

Glutamate and Multiple Sclerosis

M. Frigo¹, M.G. Cogo², M.L. Fusco², M. Gardinetti² and B. Frigeni^{*1}

¹Department of Neurology, S. Gerardo Hospital, Monza (MB), Italy

²Department of Neuroscience and Biomedical Technologies, University of Milano-Bicocca, Monza (MB), Italy

Abstract: Multiple sclerosis (MS) has been considered for a long time a typical inflammatory demyelinating disease of the central nervous system due to autoimmunity targeting oligodendrocytes with sparing of axons until advanced stages of the disease. For this reason, most of the earliest experimental studies focused on the role of cytokines and chemokines at the site of oligodendrocytes loss and on the importance in MS pathogenesis of classical inflammatory mechanisms. As a result, several attempts to treat MS through reduction of the local inflammatory milieu have been performed, leading to the current “immunomodulatory” treatment of the disease. However, more recently the importance of axonal loss and neurodegeneration even in the earliest stages of MS has been also recognized, and additional or concomitant players have been therefore searched. Evidence is now increasing that excessive glutamate is released at the site of demyelination and axonal degeneration in MS plaques, and the most probable candidates for this cellular release are infiltrating leukocytes and activated microglia. These observations are no longer simply preclinical results obtained in the MS animal model, i.e. experimental allergic encephalomyelitis, but have already been partially confirmed by *post-mortem* studies and *in vivo* analysis in MS patients, thus raising the possibility that modulation of glutamate release and transport as well as receptors blockade might be relevant targets for the development of future therapeutic interventions.

Keywords: Glutamate, multiple sclerosis, neurodegeneration, inflammation, demyelination.

INTRODUCTION

The role of the glutamatergic system is now recognized in several neurodegenerative and psychiatric diseases as well as in pain perception and even in peripheral neuropathies, and is now recognized and it has been reviewed in other sections of this Special Issue. However, besides a role in neurodegenerative diseases, glutamate has been also considered as a possible player in the course of other diseases of the central nervous system (CNS) such as multiple sclerosis (MS), where inflammation, demyelination and axonal degeneration have all been described. To perform this hypothesis, most of the data have been provided by the animal model of multiple sclerosis, i.e. experimental allergic encephalomyelitis (EAE), and *in vitro* studies, but more recently the first consistent evidence of an altered glutamate expression and metabolism in humans appeared in the literature.

These results are still preliminary, but they might be relevant not only to understand MS pathogenesis, but also to interpret if an increased glutamate concentration would result to be predictive of axonal damage, CNS atrophy and eventually patient's disability.

MS AND ITS PRECLINICAL MODELS

MS is a chronic, degenerative disease of the CNS typically affecting young adults with a predominance for females, which is characterized by focal lesions of the white matter with inflammation, demyelination, infiltration of immune cells, oligodendroglial death and axonal degeneration. Different types of MS can be identified according to the clinical course of the disease: the most common form of MS is characterized by a remitting relapsing course (RR-MS), while a minority of patients experience a chronic primary progressive course (PP-MS). A proportion of RR-MS patients eventually develops a secondary progressive course (SP-RR) clinically identical in the latest stages to PP-MS.

MS has long been considered as a primary inflammatory disease in the early relapsing phase, with neurodegeneration and axonal loss occurring only in the progressive stage that is characterized by limited inflammatory activity and global brain atrophy. However, this dichotomy is now challenged and it is accepted that axonal degeneration can be present since the earliest stages of the disease, thus suggesting that not only the classical

inflammatory soluble factors (e.g. chemokines, cytokines) but also molecules more typically involved in neurodegeneration, such as glutamate, can be relevant in MS pathogenesis.

Besides blood-borne infiltrating cells (mainly lymphocytes, monocytes and macrophages) several other cellular types are present in MS plaques. Resident astrocytes, once simply considered as the main responsible for reactive gliosis after an acute episode, have now been actively associated with inflammatory events since they can, under specific circumstances, act as partially competent antigen-presenting cells. Similarly, activated microglial cells are not simply devoted to myelin debris removal, but they are able to secrete active substances, and among them glutamate.

