Autonomic Nervous System (Re)activity and Multiple Sclerosis-Related Fatigue: A Synthesis of Evidence and a Proposed Conceptual Extension

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Summary

Fatigue is one of the most frequent and debilitating symptoms of multiple sclerosis (MS), significantly altering quality of life (Asano & Finlayson, 2014; Fisk et al., 1994; Krupp et al., 1989). Its pathophysiology is incompletely understood, but existing conceptualizations emphasize a central role of neuroimmune signaling and autonomic nervous system (ANS) dysfunction, proposing that altered functional communication, rather than structural damage, leads to fatigue (Chaudhuri & Behan, 2004; Dantzer et al., 2008; Hanken et al., 2014; Sander et al., 2020; Zielinski et al., 2019).

This dissertation is based on the sickness behavior model of MS-related fatigue (Hanken et al., 2014) and its autonomic extension (Sander et al., 2020). Through two reviews and two empirical studies using heart rate variability (HRV) as a biomarker of ANS (re)activity, this dissertation shows that ANS dysfunction, characterized by diminished parasympathetic tone and reduced autonomic adaptability, is a physiological correlate of MS-related fatigue. The studies demonstrate that while biopsychological interventions such as progressive muscle relaxation, deep breathing, and self-alert training can modulate ANS activity (Garis et al., 2023; *manuscript submitted for publication*, Sander et al., 2020), they did not significantly decrease fatigue.

The findings highlight a limitation of existing frameworks: they explain the development of MS-related fatigue but not its chronicity. To address this, the dissertation proposes an extended conceptualization. It introduces two secondary maintaining processes: "fatigue debt," the cumulative burden of MS symptoms such as pain, cognitive impairment, mood disorders, or poor sleep quality, and allostatic overload. It is argued that while primary neuroimmune-autonomic dysfunction initiates fatigue, these secondary factors create a self-reinforcing cycle that exacerbates fatigue into a chronic state.

In summary, this dissertation contributes to a more differentiated understanding of MS-related fatigue within neuroimmune-autonomic frameworks. Specifically, it 1) objectifies ANS dysfunction associated with fatigue using HRV, 2) differentiates ANS correlates of state and trait fatigue, and 3) proposes an extended conceptualization of the sickness behavior model (Hanken et al., 2014) by incorporating additional mechanisms that account not only for the initiation, but also for the maintenance and treatment resistance of MS-related fatigue.

Zusammenfassung

Fatigue ist eines der häufigsten und belastendsten Symptome der Multiplen Sklerose (MS) und beeinträchtigt die Lebensqualität der Betroffenen erheblich (Asano & Finlayson, 2014; Fisk et al., 1994; Krupp et al., 1989). Ihre Pathophysiologie ist bislang nicht vollständig verstanden, jedoch betonen bestehende Konzeptualisierungen eine zentrale Rolle neuroimmunologischer Signalprozesse sowie einer Dysfunktion des autonomen Nervensystems (ANS). Demnach wird angenommen, dass veränderte funktionelle Kommunikationsprozesse, und nicht primär strukturelle Schädigungen, zur Entstehung von Fatigue beitragen (Chaudhuri & Behan, 2004; Dantzer et al., 2008; Hanken et al., 2014; Sander et al., 2020; Zielinski et al., 2019).

Die vorliegende Dissertation basiert auf dem Sickness-Behavior-Modell der MS-assoziierten Fatigue (Hanken et al., 2014) sowie dessen autonomer Erweiterung (Sander et al., 2020). Anhand von vier Studien, darunter zwei Übersichtsarbeiten und zwei empirische Untersuchungen, in denen die Herzratenvariabilität (HRV) als Biomarker der ANS-(Re)aktivität eingesetzt wurde, wird gezeigt, dass eine ANS-Dysfunktion, gekennzeichnet durch einen verminderten parasympathischen Tonus sowie eine reduzierte autonome Adaptabilität, ein physiologisches Korrelat der MS-assoziierten Fatigue darstellt. Ferner zeigen die Studien, dass biopsychologische Interventionen wie Progressive Muskelrelaxation, Tiefenatmung und Self-Alert-Training zwar in der Lage sind, die ANS-Aktivität zu modulieren (Garis et al., 2023; Manuskript zur Publikation eingereicht; Sander et al., 2020), jedoch keine signifikante Reduktion der Fatigue bewirken.

Die Befunde verdeutlichen eine zentrale Limitation bestehender theoretischer Modelle: Sie erklären die Entstehung der MS-assoziierten Fatigue, nicht jedoch deren Chronifizierung. Zur Schließung dieser Lücke schlägt die vorliegende Dissertation eine erweiterte Konzeptualisierung vor. In diesem Zusammenhang werden das Konzept der „Fatigue Debt“, verstanden als kumulative Belastung durch MS-assoziierte Symptome wie Schmerzen, kognitive Beeinträchtigungen, affektive Störungen oder eine beeinträchtigte Schlafqualität, sowie die allostatische Überlastung als sekundäre, aufrechterhaltende Prozesse eingeführt. Es wird argumentiert, dass primäre neuroimmun-autonome Dysfunktionen zwar die Fatigue initiieren, diese sekundären Faktoren jedoch einen sich selbst verstärkenden Kreislauf begünstigen, der zur Chronifizierung der Fatigue beiträgt.

Zusammenfassend trägt die vorliegende Dissertation zu einem differenzierteren Verständnis der MS-assoziierten Fatigue im Rahmen neuroimmun-autonomer Modelle bei. Konkret 1) erfasst sie ANS-Dysfunktionen im Zusammenhang mit Fatigue objektiv mittels HRV, 2) differenziert sie ANS-

Korrelate von situativer (State-) und persistenter (Trait-) Fatigue und 3) schlägt eine erweiterte Konzeptualisierung des Sickness-Behavior-Modells (Hanken et al., 2014) vor durch die Integration zusätzlicher Mechanismen, die nicht nur die Initiierung, sondern auch die Aufrechterhaltung und Therapieresistenz der MS-assoziierten Fatigue erklären.

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List of Publications

The following can be found in the appendix of this dissertation.

Study I

Garis, G., Hildebrandt, H., & Hanken, K. (2021). Yoga als Möglichkeit zur Reduktion von Fatigue bei Multipler Sklerose. *Neurologie & Rehabilitation, 27*(1), 49–55. https://doi.org/10.14624/nr2101006

Study II

Garis, G., Haupts, M., Duning, T., & Hildebrandt, H. (2022). Heart rate variability and fatigue in MS: Two parallel pathways representing disseminated inflammatory processes? *Neurological Sciences, 44*(1), 83–98. https://doi.org/10.1007/s10072-022-06385-1

Study III

Garis, G., Dettmers, C., Hildebrandt, A., Duning, T., & Hildebrandt, H. (2023). Comparing two relaxation procedures to ease fatigue in multiple sclerosis: a single-blind randomized controlled trial. *Neurological Sciences,* (11), 4087–4098. https://doi.org/10.1007/s10072-023-07042-x

Study IV

Garis, G., Sander, C., Hildebrandt, A., & Hildebrandt, H. (Manuscript submitted for publication). *Heart rate variability as a biomarker of autonomic (re)activity and fatigue in multiple sclerosis.*

List of Figures

List of Abbreviations

ANS – Autonomic Nervous System

CNS – Central Nervous System

DB – Deep Breathing

DFA – Detrended Fluctuation Analysis

DMT(s) – Disease-Modifying Treatment/Treatments

ECG – Electrocardiography

EDSS – Expanded Disability Status Scale

FSMC – Fatigue Scale for Motor and Cognitive Functions

HF – High Frequency

HRV – Heart Rate Variability

MFIS – Modified Fatigue Impact Scale

MS – Multiple Sclerosis

PMR – Progressive Muscle Relaxation

PNS – Parasympathetic Nervous System

RCT – Randomized Controlled Trial

RMSSD – Root Mean Square of Successive Differences

SAT – Self-Alert Training

SDNN – Standard Deviation of Normal-to-Normal Intervals

SNS – Sympathetic Nervous System

Table of Contents

1. Introduction

Fatigue is a prevalent and debilitating symptom of multiple sclerosis (MS). It affects up to 90% of patients at various phases of the disease, with a recent meta-analysis indicating a global prevalence of roughly 59% (Yi et al., 2024). This type of fatigue appears to be more prevalent among female patients, individuals with lower levels of education, older adults, and those with greater disability or longer disease duration (Yi et al., 2024). Patients with MS consistently rank fatigue as one of the most bothersome symptoms they live with, often rating its burden as heavier than pain, spasticity, or cognitive difficulties (Fisk et al., 1994; Freal et al., 1984).

What fundamentally distinguishes MS-related fatigue from ordinary tiredness is its qualitative character. MS-related fatigue typically persists irrespective of daily activities or activity levels, manifesting disproportionately to any discernible trigger and often without a recognizable cause or preceding event (Braley & Chervin, 2010; Comi et al., 2001; Merkelbach et al., 2001). Moreover, in marked contrast to normal fatigue, it shows little consistent relief following rest or sleep (DeLuca, 2005; Hanken et al., 2015; Linnhoff et al., 2019). These distinctive characteristics make MS-related fatigue particularly debilitating, severely affecting everyday functioning. It substantially impairs patients' ability to sustain employment, very frequently culminating in early retirement, and it is strongly and consistently linked to considerable socioeconomic hardship (Fiest et al., 2016; Khan et al., 2014). Moreover, meta-analytic findings consistently demonstrate that higher fatigue severity correlates with increased unemployment, higher social withdrawal, and elevated rates of depression (Feinstein et al., 2014; Moore et al., 2022). Consequently, MS-related fatigue constitutes far more than a subjective complaint: it represents a substantial personal and societal burden (Braley & Chervin, 2010).

The distinction between trait and state fatigue is employed here as an empirically derived heuristic rather than a theoretically established construct, one which is currently lacking (Enoka et al., 2021). Trait fatigue is defined by a tendency to experience persistent fatigue for a prolonged period and is seen as a key symptom of MS, potentially resulting from continuous neuroimmune-autonomic dysfunction rather than irreparable damage to autonomic pathways (Malloy et al., 2021). That said, its intensity may be amplified over time by other

factors, for instance, the cumulative burden of co-occurring MS symptoms or the physiological toll of allostatic overload. Trait fatigue is usually measured with validated questionnaires capturing average fatigue severity over 1-4 weeks, such as the Modified Fatigue Impact Scale (MFIS; Fisk et al., 1994) or the Fatigue Scale for Motor and Cognitive Functions (FSMC; Penner et al., 2009).

State fatigue, by contrast, is momentary and situational, changing with task demands and environment (Boksem & Tops, 2008; Enoka et al., 2021). Researchers typically capture it in real time, using a visual analog scale before, during, or after a fatigue-inducing task (Wylie et al., 2022). Trait and state fatigue are interconnected, but appear to reflect different underlying physiology, which is why this work examines both. In particular, trait fatigue is more strongly associated with autonomic nervous system (ANS) dysfunction and diminished ANS adaptability than state fatigue is (Garis et al., 2022; *manuscript submitted for publication*).

The present dissertation focuses primarily on trait fatigue, which is the clinically dominant and persistent dimension tied to ANS dysfunction (Garis et al., 2022). The approach I have taken here serves a dual purpose. On the one hand, it examines trait-level autonomic function as a baseline characteristic. On the other hand, it draws on experimental paradigms, using a vigilance task to induce state fatigue, followed by brief biopsychological interventions, to examine how the ANS responds in real time. This allows us to examine the acute ANS reactivity that accompanies momentary fluctuations in state fatigue, and whether interventions can shift those states. Overall, the aim is to provide insights into the physiological correlates of both trait and state fatigue.

Despite its clinical importance, the biological mechanisms that underlie MS-related fatigue are not fully elucidated. Nevertheless, accumulating evidence implicates neuroimmune signaling pathways and ANS dysfunction in the development of MS-related fatigue. In this context, ANS dysfunction does not signify primary structural autonomic damage but rather modified functional neuroimmune-autonomic signaling that influences energy regulation and adaptability (Chaudhuri & Behan, 2004; Dantzer et al., 2008; Hanken et al., 2014; Sander et al., 2020; Zielinski et al., 2019). Contemporary conceptualizations, most notably the sickness behavior model developed by Hanken et al., (2014), offer a framework in which immune-to-

brain communication triggers fatigue through central and autonomic pathways. This sickness behavior model has since been extended by positioning ANS activity as a mediator between immune processes and the development of MS-related fatigue (Sander et al., 2020).

To date, no pharmacological treatment has demonstrated sufficient and consistent efficacy in alleviating MS-related fatigue. Medications such as amantadine, modafinil, and methylphenidate show minimal or placebo-level efficacy and are no longer routinely recommended (Braley & Chervin, 2010; Henze et al., 2018; Nourbakhsh et al., 2021). Moreover, disease-modifying treatments (DMTs) effectively reduce inflammatory activity but have produced variable outcomes regarding fatigue (Broch et al., 2023; Elkhooly et al., 2023). This treatment gap highlights a fundamental point: we need a deeper understanding of the mechanisms driving MS-related fatigue, and we need an expanded framework that could help guide more effective interventions. The ultimate goal of this work is to help address the personal and societal burden of MS-related fatigue.

1.1 The Sickness Behavior Model of MS-Related Fatigue (Hanken et al., 2014)

The sickness behavior model proposed by Hanken et al. (2014), reframes MS-related fatigue as a chronic manifestation of neuroimmune signaling. Early explanations of MS-related fatigue often focused primarily on secondary causes, such as motor disability, sleep difficulties, depression, or medication side effects (Comi et al., 2001). The sickness behavior model suggested that MS-related fatigue itself, rather than being a byproduct of secondary causes, stems directly from immune activation. According to this model, sickness behavior refers to the coordinated set of behavioral and physiological changes that the immune system triggers during illness or infection, such as lethargy, social withdrawal, and reduced appetite (Dantzer et al., 2008; Konsman et al., 2002). In the short term, these responses serve a purpose. They conserve energy, direct resources toward supporting immune defense, and create conditions conducive to recovery. Fatigue, in this context, is adaptive (Dantzer et al., 2008; Ron et al., 2006). In the context of a chronic autoimmune condition like MS, however, persistent immune activation may convert this normally transient state into a long-lasting and debilitating fatigue (Dantzer et al., 2018; Hanken et al., 2014).

At the neurobiological level, the model details how pro-inflammatory cytokines signal the brain via humoral pathways, such as circumventricular organs, and neural pathways,

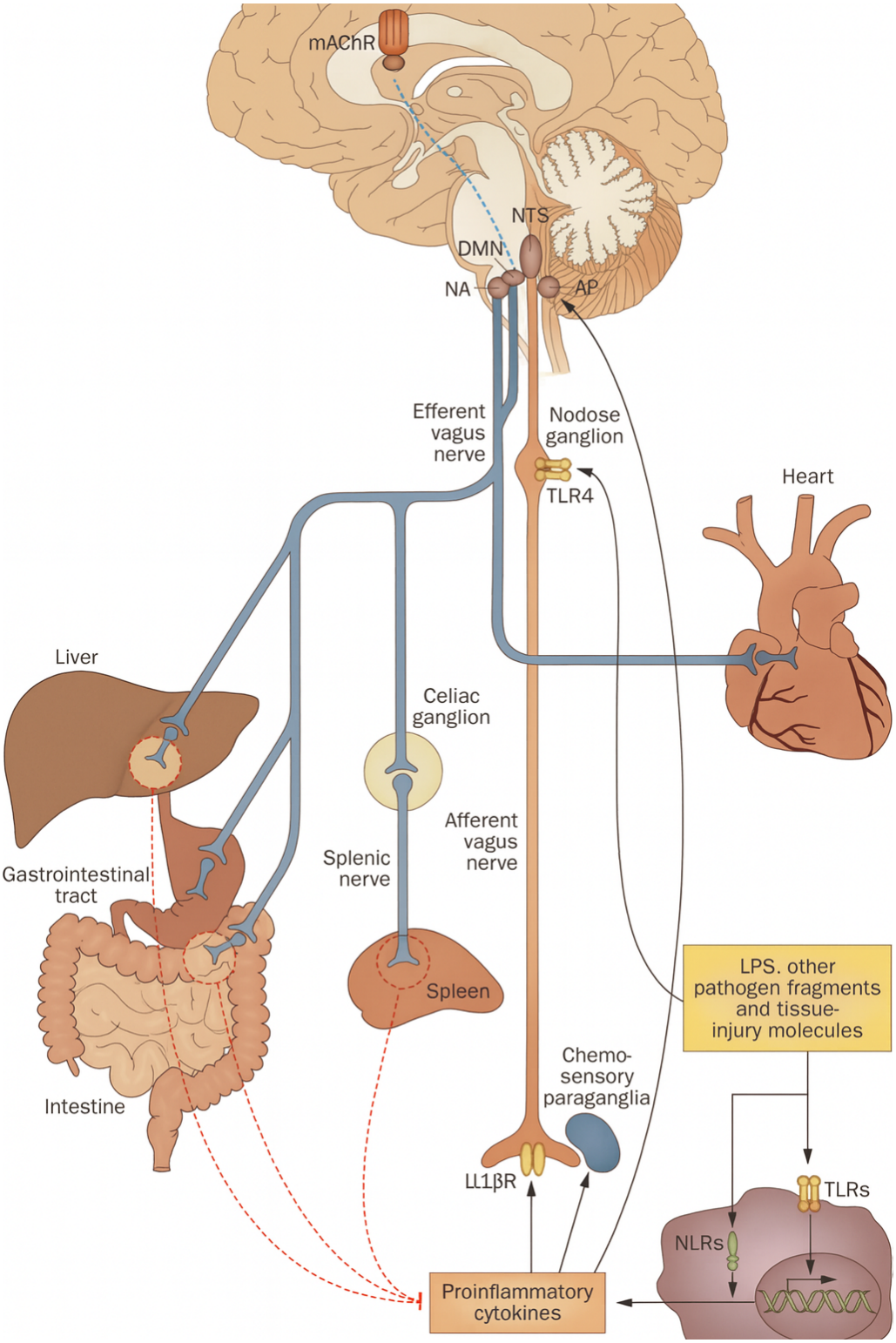
particularly via afferent vagal nerve fibers (Dantzer et al., 2008; Konsman et al., 2002; see Figure 1). Once these signals reach the brain, they do not remain in isolation, they actively modulate activity across a distributed network of regions implicated in interoception, motivation, and arousal. The brainstem, hypothalamus, insula, and anterior cingulate cortex are all part of this circuitry (Harrison et al., 2009). Neuroimaging studies corroborate that inflammatory activity disrupts cortico-subcortical networks involved in reward processing and effort valuation. The practical consequence is that the brain becomes less responsive to rewards and motivating signals (Felger & Miller, 2012; Harrison et al., 2009). Therefore, drive diminishes and effort begins to feel not worthwhile (Felger & Miller, 2012; Harrison et al., 2009). The resulting subjective fatigue, according to the sickness behavior model, creates internal interference that disrupts cognitive functions, particularly sustained attention and vigilance, a disruption that can be amplified by co-existing neurodegeneration in relevant brain networks (Hanken et al., 2014). Figure 2 provides a visual summary of the sickness behavior model as Hanken et al. (2014) originally conceived it, mapping the proposed route from peripheral inflammatory signals to the subjective experience of MS-related fatigue and the cognitive interference that follows.

From there, Sander et al., (2020) extended the model. They argued that ANS activity, vagal modulation in particular, was a mediator between immune activation and fatigue perception. That autonomic extension is what grounds the empirical work in this dissertation. It is why heart rate variability (HRV) is used hereby as a biomarker: to assess ANS function in real time, within the context of a vigilance task and biopsychological interventions that are aimed at increasing and decreasing fatigue, respectively.

Here, a distinction matters. The original sickness behavior model centers on afferent vagal signaling, that is, the transmission of inflammatory signals from the periphery to the brain. The HRV biomarkers employed by Sander et al. (2020) and in the present studies, however, predominantly reflect efferent parasympathetic nervous system (PNS) outflow from the brain to the heart. That is, increased afferent signaling is thought to trigger fatigue, while interventions designed to enhance efferent activity aim to do the opposite, to counteract it.

Figure 1

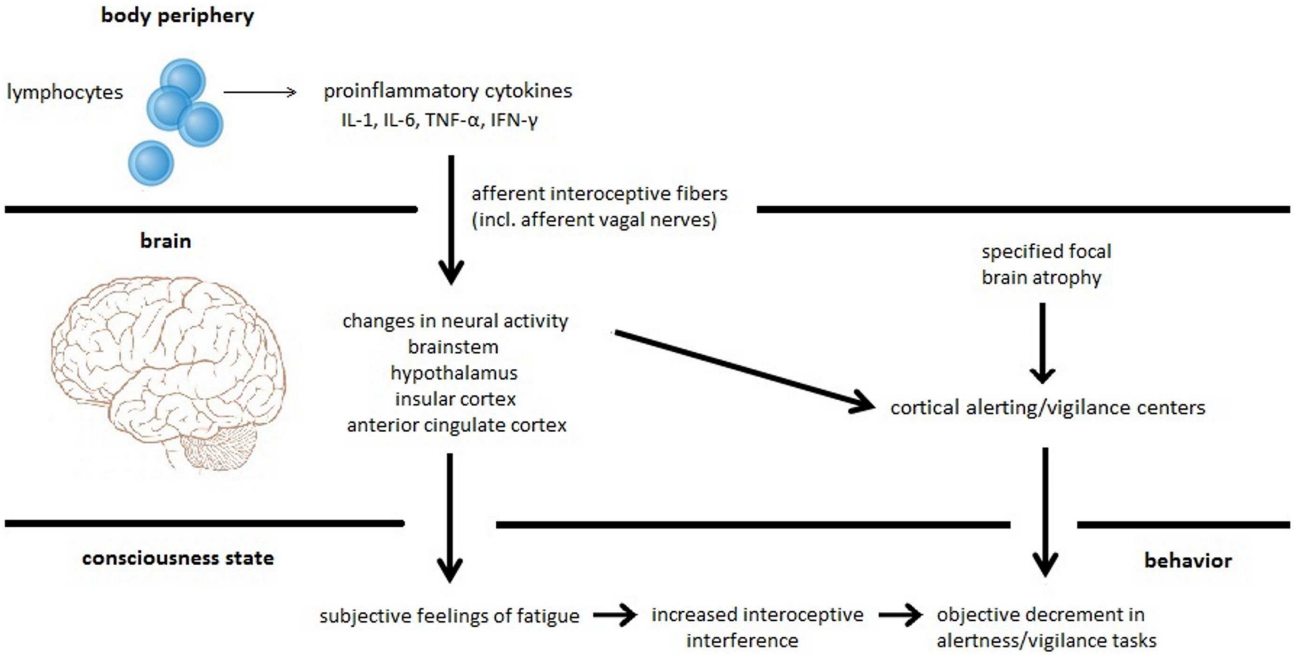
The functional anatomy of the inflammatory reflex



Note. Adapted from Pavlov, V. A., & Tracey, K. J. (2015). Neural circuitry and immunity. *Immunologic research*, *63*(1-3), 38–57. https://doi.org/10.1007/s12026-015-8718-1

Figure 2

Proposed model for MS-related fatigue by Hanken et al. (2014)



Note. Pro-inflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ), when released in the periphery, trigger signaling pathways from the immune system to the brain, primarily via afferent interoceptive nerves (especially the vagus nerve afferents). These nerves connect to brain regions that are responsible for interoception and the regulation of bodily states, such as the brainstem, hypothalamus, insula, and anterior cingulate cortex. Inflammation-related changes in neural activity within these areas contribute to the subjective experience of fatigue. Moreover, the ongoing processing of interoceptive signals creates interference that disrupts cognitive functions, particularly tasks requiring sustained alertness and vigilance, which rely on intrinsic alertness mechanisms. This inflammation-related decline in alertness and vigilance performance can be further amplified by localized brain atrophy in the neural networks supporting alertness and vigilance. Taken from Hanken, K., Eling, P., & Hildebrandt, H. (2014). The representation of inflammatory signals in the brain—A model for subjective fatigue in multiple sclerosis. *Frontiers in Neurology, 5*, 264. https://doi.org/10.3389/fneur.2014.00264 (published under the Creative Commons Attribution License (CC BY)).

1.2 Empirical Support and Extensions: Autonomic Dysfunction and HRV

The connection between ANS dysfunction and MS-related fatigue is now well documented (Garis et al., 2022). This correlation is not exclusive to MS but is characteristic of chronic systemic autoimmunity. Fatigue is a common symptom in various other autoimmune disorders as well, including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, and primary Sjögren's syndrome (Davies et al., 2021; Kawka et al., 2021; Louati & Berenbaum, 2015; Nocerino et al., 2020). In each of these disorders, fatigue often persists even when patients are in clinical remission. And across all of them, fatigue is associated reliably with objective markers of ANS dysfunction, that is, reduced HRV, and altered sympathovagal balance (Evrengül et al., 2004; Findling et al., 2020). The same patterns show up in other fatigue-heavy conditions with immunological roots, like myalgic encephalomyelitis/chronic fatigue syndrome and cancer-related fatigue (Crosswell et al., 2014; Escorihuela et al., 2020; Nelson et al., 2019).

This transdiagnostic pattern matters. It reinforces the disease behavior framework and supports a model where immune activation engages afferent vagal pathways and central autonomic networks, contributing to both the development and persistence of fatigue. HRV, a non-invasive measure modulated by autonomic input, can function as an indicator of autonomic flexibility and immune-brain communication across contexts (Hanken et al., 2014; Sander et al., 2020). This dissertation builds upon this paradigm and examines its manifestation in MS.

In MS, ANS dysfunction is more closely linked to changes in sympathovagal regulation and diminished physiological flexibility than to fixed structural lesions (Adamec et al., 2018; Flachenecker et al., 2003). Likewise, a recent comprehensive review and meta-analysis corroborated that patients with MS exhibit lower HRV across various parameters in comparison to healthy controls (Mirmosayyeb et al., 2025). This pattern indicates ANS dysfunction. MS-related fatigue has been consistently associated with decreased baseline PNS activity and autonomic adaptability (Crnošija et al., 2019; Garis et al., 2022, 2023; Merico et al., 2005; Sander et al., 2019).

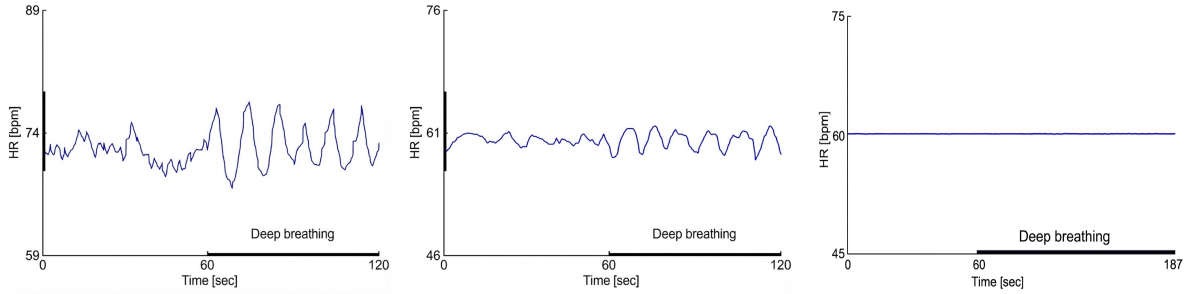
Here is why vagal pathway integrity becomes relevant. The cardiovagal reflex test, which measures the heart rate response to deep breathing (DB), is a sensitive measure of efferent

PNS function (Novak, 2011). In MS, demyelinating lesions may blunt this reflex; when that happens, it may limit the effectiveness of therapies aimed at boosting efferent vagal activity (Crnošija et al., 2019; Habek et al., 2016; Lehrer & Gevirtz, 2014) (see Figure 3 for examples of normal and abnormal cardiovagal reflex test responses). This is worth emphasizing because it is different from the afferent signaling the sickness behavior model focuses on. Afferent pathways are implicated in initiating fatigue; efferent pathways come into play during interventions (Hanken et al., 2014; Sander et al., 2020). Therefore, HRV does not only correlate with MS-related fatigue, it can also reveal functional constraints in the ANS that might affect treatment response, such as the ability to modulate efferent vagal tone.

This line of thinking leads to a question. If afferent vagal signaling is necessary to trigger fatigue, would structurally disrupting those nerves blunt it? Direct evidence from vagotomy in humans with MS does not exist, but animal studies offer clues. Research shows that while afferent vagal pathways are needed to trigger sickness behavior, subdiaphragmatic vagotomy significantly reduces, but does not eliminate, behavioral responses to peripheral immune challenges like interleukin-1 (Bluthe et al., 1996; Luheshi et al., 2000). That suggests fatigue is sustained through pathways that extend beyond the initial afferent signal. These include humoral cytokine routes (Konsman et al., 2002), immune activity confined to the central nervous system (CNS) that may evade peripheral detection and systemic treatment (Haase & Linker, 2021; Lassmann et al., 2012), and the secondary mechanisms this dissertation proposes: fatigue debt and allostatic overload (McEwen, 1998; Sterling & Eyer, 1988).

Figure 3

Examples of normal, moderately, and severely abnormal cardiovagal reflex test responses



Note. bpm = beats per minute; HR = heart rate; sec = seconds. Adapted from Novak P. (2011). Quantitative autonomic testing. *Journal of visualized experiments: JoVE*, (53), 2502. https://doi.org/10.3791/2502.

1.3 Heart Rate Variability as a Window into Autonomic Nervous System Dysfunction

HRV serves as the primary, non-invasive method in this dissertation to quantify ANS (re)activity as part of our theoretical framework. HRV reflects the subtle, beat-to-beat variations in heart rate that arise from the dynamic interplay between PNS and sympathetic nervous system (SNS) inputs on the heart's pacemaker (Shaffer & Ginsberg, 2017; Task Force, 1996). Methodologically, HRV is analyzed through complementary approaches that yield parameters with distinct physiological interpretations (see Tables 1-3 in the Appendix for a full summary):

- Time-domain parameters (e.g., standard deviation of the interbeat intervals of normal sinus beats (SDNN), root mean square of successive differences (RMSSD)), quantify the magnitude of variability within the interbeat interval time series, with certain parameters (like RMSSD) being particularly sensitive to vagal (PNS) modulation.
- Frequency-domain parameters separate the variability into component frequencies. High-frequency power (HF) is a well-established index of vagal (PNS) activity, while low-frequency power (LF) and the LF/HF ratio are more complex, reflecting a mixture of ANS influences and baroreflex activity, often interpreted as markers of sympathetic modulation or sympathovagal balance under controlled conditions (Billman, 2013).
- Nonlinear parameters (e.g., from Poincaré plots or detrended fluctuation analysis (DFA)) capture the complexity and fractal-like properties of heart rate dynamics, with reductions often associated with pathological states, stress, or reduced system adaptability (Sassi et al., 2015).

