Biological outcome measurements for behavioral interventions in multiple sclerosis

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***Abstract*:** Behavioral interventions including exercise, stress management, patient education, psychotherapy and multidisciplinary neurorehabilitation in general are receiving increasing recognition in multiple sclerosis (MS) clinical practice and research. Most scientific evaluations of these approaches have focused on psychosocial outcome measures such as quality of life, fatigue or depression. However, it is becoming increasingly clear that neuropsychiatric symptoms of MS are at least partially mediated by biological processes such as inflammation, neuroendocrine dysfunction or regional brain damage. Thus, successful treatment of these symptoms with behavioral approaches could potentially also affect the underlying biology. Rigidly designed scientific studies are needed to explore the potential of such interventions to affect MS pathology and biological pathways linked to psychological and neuropsychiatric symptoms of MS. Such studies need to carefully select outcome measures on the behavioral level that are likely to be influenced by the specific intervention strategy and should include biomarkers with evidence for an association with the outcome parameter in question. In this overview, we illustrate how biological and psychological outcome parameters can be combined to evaluate behavioral interventions. We focus on two areas of interest as potential targets for behavioral interventions: depression and fatigue.

***Keywords*:** biomarkers, depression, fatigue, multiple sclerosis, behavioral interventions, cortisol, magnetic resonance imaging (MRI), cytokines, inflammation

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Behavioral interventions in multiple sclerosis

Multiple sclerosis (MS) is a demyelinating, inflammatory disease of the central nervous system (CNS) with a presumed autoimmune origin. Neuropsychiatric symptoms including anxiety, depression, fatigue, and cognitive disturbances are very common and have a major impact on activity and participation in life.

Few drugs targeting neuropsychiatric symptoms have been tested in MS and the trials conducted have yielded disappointing results for fatigue [Brown *et al*. 2010] or cognitive impairment [Lovera *et al*. 2010; Christodoulou *et al*. 2008]. Behavioral interventions such as psychotherapy [Thomas *et al*. 2006], neurorehabilitation [Khan *et al*. 2007], and exercise [Motl *et al*. 2010] have been shown to beneficially affect quality of life and symptom domains including depression, fatigue, and possibly cognitive function. In addition, emerging evidence suggests that techniques such as meditation may be effective in decreasing depression and fatigue [Grossman *et al*. 2010].

While an increasing body of evidence supports the efficacy of behavioral interventions as symptomatic treatments in MS, little is known about potential underlying mechanisms. As we discuss in this review, symptoms such as depression and fatigue have been shown not simply to be a psychological reaction to the burden of a debilitating disease but may be caused by certain biological aspects of MS itself. If this is the case, behavioral interventions may also have direct effects on the underlying biology.

To enhance our understanding of how behavioral interventions work in MS, it is therefore essential that clinical trials include biological measures. Inclusion of biological outcome parameters is most promising if there is already evidence that the behavioral intervention has an effect on the clinical outcome variable. When selecting a potential biomarker for behavioral intervention

trials in MS, it is important to choose a parameter that meets several criteria:

a. The biomarker has to be linked to the clinical outcome variable of interest, either symptom-related (e.g. depression, fatigue, cognitive impairment) or a measure of disease activity (MRI activity, relapse, or disability progression).
b. The biological outcome parameter has to be modifiable, and has to be modifiable in the desired direction. For example, genetic markers might be useful as biomarkers of neuropsychiatric symptoms or as indicators of treatment response but are not suitable as outcomes.
c. The biomarker has to be reliably and objectively measurable.
d. The duration of the study has to be sufficiently long with an appropriate number of assessments for the biological and clinical outcome parameters to change. For example, if the outcome parameters are highly dynamic, such as markers of inflammation, shorter interventions with more frequent assessments may be most appropriate. For markers of degenerative processes (such as disability, cognitive function, and markers of brain damage such as atrophy), longer study periods will be required.

In the current review, we propose biological outcome parameters for two highly relevant symptom domains in MS: depression and fatigue.

Pathogenesis of MS-related depression

Patients with MS frequently suffer from depression. In this population, the point prevalence for major depressive disorder (MDD) has been estimated as between 13% and 30% with a lifetime risk of up to 50% [Siegert and Abernethy, 2005]. Depression in MS is associated with cognitive impairment [Heesen *et al*. 2010; Feinstein, 2006], negatively affects work performance [Vickrey *et al*. 1995] and decreases quality of life [Jonsson *et al*. 1996]. Importantly, depression can decrease treatment compliance [Mohr *et al*. 1997] and is one of the strongest predictors of suicide [Feinstein, 1997]. Despite the high clinical relevance, depression remains underdiagnosed and undertreated in MS [Goldman, 2005].

Depression as a response to MS diagnosis or therapy?

One obvious possibility is that the high rate of depression may simply reflect a psychological reaction to a chronic debilitating disease with an unpredictable disease course. However, depression in MS is not related to the severity of neurological impairment [Moller *et al*. 1994], and can occur at any stage of the disease [Sullivan *et al*. 1995]. Early studies had suggested that depression may be induced by disease-modifying drugs such as interferon beta (IFNβ). The occurrence of depression after IFNβ therapy was later found to be better explained by a previous history of depression [Feinstein *et al*. 2002]. Therefore, biological aspects of the disease itself may at least in part be responsible for the high prevalence of depression in MS.

Neurobiology of depression

Potential biological substrates of MS depression remain poorly understood (see Pucak *et al*. [2007] for a review). Lessons, however, may be learned from clinical and preclinical studies of idiopathic depression in psychiatry. It has become clear that the classical 'monoamine hypothesis' of depression is too simplistic and current pathogenetic concept of depressive disorders include a range of neurobiological mechanisms (see Krishnan and Nestler [2008] for a review). A large body of evidence has implicated hypothalamic–pituitary–adrenal (HPA) axis hyperactivity in depression [Pariante and Lightman, 2008]. Preclinical models suggest that stress and excess glucocorticoid levels may cause cellular and molecular changes in the CNS, possibly mediated by reduction of brain-derived neurotrophic factor (BDNF) [Duman and Monteggia, 2006]. These mechanisms are thought to contribute to damage in susceptible brain areas such as the hippocampus [Macqueen and Frodl, 2011]. The hippocampus plays a crucial role for learning, mood regulation and HPA axis control, and hippocampal atrophy is frequently observed in MDD [Koolschijn *et al*. 2009]. Additional biological substrates of depression may include inflammatory pathways [Dantzer *et al*. 2008] or disturbed energy homeostasis involving leptin and grehlin [Krishnan and Nestler, 2010].

Neuroanatomical substrates of MS depression

Owing to the widespread CNS involvement in MS, damage to brain regions involved in mood regulation is a promising candidate for biological correlates of MS-associated depression. Some studies using magnetic resonance imaging (MRI) have reported associations of MS depression with lesion load in frontal, parietal, or temporal areas [Feinstein *et al*. 2004; Zorzon *et al*. 2001;

Bakshi *et al.* 2000a]. However, studies have rarely implicated the same region and others have failed to show an association altogether [Zorzon *et al.* 2002]. More consistent correlations have been found with regional atrophy, in particular in the temporal lobe [Feinstein *et al.* 2004; Zorzon *et al.* 2002, 2001]. Using advanced imaging techniques, Feinstein and colleagues have found that subtle white and gray matter abnormalities in frontal and temporal regions are linked to depression in MS [Feinstein *et al.* 2010]. Together, these studies suggest that brain areas, particularly in the temporal lobe, may play an important role in MS-related depression.

A neuroendocrine–limbic pathology of MS-related depression?