To elucidate the basic mechanisms of MS several preclinical models have been developed, although so far none of them is able to reproduce the full range of events identified in MS. The most widely used animal model of MS is rodent EAE, a CNS inflammatory disease inducible in appropriated strains of mice and rats. The most widely used animal model of MS is rodent EAE. However, it should always be considered that EAE pathological and clinical features can be markedly different according to the method of disease induction (i.e. active immunization using a myelin antigen or passive transfer of activated lymphocytes obtained from EAE animals), the antigen used for immunization, the presence of co-stimulating substances, the species and strains of the animals. Using different combination of these elements it is possible to establish EAE variants with predominant inflammatory or neurodegenerative aspects and with a monophasic, recurrent or chronic progressive course. Therefore, any result obtained in EAE should always be referred to the model used and carefully interpreted before transferring its implications into the clinical disease and testing neuroprotective agents.

GLUTAMATE RECEPTORS IN EAE AND MS

Experimental results suggest an important role of glutamate in EAE and MS pathogenesis [1,2]. Several studies carried out *in vivo*, in EAE, in *post mortem* brains and in MS patients showed that excitotoxicity mediated by calcium-permeable glutamate receptors contributes to oligodendrocyte death, demyelination and tissue lesions in MS [3]. The earliest investigations suggested that NMDA receptors are implicated in MS pathogenesis [4-5] and the pharmacological modulation of receptor functioning in EAE using memantine (an NMDA receptor antagonist) resulted in disease suppression and restoration of neurovascular function.

*Address correspondence to this author at the via Pergolesi 33, 20052 Monza (MB), Italy; Tel: +39039233495; Fax: +390392332449; E-mail: barbarafrigeni@hotmail.com

Additional investigations have strongly indicated that also AMPA receptors play a part in the development of chronic and acute models of EAE [6]. The competitive antagonists NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]-quinoxaline-2,3-dione) and CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) modify the course of the disease, although a drug effect on kainate receptor-mediated events cannot be excluded due to their lack of specificity. By contrast, the non-competitive antagonists GYKI 52466 (1-(4-aminophenyl) 4-methyl-7,8-methylene dioxy-5H-2,3-benzodiazepine) and GYKI 53773 ((-)-1-(4-aminophenyl) 4-methyl-7,8-methylene-dioxy 4,5-dihydro 3-methylcarbamoyl 2,3-benzodiazepine) differentiate between the two receptors and indicate a specific AMPA involvement in disease development. More recently, a series of AMPA receptor antagonists, with structures based on 2,3- benzodiazepine, have demonstrated their competence in reducing the symptoms and pathological changes associated in an EAE model induced in Lewis rat by guinea pig myelin basic protein [7, 8]. AMPA receptor activation can also be relevant in the neurodegenerative features of MS since it inhibits axonal regeneration mediated by NG2-glia. This is potentially relevant in MS, since NG2-glia cells, a substantial population in the CNS that can be identified by their specific expression of NG2 chondroitine sulphate (CSPG), are able to generate oligodendrocytes and promote axonal regeneration inhibiting reactive gliosis. In fact, the majority of NG2-glia cells retain an oligodendrocyte precursor cell phenotype in the adult CNS and can regenerate oligodendrocytes following demyelination *in vitro* and *in vivo* in response to a chemically induced demyelinating lesion [9]. This type of cell is particularly sensitive to glutamate damage, by the expression expression of kainate receptors.

