ACS Chemical Neuroscience

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# Exploring the Role of Neurotransmitters in Multiple Sclerosis: An Expanded Review

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Cite This: *ACS Chem. Neurosci.* 2023, 14, 527−553 Read Online

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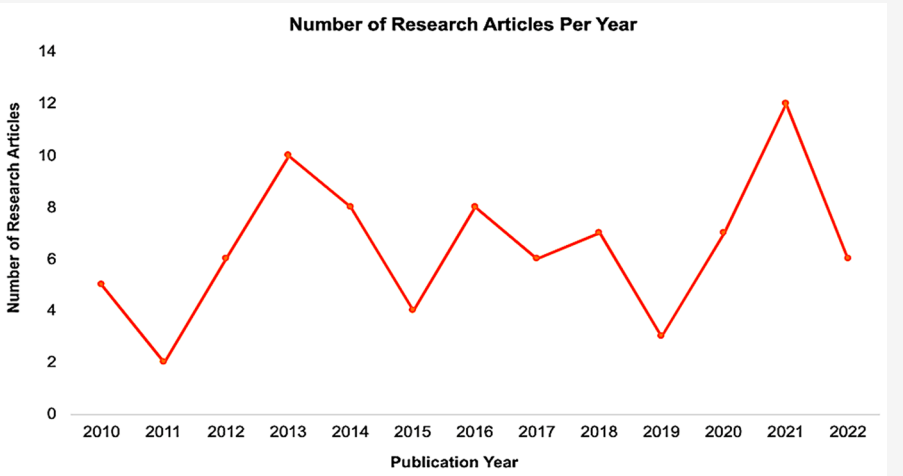


**ABSTRACT:** Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system (CNS). Although emerging evidence has shown that changes in neurotransmitter levels in the synaptic gap may contribute to the pathophysiology of MS, their specific role has not been elucidated yet. In this review, we aim to analyze preclinical and clinical evidence on the structural and functional changes in neurotransmitters in MS and critically discuss their potential role in MS pathophysiology. Preclinical studies have demonstrated that alterations in glutamate metabolism may contribute to MS pathophysiology, by causing excitotoxic neuronal damage. Dysregulated interaction between glutamate and GABA results in synaptic loss. The GABAergic system also plays an important role, by regulating the activity and plasticity of neural networks. Targeting GABAergic/glutamatergic transmission may be effective in fatigue and cognitive impairment in MS. Acetylcholine (ACh) and dopamine can also affect the T-mediated inflammatory responses, thereby being implicated in MS-related neuroinflammation. Also, melatonin might affect the frequency of relapses in MS, by regulating the sleep-wake cycle. Increased levels of nitric oxide in inflammatory lesions of MS patients may be also associated with axonal neuronal degeneration. Therefore, neurotransmitter imbalance may be critically implicated in MS pathophysiology, and future studies are needed for our deeper understanding of their role in MS.

**KEYWORDS:** *MS, glutamate, neurotransmitter receptors, ACh, nitric oxide*

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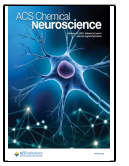
## 1. INTRODUCTION

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of the central nervous system (CNS). MS is more common in young adults and immune-mediated mechanisms are considered to play a key role in its pathogenesis. Neuropathologically, MS is characterized by demyelinating lesions in the brain and spinal cord.[1] It is estimated that 2.8 million people worldwide have MS.[2] Excessive secretion of glutamate neurotransmitter is observed in patients with progressive MS,[3] suggesting that neurotransmitters may be crucially implicated in its pathophysiology.

Changes in glutamate neurotransmission, as well as dysregulation of other neurotransmitters are implicated in several neurological conditions, including neurodegenerative diseases.[4] Detection of abnormal neurotransmitter levels may

Received: September 26, 2022
Accepted: January 12, 2023
Published: February 1, 2023



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https://doi.org/10.1021/acschemneuro.2c00589
*ACS Chem. Neurosci.* 2023, 14, 527−553

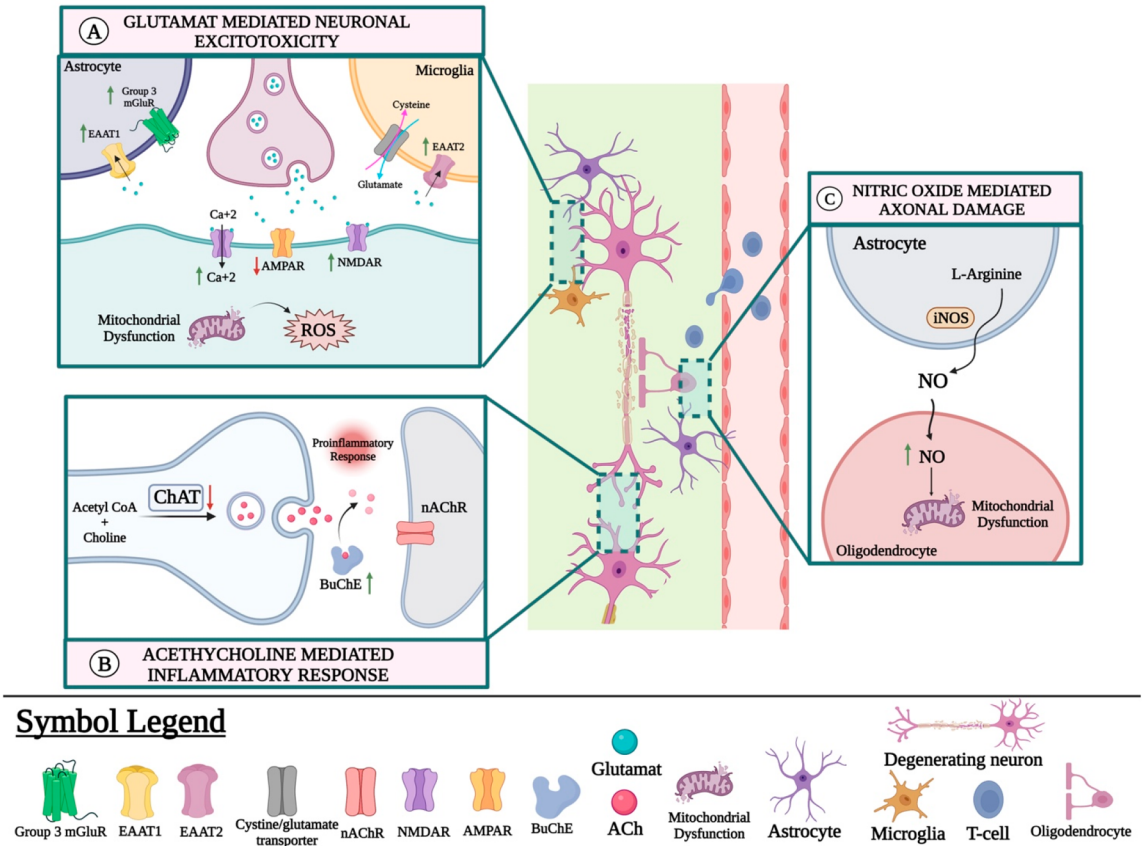


**Figure 1.** The main neurotransmitters implicated in the pathophysiology of Multiple Sclerosis. A: Glutamate concentrations are increased in acute lesions of MS patients.[13] Due to the altered NMDAR and AMPAR activities, increased intracellular $Ca^{2+}$ causes mitochondrial dysfunction and ROS production.[14] This process may damage nerve cells due to glutamate excitotoxicity. Increased expression of cysteine/glutamate antiporter causes excitotoxic damage in nerve cells.[15] In addition, the increased expression of EAAT1/2 is thought to be a compensatory response of glial cells to changing glutamate levels during neurodegeneration.[16] B: Decreased ChAT enzyme activity and ACh levels, as well as increased BuChE activity have been observed in MS patients.[17,18] These changes in ACh neurotransmission may result in proinflammatory responses. C: Increased levels of NO in MS patients may cause mitochondrial dysfunction in oligodendrocytes.[19] This leads to axonal neuronal damage. ACh = Acetylcholine, AMPAR = Alpha-amino-3-hydroxy-5-methylisoxazol4-propionic acid Receptors, BuChE = Butyrylcholinesterase, ChAT = Choline acetyltransferase, EAAT1/2 = Excitatory Amino Acid Transporters, iNOS = Inducible nitric oxide synthase, mGluR = Metabotropic glutamate Receptors, MS = Multiple Sclerosis, nAChR = Nicotinic acetylcholine receptor, NMDAR = N-methyl-D-aspartate Receptors, NO = Nitric Oxide, ROS = Reactive Oxygen Species.

aid in the diagnosis of brain diseases.[5] Imbalance in neurotransmitters is also critically involved in epilepsy;[6] however, the role of neurotransmitters' dysregulation has not been clarified in MS. In this regard, emerging preclinical evidence indicates that neurotransmitter levels may be altered in the synaptic gap in MS animal models, suggesting that this imbalance may be implicated in MS pathogenesis.

The activation of microglial cells, as well as the subsequent production and release of pro-inflammatory cytokines contribute to neuroinflammation. Microglial activation and pro-inflammatory responses in the brain can suppress the anti-inflammatory effects of norepinephrine (NE), a monoamine neurotransmitter.[7,8] In turn, inflammation in MS may alter neuronal transmission and synapse physiology, triggering the neurodegenerative process.[9] This process is associated with lower NE levels in the brain and spinal cord of animals in experimental autoimmune encephalomyelitis (EAE) models, which are widely used as MS preclinical models.[10] In addition, decreased gamma aminobutyric acid (GABA) levels have been observed in the hippocampus and sensorimotor cortex regions, which are closely related to the motor function of MS patients.[11,12]

Therefore, accumulating evidence shows that neurotransmitter levels may be altered in MS, suggesting their potential role in MS pathophysiology. This imbalance is rather complex and specific for different brain regions.

In this review, we aim to analyze preclinical and clinical evidence on the alterations in neurotransmitters in MS, and critically discuss their potential role in MS pathophysiology.

## 2. AMINO ACID NEUROTRANSMITTERS

**2.1. Glutamate.** Glutamate is the main excitatory neurotransmitter in the CNS.[21] Glutamate is majorly implicated in several functions in the brain, including neuronal differentiation, synaptic repair, plasticity, learning, and memory.[22] After its release into the synaptic cleft, glutamate exerts its functions through ionotropic and metabotropic receptors.

Glutamate receptors are classified in two groups: ionotropic glutamate receptors (iGluRs), which constitute ligand gated ion channels, and metabotropic glutamate receptors (mGluRs), which are G-protein-coupled receptors. According to their pharmacology and structural similarities, iGluRs encompass *N*-methyl-D-aspartate (NMDA) receptors, alpha-amino-3-hydroxy-5-methylisoxazol4-propionic acid (AMPA)


https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

receptors and 2 carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (Kainate) receptors. According to their pharmacology and intracellular signal transduction mechanisms, mGluRs are classified in three groups: group I (mGluR 1 and 5), group II (mGluR 2 and 3), and group III (mGluR 4, 6, 7 and 8).[23,24] mGluRs are expressed not only in neurons but also in glial cells, such as oligodendrocytes and astrocytes.

Neuronal death, accompanied by excessive activation of glutamate receptors, has been at least partially attributed to glutamate-mediated excitotoxicity.[25] Glutamate transporters in the synaptic gap prevent high glutamate concentration and excitotoxic damage in brain tissue.[26] There are 5 subtypes of glutamate transporters—called excitatory amino acid transporters (EAAT; EAAT 1−5)—according to their pharmacological properties, cellular localization and regulatory mechanisms.[27] In particular, astrocytic glutamate transporters EAAT 1 and EAAT 2 pick-up synaptic glutamate, thereby preventing excitotoxicity caused by glutamate accumulation in the synaptic gap.[28]

Glutamate can act not only as a neurotransmitter but also as an immunomodulator, since it can also bind to glutamate receptors such as GluR3[29] of the AMPA subtype, on the T cell surface.[30] Glutamate release, transport, receptor blockade, as well as modulation of glutamate metabolism play key roles in the investigation of novel therapeutic approaches in MS.[31] Glutamate concentrations have been shown to be increased in acute lesions of MS patients, while no such significant changes have been observed in chronic lesions (Figure 1.).[11] In addition, elevated glutamate levels have been identified in normal-appearing white matter.[32] This evidence suggests that altered glutamate metabolism may play a critical role in MS pathophysiology.

It has been demonstrated that neuronal glutamate concentration may be associated with cognitive function in MS patients. In this regard, glutamate levels in hippocampal, thalamic, and cingulate regions have been associated with visual−spatial memory in MS patients ($n$ = 80),[33] suggesting that glutamatergic system imbalance may affect memory function in MS. Another study demonstrated a slight reduction of cerebrospinal fluid (CSF) levels of L-glutamate (L-Glu) and inflammatory neurodegeneration in MS patients. Importantly, altered expression of L-Glu was associated with disability progression, oxidative stress, and inflammation in this study.[34] These results suggest that L-Glu in the CSF may be a promising biomarker of inflammatory neurodegeneration in MS.