Hyperactivity of the HPA axis [Pariante and Lightman, 2008] and hippocampal atrophy [Koolschijn *et al.* 2009] are among the most consistently reported biological abnormalities in idiopathic MDD. Interestingly, HPA axis hyperactivity is detectable in up to 50% of MS patients [Heesen *et al.* 2007] and gene variants involved in HPA axis regulation have recently been associated with MS [Briggs *et al.* 2010]. HPA axis hyperactivity in MS is associated with progressive disease and global neurodegeneration [Gold *et al.* 2005; Heesen *et al.* 2002; Schumann *et al.* 2002; Then Bergh *et al.* 1999]. One study recently demonstrated that subtle increases in HPA axis activity are already detectable in early disease stages [Ysrraelit *et al.* 2008].

Significant associations between HPA axis activity and depressive symptoms have been reported in relapsing–remitting (RR) MS patients during relapse [Fassbender *et al.* 1998] but not in mixed groups that included relapsing and progressive patients [Then Bergh *et al.* 1999]. In RRMS during remission, higher levels of depressive symptoms (as defined by a cut-off on the Beck Depression Inventory II [BDI-II]) are associated with normal morning cortisol but elevated evening cortisol compared with age- and sex-matched healthy controls, indicating insufficient negative feedback during the circadian nadir [Gold *et al.* 2010]. Recently, this has been confirmed in a sample of RRMS patients who met diagnostic criteria for current MDD: normal morning but elevated evening cortisol levels were found in MS patients with comorbid MDD compared to nondepressed MS patients [Gold *et al.* 2011].

Hippocampal damage and loss of volume is observable in MS patients [Dutta *et al.* 2011; Benedict *et al.* 2009; Papadopoulos *et al.* 2009; Sicotte *et al.* 2008; Geurts *et al.* 2007, 2006] as well as its animal model, experimental autoimmune encephalomyelitis (EAE) [Ziehn *et al.* 2010; Sajad *et al.* 2009]. Supporting a neuroendocrine–limbic link of MS-associated depression, smaller hippocampal volumes, particularly in the cornu ammonis (CA) 2–3 and dentate gyrus (DG) subfields, are associated with elevated levels of depressive symptoms as well as increased evening cortisol [Gold *et al.* 2010]. This is of particular interest because the CA2–3 fields are most susceptible to damage by prolonged cortisol treatment in primates [Sapolsky *et al.* 1990]. In rodents, high levels of endogenous glucocorticoids have effects localized to the CA3 region of the hippocampus [Conrad, 2008] and chronic stress has been shown to cause retraction of dendrites in the CA3 and decrease neurogenesis in the DG [McEwen, 1999]. These observations are in line with reports from experimental models and clinical studies of idiopathic MDD. However, as will be discussed later, there are also some intriguing differences in neuroendocrine–limbic correlates of idiopathic MDD and MS depression.

Are neurobiological substrates of MS depression modifiable?

There is ample evidence from psychiatric studies that HPA axis abnormalities in MDD can be modified by antidepressive therapy [Mason and Pariante, 2006]. Similarly, a normalization of HPA axis reactivity has been described in MS patients treated with the antidepressant moclobemide [Then Bergh *et al.* 2001]. Intriguingly, new evidence suggests that in addition to normalization of HPA axis responses, successful therapy of depression may also be able to reverse volume loss in brain areas such as the hippocampus. Based on experimental data in rodents and primates, smaller hippocampal volumes in MDD had long been thought to be mediated by neuronal apoptosis [Sapolsky, 2000] or by decreasing neurogenesis [Henn and Vollmayr, 2004]. However, postmortem studies failed to show significant neuronal apoptosis in the hippocampus of patients with MDD and the effect on neurogenesis is likely too small to account for the considerable decreases in hippocampal volume [Czeh and Lucassen, 2007]. Thus, it has recently been proposed that hippocampal atrophy in depression is mediated by potentially reversible

mechanisms (e.g. reduced extracellular fluid content, cellular shrinkage, and dendritic retraction) rather than neuronal apoptosis [Czeh and Lucassen, 2007]. Interestingly, dendritic remodeling is also likely to most strongly affect the CA3 and DG since incoming fibers from the entorhinal cortex to the dentate gyrus are ramified several hundredfold between the dentate gyrus and CA3 pyramidal neurons, making this an area of particularly dense synaptic connections [McEwen, 2003].

In line with this hypothesis, cross-sectional evidence suggestive of normal volumes in the CA23DG subregions of the hippocampus and normal HPA axis functioning MS patients successfully treated with selective serotonin reuptake inhibitors (SSRIs) has been found [Gold *et al.* 2010].

Similar cross-sectional observations have been reported for total hippocampal volume in psychiatric patients with MDD [Sheline *et al.* 2003]. Although this should be interpreted with caution, it is consistent with some longitudinal data showing that SSRI therapy could potentially reverse hippocampal volume loss in posttraumatic stress disorder (PTSD) [Vermetten *et al.* 2003]. However, two small longitudinal studies in MDD showed inconsistent findings [Colla *et al.* 2007; Vythilingam *et al.* 2004]. In line with reversibility of glucocorticoid (GC)-induced hippocampal damage, increases in hippocampus volume have been observed in Cushing's patients after surgical normalization of HPA axis activity [Starkman *et al.* 2003, 1999].

Alternatively, certain subtypes of depression that are not associated with hippocampal volume loss may be more responsive to pharmacological therapy, as has been shown for idiopathic MDD [Macqueen and Frodl, 2011]. The possibility to reverse hippocampal atrophy in depressed subjects, both with idiopathic MDD as well as MS-associated depression, should thus be investigated in adequately powered longitudinal studies. In this regard, behavioral as well as pharmacological strategies should be evaluated.

Is MS-associated depression neurobiologically different from idiopathic MDD?

There is some indirect evidence that, although HPA axis hyperactivity and hippocampal atrophy have been reported in MS-related depression as well as idiopathic MDD, the neurobiological correlates of depressive symptoms may not be identical.

Cortisol profiles in idiopathic MDD have been demonstrated to be characterized by an elevated morning, but normal evening cortisol concentration [Hinkelmann *et al.* 2009]. A meta-analysis of 20 studies examining salivary cortisol in MDD and healthy controls showed larger group differences in the morning than in evening samples [Knorr *et al.* 2010]. In a detailed assessment of circadian cortisol over a 24-h period in a small sample of well-characterized depressed inpatients, the largest effect sizes with the highest specificity and sensitivity for MDD were found in the morning between 10:00 and 12:00 [Paslakis *et al.* 2010]. Conversely, depression in MS patients is linked to elevated evening concentrations, but normal morning cortisol secretion [Gold *et al.* 2011, 2010].

The circadian peak levels of cortisol release are mostly dependent on low-affinity glucocorticoid receptors (GRs), while high-affinity mineralocorticoid receptors (MRs) are most important for the regulation of the circadian trough of cortisol secretion. The differential alterations in cortisol profiles may thus suggest that depression in MS is associated with relative MR dysfunction but normal GR function.

In contrast, we hypothesize that in psychiatric patients with MDD, MR signaling is largely intact while GR signaling is disturbed. Decreased GR expression has been shown in postmortem tissue from MDD patients in frontal and temporal brain regions, although notably not in the hippocampus [Webster *et al.* 2002]. In addition, functional tests of the HPA axis *in vivo* and immune cells *in vitro* have indicated GR dysfunction in MDD [Marques *et al.* 2009]. Some studies suggest intact or even enhanced MR expression and signaling in this population [Juruena *et al.* 2010, 2009, 2006; Wang *et al.* 2008; Young *et al.* 2003], although severe depression leading to suicide [Lopez *et al.* 1998] or treatment-resistant depression [Juruena *et al.* 2009] may also be linked to MR dysfunction.

In idiopathic MDD, studies using hippocampal surface mapping techniques have found evidence for hippocampal volume loss mostly clustered in the subiculum as well as the CA1 [Ballmaier *et al.* 2008; Posener *et al.* 2003]. In contrast, reduced volumes in the CA2–3 and DG subfields in

depressed RRMS patients have been reported utilizing high-resolution manual tracings of anatomically defined hippocampal subregions [Gold *et al.* 2010]. Intriguingly, within the human hippocampus, MR are highly expressed in the dentate gyrus and CA2-3 but at significantly lower levels in CA1 [Seckl *et al.* 1991].