Additional evidence for a role of glutamate dysregulation in MS can be obtained by the analysis of animal models of optic neuritis, a clinical condition that frequently occurs in MS. In fact, optic neuritis is the presenting symptom in about 20% of patients with MS, and half of the patients with isolated optic neuritis develops MS within 15 years. Patients with optic neuritis typically present with the triad of subacute unilateral loss of vision, ocular pain and impaired colour vision. Symptoms usually worsen over the course of a few days to 2 weeks and then recovery occurs in most patients [10]. However, axonal atrophy and loss are suggested in optic neuritis patients by reduction in retinal thickness in the affected eye also in those patients with a single episode and a good clinical recovery of visual acuity [11]. The injection of kainate in rat optic nerve induces AMPA-kainate activation, that results in a reactive gliosis and a reduced axonal conduction in the injection site, mimicking an acute episode of optic neuritis [12]. Activation of AMPA-kainate type ionotropic glutamate receptors in the optic nerve also evokes increased calcium concentration in NG2-glia and causes reactive gliosis with reduction of axonal conduction that clinically appears as an episode of optic neuritis. In this model, the levels of phosphorylated extracellular signal-regulated MAP kinase ERK1/2 in the isolated nerve are reduced by removal of extracellular calcium and blockade of AMPA receptors with NBQX, indicating that the release of endogenous glutamate (presumably released from axons and astrocytes) activates calcium dependent MAPK pathways [13-14]. The involvement of AMPA receptors in MS has been recently supported by a genome-wide association screening study which identified associated alleles in AMPA receptor genes in patients with MS showing a correlation between highest levels of glutamate and brain volume loss [15].

THE POSSIBLE ROLE OF GLUTAMATE TRANSPORTERS

A possible explanation for an increase in glutamate concentration in the CNS of EAE animals as well as of MS patients, might be the downregulation of its metabolizing enzymes glutamate dehydrogenases and glutamine synthetase during inflammatory conditions [16, 17]. However, abnormal changes in neuronal and

glial glutamate transporters EAATs can be observed in the spinal cord of Lewis rat EAE model and it has been suggested that under pathological conditions these transporters can be inefficient, thus leading increasing extracellular glutamate concentration [18].

As already described in detail in other chapters of this Special Issue, it has been demonstrated that glutamate uptake in the CNS is mediated by different transporters, predominantly and differentially located on neurons (EAAT3, EAAT4) and on astroglial cells (EAAT1, EAAT2) [19]. Many studies have provided compelling evidence that an impairment in the function of EAATs could amplify the sensitivity of the CNS to deleterious effects of aberrant activation of glutamate receptors under inflammatory conditions. Among other factors, oxidative stress plays an important role increasing glutamate concentrations in the extracellular space, because of the reduced efficiency of EAATs due to the free radicals [20]. *In vitro* incubation of oligodendrocytes with the proinflammatory cytokine Tumor Necrosis Factor- α (TNF α) reduced EAAT1 expression and inhibits glutamate uptake by >75%. Furthermore, in MS white matter oligodendrocytes lose expression of EAAT1 and EAAT2 receptors in the proximity of the lesion [21].

Another series of experimental studies highlighted several agents which physiologically regulate glutamate transport. Positive regulators of EAATs as Tumor Growth Factor- α (TGF α) and Epidermal Growth Factor (EGF) enhance EAAT2 expression by activating phosphoinositol-3-kinase and nuclear factor- κ B in human fetal astrocytes [22]. Remarkably, beta-lactam antibiotics are also potent activators of EAATs expression. The beta-lactam ceftriaxone increases brain expression and function of EAAT2 and is neuroprotective *in vitro* in models of ischemic injury and motor neuron degeneration and in an animal model of the fatal disease ALS as well as in a relapsing-remitting EAE rat model of MS induced by myelin oligodendrocyte glycoprotein (MOG), where astrocytes activation is prevented and motor weakness progression halted [23, 24].

In addition to glutamate transporters, levels of extracellular glutamate are controlled by cystine/glutamate antiporter x(c)⁻, an exchanger that provides intracellular cystine for production of glutathione, the major cellular antioxidant. Pampliega *et al.* showed that activated monocytes/macrophages/microglia release glutamate through cystine/glutamate antiporter by increasing xCT catalytic subunit expression, as assessed by *in vitro* assays as well as in both EAE animals and in samples from MS patients [25].

These studies suggest that it may now be possible to identify molecules capable of modulating glutamate transport that could potentially be the target for inhibit, ameliorate, or prevent MS progression.

