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Neuro-endocrine Insights into MS: Exploring the Role of HPA Axis and Sex Hormones

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Abstract

Multiple sclerosis (MS) is a complex autoimmune disease characterized by chronic inflammation and neurodegeneration within the central nervous system. Over the years, researchers have increasingly recognized the significance of neuroendocrine changes and sex hormone alterations in the pathogenesis and clinical course of MS. The neuroendocrine system, encompassing the hypothalamic-pituitary-adrenal (HPA) axis and hypothalamic-pituitary-gonadal (HPG) axis, plays a crucial role in regulating immune responses, inflammation, and tissue repair. Moreover, sex hormones, including estrogen and testosterone, exert profound effects on immune function, neuroprotection, and neuroplasticity, thereby influencing disease susceptibility, course, and progression. This review summarizes the current understanding of neuroendocrine changes observed in MS, focusing on alterations in HPA axis activity, dysregulation of cortisol levels, and disturbances in stress response mechanisms. Furthermore, it explores the impact of sex hormone fluctuations in MS, discussing the protective effects of estrogen and the potential influences of testosterone on disease activity. Further investigations are warranted to unravel the underlying mechanisms and explore targeted interventions that could positively impact the lives of individuals affected by MS.

Keywords: Multiple sclerosis, Sex hormones, HPA axis, Estrogens, Testosterone

What is Multiple Sclerosis?

Multiple sclerosis, widely known as MS, is one common mysterious neurodegenerative disease known to humankind. Mainly affecting young adults worldwide, it remains a puzzle with several missing pieces in the scientific as well as the clinical community. Looking for these missing parts, scientists are working towards finding the exact cause and best treatment, postulating different hypotheses that involve several mechanisms and risk factors like genetics, environmental, and stochastic factors. Moreover, MS is considered an inflammatory disease predominantly immune-mediated, that results in demyelination of the central nervous system (CNS). On the other hand, it is also postulated that significant neurodegenerative processes are involved in MS diagnosis, probably resulting in the development of progressive clinical disability. Having said that, an ever-increasing body of literature constantly emerges with the only goal, of solving the puzzle of MS, and providing the patients suffering from this cruel disease with the best possible treatment.

History of MS

The need for labeling a group of symptoms and their progress as a disease is a way of summarizing the cultural attitude regarding a trait or behavior. Furthermore, giving a name to the particular disorder is mostly organizing these cultural attitudes into a concept or framework called by Rosenberg (Rosenberg, 1992). In this way, all of the following beliefs are organized in the given framework of that particular disease: the biology behind the symptomatology, the cause, management, and treatment, the degree of danger to the patient or to others, as well as the stigma and blame, the cost to the patient and the medical system (Rosenberg, 1992). Over time, these

concepts and frameworks change or upgrade with the help of new scientific findings and the change in the perspective of society. Today, for many diseases there is a consensus in Western industrialized societies.

At the end of the nineteenth century, multiple sclerosis among other neurological diseases was individualized and named (Murray, 2009). However, as mentioned before the frame of a given disease can change, and it did rapidly in the twenty-first century due to the changes in treatment and the understanding of the pathology of multiple sclerosis. Probably the first description of multiple sclerosis dates from the fourteenth century (Murray, 2009). Documentation of the life and the symptoms of Saint Ludwina od Schiedam is kept in the Vatican archives. Saint Ludwina at the age of sixteen had an injury, from which she recovered partially but still had episodes of loss of balance, weakness, and visual disturbances in between remissions. Her priest was sure that her illness came from God so she accepted in the name of God to suffer for others. Her illness progressed over time and in today's medicine, it would probably be considered multiple sclerosis (Murray, 2009). Many years later, another patient is described through his diary which he kept for 26 years. His first symptoms included a transient visual impairment episode and probably optic neuritis at the age of 28. Later he experienced motor symptoms, like compromised walking ability and weakness in the legs. His illness was considered to be paraplegia, however, now will probably be a multiple sclerosis diagnosis (Murray, 2009).

At the beginning of the nineteen century, the now-known neurological and psychiatric disorders were recognized as a general class of nervous disorders. At that time the treatment was not individualized for different conditions, now recognized to be separate disorders in neurology and psychiatry. During this time, only a few illnesses were separated from each other, one of which was paraplegia. Multiple sclerosis did not exist as a separate disorder and it was considered

to be in the class of paraplegias. With a systematic investigation and advances in neuropathology, it became somewhat possible to frame individual disorders and name them. Robert Carswell (1839) was among the first to describe disseminated plaques in the nervous system through autopsy (Carswell, 1838). Jean Cruveilhier made a similar finding in four autopsy observations and thus provided a clinical description of one of the cases (Cruveilhier, 1844). Charles-Prosper Ollivier d'Angers was probably the first to separate and individualize neurological conditions from clinical presentation to pathology of the illnesses. The first modern clinical description of multiple sclerosis was done by him (Murray, 2009). Friedrich von Frerichs, a German pathologist was the first one to diagnose alive patients with what he called it at the time brain sclerosis or Hirnsklerose (1849). Unfortunately, his classification of the disease was not accepted at that time, so the framing of multiple sclerosis is linked to Charcot and his "discovery" of the illness (Murray, 2009). However, gender and age distribution has been hypothesized by Ernst Leyden, similar to what we see today. He also talked about possible inheritance in the disease (Murray, 2009). A few other important scientific findings like the myelin debris, the formation of the plaques around the cerebral blood vessels, and demyelination were observed (Murray, 2009). Finally, in 1868 Jean-Martin Charcot gave the final description for the illness which he named sclérose en plaque, now referred to as multiple sclerosis (Clanet, 2008). He was the one to connect the clinical features of the disease and the pathological changes seen in postmortem patients. After all, he was the one who diagnosed multiple sclerosis for the first time in a living patient (Murray, 2009). Almost fifty years later, the Association for Research in Nervous and Mental Diseases (ARNMD) in 1921 organized a meeting for all interested neurologists around the world, in which they reviewed the progress in those fifty years and combined the research and findings on multiple sclerosis.

Historically speaking, the impact of neuroimaging techniques on the understanding of multiple sclerosis is rather big. The technological progress in neuroimaging, especially magnetic resonance imaging made it possible to visualize the disease activity in a living brain. (Young, 1981). With the help of magnetic resonance imaging scans, we can now see that this disease can still be active even when there are no clinical signs of it, because of which multiple sclerosis is now considered to be progressive rather than an episodic disease (Murray, 2009). Nowadays, multiple sclerosis is seen as an immune-mediated disease without known etiology, that leads to an inflammatory attack on the myelin sheets and axonal damage (Podbielska, 2013).

Classification and Symptomatology of MS

At the present times, it is thought that Multiple Sclerosis has a complex underlying mechanism. Inflammation and neurodegeneration are considered to be the disease's leading principles among the not fully understood neuro-mechanisms (Hemmer et al., 2002). The clinical manifestation of the MS diagnosis includes exacerbation and progression of the disease. The term exacerbation refers to a sudden worsening in the symptomatology of the patient within 24 hours, which could possibly be remitted partially or fully after a couple of weeks or months. On the other hand, progression is a slower process, during which the symptoms increase slowly and steadier with the absence of exacerbation (C. Heesen et al., 2007). Patients diagnosed with MS can be categorized into one of the following phenotypes based on their disease course and progressions. Current MS phenotypes are Relapsing-remitting multiple sclerosis (RRMS), Primary-progressive multiple sclerosis (PPMS), and Secondary-progressive multiple sclerosis (SPMS) (Klineova & Lublin, 2018). Relapsing-remitting multiple sclerosis is defined by acute exacerbations typically followed by complete or incomplete recovery, with periods of remission. The primary-progressive multiple sclerosis is characterized by a progressive decline in neurological function from the onset

of the disease. Secondary-progressive multiple sclerosis refers to the clinical course defined by gradual progression after the initial relapsing course and decline in neurological function (Klineova & Lublin, 2018).

MS targets the central nervous system and destroys the myelin sheath which in turn leads to a wide array of symptoms that can range from mild to severe (Johnston & Joy, 2001). The symptoms may include blurred vision, weakness, balance problems, muscle spasms, numbness, tingling, and walking problems. Among patients also bladder and bowel problems, as well as emotional and cognitive changes are observed as part of the symptomatology. Symptoms of MS can appear in any region of the body because any nerves that are part of the brain and the spinal cord can be damaged. Briefly stated, every system in the brain like myelin, white matter, neurons, axons, and blood vessels can potentially be damaged due to MS (Kutzelnigg et al., 2005).

The guidelines used nowadays for the classification of MS is the McDonald 2010 diagnostic criteria (Polman et al., 2011). These diagnostic criteria for MS have two main requirements dissemination in space (DIS) and dissemination in time (DIT) which are based on the clinical presentation of the patient with the typical symptomatology and by imaging. Dissemination in space (DIS) means that lesions have occurred in multiple parts of the central nervous system. Typical CNS parts affected by MS are periventricular, juxtacortical, infratentorial, and spinal cord. Dissemination in time (DIT) refers to brain damage that occurs over time (Polman et al., 2011).

Table 1

McDonald 2010 diagnostic criteria of MS

Clinical Presentation (Possible Ms)		Additional requiremets to make diagnosis (MS)
Attacks (relapses)	Objective clinical lesions	
2 or more	2 or more	None, clinical evidence alone will suffice (additional evidence desirable but must be consistent with MS)
2 or more	1	DIS, demostrated by: >=1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord) or Further clinical attack implicating a different CNS site
1	2 or more	DIT, demostrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan or Second clinical attack
1	1 (CIS: clinically isolated syndrome)	DIS, Demostrated by: >=1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord) or second clinical attack implicating a different CNS site AND DIT, demostrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan or Second clinical attack
0 Insidious neurological progression suggestive of MS (PPMS: primary progressive MS)	0	DIT: One year of disease progression (retrospectively or prospectively determined) AND 2 or 3 of the following criteria: Evidence for DIS in the brain based on >=1 T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions or Evidence for DIS in the spinal cord based on >=2 T2 lesions in the cord or Positive CSF (evidence of oligoclonal bands and/or elevated IgG index)

Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald Criteria. Ann. Neurol. 2010; 69: 292–302

Epidemiology

Multiple sclerosis usually affects young adults, with the first diagnosis being between the age of 25-35 years. This time frame about the age of the onset of the disease is usually referred to as the peak, and then it declines slowly with age (Ascherio &Munger, 2008). We could say that MS is a pretty common disease, especially in the Western world. Pretty often cases of MS are being diagnosed in the United States, Europe, and Canada as well as Australia and New Zealand, but much less in Asia (Ascherio & Munge, 2008). Regarding sex, women are more commonly diagnosed than men in every population (Ahlgren et al., 2011), and the risk of developing MS is~

1 in 200 women. The female-to-male ratio is between 2.3 and 3.5:1 with an increase in the later years (Ahlgren et al. 2011; Orton et al. 2010; Wallin et al. 2012).

The cause of MS still remains uncertain. In fact, it is a complex disease, where most probably genes and environmental factors play a role in disease susceptibility. MS is described as an inflammatory, degenerative, and demyelinating disease with a Th-1 autoimmunity (Hemmer, et al., 2002). The mechanism of the disease involves the activation of CD4+ T cells. These particular cells target proteins of the myelin sheath of the neurons. CD4+4 T cells enter the CNS from the blood-brain barrier and start a chronic inflammatory cascade which is a result of axonal demyelination, mainly by macrophages. Other mechanisms like oligodendroglial degeneration and apoptosis, involvement of antibodies, and cellular mechanisms have also been studied in the literature (Lassmann et al., 2001). According to the literature, the cause of MS is an interaction of genetic and environmental. The genetic influence has been studied in family studies that show that monozygotic twins have higher chances of developing MS than dizygotic twins (Hansen et al., 2005; Willer et al., 2003). The risk becomes lower with a bigger genetic distance (Robertson et al., 1996). In terms of the mechanisms leading to the disorder, it is also often talked about the gender differences. MS is more prevalent in women than in men. Kantarci (2005) explains the differences might derive from the differences in gene expression of autosomal genes (Kantarci et al., 2005). Furthermore, animal studies of MS experimental autoimmune encephalomyelitis (EAE) discuss the possibility of increased risks for EAE due to the presence of two X chromosomes, regardless of hormones (Smith-Bouvier et al., 2008). Anyway, in MS studies the presence of vulnerable locations on the X chromosome has not been confirmed (Gutierrez-Roelens & Lauwerys, 2008). Thus, the higher prevalence of female patients with MS compared with males could be because of female-specific physiology and be connected to hormones (Whitacre, 2001).