The function of NMDA receptors in MS has been investigated in EAE models. In this regard, NMDA receptor levels have been shown to be increased, and this elevation depends on the astroglial activation-related glutamate release. In addition, *in vivo* blockade of NMDA receptors could ameliorate synaptic transmission defects and improve the disease course in EAE mice.[35] This evidence suggests that pharmacological targeting of NMDA receptor signaling may protect neurons against glutamate-mediated toxic effects in MS animal models.

Immuno-intervention methods may also aid in the investigation of the therapeutic effects of the pharmaceutical modulation of glutamate receptors. Targeting the GluN1 subunit of neuroendothelial NMDA receptors in EAE models with a monoclonal antibody (Glunomab (160 $\mu$g)) has been shown to inhibit inflammation and demyelination and also alleviate the severity of the disease.[36] This evidence suggests that strategies targeting the protease-regulated region of the endothelial NMDA receptor (GluN1) may be effective in MS. Another pharmacological study examined the effects of sildenafil (25 mg/kg) on the neuroinflammation and synaptic plasticity pathways in an EAE model. This study showed that sildenafil decreased the expression of NMDA and AMPA receptors,[37] highlighting the promising potential of this pharmaceutical agent to prevent glutamate-mediated excitotoxicity in MS.

In mouse models of MS, it has been demonstrated that hippocampal demyelination may be associated with increased miR-124 and decreased AMPA receptors expression.[38] Furthermore, the AMPA receptor-mediated regulation of glutamate signaling was related with worse memory function in MS preclinical models. Immunomodulatory treatments of MS in regard to glutamate-mediated excitotoxicity have also been investigated *in vitro* and *in vivo*. Monomethyl fumarate, a metabolite of dimethyl fumarate (10 nM−1 mM), has been shown to significantly reduce the release of glutamate from pathogenic Th-17 lymphocytes,[39] suggesting that these agents—currently used in the treatment of MS—may possibly prevent neuronal death by regulating glutamate-mediated excitotoxicity. Also, matrine (200 mg/kg), a quinolizidine alkaloid, has been associated with reduced NMDA and AMPA receptor levels, as well as increased glutamate transporter expression in EAE rat models.[40] Moreover, matrine therapy might also regulate the expression of glutamate-related molecules, thereby potentially exerting a neuroprotective effect in EAE.

The expression pattern changes of the Group III metabotropic glutamate receptors mGluR4 and mGluR8 has also been investigated in preclinical models of MS. In this context, the expression of Group III mGluRs has been shown to be upregulated in microglia and astrocytes during the demyelinating process in MS,[41] suggesting that group III mGluR upregulation may play an important role in neuroinflammatory responses. In this regard, a pharmacologic study in EAE mice showed that cinnabarinic acid—an endogenous mGluR 4 agonist-mediated the activation of mGlu4 against neuroinflammation.[42] Cinnabarinic acid-mediated mGlu4 activation could also suppress Th17 cells and increase regulatory T cells, suggesting that this agent may potentially limit neuroinflammation by mediating immune tolerance. In MS mouse models and human neurons, it has been also shown that neuronal mGluR 8 activations may be associated with neurodegeneration in CNS inflammatory conditions. Therefore, it has been proposed that mGluR 8 activation may exert neuroprotective effects in MS, by inhibiting glutamate-induced excitotoxic calcium accumulation in neurons.[43] Hence, this agent holds a promising potential as a therapeutic target to prevent inflammation-induced neurodegeneration in MS (Table 1).

It has been indicated that the expression of glutamate transporters, EAAT1 and EAAT2, is increased in optic nerve samples of MS patients at mRNA and protein levels.[4] It can be speculated that their increased expression may be a regulatory response of glial cells during neurodegeneration in MS (Figure 1).

In conclusion, glutamate metabolism imbalance may significantly contribute to MS pathophysiology by causing excitotoxic neuronal damage. In this context, the regulation of the expression and function of glutamate receptors and glutamate transporters is crucial, and these pathways may


https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

Table 1. Summaries of Findings Reporting the Role of Glutamate in MS[a]

| S.N. | study type | MS model | drug and dose | observations | remarks | refs |
|---|---|---|---|---|---|---|
| 1 | *In vivo* | Animal model of EAE (600 μg) induced MS in mice ($n$ = 24) | Glunomab (160 μg) | Targeting neuroendothelial NMDA receptors in the EAE model using a monoclonal antibody (Glunomab (160 μg)) alleviated the severity of the disease. | Strategies targeting the protease-regulated region of the endothelial NMDA receptor (GluN1) may be effective in MS. | 36 |
| 2 | *In vivo* | Animal model of EAE (200 μg) induced MS in mice ($n$ = 20) and control group ($n$ = 10) | Sildenafil (25 mg/kg) | Sildenafil (25 mg/kg) affected neuroinflammation and synaptic plasticity pathways by decreasing the expression of NMDA and AMPA receptors. | Sildenafil may prevent glutamate-mediated excitotoxicity in the EAE model. | 37 |
| 3 | *In vivo* | Animal model of EAE (200 μL) induced MS in mice ($n$ = 54) | Cinnabarinic acid (0.1−10 mg/kg/day) | Cinnabarinic acid (0.1−10 mg/kg/day) -mediated activation of mGluR 4 was effective in suppressing Th17 cells and increasing regulatory T cells. | Cinnabarinic acid may represent a possible "bridge" between immune tolerance aiming at limiting neuroinflammation. | 42 |
| 4 | *In vivo* | Animal model of EAE (200 μg) induced MS in mice | - | mGluR 8 activation in human neurons and preclinical MS mouse model had a protective effect on neurons by limiting glutamate-induced excitotoxic calcium deposition in neurons. | mGluR 8 activation may prevent inflammation-induced neurodegeneration in MS. | 43 |
| 5 | *In vivo* | Animal model of EAE (5.5 mg/mL) induced MS in rats ($n$ = 198) | Amantadine (100 mg/kg/day) and memantine (60 mg/kg/day) | The NMDA receptor antagonists amantadine (100 mg/kg/day) and memantine (60 mg/kg/day) reduced the expression of pro-inflammatory cytokines in the brain of EAE model rats. | NMDA receptor antagonists may reduce the expression of proinflammatory cytokines through their effects on glutamate metabolism. | 206 |
| 6 | *In vivo* | Animal model of MS TN-XXL transgenic mice ($n$ = 13) and control group ($n$ = 3) | Dimethyl fumarate (10 nM−1 mM) | Monomethyl fumarate, a metabolite of dimethyl fumarate (10 nM−1 mM), significantly reduced glutamate release from pathogenic Th-17 lymphocytes. | These agents used in the treatment of MS may prevent neuronal death by regulating glutamate-mediated excitotoxicity. | 39 |

[a]AMPA = α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, EAE = Experimental autoimmune encephalomyelitis, mGluR = Metabotropic glutamate receptor, MS = Multiple sclerosis, NMDA = *N*-methyl-D-aspartate

represent novel therapeutic targets in MS, especially for compensating for toxic glutamate levels. The increased protein expression of EAATI-2 and the mechanism of glutamate-mediated neuronal excitotoxicity are shown in Figure 1A.

**2.2. GABA.** GABA, the main inhibitory neurotransmitter in the CNS, is synthesized from glutamate by glutamate-decarboxylase (GAD). GABA plays an important role in cortical development, and alterations in GABA levels are considered to be critical for neurodevelopmental disorders. GABA participates in the myelination process, which is tightly controlled by neuron and glia communication.[44−46]

GABA is released by exocytosis or reverse transport by GABA transporters. This neurotransmitter also stimulates ligand-gated ionotropic $GABA_A$ receptors ($GABA_AR$) and G-protein coupled $GABA_B$ receptors ($GABA_BR$) in the plasma membrane of cells. The extracellular concentration of GABA decreases as it moves away from the region where it is released or when GABA transporters transfer it back to cells for reuse.[47] GABA transporters regulate extracellular GABA concentration during synaptic events in neurons and astrocytes. The varying degrees of the density, distribution, and diffusion rate modulate the strength of individual synaptic contacts and the activity of entire neuronal networks.[48] Inhibition of these synaptic contacts is largely mediated by GABA signaling. The rapid inhibitory effects of GABA are accompanied by the activation of $GABA_AR$s in the brain.

Eighteen $GABA_AR$ subunits have been identified. Based on sequence homology, they are divided into seven subunit classes with one or more members: $\alpha$ (1−6), $\beta$ (1−3), $\gamma$ (1−3), $\delta$, $\varepsilon$ (1−3), $\theta$, and $\pi$. $GABA_AR$ is usually composed of two $\alpha$ subunits, two $\beta$ subunits, and one $\gamma$ (or a $\delta$) subunit. $GABA_ARs$ composed of different subunits display different physiological and pharmacological properties. $GABA_ARs$ expression in the brain differs between subcellular regions. For example, receptors consisting of $\alpha$1, $\alpha$2, $\alpha$3, or $\alpha$5 subunits together with $\beta$ and $\gamma$ subunits are sensitive to benzodiazepines, are mostly synaptic, and mediate phasic inhibition in the brain.[49−52] Unlike $GABA_ARs$, $GABA_BRs$ have a limited number of isoforms; they have only two subunits and are associated with functional dimeric receptors.[53] Drugs acting on GABA receptors as antispasticity agents are widely used in the clinical practice for the treatment of MS (baclofen, a $GABA_B$ agonist).[54]

GABAergic neurons play a crucial role during brain development in mammals. It has been proposed that altered GABA neurotransmission may play a significant mechanistic role in neuroinflammation and neurodegeneration in MS. Therefore, a growing body of evidence has investigated the potential immunomodulatory and therapeutic effects of GABAergic drugs in MS.[55]

In this regard, a clinical study has demonstrated enhanced GABAergic and GAD67 immunoreactivity in neurons and astrocytes in cases of hippocampal inflammation.[56] The increased GABA levels may contribute to cognitive impairment in MS, by impairing hippocampal function. Reduced GABA levels in left hippocampal regions have also been associated with cognitive impairment in patients with relapsing-remitting multiple sclerosis (RRMS) (Figure 2).[57] This evidence suggests that dysregulation of the GABAergic system may be implicated in RRMS pathophysiology and highlight a potential link between cognitive impairment and disruption of regional GABA levels in patients with RRMS.


https://doi.org/10.1021/acschemneuro.2c00589
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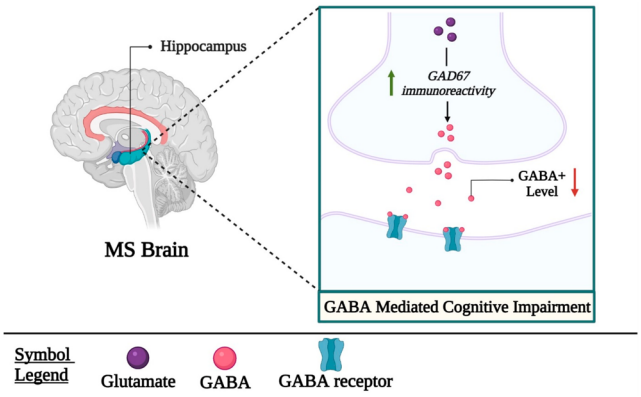


**Figure 2.** GABA-mediated cognitive impairment. GAD67 immunoreactivity is increased in hippocampal neurons of MS patients in a post-mortem study.[56] This elevation may contribute to impaired cognition in MS by impairing hippocampal function. In addition, the decrease in $GABA^{+}$ in the left hippocampus in RRMS patients has been associated with worse cognitive function.[57] GABA = Gamma-aminobutyric acid, MS = Multiple Sclerosis, RRMS = relapsing-remitting multiple sclerosis.

Cognitive impairment and memory deficits in particular are common manifestations in MS patients. Reduced GABA levels have been associated with physical disability in MS in one study. It is considered that altered GABA neurotransmission may be a marker of neurodegeneration, including cases of progressive MS.[20] However, since GABA can also mediate neuroprotection, the specific effects of GABA in the MS pathophysiology are obscure and should be further investigated. Altered GABA and glutamate levels in the brain have been associated with fatigue in MS,[58] suggesting that dysregulation of GABAergic/glutamatergic neurotransmission might contribute to MS pathophysiology. Also, $GABA_AR$ inhibition by biculline (0.025, 0.05 $\mu$g/2 $\mu$L/animal) or muscimol (0.1, 0.2 $\mu$g/2 $\mu$L/animal) could improve memory impairment and demyelination in the hippocampus. Hence, modulation of GABA neurotransmission has been proposed as a potential target for MS neuroprotection.[59] Our deeper understanding of the mechanisms underlying cognitive impairment in MS and related hippocampal pathology and dynamics may further aid in the development of novel effective GABA-related therapeutic approaches in MS-related cognitive impairment.