While HRV provides sensitive parameters of autonomic modulation, its interpretation requires nuance**.** Parameters do not measure absolute PNS or SNS outflow, and their meaning can be context-dependent. Moreover, the systematic correlation of specific parameter alterations to experimental interventions continues to be a prominent area of research (Agorastos et al., 2023). Nonetheless, HRV does something useful: it allows objective assessment of the functional state of the ANS**.** That is why, in this dissertation, it is the chosen biomarker to operationalize and test the proposed associations between ANS (re)activity and MS-related fatigue.

1.4 Limitations of the Sickness Behavior Model and the Need for an Extended Conceptualization

The sickness behavior model (Hanken et al., 2014) provides a framework for the development of MS-related fatigue via neuroimmune signaling. However, it leaves certain questions unresolved as to why fatigue becomes a chronic, treatment-resistant condition. The following observations highlight several limitations and point toward the need for an extended conceptualization.

First, there is a disconnect between systemic inflammation control and the persistence of fatigue. DMTs effectively reduce relapse rates and inflammatory activity visible on magnetic resonance imaging; however, their impact on fatigue is generally limited, and when effects do appear, they are inconsistent (Broch et al., 2023; Elkhooly et al., 2023). This suggests that peripheral inflammation may be necessary to initiate fatigue, but once fatigue takes hold, other mechanisms become dominant. So, fatigue is maintained even when the peripheral drivers are suppressed.

There are exceptions worth noting. There is some evidence that highly effective CNS-penetrant or B-cell depleting therapies, natalizumab and ocrelizumab, for instance, can improve fatigue in a subset of patients (Broch et al., 2023; Glanz et al., 2021). The likely mechanism is that these medications more effectively suppress central neuroinflammation and its downstream effects on neuroimmune signaling. If fatigue is driven in part by inflammatory processes, then suppressing those processes should help. But even here, the benefit is often incomplete. A recurrence of fatigue toward the end of a treatment cycle, a "wearing-off" effect, is reported with both medications (Broch et al., 2023; Glanz et al., 2021). This pattern is consistent with the sickness behavior model (Hanken et al., 2014): As the treatment’s effect wanes and low-grade neuroinflammation begins to re-emerge, the pathway to fatigue reactivates. The model would predict exactly this kind of fluctuation.

To reconcile why immunomodulation only alleviates fatigue in some circumstances, it is argued that relevant immune activity often becomes compartmentalized within the CNS, in the meninges, in perivascular spaces, where it is partially shielded from peripheral detection and, crucially, from the effects of systemic treatments (Haase & Linker, 2021; Lassmann et al., 2012). This would explain why peripheral markers of inflammation often fail to correlate with

fatigue, and why even highly effective therapies may not reach the relevant sites of action. This kind of clinically silent inflammation is a recognized feature of MS pathology that can independently contribute to neurodegeneration and symptomatology (Magliozzi et al., 2007; Zrzavy et al., 2017).

Second, the model does not address the cumulative burden of co-occurring MS symptoms, a concept I term "fatigue debt," which may perpetuate fatigue beyond the initial inflammatory trigger**.** Physical disability (as measured by the Expanded Disability Status Scale, EDSS; Kurtzke, 1983) correlates weakly with fatigue. Still, that is likely because the EDSS doesn't include symptoms that are psychologically and emotionally draining and persistent, such as pain, cognitive impairment, mood disorders, or sleep problems. When broader symptom burden is assessed, it consistently predicts altered ANS activity, as indicated by diminished HRV (Pilloni et al., 2024). Furthermore, symptoms such as kinesiophobia (fear of movement) are associated with fatigue severity too (Tekin et al., 2025). What this suggests is that symptoms accumulate. They create a kind of debt, and that debt may sustain fatigue even in the absence of active inflammation.

Third, the model does not account for the long-term consequences of chronic ANS dysfunction, particularly allostatic overload. When stress systems (SNS and hypothalamic-pituitary-adrenal axis) stay activated for prolonged periods without adequate recovery, the cumulative wear and tear tends to disrupt normal physiological balance leading up to allostatic overload (McEwen, 1998; Sterling & Eyer, 1988). In MS, this kind of overload may lock fatigue in place as a kind of self-reinforcing attractor state, one that persists even in the absence of active inflammation. Empirical support for this comes from studies like Sander et al. (2020), where a progressive muscle relaxation (PMR) session increased short-term HRV (efferent PNS tone) but did not reduce state fatigue. This suggests that while the acute regulatory mechanism is still accessible, something else is keeping fatigue locked in. Allostatic overload may play a role in that. Furthermore, in the study by Sander et al. (2020), PMR improved HRV solely in patients with weak-to-moderate fatigue, rather than in those with severe fatigue. That suggests severe fatigue may represent a different physiological signature altogether, a state where the ANS is so compromised that it barely responds to intervention.

Therefore, while the sickness behavior model explains how fatigue develops, it requires extension to fully explain why it becomes chronic. It is hereby proposed that MS-related fatigue is maintained by two secondary processes: (1) the cumulative burden of symptoms (hereby termed as "fatigue debt") and (2) the physiological cost of chronic adaptation (allostatic load). It is argued hereby that these secondary processes can perpetuate fatigue even when primary inflammatory drivers are partially controlled.

This proposed extended framework has effects. If fatigue debt contributes to persistence, then treating cumulative symptom burden through pain management, sleep therapy, depression treatment, and minimizing overall treatment burden (Singer et al., 2024) may mitigate fatigue severity. Measuring this debt with composite symptom scores and comparing it to HRV could help us better understand this concept.

In summary, the empirical findings of this dissertation show consistent links between reduced PNS activity, impaired autonomic adaptability, and trait fatigue. These findings align with, and extend the sickness behavior model and its extension. They describe ANS dysfunction as related to MS-related fatigue and reveal its statistically non-significant responsiveness to brief biopsychological interventions.

1.5 Toward an Extended Conceptualization of MS-Related Fatigue: Fatigue Debt and Allostatic Overload

Building on the limitations outlined in the previous section, this dissertation proposes an extended framework to explain the persistence of MS-related fatigue (see Figure 4). With this framework, thus, I aim not to reject the sickness behavior model but rather to extend it. The original model remains foundational: it has reshaped how the field understands MS-related fatigue, and its premises are retained here. The extension lies in the incorporation of secondary, maintaining factors that, I argue, may help explain why fatigue becomes chronic rather than resolving when inflammation subsides.

The primary neuroimmune-autonomic pathway remains central: persistent immune activation and cytokine signaling are fundamental to fatigue development through alterations in arousal, motivation, and interoceptive processing (Dantzer, 2018; Harrison et al., 2009). Furthermore, the empirical work of this dissertation supports the model's autonomic

component: MS-related fatigue is consistently linked to reduced HRV parameters of PNS activity and to reduced autonomic adaptability, the system's capacity to respond flexibly to challenges.

However, a primary neuroimmune-autonomic dysfunction cannot, in isolation, fully explain the observed clinical persistence and the variable effects of treatment. The interventions tested in this dissertation modulated ANS activity but led to complex outcomes: PMR alleviated state fatigue with intermediate HRV effects, DB enhanced certain SNS-related parameters, and self-alert training (SAT) suppressed overall variability. Most notably, reduced HRV adaptability (reduced SDNN change during the vigilance task) was the parameter most consistently correlated with higher trait fatigue (Garis et al., *manuscript submitted for publication*; Sander et al., 2020). What these patterns suggest is that ANS activity can be modulated, and reduced adaptability may serve as a physiological marker of MS-related fatigue.

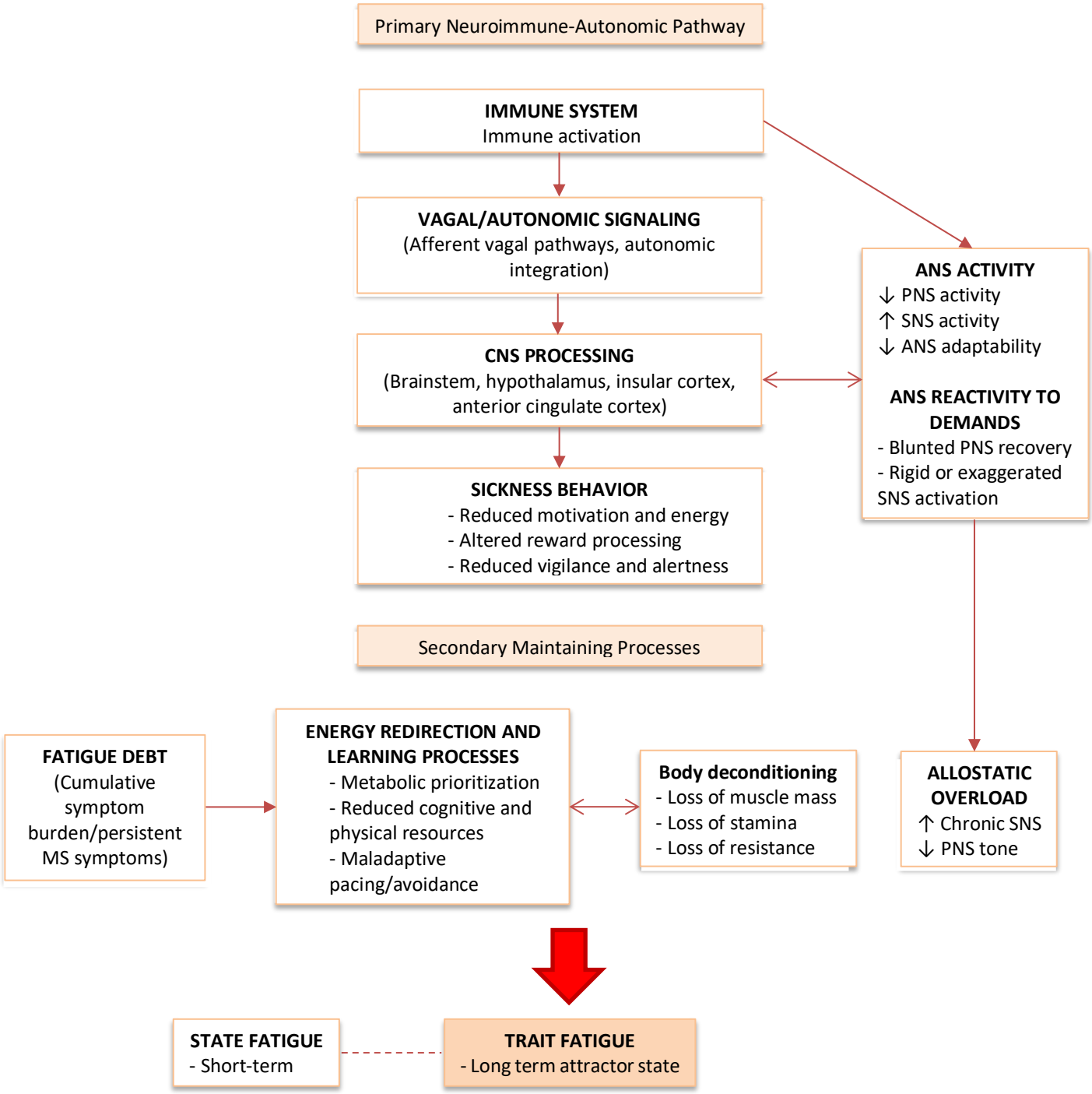
The secondary maintaining processes proposed in this framework involve cumulative symptom burden (fatigue debt) and its physiological consequences. That is, in a state of fatigue, which is intensified by cumulative burden of other persistent MS symptoms, the brain may prioritize metabolic resources away from non-essential cognitive and physical activities toward maintenance and repair, a state of metabolic prioritization consistent with sickness behavior (Dantzer et al., 2008). This subjective experience, in turn, is one of diminished cognitive and physical resources, that makes energy conservation feel like the only viable response (Koolhaas et al., 2011). In response, individuals often adopt behavioral strategies such as maladaptive pacing (overexertion followed by prolonged rest) or avoidance of activities viewed as fatiguing (Tekin et al., 2025). These behaviors make sense in context, and to the individual they may feel protective. However, what begins as a reasonable response to limited energy reserves can, over time, harden into a maladaptive pattern. The result is a cycle of physical and cognitive deconditioning (Latimer-Cheung et al., 2013). Over time, this cycle results in physiological losses, with reduction in muscle mass (atrophy), decreased cardiovascular stamina, and reduced overall resistance to physical and mental exertion (Motl et al., 2017). These effects further exacerbate fatigue debt and ANS strain (Krbot Skorić et al., 2019; Pilloni et al., 2024), establishing a self-reinforcing cycle that intensifies MS-related fatigue.

Therefore, in the proposed expanded conceptualization, MS-related fatigue arises from two interacting sources:

- A primary neuroimmune-autonomic pathway: Fatigue is initiated and perpetuated by inflammatory signaling and marked by measurable ANS dysfunction (e.g., lower HRV, poor ANS adaptability).
- Secondary maintaining processes: Fatigue is further perpetuated by the cumulative burden of co-occurring MS symptoms (fatigue debt) and the physiological cost of chronic ANS dysfunction (allostatic load).

Figure 4

Proposed illustrative integration on the pathophysiology of MS-related trait fatigue



Note. ANS = autonomic nervous system; PNS = parasympathetic nervous system; SNS = sympathetic nervous system.

1.6 Contributions of this Dissertation

This dissertation examines and refines existing ANS-based frameworks of MS-related fatigue. Furthermore, it introduces and argues for the conceptual integration of secondary

maintaining processes, such as fatigue debt and allostatic load, to better explain the chronicity of MS-related fatigue. The work primarily contributes by (a) objectifying fatigue-related ANS dysfunction using HRV; (b) differentiating autonomic correlates of state and trait fatigue; and (c) identifying reduced ANS adaptability as a physiological marker associated with trait fatigue.

The empirical contributions include: (a) an analysis of DB-focused yoga interventions and their moderate effects on fatigue, likely mediated via enhanced PNS tone (Garis et al., 2021); (b) a narrative review consolidating evidence for reduced HRV, particularly PNS-related parameters, as a correlate of fatigue severity (Garis et al., 2022); (c) a single-blind randomized controlled trial comparing short-term effects of PMR and DB after a vigilance task, showing state fatigue reduction with PMR but variable HRV modulation across both (Garis et al., 2023); and (d) a secondary analysis of linear and nonlinear HRV data from two trials, identifying separate PNS and SNS factors, differential HRV responses to interventions, and reduced SDNN change during the vigilance task as a predictor of trait fatigue (Garis et al., *manuscript submitted for publication*). In the latter study, none of the biopsychological interventions led to a significant reduction in state fatigue. Together, these studies demonstrate that biopsychological interventions can modulate ANS activity in distinct ways without consistently decreasing state fatigue, and show reduced PNS activity and autonomic adaptability as a consistent correlate of trait fatigue, without claiming to establish a novel explanatory model.

2. Empirical Studies

This dissertation brings together four studies examining how ANS function relates to MS-related fatigue. They are organized to show a conceptual progression: first summarizing the empirical and theoretical foundation, then testing interventions, and finally examining HRV parameters and predictors of MS-related fatigue across pooled data of two studies.

This dissertation builds on the sickness behavior model (Hanken et al., 2014) and its autonomic extension (Sander et al., 2020). The studies presented here collectively point to something fairly consistent: patients with MS-related fatigue tend to present with ANS dysfunction, specifically lower PNS tone and reduced ANS adaptability. At the same time, the findings also highlight a problem with current models. Even when interventions succeed in

shifting ANS activity in the short term, this does not translate into fatigue relief. This discrepancy points to the influence of likely secondary maintaining factors, such as "fatigue debt," the accumulated toll of dealing with MS symptoms on a regular basis: pain, cognitive deficits, mood disturbances, and sleep issues. Also, allostatic overload, which is the chronic wear on physiological systems from prolonged stress and dysregulation, leading to entrenched fatigue cycles. Together, these may help explain why fatigue becomes so persistent and resistant to treatment, extending the model beyond initiation to address chronicity. Each study is summarized below, with a note on how it contributes to the proposed extended framework.

2.1 Laying the Groundwork: ANS Dysfunction and Intervention Potential

2.1.1 HRV and MS-Related Fatigue (Garis et al., 2022)

This narrative review synthesized 45 studies on HRV and fatigue in MS. Patients with fatigue consistently showed reduced PNS activity and altered SNS patterns, supporting the view that ANS dysfunction is associated with MS-related fatigue. Recent meta-analyses reinforce these patterns; for instance, a 2025 systematic review and meta-analysis (Mirmosayyeb et al.) found significantly lower time-domain measures like RMSSD and the percentage of normal R-R intervals that differ by 50 msec (pNN50), as well as frequency-domain and low frequency power in patients with MS compared to healthy controls, indicating sympathetic dominance and ANS dysfunction. Another 2025 review (Penfold et al.) across medical populations, including four MS studies, consistently linked decreased HRV (e.g., reduced pNN50, SDNN, HF) to higher fatigue severity. The review also pointed out that changes in HRV typically persist independently of disease duration or disability level, which suggests that these originate from functional rather than solely structural causes.

Contribution to the proposed extension: This foundational work establishes ANS dysfunction as a primary driver in the sickness behavior model, linking neuroimmune signaling to the onset of fatigue via vagal pathways. Yet, by revealing that HRV impairments endure despite inflammation control, and drawing on recent evidence showing inconsistent correlations with acute inflammatory markers, it exposes the model's gap in addressing long-term persistence. This justifies incorporating fatigue debt and allostatic overload, leading up to a possible explanation of onset but also the self-perpetuating cycles of chronic fatigue. Also, while the

review itself did not test interventions or mechanisms of chronicity, it provides the foundation for the extended model by identifying impaired ANS adaptability as a potential correlate of MS-related fatigue.

2.1.2 Deep Breathing-Focused Yoga in MS (Garis et al., 2021)

This review summarized studies examining yoga interventions centered on DB for patients with MS. What emerged was a fairly consistent pattern: regular practice tended to reduce fatigue, and the assumption has been that this happens through enhanced PNS activity. However, the effects were variable, reflecting differences in study quality and participant characteristics.

Contribution to the proposed extension: Demonstrating that ANS-targeted practices like DB-yoga likely enhance PNS activity but struggle to produce consistent fatigue relief, this study highlights that physiological mechanisms are insufficient for explaining the persistence of MS-related fatigue.

2.2 Testing ANS Reactivity: Acute Effects of Interventions

2.2.1 Progressive Muscle Relaxation vs. Deep Breathing (Garis et al., 2023)

This randomized controlled trial (RCT) was designed to compare whether different relaxation techniques might affect fatigue differently. 34 patients with MS completed a vigilance task, a mentally draining task that generally induces fatigue, and then did either PMR or DB. PMR led to significant reductions in state fatigue, whereas DB did not, although both interventions altered HRV. The vigilance task itself reduced PNS-related HRV, indicating reduced autonomic adaptability under cognitive load.

Contribution to the proposed extension: By showing that ANS reactivity can be elicited (e.g., via PMR boosting PNS tone) yet fails to reliably alleviate state fatigue induced by the vigilance task, this study underscores the original model's strength in explaining acute sickness-like responses but its weakness in chronic maintenance. It supports the extension by illustrating how primary ANS dysfunction initiates fatigue, while secondary factors may perpetuate it.

2.3 Integrating Evidence: HRV Structure and Predicting Trait Fatigue

2.3.1 Secondary Pooled Analysis (Garis et al., *manuscript submitted for publication*)

HRV data from two RCTs (N = 78) were pooled to examine HRV structure, responses to three interventions (DB, PMR, SAT), and relationships with fatigue. Exploratory factor analysis revealed two clear factors representing PNS and SNS activity. SAT decreased HRV parameters loaded into both factors, DB increased parameters related to SNS activity, and PMR affected both factors. Importantly, reduced SDNN change during the vigilance task predicted higher trait fatigue, whereas state fatigue was not associated with HRV. These findings highlighted HRV as a sensitive marker of coordinated PNS-SNS activity and reduced autonomic adaptability in MS-related trait fatigue. While these results do not directly test secondary mechanisms, they provide empirical support for functional ANS dysfunction as a contributor to persistent fatigue.

Contribution to the proposed extension: This integrative analysis differentiates state from trait fatigue, while revealing that reduced adaptability (e.g., reduced SDNN responses) predicts chronicity. By showing intervention effects on HRV without significant fatigue reduction, and tying into recent studies linking HRV to mediated distress, it directly informs the extension: primary dysfunction starts the process, but secondary processes likely maintain it.

3. Discussion

In this dissertation, it is proposed that while immune activation in MS likely initiates and perpetuates fatigue via intact vagal signaling, persistent MS-related fatigue is further associated with functional ANS dysfunction and impaired autonomic adaptability, rather than with structural autonomic lesions. Reduced PNS activity and ANS adaptability were consistently linked to trait fatigue. In the clinical studies, biopsychological interventions, like PMR, DB, and SAT, differentially modulated ANS activity, yet these changes did not translate into reduced state fatigue. This limited effectiveness motivated the introduction of secondary mechanisms contributing to fatigue persistence. These include cumulative symptom burden (e.g., from pain, sleep problems, and low mood) and allostatic overload. Such mechanisms are best understood as secondary maintaining processes rather than primary causes of fatigue. This framing aligns with existing neuroimmune models while extending them.

Building on this, a study has highlighted the role of continuous wearable monitoring for measuring real-time HRV changes, demonstrating associations between cardiac ANS dysfunction, poor sleep quality, and increased MS-related fatigue (Gholami et al., 2020).

3.1 Integration of Empirical Findings

To test this framework, I examined three biopsychological interventions designed to influence ANS function: PMR, DB, and SAT. PMR led to both PNS and SNS changes in HRV, DB increased certain SNS parameters without reducing fatigue, and SAT suppressed overall HRV variability, consistent with heightened arousal. None of the biopsychological interventions significantly reduced state fatigue. Decreased HRV adaptability, especially the SDNN response during a vigilance task, was the parameter most strongly associated with higher trait fatigue (Garis et al., *manuscript submitted for publication*). This pattern indicates that while interventions can transiently modulate ANS activity, state fatigue is not significantly alleviated. These results underscore that interventions targeting efferent vagal pathways alone are unlikely to fully reverse trait fatigue, emphasizing the potential role of secondary mechanisms, such as fatigue debt and allostatic overload.

3.2 Theoretical Implications

These findings support a nuanced extension of the sickness behavior model. While immune activation and intact vagal afferent signaling likely initiate MS-related fatigue, trait fatigue seems to be maintained by limitations in ANS responsiveness and adaptability. By integrating fatigue debt and allostatic overload into this model, it can be explained why fatigue often persists despite controlling inflammation or acutely modulating ANS activity. This suggests that chronic MS-related fatigue arises from a combination of initiating neuroimmune processes and secondary, maintaining physiological constraints.

3.3 Clinical Implications

What the results suggest in clinical terms is that brief interventions aimed at shifting ANS activity, whether PMR, DB, or SAT, may not be enough to alleviate state fatigue, and are unlikely to fully resolve the overlapping trait fatigue. If that is the case, then relying on these approaches as treatments is probably unrealistic. Instead, effective management may require strategies that reduce cumulative symptom burden, improve physiological recovery, and address maladaptive behavioral patterns that can accidentally reinforce the fatigue cycle.

There is also a question of fit. Not every intervention will work for every patient. A patient with MS with moderate-to-severe cardiovagal impairment may respond less to DB or similar exercises. Continuous HRV monitoring could help clinicians tailor interventions to individual autonomic profiles. The bottom line is, that managing fatigue effectively may require a combination of interventions that target ANS function directly, and broader strategies that address the web of symptoms and behavior fatigue is tangled up in.

3.4 Limitations

The investigations conducted in this dissertation contain several limitations that warrant consideration.

State vs. Trait Fatigue Assessment

A primary limitation of this dissertation is the reliance on state fatigue assessments, such as visual analog scales before and after the vigilance task, and short-term ANS modulation via interventions like PMR, DB, and SAT, to inform conclusions about MS-related fatigue. The latter most closely relates to trait fatigue than to state fatigue. As noted in the introduction, trait fatigue persists over weeks or months and is typically assessed via questionnaires such as the MFIS or FSMC, whereas state fatigue is transient and task-dependent (Boksem & Tops, 2008; Enoka et al., 2021). Though correlated, they stem from distinct mechanisms: trait fatigue largely encompassing what we refer to as MS-related fatigue is linked to enduring ANS dysfunction, state fatigue to acute autonomic decrements (Garis et al., 2022; Garis et al., *manuscript submitted for publication*). Our paradigms captured acute ANS reactivity but provided limited insight into long-term effects or overlap. It is also worth acknowledging that it is not known whether the reductions in state fatigue observed in the above-mentioned studies would ever translate into lasting changes in MS-related fatigue. That gap, between temporary relief of fatigue and sustained improvement, remains unexamined.

Sample Size, Clinical Homogeneity, and Generalizability

All of this needs to be considered in light of the sample limitations. The studies here relied on relatively small groups of participants, which constrains statistical power and, more importantly, limits generalization of findings. Most participants had relapsing-remitting MS and, in the interventional trials, presented with severe fatigue. This profile may not fully represent patients with progressive MS disease courses or those with milder fatigue,

potentially biasing the observed HRV-fatigue associations toward more severely affected individuals.

Measurement of HRV

There is also the limitation of how we measured ANS function. In Garis et al. (2023), HRV was derived from a pulsimeter. While practical, this method may offer less precise data than electrocardiography (ECG) recordings (Shaffer & Ginsberg, 2017; Task Force, 1996).

Baseline Cardiovagal Impairment in Participants

Although the biopsychological interventions modulated ANS activity, only PMR produced a significant reduction in state fatigue, while DB did not (Garis et al., 2023). That is, patients with moderate-to-severe cardiovagal impairment are physiologically limited in their ability to achieve the acute vagal enhancement that DB exercises produce in healthy individuals or in MS patients with preserved pathways (Lehrer & Gevirtz, 2014), potentially being a factor into the differential efficacy observed.

3.5 Future Directions

The findings and limitations of the present dissertation delineate priority areas for subsequent investigation. First, future research should prioritize using longitudinal designs that assess both state and trait fatigue alongside ANS activity over weeks or months. That could help reveal if short-term interventions like PMR, DB, and SAT actually lead to lasting reductions in MS-related fatigue, by clarifying the overlap between acute reactivity (state fatigue) and persistent dysfunction (trait fatigue). Second, substantially larger and more heterogeneous cohorts are required, to encompass the full spectrum of MS clinical phenotypes, disease stages, and fatigue severity. Larger and more diverse samples would provide better statistical power, enable subgroup analyses, and improve the generalizability of HRV and fatigue associations. Third, HRV should ideally be measured using ECG recordings, the reference standard, preferably through validated wearable devices that allow for continuous monitoring in everyday life (Shaffer & Ginsberg, 2017). This would give a more ecologically valid picture of daily ANS fluctuations and their relation to fatigue.

Fourth, given how persistent MS-related fatigue is, broader and longer-duration interventions (at least 12-24 weeks) may be needed to determine whether sustained practice can genuinely restore ANS balance and reduce fatigue (Lehrer et al., 2020; Lehrer & Gevirtz, 2014). Lastly,

given conceptual overlaps with established constructs such as depressive symptomatology and sleep disturbance, future studies should test whether cumulative symptom burden correlates with fatigue severity by examining associations with validated measures such as the Beck Depression Inventory and the Pittsburgh Sleep Quality Index. Although such data were available in the studies included in this dissertation, these analyses were not conducted and therefore constitute future work.

Furthermore, integrating the fatigue debt concept into clinical practice could be helpful in decreasing a relevant portion of fatigue burden. This would involve systematically assessing and managing the cumulative burden of co-occurring MS symptoms, while also taking into account that many of the current first-line symptomatic medications for MS such as amitriptyline, baclofen, gabapentin, pregabalin, tetrazepam and tizanidine can themselves induce tiredness as a side effect (Henze et al., 2006).

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5. Declaration for Publication-Based Dissertation

I hereby confirm, that Guadalupe Garis, born 25th of May 1992 in Buenos Aires (Argentina), contributed to the aforementioned studies as stated below:

Article reference:

Garis, G., Hildebrandt, H., & Hanken, K. (2021). Yoga als Möglichkeit zur Reduktion von Fatigue bei Multipler Sklerose. *Neurologie & Rehabilitation, 27*(1), 49–55. https://doi.org/10.14624/nr2101006

Author contribution:

Ms. Garis (doctoral candidate) analysed and interpreted acquired data, created all figures and tables and wrote the review. Apl. Prof. Dr. phil. Helmut Hildebrandt (main supervisor) guided the whole process and participated in data search, compiling data, and writing of the final paper. Dr. rer. nat. Hanken participated in writing and reviewing the final paper.

Article reference:

Garis, G., Haupts, M., Duning, T., & Hildebrandt, H. (2022). Heart rate variability and fatigue in MS: Two parallel pathways representing disseminated inflammatory processes? *Neurological Sciences, 44*(1), 83–98. https://doi.org/10.1007/s10072-022-06385-1

Author contributions:

Guadalupe Garis (doctoral candidate) searched for and compiled all data, partly re-using data generated in the context of her earlier Master's thesis work. She created some of the figures and tables and wrote the final paper. Apl. Prof. Dr. phil. Helmut Hildebrandt (main supervisor) formulated the research question, created one figure, and contributed to writing the final paper. Prof. Dr. Michael Haupts participated in writing and critically reviewing the final paper. Prof. Dr. med. Thomas Duning critically reviewed the final paper.

Article reference:

Garis, G., Dettmers, C., Hildebrandt, A., Duning, T., & Hildebrandt, H. (2023). Comparing two relaxation procedures to ease fatigue in multiple sclerosis: a single-blind randomized controlled trial. *Neurological Sciences*, (11), 4087–4098. https://doi.org/10.1007/s10072-023-07042-x

Author contributions:

Ms. Garis (doctoral candidate) conducted the data acquisition, conducted data analysis and manuscript writing. Apl. Prof. Dr. phil. Helmut Hildebrandt (main supervisor) helped on planning the study, conducting the statistical analysis, data analysis and manuscript writing. Prof. Dr. med. Christian Dettmers helped on writing and reviewing the manuscript. Prof. Dr. med. Thomas Duning reviewed the final paper.

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Author contributions:

Ms. Garis (doctoral candidate) helped on formulating the research question, participated in the study design and planning, conducted the data acquisition, data analysis, created figures and tables and wrote the final paper. Apl. Prof. Dr. phil. Helmut Hildebrandt (main supervisor) formulated the research question, helped on planning the study design, data analysis and writing of the final paper. Prof. Dr. Andrea Hildebrandt (second supervisor) formulated the research question, helped on planning the study design, conducting statistical analyses, data analysis and writing of the final paper. Dr. rer. nat. Carina Sander-Sandersfeld participated in reviewing the final paper.

