The distinct roles of monoamines in multiple sclerosis: a bridge between the immune and nervous systems?

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Abstract

The monoaminergic neurotransmitters dopamine, noradrenaline, and serotonin are pivotal actors of the interplay between the nervous and the immune system due to their ability of binding to cell-receptors of both systems, crucially regulating their function within the central nervous system and the periphery. As monoamines are dysfunctional in many neurological and psychiatric diseases, they have been successfully used as pharmacological targets. Multiple sclerosis (MS) is one of the best examples of neurological disease caused by an altered interaction between the nervous and immune system and emerging evidence supports a dysregulation of monoaminergic systems in the pathogenesis of MS, secondary to both inflammation-induced reduction of monoamines' synthesis and structural damage to monoaminergic pathways within the brain. Here we review the evidence for monoamines being key mediators of neuroimmune interaction, affecting MS pathogenesis and course. Moreover, we discuss how the reduction/dysfunction of monoamines in MS may contribute to some clinical features typical of the disease, particularly fatigue and depression. Finally, we summarize different drugs targeting monoamines that are currently under evaluation for their potential efficacy to treat MS, as well as to alleviate fatigue and depression in MS.

Keywords

Monoamines, Multiple Sclerosis, Fatigue, Depression, Neuroimmunology, Dopamine, Noradrenaline, Serotonin

Abbreviations

5HT = serotonin; 5HTR = 5HT receptors; AR = adrenoreceptor; BDNF = brain-derived neurotropic factors; cAMP = cyclic adenosine monophosphate; CNS = central nervous system; DA = dopamine; DAT = dopamine transporter; DCs = dendritic cells; E = epinephrine; EAE = experimental autoimmune encephalomyelitis; FC = functional connectivity; HC = healthy controls; LC = *locus coeruleus*; LPS = lipopolysaccharide; MAO = monoamine oxidase; MBrN = monoaminergic brainstem nuclei; MS = multiple sclerosis; MSF = MS fatigue; NA = noradrenaline; NFkB = nuclear factor kappa-b; P = progressive; PBMC = peripheral blood mononuclear cells; PFC = prefrontal cortex; RR = relapsing-remitting; RS-fMRI = resting-state functional MRI; SSRIs = selective serotonin reuptake inhibitors; T_{reg} = T-regulatory cells; VTA = ventral tegmental area; WM = white matter.

1. Introduction

Multiple Sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), and the leading cause of non-traumatic disability in young adults in the developed world(Murray, 2006). The complex pathogenesis of MS is still largely unknown with respect to dysregulation of the immune system that pathologically targets the CNS myelin and oligodendrocytes(Weiner, 2008). Both adaptive and innate immune systems are involved to different extents during the course of the disease. The early, relapsing-remitting (RR) stage of MS is associated with antigen-specific T and B cell-mediated adaptive immune responses, whereas the progressive (P) phase is associated with innate immune responses characterized by chronic inflammation and microglial activation(Weiner, 2008). Alongside inflammation, neurodegeneration and axonal loss are also early pathophysiological processes occurring to MS brains, eventually leading to the accumulation of brain atrophy and irreversible disability(Ellwardt and Zipp, 2014).

In recent years, the investigation of MS pathogenesis has focused on the reciprocal interactions between the immune and the nervous systems(Melnikov et al., 2018). Monoamines are crucial for these interactions due to their capability of binding to cell receptors of both systems(Melnikov et al., 2020). They encompass three main types of neurotransmitters: dopamine (DA), noradrenaline (NA), and serotonin (5HT). In the brain, monoamines are produced by monoaminergic neurons, which are mainly located within the brainstem(Kandel, E. R., Schwartz, J. H., & Jessell, T. M., 2000). Monoaminergic brainstem nuclei (MBrN) project diffusely to the whole CNS, and play a crucial role in regulating normal brain function, particularly arousal, mood, reward, and cognition(Bianciardi et al., 2016). Monoamines are also synthetized outside the CNS – mainly by immune cells, intestinal enterochromaffin cells, and some gut microorganisms (such as *Clostridium* species) – where they regulate a variety of different processes(Malinova et al., 2018).

Monoaminergic systems are dysfunctional in many neurological and psychiatric diseases(Admon et al., 2017; Pavese et al., 2010; Serra et al., 2018), and represent the target for a large number of clinically efficacious drugs. In this review, we will briefly summarize the organization of the monoaminergic systems and then we will focus on the immune-regulatory properties of the monoamines. We will also review the mechanism by which MBrN damage and monoaminergic systems' dysregulation may play a role in MS pathogenesis and contribute to some clinical features of MS, particularly fatigue and depression. Finally, we will summarize different drugs targeting monoamines within and outside the CNS that are currently under evaluation for their potential efficacy in the treatment of MS.

2. General organization of the monoaminergic systems within the CNS

Brainstem monoaminergic neurons are organized in specific nuclei that belong to three main functional pathways: the dopaminergic, noradrenergic and serotoninergic pathways(Kandel, E. R., Schwartz, J. H., & Jessell, T. M., 2000). Monoaminergic cells have widespread projections to virtually every part of the CNS, through which they modulate a variety of physiological functions, such as attention, mood, cognition, and memory(Bär et al., 2016). Moreover, monoamines are essential components of the ascending arousal system and modulate the level of behavioral arousal(Bianciardi et al., 2016). A schematic of the main monoaminergic pathways within the human CNS is represented in **Fig.1**.

The catecholamines DA, NA, and epinephrine (E) are synthesized from the common precursor tyrosine, which is converted to L-DOPA by the enzyme tyrosine-hydroxylase, and to DA by the L-DOPA-decarboxylase(Kandel, E. R., Schwartz, J. H., & Jessell, T. M., 2000). Dopaminergic neurons are concentrated in the midbrain in two distinct groups: 1) the *substantia nigra pars compacta* that projects to the dorsal striatum via the nigrostriatal pathway, part of the basal ganglia loop and involved in motor control; and 2) the ventral tegmental area (VTA) that projects to the prefrontal cortex (PFC) and basal forebrain (mainly ventral striatum/nucleus accumbens and limbic system) via the mesocorticolimbic pathway(Bär et al., 2016) (**Fig.1**). VTA plays distinct roles in decision-making, working memory, reward, salience, and aversion(Serra et al., 2018). There are five different DA receptors that play different functions on target cells: D1 and D5 (D1-like family) activate their target cells by increasing the levels of cyclic adenosine monophosphate (cAMP). Conversely, D2, D3 and D4 (D2-like family) inhibit their target cells by reducing the levels of cAMP(Melnikov et al., 2020). D1-like receptors dominate in the targets of the mesocorticolimbic pathway, while the D2-like receptors are dominant in the nigrostriatal pathway(Trutti et al., 2019).

DA is converted to NA by the dopamine-β-hydroxylase in noradrenergic cells that are mainly concentrated in the pigmented pontine nucleus *locus coeruleus* (LC). LC receives afferents from and projects diffusely to the entire cortex (mostly PFC and cingulum), the hippocampus, amygdala, thalamus/hypothalamus, cerebellum, and spinal cord (**Fig.1**)(Benarroch, 2009).These reciprocal noradrenergic projections are primarily part of the ascending arousal system that modulates arousal/attention through thalamo-cortical circuits(Bär et al., 2016). Moreover, LC regulates other higher-level cognitive processes such as working memory, attention, perception, and motivation, and it also modulates pain and autonomic reflexes(Kandel, E. R., Schwartz, J. H., & Jessell, T. M., 2000). NA acts via three G-protein adrenoreceptor (AR) families (α_1, α_2 and β). In the nervous system, α_1- and β-AR are primarily distributed to postsynaptic sites, where they generally exert an excitatory action. Conversely, α_2-AR are distributed pre- and post-synaptically, and they commonly exert inhibitory effects(Benarroch, 2009). In the periphery, DA and NA are synthetized by suprarenal

glands (when stimulated by the autonomic nervous system), immune cells, the gut, and the peripheral nervous system(Kandel, E. R., Schwartz, J. H., & Jessell, T. M., 2000). NA is subsequently methylated by phenylethanolamine-N-methyltransferase to E in the chromaffin cells in the medulla of the adrenal gland(Kandel, E. R., Schwartz, J. H., & Jessell, T. M., 2000) and in a small number of medullary neurons(Malenka RC, 2009). E is mainly released into the circulation and act as an hormone on distant targets, regulating a variety of processes (e.g. it plays an important role in the fight-or-flight response by increasing blood flow to muscles, output of the heart, pupil dilation response and blood sugar level)(Malenka RC, 2009). It does this by binding to α-AR and β-AR(Kandel, E. R., Schwartz, J. H., & Jessell, T. M., 2000).