While this suggests distinct subregional hippocampal substrates and a selective dysfunction of MR and GR in MS-associated and idiopathic depression, this has not been tested directly.

If indeed biological substrates of MS depression (see Box 1) differ from those of idiopathic MDD, a different clinical phenotype of MS depression may be expected. This has rarely been investigated. However, it has been hypothesized that MS depression tends to be characterized by pervasive mood changes, diurnal variation in mood, and suicidal ideation among others [Rickards, 2005]. Depression is one of the strongest predictors of suicide in MS [Feinstein, 2002, 1997]. The suicide rate in MS may be as high as 15% [Giannini *et al.* 2010], which appears to be higher than in idiopathic MDD [Bostwick and Pankratz, 2000]. Pharmacotherapy is moderately effective in MS depression [Goldman, 2005] but there are no comparative studies with treatment response in idiopathic MDD. In general, depression in medical populations has lower treatment response and remission rates compared with patients without comorbidity [Otte, 2008]. Specific features of clinical phenotype in MS-associated depression compared with idiopathic MDD have not been well studied and it is unknown whether they correlate with the biological substrates.

Biology of MS-related fatigue

Both physical and mental fatigue are experienced by up to two thirds of MS patients and are often perceived as the most debilitating symptoms [Stuke *et al.* 2009; Fisk *et al.* 1994]. Fatigue is commonly being described as an overwhelming feeling of exhaustion or weakness during exercise and a complete lack of energy. Importantly, fatigue represents the leading cause for absence from work [Smith and Arnett, 2005]. Symptoms of fatigue seem to be strongly linked to a reduction in the quality of life of those affected, independent of physical disability [Chaudhuri and Behan, 2004].

The pathological mechanisms responsible for the high frequency in MS are still unknown. An early study found no evidence that conduction block in the patients' central motor pathways was linked to complaints of fatigue in MS [Sheean *et al.* 1997].

Sympathetic nervous system dysfunction

Autonomic dysfunction including cardiovascular abnormalities is often seen in MS. It has been hypothesized that dysregulation of the sympathetic nervous system (SNS) might partially account for development of fatigue in the sense of an impairment of sympathetic vasomotor activity [Flachenecker *et al.* 2003]. In another study, autonomic dysregulation has been observed to be associated with symptoms of fatigue only in a subgroup of MS patients [Merkelbach *et al.* 2001]. The authors therefore concluded that the autonomic cardiovascular system was of minor relevance to MS fatigue. Sympathovagal imbalance has been suggested to play a role for the development of chronic fatigue syndrome (CFS), an ill-defined condition presumably linked to impaired bodily clearance of inflammatory stimuli. In MS, Egg and colleagues were not able to find an association between fatigue and pupillary unrest, which is tightly linked to wakefulness and the ascending arousal system of the body [Egg *et al.* 2002]. Keselbrener and colleagues found evidence for an impairment of the sympathovagal balance response to standing in patients with MS who experienced fatigue and suggested premature reduction in vagal activity in these patients [Keselbrener *et al.* 2000].

Structural brain damage or dysfunction

Early studies using MRI studies have failed to find any consistent link between fatigue and quantification of MS lesion load or localization [Bakshi *et al.* 1999; Mainero *et al.* 1999; van der Werf *et al.* 1998]. However, more recently, lesion load in parietotemporal and frontal regions was found to be correlated with fatigue [Sepulcre *et al.* 2009]. A number of studies support a role for structural damage of both gray and white matter structures for fatigue [Pellicano *et al.* 2010; Penner and Calabrese, 2010; Sepulcre *et al.* 2009; Tedeschi *et al.* 2007; Codella *et al.* 2002]. One longitudinal study could demonstrate that fatigue may predict global atrophy progression [Marrie *et al.* 2005].

Functional imaging studies in MS fatigue have supported the hypothesis of cortical reorganization in MS fatigue, characterized by increased

ipsilateral and contralateral activation [Filippi *et al*. 2002]. Studies using functional MRI (fMRI) and positron emission tomography (PET) have shown associations of fatigue with altered cerebral activation patterns and glucose metabolism indicating hypofunction in frontostriatal, motor areas, limbic structures, and the basal ganglia [Tellez *et al*. 2008; Marrie *et al*. 2005; Filippi *et al*. 2002; Bakshi *et al*. 1999; Roelcke *et al*. 1997].

Neuroendocrine abnormalities

Some studies in non-MS patients with CFS [Van Houdenhove *et al*. 2009; Van Den Eede *et al*. 2007] suspected abnormalities in neuroendocrine systems such as the HPA axis to be linked to the development of fatigue. In addition, administration of pharmacological doses of cortisol has been found to ameliorate symptoms transiently in CFS patients [Cleare *et al*. 1999]. Still, there is no consistent evidence for a specific dysfunction of the HPA axis [Cleare, 2003] in CFS. Consequently in the case of MS-related fatigue involvement of the HPA axis has been hypothesized and tested. Gottschalk and colleagues reported MS patients with fatigue to exhibit enhanced HPA axis activity, shown by significantly increased adrenocorticotropic hormone (ACTH) concentrations after administration of dexamethasone [Gottschalk *et al*. 2005]. This could however not be confirmed in a later study using the combined dexamethasone–CRH suppression test [Heesen *et al*. 2006]. Tellez and colleagues found no changes in circulating cortisol levels comparing fatigued and nonfatigued MS patients [Tellez *et al*. 2006]. This latter study, however, revealed an interesting association between fatigue and low serum levels of dehydroepiandrosterone (DHEA), a cortisol antagonist with anti-inflammatory properties, and dehydroepiandrosterone sulfate (DHEAS). A small study has also suggested a contribution of low levels of melatonin in MS fatigue [Sandyk and Awerbuch, 1994].

Cytokines

A large body of evidence from animal studies suggests that cytokines, both endogenous and exogenous, can induce fatigue-like symptoms in animals [Miller *et al*. 2009]. Here, cytokines including interleukin (IL) 1α, IL-1β, IL-6, tumor necrosis factor (TNF) α and IFNγ are involved in the induction of so-called 'sickness behavior'.

In line with this hypothesis, Flachenecker and colleagues have provided evidence for a link between increases in TNFα mRNA in immune cells and MS fatigue [Flachenecker *et al*. 2004]. This association was later confirmed at the protein level by Heesen and colleagues who reported higher TNFα and IFNγ production *in vitro* by MS patients suffering from fatigue [Heesen *et al*. 2006]. In this study, TNFα production was significantly correlated with daytime sleepiness. More recently, higher frequency of IFNγ and TNFα producing CD8 T cells was shown to correlate with measures of fatigue [Gold *et al*. 2011].

It appears that these associations are specific to peripheral cytokines rather than linked to inflammatory markers in general. For example, no association was found between MS fatigue and serum C-reactive protein (CRP), soluble intercellular adhesion molecule-1 (sICAM-1), and urinary neopterin excretion [Giovannoni *et al*. 2001]. In addition, there exists no evidence that CNS inflammation as measured by gadolinium enhancing lesions is linked to fatigue [Marrie *et al*. 2005].

The importance of dissecting fatigue from depression

Fatigue and depression often co-occur in MS and most studies report moderate correlations between these symptoms [van der Werf *et al*. 2003; Voss *et al*. 2002; Bakshi *et al*. 2000b; Ford *et al*. 1998; Schwartz *et al*. 1996]. This suggests that while linked in MS, fatigue and depression may be mediated by at least partially independent pathological mechanisms. Of note, the association seems to differ between the different components of fatigue, with depression being more closely related to mental fatigue than physical fatigue [Ford *et al*. 1998].

