosis: comparison of conventional magnetic resonance imaging measures of atrophy and lesion burden. Arch Neurol. Feb;61(2):226-30.

Benedict et al. 2005: Benedict RH, Zivadinov R, Carone DA, Weinstock-Guttman B, Gaines J, Maggiore C, Sharma J, Tomassi MA, Bakshi R. (2005) Regional lobar atrophy predicts memory impairment in multiple sclerosis. AJNR Am J Neuroradiol. Aug;26(7):1824-31.

Benveniste 1997: Benveniste EN (1997) Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. J Mol Med. Mar;75(3):165-73.

Bergamaschi et al. 1997: Bergamaschi R, Romani A, Versino M, Poli R, Cosi V. (1997) Clinical aspects of fatigue in multiple sclerosis. Funct Neurol. Sep-Oct;12(5):247-51.

Bermel and Bakshi 2006: Bermel RA, Bakshi R (2006) The measurement and clinical relevance of brain atrophy in multiple sclerosis. Lancet Neurol. Feb;5(2):158-70.

Bertolone et al. 1993: Bertolone K, Coyle PK, Krupp LB, Doscher CA (1993) Cytokine correlates of fatigue in multiple sclerosis. Neurology. 43:A356,769S.

Besedovsky and Del Rey 1996: Besedovsky HO, del Rey A. (1996) Immune-neuro-endocrine interactions: facts and hypotheses. Endocr Rev. Feb;17(1):64-102.

Bjartmar et al. 2003: Bjarmar C, Wujek JR, Trapp BD. (2003) Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. J Neurol Sci. Feb 15;206(2):165-71.

Bolton and Flower 1989: Bolton C, Flower RJ (1989) The effects of the anti-glucocorticoid RU 38486 on steroid-mediated suppression of experimental allergic encephalomyelitis (EAE) in the Lewis rat. Life Sci 45(1):97-104.

Brück 2005: Brück W. (2005) Inflammatory demyelination is not central to the pathogenesis of multiple sclerosis. J Neurol. Nov;252 Suppl 5:v10-5.

Buckley and Schatzberg 2005: Buckley TM, Schatzberg AF. (2005) On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders. J Clin Endocrinol Metab. May;90(5):3106-14.

Buysse et al. 1989: Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ (1989) The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. May;28(2):193-213.

Charil and Filippi 2007: Charil A, Filippi M. (2007) Inflammatory demyelination and neurodegeneration in early multiple sclerosis. J Neurol Sci. Aug 15;259(1-2):7-15.

Charmandari et al. 2008: Charmandari E, Kino T, Ichijo T, Chrousos GP. (2008) Generalized glucocorticoid resistance: clinical aspects, molecular mechanisms, and implications of a rare genetic disorder. J Clin Endocrinol Metab. May;93(5):1563-72.

Chaudhuri and Behan 2004: Chaudhuri A, Behan PO. (2004) Fatigue in neurological disorders. Lancet. Mar 20;363(9413):978-88.

Chiaravalloti and DeLuca 2008: Chiaravalloti and DeLuca (2008) Cognitive impairment in multiple sclerosis. Lancet Neurol. Dec;7(12):1139-51.

Cleare 2003: Cleare AJ (2003). The neuroendocrinology of chronic fatigue syndrome. Endocr Rev. Apr;24(2):236-52.

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

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

Colosimo et al. 1995: Colosimo C, Millefiorini E, Grasso MG, Vinci F, Fiorelli M, Koudriavtseva T, Pozzilli C. (1995) Fatigue in MS is associated with specific clinical features. Acta Neurol Scand. Nov;92(5):353-5.

Comi et al. 2001: Comi G, Leocani L, Rossi P, Colombo B. (2001) Physiopathology and treatment of fatigue in multiple sclerosis. J Neurol. Mar;248(3):174-9. Review.

Compston and Coles 2002: Compston A, Coles A. (2002) Multiple sclerosis. Lancet Apr 6;359(9313):1221-31. Review. Erratum in: Lancet 2002 Aug 24;360(9333):648.

Confavreux et al. 2000: Confavreux C, Vukusic S, Moreau T, Adeleine P. (2000) Relapses and progression of disability in multiple sclerosis. N Engl J Med. Nov 16;343(20):1430-8.

Confavreux et al. 2003 : Confavreux C, Vukusic S, Adeleine P. (2003) Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. Brain. Apr;126(4):770-82.

Dantzer 2006: Dantzer R. (2006) Cytokine, sickness behavior, and depression. Neurol Clin. Aug;24(3):441-60.

Dantzer and Kelley 2007: Dantzer R, Kelley KW. (2007) Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun. Feb;21(2):153-60.

DeRijk et al. 2004 : DeRijk RH, Eskandari F, Sternberg EM. (2004) Corticosteroid resistance in a subpopulation of multiple sclerosis patients as measured by ex vivo dexamethasone inhibition of LPS induced IL-6 production. J Neuroimmunol. Jun;151(1-2):180-8.

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

Dunn 2000: Dunn AJ. (2000) Cytokine activation of the HPA axis. Ann N Y Acad Sci.;917:608-17.

Dunn 2006: Dunn AJ. (2006) Effects of cytokines and infections on brain neurochemistry. Clin Neurosci Res. Aug;6(1-2):52-68.

