Chapter 15
Glutamate in Multiple Sclerosis: From Pathophysiology to Treatments

Anna Pittaluga and Guendalina Olivero

Abstract Multiple sclerosis (MS) is an autoimmune disease typified by overt demyelination and inflammation that develop in selected regions of the central nervous system (CNS). Besides these signs, a diffuse loss of synaptic contacts, axonal pruning and astrocytosis are also observed, that in general correlate with the dysregulation of the glutamatergic system and with the onset of neurological symptoms. Concomitantly to the synaptic derangements, impaired glutamate homeostasis also dysregulates the immunocompetent responses, impairing the functional cross-talk between the immune system and the CNS. The study of the glutamatergic system therefore emerges as an important issue for deciphering the cellular events at the basis of MS as it would permit the proposal of new appropriate pharmacological interventions for the cure of the pathology. The chapter describes recent advances in basic research, preclinical and clinical studies concerning the impact of altered glutamate homeostasis in the course of the disease, as well as in the innovative strategies that would permit the restoration of central glutamatergic transmission.

Keywords Multiple sclerosis · EAE mice · Glutamate · Synaptopathy · Release · Uptake · Receptors

Abbreviations

2-AG	2-arachidonoylglycerol
2-PMPA	2-(phosphonomethyl) pentanedioic acid

A. Pittaluga (✉)
Department of Pharmacy (DIFAR), University of Genoa, Genoa, Italy

IRCCS Ospedale Policlinico San Martino, Genoa, Italy
e-mail: pittalug@difar.unige.it

G. Olivero
Department of Pharmacy (DIFAR), University of Genoa, Genoa, Italy
e-mail: olivero@difar.unige.it

Z. M. Pavlovic (ed.), *Glutamate and Neuropsychiatric Disorders*,
https://doi.org/10.1007/978-3-030-87480-3_15

AC	Adenylyl cyclase
CB1 receptors	Cannabinoid receptors type 1
CNS	Central nervous system
d.p.i.	Days post immunization
DMDs	Disease-modifying drugs
EAAT	Excitatory amino acid transporter
EAE	Experimental autoimmune encephalomyelitis
EC	Endogenous cannabinoids
EPSCs	Excitatory postsynaptic currents
FAAH	Fatty acid amide hydrolase
GCPII	Glutamate carboxypeptidase II
GDH	Glutamate dehydrogenase
GLS	Glutaminase
GOT	Glutamate-oxaloacetate transaminase
GPT	Glutamate-pyruvate transaminase
GS	Glutamine synthase
HCAR2	Hydroxycarboxylic acid receptor 2
IPSCs	Inhibitory postsynaptic currents
IS	Immune system
KA	Kynurenic acid
KP	Kynurenine pathway
LTD	Long-term depression
LTP	Long-term potentiation
MBP	Myelin basic protein
MGL	Monoacylglycerol lipase
mGlu receptor	Metabotropic glutamate receptor
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
MUNC-18	Mammalian uncoordinated-18
NAA	*N*-acetyl-aspartate
NAALADase	*N*-acetylated-alpha-linked acidic dipeptidase
PKA	Protein kinase A
PLP	Proteolipid protein
PPMS	Primary progressive multiple sclerosis
QA	Quinolinic acid
RMI	Resonance imaging
RRMS	Relapsing-remitting multiple sclerosis
SPMS	Secondary progressive multiple sclerosis
T	Tryptophan
TMS	Transcranial magnetic stimulation
xCT	Cystine/glutamate antiporter

15.1 Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) affecting about two to three million people worldwide. It is a progressively degenerating disorder typified by autoimmune attack directly at antigens associated with myelin, leading to the appearance of focal demyelinated plaques within the brain and the spinal cord. Despite the great efforts to investigate its aetiology and although much has been discovered about the immunobiology, genetics and epidemiology of the disease, its aetiopathogenesis remains so far unknown.

In most patients, MS develops a fluctuating course, typified by early relapsing-remitting episodes of neurological and radiological worsening, followed by symptomatologic recovers (i.e. the relapsing-remitting MS, RRMS). Epidemiological studies show that the 85% of patients suffering from MS develops the RRMS form of the disease. Within a decade, a large part of these individuals progresses to a secondary progressive MS (SPMS), that is typified by the development of neurological deficits that occur independently from relapses. A minor percentage (~15%) of patients develops a progressive course of the disease (i.e. the primary progressive MS, PPMS) for unknown reasons (Lublin et al. 2014).

Autoimmune mechanisms brought about by inflammatory lymphocytes, macrophages and activated microglia are traditionally proposed to play the major role in the development of the pathology. In particular, it is proposed that MS is initiated and maintained by the continuous migration of inflammatory immune cells from the periphery into the CNS, but also by the concomitant modulation of the autoimmune attack, probably mediated by the infiltrating T regulatory cells themselves. Recruitment of pro-inflammatory and regulatory leucocytes into inflamed tissues is controlled by chemokines and their receptors through their ability to drive gradient-dependent cell migration nearby the sites where they are actively released (Karpus and Ransohoff 1998; Ransohoff et al. 2007). Accordingly, the increased expression of selected chemokines (CCL5 and CXCL12, for instance) are predictive markers of the progression of the disease (Besong et al. 2002; Godiska et al. 1995; Pittaluga 2016; Sørensen et al. 1999).

Despite the peripheral to central immunological events are thought to play a main role in the development of the disease, they are not essential to the onset of central derangements. In fact, synaptic impairments, grey matter lesions and demyelination become evident in selected CNS areas (i.e. the cortex, the hippocampus, the cerebral cortex, the thalamus and the caudate-putamen) of MS patients starting from the earliest phases of the disease, also in the absence of infiltrating lymphocytes and macrophages (Klaver et al. 2013, Bevan et al. 2018; Eshaghi et al. 2018). The brain magnetic resonance imaging (RMI) is usually used to evidentiate these central lesions, that if present, often correlate with the onset of the early clinical neurological symptoms of the disease, consistent with their relevance in the course of the pathology.

Based on these considerations, starting from the last decade, MS has been classified also as a primary neurodegenerative disorder and two terms were proposed

to describe its course. The first term is "synaptopathy", to evidentiate the main role of synaptic disruption in the pathological framework (Mandolesi et al. 2015a, b) and the second one is "silent progression", which refers to the mode of progression of the disease, to stress the fact that neurodegeneration proceeds in MS patients largely independently from autoimmune inflammation (17; University of California, San Francisco MS-EPIC Team, and Cree 2019).

Our understanding of MS, as well as of the development of disease-modifying therapies, mostly relies on the availability of disease animal models. Among the available models, most of the preclinical data originate from studies carried out in mice suffering from the experimental autoimmune encephalomyelitis (the EAE mice). The EAE mice recapitulate many features of MS (Rangachari and Kuchroo 2013; Swanborg 1995). The demyelinating disorder is induced by immunizing mice with myelin antigens including myelin basic protein (MBP), proteolipid protein (PLP) or the myelin oligodendrocyte glycoprotein (MOG). In particular, the immunization with MOG elicits the development of a non-remitting form of disease typified by the presence of inflammatory lesions and demyelinated areas that predominate in the spinal cord. The clinical signs become evident at the early almost asymptomatic stage of the disease (at about 13 days after immunization, d.p.i.) and reach the maximal gravity at about 21 $\pm$ 1 d.p.i.

Interestingly, beside central inflammation and demyelination, EAE mice also develop altered glutamatergic transmission in selected CNS regions (i.e. the cortex), independently on the presence of clear white matter injures (Mangiardi et al. 2011). The EAE mice therefore represent a suitable model to study the neurological defects and the altered synaptic plasticity that typify the course of the disease.

15.2 The Glutamatergic System in MS and in EAE Mice

Glutamate controls the homeostasis of the CNS. It determines synaptic plasticity, i.e. the principal neuronal property involved in the ability of CNS to resist to insults, to assure an efficient neuronal response to stimuli and to build up restorative adaptations. In other terms, glutamate participates to determine the cognitive reserve, i.e. the ability of the brain to recover the maladaptation elicited by injures and/or aging during the entire lifespan. These beneficial effects however can be exhausted when pathological conditions that alter bioavailability of glutamate persist during time and/or overwhelm the mechanisms of "cognitive buffering".

In the CNS, the majority of neurons are glutamatergic and glutamate represents the principal transmitter at chemical synapsis. Derangements of glutamatergic homeostasis have a detrimental impact on the CNS and prelude to neurotoxicity as well as to synaptic dysregulation. In MS patients impaired glutamatergic (and GABAergic) synaptic transmission can be evidentiated by using the transcranial magnetic stimulation technique (TMS, Stampanoni Bassi et al. 2017). This synaptic maladaptation is proposed to play a main role in the onset and the development of the central lesions and neurological disability that typify the course of the disease. In

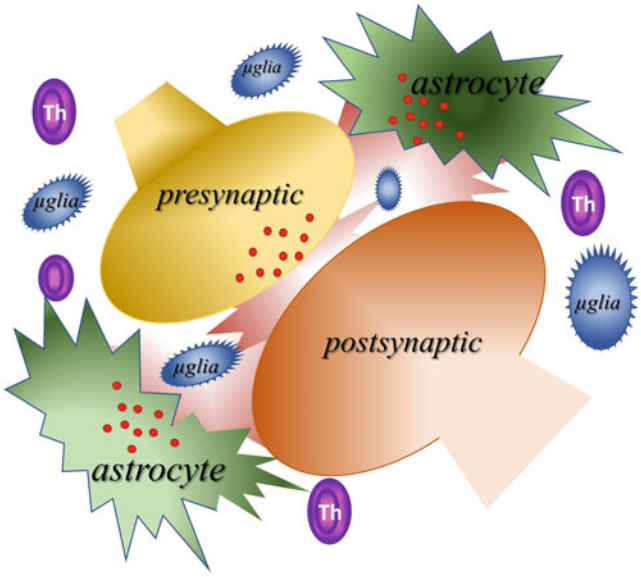

Fig. 15.1 Glutamate is the main excitatory transmitter in the central nervous system and plays a main role in controlling synaptic plasticity, to assure an efficient neuronal response to stimuli and to build up restorative adaptations. Unfortunately, when pathological conditions prevail and bioavailability of glutamate itself persists and overwhelms the mechanisms of "synaptic buffering", glutamate becomes detrimental, favouring the mechanisms of neurodegeneration and altering the efficiency of synaptic connection. In the central nervous system, besides neurons, which represent the primary physiological source of the excitatory amino acid, there are several other cells [i.e. astrocytes, microglia (μglia), brain macrophages, dendritic cells and infiltrating leucocytes and lymphocytes (Th)] that release glutamate and that control its homeostasis. These cells, in particular astrocytes and migrating lymphocytes, are important sources of the excitatory amino acid in pathological conditions associated with inflammation or immunocompetent responses, such as those observed in MS

humans, learning and memory as well as the resilience to stress and anxiety largely depend on the efficiency of central glutamate transmission and it is recognized that the deterioration of the glutamatergic innervation negatively reverberates on these functions. Consistent with this view, the neurological symptoms often observed in MS patients include difficulties in learning and remembering new information, depression and anxiety. These symptoms are observed in approximately 50% of individuals with MS and, unfortunately, in some cases (at least in early MS) are neglected or misdiagnosed (for reviews, see (Passos et al. 2014; Rao et al. 1991; Siegert and Abernethy 2005)).