Animal studies suggesting a role of cytokines for sickness behavior [Raison *et al*. 2006] may have relevance for both depression and fatigue in MS and could explain the partial overlap. 'Neuropsychiatric symptoms' such as anorexia, loss of body weight, reduced social exploration, and decreased preference for sucrose solution have been demonstrated in the animal model of MS, EAE [Pollak *et al*. 2000], were associated with inflammatory mediators including TNFα and IL-1β [Pollak *et al*. 2003a], and responded to anti-inflammatory medication [Pollak *et al*. 2003b]. One recent study suggests that in MS,

HPA abnormalities are correlated with affective symptoms of depression while INFγ and TNFα are more closely associated with measures of fatigue than with depression [Gold *et al.* 2011]. Low levels of melatonin have been reported to be a potential biomarker for MS depression [Akpinar *et al.* 2008] as well as for MS fatigue [Sandyk and Awerbuch, 1994]. Unfortunately, neither of these studies explored differential association of fatigue or depression with melatonin. Future studies should aim to better differentiate between depression (see Box 1) and fatigue (see Box 2), both biologically as well as phenomenologically.

Behavioral interventions as putative disease-modifying therapies in MS

As reviewed above, there is an increasing body of evidence that depression and fatigue are linked to biological substrates and that behavioral interventions can be effective in ameliorating the neuropsychiatric symptoms. Some of these substrates such as regional brain atrophy or markers of inflammation are also thought to be relevant in MS pathogenesis or progression [Sospedra and Martin, 2005]. Thus, behavioral interventions might not only be relevant as symptomatic treatments but could also represent putative disease-modifying therapies. However, to date, there is very little direct evidence for this possibility since behavioral intervention studies have rarely obtained biological markers. One small study showed that successful treatment of MS depression (either pharmacologically or with psychotherapy) can reduce IFNγ production by OKT3 or MBP-stimulated immune cells [Mohr *et al.* 2001]. Two small trials showed a beneficial effect of antidepressive pharmacotherapy on enhancing lesions [Mostert *et al.* 2008] and possibly gray and white matter integrity [Sijens *et al.* 2008]. Since behavioral and pharmacological therapies are comparably effective in MS depression, the effect of psychotherapy on lesion load and atrophy should be explored in future studies. A randomized controlled trial with 150 patients using patient education showed a decreased relapse rate in the intervention group [Kopke *et al.* 2009]. However, no biological or paraclinical markers of disease activity were obtained, so this should be interpreted with caution.

A large body of evidence from preclinical and clinical studies suggests that exercise may have beneficial effects on cognition and possibly underlying neuroanatomical substrates [Hillman *et al.* 2008]. In line with this literature, one recent cross-sectional study indicated that higher physical fitness levels in MS are associated with gray matter volume and white matter integrity in MS

Box 1. Biological substrates of depression suitable as outcome measures.

- ***Markers of hypothalamic–pituitary–adrenal (HPA) axis activity***, preferably circadian profiles over at least 2 days with at least three assessments: awakening, midday (11:00–15:00), and evening (20:00–22:00). A low-dose oral dexamethasone suppression test may provide a helpful functional estimate of HPA axis feedback regulation. *In vivo* and *in vitro* tests using selective agonists and antagonists of glucocorticoid receptor (GR) and mineralocortcoid receptor (MR) may help to better understand the molecular mechanisms underlying HPA axis dysregulations in multiple sclerosis (MS) depression.
- ***MRI markers*** of brain areas involved in mood regulation and neuroendocrine control, most importantly the hippocampus and frontal areas. It is advisable to use advanced imaging techniques such as diffusion tensor imaging [Feinstein *et al.* 2010] or high-resolution volumetric analyses [Gold *et al.* 2010; Sicotte *et al.* 2008] since the reported brain abnormalities in MS depression are subtle and are likely not detectable with conventional MRI.

Box 2. Biological substrates of fatigue suitable as outcome measures.

- ***Inflammatory markers,*** peripheral rather than central. Most promising candidates are cytokines such as tumor necrosis factor (TNF) α and interferon (IFN) γ, which have been linked to fatigue in several chronic disorders including multiple sclerosis (MS), cancer, and hepatitis and might thus represent a common pathway for fatigue symptomatology.
- ***Markers of brain activation*** associated with MS fatigue using functional magnet resonance imaging (fMRI) may be useful, particularly in short term studies. However, the evidence of increased activation networks as a correlate of fatigue is not conclusive. While in early stages there might be efficient compensatory coactivation (without fatigue), this may evolve into inefficient recruitment in later stages (with fatigue) and finally loss of activation in advanced MS (with fatigue). Outcome measures including regional atrophy in gray matter structures such as the basal ganglia [Pardini *et al.* 2010] may be promising if the intervention is long enough (>1 year) to reasonably expect a change in these markers. More sensitive nonconventional MRI techniques such as diffusion tensor imaging of white matter structures or spectroscopy may be able to detect changes in short-term trials.
- ***Markers of sympathetic function*** such as blood pressure responses or serum catecholamine levels to the isometric hand-grip (IHG) exercise [Khurana and Setty, 1996] or to active change of posture [Flachenecker *et al.* 2001].

[Prakash *et al*. 2010]. Exercise has been shown to partially prevent neuronal damage in EAE, the animal model of MS [Rossi *et al*. 2009].

There is also some cross-sectional [Luders *et al*. 2009] as well as preliminary longitudinal evidence [Holzel *et al*. 2008] that meditation may positively affect hippocampal volumes in healthy controls. Given the effect of meditation on depression and fatigue in MS [Grossman *et al*. 2010] and the involvement of subregional hippocampal atrophy in MS depression [Gold *et al*. 2010] these studies are in line with the possibility that meditation may affect regional atrophy in MS.

In summary, there are indications for the potential of behavioral interventions to affect MS pathology, but the few available trials have been conducted in very small samples of subjects and should be interpreted with caution. Adequately powered longitudinal studies with sensitive and pathologically relevant outcome measures are largely lacking. However, we believe that there is now sufficient indirect evidence to start testing the effect of behavioral interventions on disease-related endpoints such as those used in drug trials. A number of prospective studies have consistently indicated that psychological stress increases relapse risk in MS [Mohr *et al*. 2004], so interventions targeted at reducing stress may have the potential to affect disease activity in MS. A large randomized controlled study by Mohr and colleagues [ClinicalTrials.gov identifier: NCT00147446] using a stress management intervention has recently been completed. The primary endpoints in this study are enhancing lesions on MRI and relapse rate and results are expected shortly. Only rigidly designed trials like this will ultimately tell if behavioral interventions can affect MS pathology.

Conclusion

Despite the high clinical relevance of neuropsychiatric MS symptoms, their pathogenetic substrates are still poorly understood. This may in part explain the disappointing results of clinical trials for novel symptomatic drug therapies. Thus, it is paramount to enhance our knowledge of the underlying neurobiology of these symptoms. This is a prerequisite for designing new therapies, both pharmacological as well as behavioral, and essential for better monitoring their effectiveness in clinical trials.

Some evidence suggests behavioral interventions to affect biological pathways of neuropsychiatric MS symptoms and possibly disease mechanisms as well. These interventions thus may have therapeutic potential, not only as a symptomatic treatment but also as putative disease-modifying therapies. However, few adequately powered and well-designed studies have tested behavioral therapies in MS and even fewer have included biomarkers that could help to better understand the mechanisms underlying the therapeutic benefits.

More translational and interdisciplinary research in this area is urgently needed to expand the treatment repertoire for patients, particularly those in the progressive phase of the disease, who currently have few therapeutic options but may benefit from behavioral interventions.

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Conflict of interest statement

None declared.

References

Akpinar, Z., Tokgoz, S., Gokbel, H., Okudan, N., Uguz, F. and Yilmaz, G. (2008) The association of nocturnal serum melatonin levels with major depression in patients with acute multiple sclerosis. *Psychiatry Res* 161: 253–257.