The inhibitory effect of GABA on glutamate-mediated excitotoxicity has been investigated as a new target in the treatment of MS. Focus has been especially placed on selective $GABA_AR$ agonists or positive allosteric modulators (diazepam and phenobarbitone sodium), as well as $GABA_A$ level enhancers (sodium valproate).[60] In particular, the $GABA_B$ agonist baclofen has been demonstrated to modulate the inflammatory signaling of Toll-like receptors (TLR) in glia and immune cells, which is critically implicated in MS progression. More specifically, baclofen (10, 30, and 100 $\mu$M) could regulate TLR3 and TLR4 signaling in glia and immune cells,[61] suggesting that this agent should be further investigated as a therapeutic approach in MS. Homotaurine is an amino acid found in seaweed that interferes with the amyloid peptide's ability to form fibrils. This compound is a $GABA_AR$-specific agonist and has better pharmacokinetics than GABA. Homotaurine therapy has a good safety profile, but it is rather unable to slow cognitive decline.[62−65] In MS, homotaurine treatment (0.25 mg/mL) has been shown to limit the T cell epitope spread in the CNS,[66] suggesting that this agent may represent a promising candidate to help limit T cell autoreactivity, as well as treat MS and other inflammatory disorders of the CNS. Homotaurine can penetrate blood−brain barrier (BBB) and can ameliorate MS in mouse models. This evidence also highlights the effects of $GABA_ARs$ on lymphocytes,[64] implying that homotaurine, may have wider applications. Furthermore, it has been shown that GABA transporter-2 (GAT-2), which has been associated with neuroinflammation in MS, may be regulated by ganaxolone (GNX). GABAergic transport is altered through the induction of GAT-2 during neuroinflammation.[67] The GNX-mediated changes in GABA transporter further suggest GABAergic pathways as therapeutic targets in MS and other neuroinflammatory diseases (Table 2).

## 3. MONOAMINE NEUROTRANSMITTERS/ALKALOID NEUROTRANSMITTERS

**3.1. Dopamine.** Dopamine is a neurotransmitter involved in the regulation of cognition, emotion, and endocrine system in the CNS.[68] Dopamine receptors can be found in the CNS and immune system.[69] These receptors constitute a family of G protein-coupled receptors with five subtypes. The five subtypes are divided into two groups, D1-like and D2-like.

D1-like receptors include dopamine D1 receptor (D1R) and dopamine D5 receptor (D5R). D2-like receptors include dopamine D2 receptor (D2R), dopamine D3 receptor (D3R), and dopamine D4 receptor (D4R).[70,71] In order to understand the mechanism of actions of dopaminergic agents, it is important to understand the D1R- and D2R-signaling.[72]

D1R targeting plays an important role in psychiatric and neurological disorders.[73] In this regard, phenyl benzazepine derivatives, acting as selective D1R ligands, are crucial pharmacological tools. These chemicals include SKF compounds, which act as D1R agonists, and the D1R antagonist SCH23390.[74,75]

D2R is the most commonly targeted dopamine receptor for pharmacological purposes.[76,77] Haloperidol, an antagonist of the D2-like receptor, inhibits the differentiation of cluster of differentiation antigen 4 (CD4+) T cells in the brain into the dendritic cell-derived T helper cell 1 (Th1) phenotype, thereby affecting neuroimmune functions.[78] On the contrary, D2-like receptor antagonists induce human monocyte-derived dendritic cell-mediated Th2 phenotype differentiation. In addition, these antagonists suppress the secretion of inflammatory cytokines.[79] Risperidone, one of the D2R antagonists, can modulate the immune function of dendritic cells, by stimulating the production of proinflammatory cytokines interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-$\alpha$).[80] Therefore, this evidence suggests that D1R and D2R may be critically implicated in inflammatory processes in the CNS.

The dopamine transporter (DAT) transfers extracellular dopamine into the intracellular space, thereby contributing to the regulation of dopamine neurotransmission.[81] This transporter is a member of the neurotransmitter sodium symporters. These symporters are responsible for the termination of neurotransmission via $Na^{+}$-directed reuptake of the neurotransmitter from the extracellular space.[82] DAT contains 12 transmembrane helices, and these helices have N and C terminals extending toward the cytoplasm.[83,84] It has been found that deletion of the DAT gene may result in impaired


https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

**Table 2. Summaries of Findings Reporting the Role of GABA in MS[a]**

| S.N. | study type | MS model | drug and dose | observations | remarks | ref |
|---|---|---|---|---|---|---|
| 1 | *In vivo* | Animal model of LPC (1%) induced MS in rats | Bicuculline (0.025, 0.05 $\mu$g/2 $\mu$L/animal) and Muscimol (0.1, 0.2 $\mu$g/2 $\mu$L/animal) | $GABA_AR$ inhibition was found to improve memory impairment and local demyelination of the hippocampus. | Modulation of GABA neurotransmission may be a potential target for MS neuroprotection. | 59 |
| 2 | *In vivo* | Animal model of EAE (70 mg per kg body weight) induced MS in rats | Diazepam, (2.0 mg/kg), phenobarbitone sodium, (30.0 mg/kg), and sodium valproate (200.0 mg/kg). | Effects of selective $GABA_AR$ agonists or positive allosteric modulators and $GABA_A$ level enhancer in EAE | Sodium valproate may serve as a potential drug for the treatment of MS. | 60 |
| 3 | *In vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | Homotaurine (0.25 mg/mL) | Homotaurine is specific to $GABA_AR$, safe, and blood-brain barrier permeable, and ameliorates MS in mouse models. | These effects highlight the potential effects of $GABA_ARs$ on lymphocytes. | 64 |
| 4 | *In vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | Homotaurine (0.25 mg/mL) | Homotaurine can limit T cell autoreactivity in the CNS and ameliorate MS. | Homotaurine treatment has the ability to limit epitope spread within the CNS. | 66 |
| 5 | *Human Study* | Patients with MS ($n$ = 15) and control group ($n$ = 9) | - | GAD67 immunoreactivity is increased in neurons and astrocytes due to hippocampal inflammation. | This upregulation may result in increased GABA levels, which may contribute to impaired cognition in MS by impairing hippocampal function. | 56 |
| 6 | *Human Study* | Patients with RRMS ($n$ = 28) and control group ($n$ = 26) | - | Decreased GABA levels are associated with cognitive impairment in MS (RRMS) patients. | GABA levels associated with cognitive disorders in patients with RRMS have been found to be lower in the left hippocampus regions. | 57 |
| 7 | *Human Study* | Patients with SPMS ($n$ = 30) and control group ($n$ = 17) | - | Reduction of GABA concentration is associated with physical disability in MS. | Altered GABA neurotransmission may be a marker of neurodegeneration. | 20 |
| 8 | *Human Study* | Patients with RRMS ($n$ = 24) and control group ($n$ = 16) | - | Altered GABA and glutamate levels in the brain may be associated with central fatigue in MS. | Dysregulation of GABAergic/glutamatergic neurotransmission may underlie the pathophysiological mechanism of central fatigue in MS. | 58 |
| 9 | *Human Study* | Patients with MS ($n$ = 12), control group ($n$ = 6) and animal model of EAE (50 $\mu$g) induced MS in mice | GNX-regulated GABA transporter [low dose (15 mg/kg) and high dose (50 mg/kg)] | GABAergic transport is altered through the induction of GAT-2 during neuroinflammation. | The GNX-regulated GABA transporter highlights the therapeutic potential of GABAergic pathway targeting in neuroinflammatory diseases. | 67 |
| 10 | *Human Study* | Patients with RRMS ($n$ = 8) and control group ($n$ = 16) | - | In glia and immune cells, baclofen (10, 30, and 100 $\mu$M) can regulate TLR3 and TLR4 signaling. | The role of baclofen in the treatment of MS as a therapeutic approach could be explored in more detail. | 61 |

[a]EAE = Experimental autoimmune encephalomyelitis, GABA = gamma aminobutyric acid, GABAAR = GABAA receptors, GAD67 = glutamate decarboxylase-67, GAT-2 = gamma aminobutyric acid transporter-2, GNX = ganaxolone, LPC = Lysophosphatidylcholine, MS = Multiple Sclerosis, CNS = central nervous system, RRMS = Relapsing-remitting MS, TLR3 = Toll-like receptors-3, TLR4 = Toll-like receptors-4.



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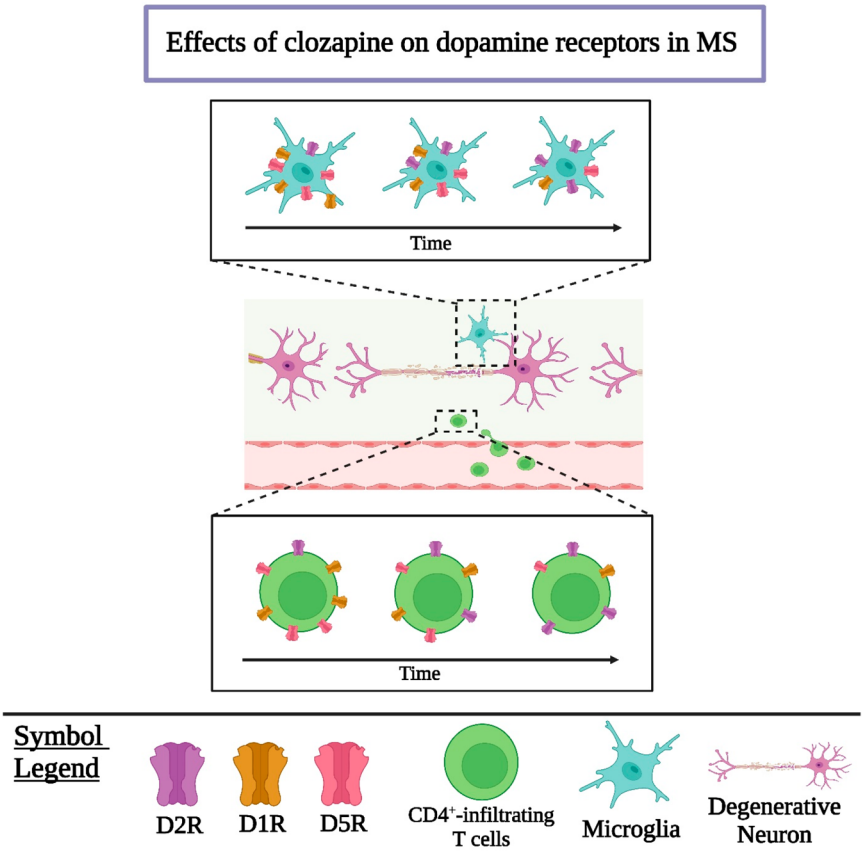


**Figure 3.** Effects of clozapine on dopamine receptors in MS. Clozapine does not affect dopamine levels. However, as a result of clozapine injection, downregulation of dopamine D1 and D5 receptors and up-regulation of dopamine D2 receptor were observed in microglia and $CD4^+$-infiltrating T cells during EAE.[94] D1R = Dopamin D1 receptor, D2R = Dopamin D2 receptor, D5R = Dopamin D5 receptor, MS = Multiple Sclerosis.

cellular immune responses.[85] Hence, DAT has been hypothesized to be involved in immune-mediated mechanisms.

Urea-like suppressors of DAT have also been studied in EAE. Urea-like DAT suppressors produced an anti-inflammatory effect in MS,[86] suggesting that DAT-suppressing agents can be an effective treatment for EAE. Dopamine can promote the glucocortoid-resistant interleukin-17 (IL-17)-producing T cells (Th17) phenotype in MS.[87] One potential underlying mechanism may be the ability of CD4+ T cells ($1 \times 10^6$ $mL^{-1}$) to trigger IL-6 production. Functional analyses of dopaminergic signaling has shown that dopaminergic system disruption can result in myelin deficits in zebrafish,[88] suggesting that impaired dopaminergic signaling may play a role in the process of demyelination. Another study has demonstrated that mood changes are associated with impaired dopaminergic neurotransmission in EAE mice models, accompanied by higher expression of interleukin-1beta (IL-1$\beta$) in the striatum of the animal models.[89] This evidence suggests that IL-1$\beta$-induced dopaminergic dysfunction signaling may play a crucial role in the pathophysiology of mood disturbances in MS. Given this evidence, dopaminergic receptors have been used as potential pharmaceutical targets in MS-related mood disorders. In particular, pramipexole (PPX) (1 mg/kg), a D2/D3 receptor-preferring agonist, has been shown to reduce depressive-like behavior in EAE animal models.[90] Therefore, D2R might be implicated in the neuroinflammatory responses in EAE-related mood disorders. Another study has demonstrated that isosibiricin, a natural coumarin compound, could inhibit microglial activation by targeting D1/D2 receptor-dependent nucleotide binding domain like receptor protein 3 (NLRP3)/caspase-1 inflammasome pathway.[91] Treatment with SCH 23390 (1 $\mu$M) or sultopride (1 $\mu$M), which are dopamine D1/D2 receptor antagonists, could also stop IL-1$\beta$ and caspase 1 cleavages in this study, highlighting the promising role of targeting D1/D2 receptors in inhibiting neuroinflammation. Moreover, PPX (0.1 and 1 mg/kg), could inhibit the development of EAE in mice, and also (1 mg/kg) block the neuroinflammatory responses and demyelination in the spinal cord.[92] Hence, PPX may be useful in MS-related motor symptoms and disability caused by spinal cord lesions. A clinical study among MS patients and healthy controls showed that dopamine could suppress the production of IL-17 by peripheral blood mononuclear cells (PBMC) in both groups, and sulpride, an antagonist of D2-like receptors, was able to inhibit this reduction.[93] This evidence shows that dopamine may have an anti-inflammatory effect in MS patients too. It has also been indicated that dopaminergic activation may result in B-cells migration into the CNS, and D3R in B cells might regulate their anti-inflammatory properties in the CNS.[94] Hence, B cell D3R signaling may significantly affect autoimmunity in the CNS. Furthermore, clozapine administration (10 mg/kg) has been associated with remarkable up-regulation of D2R and downregulation of D1R and D5R on microglia, as well as inhibition of CD4+-infiltrating T cells in EAE models (Figure 3).[95] The dual function of D5R signaling, which supports the suppressive activity of regulatory T-cells (Treg) and improves Th17-mediated immunity, has also been investigated in EAE. In this regard, the glucocorticoid-induced