Besides neurons, which represent the primary physiological source of the excitatory amino acid, several other cells (i.e. astrocytes, glial cells, brain macrophages, dendritic cells and infiltrating leucocytes and lymphocytes) release glutamate and control its homeostasis. Astrocytes and migrating lymphocytes are important sources of glutamate in pathological conditions associated with inflammation or

immunocompetent responses, such as those observed in MS (Fig. 15.1). All these aspects have been largely discussed in previous reviews and will be not further analysed in this chapter (see for exhaustive review (Centonze et al. 2009; Di Filippo et al. 2015; Fazio et al. 2018; Levite 2017; Mandolesi et al. 2015b; Matute et al. 1999; Nicoletti et al. 2011; Pittaluga 2017)).

15.2.1 Glutamate Bioavailability in the CSN of MS Patients and EAE Mice

Glutamate represents the driving force for synaptic plasticity and, as already introduced, observations in MS patients and EAE animals agree with the conclusion that severe, region-dependent alterations of glutamatergic transmission in the CNS are pivotal to disease.

In 1997, Klivényi and colleagues (Klivényi et al. 1997) quantified the levels of amino acids in the CSF of MS patients and compared the results with those obtained from the CSF of individuals suffering from lower back pain. The authors did not find significant differences in the CSF concentrations of the amino acids between the two groups. Almost concomitantly Stover et al. (1997) found that the level of different amino acids (including glutamate) was almost doubled in the CSF of MS patients at the acute symptomatic phase of the disease with respect to healthy subjects. Sarchielli et al. (2003) demonstrated a significant increase of glutamate (and aspartate) levels in the cerebrospinal fluid (CSF) of patients with the RRMS and the SPMS when compared to control individuals (subjects without central or peripheral neuronal pathology). They also observed a correlation between the phase of relapse and the concentration of glutamate in the CSF. Furthermore, they showed that the levels of glutamate in patients suffering from the RRMS but who had active lesions were higher with respect to patients without neuroradiological signs. High levels of glutamate were also detected in the CSF of patients suffering from SPMS.

In 2014 evidence was provided showing decreased level of glutamate in large areas of normal-appearing white and grey matter in MS patients (Azevedo et al. 2014). Multivoxel spectroscopy was used to quantify the glutamate and the *N*-acetylaspartate (NAA) levels and to quantify the GLU/NAA ratio in these patients. The results of the study unveiled a high GLU/NAA ratio that was considered predictive of altered neuroaxonal integrity. The result was proposed to be predictive of a decline of the brain volume and therefore of disease progression.

As to the EAE mice, data exists in the literature showing correlations between altered glutamate homeostasis and oligodendrocyte and axonal damage (Matute et al. 1999, 2001; Werner et al. 2001), among hyperglutamatergicity, neuroinflammation and synaptic degeneration (Mandolesi et al. 2010), as well as between altered glutamate release and glutamate receptor/transporters dysfunctions (as discussed below, but see (Castegna et al. 2011; Levite 2017; Pittaluga 2017)).

The excess of glutamate in the synaptic cleft is neurotoxic since it assures a pathological activation of the receptors repertoire (in particular the ionotropic glutamate receptors, namely NMDA and AMPA receptors) that is maladaptive to the synaptic network. The pathologically-relevant, increased availability of the amino acid might depend on several cascades of events, involving the impaired expression/functions of glutamate metabolizing enzymes, the dysfunction of the glutamate transporters and the hypersecretion of glutamate due to maladaptation in the synaptic machinery accounting for vesicular exocytosis, as well as to the overproduction of release-regulating factors, including cytokines. The information concerning these aspects is reviewed below.

15.2.2 Glutamate Metabolizing Enzymes in the CSN of MS Patients and EAE Mice

Studies in the literature correlate the central altered glutamate availability with the impaired expression/functions of glutamate metabolizing enzymes [i.e. the glutamate dehydrogenase (GDH) and the glutamine synthase (GS)] as well as of enzymes which tune the production of the amino acid [i.e. the glutaminase (GLS)]. As the GLS is concerned, its overexpression was reported to correlate with axonal damage. In particular, both early and chronic active lesions in brain tissue from MS patients showed high levels of the enzyme. Differently, evident GLS immunoreactivity was not observed in chronic silent lesions. The GLS-positive cells mirrored the distribution of activated macrophages and microglia cells, which are characteristic of central inflammation, suggesting a cross-linking between the overexpression of GLS-containing glutamate-producing immune cells and the development of excitotoxicity in the CNS of MS patients (Werner et al. 2001).

GS and GDH expression was dramatically decreased in the spinal cord of EAE mice with a very high score (Hardin-Pouzet et al. 1997). Increased oxidative modifications of GS in the cortex of EAE mice paralleled the severity of the clinical signs, while the GS/glutamate ratio largely decreased suggesting a correlation between EAE severity and excitotoxicity (Castegna et al. 2011). In the CNS of MS patients, the distribution of GDH and GS immunoreactivity was largely different from non-MS tissues. In particular, both enzymes were poorly expressed in oligodendrocytes but largely present in astrocytes and microglia (Werner et al. 2001).

15.2.3 Efficiency of Central Glutamatergic Transmission in the CNS of MS Patients and EAE Mice

The efficiency of synaptic transmission in MS patients is usually analysed with non-invasive techniques including the Transcranial Magnetic Stimulation (TMS) to

assess the integrity of the motor cortex plasticity, the efficiency of the cortical-spinal innervation and the efficiency of local interneurons, i.e. the GABAergic inhibitory ones for instance, in modulating synaptic signalling. Specific TMS measures and different protocols of stimulations (exhaustively described by (Stampanoni Bassi et al. 2017)) can be applied to evaluate the efficiency of glutamatergic transmission. The revision of the data in the literature suggests that the observations so far available require careful evaluation because of the heterogeneity of the modalities adopted for the recruitment of both the healthy and the MS patients, of the form of the disease they suffer from and of the concomitance of on-going therapy. Nonetheless, the results permit the conclusion that brain networks that are relevant to vision, cognition or sensory-motor functions undergo progressive modifications in the excitatory transmission (and in the GABAergic one as well) starting from the onset of the demyelinating disorder and that these modifications can be maladaptive in nature. These neuronal alterations do not cause irreversible modification in the synaptic activity, at least at the early stages of the disorder. As a matter of fact, the available results seem compatible with the conclusion that, at the onset of the disease, the brain maintains the ability to cope with the local and diffuse neuronal damages, resisting until its resilience to injures is not exhausted by the maladaptive stimuli. This synaptic flexibility accounts for the discrepancy often observed between the clinical disability and the central lesions in patients, suggesting that the ability of CNS to compensate for the central injures has an efficacy and an intensity that vary among individuals (Di Filippo et al. 2013, 2015; Weiss et al. 2014).

Synaptic plasticity, as well as glutamatergic and GABAergic transmission, was also analysed in the available animal models of demyelinating disorders, in particular in the EAE mice, by using different approaches typified by a different level of anatomical and functional complexity.

Synaptic plasticity originates from the mechanisms of the Long-Term Potentiation (LTP) and of the Long-Term Depression (LTD, Malenka and Bear 2004). LTP is the persistent increase in efficiency of transmission at excitatory synapsis (Bliss and Lomo 1973) while LTD consists of a decreased synaptic transmission that it is produced by prolonged low-frequency stimulation (Mulkey and Malenka 1992). LTP depends on glutamatergic signalling mainly involving NMDA receptors and can be manipulated pharmacologically either by controlling glutamate signalling or by modifying the GABAergic innervation. LTD involves glutamatergic signalling as well, but it is also mediated by metabotropic glutamate receptors (Jones 2017). Interventions that could affect the efficiency of glutamatergic and GABAergic transmissions permit to manage and promote the synaptic plasticity and its functional reorganization, increasing therefore the resilience of CNS to the neuronal injuries that develop in the course of several central disorders including MS.

As far as the synaptic plasticity in EAE animals is concerned, studies dedicated to quantify the efficiency of LTP and LTD in the hippocampus (Di Filippo et al. 2013; Mori et al. 2014; Mosayebi et al. 2016; Nisticò et al. 2013; Novkovic et al. 2015; Prochnow et al. 2013; Weiss et al. 2014) permitted to explore the gravity of synaptic impairments that occur during the course of the demyelinating disorder. Studies were

mainly carried out in the hippocampus of EAE mice at the acute stage of disease or soon after, at the chronic phase. The available data agree upon the reduction of LTP efficiency when compared to healthy controls, an event that strictly correlates with the gravity and the progression of the symptoms in the EAE animals. These maladaptive events are expected to play a main role in determining a progressive exhaustion of the central neuronal plastic reserve and indirectly accounts for the development of clinical signs such as cognitive defects, impaired locomotor activity, mood and social impairments that emerge when testing behavioural skills in EAE mice (Acharjee et al. 2013; Di Prisco et al. 2014a; Olechowski et al. 2013).

Beside these studies, electrophysiological recordings in slices were also carried out to evaluate specifically the efficiency of glutamate and GABA transmission in selected regions of the CNS. The studies focussed on the analysis of the excitatory (EPSCs) and the inhibitory postsynaptic currents (IPSCs) at chemical synapses. The pre- and the postsynaptic excitatory signalling was found to be increased in cortico-striatal slices from EAE mice at the early and the acute stage of disease (Grasselli et al. 2013; Haji et al. 2012; Rossi et al. 2010, 2011, 2012) and in the basolateral amygdala (Acharjee et al. 2018), but it was significantly decreased in hippocampal slices of EAE mice at the acute and the chronic stage of disorder progression (Di Filippo et al. 2013; Mosayebi et al. 2016; Ziehn et al. 2012), further suggesting the region-specificity of the synaptic impairments subserving the electrophysiological recordings. Interestingly, in the striatum of EAE at the symptomatic phase the increased EPSP signalling was paralleled by a significant reduction of the IPSP signalling (Mandolesi et al. 2013; Musumeci et al. 2011) that would amplify the altered EPSPs signalling observed in this brain region.

Finally, a direct quantification of the amount of glutamate and GABA release in nerve terminals was achieved by using purified synaptosomes. Interestingly, the dissection of the neuronal component from slices to synaptosomes unveiled a scenario even more complicated, depending on the CNS regions under study and on stage of the disease. The efficiency in glutamate exocytosis was found to be significantly increased in spinal cord synaptosomes of symptomatic EAE mice during and after the acute stage of disease (Bonfiglio et al. 2017; Di Prisco et al. 2013, 2014a, b, 2016; Marte et al. 2010), which would be consistent with the increased glutamate availability observed in the CSF of MS patients and EAE mice as well. In this region, also the GABA exocytosis was potentiated, consistent with the conclusion that the EAE-induced deregulation of exocytosis is a general event that relies on functional modifications of the intraterminal machinery subserving the recruitment of the release vesicles, independently on the neuronal population and on the neurotransmitter actively released. According to this view, it was shown that the cytosolic adenylyl cyclase (AC) dependent, protein kinase A (PKA)-mediated events, as well as the endogenous production of inositol triphosphate in spinal cord synaptosomes from EAE mice at the acute stage of disease, are largely increased when compared to healthy animals (Di Prisco et al. 2013). Considering that both events participate to determine the cytosolic calcium availability in neurons, it seems conceivable to propose that these metabolic adaptations might be

critical to determine the increased release efficiency detected at both spinal cord glutamatergic and GABAergic synaptic boutons.

