DOI 10.1007/s11055-020-00953-8
Neuroscience and Behavioral Physiology, Vol. 50, No. 6, July, 2020

The Role of Glutamate in the Pathogenesis of Multiple Sclerosis

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Translated from Zhurnal Nevrologii i Psikhiatrii imeni S. S. Korsakova, Vol. 119, No. 8, Iss. 1, pp. 160–167, August, 2019. Original article submitted July 12, 2018. Accepted September 11, 2018.

Results from studies in recent years have provided evidence that hyperactivation of the glutamatergic system plays an important role in the pathophysiology of multiple sclerosis (MS). Apart from the well known immediate toxic effects of the neurotransmitter glutamate on neurons, additional mechanisms of glutamate-induced cell injury have been described, these including actions of oligodendrocytes, astrocytes, endothelial cells, and immune cells. These toxic effects may open up a link between the various pathological components of MS, such as axon damage, oligodendrocyte death, demyelination, autoimmune reactions, and dysfunction of the blood-brain barrier. Understanding the mechanisms underlying glutamate toxicity in MS may be promoted by the development of new approaches to the diagnosis, treatment, and management of patients with MS. This review presents reports on the mechanisms leading to increases in the concentration of the neurotransmitter glutamate and excitotoxicity in the context of the pathogenesis of the disease. We also present data on drugs and therapeutic approaches, both current and under development, helping to regulate the operation of the glutamatergic system.

Keywords: glutamate, glutamate receptors, glutamate excitotoxicity, NMDA receptors, T cells, multiple sclerosis.

A number of concepts of the bases of the interaction between the nervous and immune systems have been formulated. This interaction is believed to be mediated via the hypothalamo-hypophyseal-adrenal axis, the autonomic (sympathetic and parasympathetic) nervous system, which innervates structures including the lymphoid organs, and circulating cytokines, chemokines, neuropeptides, and neurotransmitters [1]. Research interest in recent years has been focused on neurotransmitters, which apart form functioning in the central nervous system (CNS), also operate as systemic and/or local immunoregulators acting at the level of the receptors of immunocompetent cells, thus mediating connections between systems. Sufficient data have now been collected to show that immune system cells have receptors and associated signal systems components for almost all receptors, neuropeptides, and neurohormones, as well as endogenous ligands such as glutamate (Glu), substance P, and others, which directly regulate the of proliferation, differentiation, apoptosis, and migration processes of immunocompetent cells [2]. It should be noted that the dysregulatory aspects of neuroimmunopathology are of significant interest, as impairments to the mechanisms of the interregulation of the nervous and immune systems cause or make important contributions to the pathogenesis of many neurodegenerative diseases of the CNS, especially diseases with autoimmune-inflammatory components and particularly multiple sclerosis (MS) [3].

Despite significant progress in studies of the pathogenesis of MS, the etiology of the disease remains unclear. The most widely held hypothesis is that MS is a multifactorial disease whose initiation and development involve a critical role for the interaction of genetic and environmental factors. This disease is characterized by the formation of inflammatory plaques in the white matter of the brain and/or spinal cord, with infiltration of immune cells, demyelination of nerve fibers, axon and neuron degeneration, oligodendrocyte death, astrogliosis, and damage to the blood-brain barrier (BBB) [4]. The gray matter of the brain is also involved in the pathogenesis of the disease, and while damage here is not accompanied by these features (presence of immune

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cells and complexes, damage to the BBB), it is associated with the development of clinical symptomatology and neurological deficit [5–7]. Among the mechanisms initiating the disease, the primary role belongs to autoimmune reactions leading to inflammation in the CNS with subsequent demyelination and the death of neurons and oligodendrocytes [8]. In addition, neurodegenerative changes are known to have formed by the early stages of MS and to occur simultaneously with the development of inflammation, inducing atrophy of nerve fibers and neurons and producing increasing disability in patients [9].

The heterogeneity of the pathogenetic processes in MS is evidence for impairment of the link between the nervous and immune systems, whose interaction involves an important role for neurotransmitters. Glutamate is the most important of these, as well as the most intensely studied in recent years. Apart from the well-known immediate excitotoxic effects on neurons, additional mechanisms have now been described for glutamate-induced cell injury, including effects on oligodendrocytes, astrocytes, and endothelial and immune cells [10–12]. Understanding of the processes underlying the impairments to Glu metabolism and the pathophysiological processes mediated by them seen in MS will promote the development of new approaches to the diagnosis and pharmacotherapy of this disease.

Many researchers have now demonstrated increases in Glu to excitotoxic levels in MS, as well as the primary importance of excitotoxicity in the processes of neuron and axon damage and oligodendrocyte death in the brains of patients with MS and animals with experimental autoimmune encephalomyelitis (EAE), an in vivo model of the disease [10, 11, 13]. A number of studies have demonstrated increases in Glu levels in the plasma, cerebrospinal fluid, and brain in patients with MS, depending on the severity, type of course, and phase of illness [11, 14, 15]. Analogous results have been obtained in the EAE Model [16]. It has been suggested that the excitotoxic function of Glu will become dominant in the chronic phase of MS, when neurodegenerative processes become dominant, inducing further progression of disease [17]. A significant number of mechanisms leading to increased Glu levels in MS have now been described [5, 12].

