Synaptopathy connects inflammation and neurodegeneration in multiple sclerosis

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Abstract | Multiple sclerosis (MS) has long been regarded as a chronic inflammatory disease of the white matter that leads to demyelination and eventually to neurodegeneration. In the past decade, several aspects of MS pathogenesis have been challenged, and degenerative changes of the grey matter, which are independent of demyelination, have become a topic of interest. CNS inflammation in MS and experimental autoimmune encephalomyelitis (EAE; a disease model used to study MS in rodents) causes a marked imbalance between GABAergic and glutamatergic transmission, and a loss of synapses, all of which leads to a diffuse 'synaptopathy'. Altered synaptic transmission can occur early in MS and EAE, independently of demyelination and axonal loss, and subsequently causes excitotoxic damage. Inflammation-driven synaptic abnormalities are emerging as a prominent pathogenic mechanism in MS—importantly, they are potentially reversible and, therefore, represent attractive therapeutic targets. In this Review, we focus on the connection between inflammation and synaptopathy in MS and EAE, which sheds light not only on the pathophysiology of MS but also on that of primary neurodegenerative disorders in which inflammatory processes contribute to disease progression.

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Introduction

The pathogenesis of multiple sclerosis (MS) begins with an inflammatory cascade in the CNS, which is mainly caused by inappropriately activated T cells that trigger an immune response against myelin and myelin-forming cells (oligodendrocytes). Autoreactive T cells from the bloodstream penetrate into the CNS by crossing the blood–brain barrier (BBB) and produce inflammatory cytokines, causing additional damage to the myelin and surrounding tissue. The resulting formation of large demyelinating plaques in the white matter leads to neurodegeneration.[1,2]

Over the past decade, MRI studies and analysis of autopsy tissue from patients with MS have revolutionized our understanding of MS pathogenesis.[3,4] Grey matter damage is now known to occur early in the disease course,[5–12] to be partly independent of demyelination,[5–9] and to be associated with clinical disability[10–15] and cognitive dysfunction.[10,12–15] Grey matter atrophy in patients with MS seems to result from a combination of demyelination, neuronal loss and/or atrophy, neurite transaction[7] and reduced numbers of synapses and glia,[8,16] suggesting that inflammatory and neurodegenerative events are intermingled rather than sequential.[17] Several studies conducted in animals with experimental autoimmune encephalomyelitis (EAE; considered by many researchers as a validated rodent model of human MS[18]) have provided solid evidence in support of these distinct processes that affect grey matter.[19,20] Many researchers have focused on axonal damage because it is a crucial determinant of disability, and have identified several effector molecules and downstream mechanisms through which the immune system triggers the neurodegenerative process that results in axonal damage.[19,20]

Accumulating evidence from proteomic, transcriptomic, neurophysiological and histological studies of MS indicates that diffuse synaptic dysfunction and loss, collectively known as synaptopathy (Box 1), is a hallmark of MS pathophysiology. Most of the studies assessing synaptic dysfunction and loss in MS report perturbations of both excitatory (mediated by glutamate) and inhibitory (mediated by γ-aminobutyric acid [GABA]) neurotransmission, which are critically involved in the correct functioning of the CNS. Of note, a long-lasting perturbation of synaptic homeostasis can become detrimental, leading to excitotoxic damage and neuronal death. Here, we review studies relating to synaptic aspects of grey matter pathology in MS and EAE, including loss of synapses (Box 1), synaptic dysregulation as a result of perturbations of the molecular machinery responsible for neurotransmitter homeostasis, and synaptic dysfunction, including functional alteration of neurotransmission. Particular emphasis is given to inflammatory-dependent synaptopathy, which is potentially reversible and might represent a novel therapeutic target in MS.

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Key points

- Progressive synaptic loss and dysfunction—also known as synaptopathy—occur early in multiple sclerosis (MS), and in experimental autoimmune encephalomyelitis (EAE), which is used to study MS in rodent models
- Along with demyelination and axonal damage or transection, synaptopathy is a pathophysiological hallmark observed in MS and EAE; moreover, it is independent of axonal transection and demyelination
- Synaptopathy has long-lasting effects that can be detrimental for motor and cognitive functions
- In MS and EAE, neuroinflammation alters the balance between the GABAergic and glutamatergic systems in the brain and spinal cord
- Proinflammatory cytokines released during acute MS attacks increase glutamate-mediated synaptic transmission and reduce γ-aminobutyric acid-mediated synaptic signalling, resulting in unbalanced synaptic hyperexcitation and possibly also to neurodegeneration
- Targeting of mechanisms that stabilize, protect, repair or help regenerate synapses would enable clinical intervention at both early and late stages of the disease

Box 1 | Glossary

Synaptopathy
Synaptopathy refers to pathological alterations of synaptic structure and function, and has been implicated in many CNS disorders, including Alzheimer disease, Huntington disease, schizophrenia, autism and—recently—multiple sclerosis.

Synaptic loss
Loss of synapses—the structural connections between neurons—can happen in both healthy and pathological conditions. During development or learning and memory consolidation, surplus synapses are removed (synaptic pruning), but synapses can also be lost as a result of neuronal injury (synaptic stripping) caused by, for example, misfolded proteins and excitotoxic insults.

Glutamate excitotoxicity
Dysregulation of glutamate signalling, including sustained activation of ionotropic glutamate receptors or reduced glutamate uptake, impairs cellulal calcium homeostasis and activates nitric oxide synthesis, leading to free radical generation and cell death.

Excitatory postsynaptic currents (EPSCs)
Binding of glutamate to the postsynaptic glutamate-gated ion channels triggers an EPSC—a temporary influx of positive ions into the postsynaptic neuron—which results in an excitatory postsynaptic potential (EPSP). Spatial and temporal accumulation of EPSPs at the postsynaptic neuron increases the likelihood of the neuron firing an action potential.

Inhibitory postsynaptic currents (IPSCs)
Release of γ-aminobutyric acid (GABA) into the synaptic cleft induces an IPSC—a flux of negative ions—either through the GABA-gated channels localized at the postsynaptic membrane or through a shunt of cell input resistance. IPSCs result in an inhibitory postsynaptic potential (IPSP) that prevents the membrane from reaching the threshold to fire an action potential.

Loss of synapses in MS and EAE

Histochemical and biochemical analyses, as well as genetic profiling of postmortem brains, have demonstrated synaptic loss (Box 1) in patients with MS and in rodents with EAE. The inflammatory microenvironment seems to directly influence the structural modifications and loss of the presynatic and postsynaptic elements.

Studies in patients with MS

In autopsy study of patients with progressive MS, the demyelinated hippocampi showed decreased levels of proteins that are crucial to synaptic maintenance (neurexin–neuroligin complex) and synaptic function (synaptophysin, synaptotagmin, postsynaptic density protein-95 [PSD-95] and Ca^{2+}-calmodulin-dependent protein kinase II [CASK; a peripheral plasma membrane protein that is involved in synaptic vesicle release and interacts with neurexin]), even though hippocampal neuronal loss was minimal.[16] Moreover, the integrity of the cholinergic neurotransmitter system has been reported to be compromised in both demyelinated and nondemyelinated hippocampi.[9] Recently, components of the complement system—C1q and C3—have been identified as mediators of synapse elimination in the hippocampus in patients with MS,[21] suggesting a direct link between inflammation and synaptopathy in MS.

In cortical demyelinating lesions of patients with relapsing–remitting MS (RRMS) or secondary progressive MS (SPMS), the loss of both synaptophysin—a presynaptic protein involved in synaptic vesicle release—and glial excitatory amino acid transporters (EAATs; mostly involved in glutamate transport) correlates with infiltration of the CNS by activated microglia.[22] A substantial reduction in synaptophysin was also found independent of local demyelination in the neocortex.[8] Together, these studies provide new insights into synaptic pathology in MS (Figure 1), suggesting that the inflammatory microenvironment in the CNS is involved in reducing the number of synapses, which can take place independently of grey matter demyelination and neuronal loss.

Studies in EAE animal models

EAE models of MS can be induced in several rodent species and strains with immunization protocols involving injections of antigenic material against myelin proteins. EAE can reproduce several pathological features of MS, and despite some limitations (the whole spectrum of MS symptoms and pathology cannot be covered in a single EAE model, or even in several different EAE models), it is an established model to study the immune alterations that are typical of human MS,[18] and also some of the neuropathological characteristics of this disease.[23,24] Assessment of synaptic changes in different stages of the disease is easier and more controllable in rodent models of EAE than in patients.

The development of motor symptoms in EAE is dependent on the infiltration of activated mononuclear cells into the CNS from the periphery,[18] an event that is accompanied by microglial activation, astrogliosis, and abundant proinflammatory cytokine production, demyelination and macroscopic plaque formation.

In EAE models, structural synaptic alterations have been detected in several areas of the CNS, including the spinal cord, hippocampus, cerebellum, striatum and cortex. In spinal cord tissue derived from relapsing–remitting EAE rats[25,26] or mice,[27] immunohistochemical and ultrastructural analysis reveal a pronounced plastic retraction of both dendrites and synaptic terminals of spinal motor neurons during disease exacerbation, and this loss of synapses correlates with astrogliosis.[25–27] Similarly to motor deficits, most of these synaptic alterations have the potential for rapid and spontaneous