Dyment et al. 1997: Dyment DA, Sadovnick AD, Ebers GC. (1997) Genetics of multiple sclerosis. Hum Mol Genet.6(10):1693-8.

Elenkov et al. 1999: Elenkov IJ, Webster EL, Torpy DJ, Chrousos GP. (1999) Stress, corticotropin-releasing hormone, glucocorticoids, and the immune/inflammatory response: acute and chronic effects. Ann N Y Acad Sci. Jun 22;876:1-11; discussion 11-3.

Erkut et al. 2002: Erkut ZA, Endert E, Huitinga I, Swaab DF. (2002) Cortisol is increased in postmortem cerebrospinal fluid of multiple sclerosis patients: relationship with cytokines and sepsis. Mult Scler. May;8(3):229-36.

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

Fisk et al. 1994: Fisk JD, Pontefract A, Ritvo PG, Archibald CJ, Murray TJ (1994) The impact of fatigue on patients with multiple sclerosis. Can J Neurol Sci. Feb;21(1):9-14.

Flachenecker and Zettl 2002: Flachenecker P and Zettl UK (2002) Krankheitsverlauf und Prognose. In: Schmidt RM and Hoffmann F (Hrsg) Multiple Sklerose, 3. Auflage, Urban & Fischer Verlag, München Jena, Seite 52-53

Flachenecker et al. 1999: Flachenecker P, Wolf A, Krauser M, Hartung HP, Reiners K. (1999) Cardiovascular autonomic dysfunction in multiple sclerosis: correlation with orthostatic intolerance. J Neurol. Jul;246(7):578-86.

Flachenecker et al. 2002: Flachenecker P, Kumpfel T, Kallmann B, Gottschalk M, Grauer O, Rieckmann P, Trenkwalder C, Toyka KV (2002) Fatigue in multiple sclerosis: a comparison of different rating scales and correlation to clinical parameters. Mult Scler. Dec;8(6):523-6.

Flachenecker et al. 2003: Flachenecker P, Rufer A, Bihler I, Hippel C, Reiners K, Toyka KV, Kesselring J. (2003) Fatigue in MS is related to sympathetic vasomotor dysfunction. Neurology. Sep 23;61(6):851-3.

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

Fleming and Pollak 2005: Fleming WE, Pollak CP. (2005) Sleep disorders in multiple sclerosis. Semin Neurol. Mar;25(1):64-8.

Ford et al. 1998: Ford H, Trigwell P, Johnson M. (1998) The nature of fatigue in multiple sclerosis. J Psychosom Res. Jul;45(1 Spec No):33-8.

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

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

Ge et al. 2000: Ge Y, Grossman RI, Udupa JK, Wei L, Mannon LJ, Polansky M, Kolson DL. (2000) Brain atrophy in relapsing-remitting multiple sclerosis and secondary progressive multiple sclerosis: longitudinal quantitative analysis. Radiology. Mar;214(3):665-70.

Giovannoni et al. 2001: Giovannoni G, Thompson AJ, Miller DH, Thompson EJ. (2001) Fatigue is not associated with raised inflammatory markers in multiple sclerosis. Neurology. Aug 28;57(4):676-81.

Goebel et al. 2002: Goebel MU, Baase J, Pithan V, Exton M, Saller B, Schedlowski M, Limmroth V. (2002) Acute interferon beta-1b administration alters hypothalamic-pituitary-adrenal axis activity, plasma cytokines and leukocyte distribution in healthy subjects. Psychoneuroendocrinology. Nov;27(8):881-92.

Goebel et al. 2005: Goebel MU, Czolbe F, Becker H, Janssen OE, Schedlowski M, Limmroth V. (2005) Effects of interferon-beta 1a on the hypothalamic-pituitary-adrenal axis, leukocyte distribution and mood states in multiple sclerosis patients: results of a 1-year follow-up study. Eur Neurol.;53(4):182-7.

Gold and Heesen 2006: Gold SM, Heesen C. (2006) Stress and disease progression in multiple sclerosis and its animal models. Neuroimmunomodulation.;13(5-6):318-26.

Gold and Irwin 2009: Gold SM, Irwin MR (2009) Depression and Immunity: Inflammation and Depressive Symptoms in Multiple Sclerosis. Immunol Allergy Clin N Am May;29(2):309-320.

Gold et al. 2001: Gold SM, Heesen C, Schulz H, Guder U, Monch A, Gbadamosi J, Buhmann C, Schulz KH (2001) Disease specific quality of life instruments in multiple sclerosis: validation of the Hamburg Quality of Life Questionnaire in Multiple Sclerosis (HAQUAMS). Mult Scler. Apr;7(2):119-30.

Gold et al. 2005a: Gold SM, Mohr DC, Huitinga I, Flachenecker P, Sternberg EM, Heesen C. (2005) The role of stress-response systems for the pathogenesis and progression of MS. Trends Immunol. Dec;26(12):644-52.

Gold et al. 2005b: Gold SM, Raji A, Huitinga I, Wiedemann K, Schulz KH, Heesen C. (2005) Hypothalamo-pituitary-adrenal axis activity predicts disease progression in
multiple sclerosis. J Neuroimmunol. Aug;165(1-2):186-91.