Potential Sources and Mechanisms of Formation of Excess Glu in MS. The literature contains extensive data providing evidence that increases in extracellular Glu levels may result from changes in neurotransmitter homeostasis. In this regard, researchers' attention has been focused on studies of the activity of the enzymes of Glu metabolism and its protein transporters, as well as other factors affecting transmitter homeostasis in MS and EAE [12, 13]. Increases in transmitter concentrations, as noted by various authors, may result from dysfunction of activated astrocytes. In physiological conditions, these cells regulate the removal of Glu from the synaptic cleft and its utilization using the enzymes glutamate dehydrogenase and glutamine synthase – which have extremely low activity levels in inflammatory plaques in EAE [18]. Increased levels of glutaminase expression have been found in foci in patients, mainly those with the primary and secondary progressive forms of MS [18]. Decreases in glutamine synthase activity in the course of the mechanisms of inflammation may also influence changes in Glu metabolism and increases in Glu levels in EAE [19]. Several mechanisms are now known linking astrocytes and glutamate excitotoxicity in MS [12]. Activation of the microglia induces production of adenosine triphosphate, which activates metabotropic purinergic P2Y1 receptors on astrocytes, provoking Glu release. It has also been suggested that excessive transmitter formation in the brain occurs under the influence of proinflammatory cytokines (interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α)) in MS, by means of an effect on the expression of its astrocyte transporter proteins. In addition, Glu itself may increase its synthesis via metabotropic glutamate receptors on astrocytes.

Immunohistochemical studies of demyelination plaques in MS have demonstrated a lack of enzyme (glutamate dehydrogenase and glutamine synthase) activity and the expression of Glu transporters (EAAT1 and EAAT2) in oligodendrocytes in active foci and the surrounding areas [18]. Data obtained in the EAE model also support reductions in EAAT1 activity in the spinal cord and cerebellum in the acute phase of disease [20, 21]. Thus, oligodendrocytes in damaged areas are in fact unable to metabolize Glu quickly, this decreasing the ability of the white matter to maintain nontoxic extracellular Glu concentrations and contributing to the propagation of excitotoxic damage. Possible causes of enzyme deficiencies in oligodendrocytes may include proinflammatory cytokines and reactive oxygen species. In particular, histamine synthase has been shown to be sensitive to oxidative injury [22]. Decreases in EAAT1 protein content can be provoked by massive death of oligodendrocytes, which is seen mainly in the exacerbation phase of neurological symptoms [23].

A potential source of excess Glu in MS may be demyelinated axons, as indicated by the observation in active foci of demyelination of accumulations of and changes in the locations of pore-forming α1B subunits of voltage-gated Ca^{2+} channels in damaged axons (integration into the membrane facing the axoplasm) [24]. In health, channels of this type are located in presynaptic terminals and are involved in processes of vesicular transport of transmitter. This ectopic distribution of calcium channels may result in increases in calcium influx into cells, which leads to Glu release [25]. In addition, a diffuse distribution of Na^+ channels ($Na_v1.2$) on the bare axolemma is seen after demyelination, increasing intracellular Na^+ levels and activation of the Na^+/Ca^{2+} ion exchanger [26]. In these conditions, the Na^+-dependent glutamate transporter system starts to mediate reverse transport of Glu from the cell cytoplasm into the intercellular space, which is accompanied by a further decrease in its reuptake due to lack of a sufficient Na^+ gradient. The tissues

of MS patients show increased expression of $Na_v1.6$ sodium channels on damaged axons and in inflammatory foci with T-cell infiltrates and activated microglia. Published data indicate that Na^+ contents are elevated in the brains of patients with MS, mainly in plaque lesions in the white matter [12].

It is known that Glu barely penetrates the BBB, which creates a significant concentration gradient of transmitter between the blood flow and the extracellular space in the CNS. The inflammation-damaged BBB in MS has been shown to promote penetration of large quantities of Glu from the blood into the CNS [12]. Furthermore, the endothelial cells forming the BBB express glutaminase and transmitter receptors and contain a system of Na^+-dependent Glu transporter proteins (EAAT1), which is evidence for their active participation in transmitter metabolism and increases in its level in MS [5].

Accumulations of immune cells (lymphocytes, dendritic cells, macrophages, microglia) are a characteristic sign of active neuroinflammatory plaques in the CNS in MS. Activated immune cells and microglia are seen the immediate vicinity of damaged axons and neurons; furthermore, the extent of axon damage correlates with the density of these cells in the plaque, pointing to the direct involvement of excitotoxicity in axon injury in MS [2, 18]. In inflammation, these cells, under the influence of Glu, release significant quantities of Glu via connexins, i.e., proteins forming intercellular gap junctions [27] or via the cysteine/glutamate exchanger transport system [28, 29]. In addition, it has been noted that decreases in the expression of Glu transporters EAAT1 and EAAT2 by glial cells can be induced by proinflammatory cytokines (TNF-α and IL-1β) and may correlate with glial cell activation in the white matter of the brain or with activation of transmitter receptors, also leading to increases in the Glu concentration [12]. Increased nitric oxide (NO) synthesis is seen in MS, due to increase expression of NO sythase in the microglia, which leads to the formation of peroxynitrite, which inactivates Glu transporters [30]. Studies have shown that TNF-α can stimulate Glu release by the microglia via an autocrine mechanism and also via regulation of glutaminase activity. Proinflammatory and glutamate-mediated neurotoxic microglial reactions can be induced by necrotic neurons themselves via the MyD88-dependent pathway using Toll-like receptors, which mediate increases glutaminase activity [31]. A number of studies appeared not long ago, suggesting that lymphocytes penetrating into the CNS can form excess quantities of Glu. Thus, it has been reported that myelin-reactive $CD4^+$ T-lymphocytes in demyelination plaques, i.e., Th17 cells, can interact directly with nerve cells without involving T-cell receptors [32]. Strong local variations in intracellular Ca^{2+} concentrations occur in neurons during such contacts, leading to significant increases in extracellular Glu levels. The interaction of Th17 with neurons in demyelination foci has also been shown to be accompanied by increased axon injury in EAE. Existing published data also provide evidence that Glu is involved in the mechanisms of neuroinflammation mediated by, among other factors, regulation of the various functions of T-cells [10].