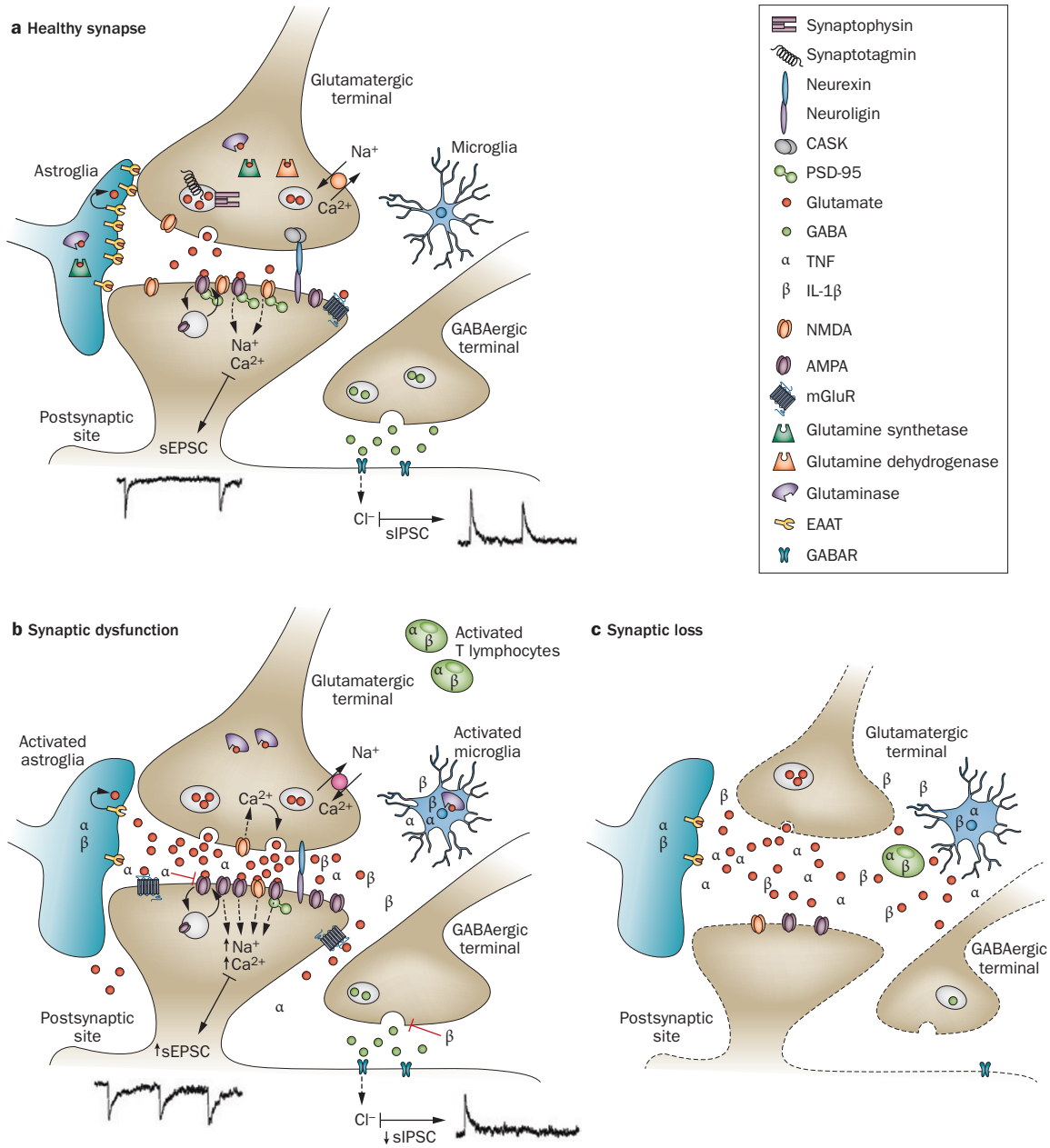

Figure 1 | Inflammatory synaptopathy in MS and EAE. Perturbations of the presynaptic and postsynaptic cellular and molecular machineries can profoundly affect synaptic transmission; moreover, altered neurotransmission can induce a maladaptive response in the CNS microenvironment. **a** | In the healthy synapse, the concentrations of excitatory (glutamate) and inhibitory (GABA) neurotransmitters at the synaptic cleft are modulated by strict control of transmitter release, degradation or reuptake. **b** | Proinflammatory cytokines released by autoreactive lymphocytes and activated microglia induce synaptic dysfunction in both glutamatergic and GABAergic systems in MS and EAE. **c** | Furthermore, inflammation can induce structural alterations, comprising synaptic loss characterized by degeneration of the presynaptic and/or postsynaptic site and alterations of the glial compartment. Representative postsynaptic electrophysiological traces (sEPSC and sIPSC) are reported in each panel. Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CASK, Ca^{2+}-calmodulin-dependent protein kinase II; EAAT, excitatory amino acid transporter; EAE, experimental autoimmune encephalomyelitis; GABA, γ-aminobutyric acid; GABAR, GABA receptor; MS, multiple sclerosis; mGluR, metabotropic glutamate receptor; NMDAR, *N*-methyl-D-aspartate receptor; PSD-95, postsynaptic density protein 95; sEPSC, spontaneous excitatory postsynaptic current; sIPSC, spontaneous inhibitory synaptic current; TNF, tumour necrosis factor.

recovery that, interestingly, is independent of the much slower remyelination process.[26] Of note, during the remission phases, the ratio between excitatory and inhibitory inputs increases, indicating the potential for development of excitotoxic neurodegenerative processes,[27] and probably presenting an impediment for the re-establishment of neural connections.[28]

In the striatum of EAE mice, neuroinflammation was found to result in a marked reduction in the spine density on striatal medium spiny neurons (MSNs), whereas the

Table 1 | Potential pharmaceutical MS treatments that target the glutamatergic system

MS type	Drugs	Trial type and number of patients	Dose/ administration	Effects
Relapsing–remitting	Amantadine (weak NMDAR antagonist)	Double-blind multicentre study; 53 patients[77]	10 mg twice daily (oral)	Reduced relapse rate
All types	Memantine (NMDAR blocker)	Double-blind, randomized, placebo-controlled trial; 114 patients[146]	10 mg twice daily (oral)	No improvement in cognitive performance
Primary progressive	Riluzole (sodium channel blocker, kainate and NMDAR antagonist)	Pilot study; 16 patients[80]	50 mg twice daily (oral)	Beneficial effect on lesion evolution and axonal loss, but no clear effect on formation of new lesions

Abbreviations: MS, multiple sclerosis; NMDAR, *N*-methyl-D-aspartate receptor.

numbers of primary dendrites and cell somata were unaffected.[29] Of note, the loss of spines could be prevented by *in vivo* treatment with a selective inhibitor for ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic glutamate receptors (AMPARs; discussed below).[29] Besides spine loss, apoptotic-like events at the synaptic level were detected in striatal synaptosomal preparations, as indicated by increased labelling of annexin A5 (a molecule that has a high specificity and affinity for phospholipid phosphatidylserine, which is exposed on the cell surface during the early stages of apoptosis).[29] The fact that synapses begin to show this sign of apoptosis in the acute phase of EAE, even in the absence of overt neuronal loss,[29] supports the idea that synapses are early targets of apoptotic events in this disease, because they are highly sensitive to the subtle inflammation-induced changes in cellular homeostasis. Moreover, in the sensorimotor cortex of EAE rats, reductions in spine density and dendritic length were observed to correlate with the inflammatory burden.[24] In the hippocampi of EAE mice, expression of certain synaptic proteins (synapsin 1, a modulator of neurotransmitter release, and PSD-95, a scaffold protein that binds to *N*-methyl-D-aspartate receptors [NMDARs] and neuroligin) and the number of presynaptic terminals in the CA1 region were substantially reduced.[30–32]

Finally, a decrease in the density of inhibitory presynaptic terminals has been repeatedly described in different brain areas of EAE models. For example, in the cerebellum, the density of basket and stellate inputs that impinge on Purkinje cells is markedly reduced,[33] and in the striatum[34] and the primary motor cortex,[35] the numbers of synaptic terminals detected by the vesicular GABA transporter (VGAT) marker are reduced. These events occur in the presence of chronic microglial activation and infiltrating blood-borne immune cells,[30,32,34,36,37] and in parallel with selective degeneration of a subpopulation of GABAergic interneurons that are detected mainly by the presence of the calcium-binding protein parvalbumin.[30,32,34–37] Of note, parvalbumin-positive interneurons seem to be particularly susceptible to degeneration not only in EAE, but also in several brain areas (including the cortex, thalamus, amygdala and hippocampus) in patients with MS.[38–40]

Dysregulation of neurotransmitters

The concentrations of glutamate, GABA and other neurotransmitters at the synaptic cleft are modulated at the levels of synthesis, release, degradation and reuptake. In both MS and EAE, the molecular machinery involved in these processes is perturbed, and the expression of receptors involved in neurotransmission is altered (Figure 1 and Supplementary Tables 1–3 online).

The glutamatergic system in MS and EAE

Abnormal accumulation of glutamate in the synaptic cleft, as a result of increased release and/or deficient reuptake of glutamate into astrocytes, causes excessive stimulation of glutamate receptors (GluRs) and, consequently, excitotoxic damage of neurons and oligodendrocytes.

In MS and EAE, glutamate excitotoxicity (Box 1) is an important link between neuroinflammation and neurodegeneration.[41–46] Accumulating evidence points to increased availability of glutamate in both MS and EAE (Figure 1 and Supplementary Tables 1 and 2 online). Despite a few controversial results,[47–51] probably reflecting differences in technique sensitivity, sampling, and MS or EAE phenotypes, high levels of glutamate have been detected in the brains of EAE model animals,[52–54] and in the cerebrospinal fluid (CSF), white matter and grey matter of patients with MS (Supplementary Table 1 online).[55–59]

Several processes that depend on the interplay between immune and nervous systems are involved in the observed increase in glutamate concentration. Besides neurons, T cells, macrophages, astroglia, and activated microglia all have the potential to synthesize and release glutamate,[60] providing a continuous local supply of this neurotransmitter. The metabolizing enzymes glutamate dehydrogenase and glutamine synthetase are downregulated in the EAE brain[53,61] and in oligodendrocytes of patients with MS,[54] whereas the glutaminase enzyme, which is responsible for glutamate synthesis, is upregulated in EAE and active MS lesions (Supplementary Tables 1 and 2 online).[54] Impaired glutamate uptake from the synaptic cleft also contributes to impaired glutamate homeostasis. Activated microglia generate reactive oxygen and nitrogen species and proinflammatory cytokines, all of which suppress the expression of glutamate transporters (GluTs) that are involved in glutamate uptake.[45] Indeed, the levels of neuronal and glial glutamate transporters (EAAT1, EAAT2 and EAAT3) are downregulated in both EAE[36,52,62–64] and MS.[22,41,54] Seemingly contradictory results—enhanced glutamate

Table 2 | EAE studies evaluating pharmaceutical treatments that target the glutamatergic system

Drug	EAE type and model animal	Administration	Effects
NBQX (AMPAR blocker)	MBP SJL/J mice	Prophylactic (SC)	Ameliorates disease Increases oligodendrocyte survival Neuroprotective in the spinal cord[41]
NBQX, fanapanel, talampanel, GYKI52466 (AMPAR antagonists)	MBP Lewis rats	Prophylactic and therapeutic (IP)	Ameliorates disease Neuroprotective in the spinal cord[75,147]
Tag-G-Gpep (peptide against GluR2–GAPDH complex)	MOG C57 mice	Therapeutic (daily IP)	Ameliorates disease Neuroprotective in the spinal cord[72]
Dizocilpine, memantine (NMDAR blockers)	Sch Lewis rats	Prophylactic and therapeutic (IP)	Ameliorates disease Reduced lesion development (only observed with prophylactic administration)[148] Reduced blood–brain barrier breakdown[149]
Memantine	MBP Lewis rats	Therapeutic (IP)	Ameliorates disease No effect on inflammation[150]
Amantadine (weak NMDAR blocker and dopamine agonist),memantine	Sch Lewis rats	Prophylactic (IP)	Ameliorates disease and glutamatergic defects[74,151,152]
Dizocilpine	MOG C57 mice	Prophylactic (ICV osmotic minipump)	Ameliorates disease Restores synaptic function[101]
NBQX–GPE (combined AMPAR + NMDAR antagonist)	MOG C57 mice	Therapeutic (50% IP and 50% IV)	Ameliorates disease Promotes CNS repair when combined with anti-inflammatory treatment[153,154]
LY367385 (mGluR1 antagonist), MPEP (mGluR5 antagonist)	Sch Lewis rats	Prophylactic (IP)	No effect on disease[74,151,152]
Riluzole (Na^+ channel blocker, kainate and NMDAR antagonist)	MOG C57 mice	Prophylactic and therapeutic (IP)	Ameliorates disease Reduced inflammation, demyelination and axonal damage[78]
Carbenoxolone (gap junction blocker) 6-diazo-5-oxo-L-norleucine (glutaminase inhibitor)	MOG C57 mice	Prophylactic (IP)	Attenuates symptoms[79]
Aniracetam (AMPAR agonist)	PLP Biozzi mice	Prophylactic (IP)	Exacerbates disease[75]
L-AP4 (mGluR4 agonist)	MBP Lewis rat	Prophylactic SC osmotic minipump	Increases recovery rate[155]

Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; EAE, experimental autoimmune encephalomyelitis, GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPE, glycine–proline–glutamic acid; ICV, intracerebroventricular; IP, intraperitoneal; IV, intravenous; MBP, myelin basic protein; mGluR, metabotropic glutamate receptor; MOG, myelin oligodendrocyte glycoprotein; NMDAR, *N*-methyl-D-aspartate receptor; PLP, proteolipid protein; PPMS, primary progressive MS; RRMS, relapsing–remitting MS; SC, subcutaneous; Sch, spinal cord homogenate.

uptake activity and increased expression of GluT mRNA—have been reported in studies assessing the optic nerves and spinal cords of patients with MS[65–67] and of EAE rodents.[63,68] These glutamatergic alterations have been interpreted as an adaptive response to enhanced release of the neurotransmitter from neurons and astrocytes in an attempt to limit excitotoxicity.[65–67]

In both MS[67,69–72] and EAE,[29,43,52,62,70,73,74] a permanent increase in proinflammatory cytokine levels and increased availability of glutamate can upregulate neuronal GluR expression and exacerbate synaptic dysfunction, thereby reinforcing the local glutamate excitotoxicity (Figure 1 and Supplementary Tables 1 and 2 online). Because this mechanism has been proposed as a major determinant of the neurodegeneration in MS and EAE, drugs that regulate the function and expression of GluRs might have a protective effect against excitotoxic cell death (Tables 1 and 2). Indeed, pharmacological treatment with GluR antagonists has been shown to ameliorate motor deficits and neuropathology in murine EAE models.[41,45,75] The beneficial effect of GluR antagonist treatment might also be mediated by the GluRs in immune cells (T cells, B cells, antigen-presenting cells, macrophages, and activated microglia).[76] Glutamate might also have an essential role in MS, not only through excitotoxic mechanisms but also by enhancing the proliferation of autoreactive T cells in response to myelin proteins, as reported in one study involving 14 patients with MS.[76]

It should be noted that pharmacological blockade of GluRs has limited clinical use, because GluRs have a vital role in maintaining normal synaptic transmission, and total blockade of ionotropic GluRs (iGluRs) could lead to numerous adverse effects. Investigators are, therefore, currently focusing on preventing excessive activation of iGluRs or metabotropic GluRs (mGluRs; Tables 1 and 2). Only a few positive human studies have been completed so far, but treatment of MS patients with amantadine (a weak NMDAR antagonist) was shown to reduce the relapse rate[60] and improve pendular nystagmus (Table 2).[77]

Agents that inhibit glutamate release from neural and immune cells have been reported to have beneficial effects in EAE (Table 2),[78,79] and suppression of glutamate

Table 3 | EAE studies evaluating pharmaceutical treatments that target the GABAergic system

Drugs	EAE model	Administration	Effects
Exogenous GABA	$MOG_{35–55}$ C57BL/6 mice	IP prophylactic	Increases disease severity Enhances MOG-dependent proliferation Increases TNF and IL-6 in spinal cord[94]
Vigabatrin (GABA-transaminase inhibitor, enhances endogenous GABA)	$MOG_{35–55}$ C57BL/6 mice	IP prophylactic	Protects from disease development[94]
Vigabatrin	$MOG_{35–55}$ C57BL/6 mice	IP therapeutic	Ameliorates disease severity[94]
Vigabatrin Topiramate	PLP SJL/J mice	Oral prophylactic	Protection or amelioration of disease development via inhibition of inflammation[92]
Vigabatrin	PLP SJL/J mice	Oral therapeutic	Ameliorates disease severity[92]
Phenobarbitone sodium ($GABA_A$ receptor allosteric modulator) and sodium valproate ($GABA_A$ level enhancer)	Immunization with spinal cord homogenate from Wistar rats	IP prophylactic	Ameliorates disease severity[95]
Diazepam ($GABA_A$ receptor agonist)	Immunization with spinal cord homogenate from Wistar rats	IP prophylactic	No effect[95]
Diazepam	Intradermal inoculation of bovine MBP in Wistar rats	IP prophylactic/ therapeutic	Ameliorates disease severity[96]

Abbreviations: GABA, γ-aminobutyric acid; IP, intraperitoneal; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein; TNF, tumour necrosis factor.

release from immune cells has also shown promising results in patients with MS. (Table 1)[80] Thus, modulation of extracellular glutamate levels seems to be one of the most promising therapeutic strategies to prevent neurodegeneration in MS.

The GABAergic system in MS and in EAE

Accumulating evidence points to dysregulation of the GABAergic system in both MS and EAE (Supplementary Table 3 online). Reduced GABA levels have been detected in the CSF of patients with MS,[49,81] as well as in the spinal cord and cerebral cortex of EAE rodents,[50,52,82] though a few contrasting results have been published.[83,84] The studies reporting reduced GABA in the CSF and CNS are in line with earlier findings of decreased GABA levels in the blood of patients with MS,[81,85] and with more-recent transcriptomic and proteomic analyses showing that the molecular machinery that controls GABA levels is dysregulated in patients with MS[71,82,86–90] and in EAE.[71,82,90,91]

Along with its neurotransmitter function, GABA is an important modulator of immunity,[92,93] affecting a variety of immune cell functions, including cytokine release, cell proliferation, and phagocytic activity.[92] The role of GABA in the regulation of immune system function has hampered our understanding of its action as a neuroprotective and antiexcitotoxic molecule, and could perhaps partly explain why GABAergic pharmacological treatment in EAE models has had seemingly contradictory effects: exogenous GABA administration exacerbates disease severity, probably because it increases immune cell activity.[92,94] By sharp contrast, enhancement of endogenous GABA activity by systemic treatment with the GABA transaminase inhibitor vigabatrin has beneficial effects on EAE symptoms (Table 3).[93,94] Sodium valproate, an enhancer of GABA levels, has also been shown to ameliorate the motor symptoms in EAE rats,[95] suggesting that neuronal and glial susceptibility to injury can be reduced by local modulation of GABA levels in the CNS. Mixed results have been obtained in EAE studies evaluating the effects of treatments with $GABA_A$ receptor agonists, such as diazepam and phenobarbitone sodium,[95,96] indicating that further studies are required. In patients with MS, GABA agonists can improve MS symptoms, such as spasticity and nystagmus (Table 4).[97–100]

Inflammatory synaptic dysfunction

The mechanisms through which the myelin-targeting autoimmune reaction results in abnormal synaptic transmission are not fully understood. However, brain-infiltrating autoreactive T cells and resident CNS immune cells—such as microglia and astroglia, which show pronounced activation in MS and EAE[29,32,33,36,37,101,102]—are known to extensively modulate synaptic transmission. Like infiltrating T cells, microglia and astroglia are crucial sensors of the local microenvironment, and can secrete substantial quantities of cytokines, growth factors and/or neurotransmitters. Therefore, these cells are considered to be potent and far-reaching regulators of the extended neuron–glia network.[103] Activated microglia can also influence synaptic structure and function by shedding microvesicles,[104] or by initiating synaptic stripping after an inflammatory insult (Box 1 and Supplementary Table 4 online).[105]

Recent studies have shown that immune cells can trigger synaptic alterations similar to those seen in EAE. Incubation of brain slices derived from healthy mice with $CD3^+$ T cells isolated from the spleens of EAE mice was found to alter glutamatergic and GABAergic synaptic transmission.[29,36,37] Remarkably, the synaptic alterations observed in striatal, hippocampal and cerebellar slices incubated with EAE $CD3^+$ T cells were reminiscent of the

Table 4 | Potential pharmaceutical MS treatments that target the GABAergic system

MS type	Drug	Trial type and number of patients	Dose/ administration	Effects
PPMS and SPMS	Gabapentin	Prospective blind crossover trial; 11 patients	1.2 g daily (oral)	Improves acquired nystagmus[98]
RRMS	Gabapentin	Single-blind crossover trial; eight patients	1.2 g daily (oral)	Improves acquired nystagmus[99]
CPMS	Gabapentin	Prospective, double-blinded, placebo-controlled crossover trial; 21 patients	0.3–0.9 g three times daily (oral)	Improves spasticity[100]
RRMS and SPMS	Gabapentin	Open-label trial; 24 patients	0.6 g daily (oral)	Reduces painful spasms[156]
RRMS	Vigabatrin	Single-blind crossover trial; eight patients	2 g daily (oral)	No effect on acquired nystagmus[99]
RRMS, SPMS and PPMS	Baclofen	Doubleblind, placebo-controlled crossover trial; 14 patients	25–50 μg injected into the lumbar subarachnoid space	No effect on gait and postural stability[157]
Not specified	Baclofen	Nine patients with spasticity	20–60 mg daily (oral)	Increases the soleus stretch reflex[158]
Not specified	Baclofen	10 patients with spasticity in the lower extremities	15–60 mg daily (oral)	Reduces ankle joint stiffness[159]

Abbreviations: CPMS, chronic progressive MS; GABA, γ-aminobutyric acid; MS, multiple sclerosis; PPMS, primary progressive multiple sclerosis; RRMS, relapsing–remitting MS; SPMS, secondary progressive MS.

defects seen in the brains of the EAE mice.[29,36,37] Similar synaptic defects characteristic of EAE have been seen in experiments where brain slices were incubated with activated microglia.[29,37] Notably, the effects of $CD3^+$ T cells and activated microglia on synaptic function can be almost completely reversed by specific cytokine antagonists,[29,37] as described below (Supplementary Table 4 online).

Proinflammatory and anti-inflammatory cytokines have an essential role in the regulation of bidirectional communication between glia and neurons, and in the modulation of synaptic transmission.[106–108] Therefore, in pathological conditions, the final downstream effects of cytokines on synaptic transmission and neuronal survival depend on the synaptic cytokine concentrations, the balance between proinflammatory and anti-inflammatory cytokines, and the subcellular expression of their receptors in specific neuronal compartments (Figure 1).[109]

Neuroinflammation can affect synaptic transmission at different levels, with a tremendous impact on synaptic excitability and, therefore, neuronal function.[106–108] Several characteristics of the spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs; Box 1) recorded from neurons are affected by inflammation. In particular, inflammation alters the frequency of presynaptic neurotransmitter release, and the amplitude and duration of the postsynaptic currents.[29,33,34,36] The effects of cytokines can be explored via administration of cytokine antagonists to prevent or reverse the synaptic deficits in EAE animals or brain slices, or by administration of a specific cytokine to a healthy animal or brain slices to replicate EAE-like alterations (Supplementary Table 4 online).