https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

**Table 3. Summaries of Findings Reporting the Role of Dopamine in MS[a]**

| S.N. | study types | MS model | drug and dose | observations | remarks | refs |
|---|---|---|---|---|---|---|
| 1 | *In vitro, in vivo* | Balb/c mice | SCH 23390 (1 $\mu$M) or sultoprid (1 $\mu$M) | Treatment with the dopamine D1/D2 receptor antagonists SCH 23390 (1 $\mu$M) or sultoprid (1 $\mu$M) can regulate caspase-1 and IL-1$\beta$ cleavages. | Therapeutic approaches against neuroinflammation can be developed by targeting dopamine D1/D2 receptors. | 91 |
| 2 | *In vitro, in vivo* | Animal model of EAE (50 $\mu$g) induced MS in mice | - | The expression of GITR correlates with the anti-inflammatory effect of D5R signaling in Tregs. | CD4+ T-cell-directed responses that improve symptoms in the late stages of EAE may have a complex role in D5R signaling. | 96 |
| 3 | *In vivo* | Animal model of EAE induced MS in mice | Compound 5 (15.0 nM) | Urea-like DAT suppressors (15.0 nM) produce an anti-inflammatory effect in MS. | DAT-suppressing agents can be an effective treatment for EAE. | 86 |
| 4 | *In vivo* | Zebrafish model | - | Dopaminergic system dysfunction causes loss of myelin sheath. | Dopaminergic signaling may be implicated in the formation of myelin sheath-related diseases. | 88 |
| 5 | *In vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | IL-1ra (150 ng/day) | Depressive behaviors and changes in the dopaminergic system are associated in EAE mice. | IL-1$\beta$-induced dopaminergic signaling dysfunction may cause mood disorders in MS patients. | 89 |
| 6 | *In vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | PPX (1 mg/kg) | D2/D3 receptor agonist PPX (1 mg/kg) improves depressive-like behaviors caused by EAE. | D2R may be implicated in the immune system dysregulation in MS. | 90 |
| 7 | *In vivo* | Animal model of EAE (200 $\mu$L) induced MS in mice | PPX (1 mg/kg) | PPX (1 mg/kg) can inhibit oxidative stress caused by EAE in the spinal cord and striatum. | PPX may be beneficial in treating motor symptoms caused by EAE. | 92 |
| 8 | *In vivo* | Animal model of EAE (50 $\mu$g) induced MS in mice | - | D3R increased the anti-inflammatory B cells in the CNS. | D3R signaling in B cells may act as a regulator of CNS autoimmunity. | 94 |
| 9 | *In vivo* | Animal model of EAE (50 $\mu$g) induced MS in mice | Pertussis toxin (200 ng/mouse) | D1R and D5R were down-regulated and D2R was up-regulated on microglia and CD4+-infiltrating T cells in EAE. | Neuroinflammation may alter the distribution of clozapine. | 95 |
| 10 | *Human study* | Patients with RRMS ($n$ = 43) and control group ($n$ = 20) | Sulpiride ($10^{-5}$ M) | Sulpiride ($10^{-5}$ M) inhibited the effect of dopamine. | Dopamine may display an anti-inflammatory role in MS. | 93 |
| 11 | *Human study* | Patients with RRMS ($n$ = 34) and control group ($n$ = 23) | SCH23390 and sulpiride ($10^{-5}$ M) and quinpirole ($10^{-7}$ M) | D2-like receptors blocked by sulpiride resulted in reduced synthesis of IL-17, IL-21 and granulocyte macrophage colony stimulating factor (GM-CSF) in MS patients. | Dopamine may exert a suppressive effect on Th17 cells of MS patients. | 97 |
| 12 | *Human study, in vitro* | Patients with RRMS ($n$ = 20) | - | Dopamine potentiated the Th17 phenotype with glucocortoid resistance in MS. | Dopamine may have the capability to induce IL-6 production by $CD4^+$ T cells. | 87 |

[a]CNS = Central nervous system, D1R = Dopamine D1 receptor, D2R = Dopamine D2 receptor, D3R = Dopamine D3 receptor, D5R = Dopamine D5 receptor, DAT = Dopamine transporter, EAE = Experimental autoimmune encephalomyelitis, GITR = Glucocorticoid-induced tumor necrosis factor receptorrelated protein, IL-1$\beta$ = Interleukin 1 beta, IL-6 = Interleukin 6, IL-1ra interleukin 1 receptor antagonist, MS = Multiple sclerosis, PPX = Pramipexole, RRMS = Relapsing–remitting multiple sclerosis, Th17 = T-helper-17, Treg = Regulatory T-Cells.



https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

**Table 4. Summaries of Findings Reporting the Role of Norepinephrine in MS[a]**

| S.N. | study type | MS model | drug and dose | observations | remarks | ref |
|---|---|---|---|---|---|---|
| 1 | *In vitro, in vivo* | Animal model of EAE (100 $\mu$L) induced MS in rats | - | NE, which acts in neurocrine and autocrine/paracrine ways, has been shown to affect microglia in EAE rat models and promote $\beta$-AR-mediated neuro-inflammation. | NE can affect microglia during EAE and promotes $\beta$-AR-mediated neuroinflammation. | 108 |
| 2 | *In vitro, In vivo* | Animal model of EAE (100 $\mu$g) induced MS in mice | Vindeburnol (20 mg/kg) | Vindeburnol exerts a protective role in the LC of the MS mouse model. | Vindeburnol decreased astrocyte activation in the LC, increased the expression of several genes involved in LC survival and maturation, as well as increased NE levels in the spinal cord. | 105 |
| 3 | *In vivo* | Animal model of cuprizone (400 mg·kg$^{-1}$) induced MS in mice | Venlafaxine (60 mg·kg$^{-1}$) | Investigation of the antagonistic effect of venlafaxine on TRPA1 calcium channels. | Venlafaxine may provide some protection against cuprizone-induced demyelination effects. | 104 |
| 4 | *In vivo* | Animal model of EAE (100 $\mu$g) induced MS in mice | - | Spinal cord COMT mRNA levels are not increase during EAE and are found to be high in the frontal cortex of MS patients. | COMT inhibitors may benefit MS patients. | 106 |
| 5 | *Human Study* | Patients with RRMS ($n$ = 11) and control group ($n$ = 12) | - | NET presence is detected in subcortical brain regions such as thalamus and amygdala in patients with MS. | NET may be a promising candidate against neuroinflammation in neuropsychiatric symptoms such as cognitive impairment. | 109 |
| 6 | *Human Study* | Patients with MS ($n$ = 75) | Duloxetine (maximum dose = 60 mg/day) | Duloxetine, a serotonin-NE dual reuptake inhibitor, is effective in the treatment of depression in MS patients. | Duloxetine is effective, well tolerated and safe in reducing both depression and fatigue in MS patients. | 102 |
| 7 | *Human Study* | Patients with MS ($n$ = 15) | - | A multidimensional, high-impact, short rehabilitation program is used among MS patients; quality of life, beta-endorphin and noradrenaline levels were increased in patients participating in the program. | NE may be a biomarker for clinical interventions in MS. | 107 |

[a]$\alpha$-1AR = $\alpha$-1 adrenergic receptors, $\beta$-AR = $\beta$-adrenergic receptors, COMT = catechol-O-methyltransferase, EAE = Experimental autoimmune encephalomyelitis, ELT = early life trauma, LC = locus coeruleus, MS = multiple sclerosis, NE = norepinephrine; NET = norepinephrine transporter, RRMS = Relapsing-remitting MS, TRPA1 = TRPA1, transient receptor potential ankyrin 1.



https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

tumor necrosis factor receptor-related protein, a steroid hormone, was found to be expressed more frequently in response to Treg D5R signaling, which has anti-inflammatory effects.[96] Accordingly, D5R signaling may be involved in CD4+ T-cell-mediated responses in EAE, which enhance effector T-cell-mediated early inflammation. The relationship between dopamine regulation of Th17 cells in MS patients and D2-like dopamine receptors has also been investigated. Blocking of D2-like receptors by sulpiride caused decreased synthesis of IL-17, IL-21 and granulocyte macrophage colony stimulating factor (GM-CSF) in MS patients.[97] Hence, dopamine may exert a suppressive effect on Th17 cells of MS patients (Table 3).

**3.2. Norepinefrin.** NE, a catecholamine, is the main neurotransmitter of the sympathetic nervous system.[98] NE can regulate the activities of neuronal cells and is involved in the regulation of immune responses, neuroplasticity, inflammation, rapid modulation of cortical circuits and cellular metabolism.[99]

NE exerts its effects by binding to G-protein-coupled $\alpha$- and $\beta$-adrenergic receptors (ARs). Each of the $\alpha$-ARs, which are divided into 2 families, $\alpha$1 and $\alpha$2, is further divided into three subfamilies. These are known as $\alpha$-1a, $\alpha$-1b, $\alpha$-1d and $\alpha$-2a, $\alpha$-2b, $\alpha$-2c. NE has the highest affinity for $\alpha$2-ARs, which inhibits adenylyl cyclases by binding to Gi and reduces cAMP production. $\beta$-ARs subtypes include $\beta$1, $\beta$2 and $\beta$3.[100] In addition, NE transporter (NET) is another member of the neurotransmitter/sodium symporter family, which also includes the serotonin transporter (SERT) and neuronal monoamine transporters for DAT.[101]

The downregulation of astrocytic $\beta$2-Adrenergic receptors (ADRB2) in MS has been demonstrated by immunohistochemical experiments. The fact that NE mediates the supportive and protective actions of astrocytes through activation of the ADRB2 suggests that NE may have a role in MS pathophysiology. By allowing astrocytes to act as facultative antigen presenting cells, NE may initiate T cell-mediated inflammatory responses that lead to characteristic demyelinating lesions.

Duloxetine, a serotonin-NE dual reuptake inhibitor, is effective in the treatment of depression in MS patients. Fatigue, which is common in MS patients, has a strong correlation with depression.[102] Duloxetine (maximum dose = 60 mg/day) has been shown to be well tolerated, safe, and effective in reducing both depression and fatigue in MS patients.

TRP ankrin 1 (TRPA1) calcium channels, belonging to the transient receptor potential (TRP) superfamily, regulate oligodendrocyte apoptosis. Pharmacological inhibition of TRPA1 may exert a protective effect in demyelination.[103] In addition, venlafaxine (60 mg·kg$^{-1}$), another serotonin-NE reuptake inhibitor, has been indicated to display an antagonistic effect on TRPA1 calcium channels during acute demyelination in cuprizone mouse models.[104] Hence, venlafaxine may provide some protection against cuprizone-induced demyelination. The vincamine derivative vindeburnol has been shown to elevate the expression and activity of tyrosine hydroxylase (TH) in Locus coeruleus (LC) neurons, which is the main source of NE in the CNS. Vindeburnol (20 mg/kg) has been demonstrated to exert beneficial effects on the LC of MS mouse models, by decreasing astrocyte activation, increasing the expression of several genes involved in LC survival and maturation, as well as elevating the NE levels in the spinal cord.[105] These results suggest that novel treatments approaches with drugs such as vindeburnol, which enhance LC survival or function, may be beneficial in MS patients.

The BBB-permeable catechol-O-methyltransferase (COMT) inhibitor dinitrocatechol can suppress the development of EAE. The reduction of NE in the CNS of MS patients has been suggested to be mediated by COMT metabolism. In particular, the use of the COMT inhibitor dinitrocatechol has been associated with increased COMT mRNA levels in the frontal cortex of MS patients.[106] Hence, COMT inhibitors may benefit MS patients. In a study conducted in MS patients in 2022, increased beta-endorphin and noradrenaline levels were investigated after a rehabilitation program. A multidimensional, high-impact, short rehabilitation program was proposed for MS patients and the difference in quality of life was measured. Quality of life, beta-endorphin and noradrenaline levels were increased in patients participating in the program.[107] Another pharmacological study has demonstrated that propranolol (10 mg/kg), a $\beta$-AR antagonist, could possibly reduce the severity of EAE by enhancing the immunomodulatory and protective properties of spinal cord microglia. Microglia are crucially implicated in the development and resolution of neuroinflammation. Interestingly, NE, which acts in neurocrine and autocrine/paracrine ways, has been shown to affect microglia in EAE rat models and promote $\beta$-AR-mediated neuroinflammation.[108] Therefore, NE may act as a key sympathetic end point mediator in the modulation of microglial phenotypic and functional properties in MS. This evidence may be the basis for further translational pharmacological research to improve MS treatment.