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

Grasser et al. 1996: Grasser A, Moller A, Backmund H, Yassouridis A, Holsboer F (1996) Heterogeneity of hypothalamic-pituitary-adrenal system response to a combined dexamethasone-CRH test in multiple sclerosis. Exp Clin Endocrinol Diabetes. 104(1):31-7.

Hadjimichael et al. 2008: Hadjimichael O, Vollmer T, Oleen-Burkey M; North American Research Committee on Multiple Sclerosis (2008) Fatigue characteristics in multiple sclerosis: the North American Research Committee on Multiple Sclerosis (NARCOMS) survey. Health Qual Life Outcomes. Nov 14;6:100.

Harbuz 2002: Harbuz M. (2002) Neuroendocrine function and chronic inflammatory stress. Exp Physiol. Sep;87(5):519-25.

Hartung et al. 2004: Hartung HP, Bar-Or A, Zoukos Y. (2004) What do we know about the mechanism of action of disease-modifying treatments in MS? J Neurol. Sep;251 Suppl 5:v12-v29.

Hein and Hopfenmüller 2000: Hein T, Hopfenmüller W. (2000) Projection of the number of multiple sclerosis patients in Germany. Nervenarzt. Apr;71(4):288-94.

Heesen et al. 2002: Heesen C, Gold SM, Raji A, Wiedemann K, Schulz KH. (2002) Cognitive impairment correlates with hypothalamo-pituitary-adrenal axis dysregulation in multiple sclerosis. Psychoneuroendocrinology. May;27(4):505-17.

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

Heesen et al. 2007: Heesen C, Gold SM, Huitinga I, Reul JM. (2007) Stress and hypothalamic-pituitary-adrenal axis function in experimental autoimmune encephalomyelitis and multiple sclerosis - a review. Psychoneuroendocrinology. Jul;32(6):604-18.

Hemmer et al. 2002: Hemmer B, Cepok S, Nessler S, Sommer N. (2002) Pathogenesis of multiple sclerosis: an update on immunology. Curr Opin Neurol. Jun;15(3):227-31.

Herrmann 1997: Herrmann C (1987) International experiences with the Hospital Anxiety and Depression Scale – a review of validation data and clinical results. J Psychosom Res. Jan;42(1):17-41.

Hobart et al. 2000: Hobart J, Freeman J, Thompson A (2000) Kurtzke scales revisited: the application of psychometric methods to clinical intuition. Brain. May;123 (5):1027-40.

Holsboer et al. 1994: Holsboer F, Grasser A, Friess E, Wiedemann K. (1994) Steroid effects on central neurons and implications for psychiatric and neurological disorders. Ann N Y Acad Sci. Nov 30;746:345-61.

Höschl and Hajek 2001: Höschl C, Hajek T. (2001) Hippocampal damage mediated by corticosteroids-a neuropsychiatric research challenge. Eur Arch Psychiatry Clin Neurosci.;251 Suppl 2:II81-8.

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

Huitinga et al. 2003: Huitinga I, Erkut ZA, van Beurden D, Swaab DF. (2003) The hypothalamo-pituitary-adrenal axis in multiple sclerosis. Ann N Y Acad Sci. May;992:118-28.

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

Inglese et al. 2002: Inglese M, Park SJ, Johnson G, Babb JS, Miles L, Jaggi H, Herbert J, Grossman RI. (2007) Deep gray matter perfusion in multiple sclerosis: dynamic susceptibility contrast perfusion magnetic resonance imaging at 3 T. Arch Neurol. Feb;64(2):196-202.

Iriarte et al. 2000 : Iriarte J, Subirá ML, Castro P. (2000) Modalities of fatigue in multiple sclerosis: correlation with clinical and biological factors. Mult Scler. Apr;6(2):124-30.

Janardhan and Bakshi 2000: Janardhan V, Bakshi R. (2000) Quality of life and its relationship to brain lesions and atrophy on magnetic resonance images in 60 patients with multiple sclerosis. Arch Neurol. Oct;57(10):1485-91.

Janardhan and Bakshi 2002: Janardhan V, Bakshi R. (2002) Quality of life in patients with multiple sclerosis: the impact of fatigue and depression. J Neurol Sci. Dec 15;205(1):51-8.

Joffe 2005: Joffe RT. (2005) Depression and multiple sclerosis: a potential way to understand the biology of major depressive illness. J Psychiatry Neurosci. Jan;30(1):9-10.

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

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

Kappos et al. 1999: Kappos L, Moeri D, Radue EW, Schoetzau A, Schweikert K, Barkhof F, Miller D, Guttmann CR, Weiner HL, Gasperini C, Filippi M. (1999) Predictive value of gadolinium-enhanced magnetic

resonance imaging for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis. Gadolinium MRI Meta-analysis Group. Lancet. Mar 20;353(9157):964-9.

Kieseier et al. 1999: Kieseier BC, Archelos JJ, Storch M, Gold R, Hartung HP. (1999) The immunopathogenesis of multiple sclerosis. A survey of recent advances and implications for future therapy. Wien Klin Wochenschr. Sep 17;111(17):728-37.