Glutamate Receptors. Along with impaired Glu homeostasis, changes in neurotransmitter receptor activity may contribute to mediating the mechanisms of excitotoxic injury. It is important to emphasize that different types of glutamate receptor have been identified in the peripheral nervous system and in non-neuronal cells in humans, where their role remains to be definitively established [10, 33, 34]. Two types of glutamate receptors are known: metabotropic and ionotropic.

Metabotropic Glu receptors (mGluR) are coupled with G-proteins and modulate synaptic transmission via intracellular second messengers. Eight subtypes have thus far been identified (mGluR1-mGluR8), which, depending on amino acid similarity, signal transduction mechanism, and pharmacological properties, are divided into three groups. mGluR1 and mGluR5 form group I metabotropic Glu receptors (mGluR1), linked with the IP3/Ca^{2+}-dependent signal transmission system. Activation of group II mGluR (mGluR2 and mGluR3) and group III mGluR (mGluR4, mGluR6, mGluR7, and mGlu8) leads to inhibition of the reaction cascade linked with cAMP formation. Group I metabotropic Glu receptors function in the postsynaptic membrane. Changes in the pattern of their expression are seen in MS, both within and around plaque lesions in the white matter. Studies of post-mortem specimens of brains from patients with MS have demonstrated an axonal location for mGluR I in areas of active demyelination, as well as in undamaged white matter, in contrast to control specimens, where mGluR mainly had a somatodendritic location [35]. The literature contains data on the ability of mGluR I receptors to modulate the operation ionotropic receptors, affecting Ca^{2+} release from the endoplasmic reticulum and the functioning of voltage-gated Ca^{2+} channels [36]. Results from a small number of studies using the EAE model have demonstrated that GluR I have both pro- and antitoxic properties [37, 38]. Metabotropic Glu receptors of groups II and III are located on the presynaptic membrane and control transmitter release into the synaptic cleft via inhibition of voltage-gated Ca^{2+} channels. Elevated mGluR II expression is seen on the surfaces of astrocytes and activated microglia in MS [35]. Experimental data provide evidence that stimulation of mGluR2 provokes neurotoxicity, while stimulation of mGluR3 provides neuroprotection [39]. Thus, simultaneous inhibition of mGluR2 and activation of mGluR3 can prevent myelin-induced microglial neurotoxicity, as demonstrated in work reported by Pinteaux-Jones et al. [40]. Particular attention is paid to the role of mGluR4 in both neurodegenerative and neuroinflammatory processes in MS. Experiments on mice with knockout of the mGluR4 gene demonstrated that this receptor subtype influences the onset of EAE and its course. mGluR4-knockout mice showed significantly increased lymphocyte infiltration of

the spinal cord as compared with wild type. There was also a displacement in the balance between Th17 and Treg cells towards the Th17 subpopulation, which is involved in maintaining inflammation and exacerbating the clinical signs of MS [41]. The high levels of expression of group III receptors (especially mGluR4 and mGluR8) seen in MS in foci are regarded as a protective-type reaction.