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Date apl. Prof. Dr. phil. Helmut Hildebrandt
(main supervisor)

6. Appendix

6.1 Table 1. Time-Domain HRV Parameters

Parameter	Description	Primary physiological reflection	Main association
SDNN	Standard deviation of all NN intervals	Overall HRV and long-term autonomic adaptability	Predominantly PNS, reflects general autonomic adaptability
RMSSD	Root mean square of successive differences between NN intervals	Short-term beat-to-beat variability, highly sensitive to vagal efferent activity	Predominantly PNS

Note. HRV = heart rate variability; PNS = parasympathetic nervous system; SNS = sympathetic nervous system. Derived from Sammito and Böckelmann (2015), Shaffer and Ginsberg (2017), and the Task Force (1996).

6.2 Table 2. Frequency-Domain HRV Parameters

Parameter	Description	Frequency band	Primary physiological reflection	Main association
HF	High-frequency power	0.15-0.40 Hz	Respiratory sinus arrhythmia, direct vagal (PNS) modulation of HR	PNS activity
LF	Low-frequency power	0.04-0.15 Hz	Includes both PNS and SNS influences, but predominantly SNS during stress/cognitive load	Predominantly SNS under stress; mixed at rest
LF/HF	Ratio of low-frequency to high-frequency power	-	Sympathovagal balance	Higher values indicate SNS dominance

Note. HR = heart rate; Hz = Hertz; PNS = parasympathetic nervous system; SNS = sympathetic nervous system. Derived from Sammito and Böckelmann (2015), Shaffer and Ginsberg (2017), and the Task Force (1996).

6.3 Table 3. Nonlinear HRV Parameters

Parameter	Description	Primary physiological reflection	Main association
SD1	Short-term variability (perpendicular spread in Poincaré plot)	Instantaneous beat-to-beat variability, predominantly vagally mediated	PNS
SD2	Long-term variability (spread along line of identity in Poincaré plot)	Longer-term HRV components, reflects both PNS and SNS influences	Primarily PNS at rest; mixed under stress
SD1/SD2	Ratio of short-term to long-term variability	Overall cardiovascular adaptability and sympathovagal balance	PNS-influenced (lower ratio = better adaptability)
DFA α1	Short-term detrended fluctuation analysis scaling exponent (4-16 beats)	Short-term fractal correlations in heart rate dynamics	SNS activation/loss of complexity under stress
DFA α2	Long-term detrended fluctuation analysis scaling exponent (>16 beats)	Long-term fractal correlations	SNS influence/reduced complexity
ApEn	Approximate entropy	Regularity and unpredictability of NN interval fluctuations	Higher values = greater complexity, generally reduced under SNS dominance
SampEn	Sample entropy	Regularity and complexity of fluctuations (more robust than ApEn)	Higher values = greater complexity, generally reduced under SNS dominance/stress

Note. HRV = heart rate variability; PNS = parasympathetic nervous system; SNS = sympathetic nervous system. Derived from Sammito and Böckelmann (2015), Shaffer and Ginsberg (2017), and the Task Force (1996).

6.4 Study I

Full bibliographic reference:

Garis, G., Hildebrandt, H., & Hanken, K. (2021). Yoga als Möglichkeit zur Reduktion von Fatigue bei Multipler Sklerose. *Neurologie & Rehabilitation, 27*(1), 49–55. https://doi.org/10.14624/nr2101006

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Abstract

Background: The causes of fatigue in patients with multiple sclerosis (MS) are still not fully understood. In addition to immunological and central nervous system mechanisms, the literature frequently discusses an association between fatigue and autonomic dysfunction, particularly reduced parasympathetic activity. Yoga practices, especially those involving breathing exercises, appear to influence the interplay between the sympathetic and parasympathetic branches of the autonomic nervous system and may therefore have a positive effect on fatigue in people with MS. The aim of this work is to identify and present existing studies that have examined the effect of yoga on fatigue in MS patients.

Methods: A systematic literature search was conducted using several online databases. All studies reporting an effect of yoga on fatigue in MS patients were included.

Results: A total of 14 studies were identified, including ten randomized controlled trials, two clinical studies, one pilot study, and one quasi-experimental study. 11 of the 14 studies demonstrate a positive effect of regular yoga practice on fatigue in MS patients. Yoga forms that place a particular emphasis on breathing exercises appear to be especially effective in improving subjective fatigue.

Discussion: In addition to physical exercise programs, yoga appears to be a promising alternative treatment option for various MS symptoms, including fatigue. Yoga should be recommended to patients suffering from fatigue as a possible complement to standard treatments.

Keywords: multiple sclerosis, fatigue, yoga, breathing exercises, parasympathetic nervous system, heart rate variability.

Introduction

In multiple sclerosis (MS) patients, fatigue is rated as one of the most common and most disabling symptoms [22]. Fatigue is often defined as a subjective feeling of lack of physical and/or mental energy [22] that severely interferes with a patient's daily activities. Fatigue is considered to be one of the main causes of impaired quality of life among MS patients, independent of depression or disability [12]. Fatigue also imposes significant socioeconomic consequences, including loss of work hours and in some instances, loss of employment [3].

The Parasympathetic Nervous System and Fatigue Recovery

The development of fatigue in MS has been regarded as a consequence of cardiovascular autonomic disturbances [23]. This is based on the assumption that subjective complaints in patients with exhaustion, dizziness, weakness, and their relationship to environmental parameters such as warm temperature, physical activity, or digestion are similar to signs and symptoms frequently reported by patients suffering from fatigue. Recently, an impairment of the sympathovagal balance was found to result in feelings of fatigue [19]. According to a spectral analysis of heart rate parameters this impairment was identified as a reduction of the vagal activity, which was described to occur earlier in MS patients complaining of fatigue [19]. This influence of the autonomic nervous system (ANS) on fatigue may offer an opportunity for treatment of MS patients. If a person can manipulate the functioning of the ANS, this may affect fatigue levels.

Stimulating the Parasympathetic Nervous System through Deep Breathing

Deep breathing exercises seem to boost a dominance of the parasympathetic autonomic system with respect to the sympathetic one, mediated by the vagal activity [6, 32]. Greater vagus nerve activity thus produces greater "cardiac vagal tone" or parasympathetic activity on the heart [5]. It has been found that in order to achieve a shift towards parasympathetic dominance, prolonged practice of deep breathing is necessary, as was observed in healthy participants who regularly practiced deep breathing exercises for 3 months [26]. That is, stimulation of the parasympathetic nervous system by deep breathing may produce body autonomic activity characteristic of relaxation.

The Practice of Yoga and Deep Breathing

One mind-body therapy that has received considerable attention in the field of science is yoga. Indeed, yoga is gaining increased popularity as a therapeutic method both in North America and Europe. Yoga traditionally is a complex intervention that comprises physical activity and meditation but is also intimately linked with the practice of breathing. In yoga, a series of breathing techniques called "Prana-Yama" are aimed at directly and consciously regulating one or more parameters of respiration (e.g., frequency, deepness, inspiration/expiration ratio).

Current research has provided evidence of the benefits and positive outcomes associated with the practice of yoga among individuals with MS, including reduced fatigue [29]. Considering the beneficial effects of deep breathing, one can see its potential as a mediator between yoga and reductions in fatigue. So, an alternative and supporting practice like yoga may overcome fatigue caused by MS, without causing side effects that medications may lead to.

Method

Search Strategy

International online databases such as PubMed, Scopus, and Google Scholar search engine were used without a publication time limit. Finally, the references of original articles were evaluated to find relevant articles. We used the following Medical Subject Headings (MeSH) keywords with all the possible combinations using "OR" and "AND" in the abovementioned databases: ‘Multiple Sclerosis’, ‘MS’, ‘Yoga’, ‘Breathing’, and ‘Fatigue’. A combined search was done in PubMed database as follows: ("Multiple Sclerosis" [Title/Abstract] OR "MS" [Title/Abstract]) AND ("Yoga" [Title/Abstract] OR "Breathing" [Title/Abstract].

Results

Data according to three pre-prepared tables has been extracted. Tables 1, 2 and 3 integrate a compilation of 14 research studies on yoga for MS (of which 10 were randomized-controlled trials, two were clinical trials, one was a pilot study and one a quasi-experimental study). Table 1 contains the first author’s name and publication year, type of study, country where participants were recruited from, patients’ information (e.g., sex, current treatment

and age), and inclusion criteria. Table 2 contains the first author's name and publication year, sample size of each group, and the total duration of study. Table 3 contains the first author's name and publication year, details of the yoga group, control group, and if applicable, of the exercise group (e.g., type, program length, frequency, duration), focus in yoga practice, fatigue scale used and results post-yoga intervention. 12 studies reported the interventional effect of yoga on fatigue to be positive [1, 2, 8, 13, 14, 15, 16, 17, 18, 24, 25, 27] while two found no such relationship in their study [32, 33]. To assess fatigue, various tools such as Fatigue Severity Scale (FFS), Modified Fatigue Impact Scale (MFIS), Rotten Fatigue Severity Scale, Multidimensional Fatigue Inventory (MFI) and Fatigue-Short Form 8a (adult version v1.0) were used.

Table 1

Reference, study design, origin, sample, and inclusion criteria

Reference	Study design	Origin	Sample	Inclusion criteria
Main author; publication year		Country; recruited from	Sample size (sex: male and female); current treatment; age details	
Ahmadi et al., 2010	A randomised trial	Iran; physiotherapy clinic	21 women; MS disease modifying drugs were allowed; mean age: 34.38 years	MS diagnosed by physician and a self assessed Kurtzke Expanded Disability Status Scale (EDSS) score between 1 and 4 (mild to moderate disability due to MS)
Ahmadi et al., 2013	A randomised trial	Iran; physiotherapy clinic	31 women; disease modifying drugs were allowed; mean age: 36.75 years	MS diagnosed by physician, a self assessed EDSS score between 1 and 4 (mild to moderate disability due to MS), being able to walk on the treadmill at a constant speed for 5 minutes with or without hand support (without human assistance)
Cohen et al., 2017	A pilot study	United States of America; National Multiple Sclerosis Society newsletters, newspaper advertisements, and flyers sent to neurologists and MS support groups	14 women; absence of a control group; age between 34 to 64 years	A neurologist-confirmed diagnosis of any sub-type of MS with moderate disability (as evidenced by a score of 3 to 6 on the self-report of Multiple Sclerosis Disease Severity (SR-MSDS)). Aged 18-64 years, ability to ambulate without the assistance of another person for a minimum of 25 feet (7.62 meters), ability to communicate in English
Garrett et al., 2012	A multi-centre, block randomised, assessor blind, controlled study	Ireland; Multiple Sclerosis Society of Ireland	314 (236 women and 78 men); no steroid treatment; mean age: 50.1 years	MS diagnosed by physician or neurologist, over 18 years of age and needed to score 0, 1 or 2 on the Mobility subscale of the Guys Neurological Disability Rating scale
Guner and Inanici, 2014	A clinical research	Turkey; University Hospitals in Ankara (Turkey)	16: 8 MS patients (intervention group) and 8 healthy subjects (control group) (14 women and 2 men); 3 patients were on Interferon beta 1a; 2 on Glatiramer acetate; 1 on Tizanidine, and 1 on Escilatopram; mean age: 36.15 years	MS patients with clinically definite relapsing remitting MS and whose EDSS ≤ 6.0 or otherwise healthy participants
Hasanpour-Dehkordi, 2016	A randomized clinical trial	Iran; not reported where participants were recruited from	90: 61 MS patients (60 of whom were women and 1 male) and 29 healthy subjects; treatment not specified; mean age: 31.9 years	Definite diagnosis of MS, patients agreed to participate in the study, ability to speak and to move and perform daily activities.
Hogan et al., 2014	An assessor blind, block randomised, and controlled study	Ireland; Multiple Sclerosis Society of Ireland	96 (58 women and 38 men); no steroid treatment in the last 12 weeks; mean age: 54.4 years	People who had their MS diagnosis confirmed by a consultant physician
Kahraman et al., 2017	Uncontrolled clinical trial (feasability study)	Turkey; through posters and brochures	12 (7 women and 5 men); treatment not specified; age beween 30 to 50 years old	Definite diagnosis of MS, relapse-free period of 6 months prior to the study, and willingness to participate in the study or otherwise healthy family members of MS patients
Karbandi et al., 2015	A randomized clinical trial	Iran; from patients referred to the neurology clinic of Mashhad University	57: 28 cases in individual yoga group and 29 in team yoga group; treatment not specified; mean age: 32.2	Diagnosis of MS based on McDonald criteria, the EDSS < 7, no history of recurrence in the last month, and lack of physical and psychological trauma in the recent 6 months. Only relapsing-recovery and secondary progressive MS patients included.
Nejati et al., 2016	A quasi-experimental study	Iran; Tehran MS Society	24 (11 women and 13 men); treatment not specified; age between 20 and 45 years	MS diagnosis by a specialist (neurologist), being in the relapse-suppression stage of the disease, independency on wheelchair, a minimum educational attainment of high school diploma, non-use of psychotropic drugs and psychological treatments throughout the study, and being aged 20-45
Oken et al., 2004	A parallel-group, randomized controlled trial	United States of America; advertisements and newsletters of MS societies and centres	57 (53 women and 4 men; no change in CNS-active medications (e.g., modafinil and antidepressants) during the course of the study; mean age: 49.0 years	A neurologist reviewed medical records for diagnostic criteria for MS; subjects with an EDSS ≤ 6.0
Razazian et al., 2015	A randomized controlled trial	Iran; MS center of the Imam Reza Hospital of Kermanshah (Iran)	54 women; immune pharmacological modulatory treatments; mean age: 33.94	MS diagnosis as ascertained by neurologists, patients' reports, and medical records; female; age between 25-50 years; EDSS < 6; having either primary-progressive secondary-progressive or relapsing-remitting progressive-relapsing MS; stable, regular, and monitored pharmacological immune modulatory treatment of MS
Velikonja et al., 2010	A randomized prospective study	Slovenia; not reported where participants were recruited from	20 (sex unknown); symptomatic and immunomodulatory therapy for MS; age between 26 and 50 years	MS patients with primary or secondary progressive MS type and an EDSS ≤ 6 and EDSS pyramidal functions score (EDSSpyr) > 2
Young et al., 2019	Three-arm randomized controlled proof-of-concept trial.	United States of America; Membership data-base of a fitness facility, physician referrals, flyers, informational mailings, and word of mouth	81 (66 women and 15 men); treatment not specified; age between 18 and 65 years	Self-reported diagnosis of MS, with a Patient Determined Disease Steps (PDDS) score between 0 and 6. The PDDS has a strong correlation (0.8) with the EDSS, ages of 18- 65 years, ability to exercise with arms and/or legs, and physician clearance

Table 2

Reference, sample sizes (yoga group, control group, exercise group), and duration

Reference	Yoga group (n)	Control group (n)	Exercise group (n)	Duration
Main author; publication year	Sample size	Sample size	Sample size	Total duration of study
Ahmadi et al., 2010	11	10	_	8 weeks
Ahmadi et al., 2013	11	10	10	8 weeks
Cohen et al., 2017	14	_	_	8 weeks
Garrett et al., 2012	63	49	Fitness instructor-led exercise: 67; Physiotherapist- led exercise: 63	10 weeks
Guner and Inanici, 2014	8	8	_	12 weeks
Hasanpour-Dehkordi, 2016	30	30	30	12 weeks
Hogan et al., 2014	13	15	83	10 weeks
Kahraman et al., 2017	12	_	_	6 months
Karbandi et al., 2015	57: 28 in individual yoga group and 29 in team yoga group	_	_	6 weeks
Nejati et al., 2016	12	12	_	8 weeks
Oken et al., 2004	22	20	15	6 months
Razazian et al., 2015	18	18	18	8 weeks
Velikonja et al., 2010	10	_	10	10 weeks (participants were asked to attend at least 9 out of 10 sports climbing or yoga sessions)
Young et al., 2019	26	28	27	12 weeks

Table 3

Reference, details (yoga group, control group, exercise group), fatigue scale used, and results

Reference	Yoga intervention	Focus in yoga practice	Exercise intervention	Control group	Fatigue scale used	Results post-intervention
Main author; publication year	Type of yoga; program length; frequency; duration	A higher focus on movement or breath in the yoga practice	Type of exercise intervention; program length; frequency; duration	Control group; (if applicable): program length; frequency; duration		Fatigue-related outcomes
Ahmadi et al., 2010	Hatha yoga; 8 weeks; 3x/weeks; 60– 70 min./session (began with calming music, postures held for 10-20 seconds each, breathing techniques, meditation)	Movement-focused	–	Waitlist control group: usual care	Fatigue Severity Scale (FFS)	Fatigue levels were significantly lower during post-test than pre-test in the yoga group by 38.69% (p= 0.01)
Ahmadi et al., 2013	Hatha yoga; 8 weeks; 3x/week; 60–70 min./session (began with calming music, postures held for 10-20 seconds each, breathing techniques, meditation)	Movement-focused	Treadmill training; 8 weeks; 3x/week; 30 min./session; which began and ended with 10 minutes of muscle stretching	Waitlist control group; usual care	Fatigue Severity Scale (FFS)	Fatigue levels were significantly lower during post-test than pre-test in the treadmill training and yoga groups by 45.08% (p=0.001) and 38.69% (p=0.01), respectively. However, no significant difference between the treadmill training group and yoga practice group (p= 0.99)
Cohen et al., 2017	Yoga; not predefined; 8 weeks; 2x/week; 90 min./session (yoga philosophy, postures, breathing practices, deep relaxation and meditation)	Movement-focused	–	–	Modified Fatigue Impact Scale (MFIS)	Pair-wise comparisons showed that the effects on fatigue were found between baseline and post-intervention (p=.010)
Garrett et al., 2012	Yoga, not predefined; 10 weeks; 1x/week; 1 hour/session (postures held for 30-90 seconds each, breathing exercises, relaxation or "body centering")	Breath-focused	10 weeks; 1x/week; 1 hour/session; 1) Physiotherapist-led exercise (aerobic, resistance); 2) Fitness instructor-led exercise (aerobic and progressive resistance)	Usual care, asked not to change exercise habits	Modified Fatigue Impact Scale (MFIS)	Fatigue levels were signifi- cantly lower during post-test than pre-test on the MFIS total (p<0.01), MFIS physical (p<0.01) and MFIS cognitive (p<0.05) subscales
Guner and Inanici, 2014	Yoga, not predefined; 12 weeks, 2x/week; 1 hour/session (18 yoga poses for 10-30 seconds each, breathing, progressive relaxation and meditation techniques)	Movement-focused	–	Usual care	Fatigue Severity Scale (FSS)	Compared to baseline measurements, fatigue decreased significantly in patients with MS (p=0.012) that atended the yoga program, but has a limitation i.e. small sample size
Hasanpour-Dehkordi, 2016	Yoga; not predefined; 12 weeks; 3x/week; 60-70 min./session	Not specified	Total body workout; 12 weeks, 3x/week; 40 min./session (5-10 minutes for warm-up, 25-30 minutes of exercise (walking), and 5 minutes for cooling down)	Usual care	Rotten Fatigue Severity Scale	Yoga decreased fatigue in MS patients (p<0.05)
Hogan et al., 2014	Yoga, not predefined; 10 weeks; 1x/week; 1 hour/session (postures, breathing, techniques, relaxation)	Breath-focused	10 weeks; 1x/week; 1 hour/session; 1) Individual physiotherapy (aerobic, resistance exercise); 2) Group led physiotherapy (aerobic, resistance exercise	Usual care	Modified Fatigue Impact Scale (MFIS)	The yoga group had significantly less fatigue than the 3 other groups (p=0.034)
Kahraman et al., 2017	Classical Hatha yoga styles; 6 months; minimum 1x/week; 1 hour/session	Movement-focused	–	–	Modified Fatigue Impact Scale (MFIS)	A 6-month yoga program can be helpful to improve fatigue, significant reduction in fatigue level (p<0.05)
Karbandi et al., 2015	Yoga, not predefined; 6 weeks; 2x/day; 10-15 min./session (mild stretching and basic yoga exercises); 1) Individualized yoga: used video CD and booklet with details of the yoga; 2) Group based yoga performed the exercises at the gym	Movement-focused	–	–	Modified Fatigue Impact Scale (MFIS)	In individualized yoga, fatigue in the physical and cognitive subscales in the group of individual were significantly different at 2 timepoints (p=0.011,p=0.017 respectively), but fatigue in the psychosocial level was not statistically significantly different. In group based yoga the perceived fatigue in physical, cognitive and psychosocial levels at 3 timepoints was not significantly different.
Nejati et al., 2016	Hatha yoga; 8 weeks; 1x/week; 120 min./session (postures, breathing techniques, along with mindfulness-based stress reduction program)	Breath-focused	–	Waitlist control group: usual care	Fatigue Severity Scale (FFS)	Yoga led to a reduction of fatigue severity in subjects of the experimental group compared to those in the control group (p<0.001)
Oken et al., 2004	Iyengar yoga; daily home practice was encouraged; 6 months; 1x/week; 90 min./session; (postures, breathing techniques, relaxation)	Breath-focused	Aerobic exercise; daily home practice was encouraged; 6 months; 1x/week; 90 min./session (bicycling on recumbent or stationary bicycles)	Waitlist control group; usual care	Multidimensional Fatigue Inventory (MFI)	The yoga intervention led to a significant reduction on the MFI general fatigue scale (p<0.01), but no significant effect on the other domains of the MFI (physical fatigue, reduced activity, reduced motivation, mental fatigue)
Razazian et al., 2015	Hatha yoga; 8 weeks; 3x/week; 60 min./session (postures, emphasis on breathing for concentration and relaxation, meditation)	Breath-focused	Aquatic training; 8 weeks; 3x/week; 60 min./session (warming up, 10 min. walking, stretching, and gymnastics; 40 min. power endurance activities; strength training; 10 min. cooling down, relaxing, stretching)	Participants met 2-3x/week in the hospital for 60-90 min./session. They were free to talk to physicians, hospital staff and other patients, to complete everyday duties and to participate in occupational therapy	Fatigue Severity Scale (FFS)	The significant time–group interaction showed that fatigue decreased over time in the yoga condition (p=0.000). Post hoc analyses with Bonferroni- Holm corrections for P values revealed a larger effect size on fatigue for yoga than aquatic training and the non-exercise group
Velikonja et al., 2010	Hatha yoga; 10 weeks; 1x/week; duration of session not specified (postures, breathing techniques)	Movement-focused	Sports climbing; 10 weeks; 1x/week; duration of session not specified	–	Modified Fatigue Impact Scale (MFIS)	There was no significant reduction of the impact of fatigue on any of the MFIS subscales in the yoga program
Young et al., 2019	Iyengar approach to Hatha yoga; 12 weeks; 3x/week; 60 min./session (isometric contraction and relaxation techniques)	Movement-focused	Movement-to-music; 12 weeks; 3x/week; 60 min./session (combinations of movement routines structured to target 3 fitness components: strength, cardiorespiratory endurance, and balance)	Waitlist control (participants received 2x/week newsletters that provided educational information on living with MS); usual care	Fatigue-Short Form 8a (adult v1.0)	There was no statistically significant group difference in change in fatigue and post hoc pairwise comparisons revealed no statistically significant difference in fatigue scores between the yoga group and control group

Discussion

The present review was carried out to evaluate the interventional effect of yoga on fatigue in patients with MS. The results indicate that yoga intervention has supportive effects for fatigue when compared with usual care. Particularly, studies that contained a higher focus on breathing in the practice of yoga significantly correlated with decreased fatigue symptoms [13, 16, 24, 25, 27], pointing to the beneficial effect of breathing exercises on MS-fatigue. This review thus asserts the notion that deep breathing is part of a feedback loop that improves vagal tone by stimulating the relaxation response of the parasympathetic nervous system [20]. It's possible therefore by voluntarily changing breathing patterns (i.e., rate, deepness, rate) to change the messages being sent from the body's respiratory system to the brain. Yoga and its breathing techniques may enable us to make a change in our autonomic response. This suggests we can send specific messages to the brain using the language of the body, a language the brain responds to.

The main limitation of this review is the small number of eligible studies, resulting in a limited overall sample size. Another limitation is the insufficient reporting of trial methodology, limiting the interpretability of the results. Because of that, authors of prospect research should improve the reporting of yoga trials and follow commonly accepted reporting guidelines (e.g., CONSORT) [27]. Among the studies, seven reported adequate random sequence generation [8, 15, 18, 24, 25, 27, 34] and three reported allocation concealment [13, 27, 34]. As inadequate allocation concealment has been demonstrated to be the most important source of bias in randomized controlled trials [28], which limits the interpretability of results. Future studies should thus ensure rigorous methodology, mainly adequate randomization and allocation concealment.

Importantly, most studies involved a synergistic combination of different components of the yoga practice (movement sequences, stretching, breathing, meditation exercises), without disclosing a distinct emphasis on one component or the other. More standardization is therefore necessary to draw clearer conclusions of the different components within yoga on fatigue (e.g., breath-focused yoga, movement-focused yoga) or according to different yoga postures which may indirectly impact breathing patterns.

Because only two of the studies investigated longer-term effects [17, 25], the results of this review are only relevant to the short-term. Moreover, safety of the yoga intervention was also insufficiently reported. Specifically, only four studies assessed adverse events [8, 17,

25, 34]. However, worsening of multiple sclerosis' symptoms were fewer or equal in number in the groups compared to the usual care or yoga groups. This is in line with previous systematic reviews of yoga interventions in other patient populations that found no evidence for serious yoga-associated adverse events [9]. Nonetheless, it should be noted that yoga has been associated with serious adverse events in case studies [10].

Mainly patients from the Middle East and Europe were included. Given that geographical factors influence the incidence and severity of multiple sclerosis [1, 2], the generalizability of these findings in other regions is limited. Moreover, since mainly women participants were included, the results might not be generalized to men patients. It should be noted that 11 studies did not report on the eligible types of multiple sclerosis and the other three [18, 27, 33] had a patient group that was a mixed cohort, including patients suffering from relapsing-remitting MS, secondary progressive MS, or primary progressive MS. Although there might be different underlying pathological mechanisms for these three types, one may agree that it is a representative patient group because in all of them autonomic abnormalities play a role. Furthermore, studies are currently limited by the lack of objective quantitative outcome measures for fatigue, as only questionnaires were used to study fatigue. When possible, future studies should consider including outcomes that address performance-based fatigue measures.

Due to its effectiveness [30], exercise is recommended for the treatment of multiple sclerosis- associated symptoms, also for reducing fatigue [20]. However, an exercise intervention should be entertaining so that individuals are more likely to remain active over time. Traditional exercises such as treadmill walking or indoor cycling can often be perceived as boring, because these exercises usually involve repetitive, continuous movements [31]. In addition, outdoor activities may be difficult for people with MS due to mobility problems and potential disease exacerbation when exposed to heat and humidity [7]. Since fatigue seems to be reduced both by yoga and exercise, yoga might be considered a preferred option for patients who do not stick to exercise modalities.

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6.5 Study II

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Abstract

Fatigue is a common and disabling symptom of patients with Multiple Sclerosis. The biological causes of fatigue are still poorly understood. Several years ago, we proposed that one origin of fatigue might be the subjective representation of inflammatory processes. An important step for a straight-forward evaluation of our model would be to show that the level of fatigue is associated with vagal activation. The heart rate is under partial control of the vagus nerve and using frequency and power spectrum analysis allows to separate, at least partly, sympathetic, and parasympathetic impact on heart rate variability. This narrative review summarizes the evidence for heart rate variability changes in MS patients, as well as their relationship with fatigue, MS disease course, disease duration and activity, risk of relapse, and brain lesions. We illustrate that (1) inflammation leads to a change in cardiac behavior during acute and chronic phases, both in animals and in humans, not suffering from MS; (2) MS patients show changes of heart rate variability (HRV) that resemble those during acute and chronic inflammation due to multiple causes; (3) existing evidence favors a set of specific predictions about fatigue and parallel HRV changes; (4) and that MS-related brainstem lesions, neurological impairments or task load do not completely explain HRV changes in multiple sclerosis, leaving place for an explanatory relation between HRV and fatigue. The few studies that already analyzed HRV and the feeling of fatigue in MS patients are summarized, and we discuss the results of this review in relation to our model of fatigue. In the last section we propose several observational and experimental studies that could be studied to gain better insight into whether fatigue and HRV can be interpreted as a common pathway, both reflecting activated autoimmune processes in MS patients.

Keywords: fatigue, heart rate variability, multiple sclerosis, neuroinflammatory reflex, parasympathetic modulation, sympathetic modulation, autonomic nervous system, vagus nerve.

0) Overview

Fatigue is a common and disabling symptom of patients with Multiple Sclerosis [1]. Patient reports support the existence of distinct components in fatigue [2], some of which are cognitive and others more motor-related. Evidence from previous studies shows that fatigue may be more prevalent amongst patients with progressive forms of the disease [3-5], than those with non-progressive forms of MS. The biological causes of fatigue are still poorly understood. Several years ago, we [6] proposed that one of several origins of fatigue might be the subjective representation of inflammatory processes. According to our model [6], activated T-and B-cells, sessile immune cells, and soluble cytokines cells are detected by the vagal nerve. This information is transmitted to the brainstem and the hypothalamus eliciting a mild form of "sickness behavior." The signal also reaches the insular cortex and the anterior cingulate, and resulting activity there represents a feeling, i.e., fatigue. The feeling of fatigue acts as an interference during attentional processes that should be directed towards the environment. Consequently, this internally interfering fatigue may affect task performance. However, the degree of interference depends on the task structure: if a task allows mind wandering (like vigilance tasks), the risk of internal interference will be high, if the task does not allow for mind wandering the impact of fatigue will be low. Behavioral impairment may also be high if the neuronal structures, subserving vigilance, suffer from neurodegeneration. This explains why some studies found a correlation between thalamic, basal ganglia and right frontoparietal lesion load and fatigue. However, the impact of lesion load on performance in persons with MS-related fatigue is indirect, i.e., defined by the specific task structure allowing for mind wandering.