5HT (5-hydroxytryptamine) is synthesized from tryptophan by the enzymes tryptophan-hydroxylase and 5-hydroxytryptophan-decarboxylase(Kandel, E. R., Schwartz, J. H., & Jessell, T. M., 2000). Serotoninergic MBrN are concentrated in two functional groups(Hornung, 2003): 1) The raphe magnus, raphe obscurus, and raphe pallidus that project from the medulla to the spinal cord, and modulate afferent pain signals, thermoregulation, cardiovascular control, and breathing; and 2) the rostral median and dorsal raphe that project from the pons/midbrain to the forebrain and all cortical areas(Hornung, 2003). The rostral nuclei send also a large contingent of fibers to the cerebellum, other MBrN and the spinal cord(Hornung, 2012) (**Fig.1**). Serotoninergic cells modulate many physiological functions, particularly arousal, attention, mood, and cognition(Kandel, E. R., Schwartz, J. H., & Jessell, T. M., 2000), by binding to 5HT receptors (5HTR). There are seven families and 15 subtypes of 5HTR (5HTR1-5HTR7), according to their different signaling mechanisms. All of them are G-protein-coupled receptors, except for 5HT3, which is a ion channel(Wan et al., 2020).

3. Monoamines modulate the immune system and contribute to MS pathogenesis

In this section the interactions between monoamines and immune systems are summarized in order to provide evidence for a major contribution of monoaminergic alteration/dysregulation to the pathogenesis of MS (**Fig.2**).

3.1 Monoamines are key mediators of neuroimmune interaction

The monoaminergic and immune systems have a reciprocal interaction that occurs through the hypothalamic-pituitary-adrenal axis and the sympathetic/parasympathetic innervations of lymphoid organs(Pacheco et al., 2014). On the one hand, monoamines modulate the activity of the immune system and the production of cytokines, by directly interacting with cells of both innate and adaptive immunity(Ben-Shaanan et al., 2016, p.; Castellani et al., 2019; Melnikov et al., 2020; Yan et al., 2015). On the other hand, immune cells are able to synthesize monoamines, regulating the

peripheral levels of these neurotransmitters(Cosentino et al., 2002). Peripheral inflammation reduces the synthesis of monoaminergic neurotransmitters, by causing a deficit of tetrahydrobiopterin, an essential cofactor of aromatic amino acid hydroxylase enzymes that are critical for the synthesis of monoamines(Harrison et al., 2015). Inflammatory cytokines also activate indoleamine-2,3-dioxygenase, which degrades tryptophan (a 5HT-precursor) along the kynurenine pathway and thus limits 5HT synthesis(Manjaly et al., 2019). Within the CNS, inflammation causes microglial cells to alter monoaminergic transporters and receptors, decreasing the overall transmission and initiating neurodegeneration(Deleidi et al., 2010).

DA-receptors are expressed by all immune cells, including effector T and B cells, dendritic cells (DCs), macrophages, microglia, neutrophils, natural killer cells, and T-regulatory cells (T_{reg})(Zhang et al., 2017). The binding of different DA-receptors to DA varies according to DA plasma concentration, and DA has different effects on immune cells, depending on the state of activation of DA-receptors(Melnikov et al., 2020). In addition, immune cells may themselves synthesize DA and express monoamine-degrading enzymes, possibly modulating DA peripheral level(Melnikov et al., 2018). For instance, DCs contain intracellular DA that is released following antigen presentation and acts in an autocrine manner to stimulate D5 expressed on DCs, thus promoting a potent production of IL-23 by DCs with a pro-inflammatory effect (**Fig.3**)(Prado et al., 2012).

When released in *in vitro* experiments, IL-23 directs to naïve CD4+-T cells, favouring their polarization toward the pro-inflammatory Th17 phenotype(Prado et al., 2012). This loop is mediated by D5 and may act as a driving force in the autoimmune response(Pacheco et al., 2014). Proliferating CD4+-T cells express D3 that contribute to destruction of dopaminergic neurons and generate IFN-γ, which prevents DA synthesis(Dobryakova et al., 2015; González et al., 2013; Pacheco et al., 2014). Human T_{reg} produce DA(Cosentino et al., 2016). In response to yet unknown physiological stimuli, T_{reg} release DA, which can interact with DA-receptors expressed on their cell surface, but also with DA-receptors present on DCs and effector CD4+-T cells(Pacheco et al., 2014). T_{reg}-derived DA interaction with D1-like-family expressed by T_{reg}, reduces the expression of IL-10 and TGF-β, and weakens the T_{reg}'s suppressive activity exerted over effector CD4+-T cells(Pacheco et al., 2014). Moreover, T_{reg}-derived DA interaction with D2-like-family acts in an autocrine inhibitory loop, resulting in T_{reg} functional suppression (**Fig.3**)(Cosentino et al., 2016). Notably, mice with genetic hypofunction of the dopamine transporter (DAT) show an altered immune-tolerance with increased functional T_{reg} and reduced microglia activation and infiltration of brain monocyte-derived macrophages(Castellani et al., 2019).

NA has both anti-inflammatory and neuroprotective effects, acting mainly on microglia and astrocytes by different mechanisms, which include reduction of the nuclear factor kappa-b (NFkB) activity and lipopolysaccharide (LPS)-induced TNF-α microglial expression(Feinstein et al., 2016). NA reduces also neurotoxicity elicited by inflammatory and excitotoxic stimuli(Madrigal et al., 2007), and lowers oxidative stress-related damage in neurons(Troadec et al., 2008). Selective NA-reuptake inhibitors reduce CNS chemokine, cytokine, and cell adhesion expression(O'Sullivan et al., 2010), and increase anti-inflammatory cytokines(McNamee et al., 2010; Polak et al., 2011). Moreover, peripheral inflammation promotes brainstem noradrenergic neurons to synthetize NA/E, which in turn activates the paraventricular nucleus of the hypothalamus, controlling the hypothalamic-pituitary-adrenal axis(Dampney et al., 2018). Particularly, the activation of hypothalamus leads to the release of corticotropin-releasing hormone and stimulates the secretion of adrenocorticotropic hormone by the pituitary gland of the anterior lobe(Capellino et al., 2020). As a consequence, the adrenal cortex releases glucocorticoids into the bloodstream, which inhibit peripheral immune activation, by suppressing the production of proinflammatory cytokines(Capellino et al., 2020).