Kieseier and Hartung 2003: Kieseier BC, Hartung HP. (2003) Multiple paradigm shifts in multiple sclerosis. Curr Opin Neurol. Jun;16(3):247-52.

Konsman et al. 2002: Konsman JP, Parnet P, Dantzer R. (2002) Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci. Mar;25(3):154-9.

Kos et al. 2008: Kos D, Kerckhofs E, Nagels G, D'hooghe MB, Ilsbroukx S. (2008) Origin of fatigue in multiple sclerosis: review of the literature. Neurorehabil Neural Repair. Jan-Feb;22(1):91-100. Epub 2007 Apr 4.

Kotterba et al. 2003: Kotterba S, Eren E, Fangerau T, Malin JP, Sindern E. (2003) Sleepiness and fatigue in multiple sclerosis - comparison of different measuring instruments. Fortschr Neurol Psychiatr. Nov;71(11):590-4.

Kremenchutzky et al. 2006: Kremenchutzky M, Rice GP, Baskerville J, Wingerchuk DM, Ebers G. (2006) The natural history of multiple sclerosis: a geographically based study 9: observations on the progressive phase of the disease. Brain. Mar;129(3):584-94.

Kroencke et al. 2000 : Kroencke DC, Lynch SG, Denney DR. (2000) Fatigue in multiple sclerosis: relationship to depression, disability, and disease pattern. Mult Scler. Apr;6(2):131-6.

Krupp and Rivzi 2002: Krupp LB, Rizvi SA. (2002) Symptomatic therapy for underrecognized manifestations of multiple sclerosis. Neurology. Apr 23;58(8 Suppl 4):S32-9.

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

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

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

Kuhlmann et al. 2002: Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Brück W. (2002) Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain. Oct;125(10):2202-12.

Kulmatycki and Jamali 2006: Kulmatycki KM, Jamali F. (2006) Drug disease interactions: role of inflammatory mediators in depression and variability in antidepressant drug response. J Pharm Pharm Sci.;9(3):292-306. Review.

Kurtzke 1983: Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. Nov;33(11):1444-52.

Kutzelnigg et al. 2005: Kutzelnigg A, Lucchinetti CF, Stadelmann C, Brück W, Rauschka H, Bergmann M, Schmidbauer M, Parisi JE, Lassmann H. (2005) Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain. 2005 Nov;128(11):2705-12.

Lange et al. 2009: Lange R, Volkmer M, Heeesen C, Liepert J (2009) Modafinil effects in multiple sclerosis patients with fatigue. J Neurol. Apr;256(4):645-650.

Lapierre and Hum 2007: Lapierre Y, Hum S (2007) Treating Fatigue. Int MS J. 14(2);64-71

Lassmann et al. 2007: Lassmann H, Brück W, Lucchinetti CF. (2007) The immunopathology of multiple sclerosis: an overview. Brain Pathol. Apr;17(2):210-8.

Limone et al. 2002: Limone P, Ferrero B, Calvelli P, Del Rizzo P, Rota E, Berardi C, Barberis AM, Isaia GC, Durelli L. (2002) Hypothalamic-pituitary-adrenal axis function and cytokine production in multiple sclerosis with or without interferon-beta treatment. Acta Neurol Scand. May;105(5):372-7.

Lisak 2007: Lisak RP. (2007) Neurodegeneration in multiple sclerosis: defining the problem. Neurology. May 29;68(22 Suppl 3):S5-12; discussion S43-54.

Lobentanz et al. 2004: Lobentanz IS, Asenbaum S, Vass K, Sauter C, Klösch G, Kollegger H, Kristoferitsch W, Zeitlhofer J. (2004) Factors influencing quality of life in multiple sclerosis patients: disability, depressive mood, fatigue and sleep quality. Acta Neurol Scand. Jul;110(1):6-13.

Lublin and Reingold 1996: Lublin FD, Reingold SC (1996) Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology. Apr;46(4):907-11.

Lucchinetti et al. 2000: Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol. Jun;47(6):707-17.

MacPhee et al. 1989: MacPhee IA, Antoni FA, Mason DW. (1989) Spontaneous recovery of rats from experimental allergic encephalomyelitis is dependent on regulation of the immune system by endogenous adrenal corticosteroids. J Exp Med. Feb 1;169(2):431-45.

Marik and Zaloga 2002: Marik PE, Zaloga GP. (2002) Adrenal insufficiency in the critically ill: a new look at an old problem. Chest. Nov;122(5):1784-96.

Marrie 2004: Marrie RA. (2004) Environmental risk factors in multiple sclerosis aetiology. Lancet Neurol. Dec;3(12):709-18.

Marrie et al. 2005: Marrie RA, Fisher E, Miller DM, Lee JC, Rudick RA. (2005) Association of fatigue and brain atrophy in multiple sclerosis. J Neurol Sci. Feb 15;228(2):161-6.

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

McDonald et al. 2001: McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS. (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol. Jul;50(1):121-7.

McEwen 1998: McEwen BS. (1998) Stress, adaptation, and disease. Allostasis and allostatic load. Ann N Y Acad Sci. May 1;840:33-44.