In MS, two approaches are typically applied to reveal inflammation-dependent modulation of synaptic function: correlation of cortical excitability and plasticity—as measured by transcranial magnetic stimulation (TMS)—with levels of cytokines in the CSF,[32] or exploration of the effects of cytokines in patient-derived CSF on synaptic transmission in brain slices from healthy rodents.[110]

Glutamatergic and GABAergic transmission in EAE

Glutamatergic transmission in the striatum

An early electrophysiological study conducted in a myelin oligodendrocyte glycoprotein peptide 33–55 ($MOG_{33–35}$) EAE mouse model (Supplementary Table 4 online) demonstrated dramatic changes in glutamate-mediated transmission in MSNs.[29] Of note, these alterations started in the presymptomatic phase of the disease (7–9 days post injection [dpi]) before the appearance of motor deficits, and were still present at the peak of the acute phase of the disease (20–25 dpi),[29] and even in the late, chronic stages of EAE (50 dpi).[111] Furthermore, these synaptic abnormalities were seen in regions devoid of overt inflammatory foci, and persisted when microglial activation declined in the late phase of EAE.[29] These findings suggest that the synaptic abnormalities represent virtually irreversible alterations, triggered by inflammation but persisting after its resolution, resulting in a chronic synaptic insult that ultimately leads to neuronal excitotoxic damage.

Both the presynaptic and postsynaptic compartments of excitatory synapses are compromised in EAE, resulting in increased frequency and enhanced duration of the spontaneous glutamate current (Supplementary Table 4 online).[29,101,111] These glutamatergic alterations are in part caused by increased presynaptic NMDAR activation and increased postsynaptic AMPA receptor expression and sensitivity (Supplementary Table 4 online),[29,101] which contribute to degeneration of dendritic spines and development of motor deficits. Pharmacological blockade of AMPA receptors[29] and preventive intracerebroventricular (ICV) NMDAR blockade[101] have been associated with reversal of these synaptic deficits and reduced EAE severity (Table 2). In line with these findings, EAE mice with genetically enhanced NMDAR signalling showed exacerbated synaptic defects (Supplementary Table 4 online).[101]

A concurrent mechanism that is responsible for enhanced synaptic release of glutamate in the EAE striatum involves a reversal in the direction of the axonal Na^+/Ca^{2+} cotransporter.[111] This reversal alters the intra-axonal Na^+/Ca^{2+} balance so that Ca^{2+} levels exceed those of Na^+, and is likely to be secondary to focal axonal demyelination and the associated abnormal functioning of voltage-dependent Na^+ channels, rather than to diffuse inflammation (Supplementary Table 4 online).[111]

Unlike the synaptic alterations triggered by inflammatory cytokines, which affect the postsynaptic compartment of neurons and are detectable in the early as well as in the late phase of EAE,[29,111] the alterations caused

by axonal demyelination affect presynaptic transmitter release, and disappear in the chronic phases of EAE, when a certain degree of remyelination presumably takes place.[111] EAE-related synaptic abnormalities that occur in other brain structures and affect other neurotransmitter systems could, therefore, be caused by a combination of diffuse and focal inflammation and axonal demyelination (Supplementary Table 4 online).

Application of either activated microglia or tumour necrosis factor (TNF) to control striatal slices increases sEPSC duration, thereby mimicking the effects of EAE, and making TNF a candidate for the cytokine responsible for the induction of the synaptic deficits seen in the striatum of EAE animal models (Supplementary Table 4 online).[29] Similar effects have been reported following *in vivo* treatment: ICV administration of the TNF signalling inhibitor etanercept prevented sEPSC alterations in EAE mice, whereas ICV treatment with TNF in healthy mice altered sEPSC duration in a way that was reminiscent of abnormal glutamate transmission in EAE (Supplementary Table 4 online).[112] IL-1β has also been implicated in synaptic modulation: it can increase sEPSC frequency via modulation of transient receptor potential vanilloid 1 (TRPV1) channels[110] in striatal MSNs, corroborating a role for IL-1β in synaptic alterations in EAE and MS.

Glutamatergic transmission in the cerebellum

Electrophysiological recordings from Purkinje cells of MOG_{33-35} EAE mice revealed enhanced glutamate transmission during the symptomatic phase of EAE (21–25 dpi; Supplementary Table 4 online).[36] The increased duration of sEPSCs is caused by reduced expression and functioning of EAAT1 (also known as GLAST), the most abundant GluT expressed by the Bergmann astroglia. A direct link between inflammation and such synaptic dysregulation has been demonstrated in *ex vivo* and *in vivo* studies: although TNF did not have any effect on cerebellar synaptic transmission, IL-1β—released by a subset of activated microglia and macrophages and infiltrating T cells—enhanced glutamatergic transmission.[36] Brief incubation of normal cerebellar slices with IL-1β replicated EAE modifications through rapid EAAT1 downregulation, whereas treatment with the IL-1β receptor antagonist (IL-1ra) blocked sEPSC alterations, and normalized EAAT1 expression.[36]

Glutamatergic transmission in the hippocampus

Neuroimaging studies suggest that hippocampal pathology is involved in MS-associated memory defects,[48] and a correlation with CNS glutamate levels and memory has recently been observed in patients with the disease.[48] Hippocampal neurodegeneration and resulting spatial learning impairment has been described to occur in MOG_{33-35} EAE mice;[30] moreover, synaptic dysfunction was reported in the CA1, which is the main output of the hippocampus to the cortex (Supplementary Table 4 online).[31,37,113,114]

In support of a role for alterations to the hippocampal glutamatergic system in EAE, earlier electrophysiological studies reported impairments in basal excitatory synaptic transmission that involved AMPAR-mediated changes in synaptic currents and faster decay rates of NMDAR-mediated currents.[31] Furthermore, altered paired-pulse facilitation (an indicator of a presynaptic alterations) has been observed.[114] Crossmodality correlation analysis revealed that deficits in excitatory synaptic transmission correlated with reductions in trans-synaptic protein binding partners that are known to modulate excitatory synaptic transmission.[114] Contrasting results were obtained in another EAE model (one boost of MOG_{35-55} immunization in female mice): no difference was found in the efficacy of glutamatergic transmission in the CA1 area, or in sEPSC amplitudes or frequency.[37] The effects of proinflammatory cytokines on hippocampal glutamatergic transmission are still unknown.

GABAergic transmission

The most established and common GABAergic alteration observed in the cerebellum, hippocampus and striatum in EAE mice with a chronic disease course is a reduction in the frequency of GABAergic spontaneous synaptic currents (Figure 1 and Supplementary Table 4 online). The reduced sIPSC frequency typically manifests during the acute phase of the disease, and strongly correlates with selective loss of GABAergic interneurons in these brain areas. Moreover, the amplitude of GABAergic sIPSCs is reduced in the striatum[34] and hippocampus,[37] but remains unaffected in the cerebellum.[33]

Although TNF does not seems to interfere with GABAergic transmission (at least in the striatum), IL-1β signalling impairs GABAergic transmission in all three studied brain structures, partly resembling the synaptic alterations in EAE.[32–34,37,115] In support of these observations, ICV delivery of IL-1ra rescues GABAergic function in EAE mice.[32,36]

Inflammation-dependent neurotransmission in MS

Cortical excitability in patients with MS

Cortical excitability and plasticity in MS can be assessed by measuring the response to different TMS protocols that target the motor cortex.[116] TMS techniques, such as paired-pulse and twin-coil TMS, enable measurement of cortical neuronal populations that are functionally connected to pyramidal cells. With these techniques, disability in MS has been shown to correlate with reduced measures of short-interval intracortical inhibition,[117,118] and with reduced transcallosal inhibitory and facilitatory connectivity.[119–121] Furthermore, intracortical facilitation, a TMS measure of glutamate transmission, correlates with a high IL-1β–IL-1ra ratio in the CSF of MS patients with active lesions,[110] suggesting a link between inflammation and excitotoxicity in MS. During a relapse, patients with MS show decreased short-interval intracortical inhibition,[122] which is known to be regulated by both NMDAR[123] and $GABA_A$ receptors.[124] *Ex vivo* experiments have demonstrated that the reduced $GABA_A$-mediated neuronal inhibition depends on IL-1β (see below).[125]