Opposite to the spinal cord, the efficiency of glutamate exocytosis was drastically reduced in cortical synaptosomes. The negative adaptation became evident very early, when the animals became symptomatic (i.e. 13 ± 1 d.p.i.), and persisted during and after the acute stage of disease. Also in this case the onset of glutamate release defects was paralleled by significative changes in AC e PKA activities, that were drastically reduced in this CNS region (Chanaday et al. 2015; Cid et al. 2011; Di Prisco et al. 2013; Vilcaes et al. 2009). Finally, impaired glutamate exocytosis emerged in hippocampal synaptosomes only after the acute stage of disease (35 ± 1 d.p.i., (Bonfiglio et al. 2017)). Evident changes in GABA exocytosis were not observed at all the stages of the disease in both cortical and hippocampal synaptosomes (Bonfiglio et al. 2017; Di Prisco et al. 2013, 2014a, b, 2016).

Studies were also dedicated to investigate whether changes in glutamate exocytosis efficiency observed in EAE mice were paralleled by modifications of the expression of proteins involved in the mobilization of transmitter vesicles at presynaptic nerve endings. It was found that the reduced exocytosis of glutamate detected in EAE animals coincides with alterations of the presynaptic machinery. In particular, the kinetic of the calcium-dependent phosphorylation of synapsin I was significantly decreased in cortical synaptosomes of EAE rats (Chanaday et al. 2015; Vilcaes et al. 2009), an event well consistent with the reduced mobility of the synaptic vesicles. Furthermore, the expression of the mammalian uncoordinated-18 (MUNC-18) protein was reduced in cortical nerve endings from EAE mice, suggesting the destabilization of the synaptic vesicle fusion complex (Bonfiglio et al. 2019). Finally, the expression of synapsin-2 and synaptotagmin-1 in the serum of EAE mice was modified (Raphael et al. 2017).

15.2.4 Glutamate Transporters Expression and Function in the CSN of MS Patients and EAE Mice

Altered glutamate availability in the synaptic cleft depends on impaired exocytosis but also on the impaired mechanisms of reuptake in astrocytes, neurons, oligodendrocytes and immune-competent cells (Fig. 15.2). In physiological conditions, the transporters expressed in all these cells rapidly remove, although to a different extent and with different efficacy, the glutamate in the synaptic cleft, strictly controlling the efficiency of the excitatory postsynaptic signalling which correlates with the concentration of the excitatory amino acid in the biophase. The main players in these cellular events are the excitatory amino acid transporters (EAATs, Danbolt et al. 2016) and the cystine/glutamate antiporter (xCT). As far as the EAATs are concerned, to date five members of the family have been described, which predominate in the CNS and that are typified by a preferential anatomical distribution. The EAAT1 transporter has a main non-neuronal expression, being preferentially

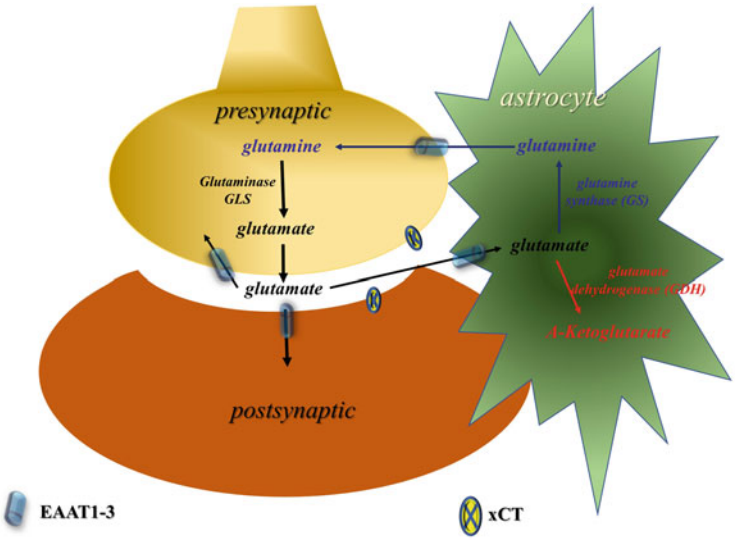

Fig. 15.2 Representation of glutamate transport and metabolism at the tripartite synapsis. Glutamate released by nerve endings and astrocytes can diffuse in the synaptic biophase but it is also actively taken up by astroglial and neuronal excitatory amino acid transporters (EAAT 1–3), as well as by cystine/glutamate antiporter (xCT) that indirectly dictate the strength and the efficiency of glutamate signalling. Glutamate taken up by astrocytes is the substrate of glutamine synthase (GS) to produce glutamine that diffuses to the presynaptic component of glutamatergic synapses to produce glutamate by means of glutaminase (GLS). Glutamate in astrocytes is also substrate of glutamate dehydrogenase (GDH) to produce α-ketoglutarate. The availability of glutamate at synapsis is therefore critically dependent upon diffusion, metabolism and active uptake through selected glutamate transporters, representing therefore a complex system that can be targeted at different levels by pathological conditions typified by inflammation and autoimmune responses

expressed in subpopulation of glial cells, i.e. the Bergmann glia and the Muller cells, while the EEAT2 locates in astrocytes and accounts for most of the glutamate uptake. Differently, the EAAT3, 4 and 5 subtypes are mainly expressed in neurons, the EEAT4 in cerebellar Purkinje cells and the EEAT5 in the retina (Dunlop 2006).

EAAT subtypes impact differently the course of central neurological diseases, including MS or EAE, and their activity depends on the phase of the pathology. In general, at the acute symptomatic stage of EAE, EAAT1 was reported to undergo adaptive modifications preferentially leading to the reduction of mRNA and protein expression, that would be consistent with a reduced efficacy of the mechanism of synaptic protection. Differently, contradictory results are available on the impact of the demyelinating disorders on EAAT2 and the EAAT3 proteins, that were reported to be either increased, decreased or unaffected during the course of disease (Azami Tameh et al. 2013; Mandolesi et al. 2015a, b; Melzer et al. 2008; Mitosek-Szewczyk et al. 2008; Ohgoh et al. 2002; Sulkowski et al. 2009; Vallejo-Illarramendi et al. 2006; Werner et al. 2001). Interestingly, by a functional point of view, glutamate was taken up more efficiently in both nerve endings (synaptosomes) and glial

particles (gliosomes) purified by the spinal cord of EAE mice at the early asymptomatic stage of disease (Marte et al. 2010).

Last but not least, in 2008 the group of Domercq reported the presence of a polymorphism in the promoter of the EAAT2 protein leading to a significant reduction of the expression of the transporter expression. The polymorphism is not associated with an increased risk to develop MS, but it is associated with high glutamate plasma levels during the course of a relapse in RRMS patients (Pampliega et al. 2008).

Besides EAATs, the xCT antiporter also influences glutamate bioavailability in the synaptic cleft. The xCT is a membrane transport system that assures the uptake of extracellular cystine (i.e. the limiting factor in the biosynthesis of glutathione, which has a key role in antioxidant defence) and the concomitant outflow of glutamate in most cells, including oligodendrocytes. xCT consists of two subunits, namely xCT and 4F2hc that heterodimerize. The xCT light chain determines the specificity of the amino acid transport, whereas the 4F2hc protein is common to several amino acid transporters and assures the correct insertion of the antiporter in membrane. Because of the main role of the two substrates in controlling the brain functions, functional maladaptation of this antiporter could be detrimental to central homeostasis and in particular to the myelinated fibres (Soria et al. 2016). In particular, upregulation of the xCT is protective to oxidative stress since indirectly potentiates the intracellular biosynthesis of glutathione to improve reactive oxygen species detoxification. The dysregulation of the xCT causes increased release of glutamate in the biophase, participating to glutamate-mediated excitotoxicity. In 2011 Pampliega and colleagues (Pampliega et al. 2011) demonstrated that the xCT light chain is overexpressed in the CNS and in peripheral blood cells (i.e. cells from monocyte-macrophage-microglia lineage) in MS patients as well as in EAE mice. These findings allowed the conclusion that upregulation of xCT antiporters represents a maladaptive event of the demyelinating disorder that may favour overt hyperglutamatergicity and excitotoxic damage to oligodendrocytes.

In a whole, the observations so far discussed agree with the conclusion that the functionalities of most of the regulatory systems that control the glutamatergic innervation are selectively altered during MS and or EAE and cannot correctly assure the excitatory transmission required to maintain synaptic plasticity.

15.2.5 Glutamate as Modulator of the Immune System: Central Nervous System Cross-Talk

Although the CNS has been long considered an immune-privileged organ, it is now widely recognized that the immune system (IS) plays a main role in the development of central neurodegenerative diseases (i.e. Alzheimer's disease or amyotrophic lateral sclerosis) as well as in classic autoimmune-inflammatory disorders (MS). In support to this conclusion evidence suggests that many endogenous

immunocompetent molecules (i.e. cytokines and chemokines) are produced and released in the CNS where they control synaptic transmission, being therefore specific signalling molecules linking the IS and the CNS (Besong et al. 2002; Centonze et al. 2009; Rostène et al. 2007).

Central resident immunocompetent cells and/or peripheral T cells migrating to the CNS can efficiently release glutamate. Furthermore, these cells also express glutamate receptors sensing therefore the changes in glutamate homeostasis in the brain. Finally, myelin-reactive T cells provoke microglia to release glutamate through the system xCT transporter promoting myelin degradation in EAE (Evonuk et al. 2020). All these aspects have been deeply revised by other authors (Levite 2017) and will not be further discussed in this chapter.

15.3 Glutamate Receptors as Potential Drug Targets for Treating Autoimmune Demyelinating Disease

Glutamate exerts its action at chemical synapses by binding glutamatergic receptors that locate synaptically, both at the pre- and at the postsynaptic components of the synapsis, as well as in surrounding cells, such as astrocytes and microglia. Glutamate receptors also exist in oligodendrocytes and oligodendrocyte progenitor cells as well as in immunocompetent cells, where they drive the myelination and the immunocompetent activities of the CNS.

Glutamate receptors consist of ionotropic and metabotropic receptors which have been proposed to participate to a different level and with different impact to the onset and the development of MS (Fig. 15.3).

15.3.1 Ionotropic Glutamate Receptors in the CSN of MS Patients and EAE Mice

In 2000, two different laboratories provided evidence showing that the treatment with an AMPA antagonist (i.e. NBQX) largely reduced the neurological deficits in EAE mice causing a substantial amelioration of the clinical scores, increasing oligodendrocytes survival and reducing axonal lesions (Pitt et al. 2000; Smith et al. 2000). Both the groups proposed that the AMPA-induced beneficial effects did not rely on anti-inflammatory activity, since NBQX-treatment had no effect on lesion size and did not reduce the degree of central inflammation. In addition, NBQX did not alter the proliferative activity of antigen-primed T cells in vitro, suggesting that an immunomodulatory activity was not primarily involved. Rather, it was proposed that AMPA receptors mediate the neurological sequelae of events that sustain the disease progression and therefore that their blockade could be beneficial to the course of the pathology (Pitt et al. 2000; Smith et al. 2000; Werner et al. 2000).