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

Merkelbach et al. 2002: Merkelbach S, Sittinger H, Koenig J. (2002) Is there a differential impact of fatigue and physical disability on quality of life in multiple sclerosis? J Nerv Ment Dis. Jun;190(6):388-93.

Metz et al. 2004: Metz LM, Patten SB, Archibald CJ, Bakker JI, Harris CJ, Patry DG, Bell RB, Yeung M, Murphy WF, Stoian CA, Billesberger K, Tillotson L, Peters S, McGowan D. (2004) The effect of immunomodulatory treatment on multiple sclerosis fatigue. J Neurol Neurosurg Psychiatry. Jul;75(7):1045-7.

Michelson et al. 1994: Michelson D, Stone L, Galliven E, Magiakou MA, Chrousos GP, Sternberg EM, Gold PW. (1994) Multiple sclerosis is associated with alterations in hypothalamic-pituitary-adrenal axis function. J Clin Endocrinol Metab. Sep;79(3):848-53.

Mohr and Pelletier 2006: Mohr DC, Pelletier D. (2006) A temporal framework for understanding the effects of stressful life events on inflammation in patients with multiple sclerosis. Brain Behav Immun. Jan;20(1):27-36.

Mohr et al. 2003: Mohr DC, Hart SL, Goldberg A. (2003) Effects of treatment for depression on fatigue in multiple sclerosis. Psychosom Med. Jul-Aug;65(4):542-7.

Multiple Sclerosis Council for Clinical Practice Guidelines 1998: Multiple Sclerosis Council for Clinical Practice Guidelines (1998) Fatigue and multiple sclerosis: evidence based management strategies for fatigue in multiple sclerosis, Multiple Sclerosis Council for Clinical Practice Guidelines, Paralyzed Veterans Association of America, October 1998

Mumford and Compston 1993: Mumford CJ, Compston A (1993) Problems with ra-ting scales for multiple sclerosis: a novel approach – the CAMBS score. J Neurol. 1993;240(4):209-15.

Mungas et al. 2005: Mungas D, Harvey D, Reed BR, Jagust WJ, DeCarli C, Beckett L, Mack WJ, Kramer JH, Weiner MW, Schuff N, Chui HC. (2005) Longitudinal volumetric MRI change and rate of cognitive decline. Neurology. Aug 23;65(4):565-71.

Murray 1985: Murray TJ. (1985) Amantadine therapy for fatigue in multiple sclerosis. Can J Neurol Sci. Aug;12(3):251-4.

Natelson 2001: Natelson BH. (2001): Chronic fatigue syndrome. JAMA. May 23-30;285(20):2557-9.

Neilley et al. 1996: Neilley LK, Goodin DS, Goodkin DE, Hauser SL. (1996) Side effect profile of interferon beta-1b in MS: results of an open label trial. Neurology. Feb;46(2):552-4.

Niepel et al. 2006: Niepel G, Tench ChR, Morgan PS, Evangelou N, Auer DP, Constantinescu CS. (2006) Deep gray matter and fatigue in MS: a T1 relaxation time study. J Neurol. Jul;253(7):896-902.

Nolte 2002: Nolte J (2002) The Human Brain – An Introduction to Its Functional Anatomy. 5th Edition, Mosby (An Affiliate of Elsevier Science), St. Louis, Missouri. 525-585.

Nortvedt et al. 1999: Nortvedt MW, Riise T, Myhr KM, Nyland HI. (1999) Quality of life in multiple sclerosis: measuring the disease effects more broadly. Neurology. Sep 22;53(5):1098-103.

Noseworthy et al. 2000: Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. (2000) Multiple sclerosis. N Engl J Med. Sep 28;343(13):938-52.

O'Connor et al. 2001: O'Connor KC, Bar-Or A, Hafler DA. (2001) The neuroimmunology of multiple sclerosis: possible roles of T and B lymphocytes in immunopathogenesis. J Clin Immunol. Mar;21(2):81-92.

Oken et al. 2004: Oken BS, Kishiyama S, Zajdel D, Bourdette D, Carlsen J, Haas M, Hugos C, Kraemer DF, Lawrence J, Mass M. (2004) Randomized controlled trial of yoga and exercise in multiple sclerosis. Neurology. Jun 8;62(11):2058-64.

Pace et al. 2007: Pace TW, Hu F, Miller AH. (2007) Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. Brain Behav Immun. Jan;21(1):9-19.

Pantano et al. 2002: Pantano P, Iannetti GD, Caramia F, Mainero C, Di Legge S, Bozzao L, Pozzilli C, Lenzi GL. (2002) Cortical motor reorganization after a single clinical attack of multiple sclerosis. Brain. Jul;125(7):1607-15.

Pariante 2004: Pariante CM. (2004) Glucocorticoid receptor function in vitro in patients with major depression. Stress. Dec;7(4):209-19.

Pirko et al. 2007: Pirko I, Lucchinetti CF, Sriram S, Bakshi R. (2007) Gray matter involvement in multiple sclerosis. Neurology. Feb 27;68(9):634-42.

Pittion-Vouyovitch et al. 2006: Pittion-Vouyovitch S, Debouverie M, Guillemin F, Vandenberghe N, Anxionnat R, Vespignani H. (2006) Fatigue in multiple sclerosis is related to disability, depression and quality of life. J Neurol Sci. Apr 15;243(1-2):39-45.