Another study has shown higher NET expression in subcortical brain regions such as thalamus and amygdala in patients with MS. Changes in monoamine signaling reported in MS may contribute to the high prevalence of comorbid depression and fatigue in MS patients.[109] Since the noradrenergic system is significantly involved in neuropsychiatric disorders, targeting NET may be a promising approach to link neuroinflammation with neuropsychiatric symptoms in MS, such as cognitive impairment (Table 4).

**3.3. Serotonin.** Serotonin (5-hydroxytryptamine (5-HT)) plays a key role in the regulation of sleep, motor activities, and cognitive function.[110] Apart from these activities, 5-HT is also involved in the immune system. Immune cells including T cells and dendritic cells may express 5-HT.[111]

Different subtypes of receptors correspond to seven families, including 5-HT (1−7).[112] There are three subtypes of 5-HT2 receptors: 5-HT2A, 5-HT2B, and 5-HT2C. 5-HT2A and 5-HT2C receptors have a widespread distribution and function in the CNS, while 5-HT2B receptors have limited expression.[113] Except for the 5-HT3A and 5-HT3B receptors, 5-HT receptors are G-protein dependent.[114] 5-HT3 receptors have ligand-gated ion channel function.[115]

Serotonin reuptake transporter (SERT) is implicated in the regulation of the serotonergic system and its receptors, by modulating the extracellular serotonin concentrations.[116] The gene encoding SERT contains a regulatory variation associated with anxiety-related traits and susceptibility to depression.[117] The increased frequency of depression in MS patients may be associated with changes in proinflammatory cytokine levels and monoaminergic neurotransmitter functions.[118] It has been proposed that the dysregulation of monoaminergic systems in MS may be at least partially caused by reduced monoamine synthesis due to inflammation and structural damage in the monoaminergic pathways in the brain.[119]


https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

A clinical study has demonstrated that 5-HT supplementation in MS patients (200 ng/mL) could reduce Th1 and Th17 cells. These effects were associated with decreased cytokine production and enhanced regulatory T cell function.[120] Hence, exogenous 5-HT administration seems to critically affect T cell behavior in MS. Fluoxetine ($10^{-6}$ M) has also been shown to affect cytokine production in RRMS patients via 5-HT2B receptors. In particular, fluoxetine-mediated 5-HT2B receptor activation inhibited the production of IL-17, interferon gamma, and GM-CSF.[121] These data suggest that fluoxetine may attenuate the inflammatory responses by activating 5-HT2B receptors in MS and regulating Th17 and Th1 function. Another study investigated the effects of fluoxetine and riluzole on the modulation of glutamatergic or serotonergic pathways in the treatment of neuropathic pain associated with secondary progressive multiple sclerosis (SPMS). In this study, fluoxetine (20 mg/day) and riluzole (50 mg/day) were not found to be effective in improving neuropathic pain symptoms in SPMS compared to placebo.[122] Furthermore, 5-HT7 expression on T lymphocytes has also been investigated in blood samples of MS patients. In particular, in MS patients treated with natalizumab, the 5-HT7 receptor was shown to be upregulated on the surface of T lymphocytes and different CD4+ T cell subsets.[123] These findings strengthen the hypothesis that 5-HT7 receptor may play a role in the immunoprotective mechanisms of natalizumab in MS. The effects of the SSRI fluvoxamine on EAE rats and neural stem cells have also been investigated. In this regard, immune cell infiltration and demyelination plaques were significantly reduced in the spinal cords of EAE rats treated with fluvoxamine (50 mg/kg). In addition, fluvoxamine could stimulate the differentiation of neuronal stem cells into oligodendrocytes *in vitro*.[124] Based on this evidence, it has been proposed that fluvoxamine might be used to reduce neuroinflammation and stimulate oligodendrogenesis in MS patients. Also, clozapine (60 mg/kg), a serotonin receptor antagonist (154), may modulate CD4+ T cell responses in EAE models, by promoting the differentiation of regulatory T cells.[125] However, regulatory T cells were not found to be responsible for the protective effects of clozapine during the induction and effector phase of EAE in another study.[126] These results suggest that the protective effects of clozapine could not be directly mediated by the regulation of CD4+ T cells. Clozapine has also been shown to regulate microglial function in cell cultures and EAE model mice, as well as attenuate (2.5−15 mg/kg) chronic EAE in mice in a dose-dependent manner.[127] A randomized clinical trial aiming to investigate the effects of the SSRI escitalopram (10 mg/day) on stress-related relapses in MS demonstrated that the risk of relapse in female patients with MS was lower in those treated with escitalopram compared to the control group.[128] This suggests that escitalopram may be an effective treatment option in the prevention of stress-related relapses in MS.

The variation of SERT levels in the brain of MS patients has also been investigated. In this regard, increased SERT was observed in the prefrontal regions in primary progressive MS (PPMS) patients, while RRMS patients displayed lower SERT in the hippocampus. In addition, the levels of SERT in the insula showed a positive correlation with depression and fatigue scores.[129] This evidence suggests that changes in SERT levels in the brain may contribute to specific symptoms of MS, such as depression and fatigue (Table 5).

In summary, serotonin plays a key role in the immune system in the CNS. In MS, serotonin transporter and receptor

**Table 5. Summaries of Findings Reporting the Role of Serotonin in MS[a]**

| S.N. | study type | MS model | drug and dose | observation | remarks | refs |
|---|---|---|---|---|---|---|
| 1 | *In vivo* | Animal model of EAE (200 μL) induced MS in rats ($n$ = 7) and control group ($n$ = 14) | Fluvoxamine (50 mg/kg) | Immune cell infiltration and demyelination plaque are reduced in the spinal cords of EAE rats treated with fluvoxamine (50 mg/kg); fluvoxamine could stimulate the differentiation of neuronal stem cells into oligodendrocytes *in vitro*. | Fluvoxamine may be used to reduce neuroinflammation and stimulate oligodendrogenesis in MS patients. | 124 |
| 2 | *In vivo* | Animal model of EAE (500 μg) induced MS in mouse ($n$ = 10) and control group ($n$ = 10) | Clozapine (60 mg/kg) | Clozapine (60 mg/kg) increased the differentiation of regulatory T cells. However, regulatory T cells were not found to be responsible for the protective effects of clozapine during the induction and effector phase of EAE. | The protective effects of clozapine may not directly alter CD4+ T cells. | 126 |
| 3 | *In vivo* | Animal model of EAE (500 μg/mL) induced MS in mouse ($n$ = 18) and control group ($n$ = 6) | Clozapine (2.5−15 mg/kg) | The effect of clozapine on microglia cell culture and EAE model mice could regulate microglial function in cell culture and attenuate dose-dependent (2.5−15 mg/kg) chronic EAE in mice. | Clozapine might be beneficial for progressive MS. | 127 |
| 4 | *Human study* | Patients with RRMS ($n$ = 30) | Fluoxetine ($10^{-6}$ M) | Blockade of 5-HT2B receptors can reduce the inhibitory effect of fluoxetine ($10^{-6}$ M) on cytokine production in RRMS patients. Moreover, 5-HT2B receptor activation can inhibit the production of IL-17, interferon gamma and GM-CSF. | Fluoxetine can attenuate the inflammatory response by activating 5-HT2B receptors in MS. | 121 |
| 5 | *Human study* | Patients with MS ($n$ = 24) and control group ($n$ = 24) | Escitalopram (10 mg/day) | The risk of relapse in female patients with MS is lower in those treated with escitalopram (10 mg/day) compared to the control group. | Escitalopram may be an effective treatment option in the prevention of stress-related relapses in MS. | 128 |
| 6 | *Human study* | Patients with MS ($n$ = 21) | - | In PPMS patients, increased SERT is observed in the prefrontal regions while RRMS patients display lower SERT in the hippocampus. In addition, the level of SERT in the insula shows a positive correlation with depression and fatigue scores. | Altered SERT levels in the brain may contribute to symptoms of MS such as depression and fatigue. | 129 |

[a]EAE = Experimental Autoimmune Encephalomyelitis, GM-CSF = Granulocyte macrophage colony stimulating factor, IL-17 = Interleukin 17, MS= Multiple Sclerosis, PPMS = Primer Progressive Multiple Sclerosis, RRMS = Relapsing-remitting multiple sclerosis, SERT = Serotonin transporter, 5-HT = 5-hydroxytryptamine.



https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

**Table 6. Summaries of Findings Reporting the Role of Histamine in MS[a]**

| S.N. | study types | MS model | drug and dose | observations | remarks | refs |
|---|---|---|---|---|---|---|
| 1 | *In vitro, in vivo* | Animal model of EAE (100 $\mu$g) induced MS in mice | - | Histamine may prevent myelin-autoreactive T cells from migrating through the inflamed BBB via H1R and H2R. | Histamine intervention may be beneficial in the treatment of MS. | 143 |
| 2 | *In vitro, in vivo* | Animal model of EAE (50 $\mu$g) induced MS in mice | Ketamine (600 mg/kg) and Xylazine (20 mg/kg) | There is a correlation between MS disease vulnerability and neurogenic regulation of T cell responses. | Therapeutic approaches to H3R may prevent the formation of new lesions in MS. | 144 |
| 3 | *In vitro, in vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | ST-1505 and ST-1478 (10−100 $\mu$M) | ST-1505 and ST-1478 (10−100 $\mu$M) can improve cognitive impairment in MS. | H3R antagonists may be useful in the treatment of MS. | 145 |
| 4 | *In vitro, in vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | Immethridine (1 $\mu$M) | Immethridine (1 $\mu$M) treatment can inhibit NF-KB p65 in dendritic cells. | Immethridine may be a useful therapeutic approach in EAE. | 146 |
| 5 | *In vivo* | Animal model of EAE (100 $\mu$g) induced MS in mice | Histamine (10 $\mu$M) | Expression of endothelial H1R affects BBB permeability. | The endothelial H1R signaling may be essential in providing cerebrovascular integrity. | 142 |
| 6 | *Human study* | Patients with SPMS ($n$ = 24) and patients with RRMS ($n$ = 12) | - | CSF samples of MS patients have higher levels of histamine (median: 35.6 pg/mL) compared to the control group (median: 5.5 pg/mL). | Histamine may be crucial in the onset and maintenance of MS disease. | 137 |
| 7 | *Human study* | Patients with RRMS ($n$ = 50) and control group ($n$ = 41) | - | Histamine levels are lower in RRMS patients. | The reason for low histamine levels in RRMS patients is unknown. | 138 |
| 8 | *Human study* | Patients with MS ($n$ = 228) and control group ($n$ = 295) | - | The frequencies of HNMT polymorphism did not differ in MS patients compared to the control group. | MS risk may not be associated with the HNMT polymorphism. | 140 |
| 9 | *Human study* | Patients with clinically isolated syndrome ($n$ = 16), RRMS ($n$ = 15), SPMS ($n$ = 9), PPMS ($n$ = 9), and control group ($n$ = 17) | - | H1R and H4R were dysregulated in in SPMS patients compared to the control group. | H1R and H4R signaling may be impaired in SPMS. | 141 |

[a]BBB = Blood−brain barrier, CSF = Cerebrospinal fluid, EAE = Experimental autoimmune encephalomyelitis, H1R = Hisamine H1 receptor, H2R = Histamine H2 receptor, H3R = Histamine H3 receptor, H4R = Histamine H4 receptor, HNMT = Histamine *N*-methyltransferase, MS = Multiple sclerosis, PPMS = Primary-progressive multiple sclerosis, RRMS = Relapsing-remitting multiple sclerosis, SPMS = Secondary-progressive multiple sclerosis.



https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

dysregulation are associated with changes in the inflammatory responses but also neuropsychiatric symptoms, such as depression. Therefore, targeting the serotoninergic system in MS represents a promising therapeutic target, and the specific effects of serotoninergic system dysregulation in MS pathophysiology should be further explored.