EVIDENCE OF ELEVATED GLUTAMATE CONCENTRATION IN MS

Data supporting the excitotoxic hypothesis in MS include the report of higher glutamate levels observed both in the CNS and in peripheral blood of affected patients. More than 20 years ago, increased glutamate levels were demonstrated in the CSF of MS patients with acute lesions, whereas glutamate levels were reported to be similar to controls in patients with silent lesions [26, 27]. Moreover, glutamate plasma levels were also found to be increased in relapsing MS patients [28].

Recently the evidence of increased glutamate concentration in the CNS of MS patients has been provided in a study using MR spectroscopy (MRS) [29]. In this study, increased glutamate concentration was demonstrated in both active demyelinating lesions as well as in normal appearing white matter (NAWM) in comparison with healthy controls. The presence of increased glutamate in acute demyelinating lesions and in near NAWM was

ascribed by the authors to increased metabolism and hypercellularity, as large quantities of glutamate are produced and released by activated leucocytes, macrophages and microglial cells in areas of active inflammation (Fig. 1) [30].

However, this result should be cautiously interpreted, since MRS is much more sensitive in the detection of intracellular than extracellular glutamate and, for this reason, it should be assumed that the glutamate levels detected by MRS in these patients is largely due only to the intracellular compartment [31]. Another intriguing observation is that the expression of the glutamate transporters EAAT1 and EAAT2 on the surface of oligodendrocytes in MS is defective in NAWM and this might contribute to excessive tissue concentration of glutamate with a possible toxic action on neurons and glial cells in the lesioned white matter [21].

Besides local production and reduced metabolism, the increase of extracellular glutamate in the CNS may be secondary to increased peripheral production and facilitated by blood-brain barrier breakdown during acute MS relapses [32]. In fact, monocytes are able to release glutamate [28] and they constitute a relevant cell type in the perivascular infiltrates that are characteristic of MS. In addition, CD11b+CD115+Ly-6Chi monocytes are precursors of CNS dendritic cells and macrophages in EAE lesions, being dynamically regulated during the course of the disease and accumulating in blood immediately prior to clinical relapses [33]. Therefore, monocytes are an attractive tool for understanding some of the CNS alterations that occur in MS [34], as confirmed by a recent study investigating the mechanism of glutamate release in monocytes both *in vitro*, in EAE and in MS patients [25].

Despite evidence is increasing to support a detrimental effect of high levels of glutamate in the CNS of MS patients, a recent study suggested that high amounts of extracellular glutamate may also

have a protective role against neuroinflammation. This study, based on an EAE model induced in metabotropic glutamate receptor-4 (mGluR4)-knockout mice and their wild-type counterpart by immunization with the MOG peptide, showed that extracellular glutamate drives a regulatory T cell response that ameliorates neuroinflammation [35]. The authors show that this increase in regulatory T cells is mediated by mGluR4 expressed on dendritic cells, another crucial cell type in immune response.

The description of an upregulation of N-acetyl-aspartyl-aspartate (NAAG), the substance hydrolyzed into glutamate and N-acetylaspartate (NAA) by the enzyme glutamate carboxypeptidase II (GCPII), in MS plaques but also in the surrounding normal-appearing white matter [36] might give further support to the hypothesis that glutamate may also have a protective and not only a detrimental effect, at least in some stages of the disease, although this awaits further investigation.

OXIDATIVE STRESS AND THE MITOCHONDRIAL HYPOTHESIS

Preclinical results support the hypothesis that mitochondrial dysfunction can contribute to axonal disruption in acute inflammatory lesions in MS and can induce the chronic axonal degeneration in the progressive form of disease. Both nitric oxide (NO) and glutamate can contribute to mitochondrial damage converging into a common pathway that alters the electron transport chain, ATP synthesis, ionic homeostasis, Protein Tyrosine Phosphatase (PTP) opening and release of pro-apoptotic factors, leading to mitochondrial dysfunction [37]. Microglial cells are able to generate reactive oxygen and nitrogen species that might impair glutamate uptake mechanisms and the increased glutamate concentration in active plaques induces upregulation of its receptors and the synthesis of mediators of neurotoxicity in EAE [18, 26, 38]. Additionally, the involvement of two enzymes that are expressed in