Pittock and Lucchinetti 2007: Pittock SJ, Lucchinetti CF. (2007) The pathology of MS: new insights and potential clinical applications. Neurologist. Mar;13(2):45-56.

Poser et al. 1983: Poser CM, Paty 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. Ann Neurol. Mar;13(3):227-31.

Prat and Martin 2002: Prat E, Martin R. (2002) The immunopathogenesis of multiple sclerosis. J Rehabil Res Dev. 2002 Mar-Apr;39(2):187-99.

Prat and Antel 2005: Prat A, Antel J. (2005) Pathogenesis of multiple sclerosis. Curr Opin Neurol. Jun;18(3):225-30.

Pucci et al. 2007: Pucci E, Branãs P, D'Amico R, Giuliani G, Solari A, Taus C. (2007) Amantadine for fatigue in multiple sclerosis. Cochrane Database Syst Rev. Jan 24;(1):CD002818.

Purba et al. 1995: Purba JS, Raadsheer FC, Hofman MA, Ravid R, Polman CH, Kamphorst W, Swaab DF. (1995). Increased number of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of patients with multiple sclerosis. Neuroendocrinology. Jul;62(1):62-70.

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

Reddy et al. 2000: Reddy H, Narayanan S, Arnoutelis R, Jenkinson M, Antel J, Matthews PM, Arnold DL. (2000) Evidence for adaptive functional changes in the cerebral cortex with axonal injury from multiple sclerosis. Brain. Nov;123(11):2314-20.

Reder and Lowy 1992: Reder AT, Lowy MT. (1992) Interferon-beta treatment does not elevate cortisol in multiple sclerosis. J Interferon Res. Jun;12(3):195-8.

Reder et al. 1987: Reder AT, Lowy MT, Meltzer HY, Antel JP. (1987) Dexamethasone suppression test abnormalities in multiple sclerosis: relation to ACTH therapy. Neurology. May;37(5):849-53.

Reder et al. 1994: Reder AT, Makowiec RL, Lowy MT. (1994) Adrenal size is increased in multiple sclerosis. Arch Neurol. Feb;51(2):151-4.

Rietberg et al. 2005: Rietberg MB, Brooks D, Uitdehaag BM, Kwakkel G. (2005) Exercise therapy for multiple sclerosis. Cochrane Database Syst Rev. Jan 25;(1):CD003980.

Rocca et al. 2005: Rocca MA, Colombo B, Falini A, Ghezzi A, Martinelli V, Scotti G, Comi G, Filippi M. (2005) Cortical adaptation in patients with MS: a cross-sectional functional MRI study of disease phenotypes. Lancet Neurol. Oct;4(10):618-26.

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

Sanfilipo et al. 2006: Sanfilipo MP, Benedict RH, Weinstock-Guttman B, Bakshi R. (2006) Gray and white matter brain atrophy and neuropsychological impairment in multiple sclerosis. Neurology. Mar 14;66(5):685-92.

Saper et al. 2005: Saper CB, Scammell TE, Lu J. (2005) Hypothalamic regulation of sleep and circadian rhythms. Nature. Oct 27;437(7063):1257-63.

Schapiro 2002: Schapiro R (2002) The pathophysiology of MS-Related Fatigue: What is the Role of Wake Promotion? Int J of MS Care. Oct Suppl. 3-7.

Schmidt 2002: Schmidt RM (2002) Geschichte der Multiplen Sklerose. In: Schmidt RM and Hoffmann F (Hrsg) Multiple Sklerose, 3. Auflage, Urban & Fischer Verlag, München Jena, Page 1-2

Schulz and Engelhardt 2005: Schulz M, Engelhardt B. (2005) The circumventricular organs participate in the immunopathogenesis of experimental autoimmune encephalomyelitis. Cerebrospinal Fluid Res. Sep 30;2:8.

Schulz et al. 2004: Schulz KH, Gold SM, Witte J, Bartsch K, Lang UE, Hellweg R, Reer R, Braumann KM, Heesen C. (2004) Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis. J Neurol Sci. Oct 15;225(1-2):11-8.

Schulz et al. 2005: Schulz KH, Heesen C, Gold SM. (2005) The concept of allostasis and allostatic load: psychoneuroimmunological findings. Psychother Psychosom Med Psychol. Nov;55(11):452-61.

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

Schwid et al. 2002: Schwid SR, Covington M, Segal BM, Goodman AD (2002) Fatigue in multiple sclerosis: current understanding and future directions. J Rehabil Res Dev. Mar-Apr;39(2):211-24.

Sharrack and Hughes 1996: Sharrack B, Hughes RA (1996) Clinical scales for mul-tiple sclerosis. J Neurol Sci. Jan;135(1):1-9.

Sharrack et al. 1999: Sharrack B, Hughes RA, Soudain S, Dunn G (1999) The psycho-metric properties of clinical rating scales used in multiple sclerosis. Brain. Jan;122 (1):141-59.

Siegel 2004: Siegel J. (2004) Brain mechanisms that control sleep and waking. Naturwissenschaften. Aug;91(8):355-65.