5HT regulates both the innate and adaptive immune systems, since almost all immune cells express 5HTR(Malinova et al., 2018). 5HT recruits innate immune cells to the inflammatory site and increases the release of inflammatory cytokines from macrophages and DCs(Wan et al., 2020). It also interacts with 5HTR2B and 5HTR7 on monocytes, influencing macrophage phenotype polarization: by suppressing LPS-induced proinflammatory cytokines and regulating M1/M2 polarization-related genes, 5HT drives monocyte polarization to M2 phenotype(de las Casas-Engel et al., 2013; Malinova et al., 2018). Notably, DCs can uptake and store 5HT from the local environment, and then release it to activate T cells and modulate their function(Arreola et al., 2015). Acting on 5HTR1B and 5HTR2B, 5HT modulates the differentiation, proliferation, and effector functions of T cells in a multidirectional manner that depends on the T cells environment and activation state(Arreola et al., 2015; Malinova et al., 2018). Lastly, by catabolizing the essential amino acid tryptophan – the 5HT-precursor – cells expressing the enzyme indoleamine-2,3-dioxygenase (IDO) can mediate different local effects on innate and adaptive immune responses to inflammatory insults(Munn and Mellor, 2013). For instance, IDO expressed by professional antigen presenting cells (macrophages and DCs) promote systemic tolerance by activating T_{reg}(Munn and Mellor, 2013). Moreover, an increased IDO activity contributes to pain perception and depression, possibly as a result of reduced 5HT levels (due to depletion of tryptophan) and/or release of neurotoxic tryptophan catabolites(Kim et al., 2012).

3.2 Monoaminergic systems modulate immune response in MS and influence MS pathogenesis

The alteration/dysregulation of monoaminergic systems play a major role in the pathogenesis of both experimental autoimmune encephalomyelitis (EAE) and MS(Feinstein et al., 2016; Levite et al., 2017; Malinova et al., 2018; Melnikov et al., 2020; Polak et al., 2011; Vidal and Pacheco, 2020).

Different studies have shown that DA modulates the pathogenesis and influences the course of EAE and MS(Levite et al., 2017; Melnikov et al., 2020; Vidal and Pacheco, 2020). As reviewed by (Levite et al., 2017), in MS patients, dopaminergic dysregulation occur at several levels (**Fig.3**): 1) reduction of D1-like and increase of D2-like-receptors with consequent predominance of D2-like stimulation on immune cells; 2) impaired DA synthesis and release from immune cells with reduced effects on neighboring cells; 3) T_{reg} overexpression of D1-like-receptors and DA overproduction with enhanced activity of the auto-inhibitory loop(Levite et al., 2017; Prado et al., 2012). The production of DA and the expression of DA-receptors is altered in peripheral blood mononuclear cells (PBMC) and T cells of MS patients(Prado et al., 2018; Vidal and Pacheco, 2020), since the early disease stages(Cosentino et al., 2016). A study including 43 MS patients compared to healthy controls (HC) revealed, in the former group, lower DA levels together with a higher proportion of Th-17 cells and higher levels of IL-17 and IFN-γ released by PBMC during clinical relapses, in comparison to remissions: these findings suggest that DA suppresses IL-17 released by PBMC(Melnikov et al., 2016). Additionally, within the same study, the suppressive effect of DA was found to be abolished in the presence of a sulpride (a D2-like family antagonist)(Melnikov et al., 2016). Protein and mRNA levels of D5 (D1-like-family), but not of D3 (D2-like-family), were found reduced in PBMCs of RR-MS patients(Giorelli et al., 2005). Upon treatment of patients with IFN-β, lymphocytes showed reduced D3 expression and restored ability of DA to regulate cell functions(Levite et al., 2017). Similarly, Zaffaroni et al. found in RR-MS that during 12-month treatment with IFN-β mRNA levels of D5 increased in PBMCs, while the D2 decreased, suggesting that IFN-β modifies DA pathways in circulating lymphocytes(Zaffaroni et al., 2008). Moreover, D5 deficiency, either whole-body or limited to DCs, reduces EAE severity(Osorio-Barrios et al., 2018; Prado et al., 2012). The administration of risperidone and clozapine – atypical antipsychotic agents that act mainly as D2-antagonists – reduces EAE severity, even if such an effect cannot be attributed to D1-like or D2-like antagonism alone(Levite et al., 2017). Taken altogether, these data indicate that the balance between pro- and anti-inflammatory roles of DA is altered in MS.

Various studies have provided evidence of a perturbation of NA levels in both EAE and MS(Feinstein et al., 2016). Within the CNS, NA levels were found to be higher in the CSF from MS patients compared to HC in one study(Barkhatova et al., 1998), while another study reported a negative correlation between CSF levels of a NA metabolite, disease duration and relapse frequency, which could reflect a diminished response of the noradrenergic system to ongoing

inflammation(Markianos et al., 2009; Polak et al., 2011). Similar findings were obtained in EAE(Polak et al., 2011). Pilipovic et al. demonstrated that β-AR are dysregulated in EAE, suggesting that NA may affect disease development by modulating interactions between distinct subtypes of CD4+-T cells and antigen presenting cells through α_1-AR, and consequently CD4+ T cell priming(Pilipović et al., 2019). Consistently, the astroglial expression of β-AR is reduced in MS, to possibly compensate for the alteration of NA levels(De Keyser et al., 1999). The experimental increase of NA levels within the CNS in EAE improves both clinical and neuropathological aspects (for a review see: Feinstein et al., 2016).

Growing evidence supports an anti-inflammatory role of 5HT in EAE and MS. Excessive levels of proinflammatory cytokines within the CNS are associated with reduced 5HT synthesis(Sacramento et al., 2018). 5HT content is lower in EAE mice, as compared to wild-types(Benson et al., 2013). The increase of CNS 5HT levels in the animal model of MS – by administering monoamine oxidase (MAO) inhibitors or selective serotonin reuptake inhibitors (SSRIs) – improves 5HT innervation of the spinal cord, reduces EAE severity, impairs T cell proliferation, and lowers inflammatory infiltration and IFN-γ production(Benson et al., 2013; Hofstetter et al., 2005). Similarly, the electrical stimulation of raphe nuclei attenuates the course of EAE, reduces inflammatory response, and increases myelinated axons(Madsen et al., 2017). In cell cultures from MS patients, 5HT reduces the release of the detrimental cytokines IFN-γ and IL-17 by $CD8^+$ T cells, increases the production of IL-10 by $CD4^+$ T cells, and favors the expansion of T_{reg}(Sacramento et al., 2018). Interestingly, the dietary intake of tryptophan – and thus the synthesis of 5HT – is lower in high-latitude areas, where there is an increased susceptibility for MS(Malinova et al., 2018; Wan et al., 2020).

3.3 Monoamines are mediators of the gut-brain axis in MS

The gut-microbiome – the totality of microorganisms (bacteria, viruses, protozoa, and fungi), and their collective genetic material present in the gastrointestinal tract – is essential for regulating human metabolism and immune system(Strandwitz, 2018). Gut-microbiome interacts with the enteric nervous system and CNS via the ‘‘gut-brain-axis”(Fung et al., 2017). Growing evidence suggests that the individual gut-microbiome affects the course of MS and contributes to its pathogenesis(Cekanaviciute et al., 2017).

The association between monoamines and gut-microbiome has been evaluated. Intestinal enterochromaffin cells produce monoamines (particularly 5HT and DA), through a process coordinated by the gut-microbiome (directly or through host biosynthesis pathways) that modulates monoamines’ central and peripheral levels, as well as the expression of monoamines’

receptors(Malinova et al., 2018; Strandwitz, 2018) (**Fig.2**). In the gut, monoamines influence different intestinal processes (such as controlling blood flow, gut motility, and nutrient absorption)(Mittal et al., 2017), regulate gastrointestinal innate and adaptive immune systems(Malinova et al., 2018), modulate gut-microbiome(Strandwitz, 2018), and in their capacity of neurotransmitters they can travel to the CNS (for a review see Mittal et al., 2017). Their effects on nervous cells may be both direct and indirect, via interaction with immune, metabolic, epigenetic, and neuroendocrine systems(Oleskin et al., 2017). For these reasons monoamines are currently under investigation as potential mediators of the gut-brain axis in MS(Malinova et al., 2018). An example is given by platelets that uptake 5HT in the gut and transport it to the peripheral blood, where they are the only available source of 5HT and constitute a link between microbial modulation of gut 5HT and its effects on immune cells at distant locations(Malinova et al., 2018). During EAE exacerbations, the depletion of platelets enhances inflammation and increases immune cell infiltration of the CNS(Starossom et al., 2015). In MS patients, platelets fail to secrete 5HT and this may be linked to the occurrence of clinical relapses(Malinova et al., 2018; Starossom et al., 2015).