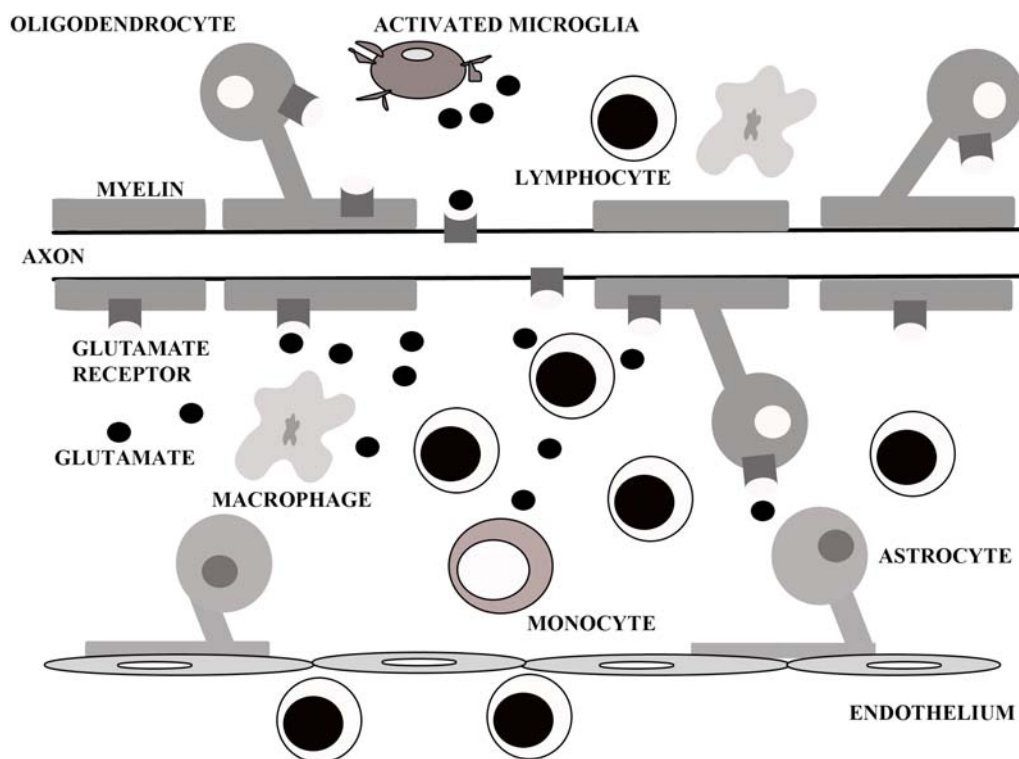


Fig. (1). schematic drawing of the proposed microenvironment at the site of active demyelination in MS based on the current knowledge of the possible origin and effects of glutamate. The main cellular types able to release glutamate are activated microglia and infiltrating peripheral blood mononuclear cells crossing the blood brain-barrier formed by endothelial cells and astrocytes. Glutamate receptors are expressed by myelinating oligodendrocytes and axons.

MS lesions has been suggested: cyclooxygenases-2 (COX-2) and inducible nitric oxide synthase (iNOS) [39]. Both these enzymes contribute to the increased concentration of reactive oxygen species (ROS) that react with NO to produce peroxynitrite (ONOO-) which inactivates the glutamate transporters [27, 40].

In acute demyelinating lesions there is an increased production of NO, that inhibits the cytochrome C activity, leading to production of ONOO- with damage of lipids, proteins and carbohydrates and eventually leading to cell apoptosis. These events are not relevant only in the inflammatory, demyelinating stages since also in chronic MS lesions mitochondrial dysfunction has been demonstrated [41, 42].