Multiple sclerosis shows different phenotypes in patients, considering sex, age of the onset, and disease progression. Genetic studies are also interested in figuring out those differences. For example, each copy of the HLA-DRB1*1501 allele decreases the age of the onset (Sawcer et al., 2011). Some of these studies argue that this effect is more found in women (Celius et al., 2000; Masterman et al., 2000). On the other hand, some magnetic resonance imaging studies show that HLA-DRB1*1501 increases the severity of MS and has an effect on cognitive performance and reduction in brain volume, and an increase in disability progression in HLA-DRB1*1501-positive patients with MS (Okuda et al., 2009). In conclusion, it is believed that in multiple sclerosis patients, the highest genetic risk factor is probably the HLA alleles.

Environmental factors

Because of the complexity of the MS disease and its epidemiology, it is worth mentioning the potential causes of the disease that are the ones connected to genetics. Talking about environmental risk factors that are mentioned in the literature very often as ones with a very high probability chance of having a role in this disease. However, until now there is not only one single factor proven to be the cause of MS. For this reason, it is believed that there are multiple factors influencing the cause of MS, and it is most probably a mix of several genetic and environmental factors. The role of sex and genes is important but environmental studies demonstrate an important role for the environment in affecting MS risk. Risk factors of an environmental nature that according to the literature have a leading role in MS and are much talked about include the Epstein-Barr virus (EBV), vitamin D, smoking, and others.

Epstein-Barr virus is a human herpesvirus also known as mononucleosis and after infection persists in latent form in B lymphocytes throughout life. There is research examining the possible correlation between this virus and MS since it is considered to play a role in the development of

MS (Jacobs et al., 2020). In a recent very important study, it was shown that the MS risk increases up to 32-fold after being infected with EBV. Bjornevik et al. (2022) conducted a 20-year-old longitudinal study done on active-duty US military personnel with a cohort of more than 10 million individuals. For the study, they used stored samples for the EBV status. They reported 955 MS cases with 3 sample serums collected before the onset of the disease and were matched with MS-free individuals of the same age, sex, race, branch of military service, and dates of collection of blood samples. In the study 801 MS cases and 1566 controls had available EBV status and only one of the 801 MS cases was not EBV-positive. They state that the infection with the virus is not a consequence but a cause of MS. The authors strongly believe that EVB-negative people have a very low risk for developing MS and almost all of the MS cases are caused by mononucleosis. It was even suggested that a potential vaccine triggering EBV could prevent MS (Bjornevik et al., 2022). However, a preventive EBV vaccine needs yet to be studied and needs to be carefully studied for us to be able to make any inferences.

Another potential environmental factor is vitamin D and sunlight exposure, which in the last two or three decades has been studied quite a lot to demonstrate more details about the connection with MS. Sun exposure reduces the risk of developing MS and has in a way a protective effect on MS (Freedman et al., 2000*).* The analysis in a large study shows that increased serum levels of vitamin D (25-hydroxyvitamin D) decrease the risk of MS. Other studies also discuss the importance of sunlight and vitamin D in early childhood. For example, when migration happens during childhood there is an increased risk of MS development which tends to decrease when the migrations occur later in life (Gale et al.,1995). Different studies are concentrated on examining the association between vitamin D during pregnancy and the risk of developing MS in children later in their lives. A lack of vitamin D (25-hydroxyvitamin D) in expectant mothers might lead to

an elevated risk of multiple sclerosis (MS) in their children. This implies a potential connection between maternal vitamin D levels and the development of MS in offspring (Munger et al.,2016). Overall, vitamin D is considered to be a part of the most influential environmental risk factors regarding MS. The exact causes of MS are still not fully understood. It is believed to involve a combination of genetic predisposition and environmental factors. The specific triggers that lead to the development of MS in susceptible individuals are still a subject of investigation.

Neuroendocrine Regulation of The Immune System in MS

The nervous and endocrine systems are interconnected, collectively forming the neuroendocrine system. Moreover, this neuroendocrine system engages in bidirectional communication with the immune system (Deckx et al., 2013). These systems exert influence on each other directly or indirectly, subsequently affecting each other's functional activities. (Besedovsky & Del Rey, 1996). The two-way interaction in the neuroendocrine-immune system is formed by hormones, neurotransmitters, and cytokines that are being released. In a state of well-being, the neuroendocrine and immune systems operate in harmony, creating a precisely balanced regulatory system. This interplay is of great importance in maintaining homeostasis and overall health. In addition, both physiological and psychological stress are being managed by the neuroendocrine control of the immune responses and, this is possible due to the HPA and HPG axis and the hormones involved in their functioning. (Miyake, 2012). MS is mainly being considered as an autoimmune or immune-mediated disease. Deviation at any level in the neuroendocrine-immunological connection may result in alterations in susceptibility and the severity of various autoimmune and inflammatory conditions, including MS (Sternberg, 2001).

Aim of the current literature review

With an estimated 1.8 million individuals worldwide affected by MS, it is a disease that knows no geographic boundaries, as noted by the World Health Organization (WHO). It is one of the most widely discussed autoimmune diseases, targeting the brain and spinal cord. Consequently, MS patients encounter challenges that encompass cognitive decline, motor and sensory issues, as well as emotional and visual impairments across various aspects of their lives. Even though MS can affect individuals of all ages, it is most common in young adults and women. Given these considerations, it's crucial to underscore the importance of research in the field of MS. Enhancing the quality of life for these individuals, many of whom are young and possess vast potential, holds immense value. This paper will explore the disease through a psycho-endocrinological lens, examining the complex interplay between psychological and hormonal factors in MS. By taking this multidisciplinary approach, we aim to gain a deeper understanding of the various aspects of MS, including its potential links to neurological, psychological factors and endocrine system functioning. This approach recognizes the intricate nature of MS and seeks to shed light on new insights into its causes, progression, and potential avenues for management and treatment. This study aims to dig deeper into the understanding of the complexity of this worldwide spread disease from a neuroendocrine perspective. The overall objective is to discuss the previous findings about the role that the HPA axis plays in the progression of the disease. Furthermore, we want to provide a glimpse into the sex differences deriving from the influence of hormonal factors, which might give us a better understanding of the involvement of sex hormones in the prognosis and development of MS. The idea is to give a better picture of the involvement of sex steroids and other hormones like cortisol in multiple sclerosis. Do the sex differences in MS occur due to the differences in sex hormones, and could the stress axis potentially be a biomarker for MS? Such

insights could provide valuable information for understanding the underlying mechanisms of MS, its clinical manifestations, and the development of more targeted approaches to address this complex neurological disorder.

HPA AXIS in Multiple sclerosis

The complexity of multiple sclerosis is substantial, characterized by a large number of older and newly discovered risk factors and mechanisms that endeavor to establish connections within the disorder. For quite some time now, there has been ongoing research on the connection between the HPA axis and MS. The HPA (hypothalamic-pituitary-adrenal) axis plays a pivotal role in the body's response to stress, orchestrating the release of hormones that regulate various physiological processes that help adapt to and cope with challenging situations. Animal models as well as clinical trials are trying to provide information about the potential involvement of the HPA axis in the course of the disease. Having said this, in this part, studies that try to shed light on this topic will be presented as well as findings that are contradictive and confusing, making it almost impossible to extract one conclusion.

The Influence of Stress in MS

Stress can impact multiple sclerosis (MS) in various ways (Mohr et al., 2004). In research and clinical practice, there is a significant interest in the relationship between stress and MS. Furthermore, stress has stood the test of time as one of the enduring risk factors associated with multiple sclerosis (MS), with its recognition tracing back to the pioneering work of Jean-Martin Charcot (Charcot, 1877). His groundbreaking proposal has paved the way for a deeper

understanding of the intricate relationship between stress and the development or exacerbation of MS. While there is no conclusive evidence that stress directly causes MS, many people with MS believe that stress worsens their symptoms. Numerous research studies indicate that individuals diagnosed with MS may experience a notable rise in the risk of exacerbations in the weeks or months following the occurrence of stressful life events (Mohr et al., 2004). However, the relationship between stress and MS is complex, and the impact of stress on MS symptoms may vary from person to person. Similar to numerous other psychological phenomena, measuring psychological stress proves challenging due to its highly personal and subjective nature. In studies examining stress and MS, the measurements vary widely are quite heterogeneous, and, are primarily focused on the environmental approach (Riis et al., 2011). This could be one of the reasons why the literature lacks definitive or conclusive results on this topic. However, a more biological measurement of stress is the HPA axis, the so-called stress management or stress axis. The hypothalamic-pituitary-adrenal (HPA) axis is a crucial neuroendocrine system that plays a central role in the body's response to stress (Smith & Vale, 2006). It regulates various processes, including the immune system, metabolism, and the body's circadian rhythm. As a biomarker for stress, cortisol levels in the blood, saliva, or urine can be measured to assess the activity of the HPA axis (Nicolson, 2008). Elevated cortisol levels may indicate chronic stress or dysregulation of the stress response system (Guilliams & Edwards, 2010). Having said that, the HPA axis is being used as a potential indicator of an individual's stress response by researchers and healthcare professionals. Abnormalities in the HPA axis have been associated with various health conditions (Guilliams & Edwards, 2010).

The Mechanism of HPA Axis

The Hypothalamic-Pituitary-Adrenal (HPA) axis is a crucial neuroendocrine system that plays a central role in the body's response to stress and maintaining homeostasis. It involves a complex interplay between the hypothalamus, the pituitary gland, and the adrenal glands (Tsigos & Chrousos. 2002). When the body encounters stress or a threatening situation, the HPA axis is activated, leading to the release of various hormones that help the body cope with the stressor (Smith & Vale, 2006). Very often the HPA axis is referred to as stress management or just the stress axis, because of its functional importance in the body's response to perceived stress (Guilliams & Edwards, 2010).

In the Hypothalamic-Pituitary-Adrenal (HPA) axis neuroendocrine system three main components are involved in a sequence of interactions between each other. These are the hypothalamus, the pituitary gland, and the adrenal glands (Papadimitriou & Priftis 2009). The activation of the HPA axis starts in the hypothalamus, which is a small brain region located above the brainstem. The corticotropin-releasing hormone (CRH) is secreted from the hypothalamus into the bloodstream. The CRH travels through the bloodstream and reaches the Pituitary gland, which serves as a crucial link between the nervous and the endocrine system. In turn, the pituitary gland releases adrenocorticotropic hormone (ACTH) into the bloodstream from where it goes to the adrenal glands (Papadimitriou & Priftis 2009). CRH helps the anterior pituitary gland to be stimulated and to release adrenocorticotropic hormone (ACTH). Furthermore, ACTH circulates towards the adrenal cortex where glucocorticoids are released in a diurnal pattern. CRH secretion is upregulated by serotonergic, cholinergic, and catecholaminergic systems, whereas GABA, GC, and ACTH can inhibit the secretion through negative feedback. (Deckx et al., 2013). In response to ACTH, the adrenal glands produce and release cortisol, which is a glucocorticoid hormone.

Cortisol is the primary stress hormone and plays a vital role in the body's response to stress, metabolism, immune function, and other essential processes (Lightman, Birnie & Conway-Campbell, 2020). With the rise of cortisol in the system, there is a negative feedback loop that occurs. The hypothalamus and the pituitary gland inhibit the release of the CRH and ACTH, respectively. This feedback loop is important for the regulation of the HPA axis by making sure the cortisol levels return to normal (Lightman, Birnie & Conway-Campbell, 2020). A diagnostic method usually used in clinical settings for examining the functioning of the Hypothalamic-Pituitary-Adrenal (HPA) axis is the Corticotropin-Releasing Hormone (CRH) stimulation test (Yanovski, 1993). The procedure involves the administration of a synthetic form of CRH called corticotropin-releasing hormone or corticotropin-releasing factor (CRF). The purpose of this hormone is to stimulate the HPA axis, by activating and releasing ACTH from the pituitary gland. In succession, cortisol is released because of ACTH triggering the adrenal glands (Yanovski, 1993). As part of the procedure, it is important to measure the patient's cortisol levels before or at baseline levels and after the administration of the CRF. Even though cortisol can have beneficial effects in managing inflammation and immune responses in MS (Gold et al., 2005), prolonged or chronic elevation of cortisol levels, often associated with chronic stress, can potentially have adverse consequences (Guilliams & Edwards, 2010). Ultimately, the assessment of cortisol levels in individuals with multiple sclerosis holds significant importance as it provides insightful measurements critical for a comprehensive understanding of the disease and its potential links to the neuroendocrine system.