**3.4. Histamine.** Beta-imidazolyl ethylamine, histamine amine, is formed by the separation of the carbon dioxide molecule from the histidine amino acid.[130] Histamine produced by mast cells is an important neurotransmitter, and it plays a significant role in several CNS disorders, such as Parkinson's disease, schizophrenia, and Alzheimer's disease.[131,132]

The functions of histamine are mediated by four G-protein coupled receptors named histamine H1 receptor (H1R), histamine H2 receptor (H2R), histamine H3 receptor (H3R), and histamine H4 receptor (H4R).[133] H1R expressed in mast cells is involved in Type 1 hypersensitivity reactions, which is an allergic reaction.[131] Importantly, this receptor has been found to be overexpressed in chronic plaques of MS patients.[134] H2R is involved in Th1 lymphocyte cytokine production, while H3R stands out with its role in the BBB.[131] H1R and H2R are excitatory, while H3R is inhibitory;[135] with the stimulation of H4R, histamine and cytokine formation in mast cells increases.[131]

In the mammalian brain, histaminergic neurons are located only in the tuberomammillary nucleus of the posterior hypothalamus.[135] Changes in the histaminergic system have been shown to be associated with MS.[136] In this context, histamine has been shown to contribute to neuroinflammation by altering the permeability of the BBB.[128]

Histamine levels in the CSF have been shown to be higher in MS patients compared to controls,[137] suggesting that histamine may play a crucial role in MS pathophysiology. Also, serum levels of histamine and diamine oxidase—required to produce this amine—have been found to be significantly lower in RRMS patients,[138] highlighting the potential role of altered histamine levels in MS.

Histamine *N*-methyltransferase (HNMT), an enzyme expressed in the brain, can metabolize the neurotransmitter histamine.[139] HNMT is the main metabolizing enzyme of histamine in the CNS. It has been demonstrated that MS patients do not have a different HNMT genotype frequency compared to controls;[140] therefore, HNMT polymorphism might not be associated with MS risk. In another study, peripheral blood mononuclear cells were obtained from individuals with different stages of MS, and gene expression analyzes of histamine receptors were performed. In this study, the H1R transcript was significantly downregulated in secondary progressive MS (SPMS) compared to healthy controls, and H4R was increased in SPMS.[141] No other difference were observed in the expression of histamine receptors between MS and healthy controls. Also, the endothelial H1R expression has been shown to reduce BBB permeability.[142] Hence, endothelial H1R signaling may be important for the preservation of cerebrovascular integrity in MS. Histamine has been demonstrated to inhibit the activation of myelin-autoreactive T cells via H1R and H2R and also their ability to pass through the inflammatory BBB.[143]

Furthermore, it has been shown that there may be a functional link between the H3R-mediated neurogenic control of T cell responses and susceptibility to MS.[144] Therefore, pharmacological targeting of H3R may aid in the prevention of the formation of new lesions and inhibit disease progression in MS. Also, the new compounds with H3R antagonist activities have shown protective effects in MS mouse models. For example, the piperidine derivatives ST-1505 and ST-1478 (10−100 $\mu$M) with H3R antagonist effects may represent new drugs for MS treatment,[145] displaying potential beneficial effects in cognitive function in MS. Moreover, signaling pathway analysis has demonstrated that the phosphorylation of nuclear factor kappa B (NF-$\kappa$B) p65 in dendritic cells was reduced after immethridine—an H3R agonist—treatment (1 $\mu$M).[146] Based on these results, immethridine may be proposed to play a therapeutic role in EAE, even though the inhibition of dendritic cells should be also considered (Table 6).

## 4. ALKALOID NEUROTRANSMITTERS/ETHANOLAMIDE NEUROTRANSMITTERS

**4.1. Acetylcholine.** Acetylcholine (ACh) acts as a neuromodulator by affecting neuron excitability, synaptic transmission and synaptic plasticity. The effects of this neurotransmitter depend on receptor subtypes and target neuronal population.[147,148] Muscarinic acetylcholine receptors (mAChRs) are G protein-dependent receptors with 5 subtypes, M1-M5.[149] Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels with 12 neuronal subunits, alpha2-alpha10 and beta2-beta4.[150] ACh controls immune cell functions through alpha7 nAChR. Nicotinic agonists have been suggested to be rather more effective than ACh in inhibiting the inflammatory responses and the production of proinflammatory cytokines.[151]

In EAE models, the expression of cholinergic system elements in the CNS has been investigated in the acute and recurrent phase of the disease. The expression of cholinergic system components has been found to vary significantly during the different phases of EAE pathology.[152] This variance suggests that altered ACh levels may diversely contribute to the neuroinflammatory process of the different phases of MS. Furthermore, it has been shown that the inhibition of ACh release by vagotomy in mice could suppress the proliferation of CD4+ T cells.[153] This evidence indicates that the regulation of ACh release may improve the course of EAE by modulating Th1, Th2, and Th17 cells.

At a clinical level, it has been demonstrated that serum ACh levels of RRMS patients are inversely correlated with the cholinergic hydrolyzing enzyme activity.[154] The dysregulation of ACh levels may contribute to the pathogenesis of MS and the maintenance of the proinflammatory state. The effect of nicotinic receptor activation on proinflammatory cytokine production in RRMS has also been investigated, and a decrease in IL-1$\beta$ and IL-17 levels in cell culture supplemented with nicotine has been observed.[155] Therefore, nicotine may contribute to the suppression of inflammation in MS by increasing the expression of alpha 7 receptor subtype in RRMS patients.

Another study has shown that the noncompetitive antagonist mecamylamine (6.5 mg/kg/day) and the silent agonist *m*-bromo PEP (6 mg/kg/day) of nicotonic receptors could ameliorate the course of the disease in EAE model mice. In addition, cytokine production was reduced in bone marrow-derived monocyte/macrophage cultures of mice.[156] Based on these results, it could be proposed that alpha7 nAChR selective silent agonists might be used for their anti-inflammatory effects


https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

**Table 7. Summaries of Findings Reporting the Role of Acetylcholine in MS[a]**

| S.N. | study types | MS model | drug and dose | observations | remarks | refs |
|---|---|---|---|---|---|---|
| 1 | *In vivo* | Animal model of EAE (100 μL) induced MS in mice ($n$ = 8) and control group ($n$ = 16) | - | Inhibition of ACh release by vagotomy in mice may suppress the proliferation of CD4+ T cells. | The regulation of ACh release may improve the course of EAE by affecting the differentiation of Th1, Th2 and Th17. | 153 |
| 2 | *In vivo* | Animal model of EAE (200 μg) induced MS in mice ($n$ = 14) | Mecamylamine (6.5 mg/kg/day) and the silent agonist *m*-bromo PEP (6 mg/kg/day) | The noncompetitive antagonist mecamylamine (6.5 mg/kg/day) and the silent agonist *m*-bromo PEP (6 mg/kg/day) of nicotonic receptors may ameliorate the course of the disease in EAE model mice. In addition, cytokine production by cells is reduced in bone marrow-derived monocyte/macrophage culture of mice. | Alpha7 nAChR selective silent agonists can be used for their anti-inflammatory effects in diseases such as MS. | 156 |
| 3 | *In vivo* | Animal model of EAE (200 μg) induced MS in mice ($n$ = 15) and control group ($n$ = 14) | - | The disease exacerbation may occur partially through alpha9 nAChR in peripheral immune cells and alpha7 nAChR may exert a protective effect in the CNS. | Alpha7 and alpha9 nAChR can be targeted to regulate inflammation. | 158 |
| 4 | *In vivo* | Animal model of EAE (100 μL) induced MS in mice model ($n$ = 12) and control group ($n$ = 6) | Donezepil (2 mg/kg/day) | Donezepil (2 mg/kg/day) treatment may improve clinical, pathological and magnetic resonance imaging results in EAE model mice. | Donepezil may be exert anti-inflammatory effects and be beneficial in the treatment of EAE. | 159 |
| 5 | *Human Study* | Patients with RRMS ($n$ = 87) | - | Balance of ACh levels in immune cells of RRMS patients show an inverse correlation between cholinergic hydrolyzing enzyme activity and ACh levels in the serum of RRMS patients. | The dysregulation of ACh levels may contribute to the pathogenesis of MS and the maintenance of the proinflammatory state. | 154 |
| 6 | *Human Study* | Patients with RRMS ($n$ = 20) and control group ($n$ = 20) | Dimethyl Fumarate | Treatment with DMF can reduce short-latency afferent inhibition, a measure of transcranial stimulation of central cholinergic transmission in RRMS patients. | The possible neuroinflammation and neuroprotection effects of DMF treatment in MS may be associated with increased cholinergic stimulation. | 160 |

[a]Ach = Acetylcholine, DMF = Dimethyl Fumarate, MS = Multiple sclerosis, nAChR = Nicotinic Acetylcholine Receptor, RRMS = Relapsing-remitting multiple sclerosis.



https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

**Table 8. Summaries of Findings Reporting the Role of Melatonin in MS[a]**

| S.N. | study type | MS model | drug and dose | observations | remarks | ref |
|---|---|---|---|---|---|---|
| 1 | *In vitro, In vivo* | Animal model of EAE (100 $\mu$g) induced MS in rats | Melatonin (5 mg/kg) | Melatonin may contribute to seasonal relapses in MS. | Although melatonin-dependent signaling may be a potential target for therapeutic immunomodulation, the pathways involved are complex and cross-regulated. | 172 |
| 2 | *In vitro* | Animal modelof EAE (100 $\mu$L) induced in rats | Melatonin (1 mg/kg) | Clinically, melatonin can reduce oxidative stress, bacterial dysbiosis and inflammation on MS. | In the treatment of MS, the role of melatonin could be further explored. | 169 |
| 3 | *In vivo* | Animal modelof EAE (300 $\mu$g) induced in mice | - | Treatment with melatonin increased $NAD^+$ metabolism, while decreasing mRNA expression of the aryl hydrocarbon receptor associated with kynurenine. | The modulation of kynurenine that is the main pathway of tryptophan metabolite in MS, can be modulated by melatonin. | 174 |
| 4 | *Human Study* | Patients with RRMS ($n$ = 12) and control group ($n$ = 14) | - | Investigation of the effects of sirtuin 1 (SIRT1) and its effect on antioxidant enzymes and inhibition of oxidative stress in MS. | Melatonin may improve impaired antioxidant defense mechanisms by rearranging key antioxidant molecules such as SIRT1, MnSOD and catalase, which may be important in MS management. | 167 |
| 5 | *Human Study* | Patients with SPMS ($n$ = 16) and control group ($n$ = 13) | Melatonin (10 mg per day) | Melatonin could be beneficial against oxidative stress in MS. | Melatonin may increase antioxidative superoxide dismutase and glutathione peroxide levels, as well as decrease malondialdehyde in the erythrocytes of SPMS patients. | 168 |
| 6 | *Human Study* | Patients with RRMS ($n$ = 18) | Natalizumab (300 mg) | In female patients with RRMS treated with natalizumab, the association with elevated melatonin levels and oxidative stress is investigated. | A reduction in the number of relapses associated with major melatonin levels in the peripheral blood of female patients with RRMS is observed. | 170 |
| 7 | *Human Study* | Patients with RRMS ($n$ = 40) | 25-hydroxyvitamin D form (low dose, 800 IU; high dose, 4.370 IU) | Melatonin secretion in MS patients treated with IFN-$\beta$ correlates negatively with changes in 25-hydroxyvitamin D. | The reciprocal effects of both melatonin and vitamin D on immune adequacy and neuroprotection can be further evaluated. | 171 |
| 8 | *Human Study* | Patients with MS ($n$ = 34) | Melatonin (2 mg) | Low-dose melatonin at bedtime is an ineffective treatment in MS patients with nocturia once a night or more frequently. | A different melatonin dosing regimen may be considered to determine whether melatonin affects nocturia in MS. | 173 |

[a]EAE = Experimental autoimmune encephalomyelitis, IFN-$\beta$ = İnterferon beta, MnSOD = manganese superoxide dismutase, MS = Multiple Sclerosis, PPMS = Primary progressive MS, RRMS = Relapsing-remitting MS, SIRT1 = Sirtuin 1, SPMS = Secondary progressive MS.



https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

in CNS inflammatory disorders such as MS. Similarly, nAChRs have been shown to regulate the infiltration of CCR2+Ly6C high proinflammatory monocytes and neutrophils into the CNS. In EAE mice, nicotine treatment (0.39 mg/day) could affect the migration of proinflammatory monocytes and neutrophils into the CNS via nAChRs-alpha7 and alpha9 subtypes.[157] Therefore, the use of selective ligands of nAChR subtypes could provide a valuable therapeutic strategy for MS. The effect of alpha7 and alpha9 nAChRs on inflammatory and autoimmune responses in EAE models has been also investigated. In this regard, disease exacerbation has been shown to occur partially through alpha9 nAChR in peripheral immune cells, and alpha7 nAChR might act protectively in the CNS.[158] This evidence suggests that alpha7 and alpha9 nAChRs could represent promising targets against neuroinflammation.

The effects of donezepil on inflammatory responses have been also explored; optimal dose of donezepil (2 mg/kg/day) treatment was shown to improve clinical, pathological and magnetic resonance imaging results in EAE models.[159] This evidence suggests that donepezil may be effective in the treatment of EAE via anti-inflammatory mechanisms. Dimethyl fumarate (DMF), which acts by upregulating the Nrf2 antioxidant pathway -which is also stimulated by ACh-, could also significantly reduce short-latency afferent inhibition (SAI), a measure of transcranial stimulation of central cholinergic transmission in RRMS patients.[160] Therefore, the possible protective effects of DMF treatment in MS may be associated with increased cholinergic stimulation (Table 7).