4. Brain monoaminergic nuclei are damaged in MS

Brain monoamines in MS are reduced not only as a direct effect of inflammation, but also as an indirect effect of structural damage(Manjaly et al., 2019). The brainstem is a major target of MS(Reich et al., 2018). Thus, monoaminergic dysfunction in MS may reflect the loss of monoaminergic neurons as a result of both direct damage by inflammation/degeneration in the MBrN – where these cells originate – or axonal loss of monoaminergic fibre tracts(Manjaly et al., 2019). Axonal loss in MS may results from: 1) direct inflammatory injury, leading to axonal transection and subsequent Wallerian retrograde degeneration; 2) axonal degeneration secondary to excitotoxicity generated by the redundant activity of sodium channels needed to propagate action potentials along denuded axons during chronic demyelination; 3) trans-synaptic degeneration resulting from the loss of inputs from degenerating afferent neuron or from the loss of efferent targets(Gajamange et al., 2018). Only a few studies have provided evidence of a structural damage of MBrN in MS, owning to the small dimension of the brainstem and its proximity to large arteries and ventricles, which place high demands on characterization of brainstem alterations in MS(Gadea, 2004; Polak et al., 2011). Among MBrN, LC has been investigated the most; LC damage in MS has been characterized using either neuropathology or MRI. In EAE, Polak et al. demonstrated the presence of LC damage, together with a reduction in cortical and spinal cord NA levels(Polak et al., 2011). They also showed that experimentally-induced lesions of LC neurons exacerbate EAE neuropathology, while treatments to compensate for NA depletion, or reduce LC neuronal damage, are beneficial(Polak et al., 2011).

Moreover, they observed an increase in glial cell activation in and around the LC in samples from MS patients – possibly reflecting local inflammation – and a reduction of NA levels within the brain(Polak et al., 2011). By using MRI spectroscopy, (Gadea, 2004) has shown a correlation between the levels of N-acetylaspartate (regarded as a neuronal marker) in the right LC and impairment of selective attention in RR-MS.

An indirect measure of brainstem neurodegeneration can be achieved by using resting-state functional MRI (RS-fMRI), a neuroimaging technique that characterises functional connectivity (FC) between brain areas at rest, by detecting the correlation between spontaneous fluctuations in neuronal activity at distant locations. Since neuronal loss within a specific nucleus alters its FC with its projection areas, RS-fMRI can be used as a marker of neuronal degeneration(Serra et al., 2018). Notably, impaired FC in MS may also result from the dissemination of white matter (WM) lesions throughout the brain leading to WM-tract disconnection and reorganization of monoaminergic brain networks(Soares et al., 2020). RS-fMRI has been used to measure FC between MBrN and various brain areas in many neurological diseases(Bär et al., 2016; Serra et al., 2018), including MS(Carotenuto et al., 2020). In a recent RS-fMRI study, Carotenuto et al. reported functional derangements of serotonergic, noradrenergic, and dopaminergic systems in MS. Particularly, these Authors described increased FC between the dorsal raphe and the frontal cortex, decreased FC between the VTA and PFC, and decreased betweenness centrality (a measure of the importance of a given node within a network) for the brainstem in MS patients, as compared to HC(Carotenuto et al., 2020). These findings suggest a damage in the ascending projections from brainstem regions in MS with a reduced activity in controlling the cortical regions of monoaminergic brain networks(Carotenuto et al., 2020).

5. Monoamines contribute to the pathophysiology of MS fatigue

Given the fundamental importance of monoamines in regulating normal brain function, dysfunction of monoaminergic systems may have a direct clinical implication and account for some clinical features of MS, particularly MS fatigue (MSF)(Dobryakova et al., 2015; Manjaly et al., 2019). Fatigue is defined as lack of energy, feelings of exhaustion that are unaided by sleep, and perception that one is unable to perform mental and physical activities(Dobryakova et al., 2015). Physical and cognitive fatigue affects up to 80% of MS patients, and represents one of the most debilitating symptoms, also due to the fact that very few therapeutic options are currently available(Induruwa et al., 2012). The pathophysiology of MSF is largely unknown and different underlying mechanisms have been proposed(Manjaly et al., 2019). Among them, growing evidence supports the role of aberrant monoaminergic neurotransmission(Dobryakova et al., 2015; Manjaly et al., 2019) (**Fig.4**).

Indeed, monoamines are crucial modulators of motivation, mood, attention, and arousal, functions that are all impaired in patients with MSF. Damage to MBrN or inflammation-induced decrease/alteration of monoamines synthesis/function may lower the neurotransmitter supply to the rest of the brain. As a consequence, the impairment of brainstem monoaminergic projections can lead to a functional reorganization of central cortical networks(Dipasquale et al., 2016; Manjaly et al., 2019; van den Brink et al., 2016).

Among monoaminergic systems, MSF has been mainly linked to an imbalance of DA and – to a lesser extent – 5HT within the CNS(Cordeiro et al., 2017; Dobryakova et al., 2015; Manjaly et al., 2019). The role of NA has been investigated in Parkinson's disease-related fatigue by Solopchuk at al., who did not identify any significant correlations between the extent of degeneration of LC and the degree of fatigue(Solopchuk et al., 2018). Moreover, a recent study demonstrated decreased NA transporter-enriched FC in several frontal and prefrontal areas of patients with high levels of MSF when compared to those with lower fatigue (Cercignani et al., 2021).

Dobryakova et al. have recently reviewed the literature that supports the so-called DA imbalance hypothesis for MSF(Dobryakova et al., 2015). Different MRI studies provided evidence of both structural and functional damage in brain structures that are critical for dopaminergic projections, particularly in the mesocorticolimbic regions and striatum(Dobryakova et al., 2015; Manjaly et al., 2019). Structural damage to the ventromedial-PFC (the PFC region that receives the largest contingent of dopaminergic fibres) was found to be more associated with increased fatigue than damage to other cortical regions(Pardini et al., 2010). In MS, Genova et al. reported WM damage in the internal capsule (through which dopaminergic fibres reach the PFC) in MSF patients as compared to non-fatigued patients(Genova et al., 2013). Similarly, a PET study demonstrated lower levels of glucose metabolism at rest in the PFC and striatum of patients with MSF, compared to those without fatigue(Roelcke et al., 1997). Resting-state FC in the mesocorticolimbic pathway is reduced in patients with MSF(Finke et al., 2015). However, the usefulness of RS-fMRI in studying fatigue may be limited by the fact that the scan is acquired at rest, when patients are not frankly experiencing fatigue(Dobryakova et al., 2015). Indeed, Genova and colleagues asked a group of MS patients to rate their level of fatigue before and after running an executive task during fMRI: activity in the striatum was found to be greater in individuals who complained of higher on-task MSF(Genova et al., 2013). Another study has shown that in MSF patients mesocorticolimbic connectivity is reduced when performing memory tasks(Engström et al., 2013). All these studies suggest that MSF results from the impaired functioning of the mesocorticolimbic pathway, which is likely due to a DA imbalance within regions of this network. Given the importance of the mesocorticolimbic pathway in reward and motivation, fatigue may arise from an altered response to reward and from a decrease in

motivation(Manjaly et al., 2019). If the DA imbalance hypothesis of fatigue is correct, restoring the appropriate DA levels within the CNS by means of dopaminergic medication would result in reduction of fatigue. Indeed, two drugs that are currently used to treat fatigue in MS – amantadine and methylphenidate – are both DA-agonists(Dobryakova et al., 2015). Methylphenidate has shown the ability to improve fatigue in other neurological conditions and is currently under evaluation for the treatment of MSF(Dobryakova et al., 2015; Mücke et al., 2015). Moreover, IFN-β treatment leads to an increased production of DA(Zaffaroni et al., 2008) and decreases MSF(Melanson et al., 2010).