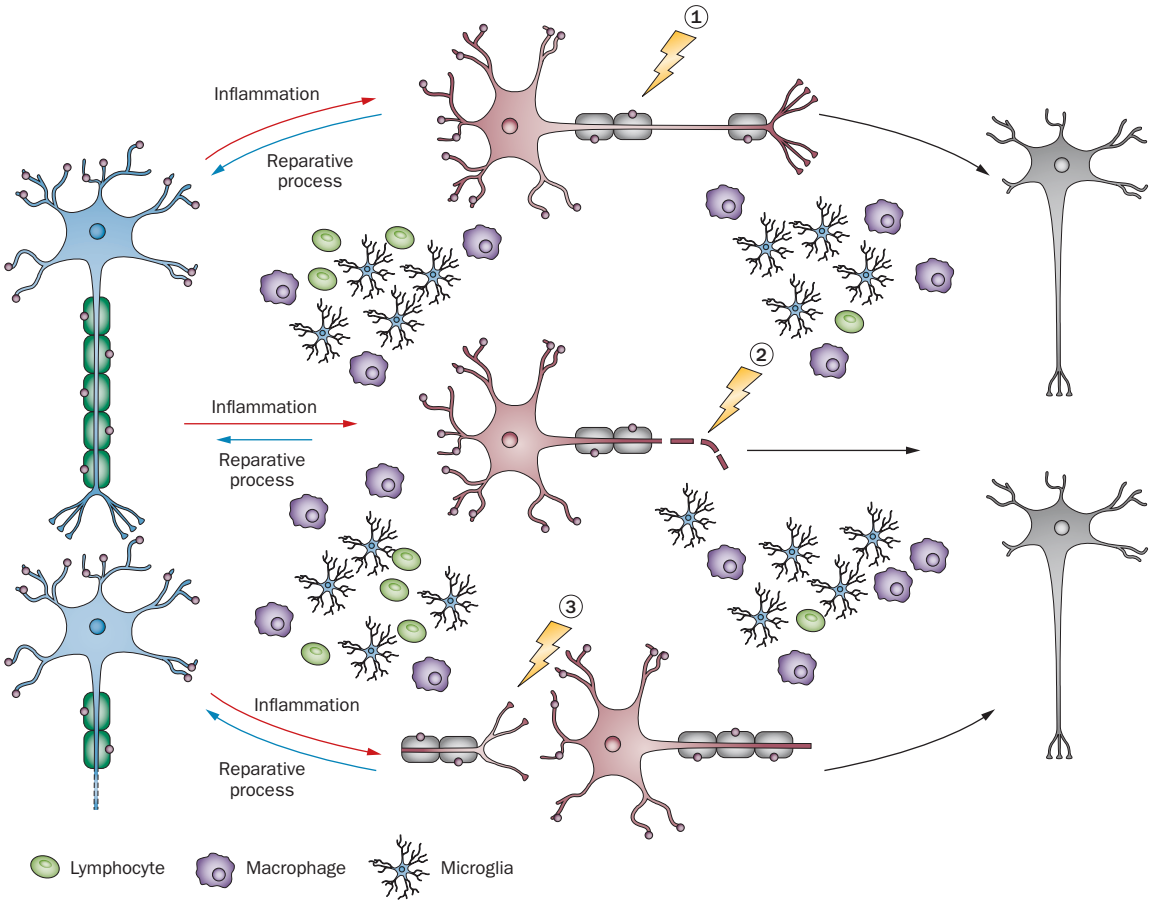

Figure 2 | Demyelination, axonal loss and synaptopathy characterize multiple sclerosis and experimental autoimmune encephalomyelitis pathophysiology. Inflammation driven by lymphocytes triggers a chain (red arrows) of inflammatory events (microglial and macrophage activation), leading to demyelination (1), axon transection (2) and synaptopathy (3) in the CNS. Axons that remain demyelinated for long periods of time (1) undergo maladaptive processes, such as redistribution of ion channels, which promote chronic neurodegeneration. Similarly, axonal loss (2) is usually not reversible and leads to neuronal death. Long-lasting but potentially reversible synaptic dysfunction (3) in the presence of chronic microglial activation can lead to excitotoxic damage and subsequent neurodegeneration.

Effects of MS CSF on synaptic transmission

Ex vivo studies in murine striatal slices incubated in CSF derived from MS patients who had active brain lesions on MRI support a role for cytokines in unbalanced synaptic hyperexcitation and, possibly, excitotoxic neurodegeneration (Supplementary Table 4 online).[110,125,126] As in EAE studies, inflammatory cytokines released during acute MS attacks can modulate both glutamatergic and GABAergic synaptic transmission. In particular, murine striatal slices incubated with MS CSF exhibit a substantial increase in sEPSC frequency and glutamate-mediated neuronal swelling, both of which are markers of excitotoxicity.[110] These effects are dependent on increased IL-1β signalling and AMPAR stimulation, as suggested by rescue experiments conducted in the presence of specific inhibitors.[110] In another *ex vivo* MS-CSF incubation study of striatal slices, TNF—which is expressed at higher levels in the CSF of patients with progressive MS than in patients with RRMS—was shown to promote glutamatergic transmission, but by increasing the duration rather than the frequency of sEPSCs, leading to neuronal swelling.[126] These data point to TNF as a primary neurotoxic molecule in progressive forms of MS. Moreoever, CSF from patients with MS who had acute brain lesions can impair GABAergic transmission in murine striatal slices.[125] IL-1β is likely to be responsible for this effect, because it could be blocked by IL-1ra incubation, and application of exogenous IL-1β mimicked the effect of MS CSF on synapses (Supplementary Table 4 online).[125]

The synapse as an MS drug target

Synaptic dysfunction could be reversible

On the basis of the findings discussed above, which point to diffuse synaptopathy (Box 1) as a pathophysiological hallmark of MS, we suggest that synaptic damage should also be considered as a target for disease-modifying treatments. Such treatments could aim for synaptic repair and regeneration. This strategy is particularly appealing, because—unlike loss of neurons—synaptic dysfunction and loss of synapses are reversible. Synapses are, in fact, highly dynamic and plastic, such that dysfunctional synapses can be repaired and new synapses can be formed, as occurs during synaptic plasticity or rehabilitative processes.[127]

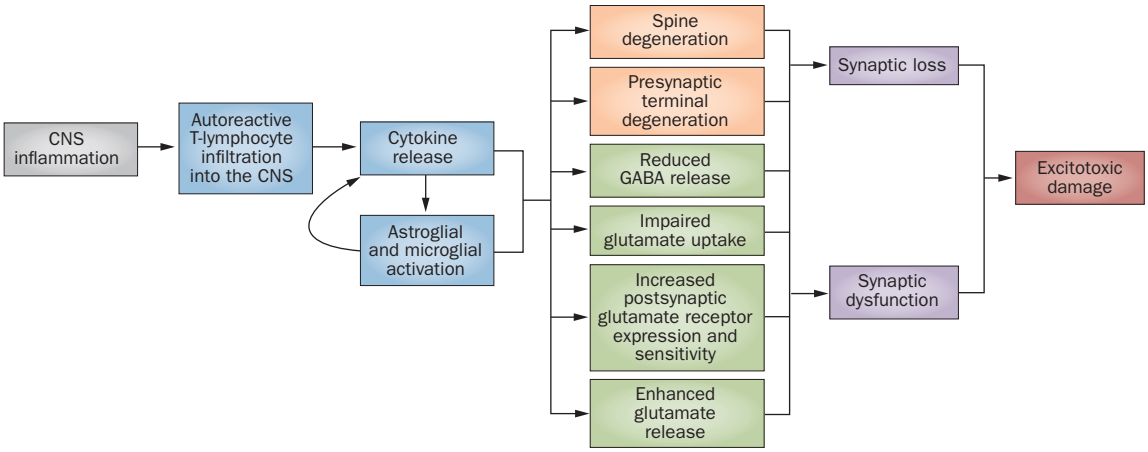

Figure 3 | Cascades leading to inflammation-induced excitotoxicity. Inflammatory events (blue) in the CNS trigger structural (orange) and functional (green) alterations to the synaptic compartment. These parallel maladaptive changes lead to synaptic dysfunction and loss of synapses and, consequently, to excitotoxic damage (red). Abbreviation: GABA, γ-aminobutyric acid.

Potential neuroprotection by current MS drugs

Currently, all disease-modifying therapies (DMTs) in MS act by restoration of a dysregulated immune response and/or by preventing the migration of autoreactive T cells into the CNS in an immunomodulatory or immunosuppressive fashion. However, MS DMTs have adverse effects associated with their immunomodulatory function, and are all ineffective in patients with progressive MS.[128–130]

In patients with RRMS, effective treatment with immunomodulatory or immunosuppressive DMTs reduces brain atrophy. In fact, treatment with some of these DMTs is associated with more-pronounced brain tissue preservation than would be expected from their activity on the immune system, raising the hypothesis that these drugs could have direct neuroprotective effects.[131–133] Fingolimod (or its analogue siponimod), laquinimod, and dimethyl fumarate are examples of small molecules that are potentially capable of crossing the BBB and exerting direct neuroprotective effects on the CNS. Clinical trials in progressive forms of MS have been designed to test the supposed neuroprotective effect of these drugs.[134] Importantly, these drugs have all been demonstrated to mitigate the effects of inflammation of synaptic transmission in rodents[135–137] and, at least in the case of laquinimod and dimethyl fumarate, these protective effects on synaptic transmission were independent of their activity on peripheral immune cells.[136,137] Both therapeutic and preventive treatment with laquinimod has been shown to ameliorate motor disability, reduce CNS inflammation, and increase the number of myelinated axons in EAE.[136,138]

Effects on GABAergic and glutamatergic pathways

In EAE, laquinimod can notably ameliorate synaptic dysfunction: the alterations in striatal GABAergic synaptic transmission can be fully reversed by both preventive and therapeutic laquinimod treatment, and the kinetic alterations of striatal glutamatergic transmission are improved by preventive treatment.[136] Given that laquinimod is a small molecule that freely diffuses across the BBB,[139] a direct neuroprotective effect is plausible. Consistent with this hypothesis, laquinimod can regulate synaptic transmission *in vitro:* direct application of laquinimod to striatal slices results in increased sIPSCs and reduced sEPSCs.[136]

Effects on microglial activation

Laquinimod,[140] dimethyl fumarate[137,141] and fingolimod[135] can inhibit microglial activation and thereby reduce axonal injury in EAE models. Dimethyl fumarate is metabolized to monomethyl fumarate, which can cross the BBB and has the potential for direct neuroprotective effects. A recent study reported that monomethyl fumarate was able to directly modulate glutamate release from presynaptic nerve endings, and to affect the synaptotoxicity of activated microglia.[137] *In vitro* studies suggest that the modulatory effect of monomethyl fumarate on microglial function and, thereby, its indirect protective effect on glutamatergic transmission is independent of its activity on peripheral immune cells.[137] In EAE mice, oral fingolimod can restore presynaptic and postsynaptic alterations of glutamatergic transmission and promote the recovery of dendritic spines,[135] probably owing to its capacity to suppress activation of microglia.[142] Fingolimod can also protect against excitotoxic insult in cultured cortical neurons[143] and, more interestingly, in patients with RRMS, by reducing glutamate-mediated intracortical excitability as measured by paired-pulse TMS.[144]

Conclusions

In the context of grey matter pathology in MS, much attention has been focused on axonal damage and transection. Insights from both clinical and experimental studies, however, are establishing a role for synaptic dysfunction in the development of disability in MS (Figure 2). First, alterations of the synaptic compartment are not only a consequence of axonal or neuronal damage, but are also part of an ongoing process that does not depend on axonal transection (because synaptic dysfunction can begin early in the MS and EAE disease course, and continues throughout the progression of the disease). Second, in both MS and EAE, the balance

between glutamatergic and GABAergic transmission is perturbed in the brain and spinal cord, and this imbalance between excitatory and inhibitory transmission is detrimental for both motor and cognitive functions. Third, the inflammatory microenvironment is a key determinant of diffuse synaptopathy in MS and EAE. Inflammatory cytokines released during acute MS attacks can both increase glutamate-mediated synaptic transmission and reduce GABA-mediated synaptic signalling, resulting in synaptic hyperexcitation and, possibly, excitotoxic neurodegeneration. Last, on the basis that synaptic dysfunction and loss are reversible, (Figure 3), targeting of mechanisms that stabilize and protect, or repair and regenerate synapses would enable clinical interventions at both early and late stages of the disease.

A deeper understanding of the fundamental roles of inflammatory and non-inflammatory mechanisms of synaptic dysfunction and degeneration could have clinical implications not only for MS, but also for other neurological disorders[145] in which an interplay between synaptopathy and neuroinflammation is implicated.

Review criteria

Literature for this Review were identified through searches of PubMed and ProteinQuest (BioDigitalValley; a platform for biomedical literature retrieval and analysis, which integrates data from scientific literature, biological images and data repositories from articles, clinical trials and patents). We searched for articles published in English up to August 2015. The keywords "multiple sclerosis" and/or "EAE" were used in combination with: "pathogenesis", "grey matter", "synaptic loss", "synaptic dysfunction", "inhibitory transmission", "GABA", "glutamate", "glutamate receptor", "NMDA", "AMPA", "pro-inflammatory cytokines", "IL-1β", "TNF", "TMS" (single or multiple items from the list). Additional filters were applied for brain regions (hippocampus, cortex, spinal cord, cerebellum, cortex, striatum) and for drugs related to clinical and preclinical treatments (including glutamate and GABA antagonist and/or agonist). We also identified articles through searches of the reference lists of the articles found with the above-cited search terms, and of the authors' own files. The final reference list was generated on the basis of originality and relevance to the scope of this Review.