Siegert and Abernethy 2005: Siegert RJ, Abernethy DA. (2005) Depression in multiple sclerosis: a review. J Neurol Neurosurg Psychiatry. Apr;76(4):469-75.

Simone et al. 2006: Simone IL, Ceccarelli A, Tortorella C, Bellacosa A, Pellegrini F, Plasmati I, De Caro MF, Lopez M, Girolamo F, Livrea P. (2006) Influence of Interferon beta treatment on quality of life in multiple sclerosis patients. Health Qual Life Outcomes. Dec 12;4:96.

Smith 1968: Smith A (1968) The symbol digit modalities test. Learn. Disabil. 3,83-91

Stankof et al. 2005: Stankoff B, Waubant E, Confavreux C, Edan G, Debouverie M, Rumbach L, Moreau T, Pelletier J, Lubetzki C, Clanet M; French Modafinil Study Group. (2005) Modafinil for fatigue in MS: a randomized placebo-controlled double-blind study. Neurology. Apr 12;64(7):1139-43.

Stanton et al. 2006: Stanton BR, Barnes F, Silber E. (2006) Sleep and fatigue in multiple sclerosis. Mult Scler. Aug;12(4):481-6.

Steinman 2001: Steinman L (2001) Multiple sclerosis: a two-stage disease. Nat Immunol. Sep;2(9):762-4.

Stefferl et al. 2001: Stefferl A, Storch MK, Linington C, Stadelmann C, Lassmann H, Pohl T, Holsboer F, Tilders FJ, Reul JM. (2001) Disease progression in chronic relapsing experimental allergic encephalomyelitis is associated with reduced inflammation-driven production of corticosterone. Endocrinology. Aug;142(8):3616-24.

Strober and Arnett 2005: Strober LB, Arnett PA. (2005) An examination of four models predicting fatigue in multiple sclerosis. Arch Clin Neuropsychol. Jul;20(5):631-46.

Tartaglia et al. 2004: Tartaglia MC, Narayanan S, Francis SJ, Santos AC, De Stefano N, Lapierre Y, Arnold DL. (2004) The relationship between diffuse axonal damage and fatigue in multiple sclerosis. Arch Neurol. Feb;61(2):201-7.

Tedeschi et al. 2005: Tedeschi G, Lavorgna L, Russo P, Prinster A, Dinacci D, Savettieri G, Quattrone A, Livrea P, Messina C, Reggio A, Bresciamorra V, Orefice G, Paciello M, Brunetti A, Coniglio G, Bonavita S, Di Costanzo A, Bellacosa A, Valentino P, Quarantelli M, Patti F, Salemi G, Cammarata E, Simone IL, Salvatore M, Bonavita V, Alfano B. (2005) Brain atrophy and lesion load in a large population of patients with multiple sclerosis. Neurology. Jul 26;65(2):280-5.

Tedeschi et al. 2007: Tedeschi G, Dinacci D, Lavorgna L, Prinster A, Savettieri G, Quattrone A, Livrea P, Messina C, Reggio A, Servillo G, Bresciamorra V, Orefice G, Paciello M, Brunetti A, Paolillo A, Coniglio G, Bonavita S, Di Costanzo A, Bellacosa A, Valentino P, Quarantelli M, Patti F, Salemi G, Cammarata E, Simone I, Salvatore M, Bonavita V, Alfano B. (2007) Correlation between fatigue and brain atrophy and lesion load in multiple sclerosis patients independent of disability.J Neurol Sci. Dec 15;263(1-2):15-9.

Tellez et al. 2005: Tellez N, Rio J, Tintore M, Nos C, Galan I, Montalban X. (2005) Does the Modified Fatigue Impact Scale offer a more comprehensive assessment of fatigue in MS? Mult Scler. Apr;11(2):198-202.

Tellez et al. 2006: Téllez N, Río J, Tintoré M, Nos C, Galán I, Montalban X. (2006) Fatigue in multiple sclerosis persists over time: a longitudinal study. J Neurol. Nov;253(11):1466-70.

The Canadian MS Research Group 1987: The Canadian MS Research Group (1987) A randomized controlled trial of amantadine in fatigue associated with multiple sclerosis. The Canadian MS Research Group. Can J Neurol Sci. Aug;14(3):273-8.

Then Bergh et al. 1999a: Then Bergh F, Grasser A, Trenkwalder C, Backmund H, Holsboer F, Rupprecht R. (1999) Binding characteristics of the glucocorticoid receptor in peripheral blood lymphocytes in multiple sclerosis. J Neurol. Apr;246(4):292-8.

Then Bergh et al. 1999b: Then Bergh F, Kümpfel T, Trenkwalder C, Rupprecht R, Holsboer F. (1999) Dysregulation of the hypothalamo-pituitary-adrenal axis is related to the clinical course of MS. Neurology. Sep 11;53(4):772-7.

Then Bergh et al. 2001: Then Bergh F, Kümpfel T, Grasser A, Rupprecht R, Holsboer F, Trenkwalder C. (2001) Combined treatment with corticosteroids and moclobemide favors normalization of hypothalamo-pituitary-adrenal axis dysregulation in relapsing-remitting multiple sclerosis: a randomized, double blind trial. J Clin Endocrinol Metab. Apr;86(4):1610-5.