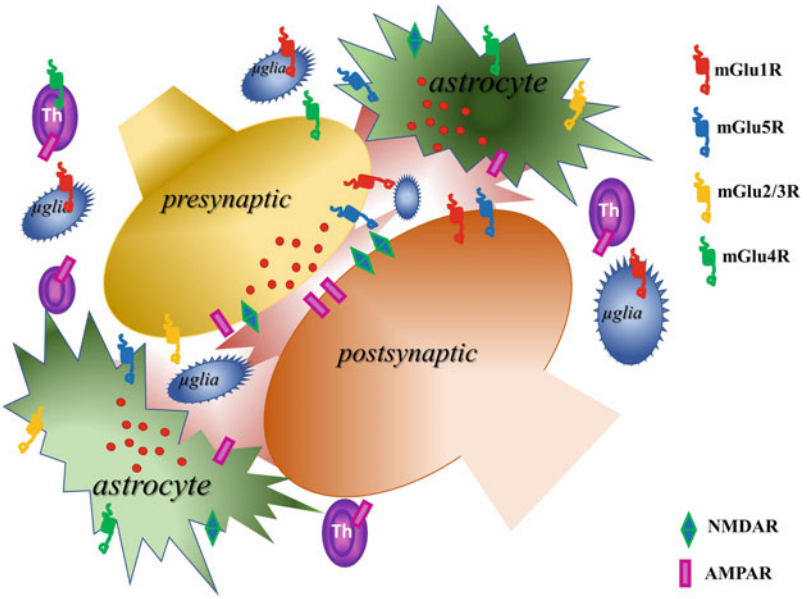

Fig. 15.3 Glutamate exerts its action by binding glutamatergic receptors that locate synaptically, both at the pre- and at the postsynaptic components of the synapsis, as well as in surrounding cells, such as astrocytes and microglia (μglia). Glutamate receptors also exist in oligodendrocytes and oligodendrocyte progenitor cells, as well as in immunocompetent cells, where they drive the myelination and the immunocompetent activities of the CNS. Glutamate receptors consist of ionotropic and metabotropic receptors which have been proposed to participate to a different level and with different impact to the onset and the development of MS. Drugs that influence the expression and the functions of glutamate receptors could permit to partly recover the central derangements and pathological signalling underlying the onset and the progression of demyelinating disorders

The role of AMPA receptors in dictating the recruitment and the migration of T cells in the CNS, however, was soon after demonstrated by Ganor et al. (Ganor et al. 2003), which provided convincing evidence that normal human T cells, human T leukaemia cell, and mouse anti-myelin basic protein T cells express high levels of GluA3-containing AMPA receptors, the activation of which drives the CXCR4-mediated T cell chemotactic migration towards the site of release of the chemokine CXCL12 in the central inflammation loci. The role of AMPA receptors in determining the progress of the disease was confirmed by Kanwar et al. (2004). In particular evidence was provided showing that GluA3 subunits are pivotal in the development of neuronal deficits in EAE mice. In fact, mice genetically modified for the expression of GluA3 subunits were more resistant to neuronal excitotoxicity and developed a milder spinal demyelination when immunized with myelin oligodendrocytes 35-55 protein (MOG35-55, Bannerman et al. 2007). Almost concomitantly, Sarchielli et al. (2007) provided evidence showing that T lymphocytes of control subjects and MS patients express both mRNA and protein of GluR3 receptors and that the activation of the GluA3-containing AMPA receptors enhances the proliferation and the

chemotactic migration of T lymphocytes from both controls and MS patients (but see also Dutta et al. (2013); Newcombe et al. (2008)).

An interesting aspect refers to the ability of AMPA receptors to undergo constitutive trafficking in neuronal plasma membranes, an event that controls the insertion and the efficiency of the AMPA receptor-mediated signalling (Henley 2003; Pittaluga et al. 2006). AMPA receptor trafficking is controlled by several agents, including the immediate early gene Arc/Arg3.1, that has a preferential postsynaptic localization and that regulates the AMPA receptor insertion in neuronal plasma membranes, prolonging LTP (Chowdhury et al. 2006). In mice with EAE between 20 and 30 d.p.i., Arc/Arg3.1 mRNA level was dramatically downregulated in the striatum. This event was proposed to participate in the reduction observed in this region of the strength of AMPA-mediated excitatory transmission and, taking into account that the Th1 cytokines (IFNg, TNFa and IL-1b) can modulate the Arc/Arg3.1 mRNA expression on primary neuronal cultures, it seems conceivable to propose a strong link between long-lasting synaptic changes and inflammation (Centonze et al. 2009).

As far as the NMDA receptors are concerned, the observations that LTP and LTD are impaired in EAE mice indirectly suggest the alteration in the expression/functioning of these receptors during the course of the disease (Di Filippo et al. 2013; Grasselli et al. 2013; Nisticò et al. 2013). The hypothesis is well in line with the results obtained in EAE mice administered with NMDA receptor antagonists. Treatment of EAE-sensitized animals with dizocilpine reduced the disease-associated increase in CNS levels of putrescine which is an endogenous negative modulator of the polyamine site at the NMDA receptors (reviewed by Bolton and Paul (2006)). Similarly, limiting NMDA receptor functions by administering EAE mice with memantine confirmed that pharmacological modulation of receptor function during EAE results in disease suppression and restoration of neurovascular function (Wallström et al. 1996).

In 2017, Lim and colleagues (Lim et al. 2017) investigated the metabolomic profile of the kynurenine pathway (KP) in MS patients. The KP is the major route of metabolism of tryptophan (T) and leads to the production of two main compounds, the quinolinic acid (QA) and the kynurenic acid (KA) which have opposite impacts on NMDA receptors. In particular, QA is an orthosteric agonist at NMDA receptor while KA is an antagonist that limits the NMDA-mediated signalling. In physiological condition, the QA/KA balance assures a correct activation of the NMDA receptors that supports the synaptic events. The activity of the pathway however is under the direct control of inflammatory agents. In particular, pro-inflammatory cytokines can elicit a dysregulation in the metabolic pathway, leading to an altered QA/KA ratio that may either favour excitotoxicity or impair the mechanisms of resilience and synaptic plasticity. QA is produced by activated microglia and infiltrating macrophages, but not by neurons or astrocytes, while KA is produced by astrocytes. The study involved two cohorts of patients suffering from the RR-SM, the SPMS and the PPMS; patients were analysed for the serum content of KA and T and identified for the KA/T ratio. In all the MS subtypes groups the K/T ratio was significantly increased compared to the healthy controls. Inasmuch, aberrant levels

of KA and QA were detected depending on the stage and on the form of the disease, leading to propose the KP metabolic signatures in patients as a marker with high sensitivity and specificity to discriminate clinical MS subtypes.

15.3.2 Metabotropic Glutamate Receptors in the CSN of MS Patients and EAE Mice

The metabotropic glutamate receptors consist of eight different receptor subtypes (namely, mGlu1 to mGlu8 receptor) that are further subdivided in main groups (group I, group II and Group III) based on the sequence homology, the coupled G protein and the associated transducing pathway(s) (Nicoletti et al. 2011; Pin and Acher 2002).

mGlu receptors are fundamental to the mechanism of synaptic plasticity and to the IS-CNS interactions, since they act as fine tuners of the functional responses of neurons and astrocytes as well as of the activation of microglia and immune-competent cells (Fazio et al. 2018).

The role of mGlu receptors in controlling chemical transmission and inflammation has been largely revised and will not be further addressed in this chapter (please refer to (D'Antoni et al. 2008; Fazio et al. 2018; Olivero et al. 2019; Pittaluga 2016; Raiteri 2008; Spampinato et al. 2018)).

Starting from the 2003, Aronica and colleagues provided evidence of changes in the expression of mGlu receptors belonging to the three groups in the CNS of MS patients. In particular, in 2003 (Geurts et al. 2003) they demonstrated that the expression of both group I and II mGlu receptors in MS tissues differed significantly from that of healthy individuals. Strong mGlu1a receptor immunoreactivity was observed in the subcortical white matter, particularly in the center of actively demyelinating lesions and in the borders of chronic active lesions. A diffuse increase in the expression of mGlu5 and mGlu2/3 receptors, but not of mGlu1a receptor, was also highlighted in reactive astrocytes, as well as in a population of microglial cells that displayed a macrophage-like morphology. Two years later, Aronica and colleagues (Geurts et al. 2005) also add insights concerning the group III. mGlu8 receptor immunoreactivity was detected in microglia/macrophage cells in the active lesions, but the expression in these cells significantly decreased in chronic active and inactive lesions. No mGlu4 receptors were detected in these lesions, but mGlu4 receptor immunopositivity emerged in a population of reactive astrocytes localized in the rim of the chronic lesions. More recently, in 2008, Fazio and colleagues demonstrated that, differently from what observed in other brain regions, the expression of the mGlu1a receptor is largely reduced in the Purkinje cells in the cerebellum of MS patients and this is paralleled by an increased expression of the mGlu5 receptors. In particular, the strong mGlu1a receptor somato-dendritic immunoreactivity in Purkinje cells of control human cerebellum was drastically reduced in the Purkinje cell/molecular cell layer of MS patients, while mGlu5 receptor

immunoreactivity, that it is not detectable in the Purkinje cell/molecular cell layer of healthy individuals, became prominent in Purkinje cells of the MS patients.

These observations in autoptic tissues from MS patients were largely replicated in the EAE mice (Besong et al. 2002). The expression of mGlu4 receptors in astroglial cells from EAE rats was significantly modified. To note, the activation of these receptors significantly reduced the production and release of the pro-inflammatory chemokine CCL5 which represent a marker of MS progression (Besong et al. 2002). Starting from 2010, however, evidence underlined the relevance of the mGlu4 receptor subtypes as potential target of therapy for the MS. Fallarino and colleagues (Fallarino et al. 2010) demonstrated that the genetic deletion of the mGlu4 receptors increases the susceptibility of mice to develop EAE, compatible with the conclusion that the overt hyperglutamatergicity observed during the progression of the disease might reflect a counter-regulatory mechanism that is protective in nature and that, by acting at mGlu4 receptors, it would provide a mechanism of defence in the progression of the pathology. Accordingly, it was demonstrated that the administration of cinnabarinic acid, an endogenous metabolite of the kynurenine pathway that acts as an orthosteric agonist of mGlu receptors, was highly protective against the development of EAE in mice (Fazio et al. 2014; Spampinato et al. 2015). Finally, besides mGlu4, also mGlu2/3 receptors were proposed to play a main role in the development of EAE signs in particular at the spinal cord level (Di Prisco et al. 2016). Group II mGlu receptors are known to have a preferential presynaptic localization in the central system and to control glutamate transmission at this level (Olivero et al. 2019). In symptomatic EAE mice, the release-regulating activity of the mGlu2/3 autoreceptors in the cortex was found to be largely reduced, but it was amplified in the spinal cord, suggesting these receptors as potential targets of new therapeutic approaches for controlling glutamate excitotoxicity in these regions.

15.4 Modulators of Glutamate Receptors for the Therapy of Autoimmune Demyelinating Disease

The relevance of the central glutamatergic transmission in the onset and the development of MS and demyelinating disorders is supported by the finding that several disease-modifying drugs (DMDs) currently in use for the cure of MS recover, at least in part, the central glutamate alterations in EAE mice.