1. Peterson, J. W., Bö, L., Mörk, S., Chang, A. & Trapp, B. D. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann. Neurol.* **50**, 389–400 (2001).
2. Compston, A. & Coles, A. Multiple sclerosis. *Lancet* **372**, 1502–1517 (2008).
3. Ciccarelli, O. *et al.* Pathogenesis of multiple sclerosis: insights from molecular and metabolic imaging. *Lancet Neurol.* **13**, 807–822 (2014).
4. Geurts, J. J. G., Calabrese, M., Fisher, E. & Rudick, R. A. Measurement and clinical effect of grey matter pathology in multiple sclerosis. *Lancet Neurol.* **11**, 1082–1092 (2012).
5. Bø, L., Vedeler, C. A., Nyland, H. I., Trapp, B. D. & Mørk, S. J. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *J. Neuropathol. Exp. Neurol.* **62**, 723–732 (2003).
6. Kutzelnigg, A. *et al.* Widespread demyelination in the cerebellar cortex in multiple sclerosis. *Brain Pathol.* **17**, 38–44 (2007).
7. Peterson, J. W., Bö, L., Mörk, S., Chang, A. & Trapp, B. D. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann. Neurol.* **50**, 389–400 (2001).
8. Wegner, C., Esiri, M. M., Chance, S. A., Palace, J. & Matthews, P. M. Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. *Neurology* **67**, 960–967 (2006).
9. Kooi, E.-J. *et al.* Cholinergic imbalance in the multiple sclerosis hippocampus. *Acta Neuropathol.* **122**, 313–322 (2011).
10. De Stefano, N. *et al.* Evidence of early cortical atrophy in MS: relevance to white matter changes and disability. *Neurology* **60**, 1157–1162 (2003).
11. Audoin, B. *et al.* Atrophy mainly affects the limbic system and the deep grey matter at the first stage of multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* **81**, 690–695 (2010).
12. Calabrese, M. *et al.* Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. *Arch. Neurol.* **66**, 1144–1150 (2009).
13. Tedeschi, G. *et al.* Brain atrophy and lesion load in a large population of patients with multiple sclerosis. *Neurology* **65**, 280–285 (2005).
14. Sanfilipo, M. P., Benedict, R. H., Sharma, J., Weinstock-Guttman, B. & Bakshi, R. The relationship between whole brain volume and disability in multiple sclerosis: a comparison of normalized gray vs. white matter with misclassification correction. *Neuroimage* **26**, 1068–1077 (2005).
15. Roosendaal, S. D. *et al.* Grey matter volume in a large cohort of MS patients: relation to MRI parameters and disability. *Mult. Scler.* **17**, 1098–1106 (2011).
16. Dutta, R. *et al.* Demyelination causes synaptic alterations in hippocampi from multiple sclerosis patients. *Ann. Neurol.* **69**, 445–454 (2011).
17. Calabrese, M. *et al.* Exploring the origins of grey matter damage in multiple sclerosis. *Nat. Rev. Neurosci.* **16**, 147–158 (2015).
18. Mix, E., Meyer-Rienecker, H., Hartung, H. P. & Zettl, U. K. Animal models of multiple sclerosis—potentials and limitations. *Prog. Neurobiol.* **92**, 386–404 (2010).
19. Friese, M. A., Schattling, B. & Fugger, L. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. *Nat. Rev. Neurol.* **10**, 225–238 (2014).
20. Ellwardt, E. & Zipp, F. Molecular mechanisms linking neuroinflammation and neurodegeneration in MS. *Exp. Neurol.* **262**, 8–17 (2014).
21. Michailidou, I. *et al.* Complement C1q-C3-associated synaptic changes in multiple sclerosis hippocampus. *Ann. Neurol.* **77**, 1007–1026 (2015).
22. Vercellino, M. *et al.* Altered glutamate reuptake in relapsing-remitting and secondary progressive multiple sclerosis cortex: correlation with microglia infiltration, demyelination, and neuronal and synaptic damage. *J. Neuropathol. Exp. Neurol.* **66**, 723–729 (2007).
23. Kornek, B. *et al.* Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. *Am. J. Pathol.* **157**, 267–276 (2000).
24. Tambalo, S. *et al.* Functional magnetic resonance imaging of rats with experimental autoimmune encephalomyelitis reveals brain cortex remodeling. *J. Neurosci.* **35**, 10088–10100 (2015).
25. Zhu, B., Luo, L., Moore, G. R., Paty, D. W. & Cynader, M. S. Dendritic and synaptic pathology in experimental autoimmune encephalomyelitis. *Am. J. Pathol.* **162**, 1639–1650 (2003).
26. Marques, K. B., Santos, L. M. & Oliveira, A. L. Spinal motoneuron synaptic plasticity during the course of an animal model of multiple sclerosis. *Eur. J. Neurosci.* **24**, 3053–3062 (2006).
27. Freria, C. M., Zanon, R. G., Santos, L. M. & Oliveira, A. L. Major histocompatibility complex class I expression and glial reaction influence spinal motoneuron synaptic plasticity during the course of experimental autoimmune encephalomyelitis. *J. Comp. Neurol.* **518**, 990–1007 (2010).
28. Kerschensteiner, M. *et al.* Remodeling of axonal connections contributes to recovery in an animal model of multiple sclerosis. *J. Exp. Med.* **200**, 1027–1038 (2004).
29. Centonze, D. *et al.* Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. *J. Neurosci.* **29**, 3442–3452 (2009).
30. Ziehn, M. O., Avedisian, A. A., Tiwari-Woodruff, S. & Voskuhl, R. R. Hippocampal CA1 atrophy and synaptic loss during experimental autoimmune encephalomyelitis, EAE. *Lab. Invest.* **90**, 774–786 (2010).
31. Ziehn, M. O. *et al.* Therapeutic testosterone administration preserves excitatory synaptic transmission in the hippocampus during autoimmune demyelinating disease. *J. Neurosci.* **32**, 12312–12324 (2012).
32. Mori, F. *et al.* Interleukin-1β promotes long-term potentiation in patients with multiple sclerosis. *Neuromolecular Med.* **16**, 38–51 (2013).
33. Mandolesi, G. *et al.* GABAergic signaling and connectivity on Purkinje cells are impaired in experimental autoimmune encephalomyelitis. *Neurobiol. Dis.* **46**, 414–424 (2012).
34. Rossi, S. *et al.* Impaired striatal GABA transmission in experimental autoimmune encephalomyelitis. *Brain Behav. Immun.* **25**, 947–956 (2011).