Ionotropic Glu receptors (iGluR), which are ligand-controlled ion channels, are divided into three subtypes depending on which selective agonist (synthetic glutamic acid analog) they interact with: 2-amino-3(3-hydroxy-5-methylisoxazol-4-yl)propionic acid receptors (AMPAR), kainate (KA) receptors, and N-methyl-D-aspartate receptors (NMDAR). All ionotropic receptors are integral proteins, which consist of subunits forming the ion channel. All types of iGluR, depending on the subunit composition, show selectivity for alkali metal cations (mainly Na^+ and K^+), and also Ca^{2+} [42]. AMPAR are homo- or heterooligomers consisting of GluA1–GluA4 subunits. In the CNS, these receptors mediate rapid voltage-gated excitation of neurotransmission and are expressed on oligodendrocytes, astrocytes, and neurons. Immunostaining with antibodies to the GluA2 subunit showed that in MS, oligodendrocytes in plaque lesions and their surrounding areas form AMPAR without GluA2, making them more sensitive to excitotoxicity and death due to intracellular Ca^{2+} overload [43]. The extreme sensitivity of oligodendrocytes to AMPAR-mediated excitotoxicity has been confirmed by data obtained by different groups of authors [5]. KA receptors are heterotetramers (GluK1–5). In contrast to AMPAR and NMDAR, KA receptors are located on post- and presynaptic membranes. Functionally active KA receptors have been seen on the surfaces of oligodendrocytes, where they can induce increases in intracellular Ca^{2+} to toxic levels, resulting in cell death [44]. In addition, Glu, activating KA receptors, increases the sensitivity of oligodendrocytes to the damaging action of components of the complement system, which are detected in the cerebrospinal fluid and CNS tissues in MS [45]. The mechanism of axon degeneration in MS can be triggered by GluK1- and GluK2-containing KA receptors located directly on axons themselves [46]. KA receptors located on endothelial cells of capillaries and blood vessels play no small role in BBB dysfunction [43]. NMDAR are of particular interest in connection with their role in controlling such important processes as neuron growth and development, the formation and maintenance of synaptic plasticity, and others. Functional NMDAR are heterotetrameric complexes consisting of three types of subunit, which contain different regulatory sites: two constitutive GluN1 and the combinations GluN2(A–D) or GluN3(A–B). it is important to note that the combination of subunits determines the biochemical and pharmacological properties of NMDAR [47, 48]. In the resting state, Mg^{2+} blocks the ion channel of the receptor, interacting with the voltage-controlled Mg^{2+}-binding part, preventing other ions from passing through the channel. Receptor activation depolarizes neuron membranes, leading to reductions in the affinity of Mg^{2+} for NMDAR channels and opening of channels to allow passage of Na^+ and Ca^{2+} ions into the cell [42]. Thus, the effectiveness of Mg^{2+} blockade may have significant importance in the processes of axon injury and neuron dysfunction in MS and EAE, as it is known that NMDAR are among the main Ca^{2+} channels in these cells [49]. The detection of Glu receptors of this subtype in other CNS cells provides additional support for the involvement of NMDAR in the pathological processes seen in MS. Wong et al. demonstrated the presence of functionally active NMDA-type Glu receptors on oligodendrocytes [50]. Some authors have noted that NMDAR on oligodendrocytes are less sensitive to Mg^{2+} blockade, so they are more susceptible to the toxic actions of high Glu concentrations [51]. NMDAR GluN1 subunits on endotheliocytes regulate signal transduction mediated by tissue plasminogen activator and control the penetration of monocytes across the BBB [52]. According to current data, T cells express different types of Glu receptors, including NMDAR [10, 34]. Study results have shown that NMDA Glu receptors have roles in regulating cytokine secretion by T cells and the mechanisms of differentiation of $CD4^+$ T-lymphocyte subpopulations, controlling the cell cycle, proliferation and apoptosis, changes in the cell membrane potential, modulation of the activity of ion channels, calcium homeostasis in T-cells, the expression of genes increasing free radical formation, and integrin-mediated adhesion to extracellular matrix glycoproteins. However, the question of the role of NMDAR in controlling the functions of immunocompetent cells in MS patients remains poorly studied [10]. The few published data and results from our own studies show various features of T-cell responses mediated by blockade of NMDAR in MS [10]. It has been suggested that a possible molecular mechanism mediating such a wide spectrum of NMDAR functions in T-cells comes from the involvement of this type of glutamate receptor in the mechanisms of calcium signaling [53]. It is important to emphasize that the literature contains data providing evidence that one possible cause of the development of the autoimmune process in MS may be dysregulation of calcium homeostasis [54].

Glu and Glu Receptors as Therapeutic Targets in MS. To date, most attention in studies of the pathogenesis of MS has been paid to inflammation and autoimmune reactions, such that the primary strategy is correction of impairments to the functions of immunocompetent cells. New data on pathological changes in MS, including in the gray matter of the brain, taken along with the known mechanisms of the involvement of Glu and excitotoxicity in neurodegeneration and demyelination, have led to a new wave of interest among researchers into this neurotransmitter and its receptors as potential targets for the pharmacotherapeutic correction of MS. One of the potential and pathogenetically grounded directions consists of actions on the glutamatergic neurotransmission system.

Studies addressing the effects of widely used drugs – interferon-β (IFN-β) and glatiramer acetate – on Glu metabolism and the activity of its receptors showed the following. IFN-β decreases excitatory postsynaptic currents in the striatum – the structure most sensitive to degeneration when MS progresses. This drug has been shown to act on Glu receptors by negative regulation of the activation of Ca^{2+}/calmodulin-dependent protein kinase II and the Ca^{2+} concentration, preventing decreases in the tolerance of excitatory synapses and, thus, leading to decreases in excitotoxicity [55]. Studies using the EAE model showed that glatiramer acetate eliminates changes in glutamate-mediated postsynaptic currents induced by TNF-α in the striatum of the experimental animals. The neuroprotective effect of glatiramer acetate in glutamate toxicity is, as suggested, due to the negative influence of the drug on activation of the microglia and TNF-α production in the gray matter of the brain in EAE. A similar mechanism has been proposed to exist in MS too [10].

It is not long since the new oral drugs fingolimod, laquinimod, and dimethylfumarate attracted the attention of MS researchers. Landi et al. [56] showed that fingolimod, which regulates the operation of sphingosine-1-phosphate receptors, apart from its main immunomodulatory action on peripheral lymphocytes, has neuromodulatory activity. It selectively decreases glutamate-mediated intracortical arousability, with negative effects on Glu excitotoxicity. In addition, studies using the EAE model have shown that fingolimod reverses presynaptic and postsynaptic changes in glutamatergic transmission and promotes the recovery of dendrites, probably due to suppression of microglial activation. It was also established that both the therapeutic and prophylactic use of laquinimod in the EAE model improves motor functions, decreases inflammation in the CNS, and increases the quantity of myelinated axons. Dimethylfumarate, which is metabolized to the pharmacologically active monomethylfumarate and fumarate, increases the activity of transcription factor Nrf2, which regulates the expression of genes encoding protein products which protect cells from oxidants, electrophiles, and genotoxic compounds. Monomethylfumarate also inhibits Glu release by pathogenic Th17 lymphocytes. Dimethylfumarate is currently being studied as a substance for the treatment of MS [57].