Animal Studies

Animal model studies are a fundamental aspect of scientific research, particularly in the fields of psychology and neuroscience. Moreover, animal research data provide the scientists with

important information regarding the MS diagnosis and progression. In the scientific literature, different animal models can be used in order to give a more thorough insight into the disease. One of the most widely used animal models for studying MS is the experimental autoimmune encephalomyelitis (EAE) (Robinson et al., 2014). Researchers induce autoimmune demyelination in animals, typically mice or rats, by injecting them with myelin proteins or peptides combined with an adjuvant (Bjelobaba et al., 2018). EAE models have helped elucidate the immunological mechanisms involved in MS and have been crucial in testing potential therapies as well (Constantinescu et al., 2011). It is important to note that although animal studies give us an important inside in studying MS, the results must be interpreted carefully and with caution since they differ from clinical human studies. Reduced responsiveness of the Hypothalamic-Pituitary-Adrenal (HPA) axis due to genetic factors is linked to an elevated susceptibility to disease. Still, this relationship is specifically observed in the MBP EAE (Myelin Basic Protein Experimental Autoimmune Encephalomyelitis) model in the Lewis rat (Heesen et al., 2007). When the disease experiences subsequent relapses following an initial clinical phase in a CR-EAE (Chronic Relapsing Experimental Autoimmune Encephalomyelitis) model, the corticosterone responses are notably reduced or diminished. This suggests that the ability to produce corticosterone, which is a hormone involved in stress response and immune regulation, is compromised during the later stages of the disease compared to the initial phase (Stefferl et al., 2001). This observation indicates a change in the body's stress response throughout the disease, which may have implications for the disease's progression and management. The Hypothalamic-Pituitary-Adrenal (HPA) axis exhibits reduced or weakened responses to inflammatory triggers or stimuli in CR-EAE (Stefferl et al., 2001). This also further indicates that the HPA axis doesn't react as strongly as it typically would in the presence of inflammatory signals. This blunted HPA axis response may have implications

for the regulation of inflammation and the progression of the CR-EAE disease model (Stefferl et al., 2001). Even though early animal model studies propose a hypoactivated HPA axis in EAE, it might not necessarily apply to other models or contexts. This effect is restricted to this particular experimental model and it doesn't strictly imply the same result in MS. Animal models are particularly important, but by any means, the results cannot be generalized for patients with MS.

As was already mentioned, animal models are particularly important, giving us insight into the possible biomarkers of MS, helping us in postulating new hypotheses, and providing us with valuable direction of thinking. However, comparing the animal with human studies it can be noted that there are numerous conflicting and contraindicative findings. More specifically, data shows that although as it was said earlier, there is a significant correlation between MS and HPA-axis functioning, the direction of this correlation differentiates between animal and human studies. That being said, data from animal studies by any means cannot be generalized for patients with MS. In the next part, this relationship will be more carefully explored.

HPA Axis in MS Patients

Even though experimental autoimmune encephalomyelitis(EAE) animal model studies support the notion that a hypoactivity of the HPA axis could be a predisposing factor for the severity of the disease as well as the susceptibility, a big number of clinical studies are talking about hyperactive HPA axis in MS patients (Heesen, 2002; Gold et al., 2005; Bergh et al., 1999), elevated basal plasma levels of cortisol (Michelson et al., 1994) and adrenocorticotropic hormone ACTH (Michelson et al., 1994) and enlarged adrenals in patients (Reder, 1994) with diagnosed multiple sclerosis. Moreover, according to some studies, the cortisol response after a CRH stimulation is potentially different in different types of MS. For example, it was shown that the cortisol response was lower in SP-MS patients when compared to PP-MS and healthy controls

(Wei & Lightman, 1997*).* On the other hand, higher β-endorphin/ACTH response was seen in RRMS patients compared with the other groups (Wei & Lightman, 1997). It also appears that higher cortisol levels usually are noted close to or during acute relapse (Fassbender, 1998). In conclusion, the contrasting observations in experimental autoimmune encephalomyelitis (EAE) models and clinical studies regarding the HPA axis and cortisol dynamics in multiple sclerosis highlight the multifaceted nature of these interactions, necessitating continued investigation to elucidate the intricate mechanisms underlying the role of the neuroendocrine system in the development and progression of the disease.

Wei and Lightman in 1997, did a study where the main investigation was basal and dynamic regulation of the hypothalamic–pituitary–adrenal (HPA), hypothalamic–pituitary–thyroid (HPT), and hypothalamic–pituitary–gonadal axes and the prolactin secretion. The study was done on 52 patients with multiple sclerosis, each of them grouped into one of the three types: relapsing-remitting multiple sclerosis, primary progressive multiple sclerosis, and secondary progressive multiple sclerosis. The majority of them were in a relapse for four weeks. The study included 10 healthy controls and 5 patients with other neurological disease. Based on the results obtained from animal studies, Wei and Lightman wanted to test the role of the HPA axis in patients with MS. To do so, among other things, baseline levels of cortisol were taken and a CRH test was performed. To obtain the measure for baseline cortisol blood samples were taken in two-hour intervals or half-hour intervals throughout the day (6:30-24:00h.). In the morning CRH hormone was injected and for one hour every 20 minutes blood was taken for the measurements. After the CRH injection gonadotropin-releasing hormone (GnRH) and synthetic ACTH were injected. Afterward, every 20 min for an hour blood samples were taken. Another measurement taking place in the study is the dexamethasone-suppression test. 1 mg of dexamethasone was orally taken by the participants at

23:00 h the night before and at 9:00 the next morning, the blood was taken. Additionally, MRI brain scans were obtained and CRP was measured and determined from the samples taken from 8-9:30 h. in the morning. The findings of this study indicated that the diurnal rhythm of cortisol secretion remained unchanged in all groups. However, when examining the cortisol response to the corticotropin-releasing hormone (CRH) stimulation test, the group of patients with secondary progressive multiple sclerosis exhibited a reduced overall cortisol response compared to both healthy participants and the group of primary progressive MS patients. For the ACTH measurements, there were no significant differences between the groups. The relapsing-remitting MS group showed a significantly higher mean total area under the curve for β-END, in comparison to primary progressive, secondary progressive, and normal controls. Only a trend towards significant differences was observed for the synthetic ACTH test, meaning the mean baseline concentrations of cortisol were lower in the relapsing-remitting MS. The dexamethasone suppression test showed that the feedback regulation was normal (Wei & Lightman, 1997). Furthermore, they conclude that the relationship between C-reactive protein (CRP) levels and MRI findings indicates that the activation of the hypothalamic-pituitary-adrenal (HPA) axis in individuals with multiple sclerosis may be a response to ongoing inflammatory stimuli (Wei & Lightman, 1997). Following this hypothesis, another study shows a positive correlation between HPA hyperactivity and Gd-enhanced lesions measured with CSF cell count and MRI scans in 23 patients with RRMS (Fassbender et al., 1998). On the other hand, Schumann et al. (2002) wanted to replicate and further establish this relationship but did not obtain the same results. This study was done on MS patients, with different disease courses (35 RRMS, 13 SPMS, 5 PPMS), but failed to prove the association between increased CNS inflammation and HPA axis hyperactivity. Instead, they found fewer Gd-enhancing lesions on MRI in patients with HPA axis hyperactivity

(Schumann et al., 2002). Furthermore, in the aforementioned study conducted by Wei and Lightman (1997), although they demonstrated a positive correlation between hyperactivity of the HPA axis and MRI lesions, it's essential to note that the sample size was notably limited. Notably, enhanced gadolinium lesions were observed in only 3 out of the 5 patients who underwent MRI scans. With this being said, any general conclusions cannot be made and further investigation is much needed.

The first longitudinal study on the prognostic significance of the HPA axis in MS was published in 2005 (Gold et al., 2005). Taking into consideration previous data on HPA hyperactivity in MS patients, during a 3-year follow-up period the main goal was to test the correlation between the HPA axis and progression as well as cognitive impairment. At the beginning of the study, 40 MS patients were included. They were first evaluated in the year 2000. The evaluation included measures for the Expanded Disability Status Scale-EDSS (Kurtzke, 1883), the Symbol Digit Modalities Test-SDMT (Smith, 1973), and the Hospital Anxiety and Depression Scale-HADS (Zigmond & Snaith, 1983). Measures were also taken from a combined Dexamethasone-CRH suppression (Dex-CRH) test. Three years later patients were contacted again, and the ones who took place in the follow-up were evaluated again. The patients were divided into ACTH high and low responders depending on their performance on the first Dex-CRH test. The high responders showed higher EDSS and CAMBS scores and progressing ratings three years later. Furthermore, those patients also had a statistically significant increase in their EDSS scores compared with their scores three years earlier, and 11 of them had a significant disease progression according to the EDSS criteria. The results of a 3-year follow-up study done by Gold and colleagues (2005) show a statistically significant connection between the HPA axis hyperactivity measured in the year 2000 and the disease progression during the follow-up. Also,

3-years later the ACTH high responders showed significantly higher EDSS scores and CAMBS ratings. Another result from this study is the statistically significant higher scores on the EDSS scale in 2003 compared to the measurements in 2000. Furthermore, the authors support earlier hypotheses about the correlations between hyperactivity in the HPA axis and increased neurological impairment, cognitive deficits, and global brain atrophy. In other words, dysregulation of the HPA axis might be caused by increased neuronal damage. The authors state that this hypothesis is also supported in their study given the results about the correlations with cognitive impairment over the 3-year follow-up period as well as the moderate stability over time. According to this study, the dex-CRH test can significantly predict the progression of the disease in the follow-up period. The explanation is that 8 out of 13 high responders or 62% showed disease progression, measured with the EDSS criteria, on the other hand only 3, or 23% of the low responders showed a clinical decline on the scale. The authors (Gold et.al, 2005) do note some of their limitations including the small sample size. They had lost 14 patients from the initial study but they do believe that their findings on the predictive value of high responders are underestimating the effect, not overestimating it. The small size didn't allow for analyses to be made on subgroups of patients with different MS courses. According to the literature, this is probably the first study that demonstrates that hyperactivity in the HPA axis might be a predictor for disease progression. It is not yet known whether the hyperactive HPA axis is a cause or just a coincidence with progression. According to the authors, Dex-CRH suppression test on MS patients could have prognostic relevance in the future (Gold et.al, 2005).

Heesen et al. (2002) hypothesized that the degree of overall brain damage in MS patients may be indicated by the degree of HPA dysregulation. To test this hypothesis, they studied the correlation between the Dex-CRH suppression test, cognitive deficit, and fatigue. The study was

done on 40 MS patients including 8 patients with RRMS, 19 diagnosed with SPMS, 13 with PPMS, and 11 controls. The variables previously referenced that were measured in this study were tested with the Dex-CRH tests for HPA axis activity, the symbol digit modalities test (SDMT) to test the cognitive impairment, and three scales to measure fatigue: Fatigues severity scale (FSS), MS fatigue scale (MSFIS) and MF-FSS. In this particular study, the progressive groups had higher scores on the Expanded Disability Status Scale (EDSS). According to the results of this study, the SDMT measure for cognitive impairment correlated with the degree of HPA axis hyperactivity, which also correlated with the EDSS scale for neurological impairment. Further, the obtained data also suggests that primary and secondary progressive MS had a hyperactivation of the HPA axis, whereas the relapsing-remitting group had similar responses to the healthy controls. Only the area under the curve analysis of ACTH stimulation was statistically significant out of the six analyzed parameters. The authors of this study imply that increased function of the HPA axis is related to increased cognitive impairment, and neuronal disability, moderately with the duration of the disease and the least with depressive symptoms and fatigue. Moreover, Heesen et al. (2002) discuss that the existence of cognitive impairments is probably more indicative of the degree of brain damage rather than a distinct disease course. Therefore, the connection between a decrease in cognitive function and a weakened cortisol suppression following dexamethasone, along with a hyper-responsive ACTH/cortisol response in the Dex-CRH test, provides additional evidence that these alterations in neuroendocrine function are a concurrent consequence of the illness and not primarily contributing to its pathogenesis (Heesen et al., 2002).

The studies whose main hypothesis includes the function of the HPA axis in MS patients and its effect on the disease date from around 30 years ago. Yet, still, there is no definitive answer. In the context of EAE, researchers have observed alterations in the hypothalamic-pituitary-adrenal

(HPA) axis, which plays a crucial role in the body's response to stress and inflammation. Studies in EAE have suggested that there can be HPA axis hypoactivity, characterized by a reduced responsiveness of the adrenal glands to produce cortisol (Heesen et al., 2007; Stefferl et al., 2001). This reduction in HPA axis activity may be linked to the overall immune response and inflammation associated with EAE (Heesen et al., 2007). Even though EAE animal studies on HPA regulation give us a starting point and some important results, the fact that these animal models do not reflect every aspect of MS human disease cannot be diminished.