Then Bergh et al. 2007: Then Bergh F, Kümpfel T, Yassouridis A, Lechner C, Holsboer F, Trenkwalder C. (2007) Acute and chronic neuroendocrine effects of interferon-beta 1a in multiple sclerosis. Clin Endocrinol (Oxf). Feb;66(2):295-303.

Thomas et al. 2006: Thomas PW, Thomas S, Hillier C, Galvin K, Baker R. (2006) Psychological interventions for multiple sclerosis. Cochrane Database Syst Rev. Jan 25;(1):CD004431.

Thompson 2001: Thompson AJ. (2001) Symptomatic management and rehabilitation in multiple sclerosis. J Neurol Neurosurg Psychiatry. Dec;71 Suppl 2:ii22-7

Tsigos and Chrousos 1994: Tsigos C, Chrousos GP. (1994) Physiology of the hypothalamic-pituitary-adrenal axis in health and dysregulation in psychiatric and autoimmune disorders. Endocrinol Metab Clin North Am. Sep;23(3):451-66.

Turnbull and Rivier 1999: Turnbull AV, Rivier CL. (1999) Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. Physiol Rev. Jan;79(1):1-71.

Van der Werf et al. 1998: van der Werf SP, Jongen PJ, Lycklama à Nijeholt GJ, Barkhof F, Hommes OR, Bleijenberg G. (1998) Fatigue in multiple sclerosis: interrelations between fatigue complaints, cerebral MRI abnormalities and neurological disability. J Neurol Sci. Oct 8;160(2):164-70.

Van Kessel et al. 2008: van Kessel K, Moss-Morris R, Willoughby E, Chalder T, Johnson MH, Robinson E. (2008) A randomized controlled trial of cognitive behavior therapy for multiple sclerosis fatigue. Psychosom Med. Feb;70(2):205-13.

Van Winsen et al. 2005: van Winsen LM, Muris DF, Polman CH, Dijkstra CD, van den Berg TK, Uitdehaag BM. (2005) Sensitivity to glucocorticoids is decreased in relapsing remitting multiple sclerosis. J Clin Endocrinol Metab. Feb;90(2):734-40.

Vercoulen et al. 1996: Vercoulen JH, Hommes OR, Swanink CM, Jongen PJ, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. (1996) The measurement of fatigue in patients with multiple sclerosis. A multidimensional comparison with patients with chronic fatigue syndrome and healthy subjects. Arch Neurol. Jul;53(7):642-9.

Vgontzas et al. 2001: Vgontzas AN, Bixler EO, Lin HM, Prolo P, Mastorakos G, Vela-Bueno A, Kales A, Chrousos GP. (2001) Chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal axis: clinical implications. J Clin Endocrinol Metab. Aug;86(8):3787-94.

Vgontzas and Chrousos 2002: Vgontzas AN, Chrousos GP. (2002) Sleep, the hypothalamic-pituitary-adrenal axis, and cytokines: multiple interactions and disturbances in sleep disorders. Endocrinol Metab Clin North Am. Mar;31(1):15-36. Review.

Vollmer 2007: Vollmer T. (2007) The natural history of relapses in multiple sclerosis. J Neurol Sci. May 15;256 Suppl 1:S5-13.

Von Bardeleben and Holsboer 1989: Von Bardeleben U, Holsboer F (1989) Cortisol response to a combined hormone challenge in patients with depression J Neuro-endocrinol 1:485-488

Vukusic and Confavreux 2007: Vukusic S, Confavreux C. (2007) Natural history of multiple sclerosis: risk factors and prognostic indicators. Curr Opin Neurol. Jun;20(3):269-74.

Walther and Hohlfeld 1999: Walther EU, Hohlfeld R. (1999) Multiple sclerosis: side effects of interferon beta therapy and their management. Neurology. Nov 10;53(8):1622-7.

Weber et al. 1998: Weber F, Polak T, Günther A, Kubuschok B, Janovskaja J, Bitsch A, Poser S, Rieckmann P. (1998) Synergistic immunomodulatory effects of interferon-beta1b and the phosphodiesterase inhibitor pentoxifylline in patients with relapsing-remitting multiple sclerosis. Ann Neurol. Jul;44(1):27-34.

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

Weinshenker 1996: Weinshenker BG. (1996) Epidemiology of multiple sclerosis. Neurol Clin. May;14(2):291-308.

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

Ysrraelit et al. 2008: Ysrraelit MC, Gaitán MI, Lopez AS, Correale J. (2008) Impaired hypothalamic-pituitary-adrenal axis activity in patients with multiple sclerosis. Neurology. Dec 9;71(24):1948-54.

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

Zigmond and Snaith 1983: Zigmond AS, Snaith RP (1983) The hospital anxiety and depression scale. Acta Psychiatr Scand. Jun;67(6):361-70.

Zimmermann and Hohlfeld 1999: Zimmermann C, Hohlfeld R. (1999) 'Fatigue' in multiple sclerosis. Nervenarzt. Jun;70(6):566-74.

Zipp and Aktas 2006: Zipp F, Aktas O. (2006) The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. Trends Neurosci. Sep;29(9):518-27.

8 Danksagung

9 Curriculum Vitae

10 Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Unterschrift: ....