Hyperactivation of Glu receptors is undoubtedly the main determinant of neurodegenerative processes in MS and EAE, and pharmacologically active agents able to regulate their operation and expression can have protective influences in conditions of excitotoxicity. In fact, the results obtained form many studies using different glutamate receptor ligands provide evidence of decreases in the manifestations of the inflammatory process and pathomorphological changes in EAE. It should be noted that pharmacological blockade of Glu receptors has limited clinical use, as they play a vitally important role in maintaining normal synaptic transmission and their full blockade can lead to many unfavorable side effects. Thus, investigators are currently focused on the search for agents preventing the overactivation of ionotropic or metabotropic Glu receptors without affecting their basic functions. Thus, mGluR II and III agonists have been shown to have protective effects, decreasing presynaptic Glu release, while agonists of group I can induce excitotoxicity. We note that in 2015, metabotropic Glu receptors were validated as pharmacological targets for the treatment of MS [55]. Blockade of Glu receptors using the competitive AMPA/KA receptor antagonist NBQX has been shown to decrease neurological deficit, which is histologically apparent as decreased axon injury and decreased oligodendrocyte death. Other AMPAR antagonists are also effective in decreasing neurological symptoms and morphological changes in animals with EAE [10, 12]. Ganor et al. [58] demonstrated that blockade of AMPAR expressed on autoreactive T-lymphocytes prevented their activation by Glu, thus decreasing the pathogenic potential of the cells. Cytokine-mediated effects can be blocked by AMPAR antagonists. KA Glu receptor antagonists such as CNQX, DNQX, etc., have antiexcitotoxic activity [55]. Research results have also shown that NMDAR antagonists have protective actions against excitotoxicity. Pharmacological inhibition of NMDAR by amantadine and memantine, along with suppression of neurological symptoms in rats with EAE, decreases the expression of anti-inflammatory cytokines in animals' brains. Riluzole, a noncompetitive NMDAR antagonist, has ben shown to prevent receptor hyperactivity and to inhibit Glu release from nerve and immune cells [10, 12].

There is currently particular interest in the regulation of CNS function by the immune system due to production of autoantibodies to the subunits of neurotransmitter receptors. On the one hand, these antibodies may be one of the causes for the development of neuroimmunopathology in the CNS, such as anti-NMDAR encephalitis [59, 60]. On the other hand, they may be endogenous agents involved in the pathogenetic mechanisms and have protective properties against, for example, the excitotoxic action of Glu. This may be supported by the anti-NMDAR antibody glunomab, developed by Macrez et al. [60] and studied in EAE, whose target is the regulatory site of the GluN1 receptor subunit, which is sensitive to the serine protease tissue plasminogen activator. This antibody has been reported to have a modulatory effect on NMDAR, without affecting its baseline activity. Glunomab has been shown to the decrease the transmission of human leukocytes in an in vitro model of the BBB and, in EAE, to block progression of neurological lesions and decrease leukocyte infiltration, which the authors felt was linked with normalization of BBB function [60].

In addition, one novel therapeutic approach consists of decreasing the level of transmitter in the CNS by "pumping" it out of the brain into the blood. The blood Glu concentration is reduced by intravenous injection of enzymes such as aspartate aminotransferase to convert it to an inactive form. Despite the fact that this technology has yet to be

validated in EAE and MS, it has potential, as it can decrease the adverse effects induced by excessive Glu [61, 62].

Overall, published data indicate that Glu may be the binding element for all primary pathogenic events in MS, such as autoimmune reactions, inflammation, demyelination, and neurodegeneration. Thus, studies of these process and the roles of Glu in them will improve diagnosis and prognosis of the course of illness, the development of new therapeutic strategies, and the creation of effective drugs for the treatment of MS.

The authors have no conflicts of interest.