In general, patients with MS experience increased HPA activity. As previously discussed Heesen et al. (2002), showed that HPA hyperactivity is connected to increased cognitive impairment and neuronal disability in MS patients. Bergh et al. (1999) further support the correlation between HPA hyperactivity and progressive disease course and disability. Their study underlines the significant relationship between heightened HPA activity and the exacerbation of disease trajectory, ultimately leading to increased levels of disability (Bergh et al., 1999). As mentioned before, Gold et al. (2005) studied HPA axis dysregulation in patients with MS and found that hyperactivity in the Dex–CRH test significantly predicted disease progression (as measured by an increase of at least 1 point on the EDSS) over a 3-year follow-up period. This longitudinal study derives the hypothesis that in the future results from the Dex–CRH tests may be of prognostic value in MS. On the other hand, research on the correlation between HPA (Hypothalamic-Pituitary-Adrenal) responses and MRI Gd-enhancing lesions, commonly utilized as indicators of inflammation, presents conflicting findings. Schumann et al. (2002) observed a negative correlation between hyperresponsiveness in the Dex–CRH test and Gd-enhanced lesions. Intriguingly, their findings diverged from expectations, as they discovered no significant correlation between hyperresponsiveness in the Dex–CRH test and inflammatory markers in both

blood and cerebrospinal fluid (CSF). The researchers, nonetheless, documented a positive correlation between HPA activity in the Dex–CRH test and an MRI measure of global atrophy (Schumann et al., 2002). In contrast, earlier studies point out a positive correlation between hyperactivity of the HPA axis and MRI brain lesions (Fassbender et al., 1998; Wei & Lightman, 1997). These contradictive discoveries add nuance to the understanding of the interplay between HPA (Hypothalamic-Pituitary-Adrenal) axis hyperresponsiveness and the inflammatory processes indicated by Gd-enhanced lesions. One possible explanation for the different results shown in different studies might be due to the dynamic nature of the disease activity at the moment of testing (Heesen et al., 2007). However, there is a compelling need for additional research endeavors aimed at unraveling the intricate relationship between HPA responses and inflammatory parameters, particularly Gd-enhancing lesions in MRI scans. Undertaking more studies will contribute to a deeper comprehension of the complexities involved in this correlation, potentially paving the way for enhanced insights into the mechanisms at play in various disease states.

In conclusion, the relationship between HPA (Hypothalamic-Pituitary-Adrenal) responses and multiple sclerosis (MS) remains a complex and delicate area of investigation. Studies, as highlighted by Heesen et al. (2002), Bergh et al. (1999), and Gold et al. (2005), reveal a notable association between increased HPA activity and cognitive impairment, neuronal disability, and disease progression in MS patients (Heesen et al., 2002; Bergh et al., 1999; Gold et al., 2005). However, when exploring the correlation with MRI Gd-enhancing lesions, the findings become contradictory, with Schumann et al. (2002) reporting a negative correlation while other studies point to a positive association (Schumann et al., 2002). Despite these challenges, the collective body of research underscores the significance of further investigations to elucidate the intricate interplay between HPA responses and inflammatory parameters, emphasizing the potential

prognostic value of Dex–CRH tests (Gold et.al, 2005). Continued efforts in this direction promise to enhance our understanding of the underlying mechanisms in MS and contribute valuable insights for future therapeutic approaches.

Changes in Sex Hormones in MS

According to the scientific data, differences between sexes are reported in multiple segments of the disease. Gender plays a significant role in creating the framework of Multiple Sclerosis (MS), aligning with patterns observed in various autoimmune diseases, where there is a pronounced prevalence of the disease among females (Kantarci & Weinshenker, 2005). Furthermore, an age gap is observed in disease onset between female and male MS patients, more so in younger patients where the female-to-male ratio is even more evident (Duquette et al., 1998). There are reported discrepancies in the symptomatology of MS, with motor symptoms being more frequently associated with the male gender (Hawkins & McDonnell, 1999). Moreover, cognitive impairment is observed to be greater also in men (Savettieri et. al., 2004). On the other side, sensory symptoms are usually connected to women more than men (Österberg et. al., 2005). MRI studies likewise explore the gender differences in MS, in fact, results show that women suffer from more inflammatory lesions but men are more susceptible to destructive brain lesions compared to women (Pozzilli et. al., 2003).

The hypothesis proposing a link between hormones and autoimmune diseases stems from two primary observations. Firstly, the impact of corticosteroids on inflammation, as these compounds exhibit influence over various autoimmune disorders responsive to corticosteroid

treatment. The second one is the prevalence of gender bias in autoimmune diseases, with a higher incidence among women, which has sparked interest in the potential role of hormones. Notably, fluctuations in hormonal levels during significant life events, such as pregnancy, contribute to varying outcomes in different autoimmune disorders, some showing improvement while others worsen (Xiang & Shi, 2023). This synthesis underscores the intricate relationship between hormonal influences and the manifestations of autoimmune diseases (Shuster, 2008).

The intricate interplay between sex hormones and the pathophysiology of Multiple Sclerosis (MS) unfolds a captivating narrative in the realm of neurological disorders. As researchers delve into the complexities of MS, the influence of sex hormones, including estrogen and testosterone, emerges as a crucial factor in shaping the risk, progression, and clinical manifestations of the disease. While estrogen is implicated in potential protective effects, modulating immune responses and offering neuroprotection (Green & Simpkins, 2000), testosterone, on the other hand, may play a role in mitigating the severity of MS, particularly in men (Bove & Chitnis, 2013). These intricate hormonal dynamics extend to critical life phases, such as pregnancy and menopause, where hormonal fluctuations wield varying impacts on the course of MS (Ghezzi & Zaffaroni, 2008). In this exploration, we delve into the nuanced relationship between sex hormones and MS, unraveling the current understanding of their roles and implications for both disease susceptibility and progression.

The Role of Estrogens

Estrogens are sex steroid hormones that are present in both men and women, but they circulate at significantly higher levels in women during reproductive age (Maglione, 2019). Three naturally occurring estrogens are present in the female body: Estrone (E1), the primary form during menopause; Estradiol (E2), predominant in non-pregnant women; and Estriol (E3), the main

estrogen during pregnancy (Deckx et al., 2013). The hypothalamus produces and releases Gonadotropin-releasing hormone (GnRH) into the hypophyseal-portal circulation. GnRH acts as a stimulant for the synthesis and secretion of gonadotropic hormones, including follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Once released, FSH and LH travel through the bloodstream to the reproductive organs, where they stimulate the release of estrogen and progesterone, essential hormones for reproductive processes (Deckx et al., 2013). Estrogen binds to two distinct estrogen receptors (ER), each with unique transcriptional properties. ERα is expressed in the endometrium, ovarian stromal cells, breast, and hypothalamus. In contrast, ERβ is widely expressed in various tissues, including the brain, kidney, bone, heart, lungs, intestine, and endothelial cells. This broad distribution suggests that ERβ may have immunomodulatory properties (Deckx et al., 2013). Furthermore, the effects of estrogen are conveyed through the activation of these receptors, which are prominently present in the central nervous system (Tomassini & Pozzilli, 2009). Estrogens, particularly estradiol, play a role in inhibiting encephalitogenic T cells, preventing the migration of cells into CNS tissue, enhancing the presence of Treg cells, and exerting neuroprotective effects that support the survival of axons and myelin. Additionally, estrogens contribute to the growth and differentiation of neurons (Tomassini & Pozzilli, 2009). However, in studies involving animals with Theiler's murine encephalomyelitis and experimental autoimmune encephalomyelitis (EAE), it has been observed that varying concentrations of estrogens elicit divergent responses. Low concentrations of estrogens appear to facilitate immune responses, while elevated concentration levels may have suppressive effects on immune responses (Tomassini & Pozzilli, 2009). With this being said, our focus now shifts to exploring the role of "female hormones" in the development and prognosis of multiple sclerosis (MS).

The concentration of circulating estrogens undergoes fluctuations throughout a woman's life, commencing in childhood and persisting until menopause. These hormonal variations exert consistent effects on the female body during each life stage. Estrogens play a pivotal role in fostering the emergence of secondary sexual characteristics in females and orchestrating the menstrual cycle (Deckx et al., 2013). Beyond their involvement in sexual development, estradiol has a profound impact on the functioning of diverse organs and tissues such as the skin, muscles, adipose tissue, the brain, cardiovascular system, and bones. Notably, it actively safeguards against conditions like osteoporosis and various cardiovascular diseases (Wend et al., 2012).

Pregnancy induces physiological transformations in all women. This transformation almost always includes increased basal metabolic rate and cardiac output, as well as elevated lipid levels and weight gain. Moreover, during pregnancy, women experience hormonal changes, and these changes are evident in hormones like estriol, progesterone, and prolactin among others. (McCombe and Greer, 2012). According to the scientific literature, the course of multiple sclerosis is significantly impacted by pregnancy, a physiological phenomenon known for its distinctive hormonal shifts. In research findings, there is frequent discussion about the reduction in the number of relapses observed in pregnant women, particularly during the third trimester. This phase is marked by notable changes in the levels of progesterone and estrogens. During pregnancy, there is a physiological shift from TH1 to TH2 immune response, leading to the production of anti-inflammatory cytokines. This shift contributes to favorable conditions for embryo implantation and development (Al-Shammri et al., 2004). The concentrations of two estrogen hormones, estradiol and estriol, along with progesterone, experience a gradual rise throughout pregnancy, reaching their highest levels in the third trimester (Bates et al., 2020). Shortly after childbirth, the levels of these hormones decrease, aligning with the temporal pattern that corresponds to the

protective effect of pregnancy on the rate of relapses in multiple sclerosis (Tulchinsky et al., 1973). To sum up, researchers agree on the fact that pregnancy has beneficial effects in terms of the progression of the disease, at least temporarily. Notably, they believe that pregnant women with MS experience fewer relapses during pregnancy compared to the pre-and postpartum periods. Furthermore, it is well known that all women during their pregnancy are prone to changes in their hormonal levels such as estrogen (Miller et al., 2014). Even with this knowledge, we don't have enough scientific evidence to accept the hypothesis that the naturally occurring changes in estrogens and potentially progesterone during pregnancy are the factors that directly contribute to less active lesions and the decline in MS relapses. In the future, the next goal is to test this hypothesis and try to prove a correlation between estrogens and MS in pregnant women.

It is noteworthy to highlight the initial investigation examining the impact of estriol on women who are not pregnant. Estriol, an estrogen produced by the fetal placental unit, experiences a gradual rise during pregnancy. However, its concentrations are significantly lower in nonpregnant women. The main hypothesis in this study is that administering the pregnancy dosage of estriol to nonpregnant females with MS would yield positive effects on relapse rates and MRI lesions, mirroring the observed outcomes in MS patients during pregnancy (Sicotte et al., 2002). The study has a crossover design, 6 months of observation during pretreatment followed by 6 months of treatment period with oral estriol (8mg/day), after that another 6 months of posttreatment period and 4 more months or re-treatment period. Additionally, MRI scans were done monthly, and neurological examinations once in 3 months on all 10 patients that completed the study. The patients were divided into RR-MS or SP-MS according to the course of the diagnosed disease. Based on the findings, the estriol serum levels during treatment and the re-treatment period resembled the estimated concentrations observed in women at the sixth month of

pregnancy. Still, they were lower than those at 8.5 months. During the treatment periods, the number of enhancing lesions decreased compared to the pretreatment baseline measures. This was significant only in the RR-MS patients and unfortunately not in the SP-MS group. An increased number of lesions were observed during the 6 months posttreatment period (Sicotte et al., 2002). These results suggest a beneficial effect of estriol hormone in nonpregnant RR-MS women with a reduction in enhanced brain lesions. This is the first study empirically investigating the pregnancy hormone estriol and its effects in nonpregnant MS females. Notably, estriol treatment had a significant impact on enhancing cognitive function, as assessed by the PASAT, within the RRMS group, although such effects were not observed in the SPMS group. Even though the sample was obviously small, the effects of estriol were proven to be statistically significant. Replication studies done on a much bigger cohort are needed to further support the protective anti-inflammatory effects of estriol and estrogens in general.

Pozzilli and colleagues (1999) conducted a study involving eight women diagnosed with relapsing-remitting multiple sclerosis (RRMS), aged between 27 and 40 years. The investigation utilized serial magnetic resonance imaging (MRI) with a triple dose of gadolinium, incorporating delayed post-contrast scanning across four consecutive menstrual cycles. On the same day as the MRI, levels of 17b-estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone (P4) were assessed. Comparable blood level measurements were performed on eight healthy women of a similar age. The study revealed no discernible difference in the frequency of enhancing lesions during the follicular (day 3–9) or luteal (day 21–28) phases of the menstrual cycles. Similarly, there was no correlation between the number and volume of gadolinium-enhancing lesions and the levels of a single hormone. However, a significant relationship ($R = 0.7$, $p = 0.009$) emerged between the number and volume of enhancing lesions and the ratio of

progesterone to estradiol (P4/E2). Higher levels of this ratio were associated with increased MRI activity, suggesting a potential link between hormonal fluctuations, particularly in the progesterone to estradiol ratio, and MS disease activity (Pozzilli et al., 1999).