Impaired glutamate release efficiency (measured as amount of transmitter release upon application of a depolarizing stimulus at the presynaptic level, as well as EPSPs frequency/intensity and as AMPA/NMDA ratio at the postsynaptic component of chemical synapses) was reported to recover following chronic administration of fingolimod (Rossi et al. 2012; Luchtman et al. 2016; Bonfiglio et al. 2017), dimethyl fumarate (Luchtman et al. 2016; Parodi et al. 2015), glatiramer (Gentile et al. 2013) and rituximab (Rossi et al. 2014).

A large part of these studies investigate the effects of the prophylactic administration of the drugs, but some of them also focussed on the therapeutic approach, i.e. the administration of the drug starting from the onset of the first symptoms, a condition well consistent with the timing experienced by MS patients.

In some cases, the possibility was discussed that the drugs could directly affect the release of glutamate, as well as the mechanism of uptake of the endogenous amino acid, by directly modulating neurons and astrocyte functions. Besides the activity at neurons and synaptic connections, however, the beneficial effects of these therapeutics were mainly related to their ability to recover the pathological activation of central glial cells as well as to reduce the infiltration of circulating lymphocytes (both B and T cells) and macrophages in the CNS, claiming for their main immunomodulatory/antiinflammatory activities.

The main targets of fingolimod and derivatives are the sphingosine receptors, that have a wide distribution throughout the CNS, being expressed in neurons, astrocytes, glial cells and oligodendrocytes (Healy and Antel 2016) and that allow to hypothesize either direct and/or indirect activity at neurons/astrocytes to control glutamate excitotoxicity. Chronic prophylactic and therapeutic fingolimod recovers glutamate impairments in selected region of the CNS of EAE mice at different stages of disease (Bonfiglio et al. 2017; Levite 2017; Pittaluga 2017; Rossi et al. 2012). More interestingly, clinical observations unveiled that oral chronic fingolimod restored glutamate-mediated intercortical excitability in patients suffering from the RRMS (Landi et al. 2015).

Differently the efficiency of laquinimod in recovering glutamate excitotoxicity was proposed to rely on a direct control of the uptake efficiency in astrocytes of EAE mice (Gentile et al. 2018). Finally, dimethyl fumarate was found to control glutamate homeostasis in the CNS of EAE mice by causing a change in the molecular and functional phenotype of activated microglia from the classically activated, pro-inflammatory type to the alternatively activated, neuroprotective one. The mechanism of detoxification of astrocytes relied on the activation of the hydroxycarboxylic acid receptor 2 (HCAR2), that, by means of an AMPK-Sirt1 axis, causes deacetylation, and thereby inhibition, of NF-κB-mediated pathways and, consequently, of the secretion of several pro-inflammatory molecules. This neuroprotective effect was exerted on neurons at presynaptic terminals and modulated glutamate release as evidentiated by measuring EPSPs in the cortex (Parodi et al. 2015).

Based on the observation that altered glutamatergic transmission seems to be a hallmark of the onset and progression of the demyelinating disorders, researchers hypothesized the use of glutamate receptors ligands to recover the aberrant glutamatergic transmission. In particular, it was hypothesized the use of NMDA and AMPA antagonists to contain the impact of excitotoxic conditions either on synaptic transmission or on macrophages and lymphocytes recruitment, microglia/astrocytes activation and oligodendrocytes toxicity (Basso et al. 2008; Bolton and Paul 2006; Lim et al. 2017). This approach is of course limited by the lack of safe orally active modulators of the ionotropic glutamate receptors. As far as the NMDA receptors are concerned, the only antagonist available is memantine that is an

uncompetitive NMDA antagonist approved for the therapy of the mild cognitive impairment in Alzheimer's disease and that in recent years has gained interest for the cure of other pathologies (migraine, epilepsy). However, although memantine was reported to provide symptomatic relief to MS patients (Starck et al. 1997), its efficacy was recently revised in the "EMERITE" (NCT01074619) study and the conclusion did not support the use of this drug in MS (Peyro Saint Paul et al. 2016). The EMERITE analysis was dedicated to evaluate the efficacy and safety of the long-term administration of memantine as a symptomatic treatment for cognitive disorders in patients with RR-MS. The results unveiled that memantine administration did not cause significant beneficial effects in the MS patients, but rather that its tolerability was significantly worse than expected. Based on these results, the possibility to approach a direct modulation of NMDA receptors through the administration of receptor antagonists, although attractive, seems unrealistic.

Perampanel is a selective AMPA receptor antagonist that was developed to treat epilepsy. The drug was approved in the USA and Europe to treat localization-related seizures in young and adult patients (Hanada et al. 2011). Studies were dedicated to assess whether perampanel was effective in treating multiple sclerosis, Parkinson's disease, or migraine prophylaxis, but the results showed that the drug was almost ineffective in these pathologies. The development of this drug for the cure of MS was definitively discontinued in 2016.

15.5 Emerging Treatments Related to Glutamate Modulating Drugs for Autoimmune Demyelinating Disease

Although so far there are no mGlu receptors ligands close to approval for entering the clinic (Nicoletti et al. 2011), mGlu receptors (including mGlu2 and 3 receptors) are still considered promising targets for the development of drugs for the treatment of CNS disorders. In particular, data were provided showing that MS patients with cognitive impairment had low hippocampal NAAG levels, suggesting that agonists at mGlu3-preferring receptors might be beneficial in this disease.

Glutamate carboxypeptidase II (GCPII), also known as *N*-acetylated-alpha-linked acidic dipeptidase (NAALADase), is a zinc-dependent peptidase that could represent a target of therapeutic interventions in a variety of neurologic disorders. It is preferentially expressed in astrocytes and Schwann cells (Berger et al. 1995; Sacha et al. 2007). This enzyme cleaves NAAG, inactivating it.

Inhibitors of CGPII are expected to increase the endogenous CNS level of NAAG and therefore to be efficacious in preventing clinical symptoms in MS patients (Rahn et al. 2012).

The first potent and selective GCPII inhibitor, the 2-(phosphonomethyl) pentanedioic acid (2-PMPA), was reported in 1996 (Jackson et al. 1996). 2-PMPA behaves as a competitive inhibitor of GCPII in the picomolar range and it is devoid

of activity at other cellular targets including glutamate transporters and receptors. The administration of 2-PMPA to the EAE mice significantly improved cognition in the animals at the acute stage of disease. To note, the drug was found to be present in the CNS, consistent with a direct central effect. Unfortunately, drugs acting at GCPII for human use with a profile of safety and efficacy are not available (Rahn et al. 2012). We have already discussed the potential use of NMDA antagonists to reduce the synaptic impairments and the excitotoxic events that occur during the development of the demyelinating disorders. We also reported how this approach is limited by the lack of drugs. It is however worth stressing that in recent years another therapeutic approach to modulate the NMDA-mediated signalling is gaining interest. This approach relies on the concept of the "metamodulation" and implies the use of ligands acting at colocalized, functionally coupled receptors, which, either directly or indirectly, control the functions of the receptors they cross-talk with.

In the case of the NMDA receptors, this approach would imply the use of ligands acting at non-glutamatergic receptors that modulate NMDA-mediated responses by controlling the glutamate release in the synaptic cleft or that colocalize and functional cross-talk with the NMDA receptors themselves, controlling their activity. It is the case of the cannabinoid receptors type 1 (CB1) receptors. These receptors exist presynaptically in glutamatergic nerve endings in several CNS regions and their activation negatively controls glutamate exocytosis (Kim and Thayer 2000). Furthermore, data exist showing that CB1 receptors colocalize and functionally couple with NMDA receptors (Neuhofer et al. 2019).

Ligands acting at the CB1 (Manterola et al. 2018; Pryce et al. 2003), as well as the modulators of the enzymatic pathways accounting for the synthesis and/or the metabolism of the endogenous cannabinoids (EC), would be of interest in this approach since they would be expected to tune NMDA-mediated functions.

As far as the MS is concerned, the EC system appears a suitable and challenging target for a therapeutic approach based on a series of evidences:

1. the EC system is deregulated in subjects suffering from demyelinating disorders, as well as in animals suffering from EAE, in line with its role in the onset and progression of the clinical symptoms of the disease (Centonze et al. 2007),
2. despite the above-mentioned deregulations, the EC system can function as a tuning system that could mediate the restoration of neuronal and astrocyte impairment (Pryce et al. 2003, 2015),
3. beside the control of central transmission, the EC system also modulates inflammatory processes involved in the pathological course of the demyelinating disorders (Rossi et al. 2011, 2015).

The evidence supports the interest around the discovery of drugs able to modulate the EC system for the cure of MS. The data so far available from clinical studies, however, indicate that cannabis-based medicines have a narrow therapeutic window, in particular because of the CB1 receptor-mediated psychoactive components of the drug(s) used in therapy (Parmar et al. 2016). Unfortunately, the possibility to avoid this CB1-mediated effect favouring the other CB1/cannabinoid receptors type 1 (CB2)-mediated activities by using broad spectrum exogenous receptor agonists

seems unlike and the probability that these unwanted events occur during treatment with cannabis derivatives or cannabinoid-like molecules is very high.

The advance in elucidating the enzymatic pathway accounting for the synthesis and the metabolism of ECs, however, has recently determined an important progress in cannabis-mediated medicine. In particular, the possibility to increase the levels of the ECs by the pharmacological inhibition of the enzymes involved in their degradation has emerged as a valuable approach that could represent a safe alternative way to strengthen the cannabinoid-mediated control of both central neurotransmission and inflammatory pathways (Lourbopoulos et al. (2011) and reference therein). Accordingly, the modulation of these enzymatic pathways may allow a fine-tuning of receptor-mediated functions, thus reducing the possibility of concomitant effects due to CB over-activation.

Two are the main ECs in the CNS, i.e. anandamide and 2-arachidonoylglycerol (2-AG). Anandamide is mainly metabolized by the well-characterized fatty acid amide hydrolase (FAAH). FAAH selective inhibitors enhance anandamide levels and induce analgesia and anxiolytic effects. However, the analgesic effects due to the irreversible inhibition of FAAH have not been replicated in phase II clinical studies and, more importantly, a phase I study on a FAAH inhibitor recently failed because of serious lethal side effects (Bonifácio et al. 2020).

Alternative to the blockade of FAAH is the inhibition of the 2-AG catabolic enzymatic pathway, the monoacylglycerol lipase (MGL). This enzyme is responsible for about 85% metabolism of 2-AG, the major EC in the CNS, which acts as a full agonist at both CB1 and CB2, with lower potency than anandamide towards the CB1/CB2 receptors. It has been suggested that MGL inhibitors could be useful for the treatment of several diseases, including pain, neuropsychiatric disorders, cancer and neurodegenerative disorders like MS. Evidence showing that the blockade of this enzyme can elicit any adverse effect is so far lacking. Moreover, some recent findings suggest that MGL inhibition can be attained without the onset of concomitant undesirable side effects mediated by central CB1 (Anderson et al. 2014). As the demyelinating diseases are concerned, data in literature demonstrated that enzyme inhibitors acting at both FAAH and MGL are active in in vivo studies in the EAE mouse model of MS, in which they clearly ameliorated the course of disease without inducing unwanted effects linked to the CB1 overstimulation (Bernal-Chico et al. 2015; Brindisi et al. 2016; Hernández-Torres et al. 2014).

It is predicted that the administration of the MGL inhibitors would be expected to increase the tuning of the endogenous cannabinoid at CB1 and CB2 receptors, including those located presynaptically that control glutamate release efficiency (Musella et al. 2014; Sánchez-Zavaleta et al. 2018), as well as those colocalized with the NMDA receptors, whose activation "metamodulates" the colocalized glutamatergic receptors (Neuhofer et al. 2019).