A dysregulation of the serotoninergic system in the pathophysiology of MSF has been hypothesized mainly because fatigue represents a major symptom of depression that is treated with SSRIs(Dobryakova et al., 2015). Moreover, 5HT has been linked to exercise-induced fatigue: physical exercise increases 5HT levels within the CNS, leading to reduced mental and physical performances, likely by modifying the tolerance to pain or discomfort(Cordeiro et al., 2017; Meeusen and Roelands, 2018). The administration of drugs that increase 5HT levels decreases performance, which is in turn increased by administration of 5HT activity inhibitors(Cordeiro et al., 2017). Conversely, a PET study conducted in patients with chronic fatigue syndrome revealed a reduction of density of 5HT-transporters in the rostral anterior cingulate cortex in comparison with HC(Yamamoto et al., 2004). Pavese et al. used ^{18}F-DOPA and ^{11}C-N-N-dimethyl-2-(2-amino-4-cyanophenylthio)-benzylamine to investigate DA storage capacity and 5HT transmission, respectively, in the brain of Parkinson's disease patients with and without fatigue, finding significant differences in 5HT transmission between the two groups(Pavese et al., 2010). Using PET imaging, Hesse et al. investigated the availability of 5HT-transporters in the brain of MS patients compared to HC, reporting a lower availability in limbic and paralimbic regions and higher availability in the frontal cortex(Hesse et al., 2014). A positive association between 5HT-transporter availability in the insula of MS patients and both their depression and fatigue scores was also observed(Hesse et al., 2014). Haghighi et al. investigated the role of OSU6162 (a monoaminergic stabilizer that modulates primarily DA and 5HT transmission) in MS patients, identifying a potential therapeutic effect in cognitive MSF(Haghighi et al., 2018). Although preliminary, these findings support the hypothesis of a role of 5HT in the pathophysiology of MSF.

6. Monoamines contribute to depression in MS

Mood disorders, and particularly depression, are common symptoms of MS with up to 35% of patients being on antidepressants(Minden et al., 2014; Peruga et al., 2011). The rate of depression in MS is higher than in other neurological diseases, supporting the hypothesis that its cause may be the result of alterations in the CNS and not simply the reaction to being diagnosed with a chronic and

potentially disabling disease(Grech et al., 2019). Although the pathophysiological basis of the correlation between the two conditions has not been clarified yet, depression in MS presumably results from a combination of both psychosocial factors and neurobiological changes resulting from brain tissue damage(Arnett et al., 2008; Grech et al., 2019).

In both conditions, active MS relapses and depression, there is an increase of inflammatory cytokines produced by immune cells, as well as a dysregulation of monoaminergic neurotransmission within the brain(Grech et al., 2019; Peruga et al., 2011). This supports the emerging hypothesis of a major contribution of monoamines in the pathogenesis of depression in MS (**Fig.5**).

Disruption of monoaminergic modulatory networks has been described also in other neurological disorders, such as Alzheimer's disease with a strict association between VTA driven disconnection and both cognitive and behavioural symptoms(De Marco and Venneri, 2018; Serra et al., 2018). Moreover, functional dysregulation of monoaminergic pathways has been extensively described in patients with depression(Admon et al., 2017), also as a result of inflammation(Felger et al., 2016). In line with this, a fascinating hypothesis may explain the occurrence of depression in MS as due to the monoaminergic dysregulation induced by the inflammatory cascade, which may persist over time. Consistently, Peruga et al. reported that EAE mice compared to controls mice exhibit increased levels of anxiety- and depression-like behaviours, which are associated to increased levels of TNF-α and neuronal loss in the hippocampus(Peruga et al., 2011). Moreover, the administration of the antidepressant amitriptyline was found to increase the levels of NA in the hippocampus and improve anxiety-like symptoms(Peruga et al., 2011). Based on this piece of evidence, it was argued that chronic inflammation in the CNS may impact on emotional responses(Peruga et al., 2011). By using RS-fMRI, Carotenuto et al. have recently reported an association between depressive symptoms in MS and both serotonergic and noradrenergic network reorganization(Carotenuto et al., 2020). Another MRI study has shown that MS patients with depression have an increased WM lesion load in the projection areas of the basal limbic system (which is a major projective target of the monoaminergic systems), but not in the basal limbic system itself, nor in the ponto-mesencephalic midline, where MBrN are anatomically located(Berg et al., 2000). However, these Authors focused their investigation on patients' burden of WM lesions, while there are several other structural factors that may contribute to monoaminergic dysfunction in MS(Berg et al., 2000). Another observation supporting the role of monoamines in MS depression comes from pharmacological studies. Fingolimod, an MS-specific anti-inflammatory medication, has antidepressant effects in EAE, by increasing brain-derived neurotropic factors (BDNF)(Grech et al., 2019). This supports the idea that neurogenesis and neuroplasticity may be facilitated by anti-inflammatory drugs within the CNS, and their importance for neuronal survival and plasticity(Grech et al., 2019). BDNF is expressed in

immune cells and have been linked to inflammation. Moreover, BDNF is reduced in patients with depression(Grech et al., 2019). Further studies are needed to better clarify the link between monoamines and depression in MS.

7. Monoaminergic systems are possible targets in MS treatment

The demonstration of altered monoamine levels' in both EAE and MS and of contributions of monoaminergic dysfunction to MS pathogenesis and symptoms provide a rationale for proposing therapeutic strategies to activate, replace or supplement monoaminergic transmission within the CNS of MS patients. Beside the well-known symptomatic effects of monoaminergic drugs that has been already mentioned when talking about fatigue and depression (see Section 5 and 6), the effects of monoaminergic compounds on inflammation are of particular interest in MS.

Current literature supports that the experimental increase of NA and 5HT levels within the CNS promote an anti-inflammatory state and has beneficial effects on the course of EAE (Benson et al., 2013; Feinstein et al., 2016; Hofstetter et al., 2005; Madsen et al., 2017). Consistently, different compounds that are known to increase NA and 5HT levels within the CNS (mainly SSRIs/SNRIs) have been tested in EAE and MS, with promising results. However, it should be noted that most human studies are small and need further replication in larger cohorts(Grech et al., 2019). The SSRIs fluoxetine and sertraline have been shown to ameliorate the EAE course(Grech et al., 2019). The MAO inhibitor phenelzine and the tricyclic clomipramine increase levels of 5HT, NA, and DA, and attenuate EAE by reducing T cell proliferation, oxidative stress, and B cell activity(Benson et al., 2013; Grech et al., 2019). Treatment with a mixture of the NA-reuptake inhibitor lofepramine and of the tyrosine-precursor phenyalanine relieved symptoms and reduced WM lesion-load in n=69 MS as compared to n=69 placebo-treated patients(Feinstein et al., 2016; Puri et al., 2001; Wade, 2002). In a placebo-controlled study, the administration of fluoxetine in clinically-active MS patients was found to reduce the frequency of new MS lesions, as compared to the placebo group(Mostert et al., 2008). In contrast, fluoxetine was found of no effect in modulating the clinical progression of patients suffering from progressive-MS(Mostert et al., 2013). Even though this latter study was under-powered, its findings suggest that inflammation is possibly the most relevant pathological substrate on which antidepressants play their disease-modifying role in MS(Grech et al., 2019). Moreover in a small study, Mostert et al. demonstrated a potential neuroprotective effect induced by the short-term use of fluoxetine by reporting an increased N-acetylaspartate/creatine ratio in patients' cerebral WM, which suggests a reversible effect of axonal damage(Mostert et al., 2006). The potential neuroprotective role of fluoxetine has been recently evaluated in a 4-arm, double-blinded, randomised placebo-controlled trial that compared three different molecules, including fluoxetine (n=111) against

placebo (n=112) in patients with secondary P-MS. However, no substantial beneficial effect was reported on disease progression(Chataway et al., 2020). The effect of restoring adequate levels of NA and 5HT within the CNS on MS needs to be tested in larger studies. Moreover, we believe that it is time to perform clinical trials aimed at testing 5HT/NA agonists in association with conventional immunomodulating MS treatments, to evaluate their efficacy.