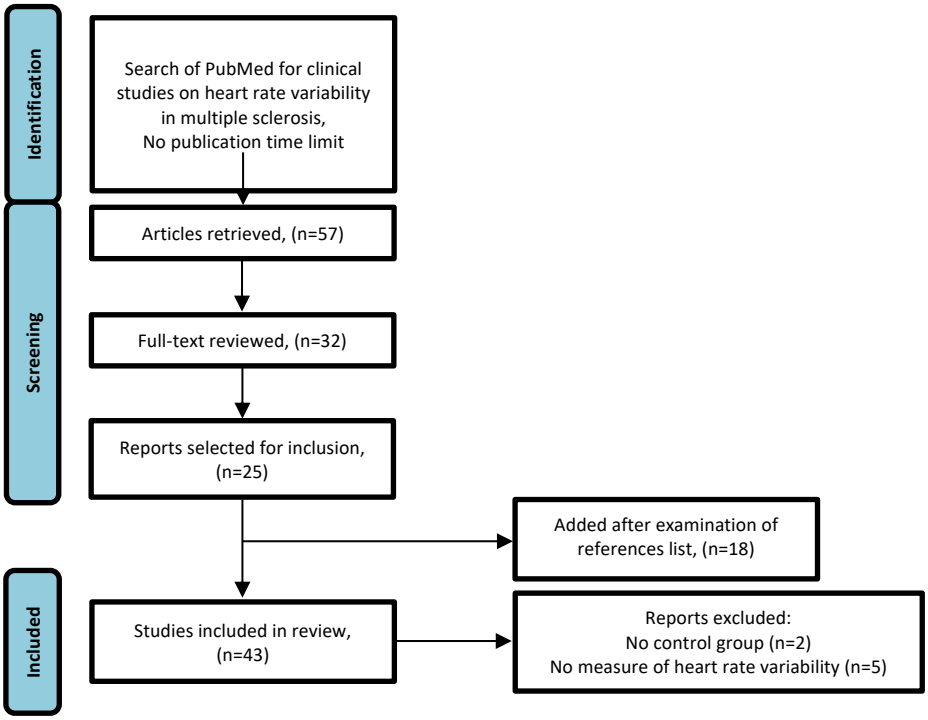
One straight-forward evaluation of our model would be to show that patients that experience a high level of fatigue systematically differ from patients with low fatigue regarding their vagal nerve activity. At present it is impossible to measure such a difference directly, because the vagus nerve system is diffusely organized with small nuclei in the brain stem acting as first relay stations. Moreover, the feeling of fatigue cannot be manipulated experimentally, which would allow us to use a subtraction method necessary for functional brain imaging. In this review about the role of the vagal nerve in fatigue in persons with MS, we will therefore use an indirect measure of its activity. The heart rate is under partial control of the vagus nerve. Using mathematical analyses allows us to separate, at least partially, sympathetic and parasympathetic components of heart rate variability. This review therefore

starts with the assumption that an activation of the inflammatory reflex interferes with vagal modulation of heart rate, and this can be measured by an electrocardiogram (ECG). To underpin this assumption, we will first show that inflammation during its acute and chronic phases leads to a change in cardiac behavior, both in animals and in humans not suffering from MS. We then will show that persons with MS show changes of heart rate variability (HRV) that resemble those during acute and chronic inflammation. Having shown that HRV is altered in MS patients, we will discuss whether brainstem lesions, neurological impairment or task load max explain these changes. As a last step, the few studies that already analyzed HRV and the feeling of fatigue in MS patients will be summarized and we will discuss the results of this review in relation to our model of fatigue.

The aim of this narrative review is to sum up current knowledge on fatigue and heart rate variability (in persons with MS) and how these may relate to an explanation of fatigue. We have followed the SANRA (Scale for the Assessment of Narrative Review Articles) statement criteria [7] and its explanations and instructions, with a view to improving the quality of this review. A search of PubMed was conducted in August 2021, using the term "multiple sclerosis" in combination with "heart rate variability", without a publication time limit. Titles, abstracts and full-text articles written in the English were considered for inclusion. Randomised clinical trials, a brief report and a pilot study reporting information on heart rate variability in MS patients were included. The search retrieved 57 articles, of which 32 were selected for review of full text. Of these, 25 were included in the review (see Figure 1). A further 18 articles were added following examination of reference list and search strategy of a review article on cardiac autonomic dysfunction [8], giving 43 articles for inclusion in the review.

Figure 1

Flowchart of the search strategy



1) What is measured by heart rate variability?

HRV is the variation in intervals between consecutive heartbeats [9]. The variation is generated by dynamic changes in the autonomic nervous system (ANS), and it serves as a marker of the combined activity of the sympathetic nervous system (SNS) and the parasympathetic nervous system on heart rate (HR) [10]. It should be noted, however, that the framework to associate the HRV frequency components (LF and HF) with the divisions (PANS and SNS) of the ANS may be restricted, as HRV cannot be completely used as a quantitative measure of the ANS components [11].

A healthy heart does not beat evenly but shows variable and non-linear oscillations in heartbeats. This variability ensures adaptability to complex and uncertain environments that are in a state of constant change [12].

The ECG of a healthy individual under resting conditions exhibits periodic variation in heartbeats known as respiratory sinus arrhythmia (RSA). RSA refers to the fluctuations of HR according to respiration phases, with HR increasing during inspiration and decreasing during expiration. RSA is predominantly mediated by respiratory gating of parasympathetic efferent activity to the heart. During expiration, vagal nerve efferent trafficking to the sinus node increases, and during inspiration it is attenuated.

HRV can be measured in time- and frequency-domains [10]. Time-domain statistical methods assess the difference between normal R-R intervals (NN), excluding ectopic beats. Time-domain units of measurement include the standard deviation of NN intervals (SDNN), which is the mean of each 5-minute segment of a 24-hour HRV recording. Another time-domain unit of measurement is the root mean square of the differences in successive R-R intervals (RMSSD), which reflects the beat-to-beat variance in HR and estimates the vagally mediated changes reflected in HRV [13]. In addition, the percentage of normal R-R intervals that differ by 50 msec (pNN50), is closely correlated with parasympathetic nervous system activity (PANS) [13] and the RMSSD. Most researchers, however, prefer the RMSSD to pNN50 [14], because it is more sensitive and less affected by recording duration, HR, or breathing rate.

HRV measures in the frequency-domain encompass high frequency (HF) and low frequency (LF) components. HF power (between 0.15 and 0.40 Hz) relates to RSA and is therefore regarded as parasympathetic in origin. LF power (between 0.04 and 0.15 Hz) is produced by the combined influence of the PANS and SNS and is influenced by baroreflex activity [15], which is mainly vagally mediated [16] LF oscillations are believed to be dominated by sympathetic efferent activity [17, 18]. Nonetheless, whether LF power generally reflects sympathetic cardiac innervation still remains controversial. That is, in resting conditions, the LF band does not reflect sympathetic tone, but baroreflex activity [13, 19-22]. The ratio of LF to HF power (LF/HF ratio) is indicative of SNS to PANS activity and an index of sympathovagal balance. The LF/HF ratio is subject to overt variation depending on activity stress levels. For instance, during rest, the absolute power of LF is less than HF, while during conditions that elicit orthostatic stress, the LF component is higher than HF. HF becomes dominant over LF (thus favoring vagal tone), at very low breathing rates (<7–8 breaths per minute).

2) Vagus nerve, the neuroinflammatory reflex, and heart rate variability

Inflammation can be detected by nociceptive fibers. The autonomous nervous system (ANS) also plays a major role in processing information about inflammation. One essential part of the ANS, which is involved in the immune response, is the vagus nerve. The afferent segment of the vagus nerve expresses receptors for cytokines, but also for tissue damage as a consequence of injuries (usually accompanied by local inflammation). The efferent part of

the vagus activates immune cells in the spleen and mediates, among others, the cholinergic anti-inflammatory pathway, by synapsing its fibers onto neurons that release acetylcholine (ACh) at the synaptic junction with macrophages [23]. When ACh binds to alpha-7-nicotinic ACh receptors of those macrophages, it suppresses the release of tumor necrosis factor, a pro-inflammatory cytokine [23].

To construct a balance between inflammation as a destructive response to immune challenges and inflammation as a necessary repair process, the vagus nerve shifts local processes into the direction of the latter [24]. The efferent action of the parasympathetic system during the response to inflammation is under control of different brainstem (e.g., the dorsal nucleus of the vagus), midbrain (e.g., the freezing center of the periaqueductal grey matter) and cortical centers (e.g., the insular cortex) [24, 25] Afferent projections of the vagus project to the solitary nucleus (SN) in the medulla oblongata. There is a topological representation of different parts of the body in the SN. Moreover, the SN is interconnected with various other nuclei of the brainstem and higher brain areas, integrating responses from other parts of the ANS, but also motor responses of the organism. In the case of inflammation, in addition to the immediate peripheral and local response there is a more global response initiated by the periaqueductal grey matter and the hypothalamus. Higher order cortical structures can nevertheless diminish the midbrain response to inflammation. This integrated behavioral response has been termed "sickness behavior" with symptoms like fever, fatigue, extended periods of sleep, loss of appetite, and motivation. Accordingly, a study by Eisenberger et al. [26] found increased reported depressed mood that resembled "sickness behavior" among participants who were exposed to an endotoxin which significantly raised interleukin 6 (IL-6) levels, and this effect correlated with greater fMRI activity in the dorsal anterior cingulate cortex and in the right anterior insula. In line with the former, increased fMRI activation of interoceptive brain regions (i.e., anterior cingulate cortex and insula) has been also observed by Harrison et al. [27] after typhoid vaccination of healthy participants induced a robust inflammatory response by increased circulating IL-6, simultaneously generating symptoms of sickness, such as fatigue, confusion, and impaired concentration. The later findings show that inflammatory activation implicates central nervous system function to elicit core symptoms of sickness behavior.

In this case, the representation of higher order goals of the organism leads to a top-down control of sickness behavior. Smith et al. [24] examined the hierarchical basis of

neurovisceral integration arguing that the interaction and interplay of increasing total organismic response to challenges of the ANS is regulated by different set points ("priors") and that long-term control of lower responses or the experience of traumatic deviations from expectations may shift such priors chronically to different states, which may be detrimental in the long run.

The vagal nerve is not only involved in the neuroinflammatory reflex, but also in many other actions of the PANS: One prominent aspect is the regulation of the heart rate. Stimulation of the right vagus nerve leads to bradykinesia, whereas stimulation of the left vagus nerve does not imply a slowing of heart rate [24]. In general, short-term changes in heart rate are triggered by the vagus nerve, whereas long-term changes (encompassing several minutes or hours) are afforded by the sympathetic nervous system (SNS). The baroreflex, for example, responsible for short-term blood pressure regulation, is under vagal control during upright standing ("orthostasis"). The central origin of the baroreflex is located in the ventrolateral superficial reticular formation of the brain stem and initiates additional activity in the SN and dorsal nucleus of the vagus as additional control centers [25]. The SN also receives excitatory stimulation from the Bötzinger complex, which is involved in the modulation of respiratory depth and rhythmicity. Deep breathing is part of the assessment of the cardiovagal reflex and results in increased short-term heart rate variability because of PANS dominance. Thus, there is a cascade of ascending brain stem centers integrating PANS and SNS control of an organism’s response to external affordances and internal goals for action.

3) Heart rate variability and inflammation

3.1 ANS disorder and Multiple Sclerosis

Our model proposes that fatigue is a subjective representation of inflammatory processes due to either activated T-cells that occasionally pass the blood brain barrier initiating immune processes against the myelin sheath of axons, or intrathecally trapped B-lymphocytes and sessile immune cells [28, 29]. One prediction that can be derived from our model is that stimulation of the vagus by inflammatory processes might also lead to changes in other autonomous functions. Many epidemiological studies demonstrated a similar intercorrelation of ANS-related symptoms in MS patients. Investigations that used the COMPASS-31 questionnaire, focusing on ANS dysfunctions, have shown that MS patients

suffer from more ANS-related symptoms than healthy controls (especially for pupillomotor symptoms and orthostatic intolerance), and that these symptoms correlate with fatigue [30-33], whereas no relationship between COMPASS-31 and EDSS, or disease duration could be established by Cortez et al. [34].

The vagal nerve is involved in digestion and bladder control. Lin et al. [35] found that fatigue, intestinal symptoms, or deficits in bladder control are correlated. A large study in the United Kingdom showed that MS patients made more visits to health practitioners in the years and sometimes decades before their diagnosis compared to the general population. Moreover, it appeared that ANS dysfunctions (including fatigue) played a major role in these visits and that ANS dysfunctions experienced early in the disease became more severe during the first two years after the diagnosis [36, 37]. In another study, Almeida et al. [38] were able to demonstrate that bowel symptoms preceded the clinical isolated syndrome by about 3 to 4 years, and they correlated with retrospectively scored fatigue.

3.2 HRV changes during acute inflammation

Subjective symptoms assessed with questionnaires are always prone to reporting bias, even when statistically controlled for. In the following, we will show that inflammatory processes alter HRV, an objective measure of ANS activity.

Infusion of lipopolysaccharides (LPS) leads to an immediate response of the immune system, and therefore it has often been used to study the biological and behavioral consequences of inflammation. Several animal studies demonstrated that shortly after LPS infusion, levels of cytokines, such as IL-6 or TNF-α etc., increased, but also changes in heart rate were observed. A few human studies have also used this design. Jan et al. [16] challenged 30 young participants with 2ng/kg endotoxin. They studied the bodily responses with serial blood probes and by monitoring heart rate. About one hour after the challenge TNF, IL-6, IL-8 an IL-10 started to increase steeply. There was a parallel sharp drop of SDANN, pNN50 and HF power of heart rate. The LF/HF ratio also changed but in a less steep manner. After 24 hours, the scores normalized. Herlitz et al. [39] repeated this study with different dosages of endotoxin to analyze the immune response at different levels. Changes in body temperature were the most sensitive marker, present already after a challenge with 0.25ng/kg. Changes In TNF-alpha and IL-6 together with an increase of heart rate were observed as a response to 0.5ng/kg. HRV changes differed significantly from placebo starting with doses of 1 and 2

ng/kg. These and other studies [40, 41] argue in favor of a common and orchestrated PANS response to inflammatory challenges. The latter results provide evidence that circulating inflammatory markers, argued to be involved in the development of sickness behavior, drop HRV parameters of PANS activity, suggesting that inflammation may indeed contribute to the pathogenesis of MS-fatigue as an expression of resulting sickness behavior.

3.3 HRV changes in chronic inflammatory diseases

Is the vagal response restricted to an acute phase or still present during chronic inflammation? Reviews examining HRV changes in chronic (auto-)immune diseases argue for a lasting change in vagal activation. Haensel et al. [42] reviewed thirteen studies on inflammatory markers in cardiovascular disease and HRV. Most studies focused on IL-6 and C-reactive protein (CRP) and found a significant negative correlation with the HRV parameter SDNN, which is most frequently for assessment. Not only SDNN and HF decreased in the patient groups, but also other parameters indicative of sympathetic modulation of HRV. Haensel et al. [42] concluded that, while inflammatory markers appear to be related to HRV, this concerns parameters reflecting both parasympathetic and sympathetic activity.

Kim et al. [43] reviewed the relation between HRV and inflammatory bowel disease. They were able to include 10 studies on this topic with 440 patients and 208 healthy controls. HF, SDNN and pNN50 measures were lower in the patient groups. Moderate to high effect sizes were present for all HRV measures except for LF. They concluded that inflammatory bowel disease is strongly associated with restricted HRV and that PANS-related HRV seems to be more closely related to chronic inflammation compared to SNS-related HRV.

Provan et al. [44] reviewed the relation between HRV and rheumatoid arthritis. They were able to include 21 papers in their review. Interestingly, they also analyzed the published results on cytokines and HRV. Their results suggested that inflammation, present at the time of the HRV measurement and measured by the concentration of cytokines in the blood, plays a greater role in determining HRV than disease duration or cumulative disease activity. Related to HRV, RMSSD and HF were used in several studies allowing for a calculation of the effect size, and both were decreased compared to healthy control. Thus, persons with rheumatoid arthritis appear to show reduced PANS-related HRV.

Crosswell et al. [45] analyzed whether fatigue in breast cancer survivors is related to HRV, CRP and IL-6 levels. They included 84 women and focused on resting HRV (no physical

or mental load during the examination). All participants had suffered from cancer before reaching the age of 50. High fatigue correlated with a lower HRV and increased concentrations of IL-6. There was also a correlation between IL-6 and RMSSD or HF. After controlling for age, physical activity, and body mass index, the correlation between HF and IL-6 remained significant as well as the correlation between fatigue and RMSSD. Crosswell et al. [45] concluded that research on the etiology of cancer-related fatigue should give attention to ANS dysfunctions.

Williams et al. [46] reviewed 51 studies (irrespective of etiology) on the relation between inflammatory markers like CRP, IL-1, IL-6, or TNF and HRV. They found that SDNN and HF showed the strongest negative associations with HRV. SDNN correlated with CRP, IL-6, and WBC, whereas HF correlated with CRP, IL-1, IL-6, IL-10, TNF, WBC, and fibrinogen. All these correlations were negative, i.e., low PANS-related HRV was associated with high counts of proinflammatory cytokines and vice versa.

3.4 HRV and fatigue in MS patients: defining some characteristics derived from the phenomenology of fatigue and structural assumptions of our model

Summarizing the results of the experimental studies and those in chronic inflammatory diseases, we conclude that there is combined evidence that inflammatory processes not only elicit a neuroinflammatory reflex but also decrease PANS-related HRV and trigger the feeling of fatigue. However, the mere presence of fatigue and PANS-related changes of HRV is only a weak argument for a causal interrelation. In the following section we will specify features of fatigue in MS (based on Sander et al. [47]), which allow a formulation of more specific predictions.

One major feature of MS-related fatigue is that it is only very loosely related to the level of mental or physical load. Fatigue can arise even after minimal effort or in the absence of effort. It can develop instantly and is unpredictable for a patient. Thus, according to these defining characteristics that separate MS-related fatigue from increased tiredness, it can be expected that HRV changes, if they are related to fatigue, are already present during rest and not only after any kind of effort.

This assumption can be stressed further: inflammatory processes do not stop during sleep, a status without any environmental load. Our model assumes that the HRV changes are

present during sleep, probably even more so than during the day, because during the night there is a circadian-mediated increase of inflammatory processes.

According to our model, fatigue is not related to neurological impairment. If the biological basis of fatigue is the neuroinflammatory reflex, changes in HRV depend on the detection of cytokines by the vagus in the periphery. The expression of cytokines can be high (even if the activated T-cells do not pass the blood brain barrier) or low during different phases of the disease course. However, a link between HRV and ongoing disease activity in MS (as reflected by EDSS) has been suggested [48] (being just another part of accumulating neurological deficits). Thus, our model predicts a constant inflammation-based association between fatigue and PANS-related HRV (see section 3.2 and 3.3 of this review). However, there might also exist an increasing accumulation-based association between disease duration and HRV.

As a correlation of this latter prediction, we assume that PANS-related HRV changes should be common (like fatigue), but should not arise due to brain lesions. This does not exclude that ANS dysfunctions related to brain lesions may be present in MS patients (due to brain stem lesions, for example) – however they should affect only a minority of the patients (with increasing frequency related to a higher duration of the disease).

4) Changes of HRV in Multiple Sclerosis

4.1 HRV adaptation in rest and during orthostatic challenge

Numerous studies have provided evidence of autonomic dysfunction involving the cardiovascular system of MS patients (reviewed in depth by Findling et al. [8]). The most prevalent finding is a lower overall parasympathetic output in relapse-remitting MS (RRMS) patients in comparison to healthy controls (HC), as demonstrated by significant reductions in long-term monitoring (24-h electrocardiogram) [49-52] and short-term HRV analysis [53]. In studies that measured the strength of each HRV frequency component at rest (while sitting or in supine position), variant-undefined MS patients were more predisposed to reduced parasympathetic activity at rest than HC [53-57]. However, Reynders et al. [48] found that even though the parasympathetic parameters SDNN and RMSSD showed a strong correlation at baseline and after three months within the MS group, they showed little intra-individual reproducibility.

Two studies also analyzed if the differences between HC and MS patients increase or decrease during sleep. Both studies found that the ANS associated difference in cardiac activity increases during the night [58, 59].

Different patterns of HRV have been observed during and outside supine resting conditions. Baroreflex activity, as reflected by the LF power component of HRV in resting conditions, was found to be lower in RRMS [49, 50] and in MS variant-undefined patients [51, 54, 60] in relation to HC. In the study by Monge-Argilés et al. [59], variant-undefined MS patients had a lower sympathovagal tone during the supine resting condition than HC, while sympathovagal tone increased outside of the rest condition of the 24 h recording. The divergent results in sympathetic tone between the rest condition and the non-rest part of the examination could be explained by more experience (and then better relaxation) for clinical examinations in MS patients, than in HC.

Expected changes in ANS, as reflected by HRV variations in response to orthostatic stress conditions (sit-to-stand transition and head-up tilt tests) and physical exercise, have been tested on MS patients in comparison to HC. During an orthostatic challenge and activity, healthy organisms typically experience an augmented sympathetic tone, along with inhibition of the cardiovagal system, in order to sustain the necessary increase in HR. Accordingly, expected HRV variations during head-up tilt test (sympathetic predomination and lower vagal tone) have been reported in the variant-undefined MS group [60]. In contrast, Rzepinski et al. [61] established that RRMS patients are more predisposed to a combined deficit in sympathetic and parasympathetic responses to the head-up tilt test, compared to HC. Moreover, Gervasoni et al. [56] found a lower RMSSD in variant-undefined MS patients than HC post-exercise recovery, which suggests that inhibition of the vagal tone may remain preserved in MS. On the other hand, others did not observe any changes in HRV variables between variant-undefined MS patients and HC after orthostatic challenge [62, 63].

Targeted changes in ANS activity are also known to occur in response to deep breathing and relaxation [64]. That is, deep breathing techniques increase vagal nerve activity, signaling the PANS to calm the body down [65] due to an entrainment between respiratory rate and vagal tone [66]. Abnormalities in vagal power in MS have been detected at slower respiratory rates during paced breathing exercises, as reflected by lower HF power in the variant-undefined MS group than HC, at 8, 12 and 15 breaths per minute (b.p.m), but not at 18 b.p.m [55]. Further, a recent review demonstrated that yoga practices, which heavily

rely on deep breathing exercises, may be particularly helpful in alleviating MS-related fatigue [67], arguing for a temporary increase in PANS activity. Judging from this evidence, increasing HRV through deep breathing exercises could presumably produce body autonomic activity characteristic of relaxation in MS patients, helping to restore parasympathetic balance.

Summarizing this section, there is clear evidence of a PANS-related HRV change during rest and sleep, unclear evidence of a lack of sympathetic modulation during orthostatic challenge and activity, and weak evidence of decreased vagal response to deep breathing in MS patients.

4.2 Relation to disease activity and risk of relapse

It has already been mentioned in section 3.1 that epidemiological studies argue that alteration of autonomic functions may define something like an MS prodrome [36, 37], these already being present years before initial inflammatory processes give rise to clinically relevant impairments. Focusing on the experience of a relapse, different patterns of HRV have been associated with an increased risk of MS disease activity [62] and HRV changes may happen in response to elevated inflammation during the prodromal phase of a MS-relapse. In active RRMS patients, relapse rate, even sub-clinically, was associated with lower sympathetic tone, whereas stable RRMS patients did not differ from HC [62].

Studer et al. [62] also observed that patients with incomplete recovery from relapse showed less sympathetic increase after head-up tilt test during the relapse, compared to subjects who completely recovered. Notably, the severity of relapse, assessed by an EDSS increase during the relapse, did not correlate with the HRV parameters [62]. These results suggest that during the relapse, the sympathetic system is most affected with a relative sparing of the parasympathetic system.

Reynders et al. [48] found that higher HRV parasympathetic parameters predicted MS relapse after 3 and 6 months. Nonetheless, they used self-reported relapses, which were not necessarily confirmed by a physician. Another study contradicted the previous results by reporting that parasympathetic HRV parameters were not significantly related to relapse rate [52]. In line with a presumed association between neuronal inflammation and HRV, Gidron et al. [68] found a negative correlation between parasympathetic tone and neurofilament light chain, an identified biomarker of disease activity in MS [69] in variant-undefined MS patients without relapse.

Due to these contradicting findings no definite conclusions can be drawn about HRV directly before and during an acute relapse. Given the modern immunohistological perspective of ongoing neuroinflammatory processes even in MS appearing clinically "stable", as well as early degenerative changes in persons with MS classified "relapsing-remitting," assorting conventional clinical MS classifications may not help for further understanding [29, 70].

a. Relation to lesions of the central nervous system

Autonomic abnormalities in MS are usually explained by widespread demyelinating lesions in areas of the brain that exert influence on cardiovascular reflexes and autonomic regulation [71]. Specifically, cardiovascular autonomic disruption may happen due to plaques distributed throughout the brainstem and spinal cord affecting autonomic regulatory areas and their connections [54, 72, 74].

Demyelination is likely to interfere with the descending ANS brainstem pathways [72, 62]. It is therefore reasonable to examine a possible association between a HRV impairment and the presence and localization of CNS lesions in MS patients.

The relationship between brain and spinal lesions as detected by magnetic resonance imaging and HRV has been researched in studies with stratified patients according to the presence or absence of demyelinating lesions, mainly involving the brainstem and cervical spinal cord. While the presence of brainstem lesions has been linked to lower parasympathetic tone [75], spinal lesions have been associated with a higher sympathetic tone [53], and insular lesions correlated with sympathovagal predominance [53]. However, Videira et al. [53] retrospectively analyzed white matter lesions near these CNS structures and did not specify where they were situated. In contrast, a recent study that investigated PANS-related HRV found no significant differences between MS patients with and without brainstem lesions [50]. Likewise, a study from the 1990s revealed no significant correlations between the total lesion area in both hemispheres or their specific localization and any of the frequency-domain HRV frequency parameters [60]. A similar finding was reported by Damla et al. [49] who found no significant relationship between brainstem, cervical and thoracic spinal lesions and time- and frequency-domain HRV analyses. Similarly, the presence of demyelinating lesions in the brainstem was not attributed to significant differences in the sympathovagal ratio [62].

We conclude that there is no clear evidence to assume an association between HRV impairment and structural damage to the brain in MS.

b. HRV, disease duration and functional impairments

Several investigators directly compared HRV differences in MS patients in relation to the duration of the illness. First, early involvement of ANS dysfunction in MS has been validated, with PANS-related HRV changes to be present already in newly diagnosed RRMS patients [49]. The duration of MS illness seems to provoke more pronounced parasympathetic deterioration, as confirmed by several investigators [51, 60, 62]. Progression of the disease was reported to correlate with increased parasympathetic dysfunction [76]. Others, however, did not observe changes in HRV parameters in prolonged MS [53, 55, 75-78]. A study including RRMS patients, determined that those with more than 5 years since formal diagnosis had a stronger decrease in PANS-related HRV than those with less than 5 years from diagnosis [51]. This is in line with the study by Studer et al. [62], who showed that more than 5 years of MS disease duration correlated with a lower parasympathetic tone.

Overall, findings indicate that the reduction in parasympathetic tone amplifies over time in MS. That is, the longer the disease goes on for, the more profound the parasympathetic dysfunction. However, sympathetic dysfunction can also be present very early as evidenced in patients with clinically isolated syndrome [75]. As the disease progresses over time, sympathetic activity becomes prevalent [56, 62], whereas expected sympathetic reactivity to orthostatic stresses weakens [55]. Others, however, did not observe a significant relationship between sympathetic reactivity to orthostatic stressors in MS and disease duration [51, 63, 75, 77]. In conclusion, sympathetic activity may be weakly reduced already throughout the early phases, but then it chronically evolves into sympathetic predominance, weaker sympathetic reactivity, and parasympathetic deficiency.

HRV impairment has also been connected to disease-related functional impairments. In MS, lower PANS activity [59, 78], and SNS hyperactivity [62] are more apparent with increasing severity of functional impairments, as measured by the Expanded Disability Status Scale (EDSS). Accordingly, less affected MS patients are found to preserve higher levels of HRV [59]. Furthermore, Studer et al. [62] revealed that SNS hyperactivity was more manifested among severely disabled PPMS patients. In the study by Studer et al. [62], disease severity was associated with increased disruption of sympathetic reactivity during head-up tilt test in

PPMS patients, while no difference was found between RRMS patients and HC. Likewise, in the study by Zawadka-Kunikowska et al. [78], a higher overall EDSS score was linked to a higher sympathovagal ratio at rest and lower parasympathetic parameters at rest in variant-undefined MS patients. Other investigators did not find a similar significant difference [34, 52, 53, 60]. Overall, functional deterioration leads to a decrease in parasympathetic activity, but maintenance of an increased sympathetic tone compared to HC. However, the increase sympathetic tone was found in PPMS patients, and we will discuss the possible course of the disease and HRV changes below.

It should be noted that in some of the studies described above [57, 60, 78], patients took an immunomodulating medication "Fingolimod" as a treatment for RRMS. Fingolimod and various other immunomodulating medications have been found to diminish cardiovagal responses to autonomic challenges as well as to reduce cardiac autonomic modulation at rest [8]. Moreover, age must be considered as a confounding factor discussing disease duration, related functional deterioration and MS-related HRV changes. In the study by Gökaslan et al. [50], all patients had been diagnosed with MS within the last 3 years, and parasympathetic HRV parameters significantly decreased as patient age increased. This is in line with McDougall and McLeod [71], who showed that HRV parameters decreased as persons with MS aged. These findings argue that parasympathetic activity decreases, and sympathetic activity becomes more dominant as patients age, regardless of disease duration.

c. HRV in different MS disease courses

A difference in patterns of dysautonomia among MS variants has been established. Compared to HC and patients with RRMS, patients with PPMS have been particularly associated with higher sympathetic predominance at rest [60, 62, 78], and lower sympathetic reactivity during head up tilt-up [62, 71, 78] even when corrected for age, sex, and disease duration [71]. Specifically, a heightened basal sympathetic tone in PPMS, related to inadequate sympathetic reactivity during orthostatic stress [7]. However, in the study by Gervasoni et al. [56] sympathovagal parameters during orthostatic challenge were similar in the PPMS and RRMS groups, and in the PPMS and HC groups. Also, Zawadka-Kunikowska et al. [78] observed impaired vagal inhibition after a head-up tilt test in the progressive MS group, comprised by 17 secondary progressive (SPMS) patients and 4 PPMS patients, indicating insufficient withdrawal of PANS modulation after orthostatic challenge. Thus, PPMS

subjects cannot adapt during orthostatic challenge, and therefore do not reach expected sympathetic increase and parasympathetic inhibition. Given the set of associations described above, the PPMS variant involves a stronger impairment in physiological sympathetic reactivity, along with a shift of the sympathovagal modulation toward sympathetic predominance, as compared to the RRMS and HC.