Finally, a new approach has been proposed to control the hyperglutamatergicity that typifies several central neuropathologies, including MS, the so-called blood glutamate scavenging approach. When glutamate concentrations are pathologically elevated in the brain, several inherent mechanisms can participate to reduce its level. One such mechanism utilizes sodium-dependent transporters located on brain

capillaries that provide an important way by which pathologically glutamate is reduced in the brain and diffuses in the blood through mechanisms of facilitated diffusion (Zhumadilov et al. 2015). The rate of brain-to-blood glutamate efflux can be increased (Gottlieb et al. 2003) by using the blood enzymes glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT), which, in the presence of their cosubstrates pyruvate and oxaloacetate, convert glutamate to 2-ketoglutarate. By injecting pyruvate and oxaloacetate in the peripheral blood, Gottlieb and colleagues successfully increased the rate of elimination of glutamate from the brain ECF, providing the first demonstration that the manipulation of blood glutamate dramatically reduces brain glutamate concentrations.

The clinical relevance of the preclinical results concerning this approach has been revised by Castillo et al. (2016). The results from clinical studies are in progress and, if positive, would allow the use in therapy of this approach to contain hyperglutamatergicity in patients.

15.6 Conclusion and Future Perspectives

Based on the findings revised in the chapter, it seems conceivable to confirm the main role of the glutamatergic system in the aetiopathogenesis of MS. The results concerning the role of glutamate derangement, either in the dysregulation of the immune system or in determining the impaired chemical transmission at central synapses, that were once obtained in MS animals models were largely confirmed in MS patients. These observations proved the main role of glutamate in controlling the immunocompetent responses, but also highlight its impact on the development of the synaptic derangements that typify the demyelinating disease.

Very interestingly, several therapeutics known to modulate the immunocompetent responses were recently reported also to significantly recover the glutamatergic central derangements both in EAE mice and in MS patients. These observations by one side further support the strict correlation and functional interaction linking the IS and the CNS, but also suggest new approaches to counteract central excitotoxicity. The efficacy of certain drugs (fingolimod, laquinimod) to recover glutamate transmission might open the road to the use of these therapeutics also for the cure of other central pathologies that are typified by overt altered glutamate homeostasis. These observations are particularly intriguing, if one considers that almost all the glutamate receptor ligands (with few exceptions, see for instance memantine) that were proposed as promising drugs for the cure of central disorders were discontinued because of the onset of unwanted side effects.

Alternative to the use of glutamate receptor ligands, some preclinical results in EAE mice also unveiled the efficacy of alternative approaches that rely on the use of indirect modulators of the glutamatergic system. It is the case the GCPII inhibitors, which would modulate the bioavailability of endogenous glutamate ligands at selective receptor subtypes. In this context, a particular attention must be paid to the repositioning of therapeutics that are currently in use for certain pathologies but

that can "metamodulate" the functions and the expression of glutamate receptors and might assure an indirect control and tuning of glutamate transmission in the CNS. We are referring to the MGL inhibitors that would "metamodulate" glutamate receptors colocalized with cannabinoid receptors, restoring their physiological role in CNS. All these approaches however deserve further investigations to translate them to clinical studies and to ascertain their safety, tolerability and efficacy.

Finally, the new approach of blood glutamate scavenging represents a revolutionary therapeutic strategy to be evaluated for its efficacy in containing hyperglutamatergicity in MS patients.

We firmly believe that in the next future the study of the impact of the available therapeutics on central glutamatergic system would improve our knowledge of the mechanisms underlying the onset and the progression of MS, also unveiling new cellular/molecular targets of new therapeutics.

References

Acharjee S, Nayani N, Tsutsui M et al (2013) Altered cognitive-emotional behavior in early experimental autoimmune encephalitis--cytokine and hormonal correlates. Brain Behav Immun 33:164–172

Acharjee S, Verbeek M, Gomez CD et al (2018) Reduced Microglial activity and enhanced glutamate transmission in the basolateral amygdala in early CNS autoimmunity. J Neurosci 38:9019–9033

Anderson WB, Gould MJ, Torres RD et al (2014) Actions of the dual FAAH/MAGL inhibitor JZL195 in a murine inflammatory pain model. Neuropharmacology 81:224–230

Azami Tameh A, Clarner T, Beyer C et al (2013) Regional regulation of glutamate signaling during cuprizone-induced demyelination in the brain. Ann Anat 195:415–423

Azevedo CJ, Kornak J, Chu P et al (2014) In vivo evidence of glutamate toxicity in multiple sclerosis. Ann Neurol 76:269–278

Bannerman P, Horiuchi M, Feldman D et al (2007) GluR2-free alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors intensify demyelination in experimental autoimmune encephalomyelitis. J Neurochem 102:1064–1070

Basso AS, Frenkel D, Quintana FJ et al (2008) Reversal of axonal loss and disability in a mouse model of progressive multiple sclerosis. J Clin Invest 118:1532–1543

Berger UV, Carter RE, McKee M et al (1995) N-acetylated alpha-linked acidic dipeptidase is expressed by non-myelinating Schwann cells in the peripheral nervous system. J Neurocytol 24: 99–109

Bernal-Chico A, Canedo M, Manterola A et al (2015) Blockade of monoacylglycerol lipase inhibits oligodendrocyte excitotoxicity and prevents demyelination in vivo. Glia 63:163–176

Besong G, Battaglia G, D'Onofrio M et al (2002) Activation of group III metabotropic glutamate receptors inhibits the production of RANTES in glial cell cultures. J Neurosci 22:5403–5411

Bevan RJ, Evans R, Griffiths L et al (2018) Meningeal inflammation and cortical demyelination in acute multiple sclerosis. Ann Neurol 84:829–842

Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232:331–356

Bolton C, Paul C (2006) Glutamate receptors in neuroinflammatory demyelinating disease. Mediat Inflamm 2006:93684

Bonfiglio T, Olivero G, Merega E et al (2017) Prophylactic versus therapeutic fingolimod: restoration of presynaptic defects in mice suffering from experimental autoimmune encephalomyelitis. PLoS One 12(1):e0170825

Bonfiglio T, Olivero G, Vergassola M et al (2019) Environmental training is beneficial to clinical symptoms and cortical presynaptic defects in mice suffering from experimental autoimmune encephalomyelitis. Neuropharmacology 145:75–86

Bonifácio MJ, Sousa F, Aires C et al (2020) Preclinical pharmacological evaluation of the fatty acid amide hydrolase inhibitor BIA 10-2474. Br J Pharmacol. https://doi.org/10.1111/bph.14973

Brindisi M, Maramai S, Gemma S et al (2016) Development and pharmacological characterization of selective blockers of 2-arachidonoyl glycerol degradation with efficacy in rodent models of multiple sclerosis and pain. J Med Chem 59:2612–2632

Castegna A, Palmieri L, Spera I et al (2011) Oxidative stress and reduced glutamine synthetase activity in the absence of inflammation in the cortex of mice with experimental allergic encephalomyelitis. Neurosci 185:97–105

Castillo J, Loza MI, Mirelman D et al (2016) A novel mechanism of neuroprotection: blood glutamate grabber. J Cereb Blood Flow Metab 36:292–301

Centonze D, Bari M, Rossi S et al (2007) The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. Brain 130:2543–2553

Centonze D, Muzio L, Rossi S et al (2009) Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. J Neurosci 29:3442–3452

Chanaday NL, Vilcaes AA, de Paul AL et al (2015) Glutamate release machinery is altered in the frontal cortex of rats with experimental autoimmune encephalomyelitis. Mol Neurobiol 51: 1353–1367

Chowdhury S, Shepherd JD, Okuno H et al (2006) Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron 52:445–459

Cid MP, Vilcaes AA, Rupil LL et al (2011) Participation of the GABAergic system on the glutamate release of frontal cortex synaptosomes from Wistar rats with experimental autoimmune encephalomyelitis. Neuroscience 189:337–344

Danbolt NC, Furness DN, Zhou Y (2016) Neuronal vs glial glutamate uptake: resolving the conundrum. Neurochem Int 98:29–45

D'Antoni S, Berretta A, Bonaccorso CM et al (2008) Metabotropic glutamate receptors in glial cells. Neurochem Res 33:2436–2443

Di Filippo M, Chiasserini D, Gardoni F et al (2013) Effects of central and peripheral inflammation on hippocampal synaptic plasticity. Neurobiol Dis 52:229–236

Di Filippo M, de Iure A, Durante V et al (2015) Synaptic plasticity and experimental autoimmune encephalomyelitis: implications for multiple sclerosis. Brain Res 1621:205–213

Di Prisco S, Merega E, Milanese M et al (2013) CCL5-glutamate interaction in central nervous system: early and acute presynaptic defects in EAE mice. Neuropharmacology 75:337–346

Di Prisco S, Merega E, Lanfranco M et al (2014a) Acute desipramine restores presynaptic cortical defects in murine experimental autoimmune encephalomyelitis by suppressing central CCL5 overproduction. Br J Pharmacol 171:2457–2467

Di Prisco S, Merega E, Pittaluga A (2014b) Functional adaptation of presynaptic chemokine receptors in EAE mouse central nervous system. Synapse 68:529–535

Di Prisco S, Merega E, Bonfiglio T et al (2016) Presynaptic, release-regulating mGlu2 -preferring and mGlu3 -preferring autoreceptors in CNS: pharmacological profiles and functional roles in demyelinating disease. Br J Pharmacol 173:1465–1477

Dunlop J (2006) Glutamate-based therapeutic approaches: targeting the glutamate transport system. Curr Opin Pharmacol 6:103–107

Dutta R, Chomyk AM, Chang A et al (2013) Hippocampal demyelination and memory dysfunction are associated with increased levels of the neuronal microRNA miR-124 and reduced AMPA receptors. Ann Neurol 73:637–645

Eshaghi A, Marinescu RV, Young AL et al (2018) Progression of regional grey matter atrophy in multiple sclerosis. Brain 141:1665–1177

Evonuk KS, Doyle RE, Moseley CE et al (2020) Reduction of AMPA receptor activity on mature oligodendrocytes attenuates loss of myelinated axons in autoimmune neuroinflammation. Sci Adv 6:eaax5936

Fallarino F, Volpi C, Fazio F et al (2010) Metabotropic glutamate receptor-4 modulates adaptive immunity and restrains neuroinflammation. Nat Med 16:897–902

Fazio F, Notartomaso S, Aronica E et al (2008) 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

Fazio F, Zappulla C, Notartomaso S et al (2014) Cinnabarinic acid, an endogenous agonist of type-4 metabotropic glutamate receptor, suppresses experimental autoimmune encephalomyelitis in mice. Neuropharmacology 81:237–843

Fazio F, Ulivieri M, Volpi C et al (2018) Targeting metabotropic glutamate receptors for the treatment of neuroinflammation. Curr Opin Pharmacol 38:16–23

Ganor Y, Besser M, Ben-Zakay N et al (2003) Human T cells express a functional ionotropic glutamate receptor GluR3, and glutamate by itself triggers integrin-mediated adhesion to laminin and fibronectin and chemotactic migration. J Immunol 170:4362–4372