As concerns DA agents, some DA-agonists selective for D1-like-receptors (e.g., fenoldopam), or D2-like receptors (e.g., pramipexole), or active on both D1- and D2-like receptors (e.g., rotigotine) – that are already used in clinics for different indications – have been shown to exert immune effects on T cells and could be potentially relevant for MS treatments(Levite et al., 2017). Indeed, the D2-agonists bromocriptine, arylpiperazine, and pramipexole have shown promising therapeutic results in EAE(Dijkstra et al., 1994; Lieberknecht et al., 2017; Popovic et al., 2015; Vidal and Pacheco, 2020). Given the complex and distinct effects of DA on immune cells – that largely depend on DA receptors activation state and on DA local concentration(Melnikov et al., 2020) – it is still a matter of debate whether D1- or D2-like-agonists might be preferred. Current literature and this review suggest that the beneficial effect on MS inflammation might come from an increase of D1-like receptor rather than D2-like receptor activation(Cosentino et al., 2016; Levite et al., 2017; Melnikov et al., 2018; Prado et al., 2012, p. 2), but further studies are needed to clarify it. Arylpiperazine ligands (which bind to D2-like and 5-HTR1A) show that a combined dopaminergic/serotoninergic stimulation has a neuroprotective effect and reduces CNS immune infiltration in EAE(Malinova et al., 2018). In a clinical trial, tri- or tetra-cylic antidepressants were administered to MS patients in combination with L-DOPA (which is converted to DA and then to NA) reporting a substantial improvement in 75% of patients within 2 months(Berne-Fromell et al., 1987). This effect was related to the increased of NA, but a contribution of DA cannot be ruled out in view of the pharmacology of the drugs employed(Levite et al., 2017). Two phase-II clinical trials evaluating the D2-like-antagonist domperidone, in patients with secondary P-MS (NCT02308137) and in RR-MS (NCT02493049) are on-going in Canada(Vidal and Pacheco, 2020). Further studies are still needed to properly assess the role of DA compounds in MS.

8. Conclusions and future perspectives

In this review we provided evidence of how monoamines are key mediators of neuroimmune interaction and influence MS pathogenesis and course. In the periphery, monoamines interact with the adaptive and innate immune cells, which express monoaminergic receptors. Vice versa, peripheral neurotransmitters' levels are regulated by immune and gut cells that are able to synthesize monoamines. Similarly, within the CNS, MBrN-derived monoamines interact with microglia and

other CNS-resident cells, regulating their functions reciprocally. The effect of monoamines on immune cells' phenotype is complex and still largely unknown, since they can alternatively promote a pro-inflammatory and anti-inflammatory phenotype, depending on their concentration and probably on their interaction mechanisms with other cells or cofactors. However, understanding these mechanisms is fundamental in developing interventions to modulate inflammation in inflammatory diseases like MS. In this context, a fascinating hypothesis concerns the scenario in which the interplay between monoaminergic and immune system takes place: the sympathetic/parasympathetic innervation of lymphoid organs. For instance, in both primary and secondary lymphoid tissues immune cells come in direct contact with the dendrites of post-ganglionic sympathetic neurons that predominantly secrete NA as their primary neurotransmitter(Sharma and Farrar, 2020). The autonomic nervous system could serve as a mirror to better understand the interactions between the two systems. If the direct effect of monoamines on immune cells is only partially assessed in MS and many studies are currently ongoing, we provided evidence of the different consequences that MS-inflammation has on the monoaminergic systems. Peripheral inflammation-induced reduction of monoamines' synthesis promotes a pro-inflammatory status in immune cells, increasing cytokine release and inflammation in an auto-enhancing loop, and suppresses anti-inflammatory pathways, such as the ones mediated by T_{reg}. The same processes occur also within the CNS of MS patients where monoamines synthesis is reduced, as a result of inflammatory cytokines released within the brain from both peripheral immune cells and microglia.

Our review suggests that the levels of brain monoamines in MS are reduced not only as a direct effect of inflammation, but also as an indirect effect of structural damage. As described in the text, axonal loss of monoaminergic fibre tracts can result from several pathological processes in MS. Moreover the brainstem is a major target of inflammation in MS and MBrN lesions are described with potential MBrN retrograde degeneration(Gadea, 2004; Polak et al., 2011). All these structural alterations can alter/reduce monoaminergic transmission in MS and cause a dysregulation of monoaminergic pathways. Quantitative MRI and fMRI are promising tools to better characterize structural alterations of monoaminergic pathways in MS.

The well-established role of monoamines as key regulators of many brain functions lead us to speculate that their reduction/dysfunction in MS may contribute to a wide range of clinical features typical of MS, such as cognitive deficits, mood disorders and fatigue. Particularly, our review provided evidence of the monoaminergic contribution of monoamines to MS fatigue and depression. The pathogenesis of MS fatigue is complex and still-largely unexplained. On the one hand, fatigue can result from the release of cytokines within and outside the CNS in the context of inflammation(Manjaly et al., 2019). On the other hand, other mechanisms independent from

inflammation (structural damage, maladaptive network recruitment, metacognition of interoception of dyshomeostatic states) promote fatigue, as testified by MS patients experiencing fatigue despite treatment with natalizumab with neither clinical nor radiological activity(Manjaly et al., 2019). Monoamines are linked to both inflammatory and non-inflammatory theories of MS fatigue: altered monoaminergic neurotransmission and/or inflammation-induced decrease of neurotransmitter synthesis – with the consequent reduction of monoaminergic inputs supply to cortical and subcortical regions and the functional reorganisation of cortical networks – could contribute to MS fatigue. Thus, a better insight of monoaminergic dysfunction in MS fatigue would add important information on its pathogenesis and, mostly, would provide new potential therapeutic targets. Many inexpensive drugs targeting monoamines are already available and their use in MS has a rationale that needs to be further explored by larger studies. Non-pharmacological approaches, such as transcranial magnetic stimulation(Medina-Fernandez et al., 2018), may also allow to manipulate monoaminergic functioning. Since fatigue has pleomorphic and extremely variable manifestations in MS patients (given the variety of factors contributing to it), a personal approach to treatment should be sought. In order to select treatments in a rational manner, novel tests coming either from quantitative MRI or electrophysiological techniques able to interfere on monoaminergic function are urgently needed. In parallel to fatigue, we speculated that, in the presence of predisposing psychosocial factors, the deregulated monoaminergic neurotransmission contributes to MS depression, alongside – and as a result of – inflammatory cascade and brain structural damage. Thus balancing the monoaminergic neurotransmission through personalized treatments may also provide benefit to mood disorders in MS.