Since it appears that mitochondrial function is crucial in preserving axonal integrity in both acute inflammatory and progressive stages of MS it is not surprising that several therapeutic attempts to treat MS have focused on mitochondrial protection, including the use of sodium channel blockers, antioxidants and PTP inhibitors. Moreover, anti-inflammatory drugs could be useful in preserving mitochondrial function. Based on the observation that the action of glutamate receptor agonists increases the expression of cyclooxygenase (COX)-2 in oligodendrocytes, a study performed by Carlson and coworkers [43] showed that the administration of COX-2 inhibitors in TMEV-IDD model of MS, characterized by a chronic persistent central nervous system infection, accompanied by inflammatory demyelinating lesions of the spinal cord, reduced the demyelination rate and protected oligodendrocytes against excitotoxic death *in vitro*. The preclinical studies demonstrate that COX-2 expression in oligodendrocytes contributes to susceptibility to excitotoxic death and that the damage is mediated by specific COX-2 generated prostanoids with prostaglandin E2 being the major responsible for neuronal death *in vivo* [44] and *in vitro* [45].

CONCLUSIONS

The findings we have outlined in this review highlight that the control of glutamate release and metabolism may offer ways to reduce the damage subsequent to excitotoxicity in MS limiting disease severity and disability progression. Moreover, besides the role in demyelination, neurodegeneration and axonal damage a glutamatergic system dysfunction has also been associated with other MS symptoms with a still unclear pathogenesis, such as memory impairment and mental fatigue [46]. For instance, in a *post mortem* study performed to investigate the possible mechanisms of memory impairment in MS patients a comparison between the morphological and molecular changes in myelinated and demyelinated hippocampi from MS brains was performed and it was evidenced that astrocytic EAAT1 and EAAT2 were significantly reduced in demyelinated hippocampi despite the presence of astrogliosis [47]. Mental fatigue in MS is an ill-defined, but potentially clinically-relevant, condition characterized by decreased concentration and often appears before physical neurological symptoms. A possible hypothesis to explain the occurrence of mental fatigue in MS is that the proinflammatory cytokines TNF α , Interleukin 1 β (IL-1 β) and IL-6 are involved through their ability to attenuate the astroglial clearance of extracellular glutamate and lead to the formation of cysteine bridges in EAAT2 resulting in impaired glutamate transport [48]. The consequence of an increased glutamate concentration in the CNS would be a loss of the "filter" function which normally selects information and prevents it from reaching the cerebral cortex. These hypothesis is supported by the demonstration of reduced glutamate transport in frontal cortex and basal ganglia in MS patients with fatigue [49].

In conclusion, a clear link has now been strongly suggested between inflammation, demyelination, axonal loss and glutamate levels in the CNS [50]. However, despite an increasing amount of preclinical data and *post-mortem* results, at least in part already

confirmed by instrumental results obtained in MS patients, the potential importance of the issue strongly indicates the need for further efforts aimed to better elucidate if the modulation of excessive glutamate levels might really become a target for neuroprotection treatments in MS