Bakshi, R., Czarnecki, D., Shaikh, Z.A., Priore, R.L., Janardhan, V., Kaliszky, Z. *et al.* (2000a) Brain MRI lesions and atrophy are related to depression in multiple sclerosis. *Neuroreport* 11: 1153–1158.

Bakshi, R., Miletich, R.S., Henschel, K., Shaikh, Z.A., Janardhan, V., Wasay, M. *et al.* (1999) Fatigue in multiple sclerosis: cross-sectional correlation with brain MRI findings in 71 patients. *Neurology* 53: 1151–1153.

Bakshi, R., Shaikh, Z.A., Miletich, R.S., Czarnecki, D., Dmochowski, J., Henschel, K. *et al.* (2000b) Fatigue in multiple sclerosis and its relationship to depression and neurologic disability. *Mult Scler* 6: 181–185.

Ballmaier, M., Narr, K.L., Toga, A.W., Elderkin-Thompson, V., Thompson, P.M., Hamilton, L. *et al.* (2008) Hippocampal morphology and distinguishing late-onset from early-onset elderly depression. *Am J Psychiatry* 165: 229–237.

Benedict, R.H., Ramasamy, D., Munschauer, F., Weinstock-Guttman, B. and Zivadinov, R. (2009)

Memory impairment in multiple sclerosis: correlation with deep grey matter and mesial temporal atrophy. *J Neurol Neurosurg Psychiatry* 80: 201–206.

Bostwick, J.M. and Pankratz, V.S. (2000) Affective disorders and suicide risk: a reexamination. *Am J Psychiatry* 157: 1925–1932.

Briggs, F.B., Bartlett, S.E., Goldstein, B.A., Wang, J., McCauley, J.L., Zuvich, R.L. *et al.* (2010) Evidence for CRHR1 in multiple sclerosis using supervised machine learning and meta-analysis in 12,566 individuals. *Hum Mol Genet* 19: 4286–4295.

Brown, J.N., Howard, C.A. and Kemp, D.W. (2010) Modafinil for the treatment of multiple sclerosis-related fatigue. *Ann Pharmacother* 44: 1098–1103.

Chaudhuri, A. and Behan, P.O. (2004) Fatigue in neurological disorders. *Lancet* 363: 978–988.

Christodoulou, C., MacAllister, W.S., McLinskey, N.A. and Krupp, L.B. (2008) Treatment of cognitive impairment in multiple sclerosis: is the use of acetylcholinesterase inhibitors a viable option? *CNS Drugs* 22: 87–97.

Cleare, A.J. (2003) The neuroendocrinology of chronic fatigue syndrome. *Endocrine Rev* 24: 236–252.

Cleare, A.J., Heap, E., Malhi, G.S., Wessely, S., O'Keane, V. and Miell, J. (1999) Low-dose hydrocortisone in chronic fatigue syndrome: a randomised crossover trial. *Lancet* 353: 455–458.

Codella, M., Rocca, M.A., Colombo, B., Rossi, P., Comi, G. and Filippi, M. (2002) A preliminary study of magnetization transfer and diffusion tensor MRI of multiple sclerosis patients with fatigue. *J Neurol* 249: 535–537.

Colla, M., Kronenberg, G., Deuschle, M., Meichel, K., Hagen, T., Bohrer, M. *et al.* (2007) Hippocampal volume reduction and HPA-system activity in major depression. *J Psychiatr Res* 41: 553–560.

Conrad, C.D. (2008) Chronic stress-induced hippocampal vulnerability: the glucocorticoid vulnerability hypothesis. *Rev Neurosci* 19: 395–411.

Czeh, B. and Lucassen, P.J. (2007) What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated? *Eur Arch Psychiatry Clin Neurosci* 257: 250–260.

Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W. and Kelley, K.W. (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 9: 46–56.

Duman, R.S. and Monteggia, L.M. (2006) A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* 59: 1116–1127.

Dutta, R., Chang, A., Doud, M.K., Kidd, G.J., Ribuado, M.V., Young, E.A. *et al.* (2011) Demyelination causes synaptic alterations in hippocampi from multiple sclerosis patients. *Ann Neurol* 69(3): 445–454. doi: 10.1002/ana.22337.

Egg, R., Hogl, B., Glatzl, S., Beer, R. and Berger, T. (2002) Autonomic instability, as measured by pupillary unrest, is not associated with multiple sclerosis fatigue severity. *Mult Scler* 8: 256–260.

Fassbender, K., Schmidt, R., Mossner, R., Kischka, U., Kuhnen, J., Schwartz, A. *et al.* (1998) Mood disorders and dysfunction of the hypothalamic–pituitary–adrenal axis in multiple sclerosis: association with cerebral inflammation. *Arch Neurol* 55: 66–72.

Feinstein, A. (1997) Multiple sclerosis, depression, and suicide. *Bmj* 315: 691–692.

Feinstein, A. (2002) An examination of suicidal intent in patients with multiple sclerosis. *Neurology* 59: 674–678.

Feinstein, A. (2006) Mood disorders in multiple sclerosis and the effects on cognition. *J Neurol Sci* 245: 63–66.

Feinstein, A., O'Connor, P., Akbar, N., Moradzadeh, L., Scott, C.J. and Lobaugh, N.J. (2010) Diffusion tensor imaging abnormalities in depressed multiple sclerosis patients. *Mult Scler* 16: 189–196.

Feinstein, A., O'Connor, P. and Feinstein, K. (2002) Multiple sclerosis, interferon beta-1b and depression A prospective investigation. *J Neurol* 249: 815–820.

Feinstein, A., Roy, P., Lobaugh, N., Feinstein, K., O'Connor, P. and Black, S. (2004) Structural brain abnormalities in multiple sclerosis patients with major depression. *Neurology* 62: 586–590.

Filippi, M., Rocca, M.A., Colombo, B., Falini, A., Codella, M., Scotti, G. *et al.* (2002) Functional magnetic resonance imaging correlates of fatigue in multiple sclerosis. *NeuroImage* 15: 559–567.

Fisk, J.D., Pontefract, A., Ritvo, P.G., Archibald, C.J. and Murray, T.J. (1994) The impact of fatigue on patients with multiple sclerosis. *Can J Neurol Sci* 21: 9–14.

Flachenecker, P., Bihler, I., Weber, F., Gottschalk, M., Toyka, K.V. and Rieckmann, P. (2004) Cytokine mRNA expression in patients with multiple sclerosis and fatigue. *Mult Scler* 10: 165–169.

Flachenecker, P., Reiners, K., Krauser, M., Wolf, A. and Toyka, K.V. (2001) Autonomic dysfunction in multiple sclerosis is related to disease activity and progression of disability. *Mult Scler* 7: 327–334.

Flachenecker, P., Rufer, A., Bihler, I., Hippel, C., Reiners, K., Toyka, K.V. *et al.* (2003) Fatigue in MS is related to sympathetic vasomotor dysfunction. *Neurology* 61: 851–853.

Ford, H., Trigwell, P. and Johnson, M. (1998) The nature of fatigue in multiple sclerosis. *J Psychosomatic Res* 45: 33–38.

Geurts, J.J., Bo, L., Roosendaal, S.D., Hazes, T., Daniels, R., Barkhof, F. *et al.* (2007) Extensive hippocampal demyelination in multiple sclerosis. *J Neuropathol Exp Neurol* 66: 819–827.

Geurts, J.J., Reuling, I.E., Vrenken, H., Uitdehaag, B.M., Polman, C.H., Castelijns, J.A. *et al.* (2006) MR spectroscopic evidence for thalamic and hippocampal, but not cortical, damage in multiple sclerosis. *Magn Reson Med* 55: 478–483.