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CRediT authorship contribution statement:

Tiziana Carandini: writing the original draft, reviewing literature, conceptualization. **Mara Cercignani**: writing the original draft, conceptualization, revision for intellectual content. **Daniela Galimberti**: revision for intellectual content. **Elio Scarpini**: revision for intellectual content. **Marco Bozzali**: writing the original draft, revision for intellectual content.

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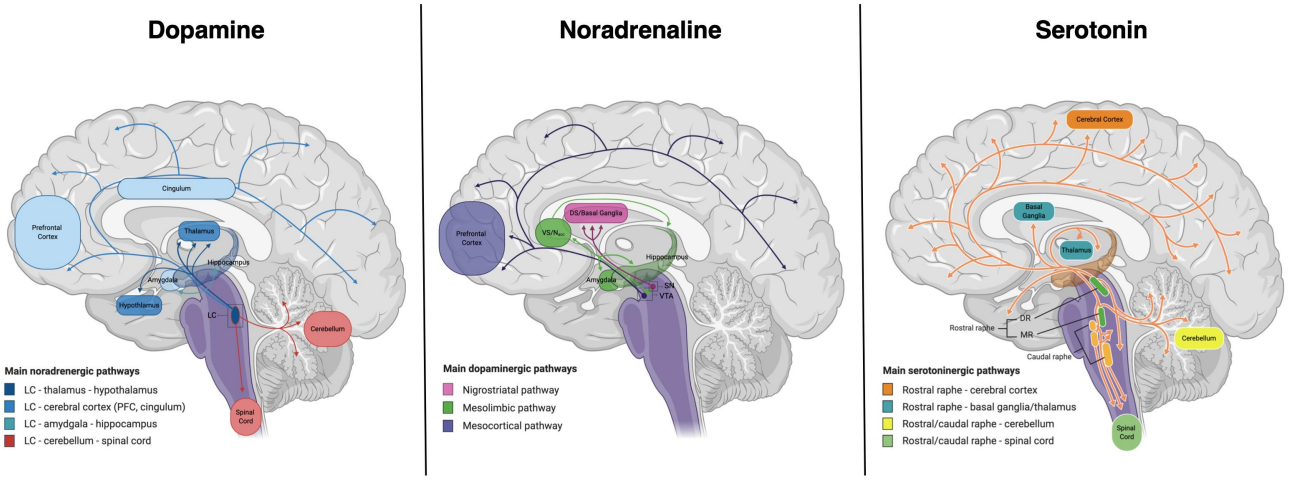
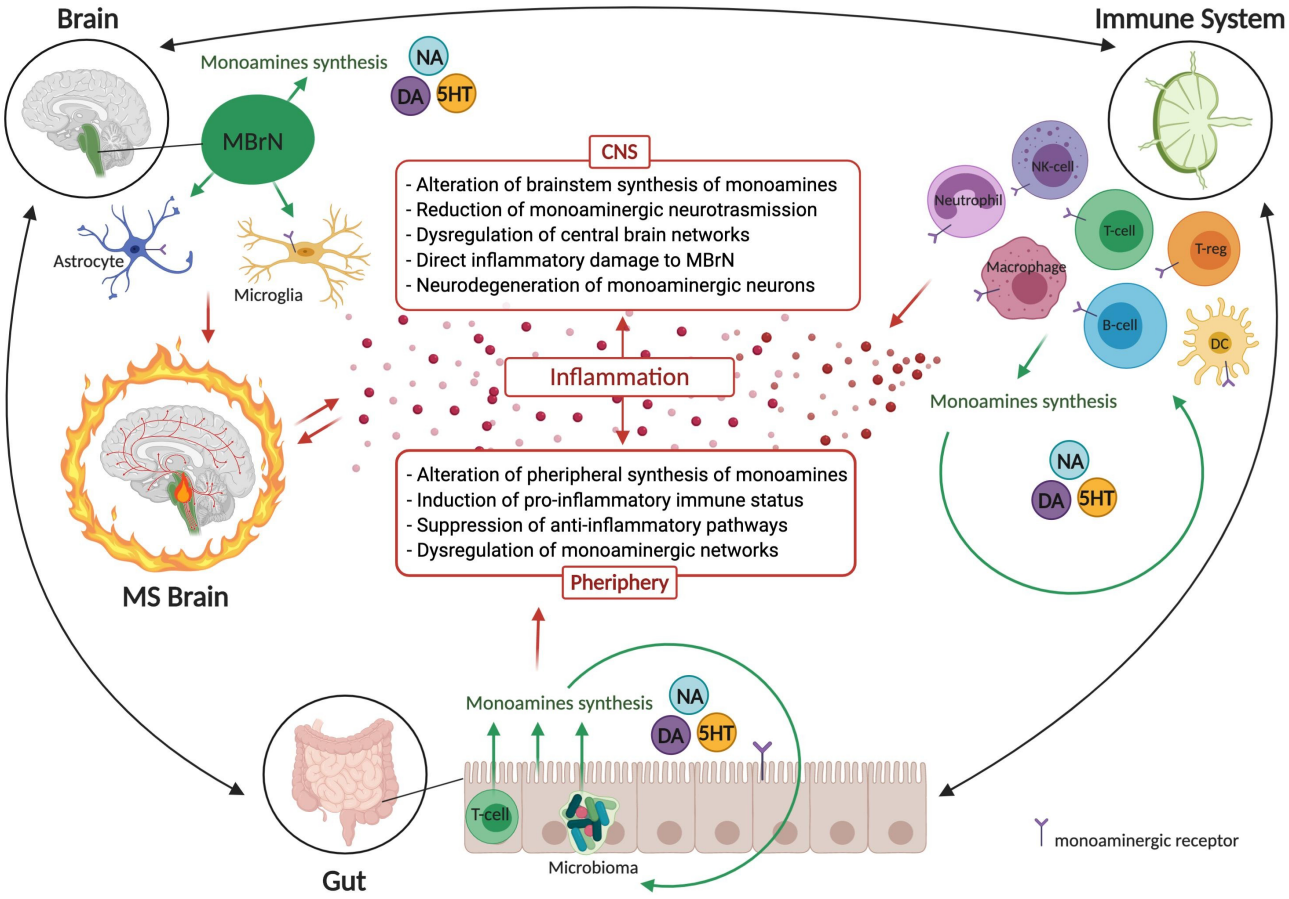
Figure Legends

Fig.1: Main dopaminergic (left), noradrenergic (centre) and serotoninergic (right) pathways within the brain.