35. Falco, A., Pennucci, R., Brambilla, E. & De Curtis, I. Reduction in parvalbumin-positive interneurons and inhibitory input in the cortex of mice with experimental autoimmune encephalomyelitis. *Exp. Brain Res.* **232**, 2439–2449 (2014).
36. Mandolesi, G. *et al.* Interleukin-1β alters glutamate transmission at purkinje cell synapses in a mouse model of multiple sclerosis. *J. Neurosci.* **33**, 12105–12121 (2013).
37. Nisticò, R. *et al.* Inflammation subverts hippocampal synaptic plasticity in experimental multiple sclerosis. *PLoS ONE* **8**, e54666 (2013).
38. Clements, R. J., McDonough, J. & Freeman, E. J. Distribution of parvalbumin and calretinin immunoreactive interneurons in motor cortex from multiple sclerosis post-mortem tissue. *Exp. Brain Res.* **187**, 459–465 (2008).
39. Freeman, L. *et al.* The neuronal component of gray matter damage in multiple sclerosis: a [^{11}C] flumazenil positron emission tomography study. *Ann. Neurol.* **78**, 554–567 (2015).
40. Nicholas, R., Magliozzi, R., Campbell, G., Mahad, D. & Reynolds, R. Temporal lobe cortical pathology and inhibitory GABA interneuron cell loss are associated with seizures in multiple sclerosis. *Mult. Scler*. http://dx.doi.org/10.1177/1352458515579445.
41. Pitt, D., Werner, P. & Raine, C. S. Glutamate excitotoxicity in a model of multiple sclerosis. *Nat. Med.* **6**, 67–70 (2000).
42. Stojanovic, I. R., Kostic, M. & Ljubisavljevic, S. The role of glutamate and its receptors in multiple sclerosis. *J. Neural Transm.* **121**, 945–955 (2014).
43. Centonze, D. *et al.* The link between inflammation, synaptic transmission and neurodegeneration in multiple sclerosis. *Cell Death Differ*. **17**, 1083–1091 (2010).
44. Azevedo, C. J. *et al. In vivo* evidence of glutamate toxicity in multiple sclerosis. *Ann. Neurol.* **76**, 269–278 (2014).
45. Bolton, C. & Paul, C. Glutamate receptors in neuroinflammatory demyelinating disease. *Mediators Inflamm.* **2006**, 93684 (2006).
46. Mehta, A., Prabhakar, M., Kumar, P., Deshmukh, R. & Sharma, P. L. Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *Eur. J. Pharmacol.* **698**, 6–18 (2013).
47. MacMillan, E. L. *et al.* Progressive multiple sclerosis exhibits decreasing glutamate and glutamine over two years. *Mult. Scler.* http://dx.doi.org/10.1177/1352458515586086.
48. Muhlert, N. *et al.* Memory in multiple sclerosis is linked to glutamate concentration in grey matter regions. *J. Neurol. Neurosurg. Psychiatry* **85**, 834–840 (2014).
49. Qureshi, G. A. & Baig, M. S. Quantitation of free amino acids in biological samples by high-performance liquid chromatography. Application of the method in evaluating amino acid levels in cerebrospinal fluid and plasma of patients with multiple sclerosis. *J. Chromatogr.* **459**, 237–244 (1988).
50. Honegger, C. G., Krenger, W. & Langemann, H. Changes in amino acid contents in the spinal cord and brainstem of rats with experimental autoimmune encephalomyelitis. *J. Neurochem.* **53**, 423–427 (1989).
51. Musgrave, T. *et al.* The MAO inhibitor phenelzine improves functional outcomes in mice with experimental autoimmune encephalomyelitis (EAE). *Brain Behav. Immun.* **25**, 1677–1688 (2011).
52. Kan, Q.-C., Zhang, S., Xu, Y.-M., Zhang, G.-X. & Zhu, L. Matrine regulates glutamate-related excitotoxic factors in experimental autoimmune encephalomyelitis. *Neurosci. Lett.* **560**, 92–97 (2014).
53. Castegna, A. *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 (2011).
54. Werner, P., Pitt, D. & Raine, C. S. Multiple sclerosis: Altered glutamate homeostasis in lesions correlates with oligodendrocyre and axonal damage. *Ann. Neurol.* **50**, 169–180 (2001).
55. Stover, J. F. *et al.* Neurotransmitters in cerebrospinal fluid reflect pathological activity. *Eur. J. Clin. Invest.* **27**, 1038–1043 (1997).
56. Sarchielli, P., Greco, L., Floridi, A., Floridi, A. & Gallai, V. Excitatory amino acids and multiple sclerosis: evidence from cerebrospinal fluid. *Arch. Neurol.* **60**, 1082–1088 (2003).
57. Srinivasan, R., Sailasuta, N., Hurd, R., Nelson, S. & Pelletier, D. Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T. *Brain* **128**, 1016–1025 (2005).
58. Vrenken, H. *et al.* MR spectroscopic evidence for glial increase but not for neuro-axonal damage in MS normal-appearing white matter. *Magn. Reson. Med.* **53**, 256–266 (2005).
59. Baranzini, S. E. *et al.* Genetic variation influences glutamate concentrations in brains of patients with multiple sclerosis. *Brain* **133**, 2603–2611 (2010).
60. Ganor, Y. & Levite, M. 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.* **121**, 983–1006 (2014).
61. Hardin-Pouzet, H. *et al.* Glutamate metabolism is down-regulated in astrocytes during experimental allergic encephalomyelitis. *Glia* **20**, 79–85 (1997).
62. Olechowski, C. J. *et al.* A diminished response to formalin stimulation reveals a role for the glutamate transporters in the altered pain sensitivity of mice with experimental autoimmune encephalomyelitis (EAE). *Pain* **149**, 565–572 (2010).
63. Ohgoh, M. Altered expression of glutamate transporters in experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **125**, 170–178 (2002).
64. Mitosek-Szewczyk, K., Sulkowski, G., Stelmasiak, Z. & Strużyńska, L. 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 (2008).
65. Vallejo-Illarramendi, A., Domercq, M., Pérez-Cerdá, F., Ravid, R. & Matute, C. Increased expression and function of glutamate transporters in multiple sclerosis. *Neurobiol. Dis.* **21**, 154–164 (2006).
66. Lieury, A. *et al.* Tissue remodeling in periplaque regions of multiple sclerosis spinal cord lesions. *Glia* **62**, 1645–1658 (2014).
67. Newcombe, J. *et al.* Glutamate receptor expression in multiple sclerosis lesions. *Brain Pathol.* **18**, 52–61 (2008).
68. Marte, A. *et al.* Alterations of glutamate release in the spinal cord of mice with experimental autoimmune encephalomyelitis. *J. Neurochem.* **115**, 343–352 (2010).
69. Geurts, J. J. G. *et al.* Altered expression patterns of group I and II metabotropic glutamate receptors in multiple sclerosis. *Brain* **126**, 1755–1766 (2003).
70. Fazio, F. *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).
71. Han, M. H. *et al.* Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. *Nature* **451**, 1076–1081 (2008).
72. Zhai, D. *et al.* Blocking GluR2-GAPDH ameliorates experimental autoimmune encephalomyelitis. *Ann. Clin. Transl. Neurol.* **2**, 388–400 (2015).
73. Wheeler, E., Bolton, C., Mullins, J. & Paul, C. Identification of the NMDA receptor subtype involved in the development of disease during experimental autoimmune encephalomyelitis. *pA*$_2$ [online], http://www.pa2online.org/Vol1Issue1abst018P.html (2003).
74. Sulkowski, G., Dąbrowska-Bouta, B., Chalimoniuk, M. & Strużyńska, L. Effects of antagonists of glutamate receptors on pro-inflammatory cytokines in the brain cortex of rats subjected to experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **261**, 67–76 (2013).
75. Groom, A. J., Smith, T. & Turski, L. Multiple sclerosis and glutamate. *Ann. N. Y. Acad. Sci.* **993**, 229–275 (2003).
76. Starck, M., Albrecht, H., Pöllmann, W., Straube, A. & Dieterich, M. Drug therapy for acquired pendular nystagmus in multiple sclerosis. *J. Neurol.* **244**, 9–16 (1997).
77. Plaut, G. S. Effectiveness of amantadine in reducing relapses in multiple sclerosis. *J. R. Soc. Med.* **80**, 91–93 (1987).
78. Gilgun-Sherki, Y., Panet, H., Melamed, E. & Offen, D. Riluzole suppresses experimental autoimmune encephalomyelitis: Implications for the treatment of multiple sclerosis. *Brain Res.* **989**, 196–204 (2003).
79. Shijie, J. *et al.* Blockade of glutamate release from microglia attenuates experimental autoimmune encephalomyelitis in mice. *Tohoku J. Exp. Med.* **217**, 87–92 (2009).
80. Killestein, J., Kalkers, N. F. & Polman, C. H. Glutamate inhibition in MS: the neuroprotective properties of riluzole. *J. Neurol. Sci.* **233**, 113–115 (2005).
81. Manyam, N. V, Katz, L., Hare, T. A., Gerber, J. C. & Grossman, M. H. Levels of gamma-aminobutyric acid in cerebrospinal fluid in various neurologic disorders. *Arch. Neurol.* **37**, 352–355 (1980).
82. Gottesfeld, Z., Teitelbaum, D., Webb, C. & Arnon, R. Changes in the GABA system in experimental allergic encephalomyelitis-induced paralysis. *J. Neurochem.* **27**, 695–699 (1976).
83. Achar, V. S., Welch, K. M., Chabi, E., Bartosh, K. & Meyer, J. S. Cerebrospinal fluid gamma-aminobutyric acid in neurologic disease. *Neurology* **26**, 777–780 (1976).
84. Zepeda, A. T., Ortiz Nesme, F. J., Méndez-Franco, J., Otero-Siliceo, E. & Pérez de la Mora, M. Free-GABA levels in the cerebrospinal fluid of patients suffering from several neurological diseases Its potential use for the diagnosis of diseases which course with inflammation and tissular necrosis. *Amino Acids* **9**, 207–216 (1995).
85. Demakova, E. V, Korobov, V. P. & Lemkina, L. M. Determination of gamma-aminobutyric acid concentration and activity of glutamate decarboxylase in blood serum of patients with multiple sclerosis [Russian]. *Klin. Lab. Diagn.* **4**, 15–17 (2003).