Gentile A, Rossi S, Studer V et al (2013) Glatiramer acetate protects against inflammatory synaptopathy in experimental autoimmune encephalomyelitis. J NeuroImmune Pharmacol 8:651–663

Gentile A, Musella A, De Vito F et al (2018) Laquinimod ameliorates excitotoxic damage by regulating glutamate re-uptake. J Neuroinflammation 15:5

Geurts JJ, Wolswijk G, Bö L et al (2003) Altered expression patterns of group I and II metabotropic glutamate receptors in multiple sclerosis. Brain 126:1755–1766

Geurts JJ, Wolswijk G, Bö L et al (2005) Expression patterns of Group III metabotropic glutamate receptors mGluR4 and mGluR8 in multiple sclerosis lesions. J Neuroimmunol 158:182–190

Godiska R, Chantry D, Dietsch GN et al (1995) Chemokine expression in murine experimental allergic encephalomyelitis. J Neuroimmunol 58:167–176

Gottlieb M, Wang Y, Teichberg VI (2003) Blood-mediated scavenging of cerebrospinal fluid glutamate. J Neurochem 87:119–126

Grasselli G, Rossi S, Musella A et al (2013) Abnormal NMDA receptor function exacerbates experimental autoimmune encephalomyelitis. Br J Pharmacol 168:502–517

Haji N, Mandolesi G, Gentile A et al (2012) TNF-α-mediated anxiety in a mouse model of multiple sclerosis. Exp Neurol 237:296–303

Hanada T, Hashizume Y, Tokuhara N et al (2011) Perampanel: a novel, orally active, noncompetitive AMPA-receptor antagonist that reduces seizure activity in rodent models of epilepsy. Epilepsia 52:1331–1340

Hardin-Pouzet H, Krakowski M, Bourbonnière L et al (1997) Glutamate metabolism is down-regulated in astrocytes during experimental allergic encephalomyelitis. Glia 20:79–85

Healy LM, Antel JP (2016) Sphingosine-1-phosphate receptors in the central nervous and immune systems. Curr Drug Targets 17:1841–1850

Henley JM (2003) Proteins interactions implicated in AMPA receptor trafficking: a clear destination and an improving route map. Neurosci Res 45:243–254

Hernández-Torres G, Cipriano M, Hedén E et al (2014) A reversible and selective inhibitor of monoacylglycerol lipase ameliorates multiple sclerosis. Angew Chem Int Ed Engl 126(50):13985–13990

Jackson PF, Cole DC, Slusher BS et al (1996) Design, synthesis, and biological activity of a potent inhibitor of the neuropeptidase N-acetylated alpha-linked acidic dipeptidase. J Med Chem 39: 619–622

Jones OD (2017) Do group I metabotropic glutamate receptors mediate LTD? Neurobiol Learn Mem 138:85–97

Kanwar JR, Kanwar RK, Krissansen GW (2004) Simultaneous neuroprotection and blockade of inflammation reverses autoimmune encephalomyelitis. Brain 127:1313–1331

Karpus WJ, Ransohoff RM (1998) Chemokine regulation of experimental autoimmune encephalomyelitis: temporal and spatial expression patterns govern disease pathogenesis. J Immunol 161:2667–2671

Kim DJ, Thayer SA (2000) Activation of CB1 cannabinoid receptors inhibits neurotransmitter release from identified synaptic sites in rat hippocampal cultures. Brain Res 852:398–405

Klaver R, De Vries HE, Schenk GJ et al (2013) Grey matter damage in multiple sclerosis: a pathology perspective. Prion 7:66–75

Klivényi P, Kékesi K, Juhász G et al (1997) Amino acid concentrations in cerebrospinal fluid of patients with multiple sclerosis. Acta Neurol Scand 95:96–98

Landi D, Vollaro S, Pellegrino G et al (2015) Oral fingolimod reduces glutamate-mediated intracortical excitability in relapsing-remitting multiple sclerosis. Clin Neurophysiol 126:165–169

Levite M (2017) Glutamate, T cells and multiple sclerosis. J Neural Transm 124:775–798

Lim CK, Bilgin A, Lovejoy DB et al (2017) Kynurenine pathway metabolomics predicts and provides mechanistic insight into multiple sclerosis progression. Sci Rep 7:41473

Lourbopoulos A, Grigoriadis N, Lagoudaki R et al (2011) Administration of 2-arachidonoylglycerol ameliorates both acute and chronic experimental autoimmune encephalomyelitis. Brain Res 1390:126–141

Lublin FD, Reingold SC, Cohen JA et al (2014) Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 83:278–286

Luchtman D, Gollan R, Ellwardt E et al (2016) In vivo and in vitro effects of multiple sclerosis immunomodulatory therapeutics on glutamatergic excitotoxicity. J Neurochem 136:971–980

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5–21

Mandolesi G, Grasselli G, Musumeci G et al (2010) Cognitive deficits in experimental autoimmune encephalomyelitis: neuroinflammation and synaptic degeneration. Neurol Sci 31:S255–S259

Mandolesi G, Musella A, Gentile A et al (2013) Interleukin-1β alters glutamate transmission at purkinje cell synapses in a mouse model of multiple sclerosis. J Neurosci 33:12105–12121

Mandolesi G, Gentile A, Musella A et al (2015a) IL-1β dependent cerebellar synaptopathy in a mouse mode of multiple sclerosis. Cerebellum 14:19–22

Mandolesi G, Gentile A, Musella A et al (2015b) Synaptopathy connects inflammation and neurodegeneration in multiple sclerosis. Nat Rev Neurol 11:711–724

Mangiardi M, Crawford DK, Xia X et al (2011) An animal model of cortical and callosal pathology in multiple sclerosis. Brain Pathol 21:263–278

Manterola A, Bernal-Chico A, Cipriani R et al (2018) Re-examining the potential of targeting ABHD6 in multiple sclerosis: Efficacy of systemic and peripherally restricted inhibitors in experimental autoimmune encephalomyelitis. Neuropharmacology 141:181–191

Marte A, Cavallero A, Morando S et al (2010) Alterations of glutamate release in the spinal cord of mice with experimental autoimmune encephalomyelis. J Neurochem 115:343–352

Matute C, Domercq M, Fogarty DJ et al (1999) On how altered glutamate homeostasis may contribute to demyelinating diseases of the CNS. Adv Exp Med Biol 468:97–107

Matute C, Alberdi E, Domercq M et al (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 24:224–230

Melzer N, Meuth SG, Torres-Salazar D et al (2008) A beta-lactam antibiotic dampens excitotoxic inflammatory CNS damage in a mouse model of multiple sclerosis. PLoS One 3(9):e3149

Mitosek-Szewczyk K, Sulkowski G, Stelmasiak Z et al (2008) 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

Mori F, Nicoletti CG, Rossi S et al (2014) Growth factors and synaptic plasticity in relapsing-remitting multiple sclerosis. NeuroMolecular Med 16:490–498

Mosayebi G, Soleyman MR, Khalili M et al (2016) Changes in synaptic transmission and long-term potentiation induction as a possible mechanism for learning disability in an animal model of multiple sclerosis. Int Neurourol J 20:26–32

Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975
Musella A, Sepman H, Mandolesi G et al (2014) Pre- and postsynaptic type-1 cannabinoid receptors control the alterations of glutamate transmission in experimental autoimmune encephalomyelitis. Neuropharmacology 79:567–572
Musumeci G, Grasselli G, Rossi S et al (2011) 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
Neuhofer D, Spencer SM, Chioma VC et al (2019) The loss of NMDAR-dependent LTD following cannabinoid self-administration is restored by positive allosteric modulation of CB1 receptors. Addict Biol 16:e12843
Newcombe J, Uddin A, Dove R et al (2008) Glutamate receptor expression in multiple sclerosis lesions. Brain Pathol 18:52–61
Nicoletti F, Bockaert J, Collingridge GL et al (2011) Metabotropic glutamate receptors: from the workbench to the bedside. Neuropharmacology 60:1017–1041
Nisticò R, Mori F, Feligioni M et al (2013) Synaptic plasticity in multiple sclerosis and in experimental autoimmune encephalomyelitis. Philos Trans R Soc Lond Ser B Biol Sci 369: 20130162
Novkovic T, Shchyglo O, Gold R et al (2015) Hippocampal function is compromised in an animal model of multiple sclerosis. Neuroscience 309:100–112
Ohgoh M, Hanada T, Smith T et al (2002) Altered expression of glutamate transporters in experimental autoimmune encephalomyelitis. J Neuroimmunol 125:170–178
Olechowski CJ, Tenorio G, Sauve Y et al (2013) Changes in nociceptive sensitivity and object recognition in experimental autoimmune encephalomyelitis (EAE). Exp Neurol 241:113–121
Olivero G, Vergassola M, Cisani F et al (2019) Presynaptic release-regulating metabotropic glutamate receptors: an update. Curr Neuropharmacol. https://doi.org/10.2174/1570159X17666191127112339
Pampliega O, Domercq M, Villoslada P et al (2008) Association of an EAAT2 polymorphism with higher glutamate concentration in relapsing multiple sclerosis. J Neuroimmunol 195:194–198
Pampliega O, Domercq M, Soria FN et al (2011) Increased expression of cystine/glutamate antiporter in multiple sclerosis. J Neuroinflammation 8:63
Parmar JR, Forrest BD, Freeman RA (2016) Medical marijuana patient counseling points for health care professionals based on trends in the medical uses, efficacy, and adverse effects of cannabis-based pharmaceutical drugs. Res Social Adm Pharm 12(4):638–654
Parodi B, Rossi S, Morando S et al (2015) Fumarates modulate microglia activation through a novel HCAR2 signaling pathway and rescue synaptic dysregulation in inflamed CNS. Acta Neuropathol 130:279–295
Passos J, Azevedo A, Salgado D et al (2014) Three decades of drift: the misdiagnosis of predominantly neuropsychiatric multiple sclerosis. J Neuropsychiatry Clin Neurosci 26:E55–E56
Peyro Saint Paul L, Creveuil C, Heinzlef O et al (2016) Efficacy and safety profile of memantine in patients with cognitive impairment in multiple sclerosis: A randomized, placebo-controlled study. J Neurol Sci 363:69–76
Pin JP, Acher F (2002) The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. Curr Drug Targets CNS Neurol Disord 1:297–317
Pitt D, Werner P, Raine CS (2000) Glutamate excitotoxicity in a model of multiple sclerosis. Nat Med 6:67–70
Pittaluga A (2016) Presynaptic release-regulating mGlu1 receptors in central nervous system. Front Pharmacol 7:295
Pittaluga A (2017) CCL5-glutamate cross-talk in astrocyte-neuron communication in multiple sclerosis. Front Immunol 8:1079
Pittaluga A, Feligioni M, Longordo F et al (2006) Trafficking of presynaptic AMPA receptors mediating neurotransmitter release: neuronal selectivity and relationships with sensitivity to cyclothiazide. Neuropharmacology 50:286–296

Prochnow N, Gold R, Haghikia A (2013) An electrophysiologic approach to quantify impaired synaptic transmission and plasticity in experimental autoimmune encephalomyelitis. J Neuroimmunol 264:48–53

Pryce G, Ahmed Z, Hankey DJ et al (2003) Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. Brain 126:2191–2202

Pryce G, Riddall DR, Selwood DL et al (2015) Neuroprotection in experimental autoimmune encephalomyelitis and progressive multiple sclerosis by cannabis-based cannabinoids. J Neuroimmune Pharmacol 10:281–292