PPMS patients are often diagnosed in higher age than RRMS patients, which may play a confounding role for the interpretation of results. Still, the vast majority of studies reviewed here matched for age [53-57, 59-61, 51, 63, 65, 67, 80], most demonstrating that cardiac parasympathetic dysfunction is a common denominator in MS. Thus, MS disease courses have been found to influence the ANS differently.

In sum, autonomic balance is substantially altered in the MS patient group compared to HC, and the alterations are more apparent in the primary progressive variant of MS. Indeed, patients with PPMS were found to have lower parasympathetic activity and higher sympathetic tone at rest and lower expected sympathetic reactivity in response to orthostatic challenges. For RRMS patients only a lower parasympathetic tone has been documented, with scores between HC and PPMS patients. An increased basal sympathetic tone was associated with the failure of sympathetic reactivity, and this is a further index of autonomic impairment in the PPMS variant, suggesting that the SNS of PPMS patients is overactive and exhausted.

It should be mentioned that a lack of reactivity of the SNS in PPMS could be associated with the limitation of physical activity and resulting cardiovascular deconditioning in severely disabled PPMS patients. For instance, Goldsmith et al. [81] found that untrained participants had a lower LF power than trained ones. Because of that, comparing hypoactive MS patients and normally active subjects could prove, in relation to the LF parameter, a difference that has been reported between endurance-trained and untrained individuals.

5) What is known about HRV and fatigue in MS

Three studies addressed the topic of fatigue and HRV indirectly. Cleland et al. [82] analyzed the role of physical activity on bone mineral density in 23 persons with MS compared to 22 control participants. They also assessed fatigue, depression, cytokines and HRV (only SDNN) in both groups, however evaluated only their relation to bone mineral content and not their interrelations. MS patients did not differ from healthy controls in HRV, but in IL-6 and TNF-alpha concentrations. Unfortunately, there is no description of how HRV was measured

(supine, during sitting or standing) and if it correlated with fatigue. Therefore, these results are difficult to interpret.

Rampichini et al. [83] studied the relation between HRV and sub-maximal physical exercise in 17 persons with MS and 17 healthy controls. They also measured fatigue. However, the fatigue measure was restricted to physical and muscle exhaustion after the sub-maximal workout, it did not concern fatigue as measured with validated questionnaires and did not fit the definition of fatigue as being not explained by prior external effort. Rampichini et al. [83] included older patients (average age of 56 years), being already impaired in walking (EDSS of 5.9) and suffering from different disease courses. RMSSD, the only HRV parameter they analyzed, was decreased in patients before the exercise and remained decreased afterwards. The difference between the two groups did not change during exercising. HRV did not correlate with fatigue measures before and after the physical exercise. The significance of this study for this review is unclear because it focused on (muscle) exhaustion not on fatigue.

Increased heat sensitivity exaggerating fatigue is a common symptom in MS and there are several reports that cooling the body leads to a reduced feeling of fatigue [84]. Meyer-Heim et al. [84] studied the impact of advanced lightweight cooling-garment technology on fatigue and HRV in MS. Cooling influenced motor, but not cognitive performance of the MS patients. In the subgroup of 6 patients with perfect ECG data, the HRV was reduced in the sham condition, whereas it approached the level of healthy controls during cooling.

Gossmann et al. [85] studied the effect of cooling on fatigue in 29 persons with MS and 10 healthy controls using a 20-minute-long vigilance task to induce fatigue and they measured HRV (focusing exclusively on the LF/HF ratio) during the assessment. Only three of the MS patients did not suffer from fatigue and all relevant statistical evaluations were repeated after their exclusion. MS patients produced more omissions during the vigilance task and their number of omissions increased significantly during the task. Concerning the LF/HF ratio, the difference between the persons with MS-related fatigue and the healthy controls approached significance. MS patients showed a lower LF/HF ratio than healthy controls. Moreover, during the vigilance task the LF/HF ratio increased in the control group but did not show any change in the MS patient group. However, wearing the cooling vest had no measurable effect on body temperature, and had no impact on HRV and fatigue. As a result, it can be argued that compared to healthy controls MS patients with fatigue show a lower

LF/HR ratio and do not respond with heart rate variability change if challenged by a vigilance task.

In one of the first studies using HRV analysis related to fatigue in MS patients, Keselbrener et al. [80] included just 10 MS patients with a Fatigue Severity Scale (FSS) score above 3.5 (which is below the cut-off score of 4 for fatigue in the FSS). They also used frequency ranges for calculating VLF, LF and HF, which are uncommon today: 0.02-0.06, 0.06-0.2 and 0.2-0.5 respectively instead of VLF: 0.0033-0.04 HZ, LF: 0.04-0.15 HZ, HF: 0.15-0.4 HZ. Compared to healthy controls, only the LF/HF ratio approached significance. Further analyses showed that MS patients showed a reduction of vagal activity related to age, whereas healthy controls did not. However, the small number of patients do not really allow for subgroup analyses.

Merico [86] analyzed different ANS parameter in 22 MS patients in supine position, during deep breathing and during standing. Unfortunately, the paper does not offer information about the results in different HRV parameters. However, Merico [86] reported that stress did not induce an increase in autonomic variation (as expected in healthy subjects) and that this concerned mostly a loss of parasympathetic modulation. Moreover, fatigue measured by the FSS correlated with autonomic parameters, but the average score of experienced fatigue was not reported.

Yu et al. [87] analyzed HRV in 17 MS patients with fatigue and 9 healthy controls. The fatigue score was moderate to severe with an average of about 6 in the FSS. They found that PANS-related HRV was reduced in the MS patients in most of the assessed conditions. Because they did not include a group of MS patients not suffering from fatigue, it is unclear whether the difference is due to the disease or to the feeling of fatigue and no specific comparison between rest and activity periods were performed. However, this study (and [85]) is one of the very few looking for the sequelae of a cognitive task on HRV. They used a learning task, presenting 20 figures that the participants had to recall subsequently. They also found that during this task without any specific body movements the HRV parameters of LF and HF differed between the patients and the controls.

Sander et al. [64] treated 50 persons with MS-related fatigue with a single trial containing two different kinds of a biofeedback intervention. They looked for the impact of the two biofeedback exercises on fatigue before and after performing a vigilance task. The MS patients were divided by those suffering from severe fatigue (defined by the total score

of the FSMC) or from weak to moderate fatigue. Sander et al. [64] focused their HRV analysis on SDNN and pNN50. They found that the group with high fatigue showed significantly lower SDNN and pNN50 scores than the group with weak to moderate fatigue. Moreover, they found that progressive muscle relaxation (one of the two biofeedback interventions) led to an increase of the SDNN and the pNN50 scores, but only for the group with weak to moderate fatigue. The group with severe fatigue did not respond with any relevant changes in the two HRV scores.

In a former study Sander et al. [88] analyzed which HRV parameter (VLF, LF, HF, SDNN, RMSSD, pNN50) predicted total fatigue, as measured with FSMC. Regression analysis showed that VLF and HF were the best predictors. In sum, these two studies found that the total score of the FSMC is related to VLF, two parameters primarily indicating short-term fluctuation, which are closely related to vagal activity. SDNN and pNN50 are sensitive to biofeedback induced PANS activation, but MS patients with high fatigue are not able to learn to induce such changes. Similarly, Merico [86], Yu et al. [87], and (in interaction with age) Keselbrener et al. [80] found primarily PANS-related HRV reduction related to fatigue, measure with the FSS.

6) HRV and fatigue in MS: a common pathway of disseminated inflammatory processes?

The present review highlights the possibility that in MS, activated T- and B-cells, sessile immune cells and soluble cytokines induce ANS activity, and this is reflected in HRV parameters. We also proposed that fatigue is associated with an altered HRV, because, according to our model 1, HRV and fatigue share a common neural substrate, i.e., vagal processing. To analyze this assumption, we developed a set of criteria for defining fatigue in section 3.6 and we predicted alterations in HRV.

Fatigue can be regarded as a feeling arising independently from environmental challenges, that can subsequently be experienced during rest. Thus, we hypothesized that changes in ANS that are reflected in HRV should be present during rest. We found that, irrespective of disease duration and disease course, MS patients demonstrate a lower PANS-related HRV during rest and during deep breathing (section 4.1). Studies directly measuring the relationship between HRV and fatigue also demonstrated that MS patients with fatigue have a lower PANS-related HRV and that the LF/HF ratio correlated with fatigue (section 5) [64, 80, 86]. A study by Sander et al. [64] compared MS groups with differing fatigue status

on HRV parameters and implemented an experimental intervention (biofeedback). Persons with MS with severe fatigue showed no expected increases in HRV levels in response to the biofeedback intervention. Those with mild to moderate fatigue responded with PANS-related HRV changes to the relaxation condition and did not show differences in SNS-related HRV. This would suggest that beyond the severe fatigue threshold, MS patients do not react in the optimal ranges to changing internal conditions.

Inflammatory processes do not cease during sleep. Cortisol production drops to its lowest point around midnight, resulting in increased inflammation. Because of this, we expect HRV changes to become more pronounced during sleep. Accordingly, Monge-Argilés et al. [59] and Ferini-Strambi et al. [77] found that during the night, parameter differences between MS patients and the control group seemed larger. Moreover, 24-hour measurements taken at an early stage of the disease revealed increased levels of PANS-related HRV activity, whereas those taken at a later stage of the disease revealed decreased PANS-related HRV activity [54-56, 77].

If the biological basis of fatigue is the neuro-inflammatory reflex, then afferent vagus nerve signaling, activated by cytokines in the body, would be accompanied by changes in HRV. Cytokine expression can be high (even if activated T-cells do not pass the blood brain barrier) or low at different phases of the disease course. Hence, fatigue feelings should not be closely related to disease duration. The results of the present review confirmed this prediction (section 4.4). We found that RRMS patients have lower PANS-related HRV activity than HCs. The lower HRV was derived from time domain parameters of HRV (SDNN and pNN50), and frequency domain parameters (HF). The association between levels of inflammatory biomarkers (interferon γ and tumour necrosis factor α) and MS-fatigue [89, 90] may have limited relevance to progressive forms of MS due to the absence of a marked inflammatory response [91, 92]. One study argued that RRMS patients suffering an acute relapse may have increased PANS-related HRV activity relative to SNS-related HRV activity. However, the latter finding has not been replicated in other studies (section 4.2). As shown in section 3.2. and 3.3, such an increase in PANS-related HRV would contradict the findings from other acute and chronic diseases. Therefore, the available evidence argues equivocally that PANS-related HRV decrease during ongoing inflammatory processes.

According to our model, focal brain atrophy may produce similar effects on behavioral performance due to the interfering feeling of fatigue, but brain atrophy does not lead to

fatigue. Thus, HRV may change in parallel with EDSS (reflecting accumulated neurological deficits) and with increasing brain lesions, however, their statistical correlation would be low. Our review revealed that both assumptions seem to be correct: (1) there was no conclusive evidence for a correlation between the EDSS score and HRV (section 4.4), (2) there was no conclusive evidence for an association between brain damage and HRV changes either (section 4.5).

Another result of our review is that, in parallel with the disease course, PANS-related HRV decreases even further. First, some studies showed age-related changes in PANS-related HRV in MS patients but not in HCs. Second, a reduction in PANS-related HRV was found more often in chronic MS patients than in RRMS patients. However, at the same time, duration of disease and disease course also led to increases in HRV indices that reflect SNS activity. Sympathetic dominance is already present during rest and is not influenced by orthostatic stress (no significant results for the head-up tilt test). Therefore, in the later phases of the disease, a more pronounced imbalance between PANS and ANS is present. It should be noted that our model does not predict any relation between SNS activity and fatigue, because the SNS seems to be unaffected by inflammatory processes in the body. Consequently, our model does not predict that a higher imbalance between PANS and ANS would lead to higher levels of fatigue nor that the disease duration or the conversion to a chronic phase would be associated with higher levels of fatigue.

In summary, it can be stated that MS patients differ from HCs by having less activity levels of PANS-related HRV and that these are inversely correlated with fatigue. At first sight, this seems to contradict our assumption, because a high state of parasympathetic activity should be related to high HRV, a causal relation used, for example, to assess the cardiovagal reflex. However, after reviewing experimental animal studies on HRV in acute and chronic human inflammatory diseases (section 3.2 and 3.3), we were able to show that (increased level of pro-inflammatory cytokines and activated T-cells), which induces vagal processing, is related to lower PANS-related total HRV even with higher parasympathetic activity. Therefore, analyses of acute and chronic inflammatory processes in various diseases fit the results published of MS patients, with a prominent decrease in PANS-related HRV, arguing for a relation between inflammation and PANS-related HRV, however, in the opposite direction as it may be expected.

We would like to propose two different explanatory frameworks for fatigue and HRV changes in MS. First, by a strictly mechanical explanation, stimulation of the vagus nerve might act in a similar manner as stimulation of other neuronal structures. As has been shown in different contexts, such stimulation follows an inverted U-shaped curve: under- and overstimulation result in a similar dysfunction, whereas moderate levels of stimulation increase the effectivity of processing. If this assumption is correct, acute and chronic stimulation of the vagal nerve in autoimmune diseases might lead to a partial loss of its ability to regulate HRV (see Figure 2, left side, vagal setpoint). Sander et al. [64] argue along the same lines. Patients with weak to moderate fatigue demonstrated expected changes in PANS-related HRV in response to a biofeedback intervention. Thus, weak to moderate levels of fatigue would belong to the upper part of the inverted U-shape. However, patients with severe fatigue did not demonstrate changes in PANS-related HRV in response to the biofeedback training. These patients would be localized on the right bow of the inverted U-shape. Second, Smith et al. [24] assume that adaption of the priors serving in the control of homoeostatic processing can take place because of a repetitive (over-)stimulation of the vagal afferents. Figure 2 offers an adapted version of our previous model 1 and at the same time an overview from previous models on the possible changes that may lead to chronic and self-stabilizing feelings of fatigue. This would not be a pure mechanical explanation for fatigue, it relies on learning processes at various biological, neuronal and conscious levels.

Although many of the results matched the predictions of our model, direct evidence from correlational studies between fatigue levels, HRV, and inflammatory processes is still weak. Most of the studies we reviewed focused on HRV in MS, and many were performed based on the assumption that HRV changes represent neural damage, which means that they did not consider HRV changes as a side aspect of a working neuro-inflammatory reflex. Therefore, they included patients with a different disease course and disease duration. Failing to differentiate MS course in the analyses may have led to discrepant data on sympathetic cardiovascular tone in MS. On the other hand, irrespective of disease course, RRMS, PPMS and variant-undefined MS patients were found to have lower PANS-related HRV than HC. This general finding argues that changes in PANS-related HRV do not depend on the existence of a lesion, brain atrophy or functional disability. The studies directly focusing on fatigue and HRV also displayed that the feeling of fatigue is related to changes in PANS-related HRV. This fits our hypothesis; however, the number of included patients was small, simultaneous

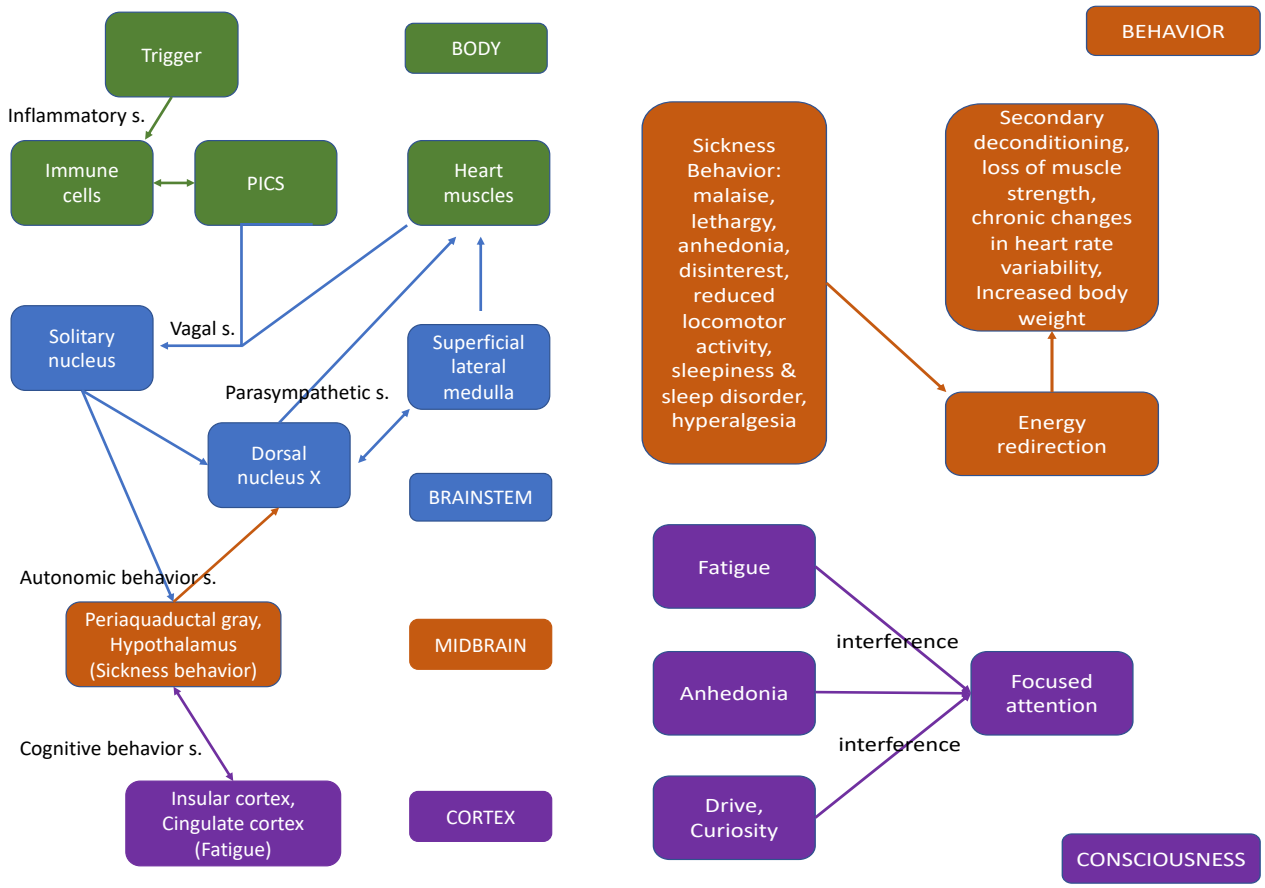
measures of cytokines, HRV changes, and fatigue have not been done yet, and experimental variations in fatigue, HRV, or cytokine levels are lacking.

Convincing evidence for our assumption would depend on cross-correlational studies on fatigue, HRV and inflammation in MS and on longitudinal studies in RRMS patients. The prediction is that in newly diagnosed MS patients, PANS-related HRV would be lower than in HCs, and decreases even further shortly before a relapse. Methylprednisolone is effective in treating MS-related relapses and therefore an easily testable prediction is that this treatment would lead to an increase in PANS-related HRV. A similarly interesting question is, whether interventions focusing on biofeedback training could boost PANS-related HRV in MS patients, and if such an effect is associated with reductions in experienced fatigue. In this respect, it would also be interesting to examine whether longitudinal studies show such an adaptation and whether treatment can induce some recovery to prior environmental settings.

We explore the possibility that processing of chronic bodily inflammation perpetuates ANS changes in order to maintain allostasis [93], leading to different patterns of HRV activity, and that fatigue might be another, subjective, representation of such processing. The results of our review do not exclude this possibility, and assuming that our hypothesis turn out to be proven, that would open a new field of development of new interventions to treat MS-related fatigue.

Figure 2

Schematic drawing of our model



Note. ***Somatic processes (left side):*** The existence of casual triggers leads to the activation of autoimmune responses. Previous exposure to these triggers might have led to a changed of the "prior ", the set-point for starting and regulating the autoimmune response. The production of pro-inflammatory cytokines leads to the activation of the neuro-inflammatory reflex and, consequently, to increased activity of the afferent vagal nerve. This activation might interfere with afferents of other compartments of the vagus nerve, originating from the mechanoreceptors of the heart. Chronic summation or interference of the combined activation might have changed the "prior" for starting and regulating the activation of the solitary nucleus and may therefore lead to reduced coupling with dorsal nucleus of the nervus vagus. Consequently, parasympathetic modulation of the heart rate decreases. Information about autoimmune activation is relayed to midbrain centres and initiates sickness behavior.

Behavior and consciousness (right side): Sickness behavior reduces locomotor activity, increases sleepiness and at the same time sleep disorder. Previous activations of sickness

behavior might have changed the prior for triggering sickness behavior, and it becomes learned as being a chronic state. This leads to secondary deconditioning of the patients, loss of muscle strength and chronic changes in heart rate variability. Further processing of the signal induces the feeling of fatigue, loss of drive and anhedonia. The conscious processes start to interfere with focused attention, leading to enduring concentration deficits, which is fostered by deconditioning and chronic changes of heart rate variability. *s.* setpoint

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6.6 Study III

Full bibliographic reference:

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Abstract

Background: Various relaxation procedures have been proposed to reduce fatigue in Multiple Sclerosis (MS). However, it is unknown, which type of relaxation has the largest effect on fatigue reduction and on autonomic nervous system (ANS) activity.

Objective: We aimed to compare two biofeedback-supported relaxation exercises: a deep breathing (DB) exercise, and progressive muscle relaxation (PMR), which may ameliorate MS-fatigue and alter ANS activity.

Methods: We performed a single-blind randomized clinical trial, introducing MS patients (*n* = 34) to the DB or PMR exercise. We first tested cardiovagal integrity, reflected by changes in heart rate variability (HRV) in response to DB. Participants then performed a fatigue-inducing vigilance task, followed by the DB or PMR. State fatigue was recorded consecutively at baseline, after the vigilance task, and after the relaxation exercise, along with HRV reflecting ANS activity.

Results: Only patients assigned to the PMR group experienced a significant drop in fatigue, whereas both relaxation exercises changed ANS activity. MS patients showed the expected autonomic response during the cardiovagal reflex test. The vigilance task elevated short-term feelings of fatigue and significantly reduced HRV parameters of parasympathetic activity. Trait fatigue was negatively correlated with HRV during the second half of the vigilance task.

Conclusion: PMR alleviates short-term feelings of fatigue in persons with MS. The vigilance task in combination with HRV measurements may be helpful for evaluating relaxation procedures as a treatment of fatigue. Hereby, Future studies should ensure longer and more frequent relaxation exercises and focus on patients with weak to moderate fatigue.

Trial registration: Trial Registry: DRKS00024358.

Keywords: multiple sclerosis; fatigue; progressive muscle relaxation; biofeedback; autonomic nervous system; heart rate variability.

Introduction

Fatigue is one of the most frequent symptoms of Multiple Sclerosis (MS) [1] and is often defined as a subjective feeling of lack of physical and/or mental energy [2] that seriously interferes with a patient's daily activities. Furthermore, it is often followed by a feeling of discomfort and decreased motivation [3]. Literature on fatigue has made the distinction between two forms of fatigue: trait fatigue, indicated by the propensity to fatigue over an extended period of time, and state fatigue, which refers to the acute experience of fatigue [4]. Having sufficient energy to perform daily activities is paramount to an individual's well-being, and for that matter, fatigue can be the most distressing aspect of the disease [5-7].

Overall, knowledge toward an effective treatment of MS fatigue remains incomplete [8]. Commonly prescribed medications for decreasing MS fatigue like amantadine, modafinil, and methylphenidate are no longer advocated given that they were shown not to be more effective than placebo in improving MS fatigue [8]. Additionally, they cause adverse events more frequently [9], and are associated with long-term safety concerns [9].

Psychological interventions such as structured fatigue management programs, cognitive behavioral therapy, energy conservation interventions as well as a mindfulness-based intervention may be beneficial [10,11]. One important aspect of structured fatigue management programs (as a psychoeducational intervention) is learning and implementing relaxation techniques. A common type of relaxation technique, progressive muscle relaxation (PMR), is an exercise that includes voluntary stretching and relaxation of large muscle groups in the human body from hands to feet [12]. PMR has been found effective in reducing fatigue in MS patients at week 12, and its beneficial impact was still observed at follow-up, two weeks after the end of the intervention [13]. Other studies have similarly revealed a positive effect of PMR in decreasing MS fatigue [14,15]. In contrast, a study by Sander et al. (2020) [16] found that biofeedback-supported PMR did not end with an instant reduction of state fatigue in MS patients.

Exercise therapy seems to ease symptoms of fatigue in MS [17]. Similarly, a recent review illustrated that yoga practices ameliorate symptoms of fatigue in MS patients when compared with usual care [18] indicating a temporary increase in parasympathetic nervous system (PANS) activity. It remains however unclear which element of yoga practices led to decreases in fatigue, since most reviewed studies involved a synergistic combination of different components of the yoga practice (movement sequences, stretching, breathing,

meditation exercises). Thus, there is no distinct emphasis and specific research on one component or the other, limiting the interpretability of the results. Nevertheless, studies that integrated breathing techniques in the practice of yoga were significantly associated with decreased symptoms of fatigue [19-23], pointing to the beneficial effect of breathing exercises on MS fatigue.

Biofeedback may be used to increase the effectivity of relaxation training, as it informs about increases in PANS activity [24, 25]. In biofeedback learning, information on bodily functions is visualized on a screen to help the person learn how to modulate these functions [26]. Increases in heart rate variability (HRV) parameters of PANS activity have been observed in relation to biofeedback-assisted relaxation in patients with hypertension [27], as well as healthy participants [28]. Through enhanced parasympathetic control, biofeedback would also be expected to result in reduction of fatigue. However, a causal relationship cannot be yet assumed, as biofeedback could nevertheless activate other systems (cognitive or limbic) that may also be influencing PANS. Given that autonomic reactivity can be impaired in MS patients [29-31], it remains unclear if biofeedback can be used in these patients.

In the current study, we aim to use biofeedback to train two different relaxation strategies and to analyze their effect on MS-related fatigue. For that purpose, we first analyze whether an effect of deep breathing (DB) on PANS activity (the cardiovagal reflex) can be measured by changes in HRV, which has to be shown before using biofeedback as part of a treatment strategy. We then carried out a single-blind randomized controlled trial with two groups of MS patients. The first group participated in a deep breathing (DB) exercise whereas the second group practiced a variant of PMR treatment that has been linked with increased parasympathetic activity (based on the results of Sander et al, 2020) [17]. Both groups first learned to practice either of the relaxation strategies, then performed a vigilance task that was aimed to increase their current (state) feelings of fatigue. Shortly afterward the patients were requested to carry out the relaxation strategies that were aimed at alleviating their fatigue.

We measured subjective feelings of fatigue in response to the vigilance task and the relaxation exercises. We further explored the association between the feeling of fatigue and the biofeedback measures. For that purpose, we measured HRV throughout the fatigue-inducing task and after practicing the relaxation techniques.

Given the study design, we expected that the DB group would bring about higher recovery from fatigue than the PMR group after the fatigue-inducing vigilance task. We further expected that the vigilance task would be related with decreased PANS activity, whereas the relaxation exercises would increase PANS activity.

Methods

Participants

Forty MS patients fulfilling inclusion criteria were recruited at the Kliniken Schmieder in Constance (Baden-Württemberg, Germany) or were otherwise referred from self-help groups in Oldenburg (Niedersachsen, Germany) and surroundings. Participants were required to have a clinical diagnosis of MS according to the McDonald criteria [32], be older than 18 years, have a clinical diagnosis of MS, no relapse or corticosteroid use in the previous four weeks, no psychoactive medication use (such as noradrenergic antidepressants, modafinil and amantadine), and no MS-independent psychiatric disease. We have also asked for the presence of ischemic heart disease, lung disease affecting the ability to perform the deep breathing exercise to prevent adverse side effects. Available information on immunomodulatory drugs and disease progress was collected from the medical record (e.g., Expanded Disability Status Scale (EDSS) [33], disease duration). If this was not available, the Patient-reported Expanded Disability Status Scale was administered instead. Our participants reported whether they were using the following disease-modifying treatments: glatiramer acetate, teriflunomide, dimethyl fumarate, interferon beta-1a, ocrelizumab, natalizumab and fingolimod. The study was approved by the medical ethics committee of the University Medicine Oldenburg (UMO) (approval number 2020-152) and was registered in the German Clinical Trials Register (registration number DRKS00024358). Written informed consent was obtained from each participant before initiation of the study. Group characteristics are summarized in Table 1.

Table 1

Characteristics of the patient groups

	Total sample	**DB group**	**PMR group**
Sex (female/male)	(23/11)	(10/7)	(13/4)
MS course (RRMS/PPMS/SPMS)	(21/8/5)	(9/4/4)	(12/4/1)
Recruiting place (Oldenburg/Konstanz)	(14/20)	(6/11)	(8/9)
	M ± SD	***M ± SD***	***M ± SD***
Age (years)	46.2 ± 12.5	47.4 ± 13.9	44.9 ± 11.3
Disease duration	12.3 ± 8.2	12.1 ± 9.3	12.4 ± 7.2
Expanded Disability Status Scale	3.4 ± 1.8	3.6 ± 1.4	3.1 ± 2.1
Multiple Sclerosis Functional Composite	-2.0 ± 2.5	-2.3 ± 1.9	-1.8 ± 3.0
Beck Depression Inventory	10.5 ± 6.7	9.8 ± 5.3	11.2 ± 8.0
Pittsburgh Sleep Quality Index	6.2 ± 3.6	6.2 ± 3.9	6.2 ± 3.4
Epworth Sleepiness Scale	9.1 ± 3.8	9.3 ± 3.9	8.8 ± 3.8
Fatigue Scale for Motoric and Cognition	72.7 ± 13.7	75.5 ± 15.9	69.8 ± 10.9
State fatigue pre vigilance task	32.4 ± 18.8	33.1 ± 14.6	31.8 ± 22.7
State fatigue post vigilance task	56.9 ± 20.4**	56.2 ± 21.1*	57.6 ± 20.4*
State fatigue post relaxation	43.3 ± 24.9**	47.5 ± 24.9	39.0 ± 24.9*

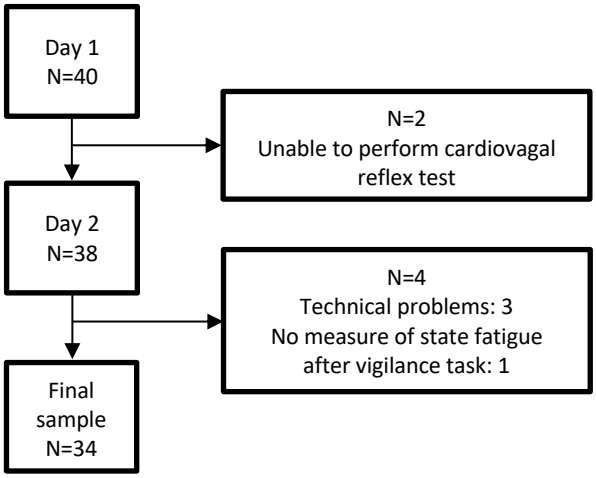
Note. DB = deep breathing; MS = multiple sclerosis; PMR = progressive muscle relaxation; PPMS = primary progressive MS; RRMS = relapse-remitting MS; SPMS = secondary progressive MS. No statistically significant differences between the two intervention groups.