In a study by Bansil and colleagues (1999) involving 30 premenopausal women diagnosed with clinically definite or laboratory-supported multiple sclerosis (MS), the researchers sought to investigate the correlation between hormonal levels and MRI findings. Since estrogen levels did not consistently align with the menstrual cycle in some participants, the study categorized patients into three groups based on distinct hormonal ratios correlated with MRI imaging. Group 1 exhibited low levels of both estradiol (E2) and progesterone (P4) during the early follicular phase, Group 2 showed high E2 and low P4 during the late follicular phase, and Group 3 had high P4 with variable E2 during the luteal phase. The findings indicated a significantly higher number of gadolinium-enhancing lesions ($p = 0.04$) in patients with high E2 and low P4 compared to those with low levels of both hormones, suggesting a pro-inflammatory effect associated with elevated estrogen combined with low progesterone. Conversely, patients with high levels of both estrogen and progesterone exhibited lower disease activity, supporting a potential protective effect. Notably, Group 2 patients, characterized by high estrogen and low progesterone, had a higher prevalence of relapsing-progressive MS, potentially influencing the results, which were based on the mean number of gadolinium-enhancing lesions in their comparisons (Bansil et. al., 1999).

All women experience a permanent cut-off of ovarian function, which is called menopause. During this period which usually happens around the age of 50, levels of sex hormones change and estrogen levels are decreased. Low levels of estrogens are connected to neuroinflammation and neurodegeneration (Christianson et al., 2015). Menopause affects the reproductive, immunological, and neurological systems (Anderson et al., 2021). According to the literature

women with MS go through menopause during the same age as healthy women (Bove et al., 2015). There is some inconclusive data in the literature regarding menopause and MS. On the one hand, two studies suggest that after menopause there is a decline in the relapse rate in MS (Baroncini et al., 2019; Ladeira, 2018). On the other hand, a longitudinal study assessing EDSS scores showed that the scores in fact increased after menopause (Bove et al., 2016). The relationship between menopause, Multiple Sclerosis (MS), and hormonal changes, particularly the decrease in estrogen levels, remains an area of limited research in the current literature. Further investigation into this topic is crucial as it may shed light on the hypothesis that menopausal transitions could contribute to the progression of patients from Relapsing-Remitting MS (RRMS) to more severe and progressive forms of the disease (Schwendimann & Alekseeva, 2007). Expanding our understanding of these connections could pave the way for more targeted interventions and improved management strategies for women experiencing both menopause and MS.

There is little evidence about the connection between estrogens and male patients with MS. Tomassini, Onesti, Mainero, et al. (2005) did a correlational study on sex hormones and MRI lesions in both men and women with MS. Based on the results, there was a positive correlation between estradiol concentration levels and brain damage in the male participants (Tomassini et al., 2005). There is an evident gap in research concentrated on the predominantly female hormones in the opposite sex. The findings of this study, indicating a connection between estradiol levels and brain damage in men with MS, highlight the need for further research in this specific area.

In summary, estrogens, vital sex hormones present in both genders, exert diverse influences beyond reproduction, with estrogen receptors distributed widely in the body. Pregnancy-induced hormonal shifts, particularly elevated estrogens, temporarily shield against MS relapses, while studies on estriol in nonpregnant women with RRMS suggest potential protective effects.

Menopause introduces complexities, with conflicting findings on its impact on MS progression. Limited research on male MS patients emphasizes the necessity for further investigation into the intricate relationship between sex hormones and brain damage. Overall, understanding these hormonal intricacies is crucial for targeted interventions and enhanced patient care, demanding continued research efforts to unravel the nuanced connections between hormones and MS.

The Role of Testosterone

Almost all reproductive hormones are secreted by the hypothalamus pituitary gonad axis (Meethal & Atwood, 2005), such as gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone. With the aging of the body, gonadal function decreases gradually (Dong et al., 2021). This means that androgen and estrogen levels decline with time and age. Androgens play a vital role in human reproductive and sexual function (Hsu et al., 2015; Davis et al., 2016). Testosterone belongs to a class of male sex hormones known as androgens and serves as the primary androgen, but it is present in both males and females. It is a hormone primarily produced in the testicles of men. Conversely, in females, the adrenal glands and ovaries serve as the principal sources of androgens. Testosterone plays a crucial role in various physiological processes, including the development of male reproductive tissues, the maintenance of muscle mass and bone density, and the promotion of facial and body hair growth (Smith, 2013). Testosterone is synthesized from cholesterol through a cascade of enzyme reactions. This biosynthetic pathway is regulated by the hypothalamic-pituitary-gonadal (HPG) axis, involving interactions between the hypothalamus, pituitary gland, and gonads. Hormones such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH) play key roles in regulating testosterone production (Swerdloff et al., 2002). On the other hand, in females, testosterone can be converted into estrogen through a process called aromatization. The enzyme aromatase, present in various

tissues, facilitates the conversion of testosterone into estradiol, which is a form of estrogen. This conversion plays a crucial role in maintaining hormonal balance in females (Simpson, 2002). Concentrations of bioavailable testosterone, a form of testosterone that is readily accessible for use by the body, can experience a significant decline of up to 50% from the ages of 25 to 75 years (Gould et al., 2000). This observation aligns with the general understanding that testosterone levels tend to decrease with age, particularly in men. According to the literature, testosterone has many beneficial effects on the CNS. Essentially it boosts the survival of neurons and astrocytes (Meydan et al., 2010), as well as activates neuronal plasticity (Matsumoto, 1997). Furthermore, testosterone increases the connectivity between hypothalamic neurons (Beyer & Hutchison, 1997), supports synaptic density (Leranth et al., 2003) and of course, it postpones aging (Zárate et al., 2017). Given all of the preceding information, the role of testosterone in Multiple Sclerosis will be elaborated.

Animal models have studied the influence of sex hormones, in this particular case, testosterone. The removal of male animals' testes, known as castration, has demonstrated harmful effects on the susceptibility and severity of Experimental Autoimmune Encephalomyelitis (Bebo et. al., 1998). In line with this, other animal studies, affirm the concept that testosterone has an anti-inflammatory effect because treatment with testosterone reduces inflammation, decreases secretion of proinflammatory cytokines (TNF-α and IFN-γ), and increases the production of Th2 cytokines (Dalal et. al., 1997; Liva et. al., 2001). Taking this into consideration, human studies further explore the effects of testosterone, starting with the hypothesis that testosterone is positively correlated with the severity of MS and has a potential effect on reducing inflammation.

Bove et al. (2014) supported the hypothesis that low levels of testosterone in men are associated with disease severity. They essentially wanted to dig deeper into the already postulated fact that men develop MS later in life, but are more likely to experience progressive forms of MS

and present increased brain atrophy, disability, and cognitive impairment in comparison to women with MS (Bove & Chitnis, 2013; Savettieri et al., 2004). This is a longitudinal study that included 96 males, which were diagnosed with either RRMS or clinically isolated syndrome (CIS). For measuring clinical disability Expanded Disability Status Scale (EDSS) was used every 6 months, moreover for annual testing of executive function, processing speed, and early markers for longitudinal cognitive changes they used the Symbol Digit Modalities Test (SDMT). Additionally, testosterone levels were measured. Firstly, according to their analyses, 39% of the participants showed hypogonadism or low testosterone levels early in the disease. Secondly, low testosterone levels were associated with increased EDSS and SDMT scores longitudinally. To sum up, in compliance with these findings, men with MS may experience low levels of testosterone which may be associated with the progression of the disease (Bove et al., 2014). Nevertheless, we cannot conclude causality, but it opens a discussion about testosterone levels potentially being used as biomarkers not only as a consequence of the disease. However, we are far from giving definitive answers on this, and future research will give us a better idea of the importance of testosterone in MS and its involvement in the progression of the disease in men.

The question of the involvement of testosterone in women with MS is not commonly investigated. There are just a few studies exploring this subject. One of those studies is by Tomassini, Onesti, Mainero, et al. (2005), who investigated the connection between sex hormone concentrations and MRI features in RRMS. The data indicates that women with MS had lower concentration levels of testosterone than controls in both follicular and luteal phases of their menstrual cycles. Additionally, the women who had abnormally low testosterone levels also had a significantly higher number of Gd-enhancing lesions in comparison with the rest of the women with normal testosterone levels. Moreover, in women testosterone levels showed a trend towards

significant association with neurological disability on the EDSS scale (Tomassini et al., 2005). These findings indicate the importance of studying testosterone in women as in men. The authors suggest that in women testosterone has a role in the increased brain damage in MS (Tomassini et al., 2005). Both of these studies affirm the idea that reduced testosterone levels are linked to increased disability and brain damage both in men and women (Tomassini et al., 2005; Bove, et al., 2014).

The results from two limited clinical trials involving men with RRMS indicate a neuroprotective impact of testosterone, as evidenced by enhancements in cognitive performance and a deceleration in brain atrophy (Sicotte et. al., 2007; Kurth et al., 2014). Hence, a total of 10 men diagnosed with relapsing-remitting MS participated in the study done by Sicotte et. al. (2007). They implemented a crossover design where each patient functioned as their control. The study consisted of a 6-month pretreatment phase, succeeded by a 12-month period during which participants received daily treatment with a 10 g gel containing 100 mg of testosterone. After one year of testosterone gel treatment, there was a notable enhancement in cognitive performance ($P = 0.008$) and a deceleration in brain atrophy ($P = 0.001$). However, there was no significant impact of testosterone treatment on gadolinium-enhancing lesion numbers ($P = 0.31$) or volumes ($P = 0.94$). Notably, there was an increase in lean body mass (muscle mass) ($P = 0.02$) (Sicotte et. al., 2007). This trial study opened up the discussion of treatment with testosterone for improvement in cognitive function in men with RRMS. Additional studies should be done in the future, and optimistically give us a bigger picture of testosterone treatment.

Furthermore, the second pilot clinical trial, explored the potential neuroprotective effects of testosterone on cerebral gray matter, using voxel-based morphometry, to assess focal gray matter loss as a marker for neurodegeneration (Kurth et al., 2014). Ten men with RRMS took part in the

trial that 6-month observation period without treatment, followed by a 12-month phase of testosterone treatment. During the non-treatment phase, significant voxel-wise gray matter decreases were widespread ($p \leq 0.05$ corrected). However, when subjects underwent testosterone treatment, gray matter loss was no longer evident. Instead, a noteworthy gray matter increase in the right frontal cortex was observed ($p \leq 0.05$ corrected). These findings suggest the potential of testosterone treatment to halt, and possibly reverse, neurodegeneration associated with MS. Additionally, the authors underscore the need for further exploration of testosterone's neuroprotective effects through larger, placebo-controlled MS trials and investigations in other neurodegenerative diseases (Kurth et al., 2014).

An important life-changing process in all men is what is most commonly called andropause. Andropause or “male menopause” represents a slow decrease in testosterone concentration levels, caused by reduced function of the HPG axis and the testicles in men (Ysrraelit et al., 2021). This phenomenon is characterized by reduced sexual desire and erectile capacity, loss of muscle mass, increase in fat, decrease in bone mineral density, and falling hair. Among other symptoms, a decline in intellectual activity has also been reported (Bianchi et al., 2020). In recent years, there has been an emerging discourse surrounding andropause in individuals diagnosed with MS. This topic has garnered attention because of factors we have already mentioned before. One of them is the positive results in testosterone treatment trials (Sicotte et al., 2007; Kurth et al., 2014).). Also, the already known fact that the onset of MS in men is later than in women but the progression is faster and the outcomes are more severe, which leads to the hypothesis that all of this may be connected to the gradual loss of testosterone during the same period (Bove, et al., 2014). According to a review study published in 2021, pilot studies on andropause in MS patients and testosterone are in the process of being completed (Ysrraelit et al., 2021). Those results and

hopefully even more in the upcoming future, will give us a better understanding and a chance to slow down the severity and the course of older men with MS, and provide them with better care and treatment.

In summary, the intricate interplay between reproductive hormones, particularly testosterone, and Multiple Sclerosis (MS) has become a focal point of research. Governed by the hypothalamus-pituitary-gonad axis, testosterone, a key player in both men and women, exhibits age-related declines with potential implications for neurological health. Studies suggest that testosterone plays a crucial role in supporting the central nervous system, influencing neuronal survival, and cognitive functions, and potentially exerting anti-inflammatory effects. Clinical trials in men with MS show promising indications of testosterone's neuroprotective impact, suggesting a link between testosterone decline and disease severity, especially in the context of andropause. Limited but suggestive evidence in women points to a potential association between lower testosterone levels and heightened disease activity. This evolving landscape underscores the need for further research to unravel the nuanced mechanisms and therapeutic possibilities, paving the way for personalized interventions in managing MS and its complex interactions with hormonal dynamics.