86. Lock, C. *et al.* Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat. Med.* **8**, 500–508 (2002).
87. Massella, A. *et al.* Gender effect on neurodegeneration and myelin markers in an animal model for multiple sclerosis. *BMC Neuroscience* **13**, 12 (2012).
88. Vanheel, A. *et al.* Identification of protein networks involved in the disease course of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. *PLoS ONE* **7**, e35544 (2012).
89. Wang, Y. *et al.* γ-Aminobutyric acid transporter 1 negatively regulates T cell-mediated immune responses and ameliorates autoimmune inflammation in the CNS. *J. Immunol.* **181**, 8226–8236 (2008).
90. Paul, A. M. *et al.* GABA transport and neuroinflammation are coupled in multiple sclerosis: Regulation of the GABA transporter-2 by ganaxolone. *Neuroscience* **273**, 24–38 (2014).
91. Tajouri, L. *et al.* Quantitative and qualitative changes in gene expression patterns characterize the activity of plaques in multiple sclerosis. *Brain Res. Mol. Brain Res.* **119**, 170–183 (2003).
92. Bhat, R. *et al.* Inhibitory role for GABA in autoimmune inflammation. *Proc. Natl Acad. Sci. USA* **107**, 2580–2585 (2010).
93. Jin, Z., Mendu, S. K. & Birnir, B. GABA is an effective immunomodulatory molecule. *Amino Acids* **45**, 87–94 (2013).
94. Carmans, S. *et al.* Systemic treatment with the inhibitory neurotransmitter gamma-aminobutyric acid aggravates experimental autoimmune encephalomyelitis by affecting proinflammatory immune responses. *J. Neuroimmunol*. **255**, 45–53 (2013).
95. Gilani, A. A., Dash, R. P., Jivrajani, M. N., Thakur, S. K. & Nivsarkar, M. Evaluation of GABAergic transmission modulation as a novel functional target for management of multiple sclerosis: Exploring inhibitory effect of GABA on glutamate-mediated excitotoxicity. *Adv. Pharmacol. Sci.* **2014**, 632376 (2014).
96. Bibolini, M. J. *et al.* Inhibitory role of diazepam on autoimmune inflammation in rats with experimental autoimmune encephalomyelitis. *Neuroscience* **199**, 421–428 (2011).
97. Rode, G., Maupas, E., Luaute, J., Courtois-Jacquin, S. & Boisson, D. Medical treatment of spasticity [French]. *Neurochirurgie* **49**, 247–255 (2003).
98. Starck, M., Albrecht, H., Pöllmann, W., Dieterich, M. & Straube, A. Acquired pendular nystagmus in multiple sclerosis: an examiner-blind cross-over treatment study of memantine and gabapentin. *J. Neurol.* **257**, 322–327 (2010).
99. Bandini, F., Castello, E., Mazzella, L., Mancardi, G. L. & Solaro, C. Gabapentin but not vigabatrin is effective in the treatment of acquired nystagmus in multiple sclerosis: how valid is the GABAergic hypothesis? *J. Neurol. Neurosurg. Psychiatry* **71**, 107–110 (2001).
100. Cutter, N. C., Scott, D. D., Johnson, J. C. & Whiteneck, G. Gabapentin effect on spasticity in multiple sclerosis: a placebo-controlled, randomized trial. *Arch. Phys. Med. Rehabil*. **81**, 164–169 (2000).
101. Grasselli, G. *et al.* Abnormal NMDA receptor function exacerbates experimental autoimmune encephalomyelitis. *Br. J. Pharmacol.* **168**, 502–517 (2013).
102. Mallucci, G., Peruzzotti-Jametti, L., Bernstock, J. D. & Pluchino, S. The role of immune cells, glia and neurons in white and grey matter pathology in multiple sclerosis. *Prog. Neurobiol.* **127–128**, 1–22 (2015).
103. Wrona, D. Neural-immune interactions: an integrative view of the bidirectional relationship between the brain and immune systems. *J. Neuroimmunol.* **172**, 38–58 (2006).
104. Verderio, C. *et al.* Myeloid microvesicles are a marker and therapeutic target for neuroinflammation. *Ann. Neurol.* **72**, 610–624 (2012).
105. Kettenmann, H., Kirchhoff, F. & Verkhratsky, A. Microglia: new roles for the synaptic stripper. *Neuron* **77**, 10–18 (2013).
106. Vitkovic, L., Bockaert, J. & Jacque, C. "Inflammatory" cytokines: neuromodulators in normal brain? *J. Neurochem.* **74**, 457–471 (2000).
107. Bauer, S., Kerr, B. J. & Patterson, P. H. The neuropoietic cytokine family in development, plasticity, disease and injury. *Nat. Rev. Neurosci.* **8**, 221–232 (2007).
108. Schäfers, M. & Sorkin, L. Effect of cytokines on neuronal excitability. *Neurosci. Lett.* **437**, 188–193 (2008).
109. Steinman, L. Inflammatory cytokines at the summits of pathological signal cascades in brain diseases. *Sci. Signal.* **6**, pe3 (2013).
110. Rossi, S. *et al.* Interleukin-1β causes synaptic hyperexcitability in multiple sclerosis. *Ann. Neurol.* **71**, 76–83 (2012).
111. Rossi, S. *et al.* Abnormal activity of the Na/Ca exchanger enhances glutamate transmission in experimental autoimmune encephalomyelitis. *Brain Behav. Immun.* **24**, 1379–1385 (2010).
112. Haji, N. *et al.* TNF-α-mediated anxiety in a mouse model of multiple sclerosis. *Exp. Neurol.* **237**, 296–303 (2012).
113. Weiss, S., Mori, F., Rossi, S. & Centonze, D. Disability in multiple sclerosis: when synaptic long-term potentiation fails. *Neurosci. Biobehav. Rev.* **43**, 88–99 (2014).
114. Ziehn, M. O., Avedisian, A. A., Dervin, S. M., O'Dell, T. J. & Voskuhl, R. R. Estriol preserves synaptic transmission in the hippocampus during autoimmune demyelinating disease. *Lab. Invest.* **92**, 1234–1245 (2012).
115. Musumeci, G. *et al.* Transient receptor potential vanilloid 1 channels modulate the synaptic effects of TNF-α and of IL-1β in experimental autoimmune encephalomyelitis. *Neurobiol. Dis.* **43**, 669–677 (2011).
116. Rossini, P. M. *et al.* Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr. Clin. Neurophysiol.* **91**, 79–92 (1994).
117. Conte, A. *et al.* Intracortical excitability in patients with relapsing-remitting and secondary progressive multiple sclerosis. J. *Neurol.* **256**, 933–938 (2009).
118. Vucic, S. *et al.* Cortical dysfunction underlies disability in multiple sclerosis. *Mult. Scler.* **18**, 425–432 (2012).
119. Schmierer, K., Irlbacher, K., Grosse, P., Röricht, S. & Meyer, B.-U. Correlates of disability in multiple sclerosis detected by transcranial magnetic stimulation. *Neurology* **59**, 1218–1224 (2002).
120. Codecà, C. *et al.* Differential patterns of interhemispheric functional disconnection in mild and advanced multiple sclerosis. *Mult. Scler.* **16**, 1308–1316 (2010).
121. Mori, F. *et al.* Short interval intracortical facilitation correlates with the degree of disability in multiple sclerosis. *Brain Stimul.* **6**, 67–71 (2013).
122. Caramia, M. D. *et al.* 'Excitability changes of muscular responses to magnetic brain stimulation in patients with central motor disorders. *Electroencephalogr. Clin. Neurophysiol.* **81**, 243–250 (1991).
123. Schwenkreis, P. *et al.* Influence of the *N*-methyl-D-aspartate antagonist memantine on human motor cortex excitability. *Neurosci. Lett.* **270**, 137–140 (1999).
124. Ziemann, U., Lönnecker, S., Steinhoff, B. J. & Paulus, W. Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. *Ann. Neurol.* **40**, 367–378 (1996).
125. Rossi, S. *et al.* Inflammation inhibits GABA transmission in multiple sclerosis. *Mult. Scler.* **18**, 1633–1635 (2012).
126. Rossi, S. *et al.* Tumor necrosis factor is elevated in progressive multiple sclerosis and causes excitotoxic neurodegeneration. *Mult. Scler.* **20**, 304–312 (2014).
127. Morgen, K. *et al.* Training-dependent plasticity in patients with multiple sclerosis. *Brain* **127**, 2506–2517 (2004).
128. Vosoughi, R. & Freedman, M. S. Therapy of MS. *Clin. Neurol. Neurosurg.* **112**, 365–385 (2010).
129. Ransohoff, R. M., Hafler, D. A. & Lucchinetti, C. F. Multiple sclerosis—a quiet revolution. *Nat. Rev. Neurol.* **11**, 134–142 (2015).
130. Franklin, R. J., ffrench-Constant, C., Edgar, J. M. & Smith, K. J. Neuroprotection and repair in multiple sclerosis. *Nat. Rev. Neurol.* **8**, 624–634 (2012).
131. Arnold, D. L. *et al.* Effects of delayed-release dimethyl fumarate on MRI measures in the Phase 3 DEFINE study. *J. Neurol.* **261**, 1794–1802 (2014).
132. Filippi, M. *et al.* Placebo-controlled trial of oral laquinimod in multiple sclerosis: MRI evidence of an effect on brain tissue damage. *J. Neurol. Neurosurg. Psychiatry* **85**, 851–858 (2014).
133. Cohen, J. a. *et al.* Long-term (up to 4.5 years) treatment with fingolimod in multiple sclerosis: results from the extension of the randomised TRANSFORMS study. *J. Neurol. Neurosurg. Psychiatry* http://dx.doi.org/10.1136/jnnp-2015-310597.
134. Ontaneda, D., Fox, R. J. & Chataway, J. Clinical trials in progressive multiple sclerosis: lessons learned and future perspectives. *Lancet Neurol.* **14**, 208–223 (2015).
135. Rossi, S. *et al.* Oral fingolimod rescues the functional deficits of synapses in experimental autoimmune encephalomyelitis. *Br. J. Pharmacol.* **165**, 861–869 (2012).
136. Ruffini, F. *et al.* Laquinimod prevents inflammation-induced synaptic alterations occurring in experimental autoimmune encephalomyelitis. *Mult. Scler.* **19**, 1084–1094 (2013).
137. Parodi, B. *et al.* Fumarates modulate microglia activation through a novel HCAR2 signaling pathway and rescue synaptic dysregulation in inflamed CNS. *Acta Neuropathol.* **130**, 279–295 (2015).
138. Moore, S. *et al.* Therapeutic laquinimod treatment decreases inflammation, initiates axon remyelination, and improves motor deficit in a mouse model of multiple sclerosis. *Brain Behav.* **3**, 664–682 (2013).
139. Brück, W. & Wegner, C. Insight into the mechanism of laquinimod action. *J. Neurol. Sci.* **306**, 173–179 (2011).
140. Mishra, M. K. *et al.* Laquinimod reduces neuroaxonal injury through inhibiting microglial activation. *Ann. Clin. Transl. Neurol.* **1**, 409–422 (2014).
141. Wilms, H. *et al.* Dimethylfumarate inhibits microglial and astrocytic inflammation by suppressing the synthesis of nitric oxide, IL-1β, TNF-α and IL-6 in an in-vitro model of brain inflammation. *J. Neuroinflammation* **7**, 30 (2010).

142. Noda, H., Takeuchi, H., Mizuno, T. & Suzumura, A. Fingolimod phosphate promotes the neuroprotective effects of microglia. *J. Neuroimmunol.* **256**, 13–18 (2013).
143. Di Menna, L. *et al.* Fingolimod protects cultured cortical neurons against excitotoxic death. *Pharmacol. Res.* **67**, 1–9 (2013).
144. Landi, D. *et al.* Oral fingolimod reduces glutamate-mediated intracortical excitability in relapsing-remitting multiple sclerosis. *Clin. Neurophysiol.* **126**, 165–169 (2015).
145. Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C. & Gage, H. Mechanisms underlying inflammation in neurodegeneration. *Cell* **140**, 918–934 (2010).
146. Lovera, J. F. *et al.* Memantine for cognitive impairment in multiple sclerosis: a randomized placebo-controlled trial. *Mult. Scler.* **16**, 715–723 (2010).
147. Smith, T., Groom, A., Zhu, B. & Turski, L. Autoimmune encephalomyelitis ameliorated by AMPA antagonists. *Nat. Med.* **6**, 62–66 (2000).
148. Bolton, C. & Paul, C. MK-801 limits neurovascular dysfunction during experimental allergic encephalomyelitis. *J. Pharmacol. Exp. Ther.* **282**, 397–402 (1997).
149. Paul, C. & Bolton, C. Modulation of blood-brain barrier dysfunction and neurological deficits during acute experimental allergic encephalomyelitis by the *N*-methyl-D-aspartate receptor antagonist memantine. *J. Pharmacol. Exp. Ther.* **302**, 50–57 (2002).
150. Wallström, E. *et al.* Memantine abrogates neurological deficits, but not CNS inflammation, in Lewis rat experimental autoimmune encephalomyelitis. *J. Neurol. Sci.* **137**, 89–96 (1996).
151. Sulkowski, G., Dąbrowska-Bouta, B., Salińska, E. & Strużyńska, L. Modulation of glutamate transport and receptor binding by glutamate receptor antagonists in EAE rat brain. *PLoS ONE* **9**, e113954 (2014).
152. Sulkowski, G., Dąbrowska-Bouta, B., & Strużyńska, L. Modulation of neurological deficits and expression of glutamate receptors during experimental autoimmune encephalomyelitis after treatment with selected antagonists of glutamate receptors. *Biomed. Res. Int.* **2013**, 186068 (2013).
153. Kanwar, J. R., Kanwar, R. K. & Krissansen, G. W. Simultaneous neuroprotection and blockade of inflammation reverses autoimmune encephalomyelitis. *Brain* **127**, 1313–1331 (2004).
154. Kanwar, J. R. Anti-inflammatory immunotherapy for multiple sclerosis/experimental autoimmune encephalomyelitis (EAE) disease. *Curr. Med. Chem.* **12**, 2947–2962 (2005).
155. Besong, G. *et al.* Activation of group III metabotropic glutamate receptors inhibits the production of RANTES in glial cell cultures. *J. Neurosci.* **22**, 5403–5411 (2002).
156. Solaro, C., Uccelli, M. M., Guglieri, P., Uccelli, A. & Mancardi, G. L. Gabapentin is effective in treating nocturnal painful spasms in multiple sclerosis. *Mult. Scler.* **6**, 192–193 (2000).
157. Ørsnes, G., Crone, C., Krarup, C., Petersen, N. & Nielsen, J. The effect of baclofen on the transmission in spinal pathways in spastic multiple sclerosis patients. *Clin. Neurophysiol.* **111**, 1372–1379 (2000).
158. Nielsen, J. F., Anderson, J. B. & Sinkjaer, T. Baclofen increases the soleus stretch reflex threshold in the early swing phase during walking in spastic multiple sclerosis patients. *Mult. Scler.* **6**, 105–114 (2000).
159. Nielsen, J. F. & Sinkjær, T. Peripheral and central effect of baclofen on ankle joint stiffness in multiple sclerosis. *Muscle Nerve* **23**, 98–105 (2000).

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D.C., G.M., A.G., A.M., D.F., F.D.V., S.B. and H.S. researched data for the article. D.C. and G.M. wrote the article. D.C., G.M., A.G., A.M. and G.A.M. provided substantial contributions to discussion of content. All authors participated in reviewing and/or editing of the manuscript before submission.

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