REFERENCES

1. R. Dantzer, "Neuroimmune interactions: From the brain to the immune system and vice versa," *Physiol. Rev.*, **98**, No. 1, 477–504 (2018), https://doi.org/10.1152/physrev.00039.2016.
2. M. Levite, "Neurotransmitters activate T-cells and elicit crucial functions via neurotransmitter receptors," *Curr. Opin. Pharmacol.*, **8**, No. 4, 460–471 (2008), https://doi.org/10.1016/j.coph.2008.05.001.
3. S. Talbot, S. L. Foster, and C. J. Woolf, "Neuroimmunity: Physiology and Pathology," *Annu. Rev. Immunol.*, **34**, 421–447 (2016), https://doi.org/10.1146/annurev-immunol-041015-055340.
4. T. E. Shmidt and N. N. Yakhno, *Multiple Sclerosis. A Guide for Doctors*, MEDpress-inform, Moscow (2016), 5th ed.
5. C. Rajda, D. Pukoli, Z. Bende, et al., "Excitotoxins, mitochondrial and redox disturbances in multiple sclerosis," *Int. J. Mol. Sci.*, **18**, No. 2, 353 (2003), https://doi.org/10.3390/ijms18020353.
6. M. Prins, E. Schul, J. Geurts, et al., "Pathological differences between white and grey matter multiple sclerosis lesions," *Ann. N. Y. Acad. Sci.*, **1351**, 99–113 (2015), https://doi.org/10.1111/nyas.12841.
7. M. Calabrese, R. Magliozzi, O. Ciccarelli, et al., "Exploring the origins of grey matter damage in multiple sclerosis," *Nat. Rev. Neurosci.*, **16**, 147–158 (2015), https://doi.org/10.1038/nrn3900.
8. J. M. Frischer, S. Bramow, A. Dal-Bianco, et al., "The relation between inflammation and neurodegeneration in multiple sclerosis brains," *Brain*, **132**, 1175-1189 (2009), https://doi.org/10.1093/brain/awp070.
9. A. N. Boiko, S. V. Petrov, V. A. Nesterova, and E. I. Gusev, "Mechanisms of development of neurodegenerative processes in multiple sclerosis: neuroprotective influence of beta-interferons today and neurotrophic factors tomorrow," *Zh. Nevrol. Psikhiat. (spec. iss.)*, No. 3, 83–98 (2003).
10. M. Levite, "Glutamate, T cells and multiple sclerosis," *J. Neural Transm. (Vienna)*, **124**, No. 7, 775–798 (2003), https://doi.org/10.1007/s00702-016-1661-z.
11. I. R. Stojanovic, M. Kostic, and S. Ljubisavljevic, "The role of glutamate and its receptors in multiple sclerosis," *J. Neural. Transm. (Vienna)*, **121**, 945–955 (2014), https://doi.org/10.1007/s00702-014-1188-0.
12. R. Macrez, P. K. Stys, D. Vivien, et al., "Mechanisms of glutamate toxicity in multiple sclerosis: biomarker and therapeutic opportunities," *Lancet Neurol.*, **15**, No. 10, 1089–1102 (2016), https://doi.org/10.1016/S1474-4422(16)30165-X.
13. M. Kostic, N. Zivkovic, and I. Stojanovic, "Multiple sclerosis and glutamate excitotoxicity," *Rev. Neurosci.*, **24**, No. 1, 71–88 (2013), https://doi.org/10.1515/revneuro-2012-0062.
14. J. F. Stover, U. E. Pleines, M. C. Morganti-Kossmann, et al., "Neurotransmitters in cerebrospinal fluid reflect pathological activity," *Eur. J. Clin. Invest.*, **27**, 1038–1043 (1997).
15. R. Srinivasan, N. Sailasuta, R. Hurd, et al., "Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T," *Brain*, **128**, 1016–1025 (2005), https://doi.org/10.1093/brain/awh467.
16. D. Pitt, P. Werner, and C. S. Raine, "Glutamate excitotoxicity in a model of multiple sclerosis," *Nat. Med.*, **6**, 67–70 (2000), https://doi.org/10.1038/71555.
17. I. A. Zavalishin and A. V. Peresedova, "Multiple sclerosis: current concepts of pathogenesis and pathogenetic therapy," *Ann. Klinich. Eksperim. Nevrol.*, **1**, No. 1, 32–40 (2007).
18. P. Werner, D. Pitt, and C. S. Raine, "Multiple sclerosis: altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage," *Ann. Neurol.*, **50**, 169–180 (2003).
19. A. Castegna, L. Palmieri, I. Spera, et al., "Oxidative stress and reduced glutamine synthetase activity in the absence of inflammation in the cortex of mice with experimental allergic encephalomyelitis," *Neuroscience*, **185**, 97–105 (2003), https://doi.org/10.1016/j.neuroscience.2011.04.041.
20. M. Ohgoh, T. Hanada, T. Smith, et al., "Altered expression of glutamate transporters in experimental autoimmune encephalomyelitis," *J. Neuroimmunol.*, **125**, 170–178 (2002).
21. K. Mitosek-Szewczyk, G. Sulkowski, Z. Stelmasiak, and L. Struzyńska, "Expression of glutamate transporters GLT-1 and GLAST in different regions of rat brain during the course of experimental autoimmune encephalomyelitis," *Neuroscience*, **155**, 45–52 (2003), https://doi.org/10.1016/j.neuroscience.2008.05.025.
22. E. R. Stadtman, "Protein oxidation and aging," *Science*, **257**, 1220–1224 (1992), https://doi.org/10.1080/10715760600918142.
23. M. Domercq, M. V. Sanchez-Gomez, C. Sherwin, et al., "System xc- and glutamate transporter inhibition mediates microglial toxicity to oligodendrocytes," *J. Immunol.*, **178**, 6549–6556 (2007).
24. B. Kornek, M. K. Storch, J. Bauer, et al., "Distribution of a calcium channel subunit in dystrophic axons in multiple sclerosis and experimental autoimmune encephalomyelitis," *Brain*, **124**, 1114–1124 (2003).
25. S. Doyle, D. B. Hansen, J. Vella, et al., "Vesicular glutamate release from central axons contributes to myelin damage," *Nat. Commun.*, **9**, No. 1, 1032 (2018), https://doi.org/10.1038/s41467-018-03427-1.
26. M. J. Craner, J. Newcombe, J. A. Black, et al., "Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na^+/Ca^{2+} exchanger," *Proc. Natl. Acad. Sci. USA*, **101**, 8168–8173 (2004), https://doi.org/10.1073/pnas.0402765101.
27. I. Yawata, H. Takeuchi, Y. Doi, et al., "Macrophage-induced neurotoxicity is mediated by glutamate and attenuated by glutaminase inhibitors and gap junction inhibitors," *Life Sci.*, **82**, 1111–1116 (2008), https://doi.org/10.1016/j.lfs.2008.03.010.
28. O. Pampliega, M. Domercq, F. N. Soria, et al., "Increased expression of cystine/glutamate antiporter in multiple sclerosis," *J. Neuroinflammation*, **8**, 63 (2011), https://doi.org/10.1186/1742-2094-8-63.
29. K. S. Evonuk, B. J. Baker, R. E. Doyle, et al., "Inhibition of system Xc-transporter attenuates autoimmune inflammatory demyelination," *J. Immunol.*, **195**, 450–463 (2015), https://doi.org/10.4049/jimmunol.1401108.
30. C. Szabó, H. Ischiropoulos, and R. Radi, "Peroxynitrite: biochemistry, pathophysiology and development of therapeutics," *Nat. Rev. Drug Discov.*, **6**, 662–680 (2007), https://doi.org/10.1038/nrd2222.
31. T. F. Pais, C. Figueiredo, Peixoto, et al., "Necrotic neurons enhance microglial neurotoxicity through induction of glutaminase by a MyD88-dependent pathway," *J. Neuroinflammation*, **5**, 43 (2008), https://doi.org/10.1186/1742-2094-5-43.
32. V. Siffrin, H. Radbruch, R. Glumm, et al., "In vivo imaging of partially reversible th17 cell-induced neuronal dysfunction in the course of encephalomyelitis," *Immunity*, **33**, 424–436 (2010), https://doi.org/10.1016/j.immuni.2010.08.018.
33. M. Julio-Pieper, P. J. Flor, T. G. Dinan, and J. F. Cryan, "Exciting times beyond the brain: metabotropic glutamate receptors in periph-