Giannini, M.J., Bergmark, B., Kreshover, S., Elias, E., Plummer, C. and O'Keefe, E. (2010) Understanding suicide and disability through three major disabling conditions: Intellectual disability, spinal cord injury, and multiple sclerosis. *Disabil Health J* 3: 74–78.

Giovannoni, G., Thompson, A.J., Miller, D.H. and Thompson, E.J. (2001) Fatigue is not associated with raised inflammatory markers in multiple sclerosis. *Neurology* 57: 676–681.

Gold, S.M., Kern, K.C., O'Connor, M.F., Montag, M., Kim, A., Yoo, Y.S. *et al.* (2010) Smaller cornu ammonis (CA) 2-3/dentate gyrus volumes and elevated cortisol in multiple sclerosis patients with depressive symptoms. *Biol Psychiatry* 68: 553–559.

Gold, S.M., Kruger, S., Ziegler, K.J., Krieger, T., Schulz, K.H., Otte, C. *et al.* (2011) Endocrine and immune substrates of depressive symptoms and fatigue in multiple sclerosis patients with comorbid major depression. *J Neurol Neurosurg Psychiatry*, in press.

Gold, S.M., Raji, A., Huitinga, I., Wiedemann, K., Schulz, K.H. and Heesen, C. (2005) Hypothalamo-pituitary-adrenal axis activity predicts disease progression in multiple sclerosis. *J Neuroimmunol* 165: 186–191.

Goldman, 0. (2005) The Goldman Consensus statement on depression in multiple sclerosis. *Mult Scler* 11: 328–337.

Gottschalk, M., Kumpfel, T., Flachenecker, P., Uhr, M., Trenkwalder, C., Holsboer, F. *et al.* (2005) Fatigue and regulation of the hypothalamo–pituitary–adrenal axis in multiple sclerosis. *Arch Neurol* 62: 277–280.

Grossman, P., Kappos, L., Gensicke, H., D'Souza, M., Mohr, D.C., Penner, I.K. *et al.* (2010) MS quality of life, depression, and fatigue improve after mindfulness training: a randomized trial. *Neurology* 75: 1141–1149.

Heesen, C., Gold, S.M., Huitinga, I. and Reul, J.M. (2007) Stress and hypothalamic-pituitary-adrenal axis function in experimental autoimmune encephalomyelitis and multiple sclerosis - a review. *Psychoneuroendocrinology* 32: 604–618.

Heesen, C., Gold, S.M., Raji, A., Wiedemann, K. and Schulz, K.H. (2002) Cognitive impairment correlates with hypothalamo–pituitary–adrenal axis dysregulation in multiple sclerosis. *Psychoneuroendocrinology* 27: 505–517.

Heesen, C., Nawrath, L., Reich, C., Bauer, N., Schulz, K.H. and Gold, S.M. (2006) Fatigue in multiple sclerosis: an example of cytokine mediated sickness behaviour? *J Neurol Neurosurg Psychiatry* 77: 34–39.

Heesen, C., Schulz, K.H., Fielder, J., Von der Mark, U., Otte, C., Jung, R. *et al.* (2010) Correlates of cognitive dysfunction in multiple sclerosis. *Brain Behav Immunity* 24: 1148–1155.

Henn, F.A. and Vollmayr, B. (2004) Neurogenesis and depression: etiology or epiphenomenon? *Biol Psychiatry* 56: 146–150.

Hillman, C.H., Erickson, K.I. and Kramer, A.F. (2008) Be smart, exercise your heart: exercise effects on brain and cognition. *Nat Rev Neurosci* 9: 58–65.

Hinkelmann, K., Moritz, S., Botzenhardt, J., Riedesel, K., Wiedemann, K., Kellner, M. *et al.* (2009) Cognitive impairment in major depression: association with salivary cortisol. *Biol Psychiatry* 66: 879–885.

Holzel, B.K., Ott, U., Gard, T., Hempel, H., Weygandt, M., Morgen, K. *et al.* (2008) Investigation of mindfulness meditation practitioners with voxel-based morphometry. *Soc Cogn Affect Neurosci* 3: 55–61.

Jonsson, A., Dock, J. and Ravnborg, M.H. (1996) Quality of life as a measure of rehabilitation outcome in patients with multiple sclerosis. *Acta Neurol Scand* 93: 229–235.

Juruena, M.F., Cleare, A.J., Papadopoulos, A.S., Poon, L., Lightman, S. and Pariante, C.M. (2006) Different responses to dexamethasone and prednisolone in the same depressed patients. *Psychopharmacology (Berl)* 189: 225–235.

Juruena, M.F., Cleare, A.J., Papadopoulos, A.S., Poon, L., Lightman, S. and Pariante, C.M. (2010) The prednisolone suppression test in depression: dose–response and changes with antidepressant treatment. *Psychoneuroendocrinology* 35: 1486–1491.

Juruena, M.F., Pariante, C.M., Papadopoulos, A.S., Poon, L., Lightman, S. and Cleare, A.J. (2009) Prednisolone suppression test in depression: prospective study of the role of HPA axis dysfunction in treatment resistance. *Br J Psychiatry* 194: 342–349.

Keselbrener, L., Akselrod, S., Ahiron, A., Eldar, M., Barak, Y. and Rotstein, Z. (2000) Is fatigue in patients with multiple sclerosis related to autonomic dysfunction? *Clin Autonomic Res* 10: 169–175.

Khan, F., Turner-Stokes, L., Ng, L. and Kilpatrick, T. (2007) Multidisciplinary rehabilitation for adults with multiple sclerosis. *Cochrane Database Syst Rev* 2: CD006036.

Khurana, R.K. and Setty, A. (1996) The value of the isometric hand-grip test—studies in various autonomic disorders. *Clin Autonomic Res* 6: 211–218.

Knorr, U., Vinberg, M., Kessing, L.V. and Wetterslev, J. (2010) Salivary cortisol in depressed patients versus control persons: a systematic review and meta-analysis. *Psychoneuroendocrinology* 35: 1275–1286.

Koolschijn, P.C., van Haren, N.E., Lensvelt-Mulders, G.J., Hulshoff Pol, H.E. and Kahn, R.S. (2009) Brain volume abnormalities in major depressive

disorder: a meta-analysis of magnetic resonance imaging studies. *Human Brain Mapping* 30: 3719–3735.

Kopke, S., Kasper, J., Muhlhauser, I., Nubling, M. and Heesen, C. (2009) Patient education program to enhance decision autonomy in multiple sclerosis relapse management: a randomized-controlled trial. *Mult Scler* 15: 96–104.

Krishnan, V. and Nestler, E.J. (2008) The molecular neurobiology of depression. *Nature* 455: 894–902.

Krishnan, V. and Nestler, E.J. (2010) Linking molecules to mood: new insight into the biology of depression. *Am J Psychiatry* 167: 1305–1320.

Lopez, J.F., Chalmers, D.T., Little, K.Y. and Watson, S.J. (1998) A.E. Bennett Research Award. Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. *Biol Psychiatry* 43: 547–573.

Lovera, J.F., Frohman, E., Brown, T.R., Bandari, D., Nguyen, L., Yadav, V. *et al.* (2010) Memantine for cognitive impairment in multiple sclerosis: a randomized placebo-controlled trial. *Mult Scler* 16: 715–723.

Luders, E., Toga, A.W., Lepore, N. and Gaser, C. (2009) The underlying anatomical correlates of long-term meditation: larger hippocampal and frontal volumes of gray matter. *Neuroimage* 45: 672–678.

Macqueen, G. and Frodl, T. (2011) The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? *Mol Psychiatry* 16: 252–264.

Mainero, C., Faroni, J., Gasperini, C., Filippi, M., Giugni, E., Ciccarelli, O. *et al.* (1999) Fatigue and magnetic resonance imaging activity in multiple sclerosis. *J Neurol* 246: 454–458.