*$p < .05$; **$p < .01$ for intragroup comparisons.

After the drop out of 6 patients the final study group consisted of 34 MS patients (see Figure 1 for drop out reasons). The PMR group and the DB group consisted of 17 participants each.

Figure 1

Flow-chart illustrating the dropouts



Design

The study was a single-blind, randomized controlled trial with two arms: (a) the DB group, performing the deep breathing exercise, (b) the PMR group, being instructed to relax muscles on distal body parts, involving the muscles of the arms, shoulders, legs and feet. This study was designed (a priori) as a superiority trial with a null effect expected on the PMR group, based on the findings from Sander et al [17]. Subjects were assigned to either of the groups by a computer-generated simple block randomization method. An investigator that was not involved in data collection prepared the assignment of participants in A4 paper sheets that were twice folded in half. The main investigator knew which group a participant belonged to by opening a paper sheet with a label "true" (referring to the intervention group, that is, DB) or "false" (referring to the active control group, that is, PMR), indicating the allocation group, so as to prevent "selection bias." In addition, the main investigator kept participants unaware of group allocations.

Procedure

The assessments and the relaxation exercises were performed in a quiet room at two different times on the same day. An evaluation of the ANS by means of HRV was carried out during the whole procedure. Before each task, the instructions were read out loud to the participant from a protocol sheet. See Figure 2 for CONSORT diagram that represents the participant flow through the trial.

In the first session, which took place in the morning, participants filled out questionnaires, followed by two cardiovagal reflex tests (reflected by HRV levels in response to DB). Participants rested for a minute before the cardiovagal reflex test. The test involved participants sitting quietly and being instructed to breathe slowly and smoothly at a frequency of five cycles per minute (five seconds of inhalation and five to eight seconds of exhalation). The first assessment of HRV was obtained during baseline condition with normal breathing. Participants were then instructed to carry out the cardiovagal reflex test at three separate consecutive times. The first test was meant as a learning session. HRV was subsequently recorded during the second and third cardiovagal reflex tests. Afterwards, the investigator checked the group allocation of the participant and trained participants to the DB or PMR exercise (on distal body parts), for the purpose of familiarizing them with the task.

MS patients in the DB group were instructed to breathe continuously through the nostrils and with the mouth closed, with respiratory movements that should resemble a wave. They were also advised not to take sudden inhalations/exhalations or hold the breath to prevent hyperventilation. During the biofeedback sessions, participants were instructed to visualize their breathing rate and breathing depth on a computer screen.

MS patients in the PMR group were instructed to increase muscle tension in distal body parts vigorously for about 20 seconds each, but without really straining them, and then release this tension for separate muscles of those body parts. They were directed to rest on their back and concentrate on their body. Participants needed to tense up and release each distal body part three times consecutively. Specifically, participants were directed to tightly clench the right fist, bend their right arm, stretch their right arm by extending it, lift their shoulders as if they could touch their ears, tighten their thighs by squeezing their two knees together as if they were holding a coin between them and to flex their feet and pull their toes toward themselves and feel the tension in their calves.

The PMR exercise did not involve muscles of the face and neck, and abdomen, as participants had to wear a protective face mask and a respiration sensor over the abdomen. Inhalation and exhalation patterns were displayed on the computer screen. Lastly, participants of both groups were asked not to smoke, drink coffee or black tea for at least two hours prior to the second session. The first session lasted approximately 90 minutes.

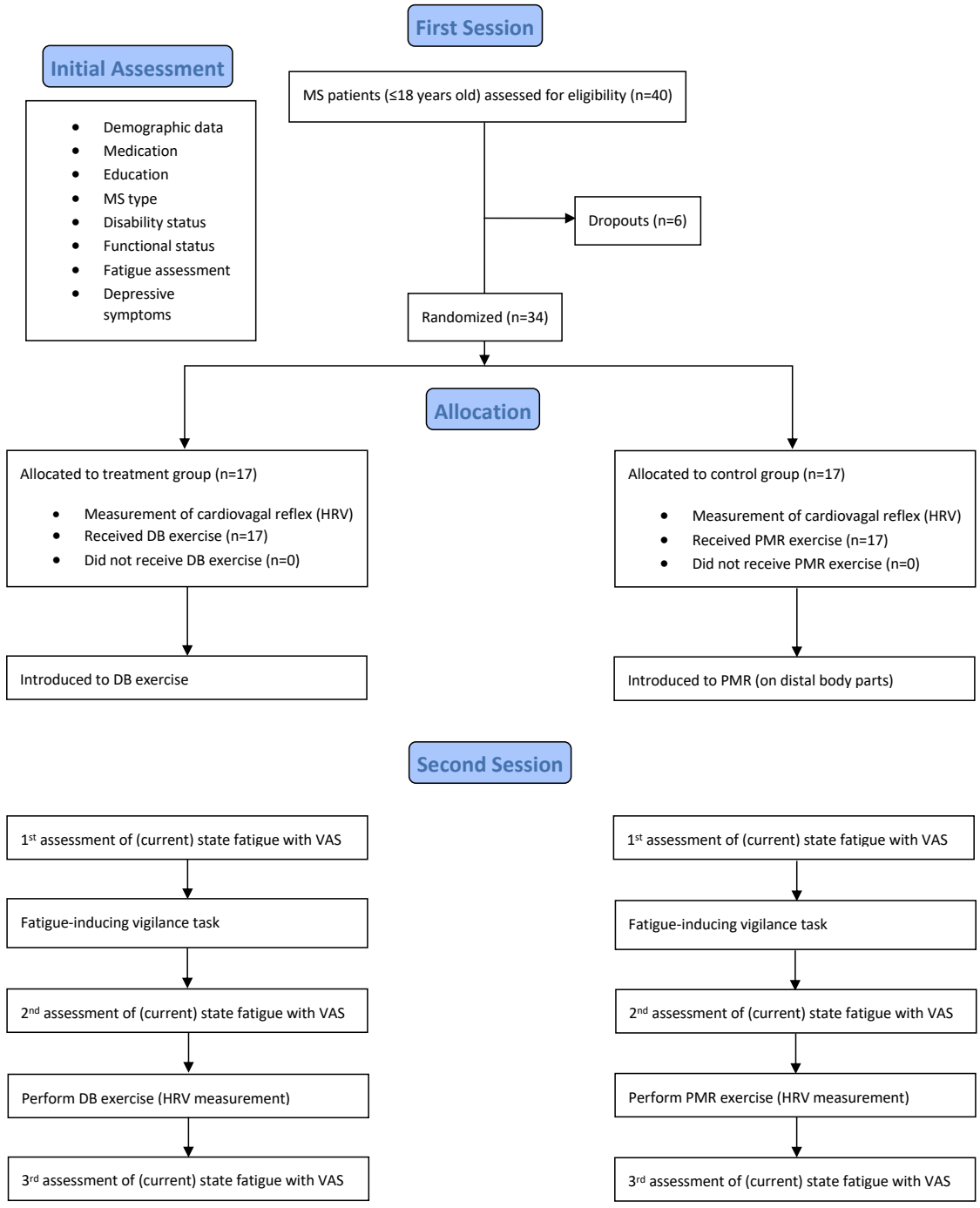
The second session, which took place in the afternoon, on the same day as the first session, started with the rating of current (state) fatigue. Afterwards, participants performed

a fatigue-inducing and monotonous acoustic vigilance task from the Test Battery for Attentional Performance (TAP) [34] lasting 30 minutes. Current (state) fatigue levels were assessed again. Afterwards, participants performed the DB or PMR exercise for five minutes, followed by a five-minute break.

Both relaxation exercises began with a short resting period that gave patients time to relax. Before starting each procedure, the investigator made sure heart rate was not trending up by ensuring a private and quiet experimental setting. As participants performed the exercise, they could visualize their respiration pattern and heart rate on a computer screen. Then, the current level of fatigue was rated for the last time to assess whether the relaxation exercises would positively impact fatigue recovery after the vigilance task. The second session lasted approximately 60 minutes.

Figure 2

CONSORT diagram showing the flow of participants throughout the trial



Assessment

The primary outcome, current (state) fatigue level, before the vigilance task, after the vigilance task, and after the relaxation exercise, was assessed with a visual analog scale (VAS). The Fatigue Scale for Motor Skills and Cognition (FSMC) was used as a measure of cognitive and motor trait fatigue in people with MS [35]. To assess depressive symptoms, the Beck

Depression-Inventory (BDI) was administered [36]. Additionally, we applied the Patient-Reported Expanded Disability Status Scale (PREDSS) to quantify disability [37], and the Multiple Sclerosis Functional Composite (MSFC) [38] to assess clinical status. All three subtests of the MSFC were administered, that is, the Timed 25-Foot Walk (T25W) for assessing leg function, 9-Hole Peg Test (9HPT) for assessing arm and hand function, and the Paced Auditory Serial Addition Test (PASAT-3) for cognitive function [38]. In addition, the assessment of sleep quality and daytime sleepiness was carried out by the Pittsburgh Sleep Quality Index (PSQI) [39] and the Epworth Sleepiness Scale (ESS) [40], respectively.

We chose the monotonous low event rate vigilance task of the TAP as it is an experimental task that systematically triggers fatigability and was shown to be correlated with MS-related fatigue [41] due to the demanding nature of the sustained attention induced by the task. Beeps of 440 and 1000 Hz were played alternatingly with an inter-stimulus interval of 1.3 seconds. On 24 occasions another stimulus of the same frequency was presented consecutively, and the participant had to press a button immediately after detecting such a beep. A two-minute practice session was administered prior to the task.

Heart rate was measured with the Blood Volume Pulse Sensor of the NeXus-4 Biofeedback-System of Hasomed. A sensor was placed on the index finger of the non-dominant hand of the patient. We chose to apply the Blood Volume Pulse Sensor Sensor rather than a standard electrocardiography (ECG) because it is a more convenient, quicker to install and less intrusive method than a standard ECG. Breathing rate and relative depth of abdominal or thoracic breathing were measured with the Respiration Sensor of the NeXus-4 Biofeedback-System of Hasomed, provided with an adjustable elastic band worn over the abdomen and over clothing.

The beginning and end of the vigilance task, the cardiovagal reflex test and its corresponding baseline preceded the test, and the relaxation exercises were marked with the NeXus Biotrace Software. Obtained interbeat (RR) intervals were further processed by using Kubios HRV Standard analysis software (version 3.5.0). Pre-processing was done with the threshold-based artefact correction algorithm. Artefacts were identified in the data and the most suitable correction level (being the lowest possible to avoid overcorrection of the data) was selected. A medium threshold (0.25 s) artefact reduction was applied. The identified artefacts were replaced by interpolated values.

Based on previous results [42], we focused on specific measures in the HRV frequency-domain and time-domain which reflect PANS activity [43], that is, the root mean square of successive differences between normal heartbeats (RMSSD), and standard deviation of all interbeat intervals (SDNN).

Statistical Analyses

Statistical analyses were performed with SPSS 25 [44]. We checked the data for normal distribution with the Shapiro–Wilk test, and for Equality of Variances with Levene's test. The Greenhouse-Geisser correction was used when the assumption of sphericity was violated for repeated measures analyses of variance. Differences between the intervention groups were compared using a t-test. Pearson correlation was used to analyze the relation between HRV data, demographic and baseline data and trait and state fatigue levels. For post hoc testing, we used the Bonferroni correction for multiple comparisons.

Effect of the vigilance task and the relaxation group on state fatigue: We further tested the impact of the vigilance task and the relaxation intervention on current (state) fatigue, and whether any changes are modulated by the relaxation group. Hereby, Time (comprised by baseline state fatigue, state fatigue after the vigilance task and state fatigue after the relaxation intervention) served as the within-subjects variable, and Group (DB or PMR) served as the between-subjects variable.

Effect of deep breathing on heart rate variability: A one-way repeated measure analysis of variance (ANOVA) was conducted to analyze whether the cardiovagal reflex tests brought about expected changes in HRV parameters. Hereby, Time (comprised by baseline, first cardiovagal reflex test and second cardiovagal reflex test) served as the within-subjects variable on mean levels of HRV parameters (SDNN and RMSSD).

Effect of the vigilance task on heart rate variability: Another one-way repeated measures ANOVA served to test whether performing the vigilance task affected HRV levels. Hereby, Time (first half of the vigilance task, second half of the vigilance task) served as the within-subjects variable on mean levels of HRV parameters (SDNN and RMSSD).

Effect of the vigilance task on reaction time: To test whether practicing the relaxation techniques affected HRV levels, Time (second half of vigilance task and post relaxation exercise) served as the within-subjects variable, and Group (DB or PMR) served as the between-subjects variables on mean levels of HRV parameters (SDNN and RMSSD).

Effect of relaxation exercises on heart rate variability: Lastly, we tested whether reaction time changed throughout the vigilance task. Hereby, Time (first half of vigilance task and second half of vigilance task) served as the within-subjects variable, and Group (DB or PMR) served as the between-subjects variables on mean levels of reaction time (in milliseconds).

Results

Relaxation group characteristics

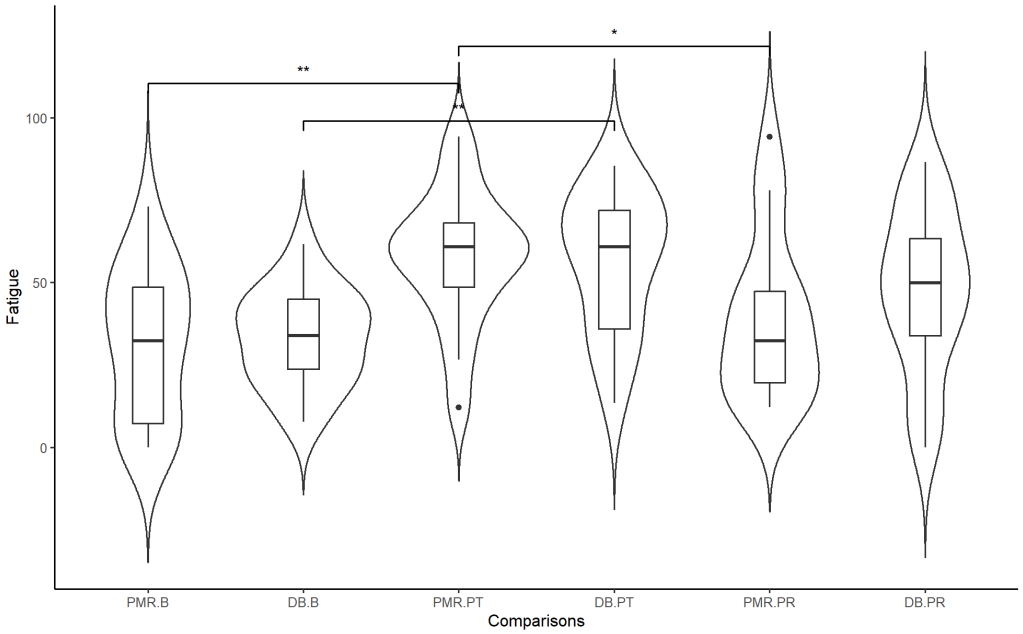
The DB and PMR groups did not differ in age, disease duration, disease course, functional (MSFC) and disability (EDSS) status, fatigue level (FSMC), depression (BDI), daytime sleepiness (ESS), and sleep quality (PSQI).

Effect of the vigilance task and the relaxation intervention on state fatigue

The relaxation groups did not differ on state fatigue during baseline, before the vigilance task began. A main effect of Time was found on state fatigue [$F(2,64) = 23.172$, $p = < .001$]. Post hoc independent t-test revealed that experienced state fatigue increased significantly after performing the vigilance task when compared to baseline [$\Delta = 24{,}456$, $p = < .001$], and decreased after carrying out the relaxation exercise [$\Delta = -13.597$, $p = .004$]. In this respect, the PMR exercise led to a statistically significant drop in state fatigue [$\Delta = -18.561$, $p = .005$], while the DB exercise did not (Figure 3, Table 2). However, there was no significant Group effect or Group * Time interaction.

Figure 3

Violin plot showing density data of state fatigue at baseline, after the vigilance task, and after the relaxation exercise for the two relaxation groups



Note. PMR.B = during baseline in the progressive muscle relaxation group; DB.B = during baseline in the deep breathing group; PMR.PT = after vigilance test in the progressive muscle relaxation group; DB.PT = after vigilance test in the deep breathing group; PMR.PR = after relaxation exercise in the progressive muscle relaxation group; DB.PR = after relaxation exercise in the deep breathing group.

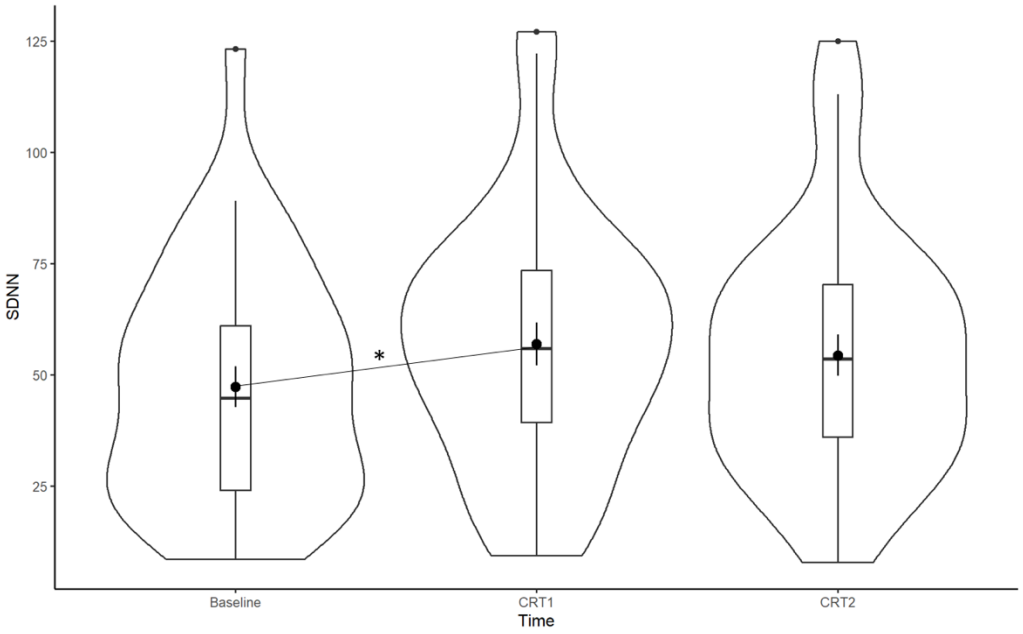
*p < .05; **p < .01.

Effect of deep breathing on heart rate variability

A main effect of Time was found on SDNN [F(1.336, 64) = 4.004, p = .041]. SDNN was higher during the first cardiovagal reflex test, followed by the second cardiovagal reflex test and during the baseline (Figure 4). RMSSD did not differ statistically significantly between Time points.

Figure 4

Violin plot showing density data of SDNN at baseline, during the first cardiovagal reflex test, and during the second cardiovagal reflex test



Note. CRT1 = first cardiovagal reflex test; CRT2 = second cardiovagal reflex test.

*$p < .05$.

Effect of the vigilance task on heart rate variability

No main effect of Time was found on SDNN and on RMSSD for the vigilance task.

Effect of the vigilance task on reaction time

A main effect of Time was found on reaction time [F(1,30) = 15.273, p = < .001]. Reaction time was higher during the second half of the vigilance task than during the first half of the vigilance task (Table 2).

Effect of relaxation exercises on heart rate variability

There was a main effect of Time on SDNN [F(1,32) = 6.612, p = .015]. Post hoc analysis showed that patients had significantly higher SDNN levels following the relaxation exercise than prior to it [Δ = 11.465, *p* = .015] (Table 2). A main effect of Time was not found on RMSSD. Post hoc analysis showed that patients also had significantly higher RMSSD levels because of the relaxation exercise than prior to it [Δ = 14.101, *p* = .003]. There was no significant Group effect or Group * Time interaction, demonstrating no significant difference between the DB and PMR groups in their HRV levels following the relaxation exercise.

Table 2

State fatigue, SDNN and RMSSD for the treatment groups

	DB group	PMR group
	M ± SD	***M ± SD***
State fatigue: after vigilance task	56.21 ± 21.18	57.64 ± 20.41
State fatigue: after intervention	47.58 ± 24.94	39.08 ± 24.92*
SDNN: during vigilance task	44.63 ± 22.09	47.68 ± 31.20
SDNN: after intervention	53.42 ± 31.85*	61.82 ± 46.89*
RMSSD: during vigilance task	45.91 ± 26.54	49.72 ± 33.37
RMSSD: after intervention	56.63 ± 38.49	54.77 ± 32.54
Reaction time: first half vigilance task	781.87 ± 312.41	676.43 ± 135.65
Reaction time: second half vigilance task	851.21 ± 377.68	749.31 ± 147.93

Note. DB = deep breathing; PMR = progressive muscle relaxation. *p < .05 for intragroup comparisons.

Sub-group analysis

Autonomic disorders and HRV may differ according to disease course in MS [45], therefore MS disease course specific subgrouping is of interest. Table 1 showed that 21 MS patients with relapse-remitting disease course were included in the study, 8 with primary progressive, and 5 with secondary progressive. The small number of progressive patients in each group did not allow for sub-group analyses. In RRMS patients, deep breathing elicited an increase in SDNN levels [F(1.436) = 5.420, p = .017]. Post hoc testing showed that both interventions differed significantly from baseline SDNN. However, there was no effect of deep breathing on RMSSD.

Vigilance testing induced higher levels of fatigue, that dropped significantly after PMR (using intragroup comparison), and not after DB. The relaxation interventions increased SDNN [F(1,19) = 12.221, p = .002] and RMSSD levels [F(1,19) = 7.302, p = .014], but there were no significant group effects for both HRV parameters.

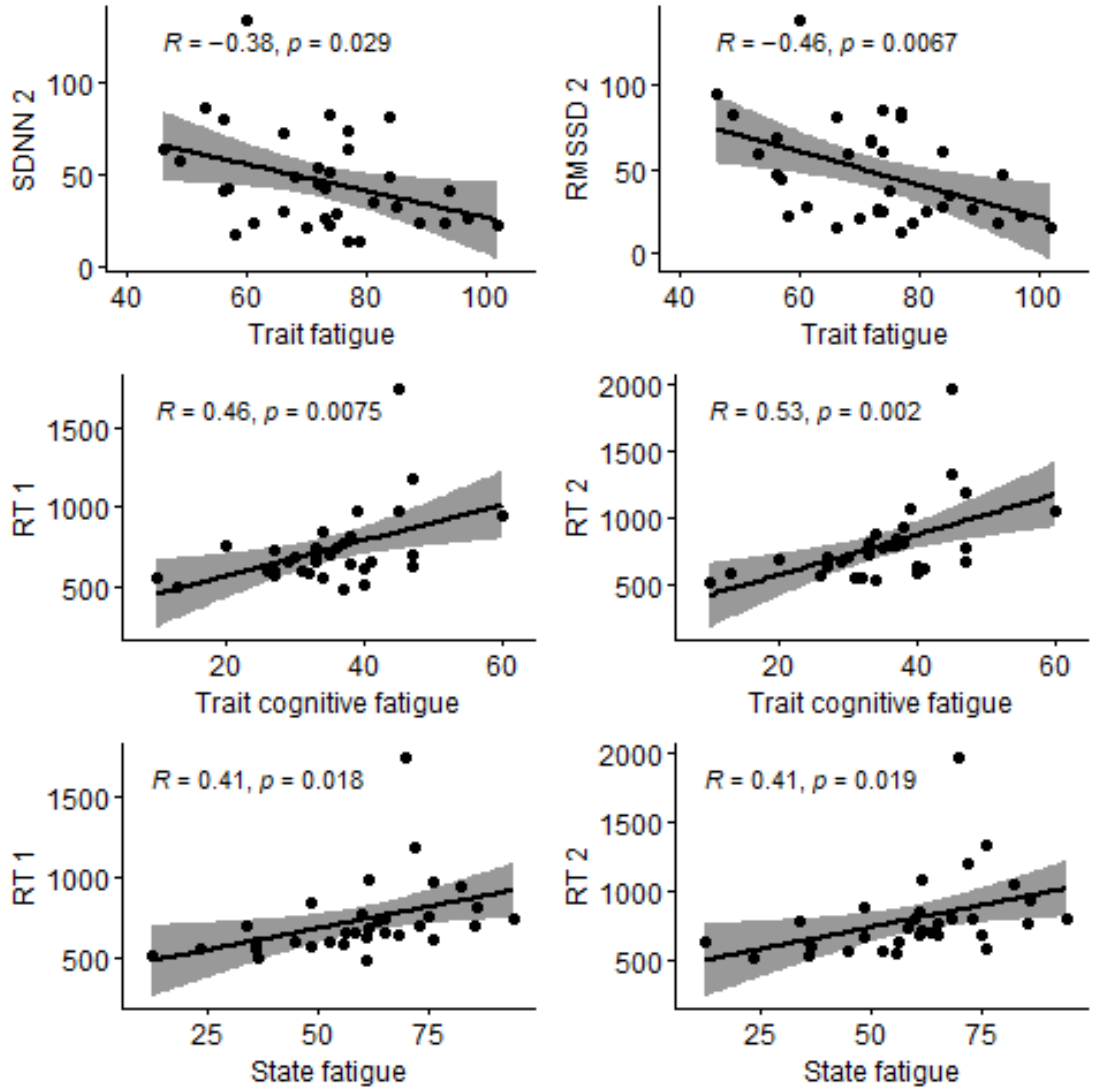
Correlations

General trait fatigue negatively correlated with SDNN (r = -0.375, p = .029) and RMSSD (r = -0.456, p = .007) during the second half of the vigilance task. Moreover, trait cognitive fatigue positively correlated with *median* reaction time during the first half of the vigilance task (r = 0.462, p = .008), and during the second half of the vigilance task (r = 0.532, p = .002). Similarly, state fatigue following the vigilance task positively correlated with median reaction

time during the first half of the vigilance task (r = 0.415, p = .020), and during the second half of the vigilance task (r = 0.415, p = .020).

Figure 5

Correlation plots



Note. RT = reaction time; 1 = first half of the vigilance task; 2 = second half of the vigilance task.

Discussion

The aim of the study was to investigate whether one of two different biofeedback-supported relaxation exercises (DB versus PMR) can alleviate MS related fatigue and whether changes of current (state) fatigue correlate with alterations in the autonomic nervous system. As a corollary, we also investigated if biofeedback interventions would elicit changes of ANS

activity in MS patients. We found that (1) changes in biofeedback-supported autonomic nervous system activity induced by deep breathing can be objectively measured through HRV; (2) performing a vigilance task induces state fatigue, (3) increasing the feeling of fatigue through the vigilance task is associated with decreased parasympathetic components of HRV and recovering from fatigue is associated with a subsequent increase in these components, (4) there is some evidence that PMR may be beneficial for fatigue recovery after the vigilance task, while DB is not.

There is evidence of autonomic dysfunction in MS patients with fatigue, more specifically, altered HRV [45]. Specifically, patients with high levels of fatigue have been found to exhibit greater autonomic dysfunction, including a reduced HRV that reflects parasympathetic activity. These has been shown by Sander et al. [16], whereby the group with high fatigue showed significantly lower SDNN and pNN50 scores than the group with weak to moderate fatigue. Moreover, Flachenecker et al. (2003) [46] found that a pattern of sympathetic dysfunction was more pronounced in patients with MS-related fatigue than in those with MS without fatigue. The grouping of patients with relapsing-remitting and progressive MS subtypes, the majority being relapsing-remitting, may have limited our results, as the burden of autonomic dysfunction has been shown to be higher in progressive MS patients [29-31]. Another limitation is the lack of knowledge on whether subjects had plaques in brain regions known to influence heart rate variability. Nonetheless, autonomic dysfunction was not the focus of our study – but rather the possibility to apply biofeedback to alleviate fatigue.

Our first goal was to show that in the case where an autonomic dysfunction may be present in MS patients, biofeedback could still be applied to improve teaching relaxation strategies and may help balancing autonomic activity in patients with an autonomic dysfunction. We found that MS patients show the expected autonomic response during the cardiovagal reflex test (i.e., after carrying out deep breathing exercises) (Figure 3). That is, SDNN levels significantly increased while stimulating the cardiovagal reflex. This is consistent with a previous study showing that deep breathing results in increased short-term levels of SDNN along with intensified feelings of relaxation compared with spontaneous breathing [47]. The cardiovagal tests lasted a minute and not the conventional recording of 5 minutes because the practice of deep breathing may be rather challenging for a longer amount of time, and shorter recording periods of 10 s, 30 s, and 60 s for RMSSD [48-50] and SDNN [48]

have been considered adequate**.** The absence of an effect on RMSSD during the cardiovagal reflex test may be explained by the shorter duration for recording than the conventional minimum period of 5 minutes [40]. We conclude that biofeedback exercises may be advised for MS patients as a means of relaxation.

Studies showing behavioral indicators of feelings of fatigue are few [41, 51]. Hanken et al. (2015) [41] emphasized in their review that fatigue can only be measured behaviorally by applying sustained cognitive tasks assessing alerting or vigilance. Vigilance tasks draw attention away from the environment, eliciting mind wandering. According to the model of Hanken et al. 41, 51], fatigue starts to interfere with processes that should be directed toward the environment especially during such monotonous tasks.

In this respect, our results demonstrate that the vigilance task can be reliably used to induce fatigue (Table 1, Figure 4). Performing the vigilance task led to a highly significant increase in fatigue. This is in line with the study of Sander et al. (2020) [16] in which the vigilance task also led to increased feelings of fatigue. The notion that the vigilance task is suitable for inducing fatigue (by testing the immediate effects of relaxation groups on levels of fatigue) is endorsed by yet other findings from our study: Both trait and state fatigue correlated with a decreased *performance* (that is, a longer reaction time) during the vigilance task.