In conclusion, estrogens, pivotal sex steroid hormones present in both men and women, play multifaceted roles in various physiological processes. While circulating at higher levels in women, their impact extends beyond reproductive functions, influencing organs and tissues throughout life. Estrogen receptors, ERα and ERβ, are widely distributed in the body, suggesting potential immunomodulatory properties of ERβ. During pregnancy, hormonal fluctuations, including elevated estrogens, contribute to a shift in the immune response, creating a temporary protective environment against MS relapses. Yet, the exact mechanisms and the role of estrogens

in this context require further exploration. Studies on estriol, an estrogen associated with pregnancy, revealed potential benefits in reducing and enhancing brain lesions in nonpregnant women with RRMS. However, larger-scale replication studies are essential to confirm these findings. Menopause, characterized by declining estrogen levels, introduces complexities in the relationship between hormonal changes and MS progression. Conflicting data exists regarding the impact of menopause on MS, with some studies suggesting a decline in relapse rates post-menopause, while others report increased disability scores. In male MS patients, limited research on the connection between sex hormones, particularly estradiol, and brain damage emphasizes the need for further investigation. Overall, understanding the intricate interplay between hormones and MS is crucial for developing targeted interventions and advancing patient care. Further research, including clinical trials and longitudinal studies, is necessary to solve the complexities of hormonal influences on MS, providing valuable insights for future therapeutic strategies.

To sum up, the role of testosterone in the context of Multiple Sclerosis (MS) has garnered increasing attention in recent years. Reproductive hormones, including testosterone, are predominantly regulated by the hypothalamus-pituitary-gonad axis. With aging, gonadal function gradually declines, leading to a decrease in androgen and estrogen levels. Testosterone, a key androgen, plays a vital role in human reproductive and sexual function, influencing various physiological processes.

In men, testosterone is primarily produced in the testicles, while in women, the adrenal glands and ovaries serve as the principal sources of androgens. Testosterone synthesis is regulated by the hypothalamic-pituitary-gonadal axis, involving hormones such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Additionally, testosterone in females can be converted into estrogen through aromatization, maintaining hormonal balance. The concentration of

bioavailable testosterone, readily accessible for use by the body, can decline significantly with age. Studies suggest that testosterone has beneficial effects on the central nervous system (CNS), supporting the survival of neurons and astrocytes, activating neuronal plasticity, and influencing various physiological processes. Animal models, particularly studies involving castration, indicate that testosterone may have anti-inflammatory effects. Limited clinical trials in men with MS suggest a potential neuroprotective impact of testosterone, with improvements in cognitive performance and a slowing of brain atrophy. Concerning andropause in men with MS, there is an emerging discourse, and ongoing studies aim to explore the potential links between testosterone decline and disease severity. Preliminary findings suggest a connection between low testosterone levels in men and increased disability, brain damage, and disease progression. In women with MS, limited studies indicate a potential association between lower testosterone levels and higher disease activity, as evidenced by an increased number of gadolinium-enhancing lesions and a trend toward neurological disability.

Overall, while there is growing evidence supporting the importance of testosterone in the context of MS, further research, especially in women, is needed to elucidate the precise mechanisms and potential therapeutic implications. Understanding the intricate relationship between hormonal fluctuations and MS may contribute to the development of targeted interventions and personalized treatment strategies for individuals affected by this complex neurological disorder.

General Discussion

Within the realm of multiple sclerosis (MS), this review seeks to unravel the complex tapestry of hormonal changes, serving as a critical exploration into their potential influence on disease dynamics. By scrutinizing current research findings, we endeavor to unveil patterns and associations that could elucidate the nuanced interactions between hormones and MS progression. This review aspires to contribute a thoughtful background of what we know so far, that not only synthesizes existing knowledge but hopefully also prompts further investigation into the intricate mechanisms underlying the role of hormonal fluctuations in the context of MS. In delving into the intricate web of hormonal changes in MS, this review aspires to bridge existing gaps in understanding, paving the way for a comprehensive grasp of the multifaceted relationship between hormones and the disease. Through a meticulous examination of the literature, we aim to foster a foundation that not only consolidates current knowledge but also sparks curiosity and instigates future inquiries into the complexities that govern the interplay of hormones and multiple sclerosis.

The complexity of the disease itself makes it hard for researchers to fully understand the immunological, psychological, and endocrinological mechanisms that contribute to the progressive course of the disease. But, taking into account all of the potential factors that take part in this challenging neurological disease provides us with more in-depth clinical characteristics of MS. The current review is primarily centered on examining the functionality of the HPA axis and certain sex hormones. Discussing the existing literature about sex hormone differences observed in the two sexes, and focusing on the importance of the stress axes we wanted to highlight the significance of the psycho-endocrinological aspect of Multiple Sclerosis.

As already mentioned, multiple sclerosis (MS) is a perplexing neurodegenerative disease predominantly affecting young adults (Hemmer et al.,2002), characterized by immune-mediated inflammation leading to demyelination in the central nervous system (Heesen et al., 2007). The disease, with complex and varied symptoms, remains a scientific puzzle, prompting ongoing research to decipher its causes and optimal treatment. Historical perspectives trace MS recognition back to the nineteenth century, culminating in Jean-Martin Charcot's comprehensive description in 1868. Contemporary diagnostic criteria, such as the McDonald 2010 criteria, rely on the spatial and temporal dissemination of lesions in the central nervous system. Epidemiologically, MS is more prevalent in women, with onset typically between 25-35 years (Ascherio et al., 2012). The etiology involves a complex interplay of genetic and environmental factors, with risk factors including Epstein-Barr virus infection (Jacobs et al., 2020), vitamin D deficiency (Freedman et al., 2000*)*, and others. Neuroendocrine regulation, specifically the interconnectedness of the nervous, endocrine, and immune systems, plays a crucial role in MS pathogenesis. The bidirectional communication between these systems, orchestrated by hormones, neurotransmitters, and cytokines, affects susceptibility and disease severity (Deckx et al.,2013). Understanding these intricate connections holds promise for unraveling the mysteries of MS and developing targeted therapeutic interventions.

Implications for Future Studies

The first objective of this review paper was to elaborate on the connection between MS and HPA axis. Animal model studies, notably using the experimental autoimmune encephalomyelitis (EAE) model, are crucial for gaining insights into multiple sclerosis (MS) diagnosis and progression. However, caution is warranted when interpreting results, as animal

studies differ from clinical human studies. In the MBP EAE model, a compromised Hypothalamic-Pituitary-Adrenal (HPA) axis, linked to genetic factors, increases disease susceptibility (Heesen et al., 2007). In the Chronic Relapsing EAE model, corticosterone responses diminish during subsequent relapses, indicating altered stress response throughout the disease. The weakened HPA axis response to inflammatory triggers in CR-EAE suggests implications for inflammation regulation and disease progression (Stefferl et al.,2001). While EAE animal models suggest a hypoactive HPA axis, clinical studies in MS patients present contrasting findings, indicating hyperactivity of the HPA axis.

Wei and Lightman's (1997) study on 52 MS patients demonstrated varied cortisol responses based on disease types. However, contrasting observations exist, as some studies propose HPA hyperactivity in MS patients, particularly during acute inflammatory episodes. The first longitudinal study by Gold et al. in 2005 indicated a significant correlation between HPA axis hyperactivity and disease progression over a 3-year follow-up. The Dex-CRH test, measuring cortisol response, emerged as a potential prognostic tool for predicting disease advancement. Heesen et al. in 2002 explored the link between HPA dysregulation, cognitive deficits, and fatigue in MS patients. The study revealed correlations between HPA axis hyperactivity and cognitive impairment, neuronal disability, and disease duration.

Despite these insights, conflicting results arise regarding the correlation between HPA responses and MRI Gd-enhancing lesions, commonly used as inflammation indicators. While some studies report positive associations, others suggest negative correlations or no significant link (Wei & Lightman, 1997; Fassbender et al., 1998; Schumann et al., 2002). The discrepancies observed in research findings may be influenced by the dynamic nature of disease activity during testing. Furthermore, the HPA axis operates on a circadian rhythm, influencing daily cortisol level

fluctuations. In multiple sclerosis (MS), disruptions in circadian rhythms could contribute to variations in symptom severity and disease activity. It is essential to comprehend the interaction between HPA axis dynamics and symptoms to develop targeted interventions for managing both disease progression and symptom severity. Furthermore, stress is often considered a potential trigger and exacerbating factor in MS, as the HPA axis releases cortisol in response to stressors (Mohr et al., 2004). However, chronic stress may result in sustained activation and dysregulation of the HPA axis (Guilliams & Edwards,2010). Investigating specific stressors triggering HPA axis responses and understanding their impact on disease activity is critical for developing comprehensive management strategies (Heesen et al., 2007).

Gender differences in the hypothalamic-pituitary-adrenal (HPA) axis have been observed in both animals and humans. When faced with a psychological stressor, males generally exhibit greater HPA responses than females (Jobin et al., 2010). In the realm of multiple sclerosis (MS) research, it is important to consider also these gender disparities. On top of that, studies often face limitations due to the relatively small number of participants, making it challenging for authors to extrapolate generalized recommendations beyond the specific results observed in the study's participants.

Longitudinal studies investigating the temporal dynamics of HPA axis function in MS are limited. In this paper, only one longitudinal study has been discussed. Understanding how HPA axis dysregulation evolves over time, especially concerning disease progression and remission, is essential for identifying critical periods of hormonal dysregulation and potential intervention points. In the aforementioned study by Gold et al. (2005), the authors propose the potential prognostic value of the HPA axis. However, it is important to note that, at present, this cannot be considered a universally definitive conclusion. This is because other studies imply that disruptions

in the HPA axis likely result from other immunological changes in multiple sclerosis and the hyperactivation of the HPA axis is a secondary process that occurs. Future longitudinal studies assessing cortisol responses over an extended period and unraveling the underlying mechanisms will bring us closer to addressing whether a dysfunctional HPA axis and cortisol response hold prognostic value for multiple sclerosis progression.

Finally, recognizing the role of the HPA axis in MS has broader implications for patient care and well-being. In addition to the current pharmaceutical therapies for MS patients and the potential new ones on the horizon, it's essential to incorporate other supportive approaches to enhance their well-being. Incorporating stress management strategies and psychosocial interventions into comprehensive treatment plans may positively impact the disease course and enhance the overall quality of life for individuals with MS. Targeting potential stress triggers and including biomarkers as well as psychological markers could help develop better stress management strategies (Reynard, 2014).

The intricate interplay between sex hormones and the pathophysiology of Multiple Sclerosis (MS) unfolds a captivating narrative in the realm of neurological disorders. As researchers delve into the complexities of MS, the influence of sex hormones, including estrogen and testosterone, emerges as a crucial factor in shaping the risk, progression, and clinical manifestations of the disease. Estrogen is implicated in potential protective effects, modulating immune responses and offering neuroprotection, while testosterone may play a role in mitigating MS severity, especially in men. These hormonal dynamics extend to critical life phases such as pregnancy and menopause, where fluctuations wield varying impacts on the course of MS. Estrogen's diverse roles, including immunomodulation, are highlighted, with studies on estriol suggesting benefits in reducing brain lesions. Menopause's effect on MS remains inconclusive,

with conflicting data on relapse rates and disability. Limited research on male MS patients underscores the need for more investigation into sex hormones and brain damage. Understanding these hormonal intricacies is vital for personalized interventions in MS management, urging continued research efforts. Similarly, testosterone's role gains attention, particularly its decline with age and potential neuroprotective effects in MS. Clinical trials suggest links between low testosterone levels and increased disability, emphasizing the need for further exploration, especially in women. The evolving landscape calls for comprehensive research to unravel mechanisms and therapeutic possibilities, paving the way for improved MS care.

Concerning alterations in sex hormones within the context of MS, a notable research gap exists in longitudinal studies. Tracking changes in sex hormone levels over extended periods is limited. Investigating how hormonal fluctuations correlate with disease course and progression over time would provide valuable insights into the dynamic interplay between sex hormones and MS. Furthermore, one can conclude that During pregnancy, a notable physiological shift occurs in the immune response, transitioning from TH1 to TH2 dominance. This shift results in the production of anti-inflammatory cytokines, creating a conducive environment for embryo implantation and development (Al-Shammri et al, 2004). Simultaneously, concentrations of two estrogen hormones, estradiol and estriol, along with progesterone, undergo a gradual increase throughout pregnancy, peaking in the third trimester (Bates et al., 2020). Following childbirth, these hormone levels decrease, aligning with a temporal pattern that corresponds to the protective effect of pregnancy on the rate of relapses in multiple sclerosis (Tulchinsky et al., 1972). This hormonal fluctuation during pregnancy contributes to a temporary reduction in the risk of relapses in women with multiple sclerosis. However, pregnancy is associated with hormonal changes that impact MS, but the specific mechanisms and long-term consequences remain unclear. Further

research is needed to explore how hormonal shifts during pregnancy influence disease progression, relapse rates, and long-term outcomes in women with MS.