Marques, A.H., Silverman, M.N. and Sternberg, E.M. (2009) Glucocorticoid dysregulations and their clinical correlates. From receptors to therapeutics. *Ann N Y Acad Sci* 1179: 1–18.

Marrie, R.A., Fisher, E., Miller, D.M., Lee, J.C. and Rudick, R.A. (2005) Association of fatigue and brain atrophy in multiple sclerosis. *J Neurol Sci* 228: 161–166.

Mason, B.L. and Pariante, C.M. (2006) The effects of antidepressants on the hypothalamic–pituitary–adrenal axis. *Drug News Perspect* 19: 603–608.

McEwen, B.S. (1999) Stress and hippocampal plasticity. *Annu Rev Neurosci* 22: 105–122.

McEwen, B.S. (2003) Interacting mediators of allostasis and allostatic load: towards an understanding of resilience in aging. *Metabolism* 52(10 Suppl. 2): 10–16.

Merkelbach, S., Dillmann, U., Kolmel, C., Holz, I. and Muller, M. (2001) Cardiovascular autonomic dysregulation and fatigue in multiple sclerosis. *Mult Scler* 7: 320–326.

Miller, A.H., Maletic, V. and Raison, C.L. (2009) Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 65: 732–741.

Mohr, D.C., Goodkin, D.E., Islar, J., Hauser, S.L. and Genain, C.P. (2001) Treatment of depression is associated with suppression of nonspecific and antigen-specific T(H)1 responses in multiple sclerosis. *Arch Neurol* 58: 1081–1086.

Mohr, D.C., Goodkin, D.E., Likosky, W., Gatto, N., Baumann, K.A. and Rudick, R.A. (1997) Treatment of depression improves adherence to interferon beta-1b therapy for multiple sclerosis. *Arch Neurol* 54: 531–533.

Mohr, D.C., Hart, S.L., Julian, L., Cox, D. and Pelletier, D. (2004) Association between stressful life events and exacerbation in multiple sclerosis: a meta-analysis. *Bmj* 328: 731.

Moller, A., Wiedemann, G., Rohde, U., Backmund, H. and Sonntag, A. (1994) Correlates of cognitive impairment and depressive mood disorder in multiple sclerosis. *Acta Psychiatr Scand* 89: 117–121.

Mostert, J.P., Admiraal-Behloul, F., Hoogduin, J.M., Luyendijk, J., Heersema, D.J., van Buchem, M.A. *et al.* (2008) Effects of fluoxetine on disease activity in relapsing multiple sclerosis: a double-blind, placebo-controlled, exploratory study. *J Neurol Neurosurg Psychiatry* 79: 1027–1031.

Motl, R.W., Goldman, M.D. and Benedict, R.H. (2010) Walking impairment in patients with multiple sclerosis: exercise training as a treatment option. *Neuropsychiatric Disease Treatment* 6: 767–774.

Otte, C. (2008) Incomplete remission in depression: role of psychiatric and somatic comorbidity. *Dialogues Clin Neurosci* 10: 453–460.

Papadopoulos, D., Dukes, S., Patel, R., Nicholas, R., Vora, A. and Reynolds, R. (2009) Substantial archaeocortical atrophy and neuronal loss in multiple sclerosis. *Brain Pathol* 19: 238–253.

Pardini, M., Bonzano, L., Mancardi, G.L. and Roccatagliata, L. (2010) Frontal networks play a role in fatigue perception in multiple sclerosis. *Behav Neurosci* 124: 329–336.

Pariante, C.M. and Lightman, S.L. (2008) The HPA axis in major depression: classical theories and new developments. *Trends Neurosci* 31: 464–468.

Paslakis, G., Krumm, B., Gilles, M., Schweiger, U., Heuser, I., Richter, I. *et al.* (2010) Discrimination between patients with melancholic depression and healthy controls: Comparison between 24-h cortisol profiles, the DST and the Dex/CRH test. *Psychoneuroendocrinology*, in press.

Pellicano, C., Gallo, A., Li, X., Ikonomidou, V.N., Evangelou, I.E., Ohayon, J.M. *et al.* (2010) Relationship of cortical atrophy to fatigue in patients with multiple sclerosis. *Arch Neurol* 67: 447–453.

Penner, I. and Calabrese, P. (2010) Managing fatigue: clinical correlates, assessment procedures and therapeutic strategies. *Int MS J/MS Forum* 17: 28–34.

Pollak, Y., Ovadia, H., Goshen, I., Gurevich, R., Monsa, K., Avitsur, R. *et al.* (2000) Behavioral aspects of experimental autoimmune encephalomyelitis. *J Neuroimmunol* 104: 31–36.

Pollak, Y., Ovadia, H., Orion, E., Weidenfeld, J. and Yirmiya, R. (2003a) The EAE-associated behavioral syndrome: I. Temporal correlation with inflammatory mediators. *J Neuroimmunol* 137: 94–99.

Pollak, Y., Ovadia, H., Orion, E. and Yirmiya, R. (2003b) The EAE-associated behavioral syndrome: II. Modulation by anti-inflammatory treatments. *J Neuroimmunol* 137: 100–108.

Posener, J.A., Wang, L., Price, J.L., Gado, M.H., Province, M.A., Miller, M.I. *et al.* (2003) High-dimensional mapping of the hippocampus in depression. *The Am J Psychiatry* 160: 83–89.

Prakash, R.S., Snook, E.M., Motl, R.W. and Kramer, A.F. (2010) Aerobic fitness is associated with gray matter volume and white matter integrity in multiple sclerosis. *Brain Res* 1341: 41–51.

Pucak, M.L., Carroll, K.A., Kerr, D.A. and Kaplin, A.I. (2007) Neuropsychiatric manifestations of depression in multiple sclerosis: neuroinflammatory, neuroendocrine, and neurotrophic mechanisms in the pathogenesis of immune-mediated depression. *Dialogues Clin Neurosci* 9: 125–139.

Raison, C.L., Capuron, L. and Miller, A.H. (2006) Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 27: 24–31.

Rickards, H. (2005) Depression in neurological disorders: Parkinson's disease, multiple sclerosis, and stroke. *J Neurol Neurosurg Psychiatry* 76(Suppl. 1): i48–i52.

Roelcke, U., Kappos, L., Lechner-Scott, J., Brunnschweiler, H., Huber, S., Ammann, W. *et al.* (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* 48: 1566–1571.

Rossi, S., Furlan, R., De Chiara, V., Musella, A., Lo Giudice, T., Mataluni, G. *et al.* (2009) Exercise attenuates the clinical, synaptic and dendritic abnormalities of experimental autoimmune encephalomyelitis. *Neurobiol Dis* 36: 51–59.

Sajad, M., Zargan, J., Chawla, R., Umar, S., Sadaqat, M. and Khan, H.A. (2009) Hippocampal neurodegeneration in experimental autoimmune encephalomyelitis (EAE): potential role of inflammation activated myeloperoxidase. *Mol Cell Biochem* 328: 183–188.

Sandyk, R. and Awerbuch, G.I. (1994) Pineal calcification and its relationship to the fatigue of multiple sclerosis. *Int J Neurosci* 74: 95–103.

Sapolsky, R.M. (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry* 57: 925–935.

Sapolsky, R.M., Uno, H., Rebert, C.S. and Finch, C.E. (1990) Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *J Neurosci* 10: 2897–2902.

Schumann, E.M., Kumpfel, T., Then Bergh, F., Trenkwalder, C., Holsboer, F. and Auer, D.P. (2002) Activity of the hypothalamic–pituitary–adrenal axis in multiple sclerosis: correlations with gadolinium-enhancing lesions and ventricular volume. *Ann Neurol* 51: 763–767.

Schwartz, C.E., Coulthard-Morris, L. and Zeng, Q. (1996) Psychosocial correlates of fatigue in multiple sclerosis. *Arch Phys Med Rehabil* 77: 165–170.