REFERENCES

- [1] Flanagan, E.M.; Erickson, J.B.; Viveros, O.H.; Chang, S.Y.; Reinhard, J.F. Jr. Neurotoxin quinolinic acid is selectively elevated in spinal cords of rats with experimental allergic encephalomyelitis. *Journal of Neurochemistry*, **1995**, 64(3):1192–1196.
- [2] Sarchielli, P.; Greco, L.; Floridi, A.; Floridi, A.; Galla, V. Excitatory amino acids and multiple sclerosis: evidence from cerebrospinal fluid. *Archives of Neurology*, **2003**, 60(8):1082–1088.
- [3] Matute, C. Glutamate and ATP signalling in white matter pathology. *J Anat*, **2011** Jul, 219(1):53–64.
- [4] Bolton, C.; Lees, P.; Paul, C.; Scott, G.S.; Williams, K.I.; Woodyer, P. Aspects of the biochemical pharmacology of neurovascular disruption in experimental allergic encephalomyelitis (EAE). *Journal of Neuroimmunology*, **1994**, 52(2):113.
- [5] Starck, M.; Albrecht, H.; Pollmann, W.; Straube, A.; Dieterich, M. Drug therapy for acquired pendular nystagmus in multiple sclerosis. *Journal of Neurology*, **1997**, 244(1):9–16.
- [6] Matute, C.; Alberdi, E.; Domercq, M.; Perez-Cerda, F.; Perez-Samartin, A.; Sanchez-Gomez, M.V. The link between excitotoxic oligodendroglial death and demyelinating diseases. *Trends Neurosci*, **2001**, 24:224–230.
- [7] Mòricz, K.; Gigler, G.; Albert, M. Effects of EGIS-8332, an AMPA antagonist, in transient focal ischemia and multiple sclerosis in rats. *Journal of Neuroimmunology*, **2004**, 154(1-2):86.
- [8] Szabó, H.; Berzsenyi, P.; Nemét, L.; András, F.; Horváth, K. Investigations with AMPA antagonist 2,3-benzodiazepines in the experimental autoimmune encephalomyelitis model in rats. *Journal of Neuroimmunology*, **2004**, 154(1-2):86.
- [9] Polito, A.; Reynolds, R. NG2-expressing cells as oligodendrocyte progenitors in the normal and demyelinated adult central nervous system. *J Anat*, **2005**, 207, 707–716.
- [10] Voss, E.; Raab, P.; Trebst, C.; Stangel, M. Clinical approach to optic neuritis: pitfalls, red flags and differential diagnosis. *Adv Neurol Disord*, **2011**, 4(2): 123–134.
- [11] Bertuzzi F.; Suzani M.; Tagliabue E.; Cavaletti G.; Angeli R.; Balgera R.; Rulli E.; Ferrarese C.; Miglior S. Diagnostic validity of optic disc and retinal nerve fiber layer evaluations in detecting structural changes after optic neuritis. *Ophthalmology*, **2010** Jun;117(6):1256–1264.
- [12] Hamilton, N.; Hubbard, P.; Butt, A. Effects of Glutamate receptors activation on NG2-glia in the rat optic nerve. *J Anat*, **2009**, 214, 208–218.
- [13] Kukley, M.; Capetillo-Zarate, E.; Dietrich, D. Vesicular glutamate release from axons in white matter. *Nat Neurosci*, **2007**, 10, 311–320.
- [14] Hamilton, N.; Vayro, S.; Kirchhoff, E.; Verkhatsky, A.; Robbins, J.; Gorecki, D.C.; Butt, A.M. Mechanisms of ATP- and glutamate-mediated calcium signaling in white matter astrocytes. *Glia*, **2008**, 56, 734–749.
- [15] Baranzini, S.E.; Srinivasan, R.; Khankhanian, P.; Okuda, D.T.; Nelson, S.J.; Matthews, P.M.; Hauser, S.L.; Oksenberg, J.R.; Pelletier D. Genetic variation influences glutamate concentrations in brains of patients with multiple sclerosis. *Brain*, **2010** Sep, 133(9):2603–11.
- [16] Hardin-Pouzet, H.; Krakowski, M.; Bourbonniere, L.; Dider-Bazes, M.; Tran, E.; Owens, T. Glutamate metabolism is downregulated in astrocytes during experimental allergic encephalomyelitis. *Glia*, **1997**, 20(1):79–85.
- [17] Rothstein, J.D. Neurobiology. Bundling up excitement. *Nature*, **2000**, 407(6801):141–142.
- [18] Ohgoh, M.; Hanada, T.; Smith, T.; Hashimoto, T.; Ueno, M.; Yamanishi, Y.; Watanabe, M.; Nishizawa, Y. *et al.* Altered expression of glutamate transporters in experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology*, **2002**, 125(1-2):170–178.
- [19] Kanai, Y.; Trotti, D.; Nussberger, S.; Hediger, M.A. High affinity glutamate transporter family: structure, function and physiological relevance. In: *Reith MEA, Totowa, ed. Neurotransmitter Transporters: Structure, Function, and Regulation*. NJ:Humana Press; **1997**, pp. 171–214.
- [20] Volterra, A.; Trotti, D.; Tromba, C.; Floridi, S.; Racagni, G. Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. *The Journal of Neuroscience*, **1994**, 14(5): 2924–2932.
- [21] Pitt, D.; Nagelmeier, I.E.; Wilson, H.C.; Raine, C.S. Glutamate uptake by oligodendrocytes: Implications for excitotoxicity in multiple sclerosis. *Neurology*, **2003**, 61: 1113–20.
- [22] Su, Z.Z.; Leszczyniecka, M.; Kang, D.C.; Sarkar, D.; Chao, W.; Volsky, D.J.; Fisher, P.B. Insights into glutamate transport regulation in human astrocytes: cloning of the promoter for excitatory aminoacid transporter2 (EAAT2). *Proc Natl Acad Sci USA*, **2003**, 100 (4), 1955–1960.
- [23] Rothstein, J.D.; Patel, S.; Regan, M.R.; Haenggeli, C.; Huang, Y.H.; Bergles, D.E.; Jin, L.; Hoberg, M.D.; Vidensky, S.; Chung, D.S.; Toan S.V.; Bruijn, L.I.; Su, Z.Z.; Gupta, P.; Fisher, P.B. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature*, **2005**, 433, 73–77.