eral and non-neural tissues," *Pharmacol. Rev.*, **63**, No. 1, 35–58 (2011), https://doi.org/10.1124/pr.110.004036.
34. A. A. Boldyrev, E. A. Bryushkova, and E. A. Vladychenskaya, "NMDA receptors in immune system cells," *Biokhimiya*, **767**, No. 2, 128–134 (2012).
35. J. J. Geurts, G. Wolswijk, L. Bo, et al., "Altered expression patterns of group I and II metabotropic glutamate receptors in multiple sclerosis," *Brain*, **126**, 1755–1766 (2003), https://doi.org/10.1093/brain/awg179.
36. E. Nanou, A. Kyriakatos, P. Kettunen, and A. El Manira, "Separate signalling mechanisms underlie mGluR1 modulation of leak channels and NMDA receptors in the network underlying locomotion," *J. Physiol.*, **587**, 3001–3008 (2009), https://doi.org/10.1113/jphysiol.2009.172452.
37. F. Fazio, S. Notartomaso, E. Aronica, et al., "Switch in the expression of mGlu1 and mGlu5 metabotropic glutamate receptors in the cerebellum of mice developing experimental autoimmune encephalomyelitis and in autoptic cerebellar samples from patients with multiple sclerosis," *Neuropharmacology*, **55**, 491–499 (2008), https://doi.org/10.1016/j.neuropharm.2008.06.066.
38. G. Sulkowski, B. Dabrowska-Bouta, B. Kwiatkowska-Patzer, and L. Struzyńska, "Alterations in glutamate transport and group I metabotropic glutamate receptors in the rat brain during acute phase of experimental autoimmune encephalomyelitis," *Folia Neuropathol.*, **47**, 329–337 (2009).
39. D. L. Taylor, L. T. Diemel, M. L. Cuzner, and J. M. A. Pocock, "Activation of group II metabotropic glutamate receptors underlies microglial reactivity and neurotoxicity following stimulation with chromogranin, a peptide up-regulated in Alzheimer's disease," *J. Neurochem.*, **82**, 1179–1191 (2002).
40. F. Pinteaux-Jones, I. G. Sevastou, V. A. Fry, et al., "Myelin-induced microglial neurotoxicity can be controlled by microglial metabotropic glutamate receptors," *J. Neurochem.*, **106**, 442–454 (2008), https://doi.org/10.1111/j.1471-4159.2008.05426.x.
41. F. Fallarino, C. Volpi, F. Fazio, et al., "Metabotropic glutamate receptor-4 modulates adaptive immunity and restrains neuroinflammation," *Nat. Med.*, **16**, 897–902 (2010), https://doi.org/10.1038/nm.2183.
42. S. F. Traynelis, L. P. Wollmuth, C. J. McBain, et al., "Glutamate receptor ion channels: structure, regulation, and function," *Pharmacol. Rev.*, **62**, 405–496 (2010), https://doi.org/10.1124/pr.109.002451.
43. J. Newcombe, A. Uddin, R. Dove, et al., "Glutamate receptor expression in multiple sclerosis lesions," *Brain Pathol.*, **18**, No. 1, 52–61 (2003), https://doi.org/10.1111/j.1750-3639.2007.00101.x.
44. E. Alberdi, M. V. Sanchez-Gomez, A. Marino, and C. Matute, "Ca^{2+} influx through AMPA or kainate receptors alone is sufficient to initiate excitotoxicity in cultured oligodendrocytes," *Neurobiol. Dis.*, **9**, 234–243 (2002), https://doi.org/10.1006/nbdi.2001.0457.
45. E. Alberdi, M. V. Sanchez-Gomez, I. Torre, et al., "Activation of kainate receptors sensitizes oligodendrocytes to complement attack," *J. Neurosci.*, **26**, 3220–3228 (2006), https://doi.org/10.1523/JNEUROSCI.3780-05.2006.
46. M. Ouardouz, E. Coderre, G. W. Zamponi, et al., "Glutamate receptors on myelinated spinal cord axons: II. AMPA and GluR5 receptors," *Ann. Neurol.*, **65**, No. 2, 160–166 (2009), https://doi.org/10.1002/ana.21539 .
47. S. Pachernegg, N. Strutz-Seebohm, and M. Hollmann, "GluN3 subunit-containing NMDA receptors: not just one-trick ponies," *Trends Neurosci.*, **35**, 240–249 (2012), https://doi.org/10.1016/j.tins.2011.11.010.