An attempt was made to measure autonomic nervous system activity in relation to mental strain during the vigilance task, by investigating whether HRV indices would change when participants are engaged in the task. We found that SDNN during the second half of the vigilance task was negatively associated with trait fatigue. We also found evidence that changes in fatigue correlate with alterations in the autonomic nervous system. SDNN increased significantly after recovery from the vigilance task and there was a marginally significant increase of RMSSD (Table 2). We have recently argued that HRV might be related to the feeling of fatigue in MS patients, due to vagal nerve signaling that controls heart rate and processes bodily inflammation to the brainstem and the hypothalamus, thus eliciting a mild form of "sickness behavior," which encompasses fatigue as one of its core symptoms [45]. In this study we show that recovery from fatigue is associated with changes in HRV and/or vice versa, especially for relapsing-remitting patients, as the progressive MS groups were too small for a group specific analysis. Granting that this observation does not allow any

causal interpretation, it still allows to speculate about the possibility of a relationship between vagal activity and subjective experience of fatigue.

The primary goal of our study was to compare two different biofeedback groups and their effect on fatigue in MS patients. Apparently, only PMR significantly improved patients' fatigue level, while DB did not. We can only speculate as to why fatigue effectively decreased after PMR, and not after DB. Following the model of Hanken et al. (2014, 2015) [51, 41], fatigue draws attention to internal states and interferes with externally directed attention as soon as the focus of attention is allowed to wander. When the focus of an individual's attention is predominantly allocated to fatigue, it becomes difficult to re-orient the attention again to external events. Now, a common characteristic of both relaxation techniques is that fatigue induced by the vigilance task could be alleviated by introducing a new focus of attention on bodily processes. However, both relaxation strategies are supposed to direct inward attention to the body. During the PMR exercise, distal body parts were likely the focus of attention, whereas DB may have directed the focus to the homeostatic state and autonomic functioning. In this behalf, PMR might have a higher potential to detract attention away from the feeling of fatigue.

Other investigators have also reported a positive effect of PMR in reducing fatigue for in MS [52] and in other chronic diseases such as chronic obstructive pulmonary disease [53, 54]. However, our goal was not to document a global effect of biofeedback on fatigue (as in almost all studies conducted on this topic), but to propose a short-term strategy for decreasing fatigue by refocusing attentional control, that could be implemented at any point during the day. Moreover, it should be taken into consideration that a direct comparison of state fatigue did not reveal any significant results in the PMR and DB groups, but only in intra-group comparisons of state fatigue. Therefore, attempts to explain possible differences between DB and PMR for controlling fatigue appear to be premature given the current empirical evidence.

The average level of fatigue of our total group was severe with an average FSMC total score of 72.7 ± 13.7, and only 9 patients scored below the cut-off score for severe trait fatigue. In the study by Sander et al. (2020) [16], PMR therapy enhanced parasympathetic nervous system activity, and these mainly concerned patients with weak to moderate levels of fatigue and not those with severe fatigue. A review on the efficacy of relaxation interventions indicated that these practices are not effective in patients with severe forms of several

psychological disorders such as depression/dysthymia, anxiety disorders, alcoholism, and sleep disorders [55]. Including patients that suffer from lower levels of experienced fatigue might provide stronger results.

We measured state fatigue severity with a visual analog scale and did not track how long it took for fatigue to completely return to baseline levels. Visual analog scales are more sensitive to changes in fatigue states than fatigue questionnaires [56]. However, at the same time, they focus on short-term changes, which include spontaneous and reactive fluctuations like increasing tiredness, whereas fatigue questionnaires are designed to measure an enduring mental state, which per definition should be roughly related to environmental strain. Therefore, our results need to be replicated in a treatment study investigating the effect of learned strategies of short-term relaxation on fatigue that is being measured with questionnaires.

Drawbacks of our study are the small sample size, the lack of a healthy control group, and that only one session for instruction of the relaxation procedure was implemented. Future studies with a similar approach should include longer and more frequent PMR and DB exercises and focus on patients suffering from weak to moderate fatigue to uncover possible effects of DB on MS-fatigue.

To sum up, we illustrate that engaging in PMR exercises may help alleviate symptoms of fatigue in persons with MS. If this holds true, short breaks of PMR after situations that evoke high fatigue during the day may help patients to recover faster and to decouple from the expectation of fatigue and the self-restriction in executed activities leading to a vicious circle of further deconditioning. Integrating short breaks of PMR may potentially improve their quality of life. Within this framework, using a vigilance task in combination with a measurement of HRV may help evaluate relaxation procedures as a treatment of fatigue.

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6.7 Study IV

Full bibliographic reference:

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The content of this chapter is identical to the submitted manuscript.

Abstract

Fatigue is a debilitating symptom in multiple sclerosis (MS) associated with autonomic nervous system (ANS) dysfunction. Heart rate variability (HRV) provides a non-invasive measure of ANS function; yet the relative sensitivity of linear and nonlinear HRV parameters in capturing specific ANS (dys)function remains unclear. This study investigated the factorial structure of HRV parameters, their response to biopsychological interventions, and their relationship with MS-related fatigue.

Data from two clinical trials (*N* = 78) were pooled. Exploratory factor analysis (EFA) was applied to time-, frequency-domain, and nonlinear HRV parameters. Repeated-measures ANOVAs examined the effects of self-alert training (SAT; activating), deep breathing (DB; relaxing), and progressive muscle relaxation (PMR; intermediate) on HRV, controlling for age, depression, and disability status. Subsequent regression analyses investigated whether HRV changes during a vigilance task predict MS-related fatigue.

EFA revealed two factors, reflecting parasympathetic (PNS) or sympathetic (SNS) activity. SAT decreased HRV parameters loaded into both factors, DB increased parameters related to SNS activity, and PMR affected both factors. Reduced SDNN change during the vigilance task predicted higher trait fatigue, whereas state fatigue was not associated with HRV. These findings highlight HRV as a sensitive marker of coordinated PNS-SNS activity and reduced autonomic adaptability in MS-related trait fatigue.

Keywords: multiple sclerosis, fatigue, autonomic nervous system, heart rate variability, nonlinear HRV, exploratory factor analysis

Fatigue is a debilitating symptom of multiple sclerosis (MS; Fisk et al., 1994) and belongs to the most frequently reported symptoms (Ayache & Chalah, 2017). It likely has multiple causes, one of which may be sustained inflammatory processes as part of the autoimmune background of MS. This means that MS-related fatigue can be considered a chronic manifestation of sickness behavior resulting from persistent systemic inflammation (Dantzer, 2001; Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Hanken et al., 2014; Zielinski, Systrom, & Rose, 2019). Sickness behavior involves autonomic nervous system (ANS) dysfunction, which is believed to interfere with ANS regulation and may ultimately impair physiological adaptability (Coyle et al., 2006; Crosswell, Lockwood, Ganz, & Bower, 2014; Haase & Linker, 2021; Kim et al., 2020; Provan et al., 2018; Williams et al., 2019).

Heart rate variability (HRV) is essential for ANS' capacity to adjust to internal and external stimuli (McCraty & Shaffer, 2015; Shaffer & Ginsberg, 2017). It is influenced by the dynamic interplay between the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS), with the former promoting restorative processes and the latter preparing the body for action (McCraty & Shaffer, 2015). Mathematical analysis of HRV is therefore a prevalent, non-invasive technique for evaluating ANS activity (Shaffer & Ginsberg, 2017).

Linear models remain the most widely used approach for HRV analysis. These were comprehensively reviewed by Sammito and Böckelmann (2015), Shaffer and Ginsberg (2017), and the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Time-domain parameters include the root mean square of successive differences (RMSSD) between normal heartbeats, and the standard deviation of the interbeat intervals of normal sinus beats (SDNN). RMSSD is calculated by taking the square root of the mean of the squared differences between successive normal-to-normal (NN) intervals (Berntson et al., 1997; Eckberg, 1983; Wolf et al., 1978). SDNN reflects the standard deviation of all NN intervals (Shaffer & Ginsberg, 2017; Task Force, 1996). In the frequency domain, high-frequency (HF) power is computed as the spectral power within the 0.15-0.40 Hz band of the NN interval time series (Akselrod et al., 1981; Bigger et al., 1988; Kleiger et al., 2005; Pomeranz et al., 1985; Task Force, 1996), typically using Fast Fourier Transform (FFT) or autoregressive modeling. Low-frequency (LF) power is analogously calculated as the spectral power within the 0.04-0.15 Hz band of the NN interval time series (Billman, 2013; Brennan et al., 2001; Reyes del Paso et al., 2013; Tulppo et al., 1996). LF/HF, a ratio-based frequency domain parameter, is calculated by dividing LF power by HF power (Sammito & Böckelmann, 2015; Task Force, 1996).

For nonlinear HRV analysis, the standard deviation of instantaneous beat-to-beat variability (SD1), as derived from Poincaré plot analysis, is computed as the standard deviation of NN intervals perpendicular to the line of identity (Malliani et al., 1991; Pagani et al., 1986; Stein and Reddy, 2005).

The standard deviation of long-term HRV (SD2) is also derived from the Poincaré plot and computed as the standard deviation along the line of identity, representing long-term HRV (Malliani et al., 1991; Pagani et al., 1986; Stein and Reddy, 2005). The ratio of short- to long-term variability (SD1/SD2) is calculated by dividing SD1 by SD2 (Malliani et al., 1991; Pagani et al., 1986; Stein and Reddy, 2005). Detrended fluctuation analysis (DFA) α1, another nonlinear HRV parameter, is computed using detrended fluctuation analysis to determine the short-term fractal scaling exponent (typically over 4-16 beats) of the NN interval time series (Peng et al., 1995; Sassi et al., 2015; Shaffer & Ginsberg, 2017). The DFA α2, which is also derived through detrended fluctuation analysis, indicates the long-term scaling exponent over periods longer than 16 beats (Sammito & Böckelmann, 2015; Sassi et al., 2015; Shaffer & Ginsberg, 2017). Sample entropy (SampEn) is computed as the negative natural logarithm of the conditional probability that two similar sequences of NN intervals remain similar after the addition of one point (Sammito & Böckelmann, 2015). Approximate entropy (ApEn) estimates the regularity and unpredictability of NN interval fluctuations using a similar but less robust algorithm (Sammito & Böckelmann, 2015).

Previous research has shown that HRV is modulated by various factors, with the primary components of top-down autonomic control being PNS and SNS activity. Although HRV cannot provide a direct measurement of PNS or SNS activity, it offers valuable indirect insights. In this context, RMSSD, SDNN, HF, SD1, and SD2 are the most effective parameters due to their substantial correlation with vagal modulation and their responsiveness to fluctuations in PNS (Akselrod et al., 1981; Berntson et al., 1997; Bigger et al., 1988; Eckberg, 1983; Kleiger et al., 2005; Malliani et al., 1991; Pomeranz et al., 1985; Shaffer & Ginsberg, 2017; Task Force, 1996; Wolf et al., 1978). In terms of nonlinear parameters, SD1 captures short-term, vagally mediated HRV (Malliani et al., 1991; Pagani et al., 1986; Stein and Reddy, 2005). SD2 has been shown to correlate with vagal tone at rest or mildly stressful states (Malliani et al., 1991; Pagani et al., 1986; Stein and Reddy, 2005), whereas SD1/SD2 provides a more detailed analysis of the balance between short- and long-term variability, and has been used as an indicator of cardiovascular adaptability influenced by PNS regulation (Malliani et al., 1991; Pagani et al., 1986; Stein and Reddy, 2005). LF power has been predominantly linked to SNS activation during stress-related states (Billman, 2013; Brennan et al., 2001; Reyes del Paso et al., 2013; Tulppo et al., 1996). The LF/HF ratio is commonly used to assess sympathovagal balance. Higher values indicate increased SNS dominance. However, its interpretation remains complex (Sammito & Böckelmann, 2015; Task Force, 1996), as is the case for other parameters as well. The parameters LF, DFA α1, DFA α2, SampEn and ApEn have been interpreted as reflecting SNS activity, particularly under conditions of stress or cognitive load (Sammito & Böckelmann, 2015; Sassi et al., 2015; Shaffer & Ginsberg, 2017).

To date, most studies investigating HRV as an indicator of ANS activity have relied on linear parameters. Although there has been an increase in studies that combine linear and nonlinear analytic approaches systematically, their respective associations with PNS and SNS remain unclear in many respects (Sammito & Böckelmann, 2015; Young and Benton, 2015). There is a lack of research that has systematically altered ANS status through experimental interventions in order to gain a better understanding of how HRV parameters relate to PNS and SNS activity (Agorastos et al., 2023; Quintana and Heathers, 2014). Most comparative studies which have systematically altered ANS status were aimed at testing ANS response of patients with MS and healthy controls (HC) to orthostatic stress conditions, by employing a head-up tilt test (Frontoni et al., 1996; Garis et al., 2022; Studer et al., 2017; Vlcek et al., 2018) or sit-to-stand transition (Rzepiński et al., 2021).

Biopsychological interventions, such as deep breathing (DB), progressive muscle relaxation (PMR), self-alert training (SAT), and also vigilance tasks have been shown to modulate ANS activity (Gervasoni et al., 2018; Sander et al., 2020), yet they have been applied only rarely in experimental research. This is unfortunate because these interventions target specific branches of the ANS, providing a framework for assessing ANS (re)activity in MS. DB involves slow and smooth breathing, at a frequency of five cycles per minute, consisting of five seconds of inhalation followed by five to eight seconds of exhalation (Garis et al., 2023). This breathing pattern stimulates respiratory sinus arrhythmia, a physiological response that is directly linked to vagal (PNS) activation of the heart (Shaffer & Ginsberg, 2017). PMR engages muscle groups in a cycle of tension and release to reduce stress and promote relaxation (Gao et al., 2017; Garis et al., 2023). It is supposed to increase PNS, thereby increasing HRV (Shields, 2009; Toussaint et al., 2021). In contrast, SAT is aimed at training to elicit the galvanic skin response voluntarily (Robertson et al., 1995), a measure regulated by the SNS. Unlike DB and PMR, which aim to promote calmness and relaxation, SAT is meant to enhance physiological arousal and alertness (Braun et al., 2015; Robertson et al., 1995). A comparison of such interventions may therefore provide valuable insights into how HRV parameters reflect PNS and SNS responses.

Biopsychological interventions are central for fatigue management. Fatigue related ANS dysfunction in MS are reflected by reduced HRV parameters of PNS activity and increased HRV parameters of SNS activity (Keselbrener et al., 2000; Merico et al., 2005; Sander et al., 2019; Yu et al., 2013). Specifically, Merico et al. (2005) found that patients with MS demonstrated reduced HRV responses to ANS challenges (DB and standing), establishing an association between fatigue and compromised ANS adaptability. Yu et al. (2013) demonstrated significant PNS suppression (lower HF) and SNS activation (elevated LF/HF) in patients with MS with moderate to severe fatigue. Sander et al. (2019) found that the MS group with severe fatigue had significantly lower SDNN compared to the

MS group with weak to moderate fatigue. Keselbrener et al. (2000) identified SNS dominance (elevated LF/HF) in MS patients with fatigue.

All of the above findings highlight HRV as a reliable indicator of ANS modulation in MS-related fatigue. This allows ANS functioning to be studied under different environmental conditions. Building on this background, our previous research has demonstrated that biopsychological interventions, such as DB, PMR, and SAT, can indeed shift ANS activity in patients with MS. In this secondary analysis of the pooled data from two randomized controlled trials (Sander et al., 2020; Garis et al., 2023), we focus on three central research questions: How can the patterns of association between different HRV parameters be explained within a factorial model? Do HRV parameters that load on different factors respond differently to biopsychological interventions? Can they capture ANS changes during a vigilance task that is positively associated with increasing impact of trait and state fatigue in MS?

Method

This study is a reanalysis of pooled data from Sander et al. (2020) and Garis et al. (2023). Because the study by Garis et al. (2023) was designed as a follow-up to Sander et al. (2020), the two datasets are fairly homogeneous, and thus suitable for a pooled analysis. Characteristics of the pooled sample are summarized in Table 1.

Table 1

Summarized characteristics of the patient sample

	Total sample	**DB group**	**PMR group**	**SAT group**
Sample size (*n*)	78	15	37	26
Sex (female/male)	55/23	8/7	28/9	19/7
MS course (RRMS/PPMS/SPMS/PRMS)	48/11/17/1	7/4/4/-	24/4/9/-	17/3/4/1
	M* ± *SD	***M* ± *SD***	***M* ± *SD***	***M* ± *SD***
Age (years)	49.05 ± 10.05	48.00 ± 12.81	48.43 ± 10.77	50.54 ± 6.94
Fatigue Scale for Motoric and Cognition (FSMC; trait fatigue)	72.94 ± 14.76	78.33 ± 14.82	70.24 ± 15.26	73.65 ± 13.56
Baseline state fatigue	36.53 ± 26.14	32.30 ± 15.26	38.13 ± 28.49	36.68 ± 28.10
Beck's Depression Inventory (BDI)	11.08 ± 6.86	9.36 ± 5.24	11.38 ± 7.71	11.58 ± 6.40
Expanded Disability Status Scale (EDSS)	3.38 ± 1.70	3.75 ± 1.47	3.28 ± 1.83	3.34 ± 1.65

Note. RRMS = relapse-remitting MS; PPMS = primary progressive MS; SPMS = secondary progressive MS; PRMS = progressive-relapsing MS; *M ± SD* = mean ± standard deviation.

Procedure

Both studies were single-blind randomized controlled trials: participants in Sander et al. (2020) were randomly assigned to either the SAT or PMR group, whereas participants in Garis et al. (2023) were randomly assigned to either the PMR or DB group. Group assignment was prepared by an independent investigator and allocated by the principal investigator.

Both studies were conducted in a quiet room at the Carl von Ossietzky Universität Oldenburg and collaborating clinics. The acoustic vigilance task from the German Test of Attentional Performance (*Testbatterie zur Aufmerksamkeitsprüfung*; TAP; Zimmermann & Fimm, 2002) was administered to participants in both studies. Afterwards, participants conducted their assigned biopsychological intervention.

In Garis et al. (2023), PMR was focused on distal muscle tension and release, including the arms, shoulders, legs, and feet. In Sander et al. (2020), it included relaxation phases with the cue word "relax." Garis et al. (2023) also included a DB exercise at a rate of five cycles per minute (5 s inhalation, 5-8 s exhalation), which was guided by visual feedback of breathing rate and depth on a computer screen. Participants inhaled through the nose with their mouths closed and were told to keep their breathing smooth and wave-like, without holding their breath or breathing too fast, to avoid hyperventilation. In the SAT exercise (Sander et al., 2020), participants learned to change their skin conductance response (SCR) based on cues between trials with and without feedback. In phase one; participants were presented with an unpredictable auditory stimulus and the word “now”. In phase two, the word “now” was presented without an auditory stimulus. In the third and fourth phase, participants were instructed to say the word “now” and try to increase their SCR. In the third phase, they received the visual biofeedback information and this feedback was eliminated in the fourth by turning off the screen.

In both studies, HRV was continuously monitored during all phases, encompassing rest, vigilance tasks, and biopsychological interventions. The literature distinguished between two forms of fatigue: trait fatigue, indicated by the propensity to fatigue over extended periods, and state fatigue, which refers to the acute experience of fatigue (Malloy et al., 2021). Trait fatigue was assessed in both studies using the Fatigue Scale for Motor and Cognitive Functions (FSMC; Penner et al., 2009), and state fatigue was similarly measured with a visual analog scale before and after each task (vigilance task and biopsychological intervention; see Table 1). The same questionnaires were further used in both studies: depressive symptoms with the Beck Depression Inventory (BDI; Beck et al., 1996), and disability status with the Expanded Disability Status Scale (EDSS) or, when unavailable, the Patient-Reported EDSS (PREDSS; Bowen et al., 2001).

The tasks differed in length between the two studies, 20-min vigilance task/PMR/SAT in Sander et al. (2020) vs. 30-min vigilance task, 5-min DB/PMR in Garis et al. (2023), and these differences were controlled for by extracting HRV data from 5-min segments obtained during each condition. Table 2 provides an overview of measurements and tasks in both studies, and their use in the subsequent statistical analyses. For more detailed information, we refer to the original publications.

Table 2

Overview of measurements, tasks, and their use in the statistical analyses

Measurement section	Use in statistical analysis
Fatigue measures	
Fatigue Scale for Motor and Cognitive Functions (FSMC)	Regression analysis predicting trait fatigue from HRV parameters
VAS 1 (baseline state fatigue)	Not used in statistical analyses
VAS 2 (state fatigue after vigilance task)	Not used in statistical analyses
Δ state fatigue (VAS 2–VAS 1)	Regression analysis predicting change in state fatigue from HRV parameters
Vigilance task	
Block 1 (5-min HRV)	Indicators submitted to exploratory factor analysis (EFA)
	Correlational analysis between HRV parameters and trait and state fatigue
Biopsychological interventions	
HRV at rest	Repeated-measures ANOVA (intervention effects on HRV)
HRV during intervention	Repeated-measures ANOVA (intervention effects on HRV)
Vigilance task	
Block 2 (5-min HRV)	Correlational analysis between HRV parameters and trait and state fatigue
Block 3 (5-min HRV)	Not used in statistical analyses
Block 4 (5-min HRV)	Correlational analysis between HRV parameters and trait and state fatigue
Δ HRV (block 4–block 2)	Regression analyses predicting trait and state fatigue from HRV parameters' change selected by EFA

Note. VAS = visual analog scale; HRV = heart rate variability; EFA = exploratory factor analysis. VAS 1 (baseline state fatigue); VAS 2 (state fatigue after the vigilance task); block 3 (5-min HRV) of the vigilance task was recorded for descriptive purposes only and were not entered into the statistical analyses; Δ indicates change between two measurements.

HRV measurement

HRV corresponding to each task was derived from 5-min segments of pulse wave recordings obtained with a Blood Volume Pulse (BVP) sensor (NeXus-4 biofeedback system, Hasomed, Magdeburg, Germany) placed on the index finger of the patient's non-dominant hand. Both studies had the beginning and end of each task recorded and marked using the Biotrace software from the same system. We performed new artifact control on the raw data from each study through manual inspection and correction to ensure consistency across datasets for the following analysis. Subsequently, interbeat (RR) intervals were analyzed using the Kubios HRV Scientific Lite software (version 4.2.0). For pre-processing, we applied the threshold-based artifact correction method implemented in Kubios HRV Scientific Lite. In this approach, RR intervals that differ from the local average by more than a specified threshold (e.g., .25s for medium correction) are automatically detected as artifacts. These may result from ectopic beats, motion artifacts, or signal noise. Once detected, artifact-contaminated intervals are corrected by interpolating values based on adjacent typical RR intervals using cubic spline interpolation. In most cases, a medium correction level (threshold = .25s) provided adequate cleaning while minimizing overcorrection.

Statistical Analyses

All analyses were performed in JASP (Version 0.18.3; JASP Team, 2024; Abhishekh et al., 2013). Normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was investigated using Levene's test. Outliers were inspected for all HRV parameters. In the case of LF/HF, participants with values above 15 during DB (outliers) were excluded from the analyses. To account for the influence of certain variables on HRV, age, depression, and disability status were included as covariates (except for factor analysis). Age is known to be associated with reduced HRV (Almeida-Santos et al., 2016; Boeschoten et al., 2017; Escorihuela et al., 2020; Shrestha, 2020). Depression correlates with decreased HRV due to lower vagal tone (Brezinova et al., 2004; Gervasoni et al., 2018; Linden et al., 1997; Videira et al., 2016), and disability status in MS is linked to reduced PNS and elevated SNS activity (Brown et al., 2018; Kemp et al., 2010; Koch et al., 2019; Koenig et al., 2016).

EFA of HRV Parameters

EFA was conducted on HRV data from the first block of the vigilance task (see Table 2) to explore the association pattern and factorial structure of time- and frequency-domain, as well as nonlinear HRV parameters. Promax rotation was applied to enhance factor interpretability. We included the following HRV parameters: SDNN, RMSSD, LF, HF, LF/HF, SD1, SD2, SD1/SD2, α1, α2, SampEn, and ApEn. To improve factor homogeneity and reduce the number of variables for later analyses, the three HRV parameters with the highest factor loading and no cross-loading were

retained. This was done to reduce the number of comparisons in later analyses given the sample size, which is not very large. Subsequent analyses did not consider HRV parameters belonging to factors with fewer than three uniquely loading indicators.

Effect of Biopsychological Intervention on HRV

To examine the sensitivity of the selected HRV parameters based on the results of the EFA to PNS and SNS activity, repeated-measures ANOVAs were conducted with TIME (at rest vs. during biopsychological intervention) as a within-subjects factor, and GROUP (DB, PMR, SAT) as a between-subjects factor. Post hoc comparisons were adjusted using Bonferroni correction.

Prediction of Trait Fatigue and State Fatigue

Difference scores of the EFA-selected parameters were computed between the second (5–10 min) and fourth block (15–20 min) of the vigilance task. All scores were entered simultaneously into stepwise regression, with trait fatigue as the dependent variable. Stepwise regressions used $p < .05$ for entry and $p > .10$ for removal; multicollinearity was accepted at VIF < 5 (Shrestha, 2020). All predictors included in the final models showed VIF values below this threshold, indicating no multicollinearity problems.

We repeated the same analysis with state fatigue as the dependent variable. Difference scores (second block vs. fourth block of the vigilance task) were computed for the EFA-selected parameters. All scores were entered into a stepwise regression.

Post Hoc Correlational Analyses of Trait and State Fatigue and HRV Parameters

Additionally, post hoc correlational analyses were performed to further explore the relationships between fatigue measures and individual absolute HRV parameters. We calculated Pearson correlations between trait and state fatigue scores and HRV parameters derived from vigilance task data (blocks 2 and 4), controlling for age, depression, and disability status.

Results

Biofeedback group characteristics

The DB, PMR, and SAT groups did not differ in age, sex, disease course, fatigue level (FSMC), depression (BDI), and disability status (EDSS).

Exploratory Factor Analysis of HRV Parameters

EFA revealed that the correlation structure of all HRV parameters was best explained by a model with three factors. Linear and nonlinear HRV parameters turned out to be significant indicators that loaded onto factors 1 and 2. SD2, SDNN, and HF had the highest loading onto the first factor. Although LF was among the three variables with highest loadings on this factor, it also showed a

substantial cross-loading (> .4) on a second factor. Because HF had a similarly high loading but no notable cross-loading, we selected this HRV parameter for subsequent analyses. This factor explained 36% of the variance. The second factor encompassed SD1/SD2, α1, and LF/HF and explained 26% of the total variance. The third factor, indicated by SampEn and ApEn, was not retained for further analysis, as it contained fewer than three HRV parameters as indicators.

HRV Parameter Selection and Assumption Testing

For subsequent analyses, the HRV parameters identified as best indicators of the first (SD2, SDNN, HF) and second factors (SD1/SD2, α1, LF/HF) were used. Shapiro-Wilk tests of the six parameters at rest and during the biopsychological intervention indicated moderate deviations from normality; however, repeated-measures ANOVA is considered robust to such deviations with the present sample size (N = 78). We further tested the assumption of homogeneity of variance using Levene's test for all dependent variables included in the repeated-measures ANOVAs. All variables met the assumption (p > .05), except for SD1/SD2 (at rest and during intervention).

Effect of Biopsychological Intervention on HRV

Tables 3 and 4, and Figure 1 summarize the results. For SD2, there was no main effect of GROUP or TIME, but there was a significant GROUP × TIME interaction, $F(2, 69) = 3.93$, $p = .024$. Post hoc testing of intergroup differences showed that participants in the DB group exhibited higher SD2 compared to PMR (Δ = 22.76, $p = .032$, see Table 3 and Figure 1) and SAT (Δ = 24.40, $p = .027$, see Table 3 and Figure 1). Post hoc testing of intragroup differences showed a significant decrease in SD2 during PMR (Δ = -15.29, $p = .017$; see Tables 3 and 4). None of the covariates revealed significant associations.

For SDNN, there was no main effect of GROUP, no main effect of TIME, and no significant GROUP × TIME interaction (see Tables 3 and 4). Post hoc testing of intragroup differences showed a significant decrease in SDNN during PMR (Δ = -14.64, $p = .007$; see Tables 3 and 4). None of the covariates revealed significant associations. Also, no significant group differences at rest were found (see Figure 1).

For HF, there was no main effect of GROUP, no main effect of TIME, and no significant GROUP × TIME interaction (see Tables 3 and 4). Post hoc testing of intragroup differences showed a significant decrease in HF during PMR (Δ = -.81, $p = .013$; see Tables 3 and 4). There was a significant DISABILITY STATUS X TIME interaction, $F(1, 69) = 5.77$, $p = .019$. Disability status was significantly negatively associated with HF induced by the intervention (B = -.39, SE = .16, $p = .022$).

For SD1/SD2, there was a significant main effect of GROUP, $F(2, 69) = 11.45$, $p < .001$. Post hoc testing of intergroup differences showed that participants in the DB group showing higher SD1/SD2 compared to PMR (Δ = 1.00, $p < .001$, see Table 3 and Figure 1) and SAT (Δ = 1.51, $p < .001$, see Table

3 and Figure 1). There was also a significant GROUP × TIME interaction, $F(2, 69) = 19.11$, $p < .001$. Post hoc testing of intragroup differences showed significant increases in SD1/SD2 during PMR ($\Delta = .34$, $p = .028$, see Tables 3 and 4) and DB ($\Delta = 1.17$, $p < .001$; see Tables 3 and 4). None of the covariates revealed significant associations.

For α1, there was a significant main effect of GROUP, $F(2, 69) = 12.56$, $p < .001$. Post hoc testing of intergroup differences showed that participants in the DB group had higher α1 compared to PMR ($\Delta = 0.407$, $p < .001$, see Table 3 and Figure 1) and SAT ($\Delta = .574$, $p < .001$, see Table 3 and Figure 1). There was also a significant GROUP × TIME interaction, $F(2, 69) = 19.01$, $p < .001$. Post hoc testing of intragroup differences revealed significant increases in α1 during DB ($\Delta = .39$, $p < .001$; see Table 3 and Figure 1). The covariate age had a significant main effect, $F(1, 69) = 9.84$, $p = .003$.

For LF/HF, there was a significant main effect of TIME, $F(1, 63) = 8.14$, $p = .006$. Post hoc testing of time differences showed a significant increase in LF/HF during the biopsychological intervention compared with rest ($\Delta = 1.33$, $p < .001$; see Tables 3 and 4). There was a significant main effect of GROUP, $F(2, 63) = 6.67$, $p = .002$. Post hoc testing of intergroup differences showed that participants in the DB group had higher LF/HF compared to PMR ($\Delta = 3.36$, $p = .008$, see Table 3 and Figure 1) and SAT ($\Delta = 4.67$, $p < .001$, see Table 3 and Figure 1). There was also a significant GROUP × TIME interaction, $F(2, 63) = 12.56$, $p < .001$. Post hoc testing of intragroup differences showed a significant increase in LF/HF during DB ($\Delta = 3.75$, $p < .001$; see Tables 3 and 4). Lastly, there was also a significant AGE × TIME interaction, $F(1, 63) = 10.11$, $p = .032$. Age was significantly negatively associated with LH/HF induced by the intervention ($B = -.32$, $SE = .47$, $p = .012$). None of the covariates revealed significant associations.