The focus on estrogen's role in women of reproductive age has overshadowed its potential relevance in postmenopausal women. Understanding the ongoing influence of estrogens beyond reproductive age is crucial for comprehending the hormone's multifaceted impact on MS pathology. Menopause introduces complexities, with conflicting findings on its impact on MS progression. On the one hand, two studies suggest a potential decline in the relapse rate in MS after menopause (Baroncini et al., 2019; Ladeira, 2018). Conversely, a longitudinal study assessing Expanded Disability Status Scale (EDSS) scores demonstrated an increase in scores after menopause (Bove et al., 2016). Likewise, even less research has been done on “male menopause”. Even though andropause in men and menopause in women are not the same process just in the opposite sex, better exploration of these changes in men with MS is still very important. R Bove, A Musallam et al. (2014) suggested the hypothesis that the later onset of MS in men and the more aggressive course of disease compared with women might be influenced by the lower testosterone concentration levels due to andropause. This theory requires additional exploration and development. If confirmed, it has the potential to positively impact the care and treatment of men.

The therapeutic use of sex hormones in MS patients is another area that requires further development and exploration. Two clinical trials with testosterone treatment were discussed, which opened the door for future research first of all because the authors concluded that testosterone didn’t have negative side effects and was safe to use (Sicotte et al., 2007; Kurth et al., 2014). In terms of estrogens, Sicotte et al. (2002) suggested a beneficial effect of estriol hormone in nonpregnant RR-MS women with a reduction in enhanced brain lesions. Replication studies must

be done to support the results of this study. Additionally, more comprehensive research is needed to investigate the potential causal significance of hormonal replacement therapy and oral contraception concerning MS (Gilli et al., 2020).

It seems like there's a gap in the literature regarding the connection between the HPA axis and sex hormones in the context of multiple sclerosis (MS). While, in this paper, the individual aspects of the HPA axis and sex hormones in MS were discussed, the specific interconnections between these two systems might need further exploration. To address this gap, future research could focus on investigating how the HPA axis interacts with sex hormones in the context of MS. This could involve studying the influence of stress and cortisol regulated by the HPA axis, on sex hormone levels, as well as understanding how sex hormones may, in turn, affect the activity of the HPA axis in individuals with MS. Viau V. talks about the Functional cross-talk between the hypothalamic-pituitary-gonadal and-adrenal axes (2002). According to his work, the normal functioning of the adrenal glucocorticoids, which are the end product of the hypothalamic-pituitary-adrenal (HPA) axis, serves as a frontline defense against threats to homeostasis, particularly stress. However, chronic activation of the HPA axis and excessive secretion of glucocorticoids have been implicated in the pathogenesis of various systemic, neurodegenerative, and affective disorders (Viau, 2002). Future research could explore the reciprocal influences, investigating the impact of stress and cortisol on sex hormone levels and vice versa, shedding light on the intricate relationship in individuals with MS. Viau V.'s insights highlight the crucial role of adrenal glucocorticoids in maintaining homeostasis and emphasize the potential implications of dysregulation in the HPA axis for various disorders (Viau, 2002).

Addressing these research gaps will contribute to a more nuanced understanding of how sex hormones and HPA dysfunction influence MS, potentially leading to tailored therapeutic

approaches and improved outcomes for individuals affected by this complex neurological disorder. It is non-negotiable that MS is very complex. Risk factors for MS today are numerous. Advances in immunology, genetics, neurobiology, and their interconnections with the endocrine system, coupled with the refinement of epidemiological methods, should lead to explanations for the tremendous increase in MS susceptibility (Jobin, 2010). That is also why, a multidisciplinary approach is probably the right way to go. In this particular paper, a neuro-endocrinological approach was taken with a focus on cortisol, estrogens, and testosterone. In the future, the best we can do is probably find a way to connect as much as we can the immunological mechanics with genetical aspects as well as the neuro-endocrine characteristics, and also with environmental and social elements.

Conclusion

In conclusion, this comprehensive review delves into the intricate relationships between hormonal changes, specifically those involving the HPA axis and sex hormones, and the progression of multiple sclerosis (MS). The multifaceted nature of MS, encompassing immunological, psychological, and endocrinological components, has been explored with a focus on cortisol, estrogens, and testosterone. The review underscores existing gaps in the literature, particularly regarding the interconnections between the HPA axis and sex hormones in the context of MS, signaling the need for further research. The influence of hormones during critical life phases, such as pregnancy and menopause, adds layers of complexity, demanding nuanced investigations. This thesis serves as a stepping stone, advocating for future research endeavors that bridge diverse disciplines to unravel the mysterious parts of the puzzle that is MS.

References

Al-Shammri, S., Rawoot, P., Azizieh, F., AbuQoora, A., Hanna, M., Saminathan, T. R., & Raghupathy, R. (2004). Th1/Th2 cytokine patterns and clinical profiles during and after pregnancy in women with multiple sclerosis. *Journal of the neurological sciences*, *222*(1-2), 21-27.

Anderson, A., Krysko, K. M., Rutatangwa, A., Krishnakumar, T., Chen, C., Rowles, W., ... & Bove, R. (2021). Clinical and radiologic disease activity in pregnancy and postpartum in MS. *Neurology: Neuroimmunology & Neuroinflammation*, *8*(2), e959.

Angelo Ghezzi, A., & Zaffaroni, M. (2008). Female-specific issues in multiple sclerosis. *Expert Review of Neurotherapeutics*, *8*(6), 969-977.

Ascherio, A., Munger, K. L., & Lünemann, J. D. (2012). The initiation and prevention of multiple sclerosis. *Nature Reviews Neurology*, *8*(11), 602-612.

Bansil, S., Lee, H. J., Jindal, S., Holtz, C. R., & Cook, S. O. (1999). Correlation between sex hormones and magnetic resonance imaging lesions in multiple sclerosis. *Acta neurologica scandinavica*, *99*(2), 91-94.

Baroncini, D., Annovazzi, P. O., De Rossi, N., Mallucci, G., Clerici, V. T., Tonietti, S., ... & Zaffaroni, M. (2019). Impact of natural menopause on multiple sclerosis: a multicentre study. *Journal of Neurology, Neurosurgery & Psychiatry*, *90*(11), 1201-1206.

Bates, K., & Herzog, E. D. (2020). Maternal-fetal circadian communication during pregnancy. *Frontiers in endocrinology*, *11*, 519328.

Bebo Jr, B. F., Schuster, J. C., Vandenbark, A. A., & Offner, H. (1998). Gender differences in experimental autoimmune encephalomyelitis develop during the induction of the immune response to encephalitogenic peptides. *Journal of neuroscience research*, *52*(4), 420-426.

Bergh, F. T., Kumpfel, T., Trenkwalder, C., Rupprecht, R., & Holsboer, F. (1999). Dysregulation of the hypothalamo-pituitary-adrenal axis is related to the clinical course of MS. *Neurology*, *53*(4), 772-772.

Besedovsky, H. O., & del Rey, A. (1996). Immune-neuro-endocrine interactions: facts and hypotheses. *Endocrine reviews*, *17*(1), 64-102.

Beyer, C., & Hutchison, J. B. (1997). Androgens stimulate the morphological maturation of embryonic hypothalamic aromatase-immunoreactive neurons in the mouse. *Developmental brain research*, *98*(1), 74-81.

Bianchi, V. E., Rizzi, L., Bresciani, E., Omeljaniuk, R. J., & Torsello, A. (2020). Androgen therapy in neurodegenerative diseases. *Journal of the Endocrine Society*, *4*(11), bvaa120.

Bjelobaba, I., Begovic-Kupresanin, V., Pekovic, S., & Lavrnja, I. (2018). Animal models of multiple sclerosis: Focus on experimental autoimmune encephalomyelitis. *Journal of neuroscience research*, *96*(6), 1021-1042.

Bove, R., & Chitnis, T. (2013). Sexual disparities in the incidence and course of MS. *Clinical Immunology*, *149*(2), 201-210.

Bove, R., & Chitnis, T. (2014). The role of gender and sex hormones in determining the onset and outcome of multiple sclerosis. *Multiple Sclerosis Journal*, *20*(5), 520-526.

Bove, R., Healy, B. C., Musallam, A., Glanz, B. I., De Jager, P. L., & Chitnis, T. (2016). Exploration of changes in disability after menopause in a longitudinal multiple sclerosis cohort. *Multiple Sclerosis Journal*, *22*(7), 935-943.

Bove, R., Healy, B. C., Secor, E., Vaughan, T., Katic, B., Chitnis, T., ... & De Jager, P. L. (2015). Patients report worse MS symptoms after menopause: findings from an online cohort. *Multiple sclerosis and related disorders*, *4*(1), 18-24.

Bove, R., Musallam, A., Healy, B. C., Raghavan, K., Glanz, B. I., Bakshi, R., ... & Chitnis, T. (2014). Low testosterone is associated with disability in men with multiple sclerosis. *Multiple Sclerosis Journal*, *20*(12), 1584-1592.

Charcot, J. M. (1877). Lectures on Diseases on the Nervous System (G Sigerson, Trans.). *New Sydenham Society, London*.

Christianson, M. S., Mensah, V. A., & Shen, W. (2015). Multiple sclerosis at menopause: Potential neuroprotective effects of estrogen. *Maturitas*, *80*(2), 133-139.

Constantinescu, C. S., Farooqi, N., O'Brien, K., & Gran, B. (2011). Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *British journal of pharmacology*, *164*(4), 1079-1106.

Dalal, M., Kim, S., & Voskuhl, R. R. (1997). Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in the autoantigen-specific T lymphocyte response. *Journal of immunology (Baltimore, Md.: 1950)*, *159*(1), 3-6.

Damek, D. M., & Shuster, E. A. (1997, October). Pregnancy and multiple sclerosis. In *Mayo Clinic Proceedings* (Vol. 72, No. 10, pp. 977-989). Elsevier.

Davis, S. R., Worsley, R., Miller, K. K., Parish, S. J., & Santoro, N. (2016). Androgens and female sexual function and dysfunction—findings from the Fourth International Consultation of Sexual Medicine. *The journal of sexual medicine*, *13*(2), 168-178.

Deckx, N., Lee, W. P., Berneman, Z. N., & Cools, N. (2013). Neuroendocrine immunoregulation in multiple sclerosis. *Journal of Immunology Research*, *2013*.

Dong, X., Jiang, H., Li, S., & Zhang, D. (2021). Low serum testosterone concentrations are associated with poor cognitive performance in older men but not women. *Frontiers in aging neuroscience*, *13*, 712237.

Duquette, P. (1998). The increased susceptibility of women to multiple sclerosis. *Multiple Sclerosis Journal*, *4*(6), 511-512.

Fassbender, K., Ragoschke, A., Rossol, S., Schwartz, A., Mielke, O., Paulig, A., & Hennerici, M. (1998). Increased release of interleukin-12p40 in MS: association with intracerebral inflammation. *Neurology*, *51*(3), 753-758.

Fassbender, K., Schmidt, R., Mößner, R., Kischka, U., Kühnen, J., Schwartz, A., & Hennerici, M. (1998). Mood disorders and dysfunction of the hypothalamic-pituitary-adrenal axis in multiple sclerosis: association with cerebral inflammation. *Archives of neurology*, *55*(1), 66-72.

Freedman, D. M., Dosemeci, M., & Alavanja, M. C. (2000). Mortality from multiple sclerosis and exposure to residential and occupational solar radiation: a case-control study based on death certificates. *Occupational and environmental medicine*, *57*(6), 418-421.

Gale, C. R., & Martyn, C. N. (1995). Migrant studies in multiple sclerosis. *Progress in neurobiology*, *47*(4-5), 425-448.

Gilli, F., DiSano, K. D., & Pachner, A. R. (2020). SeXX matters in multiple sclerosis. *Frontiers in neurology*, *11*, 529064.

Gold, S. M., Mohr, D. C., Huitinga, I., Flachenecker, P., Sternberg, E. M., & Heesen, C. (2005). The role of stress-response systems for the pathogenesis and progression of MS. *Trends in immunology*, *26*(12), 644-652.

Gould, D. C., Jacobs, H. S., & Petty, R. (2000). The male menopause—does it exist? ForAgainst. *Bmj*, *320*(7238), 858-861.

Green, P. S., & Simpkins, J. W. (2000). Neuroprotective effects of estrogens: potential mechanisms of action. *International Journal of Developmental Neuroscience*, *18*(4-5), 347-358.

Greer, J. M., & McCombe, P. A. (2012). The role of epigenetic mechanisms and processes in autoimmune disorders. *Biologics: Targets and Therapy*, 307-327.

Guilliams, T. G., & Edwards, L. (2010). Chronic stress and the HPA axis. *The standard*, *9*(2), 1-12.

Guilliams, T. G., & Edwards, L. (2010). Chronic stress and the HPA axis. *The standard*, *9*(2), 1-12.

Hawkins, S. A., & McDonnell, G. V. (1999). Benign multiple sclerosis? Clinical course, long term follow up, and assessment of prognostic factors. *Journal of Neurology, Neurosurgery & Psychiatry*, *67*(2), 148-152.