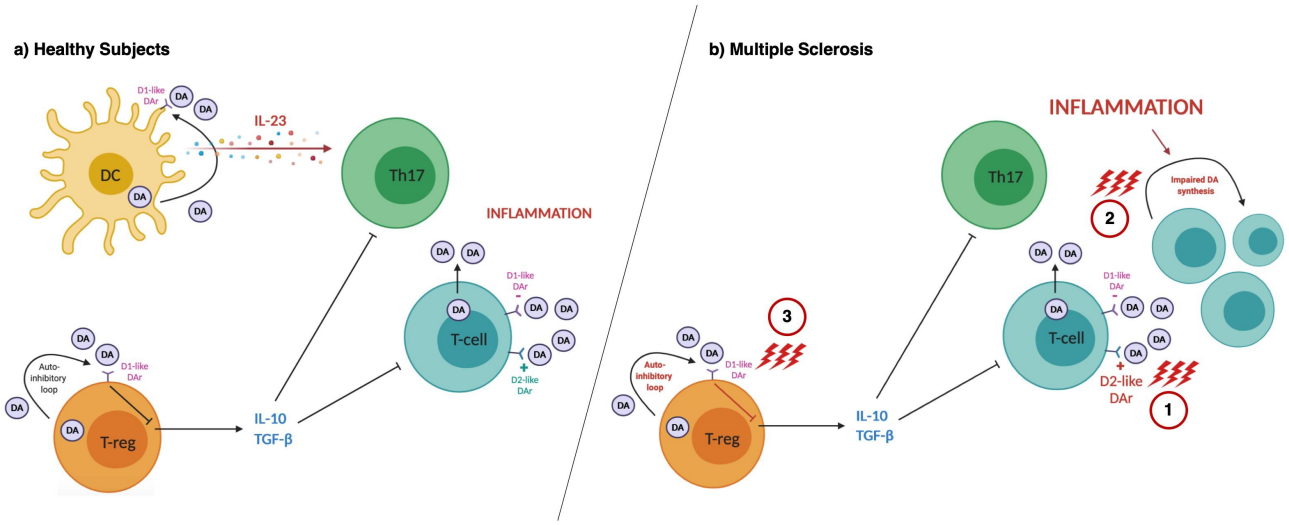
Left: dopaminergic neurons are concentrated in the midbrain in two distinct groups: 1) the *substantia nigra* (SN) that projects to the dorsal striatum (DS) via the nigrostriatal pathway, which is part of the basal ganglia loop; 2) the ventral tegmental area (VTA) that is the origin of the mesocorticolimbic pathway. The mesolimbic portion projects from the VTA to the ventral striatum (VS)/nucleus accumbens (N_{acc}) and limbic system (mainly hippocampus and amygdala). The mesocortical part connects the VTA with the prefrontal cortex and the entire neocortex. *Centre*: noradrenergic cells are mainly concentrated in the pontine nucleus *locus coeruleus* (LC) that projects diffusely to the entire cortex (mostly prefrontal cortex and cingulum) and the limbic system (mainly hippocampus and amygdala), thalamus/hypothalamus, cerebellum, and spinal cord. *Right*: serotoninergic neurons are concentrated in the brainstem in two functional groups: 1) the caudal raphe nuclei (raphe magnus, raphe obscurus, and raphe pallidus) project to the lower brainstem and spinal cord; 2) the rostral raphe nuclei (median raphe [MR] and dorsal raphe [DR]) project to the entire neocortex, as well as to the thalamus and basal ganglia. The rostral raphe nuclei send also a large contingent of fibers to the cerebellum and spinal cord. Figure was created with BioRender.com.

Fig.2 Monoamines mediate neuroimmune interaction and influence the pathogenesis and course of multiple sclerosis.



The monoamines dopamine (DA), noradrenaline (NA) and serotonin (5HT) are synthetized within the central nervous system (CNS) by monoaminergic brainstem nuclei (MBrN). In the CNS they interact with different resident cells (neurons, astrocytes, microglia) crucially regulating normal brain function through the widespread monoaminergic pathways. CNS-derived monoamines also travel to the periphery where they interact with the monoaminergic receptors expressed by cells of both the innate and adaptive immune systems, modulating inflammatory response. Vice versa, peripheral neurotrasmitters' levels are regulated by immune and gut cells that are able to synthetize monoamines and reciprocally regulate their functions. In MS, inflammation has different effect on the monoaminergic systems. Inflammation-induced reduction of monoamines' synthesis promotes a pro-inflammatory status in immune cells (both in the periphery and within the inflamed CNS), increasing cytokine release and inflammation in an auto-enhancing loop, and suppresses anti-inflammatory pathways. Moreover, brain structural damage alters neurotransmission through monoaminergic pathways, possibly leading to a functional reorganization of cortical brain networks. NK: natural-killer; DC: dendritic cells; T-reg: regulatory T cells. Figure was created with BioRender.com.

Fig.3 Examples of dopaminergic immune modulation in healthy subjects and multiple sclerosis (schematic).



a) In healthy subjects, DA interact with D1-like (mainly inhibitory) and D2-like receptors (mainly stimulatory) expressed by T-cells (the binding varies according to DA plasma concentration) and modulate cell activation processes. Dendritic cells (DCs) synthetize and accumulate dopamine (DA), which is released in response to antigen presentation and modulate CD4+ T cell responses in a paracrine manner (not shown); at certain concentrations, DCs-derived DA interacts with D1-like receptors (mainly D5) expressed by DCs, promoting IL-23 production and enhancing Th-17 responses. Moreover, T_{reg} synthetize and accumulate DA in substantial amounts. In response to yet unknown stimuli, T_{reg} release DA, which can interact with D1-like receptors (mainly D5) expressed by T_{reg} and this interaction reduces the expression of IL-10 and TGF-β, and weakens the T_{reg} suppressive activity exerted over effector CD4+-T cells. **b)** In multiple sclerosis dopaminergic dysregulation occur at several levels: (1) reduction of D1-like and increase of D2-like-receptors with consequent predominance of D2-like stimulation on immune cells; (2) impaired DA synthesis and release from immune cells with reduced effects on neighboring immune cells; (3) T_{reg} overexpression of D1-like-receptors and DA overproduction with enhanced activity of the auto-inhibitory loop. Figure was adapted from (Levite et al., 2017; Pacheco et al., 2014) and created with BioRender.com.

Fig.4 Summary of the putative pathophysiological mechanisms through which the reduction/dysregulation of monoamines contributes to fatigue in multiple sclerosis (MS).

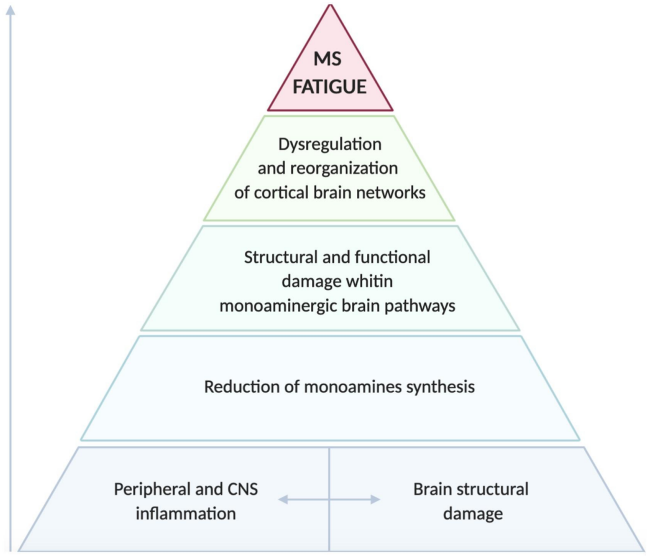


Figure was created with BioRender.com.

Fig.5 Summary of the putative pathophysiological mechanisms of depression in multiple sclerosis (MS).

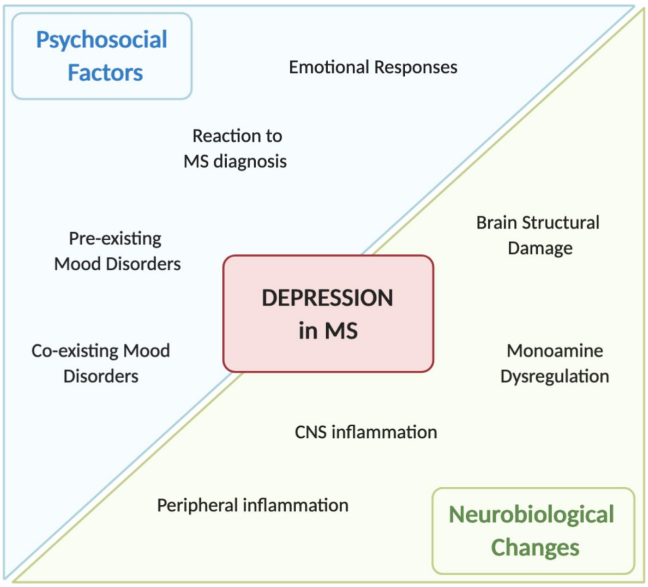


Figure was created with BioRender.com.