Collectively, altered ACh levels and cholinergic system dysregulation may contribute to the neuroinflammatory process in MS. Treatments targeting ACh receptor and disrupted enzyme levels may be beneficial as therapeutic approaches in MS, and their clinical role should be further investigated. Collectively, altered ACh levels and cholinergic system dysregulation, as shown in Figure 1B, may contribute to the neuroinflammatory process in MS.

## 5. ETHANAMIDE-STRUCTURAL NEUROTRANSMITTERS

**5.1. Melatonin.** Melatonin (5-methoxy-N-acetyltryptamine), which has indolamine properties, is a neurohormone synthesized and secreted mainly by the pineal gland, following the circadian rhythm but also by extrapineal sources including immune system cells, brain, skin, and gastrointestinal tract. Melatonin receptors (MT) display pleiotropic activities; they are expressed in several tissues, and they are produced in multiple sites. The melatonin neurotransmitter is used as a therapeutic agent in neurological diseases, such as MS, due to the sleep−wake rhythm regulation.[161−163]

In humans, MT exist as MT1 and MT2. G protein-coupled receptors respond to melatonin to regulate circadian rhythm and sleep. Agents targeting MT are being developed for the treatment of insomnia and circadian rhythm disorders.[164] Regulation of the sleep-wake cycle in MS may affect disease activity and relapse frequency in patients.[165] The dysregulation of melatonin secretion in MS suggests melatonin as a potential target for therapeutic interventions.[162]

The investigation of the protective effects of melatonin in EAE models has revealed that melatonin (1 mg/kg body weight) may protect effectively against oxidative stress.[166] Sirtuin 1 (SIRT1) plays an important antioxidant role, and it has been proposed to be regulated by melatonin. In this regard, melatonin has been demonstrated to improve the impaired antioxidant defense mechanisms by rearranging key antioxidant molecules, such as SIRT1, manganese superoxide dismutase (MnSOD), and catalase, in peripheral blood mononuclear cells (PBMC) of MS patients.[167] Melatonin has been shown to reduce oxidative stress in erythrocytes of patients with SPMS, exerting also neuroprotective properties (10 mg per day). Melatonin has also been associated with increased antioxidative superoxide dismutase and glutathione peroxide, as well as a decreased malondialdehyde in the erythrocytes of SPMS patients.[168] These results suggest that melatonin supplementation should be considered in MS patients, especially in the progressive form. In another study conducted in 2022, the protective effects of melatonin were also investigated in EAE models, regarding its effects in inflammation, oxidative stress, and indirect biomarkers of bacterial dysbiosis. Melatonin could reduce oxidative stress, bacterial dysbiosis, and inflammation in MS.[169] Hence, melatonin represents another potential therapeutic agent in MS that deserves further study. In female patients with RRMS treated with natalizumab, reduced relapses were associated with higher melatonin levels in the peripheral blood.[170] This observation has been proposed to be related to the reduction of adhesion factors, such as vascular cellular adhesion moleculea, and reduced oxidative damage.

Melatonin is considered as a potential mediator of the neuro-immunomodulatory effects of vitamin D. In this regard, melatonin secretion in MS patients treated with IFN-$\beta$ has been shown to be negatively correlated with changes in 25-hydroxyvitamin D levels.[171] The reciprocal effects of both melatonin and vitamin D on immune adequacy and neuroprotection should be further evaluated, based on their light-dependent and neuroimmunomodulatory properties.

Melatonin (5 mg/kg) may contribute to seasonal relapses in MS. Although the data have identified melatonin-dependent signaling as a potential target for therapeutic immunomodulation, the pathways involved are complex and cross-regulated.[172] Caution is needed when assessing the translational potential of these findings. Nocturia negatively affects quality of life in MS patients. Low-dose melatonin (2 mg) at bedtime has been an ineffective treatment in MS patients with nocturia.[173] A different melatonin dosing regimen may be considered to determine whether melatonin affects nocturia in MS. Kynurenine is the main pathway of tryptophan metabolite implicated in the neuropathogenesis of MS; interestingly, kynurenine can be modulated by melatonin. In this regard, treatment with melatonin could enhance $NAD^+$ metabolism and reduce mRNA expression of the aryl hydrocarbon receptor associated with kynurenine.[174] Melatonin therapy might reduce the severity of EAE. Collectively, these results show that melatonin may be one of the most important neurotransmitters in MS, with a promising therapeutic potential (Table 8).

## 6. GAS NEUROTRANSMITTERS

**6.1. Nitric Oxide.** Nitric oxide (NO) is a chemical signaling molecule, acting as a mediator between neurons; it is involved in the regulation of synaptic plasticity and secretion of neurohormones in the CNS.[175] This molecule, which is a diffusible second messenger, binds to specific guanylyl cyclase-coupled receptors, resulting in the formation of cGMP in target cells.[176] In addition, NO can mediate signaling between neurons.[177] High levels of NO biosynthesis are associated with inflammatory autoimmune diseases including MS (Figure 1).[8]


https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

**Table 9. Summaries of Findings Reporting the Role of Nitric Oxide in MS[a]**

| S.N. | study types | MS model | drug and dose | observations | remarks | refs |
|---|---|---|---|---|---|---|
| 1 | *In vitro, in vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | Phosphodiesterase inhibitor (100 $\mu$M) | Nitrotyrosine immunoreactivity, showing peroxynitrite formation, is increased in white matter of spinal cord. | Inhibiting peoxynitrite instead of NO may protect against oligodendrocytes from oxidative stress-induced toxicity. | 183 |
| 2 | *In vitro, in vivo* | Animal model of EAE (300 $\mu$g) induced MS in mice | PTx (400 ng) | Microglia of EAE mice block differentiation of Th1 cells. | Microglia may suppress differentiation of Th1 cells through PD-L1 and NO pathways. | 184 |
| 3 | *In vivo* | Animal model of EAE (100 $\mu$g) induced MS in mice | Sodium azide | Mitochondrial complex IV protects energy homeostasis in EAE. | Activation of the NO pathway may cause increased neuronal sensitivity against mitochondrial complex IV inhibition. | 182 |
| 4 | *In vivo* | Animal model of EAE (100 $\mu$g) induced MS in mice | Saline (1 mL/day) | Impaired T cell responses of OGR1 knockout mice are associated with lower numbers of dendritic cells in EAE as well as increased NO production by macrophages. | OGR1 may be involved in the regulation of T cell responses. | 185 |
| 5 | *In vivo* | Animal model of EAE (100 $\mu$g) induced MS in rats | AG (100 mg/kg) and NAC (150 mg/kg) | NO production is reduced in animals treated with AG (100 mg/kg) and NAC (150 mg/kg) compared to untreated animals. | NO-containing arginase can modulate the inflammatory responses in MS patients in the acute phase. | 186 |
| 6 | *In vivo* | Animal model of EAE (100 $\mu$L) induced MS in mice | GSNO (0.5 or 1 mg/kg) | GSNO (0.5 or 1 mg/kg) inhibits EAE by Th17 modulation independent of the Th1 and Th2 functions. | GSNO may be implicated in Th17-mediated autoimmune diseases. | 188 |
| 7 | *In vivo* | Animal model of EAE (100 ng) induced MS in mice | N6022 (1 mg/kg) | Transfer of B cells in EAE mice treated with GSNOR inhibitor N6022 (1 mg/kg) regulates T cell balance. | N6022 could be a novel therapeutic agent for MS. | 189 |
| 8 | *Human study* | Patients with MS ($n$ = 70) and control group ($n$ = 40) | - | Patients with a history of MS relapse in the last 30 days 84.87 $\pm$ 29.6 nmol/$\mu$L) have higher serum NO levels than patients without a history of MS relapse in the last 3 months (41.99 $\pm$ 24.2 nmol/$\mu$L) and the control group (12.03 $\pm$ 3.59 nmol/$\mu$L). | The concentration of NO in serum may be a biomarker for disease activity in MS patients. | 179 |
| 9 | *Human study* | Patients with MS ($n$ = 47) and control group ($n$ = 37) | - | NO metabolites levels (134.5 $\pm$ 30.8 $\mu$M) are increased in the serum of MS patients who did not receive medication, compared with healthy controls (113.9 $\pm$ 19.9 $\mu$M). | MS disease status may be associated with NO levels in readily accessible body fluids. | 180 |
| 10 | *Human study* | Untreated MS patients ($n$ = 15) and INF-$\beta$1b treated MS patients ($n$ = 12) | INF-$\beta$1b (250 $\mu$g) | In MS patients treated with INF-$\beta$1b (250 $\mu$g), plasma concentrations of nitrite + nitrate, markers of NO production, are lower compared to those at treatment initiation. | Low nitrite + nitrate concentrations may be useful as new biomarkers. | 181 |
| 11 | *Human study* | Patients with MS ($n$ = 142) and control group ($n$ = 140) | - | Increased MS risk is associated with the C/C genotype and the C allele of the NOS2 gene. | Polymorphism in nitrate/oxidative stress genes may increase the risk of MS. | 187 |

[a]AG = Aminoguanidine, EAE = Experimental autoimmune encephalomyelitis, GSNO = S-nitrosoglutathione, GSNOR = S-nitrosoglutathione reductase, INF-$\beta$1b = Interferon-$\beta$1b, MS = Multiple sclerosis, NAC = N-acetyl-L-cysteine, NO = Nitric oxide, PD-L1 = Programmed death ligand-1, OGR1 = G protein-coupled receptor 1, SNP = single-nucleotide polymorphism, Th1 = T-helper-1 cell, Th2 = T-helper-2 cell, Th17 = T-helper-17 cell.



https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

Axonal damage due to increased nitric oxide levels in MS are shown in Figure 1C. High concentrations of molecules, such as nitrate and nitrite, which are markers of NO production, have been observed in the CSF and blood of MS patients. In addition, NO has been found to be higher in the inflammatory lesions of MS patients, as compared to controls.[178]

Clinical evidence suggests that serum NO-related molecules may be useful biomarkers in MS. In particular, MS patients without a history of MS relapse within the last 3 months had significantly lower serum NO levels.[179] The levels of NO metabolites were also increased in the serum of MS patients.[180] Hence, serum NO metabolites may be promising biomarker for disease activity and relapses in MS patients.

Modulation of NO metabolism has also been proposed to be implicated in MS pathophysiology. Interferon-$\beta$1b (INF-$\beta$1b) treatment has been shown to modulate NO metabolism in RRMS patients and nitrite + nitrate plasma concentrations were significantly lower during INF-$\beta$1b treatment compared to those at the start of treatment.[181] Microglial activation and NO/cGMP/PKG pathway have been indicated to be play key roles in neuronal vulnerability against mitochondrial dysfunction in MS. In particular, mitochondrial complex IV could regulate the internal balance of energy in neurons during EAE.[182] Therefore, inhibition of mitochondrial complex IV in MS may represent an effective pharmacological target.

The role of NO in mediating oligodendrocyte (OL) toxicity also plays a critical role in MS pathophysiology. Experimental EAE and cell culture studies have shown that nitrotyrosine immunoreactivity was higher in the white matter of the spinal cord of mice, and this increase was associated with loss of mature OLs.[183] Blocking peroxynitrite instead of NO may possibly produce more meaningful results for the prevention of oxidative stress-induced toxicity against OLs. Programmed death ligand-1 (PD-L1) in microglia of EAE mice controls Th1 differentiation via NO, and microglia can inactivate the differentiation of Th1 cells.[184] Therefore, microglia may possibly prevent differentiation in Th1 cells through PD-L1-NO pathway, but this mechanism should be further examined.

Another molecule, the ovarian cancer G protein-coupled receptor 1 (OGR1)/GPR68, encoded by the GPR68 gene, regulates the synthesis of NO through macrophages. Activity of dysfunctional T cells is associated with fewer dendritic cells in EAE, as well as increased NO production by macrophages.[185] OGR1 may be involved in the regulation of T cell responses. NO synthase can be modulated via arginase, which is the last enzyme of the urea cycle. Aminoguanidine (100 mg/kg) and N-acetyl-L-cysteine (150 mg/kg) have been associated with a markedly reduced clinical score of EAE rat models.[186] This evidence suggests that arginase may be a key regulator of the inflammatory responses in MS.

Genes encoding NO synthase (NOS) have also been investigated in MS. In this regard, a higher risk for MS has been associated with the C/C genotype and the C allele SNP of the NOS2 gene.[187] Therefore, polymorphisms in nitrate/oxidative stress genes might also affect MS risk.

The effect of NO transporter S-nitrosoglutathione (GSNO) (0.5 or 1 mg/kg) on EAE has also been explored; GSNO could inhibit the effects of EAE by modulating Th17 cells, without affecting the activities of Th1 and Th2 cells.[188] According to these results, targeting GSNO may represent another promising approach for the treatment of Th17-mediated autoimmune disorders. Furthermore, GSNO has been shown to play a key role in the regulation of B cell immune imbalance in EAE mice, and the GSNO reductase (GSNOR) inhibitor N6022 exerted therapeutic benefits in this study (1 mg/kg) (Table 9).[189]

Thus, NO stands out as a critical neurotransmitter both in terms of regulating other molecules and directly playing a role in the MS pathophysiology.