Heesen, C., Gold, S. M., Huitinga, I., & Reul, J. M. H. M. (2007). Stress and hypothalamic–pituitary–adrenal axis function in experimental autoimmune encephalomyelitis and multiple sclerosis—a review. *Psychoneuroendocrinology*, *32*(6), 604-618.

Heesen, C., Gold, S. M., Raji, A., Wiedemann, K., & Schulz, K. H. (2002). Cognitive impairment correlates with hypothalamo–pituitary–adrenal axis dysregulation in multiple sclerosis. *Psychoneuroendocrinology*, *27*(4), 505-517.

Hemmer, B., Cepok, S., Nessler, S., & Sommer, N. (2002). Pathogenesis of multiple sclerosis: an update on immunology. *Current opinion in neurology*, *15*(3), 227-231.

Hsu, B., Cumming, R. G., Blyth, F. M., Naganathan, V., Le Couteur, D. G., Seibel, M. J., ... & Handelsman, D. J. (2015). The longitudinal relationship of sexual function and androgen status in older men: the Concord Health and Ageing in Men Project. *The Journal of Clinical Endocrinology & Metabolism*, *100*(4), 1350-1358.

Jacobs, B. M., Giovannoni, G., Cuzick, J., & Dobson, R. (2020). Systematic review and meta-analysis of the association between Epstein–Barr virus, multiple sclerosis and other risk factors. *Multiple sclerosis journal*, *26*(11), 1281-1297.

Jobin, C., Larochelle, C., Parpal, H., Coyle, P. K., & Duquette, P. (2010). Gender issues in multiple sclerosis: an update. *Women's Health*, *6*(6), 797-820.

Kantarci, O. H., & Weinshenker, B. G. (2005). Natural history of multiple sclerosis. *Neurologic clinics*, *23*(1), 17-38.

Kurth, F., Luders, E., Sicotte, N. L., Gaser, C., Giesser, B. S., Swerdloff, R. S., ... & Mackenzie-Graham, A. (2014). Neuroprotective effects of testosterone treatment in men with multiple sclerosis. *NeuroImage: Clinical*, *4*, 454-460.

Kurtzke, J. F. (1983). Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*, *33*(11), 1444-1444.

Kutzelnigg, A., Lucchinetti, C. F., Stadelmann, C., Brück, W., Rauschka, H., Bergmann, M., ... & Lassmann, H. (2005). Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain*, *128*(11), 2705-2712.

Ladeira, F., Salavisa, M., Caetano, A., Barbosa, R., Sá, F., & Correia, A. S. (2019). The influence of menopause in multiple sclerosis course: a longitudinal cohort study. *European Neurology*, *80*(3-4), 223-227.

Leranth, C., Petnehazy, O., & MacLusky, N. J. (2003). Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *Journal of Neuroscience*, *23*(5), 1588-1592.

Lightman, S. L., Birnie, M. T., & Conway-Campbell, B. L. (2020). Dynamics of ACTH and cortisol secretion and implications for disease. *Endocrine reviews*, *41*(3), bnaa002.

Liva, S. M., & Voskuhl, R. R. (2001). Testosterone acts directly on CD4+ T lymphocytes to increase IL-10 production. *The Journal of Immunology*, *167*(4), 2060-2067.

Maglione, A., Rolla, S., Mercanti, S. F. D., Cutrupi, S., & Clerico, M. (2019). The adaptive immune system in multiple sclerosis: an estrogen-mediated point of view. *Cells*, *8*(10), 1280.

Matsumoto, A. (1997). Hormonally induced neuronal plasticity in the adult motoneurons. *Brain research bulletin*, *44*(4), 539-547.

Meethal, S. V., & Atwood, C. S. (2005). The role of hypothalamic-pituitary-gonadal hormones in the normal structure and functioning of the brain. *Cell Mol Life Sci*, *62*(3), 257-270.

Meydan, S., Kus, I., Tas, U., Ogeturk, M., Sancakdar, E., Dabak, D. O., ... & Sarsılmaz, M. (2010). Effects of testosterone on orchiectomy-induced oxidative damage in the rat hippocampus. *Journal of chemical neuroanatomy*, *40*(4), 281-285.

Michelson, D., Stone, L., Galliven, E. L. I. S. E., Magiakou, M. A., Chrousos, G. P., Sternberg, E. M., & Gold, P. W. (1994). Multiple sclerosis is associated with alterations in hypothalamic-pituitary-adrenal axis function. *The Journal of Clinical Endocrinology & Metabolism*, *79*(3), 848-853.

Miller, D. H., Fazekas, F., Montalban, X., Reingold, S. C., & Trojano, M. (2014). Pregnancy, sex and hormonal factors in multiple sclerosis. *Multiple Sclerosis Journal*, *20*(5), 527-536.

Miyake, S. (2012). Mind over cytokines: Crosstalk and regulation between the neuroendocrine and immune systems. *Clinical and Experimental Neuroimmunology*, *3*(1), 1-15.

Mohr, D. C., Hart, S. L., Julian, L., Cox, D., & Pelletier, D. (2004). Association between stressful life events and exacerbation in multiple sclerosis: a meta-analysis. *Bmj*, *328*(7442), 731.

Mohr, D. C., Hart, S. L., Julian, L., Cox, D., & Pelletier, D. (2004). Association between stressful life events and exacerbation in multiple sclerosis: a meta-analysis. *Bmj*, *328*(7442), 731.

Munger, K. L., Åivo, J., Hongell, K., Soilu-Hänninen, M., Surcel, H. M., & Ascherio, A. (2016). Vitamin D status during pregnancy and risk of multiple sclerosis in offspring of women in the Finnish Maternity Cohort. *JAMA neurology*, *73*(5), 515-519.

Murray, T. J. (2009). The history of multiple sclerosis: the changing frame of the disease over the centuries. *Journal of the neurological sciences*, *277*, S3-S8.

Nicolson, N. A. (2008). Measurement of cortisol. *Handbook of physiological research methods in health psychology*, *1*, 37-74.

Österberg, A., Boivie, J., & Thuomas, K. Å. (2005). Central pain in multiple sclerosis–prevalence and clinical characteristics. *European journal of pain*, *9*(5), 531-542.

Papadimitriou, A., & Priftis, K. N. (2009). Regulation of the hypothalamic-pituitary-adrenal axis. *Neuroimmunomodulation*, *16*(5), 265-271.

Pozzilli, C., Falaschi, P., Mainero, C., Martocchia, A., D'Urso, R., Proietti, A., ... & Filippi, M. (1999). MRI in multiple sclerosis during the menstrual cycle: relationship with sex hormone patterns. *Neurology*, *53*(3), 622-622.

Pozzilli, C., Tomassini, V., Marinelli, F., Paolillo, A., Gasperini, C., & Bastianello, S. (2003). 'Gender gap'in multiple sclerosis: magnetic resonance imaging evidence. *European journal of neurology*, *10*(1), 95-97.

Reder, A. T., Makowiec, R. L., & Lowy, M. T. (1994). Adrenal size is increased in multiple sclerosis. *Archives of neurology*, *51*(2), 151-154.

Reynard, A. K., Sullivan, A. B., & Rae-Grant, A. (2014). A systematic review of stress-management interventions for multiple sclerosis patients. *International journal of MS care*, *16*(3), 140-144.

Riise, T., Mohr, D. C., Munger, K. L., Rich-Edwards, J. W., Kawachi, I., & Ascherio, A. (2011). Stress and the risk of multiple sclerosis. *Neurology*, *76*(22), 1866-1871.

Robinson, A. P., Harp, C. T., Noronha, A., & Miller, S. D. (2014). The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. *Handbook of clinical neurology*, *122*, 173-189.

Savettieri, G., Messina, D., Andreoli, V., Bonavita, S., Caltagirone, C., Cittadella, R., ... & Quattrone, A. (2004). Gender-related effect of clinical and genetic variables on the cognitive impairment in multiple sclerosis. *Journal of neurology*, *251*, 1208-1214.

Savettieri, G., Messina, D., Andreoli, V., Bonavita, S., Caltagirone, C., Cittadella, R., ... & Quattrone, A. (2004). Gender-related effect of clinical and genetic variables on the cognitive impairment in multiple sclerosis. *Journal of neurology*, *251*, 1208-1214.

Schumann, E. M., Kümpfel, T., Then Bergh, F., Trenkwalder, C., Holsboer, F., & Auer, D. P. (2002). Activity of the hypothalamic–pituitary–adrenal axis in multiple sclerosis: correlations with gadolinium-enhancing lesions and ventricular volume. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *51*(6), 763-767.

Schwendimann, R. N., & Alekseeva, N. (2007). Gender issues in multiple sclerosis. *International review of neurobiology*, *79*, 377-392.

Shuster, E. A. (2008). Hormonal influences in multiple sclerosis. *Advances in multiple Sclerosis and Experimental Demyelinating Diseases*, 267-311.

Sicotte, N. L., Giesser, B. S., Tandon, V., Klutch, R., Steiner, B., Drain, A. E., ... & Voskuhl, R. R. (2007). Testosterone treatment in multiple sclerosis: a pilot study. *Archives of neurology*, *64*(5), 683-688.

Sicotte, N. L., Liva, S. M., Klutch, R., Pfeiffer, P., Bouvier, S., Odesa, S., ... & Voskuhl, R. R. (2002). Treatment of multiple sclerosis with the pregnancy hormone estriol. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *52*(4), 421-428.

Simpson, E. R. (2002). Aromatization of androgens in women: current concepts and findings. *Fertility and sterility*, *77*, 6-10.

Smith, A. (1973). Symbol digit modalities test. *The Clinical Neuropsychologist*.

Smith, L. B., Mitchell, R. T., & McEwan, I. J. (2013). *Testosterone: From basic research to clinical applications*. Berlin: Springer.

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in clinical neuroscience*, *8*(4), 383-395.

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in clinical neuroscience*, *8*(4), 383-395.

Stefferl, A., Storch, M. K., Linington, C., Stadelmann, C., Lassmann, H., Pohl, T., ... & Reul, J. M. (2001). Disease progression in chronic relapsing experimental allergic encephalomyelitis is associated with reduced inflammation-driven production of corticosterone. *Endocrinology*, *142*(8), 3616-3624.

Sternberg, E. M. (2001). Eurosterone meeting. Neuroendocrine regulation of autoimmune/inflammatory disease. *Journal of Endocrinology*, *169*(3), 429-435.

Swerdloff, R. S., Wang, C., & Hikim, A. P. S. (2002). Hypothalamic-pituitary-gonadal axis in men. In *Hormones, brain and behavior* (pp. 1-36). Academic Press.

Tomassini, V., & Pozzilli, C. (2009). Sex hormones, brain damage and clinical course of Multiple Sclerosis. *Journal of the neurological sciences*, *286*(1-2), 35-39.

Tomassini, V., Onesti, E., Mainero, C., Giugni, E., Paolillo, A., Salvetti, M., ... & Pozzilli, C. (2005). Sex hormones modulate brain damage in multiple sclerosis: MRI evidence. *Journal of Neurology, Neurosurgery & Psychiatry*, *76*(2), 272-275.

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of psychosomatic research*, *53*(4), 865-871.

Tulchinsky, D., Hobel, C. J., Yeager, E., & Marshall, J. R. (1972). Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy: I. Normal pregnancy. *American journal of obstetrics and gynecology*, *112*(8), 1095-1100.

Viau, V. (2002). Functional cross-talk between the hypothalamic-pituitary-gonadal and-adrenal axes. *Journal of neuroendocrinology*, *14*(6), 506-513.

Wei, T., & Lightman, S. L. (1997). The neuroendocrine axis in patients with multiple sclerosis. *Brain: a journal of neurology*, *120*(6), 1067-1076.

Wend, K., Wend, P., & Krum, S. A. (2012). Tissue-specific effects of loss of estrogen during menopause and aging. *Frontiers in endocrinology*, *3*, 13119.

Xiang, Y., & Shi, J. (2023). The role of inflammation in autoimmune disease: a therapeutic target. *Frontiers in Immunology*, *14*, 1267091.

Yanovski, J. A., Cutler, G. B., Chrousos, G. P., & Nieman, L. K. (1993). Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration: a new test to distinguish Cushing's syndrome from pseudo-Cushing's states. *Jama*, *269*(17), 2232-2238.

Young, I. R., Hall, A. S., Pallis, C. A., Bydder, G. M., Legg, N. J., & Steiner, R. E. (1981). Nuclear magnetic resonance imaging of the brain in multiple sclerosis. *The Lancet*, *318*(8255), 1063-1066.

Ysrraelit, M. C., & Correale, J. (2021). Impact of Andropause on multiple sclerosis. *Frontiers in neurology*, *12*, 766308.

Zárate, S., Stevnsner, T., & Gredilla, R. (2017). Role of estrogen and other sex hormones in brain aging. Neuroprotection and DNA repair. *Frontiers in aging neuroscience*, *9*, 322754.

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta psychiatrica scandinavica*, *67*(6), 361-370.