Rahn KA, Watkins CC, Alt J et al (2012) Inhibition of glutamate carboxypeptidase II (GCPII) activity as a treatment for cognitive impairment in multiple sclerosis. Proc Natl Acad Sci U S A 109:20101–20106

Raiteri M (2008) Presynaptic metabotropic glutamate and $GABA_B$ receptors. Handb Exp Pharmacol 184:373–407

Rangachari M, Kuchroo VK (2013) Using EAE to better understand principles of immune function and autoimmune pathology. J Autoimmun 45:31–39

Ransohoff RM, Liu L, Cardona AE (2007) Chemokines and chemokine receptors: multipurpose players in neuroinflammation. Int Rev Neurobiol 82:187–204

Rao SM, Leo GJ, Bernardin L, Unverzagt F (1991) Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. Neurology 41:685–691

Raphael I, Webb J, Gomez-Rivera F et al (2017) Serum neuroinflammatory disease-induced central nervous system proteins predict clinical onset of experimental autoimmune encephalomyelitis. Front Immunol 8:812

Rossi S, De Chiara V, Furlan R et al (2010) Abnormal activity of the Na/Ca exchanger enhances glutamate transmission in experimental autoimmune encephalomyelitis. Brain Behav Immun 24:1379–1385

Rossi S, Furlan R, De Chiara V et al (2011) Cannabinoid CB1 receptors regulate neuronal TNF-α effects in experimental autoimmune encephalomyelitis. Brain Behav Immun 25:1242–1248

Rossi S, Lo Giudice T, De Chiara V et al (2012) Oral fingolimod rescues the functional deficits of synapses in experimental autoimmune encephalomyelitis. Br J Pharmacol 165:861–869

Rossi S, Motta C, Studer V et al (2014) Tumor necrosis factor is elevated in progressive multiple sclerosis and causes excitotoxic neurodegeneration. Mult Scler 20:304–312

Rossi S, Motta C, Musella A et al (2015) The interplay between inflammatory cytokines and the endocannabinoid system in the regulation of synaptic transmission. Neuropharmacology 96: 105–112

Rostène W, Kitabgi P, Parsadaniantz SM (2007) Chemokines: a new class of neuromodulator? Nat Rev Neurosci 8:895–903

Sacha P, Zamecnik J, Barinka C et al (2007) Expression of glutamate carboxypeptidase II in human brain. Neuroscience 144(4):1361–1372

Sánchez-Zavaleta R, Cortés H, Avalos-Fuentes JA et al (2018) Presynaptic cannabinoid CB2 receptors modulate [3 H]-Glutamate release at subthalamo-nigral terminals of the rat. Synapse 72:e22061

Sarchielli P, Greco L, Floridi A et al (2003) Excitatory amino acids and multiple sclerosis: evidence from cerebrospinal fluid. Arch Neurol 60:1082–1088

Sarchielli P, Di Filippo M, Candeliere A et al (2007) Expression of ionotropic glutamate receptor GLUR3 and effects of glutamate on MBP- and MOG-specific lymphocyte activation and chemotactic migration in multiple sclerosis patients. J Neuroimmunol 188:146–158

Siegert RJ, Abernethy DA (2005) Depression in multiple sclerosis: a review. J Neurol Neurosurg Psychiatry 76:469–475

Smith T, Groom A, Zhu B et al (2000) Autoimmune encephalomyelitis ameliorated by AMPA antagonists. Nat Med 6:62–66

Sørensen TL, Tani M, Jensen J et al (1999) Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. J Clin Invest 103:807–815

Soria FN, Zabala A, Pampliega O et al (2016) Cystine/glutamate antiporter blockage induces myelin degeneration. Glia 64:1381–1395
Spampinato SF, Merlo S, Chisari M et al (2015) Glial metabotropic glutamate receptor-4 increases maturation and survival of oligodendrocytes. Front Cell Neurosci 8:462
Spampinato SF, Copani A, Nicoletti F et al (2018) Metabotropic glutamate receptors in glial cells: a new potential target for neuroprotection? Front Mol Neurosci 11:414
Stampanoni Bassi M, Mori F, Buttari F et al (2017) Neurophysiology of synaptic functioning in multiple sclerosis. Clin Neurophysiol 128:1148–1157
Starck M, Albrecht H, Pollmann W et al (1997) Drug therapy for acquired pendular nystagmus in multiple sclerosis. J Neurol 244:9–16
Stover JF, Pleines UE, Morganti-Kossmann MC et al (1997) Neurotransmitters in cerebrospinal fluid reflect pathological activity. Eur J Clin Investig 27(12):1038–1043
Sulkowski G, Dabrowska-Bouta B, Kwiatkowska-Patzer B et al (2009) 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
Swanborg RH (1995) Experimental autoimmune encephalomyelitis in rodents as a model for human demyelinating disease. Clin Immunol Immunopathol 77:4–13
University of California, San Francisco MS-EPIC Team, Cree BAC et al (2019) Silent progression in disease activity-free relapsing multiple sclerosis. Ann Neurol 85:653–666
Vallejo-Illarramendi A, Domercq M, Pérez-Cerdá F et al (2006) Increased expression and function of glutamate transporters in multiple sclerosis. Neurobiol Dis 21:154–164
Vilcaes AA, Furlan G, Roth GA (2009) Inhibition of Ca2+-dependent glutamate release from cerebral cortex synaptosomes of rats with experimental autoimmune encephalomyelitis. J Neurochem 108:881–890
Wallström E, Diener P, Ljungdahl A et al (1996) Memantine abrogates neurological deficits, but not CNS inflammation, in Lewis rat experimental autoimmune encephalomyelitis. J Neurol Sci 137: 89–96
Weiss S, Mori F, Rossi S et al (2014) Disability in multiple sclerosis: when synaptic long-term potentiation fails. Neurosci Biobehav Rev 43:88–99
Werner P, Pitt D, Raine CS (2000) Glutamate excitotoxicity--a mechanism for axonal damage and oligodendrocyte death in multiple sclerosis? J Neural Transm Suppl 60:375–385
Werner P, Pitt D, Raine CS (2001) Multiple sclerosis: altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. Ann Neurol 50:169–180
Zhumadilov A, Boyko M, Gruenbaum SE et al (2015) Extracorporeal methods of blood glutamate scavenging: a novel therapeutic modality. Expert Rev Neurother 15:501–508
Ziehn MO, Avedisian AA, Dervin SM et al (2012) Therapeutic testosterone administration preserves excitatory synaptic transmission in the hippocampus during autoimmune demyelinating disease. J Neurosci 32:12312–12324

Suggested Reading

Bolton C, Paul C (2006) Glutamate receptors in neuroinflammatory demyelinating disease. Mediat Inflamm 2006:93684. The review considers the relevance of the glutamate receptors in EAE and MS pathogenesis, focussing particularly on the ionotropic receptors. The use of receptor antagonists/ receptor modulators to control EAE is also discussed together with the possibility of their therapeutic application in demyelinating disease
Klaver R, De Vries HE, Schenk GJ et al (2013) Grey matter damage in multiple sclerosis: a pathology perspective. Prion 7:66–75. Starting from evidence obtained in the last decade from immunohistochemical studies showing that grey matter (GM) pathology in multiple sclerosis is extensive, the authors focussed on the results obtained in the last decade with magnetic

resonance imaging technique which unveiled the GM damage is present from the earliest stages of the disease and accrues with disease progression. These observations support the conclusion that GM pathology is clinically relevant as it correlates with multiple sclerosis associated motor deficits and cognitive impairment

Mandolesi G, Gentile A, Musella A et al (2015) Synaptopathy connects inflammation and neurodegeneration in multiple sclerosis. Nat Rev Neurol 11:711–724. The review resumes the work of the group headed by Diego Centonze concerning the role of synaptic alterations in the onset and the development of multiple sclerosis. Based on the observations supporting the pathological impact of proinflammatory cytokine on synaptic efficiency, including the glutamate-GABA cross-talk, the authors propose the term synaptopathy to describe the complex interaction linking the immune system and the CNS in the course of the disease

Di Filippo M, de Iure A, Durante V et al (2015) Synaptic plasticity and experimental autoimmune encephalomyelitis: implications for multiple sclerosis. Brain Res 1621:205–213. Starting from the concept that synaptic plasticity assures the ability of the CNS to cope with injuries in an adaptive or maladaptive manner, the authors discuss the impact of peripheral and central inflammation on neuroplasticity in the course of multiple sclerosis from this point of view. By reviewing the available literature concerning the synaptic derangements in the course of the experimental autoimmune encephalomyelitis in mice, the review aims at deciphering the complex pathways involved in the progression of the disease, discussing their relevance to clinical outcomes

Danbolt NC, Furness DN, Zhou Y (2016) Neuronal vs glial glutamate uptake: resolving the conundrum. Neurochem Int 98:29–45. The review deals with excitatory amino acid transporters (EAATs) in the central nervous system, particularly focussing on the transporters that are expressed in neurons and in astrocytes, on their role in controlling glutamate bioavailability and their main regional and cellular distribution. The manuscript tackles with emphasis the role of EAAT on the glutamate-glutamine cycle as well as on the mechanism of heteroexchange compared to net uptake. Finally, the review also discusses the role of EAAT in controlling glutamate releasing probability

Levite M (2017) Glutamate, T cells and multiple sclerosis. J Neural Transm 124:775–798. The review offers a wide overview of the involvement of the glutamatergic system in the onset and the development of demyelinating disorders, particularly focusing on the pathological cross-talk linking glutamate and immune system. This is an interesting review that describes item by item the literature available on the events accounting for glutamatergic derangements in MS

Stampanoni Bassi M, Mori F, Buttari F et al (2017) Neurophysiology of synaptic functioning in multiple sclerosis. Clin Neurophysiol 128:1148–1157. The review focusses on the pathological relevance of the cross-talk linking the central nervous system and the immune system in the development and the progression of multiple sclerosis starting from the results from animal models to the clinical studies supporting the functional link between alterations of central transmission in MS patients in relation to different phenotypes and disease phases. The review also deals with explorative studies on neuronal plasticity in MS patients using the transcranial magnetic stimulation. Emphasis is dedicated to the pathological overproduction of proinflammatory cytokines and chemokines and neuronal survival and neurological manifestations in MS patients, owing to support the main involvement of inflammatory-driven, synaptic dysfunctions in the development of MS

Fazio F, Ulivieri M, Volpi C et al (2018) Targeting metabotropic glutamate receptors for the treatment of neuroinflammation. Curr Opin Pharmacol 38:16–23. The review provides a synthetic and current overview of the role and the involvement of metabotropic glutamate receptors in the onset and development of demyelinating disorders in animals as well as in MS patients

University of California, San Francisco MS-EPIC Team, Cree BAC et al (2019) Silent progression in disease activity-free relapsing multiple sclerosis. Ann Neurol 85:653–666. Based on clinical data the authors introduce the term “silent progression” to describe the insidious disability that accrues in many patients who satisfy traditional criteria for relapsing–remitting MS. In particular

the authors provide evidence that the silent progression during the RRMS phase is associated with brain atrophy suggesting that the same process that underlies SPMS likely begins far earlier than is generally recognized. This conclusion supports a unitary view of MS biology, with both focal and diffuse tissue destructive components, and with inflammation and neurodegeneration occurring throughout the disease spectrum playing a role in the disease development