## 7. PURINE NEUROTRANSMITTERS

**7.1. Adenosine.** Adenosine, the degradation molecule of extracellular ATP, is a purine nucleoside that regulates several neuronal functions.[190] This nucleoside is a compensatory molecule that has been shown to inhibit inflammation in MS.[191] Adenosine and its receptors can regulate the inflammatory cell activity in the active phase of inflammation.[192] Four G protein-coupled adenosine receptors, A1, A2A, A2B, and A3, are expressed by cells of the immune system,[193] and the role of adenosine receptors has been investigated in the pathophysiology and treatment of MS.

A2A receptor signaling inhibits the pro-inflammatory activity of Th lymphocytes and regulates their recruitment into the CNS. A2A receptor expression can promote tissue damage and repair in chronic neuroinflammation. A2A receptors may have a complex role in EAE, with the ability to alleviate as well as exacerbate disease severity.[194]

Preclinical evidence has demonstrated that the inhibition of CX3CL1 via anti-CX3CL1 IgG (4 $\mu$g/mouse) could block lymphocytes from entering the CNS and prevent the development of EAE,[195] suggesting that extracellular adenosine could be an endogenous regulator of neuroinflammation in EAE. Also, SCH58261 (2 mg/kg/d), an A2A receptor antagonist, could eliminate the neurological behavioral deficits during EAE.[196] Elucidating the therapeutic role of the A2A receptor antagonist may pave the way for the use of SCH58261 for MS therapy. Another study showed that inosine (1 or 40 mg/kg), an endogenous purine nucleoside, could downregulate the A2A receptor in the spinal cord of EAE models.[197] Therefore, inosine may have an important place in the treatment of MS in the future. A2A receptor-mediated signaling in the CNS and lymphocytes is also critical for regulating inflammation in EAE. A2A receptor expression in nonimmune cells has been shown to be crucial for EAE.[198] Expression of the A2A receptor on lymphocytes may be required for limiting inflammation. The aggravation of brain damage during EAE due to inactivation of the A2A receptor and extracellular adenosine has been indicated to stimulate a prominent neuroprotective mechanism,[199] suggesting that A2A receptor could be an effective novel therapeutic target in MS. The A2A receptor activity could suppress EAE. Moreover, the intracellular $Ca^{2+}$ concentration in murine lymphocytes could be increased by CGS21680 (0.05 mg/kg), a selective A2A receptor agonist.[200] These results show that targeting A2A receptor may lead to the development of new treatments for MS.

A2A receptors in SPMS have also been explored with [$^{11}$C] TMSX brain PET, which uses a selective radioligand that binds to these receptors. In this study, the expression of A2A receptors in the brain of SPMS patients was enhanced,[201] and [$^{11}$C] TMSX-PET may help us deeper understand SPMS pathophysiology *in vivo*.

It has been indicated that lymphocytes from MS patients with upregulated A2A receptors may also downregulate IL-1$\beta$, IL-6, IL-17, interferon $\gamma$ (IFN-$\gamma$), and TNF-$\alpha$,[202] highlighting their potential role as novel therapeutic targets in MS. The p38


https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

**Table 10. Summaries of Findings Reporting the Role of Adenosine in MS[a]**

| S.N. | Study types | MS model | drug and dose | observations | remarks | refs |
|---|---|---|---|---|---|---|
| 1 | *In vivo* | Animal model of EAE (50 $\mu$L) induced MS in mice | CX3CL1 inhibition (4 $\mu$g/mouse) | CX3CL1 inhibition (4 $\mu$g/mouse) can block lymphocyte entrance into the CNS. | Extracellular adenosine may represent an endogenous neuroinflammatory modulator that enhances lymphocyte entrance into the brain. | 195 |
| 2 | *In vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | SCH58261 (2 mg/kg/d) | Neurobehavioral deficits caused by EAE can be alleviated by SCH58261 (2 mg/kg/d). | Elucidating the therapeutic role of the $A_{2A}$ receptor antagonist may enable the use of SCH58261 for the treatment of MS. | 196 |
| 3 | *In vivo* | Animal model of EAE (200 $\mu$L) induced MS in mice | Inosine (1 or 40 mg/kg) | Inosine (1 or 40 mg/kg) may prevent the upregulation of the $A_{2A}$ receptor in the spinal cord. | Inosine may display a therapeutic potential in MS | 197 |
| 4 | *In vivo* | Animal model of EAE (50 $\mu$L) induced MS in mice | Pertussis toxin (20 ng) | The development of EAE was affected by the expression of the $A_{2A}$ receptor in nonimmune cells. | Expression of the A2A receptor on lymphocytes may be crucial in neuroinflammatory responses. | 198 |
| 5 | *In vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | - | $A_{2A}$ receptors may act protectively in EAE. | The $A_{2A}$ receptor may be a target for MS treatment. | 199 |
| 6 | *In vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | CGS21680 (0.01 and 0.05 mg/kg) | The $A_{2A}$ receptor agonist CGS21680 (0.01 and 0.05 mg/kg) can enhance the intracellular calcium content in lymphocytes of murine. | The $A_{2A}$ receptor may be a therapeutic target for MS. | 200 |
| 7 | *In vivo* | Animal model of EAE (200 $\mu$g) induced MS in mice | Saline (100 $\mu$L/mouse) | IL-6 production is affected by the $A_{2B}$ receptor through the phospholipase C$\beta$-protein kinase C pathway. | The A2B receptor may be a potential target for MS treatment. | 203 |
| 8 | *Human study* | Patients with SPMS ($n$ = 8) and control group ($n$ = 7) | - | Brains of patients with SPMS have more adenosine $A_{2A}$ receptors. | [$^{11}$C]TMSX-PET may aid in our understanding of CNS pathophysiology in SPMS. | 201 |
| 9 | *Human study* | Patients with MS ($n$ = 46) and control group ($n$ = 50) | $^{3}$H-DPCPX (120 Ci/mmol) | IL-1$\beta$, IL-6, IL-17, IFN-$\gamma$ and TNF-$\alpha$ are downregulated by adenosine $A_{2A}$ receptor stimulation. | Adenosine $A_{2A}$ receptor agonists may be ~~a~~ new therapeutic agents for the treatment of MS. | 202 |

[a]EAE = Experimental autoimmune encephalomyelitis, IFN-$\gamma$ = Interferon $\gamma$, IL-1$\beta$ = Interleukin 1 beta, IL-6 = Interleukin 6, IL-17 = Interleukin 17, MS = Multiple sclerosis, SPMS = Secondary-progressive multiple sclerosis, TNF-$\alpha$ = tumor necrosis factor alfa.



https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

and phospholipase C$\beta$-protein kinase C pathways are involved in the production of IL-6.[203] Hence, targeting A2B receptor could be a possibly useful tool for MS treatment (Table 10).

Although there is literature evidence on the role of A2A in MS, existing evidence on the implication of A2B in MS pathogenesis is lacking. Hence, further studies should also focus on this molecule and its potential for the development of future treatments.

## 8. DISCUSSION

Neurotransmitters have increasingly gained great interest regarding their role in MS pathophysiology, given their crucial implication in immune responses affecting neuronal functions in the brain.[204] The interaction between the nervous system and immune responses is critical in the pathogenesis of MS. In this context, glutamate and GABA are considered as the main neurotransmitters involved in MS pathophysiology.

First, excitotoxicity mediated by excessive glutamate release is one of the main mechanisms proposed for neurodegeneration in MS. This glutamate-mediated excitotoxicity can be triggered by energy deficiency, oxidative stress, mitochondrial dysfunction, and calcium overload.[205] Thus, targeting glutamate signaling and its receptors is important for future studies in MS treatment. Amantadine and memantine, which are NMDA receptor antagonists, inhibit the pro-inflammatory response in EAE model rats.[206] However, amantadine is not effective in the treatment of MS-related fatigue and causes side effects in MS patients.[207] Also, blockade of NMDA receptor suppressed oxidative stress, reduced inflammation, and inhibited demyelination in EAE models.[35,36] In this context, pharmacological targeting of NMDA receptor signaling may protect neurons against glutamate-mediated toxic effects in MS.

Second, dysregulated interaction between glutamate and GABA may lead to neurodegeneration, resulting in synaptic loss. Targeting glial proteins, including the transporters and receptors of these two neurotransmitters, may be beneficial.[208] The inhibitory effect of GABA on glutamate-mediated excitotoxicity can be used as a novel target in the treatment of MS. Glutamate overexpression and GABA dysregulation in MS may worsen disease progression. Altered brain GABA and glutamate levels and subsequently dysregulation in GABAergic and glutamatergic neurotransmisstion have been associated with central fatigue in MS.[58]

## 9. CONCLUSION

Herein, we aimed to provide a comprehensive overview of the role of neurotransmitters in MS pathophysiology. We discussed evidence from studies using antagonists and/or agonists of neurotransmitters in *in vivo* and *in vitro* models, as well as latest developments in human studies. Altered levels and dysregulation of neurotransmitters have been observed in the pathophysiology of inflammatory diseases of the CNS including MS. Accumulating preclinical and clinical evidence suggests that neurotransmitter targeting via for instance receptor antagonists holds promising potential for future therapeutic approaches in MS. In this context, the regulation of neurotransmitters expression, transporters and receptors are crucial. Neurotransmitter signaling pathways may represent novel targets in the field of MS treatment.

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### Author Contributions

E.A. wrote, edited, reviewed, prepared the original draft, and supervised. B.R.C, F.S.A., and H.S. wrote, edited, reviewed, drew the figures, and prepared the original draft. Ef.A. wrote, edited, and prepared the original draft. All authors discussed the results and revised the paper.

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

Figures 1−3 were created with BioRender.com.

## ABBREVIATIONS:

5-HT, serotonin; 5-HTTLPR, serotonin-transporter-linked polymorphic region; ACh, acetylcholine; AChE, acetylcholinesterase; ADRB2, beta-2 adrenergic receptor; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ARs, adrenergic receptors; BBB, blood−brain barrier; CNS, central nervous system; COMT, catechol-O-methyltransferase; CSF, cerebrospinal fluid; DAT, dopamine transporter; dLNs, draining lymph nodes; DMF, dimethyl fumarate; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; D3R, dopamine D3 receptor; D4R, dopamine D4 receptor; D5R, dopamine D5 receptor; EAAT, excitatory amino acid transporters; EAE, experimental autoimmune encephalomyelitis; eNOS, endothelial nitric oxide synthase; ELT, early life trauma; GABA, gamma-aminobutyric acid; $GABA_A$Rs, $GABA_A$ receptors; GAD, glutamate decarboxylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GM-CSF, granulocyte-macrophage colony-stimulating factor; GNX, ganaxolone; GSNO, S-nitrosoglutathione; GSNOR, S-nitrosoglutathione reductase; H1R, histamine H1 receptor; H2R, histamine H2 receptor; H3R, histamine H3 receptor; H4R, histamine H4 receptor; HNMT, histamine *N*-methyltransferase; IFN-$\beta$, interferon beta; IFN-$\beta$1b, interferon-$\beta$1a; IFN-$\gamma$, interferon gamma; iGluRs, ionotropic glutamate receptors; IL-1$\beta$, interleukin 1 beta; IL-6, interleukin 6; IL-17, interleukin 17; iNOS, inducible nitric oxide synthase; Kainate, 2-carboxy-3-carboxymethyl-4-isopropenyl-pyrrolidine; LC, locus coeruleus; mAChR, muscarinic acetylcholine receptor; MDA, malondialdehyde; mGluRs, metabotropic glutamate receptors;



https://doi.org/10.1021/acschemneuro.2c00589
ACS Chem. Neurosci. 2023, 14, 527−553

MnSOD, manganese superoxide dismutase; MS, Multiple Sclerosis; MT, melatonin receptor; NA, noradrenaline; nAChRs, nicotinic acetylcholine receptors; NBRP3, nucleotide binding domain-like receptor protein 3; NE, norepinephrine; NET, norepinephrine transporter; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; OL, oligodendrocyte; OGR1, ovarian cancer G-protein coupled receptor 1; PBMC, peripheral blood mononuclear cell; PPMS, primary progressive Multiple Sclerosis; PPX, pramipexole; PD-1L, programmed death ligand-1; RRMS, relapsing-remitting Multiple Sclerosis; ROS, reactive oxygen species; SAI, short-latency afferent inhibition; SERT, serotonin transporter; −SH, sulfhydryl; SIRT1, sirtuin 1; SLC1A1, excitatory amino acid transporter 3; SNS, sympathetic nervous system; SPMS, secondary progressive Multiple Sclerosis; SSRIs, selective serotonin reuptake inhibitors; system $Xc^-$, cystine/glutamate antiporter; Th1, T helper 1 cells; Th-17, T helper 17 cells; TLR, toll-like receptor; TNF-$\alpha$, tumor necrosis factor alpha; TPH, tryptophan hydroxylase; Treg, regulatory T cell; TRP, transient receptor potential; TRPA1, transient receptor potential ankyrin 1; VGLUT, vesicular glutamate transporter

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