Table 3

SD2, SDNN, HF, SD1/SD2, α1, and LF/HF for the intervention groups and the time effects

	DB group	PMR group	SAT group
	M ± SE	***M ± SE***	***M ± SE***
SD2: at rest *², a, b	56.66 ± 7.59	56.17 ± 5.12	51.92 ± 5.09
SD2: during exercise *², a, b	64.08 ± 8.27	41.59 ± 3.70	38.18 ± 3.40
SDNN: at rest **²	46.78 ± 6.50	47.85 ± 4.46	43.72 ± 4.52
SDNN: during exercise **²	49.50 ± 6.99	33.83 ± 3.05	33.51 ± 3.08
HF: at rest *²	6.01 ± .39	6.12 ± .22	6.07 ± .26
HF: during exercise *²	4.94 ± .39	5.35 ± .19	5.52 ± .23
SD1/SD2: at rest **¹, *², aa, bb	1.81 ± .13	1.66 ± .10	1.66 ± .06
SD1/SD2: during exercise **¹, *², aa, bb	2.98 ± .28	2.01 ± .11	1.46 ± .06
α1: at rest **¹, aa, bb	1.07 ± .06	.96 ± .03	1.02 ± .03
α1: during exercise **¹, aa, bb	1.47 ± .07	1.07 ± .04	.88 ± .04
LF/HF: at rest **¹, aa, bb	2.02 ± .69	1.72 ± .28	1.84 ± .19
LF/HF: during exercise **¹, aa, bb	6.01 ± 1.86	2.59 ± .36	1.14 ± .12

Note. DB = deep breathing; PMR = progressive muscle relaxation; SAT = self-alert training; SD2 = standard deviation of long-term variability; SDNN = standard deviation of NN intervals; HF = high frequency power; SD1/SD2 = SD1 to SD2 ratio; α1 = detrended fluctuation analysis; LF/HF = low-to-high frequency ratio; *M ± SE* = mean ± standard error.

*p < .05; **p < .01 for intragroup comparisons; ¹DB; ²PMR.

[a]p < .05; [aa]p < .01 (DB vs. SAT); [b]p < .05; [bb]p < .01 (DB vs. PMR) for intergroup comparisons.

Table 4

Visual representation of significant intragroup changes on SD2, SDNN, HF, SD1/SD2, α1, and LF/HF

	HRV parameter	Group	Direction of change
Factor 1	SD2	PMR*	↓
	SDNN	PMR**	↓
	HF	PMR*	↓
Factor 2	SD1/SD2	PMR*	↑
		DB **	↑
	α1	DB**	↑
	LF/HF	DB**	↑

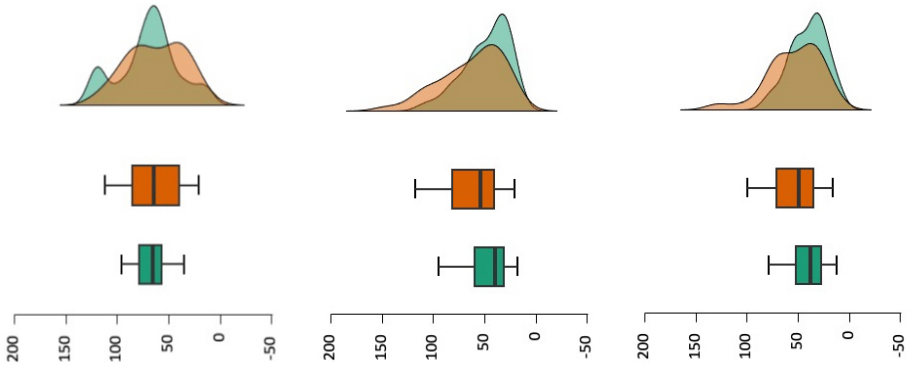
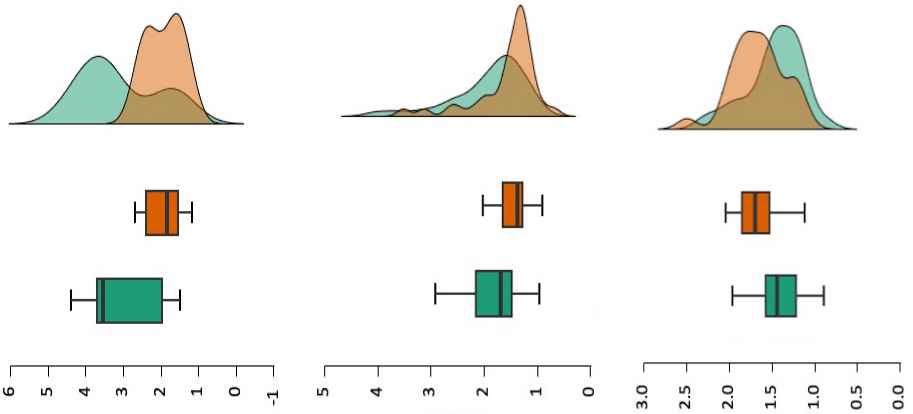
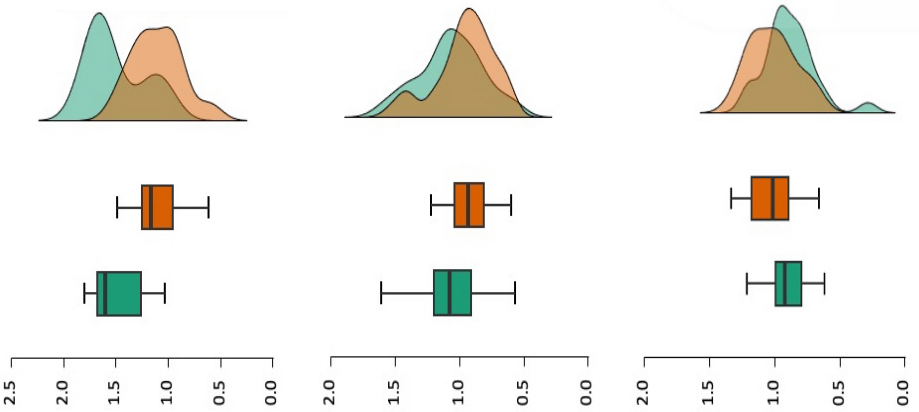
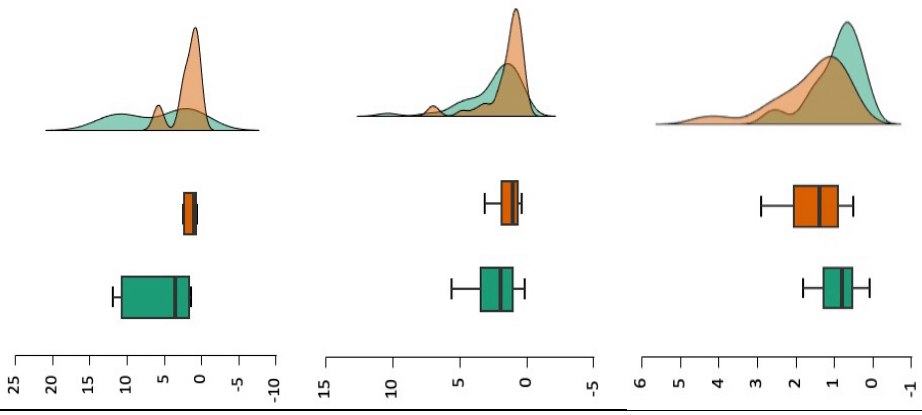
Note. PMR = progressive muscle relaxation; DB = deep breathing; SD2 = standard deviation of long-term variability; SDNN = standard deviation of NN intervals; HF = high frequency power; SD1/SD2 = SD1 to SD2 ratio; α1 = detrended fluctuation analysis; LF/HF = low-to-high frequency ratio.

*$p < .05$; **$p < .01$ for intragroup comparisons.

Intragroup SAT showed no significant test results.

Figure 1

Effects of biopsychological interventions on HRV parameters in factors 1 and 2, and significant intergroup differences

Parameter	DB	PMR	SAT	Significant intergroup differences
SD2				DB > SAT [a] DB > PMR [b]
SD1/SD2				DB > SAT [aa] DB > PMR [bb]
α1				DB > SAT [aa] DB > PMR [bb]
LF/HF				DB > SAT [aa] DB > PMR [bb]

Note. Boxplots with overlaid violin plots illustrate the distribution of values at rest (dark red) and during intervention (green) across groups. Significant effects are described in the Results section under *Effect of*

Biopsychological Intervention on HRV. DB = deep breathing; SAT = self-alert training; PMR = progressive muscle relaxation; SD2 = standard deviation of long-term variability; SD1/SD2 = SD1 to SD2 ratio; α1 = detrended fluctuation analysis; LF/HF = LF to HF ratio.

[a]*p* < .05; [aa]*p* < .01 (DB vs. SAT); [b]*p* < .05; [bb]*p* < .01 (DB vs. PMR) for intergroup comparisons.

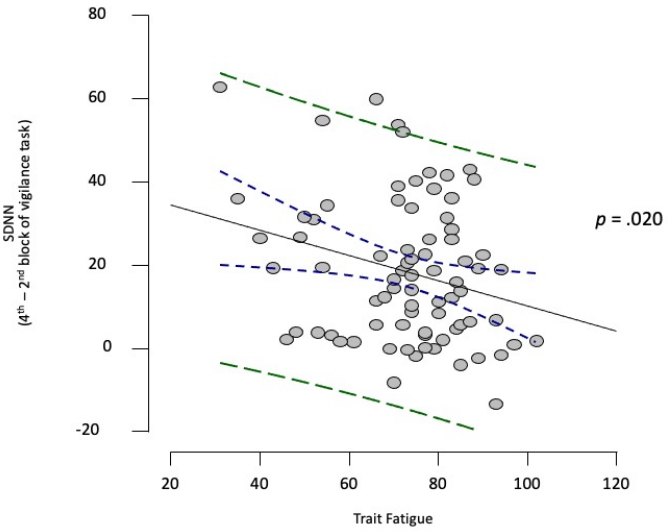
No significant group differences at rest.

Prediction of Trait Fatigue

In the stepwise regression, depression entered the model first and was a significant predictor of trait fatigue (β = .43 *p* < .001), accounting for 18% of the variance (R^2 = .18, adjusted R^2 = .17). In the second step, the six EFA-selected HRV change scores *(SD2, SDNN, HF, SD1/SD2, α1, and LF/HF; block 4–block 2 of the vigilance task) were entered as candidate predictors*. Of these, only SDNN change met the inclusion criteria and was added to the model as a significant predictor of trait fatigue (β = -.28 *p* = .007; see Figure 2 for the association plot). The inclusion of this predictor increased the explained variance by 8%. The final model including both depression and SDNN change explained 26% of the variance in trait fatigue, *F*(2, 73) = 13.08, *p* < .001, R^2 = .26, adjusted R^2 = .24. Regression assumptions were evaluated by inspecting residuals. Shapiro-Wilk tests, skewness, kurtosis, and Q-Q plots indicated approximate normality (*p* = .09). Homogeneity of variance was assessed by plotting residuals against predicted values, which showed no systematic pattern, suggesting constant variance.

Figure 2

Scatterplot of the relationship between trait fatigue and the difference in SDNN between the fourth block and second block of the vigilance task



Note. SDNN = standard deviation of NN intervals.

Prediction of State Fatigue

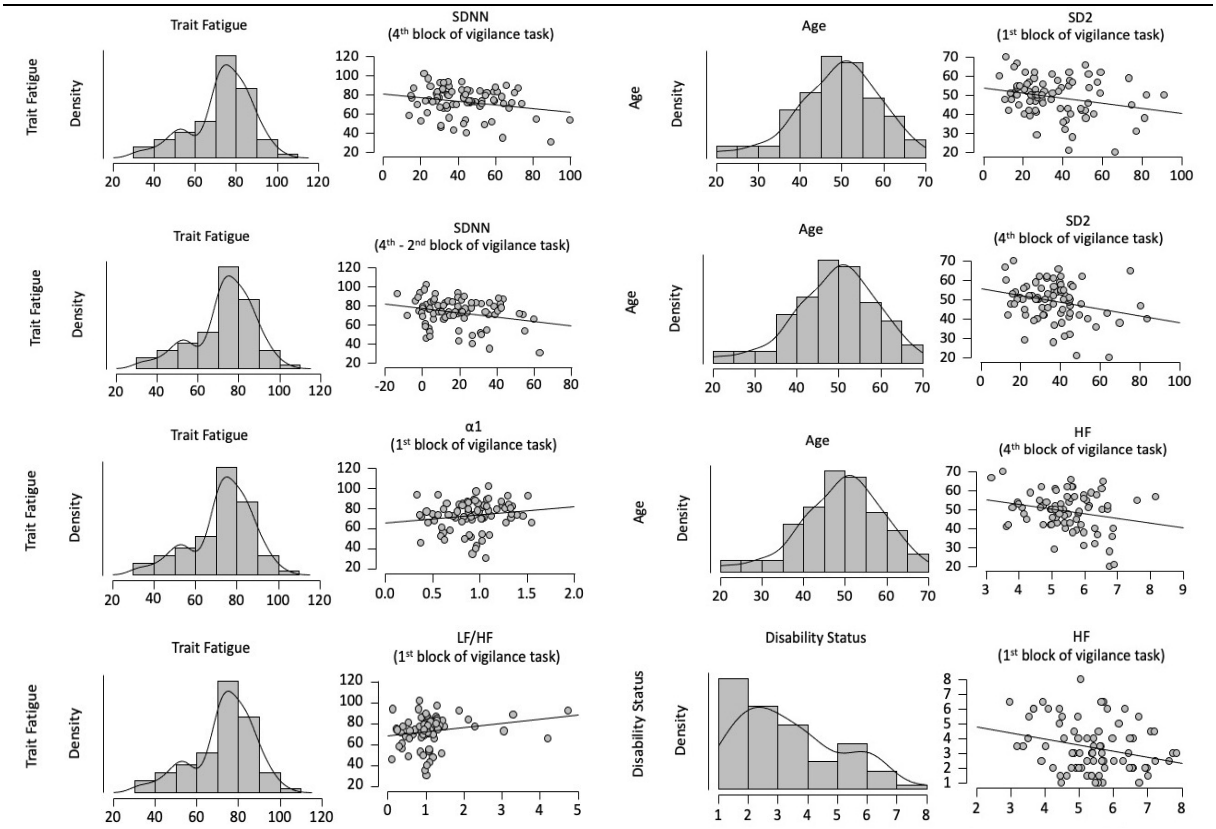
The final model explained 6% of the variance in state fatigue, $F(1, 74) = 5.00$, $p = .028$, $R^2 = .06$, adjusted $R^2 = .05$. Depression emerged as the only significant predictor of state fatigue (β = .95, $p = .028$).

Post Hoc Correlational Analyses of Trait Fatigue and HRV Parameters

Because the stepwise regression retained only SDNN change score across the vigilance task (block 4–block 2) as a significant HRV predictor of trait fatigue, post hoc correlation analyses were conducted to examine the associations between trait fatigue and the six HRV parameters individually. These correlations were based on the absolute HRV values from blocks 2 and 4 of the vigilance task, and are therefore considered post hoc tests. Normality and linearity were confirmed through Shapiro-Wilk tests, Q-Q plots, and visual inspection of scatterplots. Although some variables showed mild deviations, given the relatively large sample size, Pearson correlations can be assumed to be sufficiently robust. All correlations reported in this subsection are partial correlations controlling for age, depression, and disability status. The scatterplot matrix (see Figure 3) visualizes the significant partial correlations between trait fatigue and the HRV parameters. For HRV parameters derived from the second block of the vigilance task, LF/HF showed a significant positive correlation with trait fatigue ($r = .30$, $p = .009$), and α1 was also positively associated with trait fatigue ($r = .24$, $p = .037$). Disability status showed significant negative correlation with HF ($r = -.26$, $p = .019$), and age showed a significant negative correlation with SD2 ($r = -.25$, $p = .026$). For HRV values derived from the fourth block, age again correlated negatively with HF ($r = -.23$, $p = .040$) and SD2 ($r = -.26$, $p = .021$). Trait fatigue showed a significant negative correlation with SDNN ($r = -.23$, $p = .042$). Finally, trait fatigue was negatively associated with the SDNN change across the vigilance task (block 4–block 2) ($r = -.26$, $p = .020$, see Figure 2). In summary, across blocks 2 and 4, trait fatigue showed significant partial correlations with multiple HRV parameters, including SDNN, LF/HF, and α1.

Figure 3

Scatterplot matrix of correlations between trait fatigue, HRV parameters, and covariates



Note. SDNN = standard deviation of NN intervals; SD2 = standard deviation of long-term variability; α1 = detrended fluctuation analysis; HF = high frequency power; LF/HF = low-to-high frequency ratio.

Post Hoc Correlational Analyses of State Fatigue and HRV Parameters

Because no HRV parameter emerged as a significant predictor of state fatigue in the stepwise regression model, additional post hoc correlations were performed to explore potential associations between state fatigue measures and the six HRV parameters individually. These correlations were based on the absolute HRV values from blocks 2 and 4 of the vigilance task, and are therefore considered post hoc tests. No significant correlations were found between baseline state fatigue, state fatigue after the vigilance task, and HRV parameters. Normality and linearity were confirmed through Shapiro-Wilk tests, Q-Q plots, and scatterplots.

Discussion

This study addressed three questions: First, we examined whether linear and nonlinear HRV parameters, used to assess ANS activity, would cluster into meaningful factors, providing insight into which HRV parameters could serve as physiological markers of fatigue and intervention effects. Second, we tested how these HRV parameters respond to biopsychological interventions (DB, PMR, SAT) that are assumed to modulate ANS differently. Third, we explored how trait fatigue and state fatigue in MS relate to HRV changes during a vigilance task.

Using exploratory factor analysis to address the first question, two main factors emerged. The first factor was primarily indicated by SD2, SDNN, and HF, while the second factor reflected the commonality between SD1/SD2, α1, and LF/HF. The first factor thus reflected the covariation between HRV parameters primarily associated with PNS activity, whereas the second factor encompassed HRV parameters associated with SNS activity, supporting their use as physiological markers of PNS and SNS activity. Linear and nonlinear parameters serving as substantive indicators of the same factor suggest convergent information in capturing aspects of ANS regulation from both analytic approaches. In contrast, entropy parameters did not cluster with linear parameters, leaving their meaning and physiological interpretation an open question.

The interventions targeted ANS modulation by relaxation (DB) vs. activation (SAT), with PMR representing an intermediate condition combining mild activation with subsequent relaxation. In terms of *group differences*, SAT reduced HRV across all parameters, whereas DB increased HRV in most parameters (see Table 3). This pattern was expected, since the two interventions are presumed to elicit opposing ANS effects. It is consistent with previous findings showing that SAT-like activation reduces HRV (Järvelin-Pasanen et al., 2018), whereas DB-like relaxation increases HRV (Groß & Kohlmann, 2021). Unexpectedly, DB-related increases reached significance only for the second factor (representing SNS-related parameters) and not for the first factor (representing PNS-related parameters) (see Table 4).

A significant intergroup difference also emerged for SD2, with higher values in the DB group compared to SAT and PMR during the intervention. This finding is in line with the expected vagal effects of DB, since SD2 is closely linked to PNS modulation. PMR revealed intermediate effects on the second factor variables (LF/HF, SD1/SD2, and α1). Compared to DB, PMR scores were significantly lower. This positions PMR between DB (assumed as a relaxation intervention) and SAT (assumed as an activating intervention) in terms of ANS modulation.

HRV parameters clustering as suggested by the exploratory factor analysis also proved useful for analyzing intervention-related *intragroup changes* (see Table 3). DB selectively increased the HRV parameters loaded onto the second factor, whereas the HRV parameters loaded onto the first factor showed no significant changes. This is an unexpected finding, since DB is usually linked to vagal activation (Shaffer & Ginsberg, 2017; Shields, 2009). Raw scores of the HRV parameters belonging to the first factor nevertheless increased during DB, leaving the option that the limited sample size may have prevented to statistically identify the association. PMR decreased SD2, SDNN, and HF (first factor), but increased SD1/SD2 (HRV parameters loaded onto the second factor). Because SD1/SD2 is commonly linked to SNS, this increase may reflect repeated tensing and releasing of muscles groups. The anticipated relaxation effect of PMR on HRV parameters belonging to the first factor might instead emerge after the exercise, when muscles return to a rest and vagal activity can rebound (Conrad & Roth, 2007; Lehrer & Gevirtz, 2014; McCallie et al., 2006). Regarding the intragroup comparisons, PMR showed significant decreases in SDNN and HF. However, these effects were not supported by a significant GROUP × TIME interaction and should therefore be considered speculative given the empirical evidence at hand.

In sum, the biopsychological interventions modulated HRV parameters that clustered differently in the exploratory factor analysis in distinct ways, which is a suggestive result. PMR decreased the HRV parameters belonging to the first factor, whereas DB increased the HRV parameters belonging to the second factor, a dissociation that highlights the utility of HRV parameters for evaluating ANS (re)activity. At the same time, the observed effects suggest that the biopsychological interventions did not elicit a simple, antagonistic, and independent modulation of HRV of SNS and PNS branches (e.g., DB increasing vagal while SAT increases SNS modulation). Rather, they may result in a synergistic, state-dependent modulation of HRV. Specifically, DB increased parameters associated with both PNS and SNS influences on HRV, whereas SAT decreased both, with PMR showing intermediate and more selective effects. This pattern implies the existence of *higher-order states defined by complementary HRV changes* defined more functionally, rather than strictly physiologically. This possibility of dynamically defined states needs to be explored in future studies.

Among the covariates, age showed a significant association with α1. This pattern suggests an age-related decline in fractal scaling and ANS complexity, which aligns with prior evidence of reduced adaptability of ANS regulation across the lifespan (Abhishekh et al., 2013; Almeida-Santos et al., 2016; Bonnemeier et al., 2003; Nunan et al., 2010). Correlational analysis also showed that age significantly correlated with HRV parameters. The negative association between age, SD2, and HF indicates that older individuals exhibit reduced ANS complexity and vagal control, particularly by the end of the vigilance task. These findings align with research that demonstrates age-related reductions in HRV (Abhishekh et al., 2013; Almeida-Santos et al., 2016; Bonnemeier et al., 2003; Nunan et al., 2010). Aging in MS may thus further limit ANS flexibility.

Disability status is also associated with HRV, such that higher disability status was associated with lower HF responsiveness during all biopsychological interventions. This suggests that patients with greater MS disability exhibit decreased HF responsiveness when performing ANS modulation tasks. Moreover, disability status showed significant negative correlation with HF. Both findings are in line with reports linking higher MS disability status to reduced PNS activity (Monge-Argilés et al., 1998; Studer et al., 2017; Zawadka-Kunikowska et al., 2020). In the context of MS, where ANS dysfunction is already present, the additional burden of aging and increased MS disability may further constrain ANS (re)activity. However, this interpretation needs further empirical support by assessing a healthy control group of similar age in future studies.

We also investigated how a vigilance task modulates HRV in relation to trait fatigue in MS. Vigilance tasks, by design, are monotonous and cognitively demanding, allowing fatigue to act as an attractor state (DeLuca, 2005; Hanken et al., 2016; Thiffault & Bergeron, 2003). Vigilance tasks permit mind wandering, making them ideal for studying the severity of trait fatigue through a behavioral measure. Regression analyses confirmed that depression (BDI scores) and reduced SDNN change during the task were significant predictors of trait fatigue, with the latter showing a moderate effect size.

It is relevant that several BDI items predominantly assess somatic symptoms (e.g., tiredness, loss of energy, loss of sexual interest, sleep disturbances), which conceptually overlap with MS-related fatigue. In

our pooled sample, half of the participants did not even reach the threshold for mild depression (BDI < 10). As a result, controlling for depression with the BDI sum score probably attenuated some of the variance that is naturally linked to fatigue, which may have made the unique contribution of HRV parameters in the regression model less apparent. Consequently, the negative correlation between SDNN change and trait fatigue may be understood as the variance uniquely related to HRV after accounting for this shared symptom domain.

SDNN changes were specific to *trait* fatigue and not observed for *state* fatigue. Compared to trait fatigue, *state* fatigue also reflects transient increases in tiredness induced by the vigilance task. This suggests that it is not simply getting tired during the vigilance task that explains the predictive value of SDNN changes, but rather the way in which the ANS of patients with higher trait fatigue responds to the specific demands of fatigue-inducing tasks. Our results showed that a reduced SDNN change during the vigilance task predicted higher trait fatigue. This suggests that individuals with more chronic fatigue have reduced flexibility in adjusting their ANS responses. This pattern is also consistent with the correlational results, where several HRV parameters (including SDNN, LF/HF, and α1) were significantly associated with trait fatigue, suggesting that the SDNN association is not isolated but reflects a broader change in autonomic functioning in more fatigued MS patients.

In line with this interpretation, trait fatigue exhibited a positive correlation with SNS parameters (LF/HF and α1) during the second block of the vigilance task, and a negative correlation with PNS (SDNN) during the fourth block, indicating that the predictive effect pertains to trait fatigue-related ANS activity rather than state fatigue-related. The vigilance task may elevate stress due to the persistent attentional demands and lack of reward (Ward et al., 2018). Similar ANS findings have been observed in healthy individuals under occupational stress (Peabody et al., 2023).

Chronic fatigue in MS may indeed present as increased SNS activity and reduced PNS modulation, depending on attentional demands and fatigue accumulation, which is consistent to our proposed model (Garis et al., 2022). Likewise, other studies have similarly reported decreased SDNN and HF, along with elevated LF/HF and α1 both in patients with MS-related fatigue and chronic fatigue syndrome (Escorihuela et al., 2020; Keselbrener et al., 2000; Matuz et al., 2022; Merico et al., 2005; Sander et al., 2019; Srinivasan et al., 2024; Yu et al., 2013).

The present findings support the hypothesis that HRV changes are an integral component of MS-related fatigue because they reflect the reduced adaptability of the ANS under conditions of chronic inflammation. Based on the model proposed in our review (Garis et al., 2022), such HRV alterations can be understood as part of a broader neuroimmune-autonomic mechanism: autoimmune activation and cytokine release alter vagal signaling and reset the “set-point” for ANS regulation, leading to chronic reductions in PNS modulation, SNS dominance, and the emergence of fatigue as a sustained symptom of sickness behavior. The prediction of trait fatigue by ANS activity (SDNN), even after controlling for depression, is a strong argument

for our model positing that fatigue is a syndrome, a multi-faceted endogenous response by the brain to an inflammatory process in the body (Dantzer, 2001; Harrison et al., 2009; Sammito & Böckelmann, 2015).

It is important to acknowledge several limitations of the analyses described in this paper. Separate analyses comparing trait fatigue severity groups were not performed due to the limited number of participants in the mild (*n* = 6) and moderate (*n* = 7) groups, as well as the minimal representation in the no-fatigue group (*n* = 3), relative to the severe fatigue group (*n* = 62). Consequently, the range of fatigue in our sample was restricted, with most participants experiencing severe fatigue, and we cannot determine whether MS patients with lower fatigue levels would exhibit the same ANS-fatigue relationships observed in this study. Another limitation concerns the unequal group sizes across interventions: the DB and SAT groups were relatively small compared to the PMR group, which may have reduced statistical power and contributed to less stable estimates of intervention-related effects. Furthermore, HRV was derived from a pulsimeter rather than an electrocardiography (ECG), which may provide less fine-grained information (Shaffer & Ginsberg, 2017; Task Force, 1996). Future studies are needed to replicate these findings using more precise HRV measurement methods such as ECG. In addition, most of our participants were on immunomodulatory MS medication, which might have influenced HRV. However, because our analyses primarily relied on intrasubject changes, medication effects on the main findings are likely to be limited.

Conclusions and prospective research

First, exploratory factor analysis revealed that HRV parameters can be grouped into two factors, thereby endorsing their application as indicators of ANS (re)activity. Second, the opposing biopsychological interventions modulated HRV parameters belonging to the two factors in distinct ways. Finally, trait fatigue was consistently associated with a reduced SDNN change cross the vigilance task (indicative of reduced PNS adaptability over time on task). Nonlinear parameters complemented linear parameters by providing additional information on ANS activity in MS, even though they were not as central for specifically explaining trait fatigue.

We conclude that for biopsychological intervention studies, HRV may serve as a significant biomarker to evaluate the efficacy of approaches in modulating ANS balance. In particular, our findings highlight SDNN as a key parameter, since lower SDNN was associated with trait fatigue, indicating that modifying this parameter might be a goal worth focusing on for interventions.

Statements and Declarations

Disclosure of Interest: The authors declare that they have no relevant financial or non-financial competing interests regarding the present pooled analysis.

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Data availability: The dataset is now openly available in Figshare with the identifier https://doi.org/10.6084/m9.figshare.30643157.v2

Ethics Approval and Consent to Participate: Both contributing randomized controlled trials received ethical approval from the Ethics Committee of University Medicine Oldenburg (Sander et al., 2020: approval number 009/2017; Garis et al., 2023: approval number 2020-152). Written informed consent was obtained from all participants in both studies.

Health and Safety: All experimental procedures were conducted in accordance with laboratory health and safety requirements of the Carl von Ossietzky Universität Oldenburg.

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7. Eidesstattliche Erklärung

Hiermit versichere ich, Guadalupe Garis, geboren am 25.05.1992 in Buenos Aires (Argentinien), an Eides statt, dass ich

I. diese eingereichte Dissertation selbstständig und ohne unzulässige fremde Hilfe verfasst und keine anderen als die angegeben Quellen und Hilfsmittel benutzt habe.

II. die veröffentlichten Artikel vollständig in der Publikationsliste genannt habe.

III. die beigefügte Dissertation nur in diesem und keinem anderen Publikationsverfahren eingereicht habe und, dass diesem Promotionsverfahren keine endgültig gescheiterten Promotionsverfahren vorausgegangen sind.

IV. die allgemeinen Prinzipien wissenschaftlicher Arbeit und Veröffentlichung, wie sie in den Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg festgelegt sind befolgt habe.

V. im Zusammenhang mit dem Promotionsvorhaben keine kommerziellen Vermittlungs- oder Beratungsdienste (Promotionsberatung) in Anspruch genommen habe.

Oldenburg, 12. März 2026

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(Doktorandin)