Seckl, J.R., Dickson, K.L., Yates, C. and Fink, G. (1991) Distribution of glucocorticoid and mineralocorticoid receptor messenger RNA expression in human postmortem hippocampus. *Brain Res* 561: 332–337.

Sepulcre, J., Masdeu, J.C., Goni, J., Arrondo, G., Velez de Mendizabal, N., Bejarano, B. *et al.* (2009) Fatigue in multiple sclerosis is associated with the disruption of frontal and parietal pathways. *Mult Scler* 15: 337–344.

Sheean, G.L., Murray, N.M., Rothwell, J.C., Miller, D.H. and Thompson, A.J. (1997) An electrophysiological study of the mechanism of fatigue in multiple sclerosis. *Brain* 120: 299–315.

Sheline, Y.I., Gado, M.H. and Kraemer, H.C. (2003) Untreated depression and hippocampal volume loss. *Am J Psychiatry* 160: 1516–1518.

Sicotte, N.L., Kern, K.C., Giesser, B.S., Arshanapalli, A., Schultz, A., Montag, M. *et al.* (2008) Regional hippocampal atrophy in multiple sclerosis. *Brain* 131: 1134–1141.

Siegert, R.J. and Abernethy, D.A. (2005) Depression in multiple sclerosis: a review. *J Neurol Neurosurg Psychiatry* 76: 469–475.

Sijens, P.E., Mostert, J.P., Irwan, R., Potze, J.H., Oudkerk, M. and De Keyser, J. (2008) Impact of fluoxetine on the human brain in multiple sclerosis as quantified by proton magnetic resonance spectroscopy and diffusion tensor imaging. *Psychiatry Res* 164: 274–282.

Smith, M.M. and Arnett, P.A. (2005) Factors related to employment status change in individuals with multiple sclerosis. *Mult Scler* 11: 602–609.

Sospedra, M. and Martin, R. (2005) Immunology of multiple sclerosis. *Annu Rev Immunol* 23: 683–747.

Starkman, M.N., Giordani, B., Gebarski, S.S., Berent, S., Schork, M.A. and Schteingart, D.E. (1999) Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biol Psychiatry* 46: 1595–1602.

Starkman, M.N., Giordani, B., Gebarski, S.S. and Schteingart, D.E. (2003) Improvement in learning associated with increase in hippocampal formation volume. *Biol Psychiatry* 53: 233–238.

Stuke, K., Flachenecker, P., Zettl, U.K., Elias, W.G., Freidel, M., Haas, J. *et al.* (2009) Symptomatology of MS: results from the German MS registry. *J Neurol* 256: 1932–1935.

Sullivan, M.J., Weinshenker, B., Mikail, S. and Edgley, K. (1995) Depression before and after diagnosis of multiple sclerosis. *Mult Scler* 1: 104–108.

Tedeschi, G., Dinacci, D., Lavorgna, L., Prinster, A., Savettieri, G., Quattrone, A. *et al.* (2007) Correlation between fatigue and brain atrophy and lesion load in multiple sclerosis patients independent of disability. *J Neurol Sci* 263: 15–19.

Tellez, N., Alonso, J., Rio, J., Tintore, M., Nos, C., Montalban, X. *et al.* (2008) The basal ganglia: a substrate for fatigue in multiple sclerosis. *Neuroradiology* 50: 17–23.

Tellez, N., Comabella, M., Julia, E., Rio, J., Tintore, M., Brieva, L. *et al.* (2006) Fatigue in progressive multiple sclerosis is associated with low levels of dehydroepiandrosterone. *Mult Scler* 12: 487–494.

Then Bergh, F., Kumpfel, T., Grasser, A., Rupprecht, R., Holsboer, F. and 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* 86: 1610–1615.

Then Bergh, F., Kumpfel, T., Trenkwalder, C., Rupprecht, R. and Holsboer, F. (1999) Dysregulation of the hypothalamo–pituitary–adrenal axis is related to the clinical course of MS. *Neurology* 53: 772–777.

Thomas, P.W., Thomas, S., Hillier, C., Galvin, K. and Baker, R. (2006) Psychological interventions for multiple sclerosis. *Cochrane Database Syst Rev* 1: CD004431.

Van Den Eede, F., Moorkens, G., Van Houdenhove, B., Cosyns, P. and Claes, S.J. (2007) Hypothalamic–pituitary–adrenal axis function in chronic fatigue syndrome. *Neuropsychobiology* 55: 112–120.

van der Werf, S.P., Evers, A., Jongen, P.J. and Bleijenberg, G. (2003) The role of helplessness as mediator between neurological disability, emotional instability, experienced fatigue and depression in patients with multiple sclerosis. *Mult Scler* 9: 89–94.

van der Werf, S.P., Jongen, P.J., Lycklama a Nijeholt, G.J., Barkhof, F., Hommes, O.R. and Bleijenberg, G. (1998) Fatigue in multiple sclerosis: interrelations between fatigue complaints, cerebral MRI abnormalities and neurological disability. *J Neurol Sci* 160: 164–170.

Van Houdenhove, B., Van Den Eede, F. and Luyten, P. (2009) Does hypothalamic-pituitary-adrenal axis hypofunction in chronic fatigue syndrome reflect a 'crash' in the stress system? *Med Hypotheses* 72: 701–705.

Vermetten, E., Vythilingam, M., Southwick, S.M., Charney, D.S. and Bremner, J.D. (2003) Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. *Biol Psychiatry* 54: 693–702.

Vickrey, B.G., Hays, R.D., Harooni, R., Myers, L.W. and Ellison, G.W. (1995) A health-related quality of life measure for multiple sclerosis. *Qual Life Res* 4: 187–206.

Voss, W.D., Arnett, P.A., Higginson, C.I., Randolph, J.J., Campos, M.D. and Dyck, D.G. (2002) Contributing factors to depressed mood in multiple sclerosis. *Arch Clin Neuropsychol* 17: 103–115.

Vythilingam, M., Vermetten, E., Anderson, G.M., Luckenbaugh, D., Anderson, E.R., Snow, J. *et al.* (2004) Hippocampal volume, memory, and cortisol status in major depressive disorder: effects of treatment. *Biol Psychiatry* 56: 101–112.

Wang, S.S., Kamphuis, W., Huitinga, I., Zhou, J.N. and Swaab, D.F. (2008) Gene expression analysis in the human hypothalamus in depression by laser microdissection and real-time PCR: the presence of multiple receptor imbalances. *Mol Psychiatry* 13: 786–799, 741.

Webster, M.J., Knable, M.B., O'Grady, J., Orthmann, J. and Weickert, C.S. (2002) Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders. *Mol Psychiatry* 7: 985–994, 924.

Young, E.A., Lopez, J.F., Murphy-Weinberg, V., Watson, S.J. and Akil, H. (2003) Mineralocorticoid receptor function in major depression. *Arch Gen Psychiatry* 60: 24–28.

Ysrraelit, M.C., Gaitan, M.I., Lopez, A.S. and Correale, J. (2008) Impaired hypothalamic–pituitary–adrenal axis activity in patients with multiple sclerosis. *Neurology* 71: 1948–1954.

Ziehn, M.O., Avedisian, A.A., Tiwari-Woodruff, S. and Voskuhl, R.R. (2010) Hippocampal CA1 atrophy and synaptic loss during experimental autoimmune encephalomyelitis, EAE. *Lab Invest* 90: 774–786.

Zorzon, M., de Masi, R., Nasuelli, D., Ukmar, M., Mucelli, R.P., Cazzato, G. *et al.* (2001) Depression and anxiety in multiple sclerosis. A clinical and MRI study in 95 subjects. *J Neurol* 248: 416–421.

Zorzon, M., Zivadinov, R., Nasuelli, D., Ukmar, M., Bratina, A., Tommasi, M.A. *et al.* (2002) Depressive symptoms and MRI changes in multiple sclerosis. *Eur J Neurol* 9: 491–496.