48. G. E. Hardingham and H. Bading, "Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders," *Nat. Rev. Neurosci.*, **11**, No. 10, 682–696 (2003), https://doi.org/10.1038/nrn2911.
49. H. Bading, M. M. Segal, N. J. Sucher, et al., "N-methyl-D-aspartate receptors are critical for mediating the effects of glutamate on intracellular calcium concentration and immediate early gene expression in cultured hippocampal neurons," *Neuroscience*, **64**, No. 3, 653–664 (1995).
50. R. Wong, "NMDA receptors expressed in oligodendrocytes," *Bioessays*, **28**, 460–464 (2003), https://doi.org/10.1002/bies.20402.
51. R. Karadottir, P. Cavelier, L. H. Bergersen, and D. Attwell, "NMDA receptors are expressed in oligodendrocytes and activated in ischaemia," *Nature*, **438**, 1162–1166 (2005), https://doi.org/10.1038/nature04302.
52. A. Reijerkerk, G. Kooij, S. M. A. Van Der Pol, et al., "The NR1 subunit of NMDA receptor regulates monocyte transmigration through the brain endothelial cell barrier," *J. Neurochem.*, **113**, No. 2, 447–453 (2010), https://doi.org/10.1111/j.1471-4159.2010.06598.x.
53. L. F. Zainullina, R. S. Yamidanov, V. A. Vakhitov, and Yu. V. Vakhitova, "NMDA receptors – possible components of the depot-dependent influx of Ca^{2+} into human T-lymphocytes," *Biokhimiya*, **76**, No. 11, 1517–1524 (2011), https://doi.org/10.1134/S0006297911110034.
54. J. H. Izquierdo, F. Bonilla-Abadia, C. A. Cañas, and G. J. Tobón, "Calcium, channels, intracellular signaling and autoimmunity," *Reumatol. Clin.*, **10**, No. 1, 43–47 (2014), https://doi.org/10.1016/j.reuma.2013.05.008.
55. M. M. Shafaroudi, H. Zarei, A. M. Shafaroudi, et al., "The relationship between glutamate and multiple sclerosis," *J. Int. Biol. Biomed.*, **4**, No. 1, 1–13 (2003), http://ibbj.org/article-1-148-en.html (2003). Accessed June 25, 2018.
56. D. Landi, S. Vollaro, G. Pellegrino, et al., "Oral fingolimod reduces glutamate-mediated intracortical excitability in relapsing-remitting multiple sclerosis," *Clin. Neurophysiol.*, **126**, 165–169 (2015), https://doi.org/10.1016/j.clinph.2014.05.031.
57. D. Luchtman, R. Gollan, E. Ellwardt, et al., "In vivo and in vitro effects of multiple sclerosis immunomodulatory therapeutics on glutamatergic excitotoxicity," *J. Neurochem.*, **136**, 971–980 (2016), https://doi.org/10.1111/jnc.13456.
58. Y. Ganor and M. Levite, "The neurotransmitter glutamate and human T cells: glutamate receptors and glutamate-induced direct and potent effects on normal human T cells, cancerous human leukemia and lymphoma T cells, and autoimmune human T cells," *J. Neural. Transm. (Vienna)*, **121**, No. 8, 983–1006 (2014), https://doi.org/10.1007/s00702-014-1167-5.
59. T. Vyshkina and B. Kalman, "Autoantibodies and neurodegeneration in multiple sclerosis," *Lab. Invest.*, **88**, 796–807 (2008), https://doi.org/10.1038/labinvest.2008.53.
60. R. Macrez, M. C. Ortega, I. Bardou, et al., "Neuroendothelial NMDA receptors as therapeutic targets in experimental autoimmune encephalomyelitis," *Brain*, **139**, 2406–2419 (2003), https://doi.org/10.1093/brain/aww172.
61. A. Leibowitz, M. Boyko, Y. Shapira, and A. Zlotnik, "Blood glutamate scavenging: insight into neuroprotection," *Int. J. Mol. Sci.*, **13**, 10041–10066 (2012), https://doi.org/10.3390/ijms130810041.
62. Y. Li, X. Hou, Q. Qi, et al., "Scavenging of blood glutamate for enhancing brain-to-blood glutamate efflux," *Mol. Med. Rep.*, **9**, 305–310 (2003), https://doi.org/10.3892/mmr.2013.1793.