- [24] Ramos, K.M.; Lewis, M.T.; Morgan, K.N.; Crysdale, N.Y.; Kroll, J.L.; Taylor, F.R.; Harrison, J.A.; Sloane, E.M.; Maier, S.F.; Watkins, L.R. Spinal upregulation of glutamate transporter GLT-1 by ceftriaxone: therapeutic efficacy in a range of experimental nervous system disorders. *Neuroscience*, **2010 Sep 15**, 169(4):1888-900.
- [25] Pampliega, O.; Domercq, M.; Soria, F.N.; Villoslada, P.; Rodríguez-Antigüedad, A.; Matute, C. Increased expression of cystine/glutamate antiporter in multiple sclerosis. *J Neuroinflammation*, **2011**, 8:63.
- [26] Stover, J.F.; Pleines, U.E.; Morganti-Kossmann, M.C.; Kossmann, T.; Lowitzsch, K.; Kempfski, O.S. Neurotransmitters in cerebrospinal fluid reflect pathological activity. *European Journal of Clinical Investigation*, **1997**, 27(12):1038-1043.
- [27] Gurwitz, D. and Kloog, Y. Peroxynitrite generation might explain elevated glutamate and aspartate levels in multiple sclerosis cerebrospinal fluid. *European Journal of Clinical Investigation*, **1998**, 28(9):760-761.
- [28] Pampliega, O.; Domercq, M.; Villoslada, P.; Sepulcre, J.; Rodríguez-Antigüedad, A.; Matute, C. Association of an EAAT2 polymorphism with higher glutamate concentration in relapsing multiple sclerosis. *J Neuroimmunol*, **2008**, 195, 194-198.
- [29] Srinivasan, R.; Sailasuta, N.; Hurd, R.; Nelson, S.; Pelletier, D. Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T. *Brain*, **2005**, 128 (5), 1016-1025.
- [30] Piani, D.; Frei, K.; Do, K.Q.; Cuenod, M.; Fontana, A. Murine brain macrophages induced NMDA receptor mediated neurotoxicity *in vitro* by secreting glutamate. *Neurosci Lett*, **1991**, 133 (2): 159-62.
- [31] Leighton, M.P.; Prost, R.W.; Ulmer, J.L.; Smith, M.M.; Daniels, D.L.; Strottmann, J.M.; Brown, W.D.; Haein-Bey L.. Pictorial review of glutamate excitotoxicity: fundamental concepts for neuroimaging. *Am J Neuroradiol*, **2001**, 22: 1813-24.
- [32] Nedergaard, M.; Takano, T.; Hansen, A.J. Beyond the role of glutamate as a neurotransmitter. *Nat Rev Neurosci*, **2002**, 3, 748-755.
- [33] King, I.L.; Dickendesher, T.L.; Segal, B.M. Circulating Ly-6C⁺ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. *Blood*, **2009**, 113, 3190-3197.
- [34] Kouwenhoven, M.; Teleshova, N.; İzenci, V.; Press, R.; Link, H. Monocytes in multiple sclerosis: phenotype and cytokine profile. *J Neuroimmunol*, **2001**, 112(1-2), 197-205.
- [35] Fallarino, F.; Volpi, C.; Fazio, F.; Notartomaso, S.; Vacca, C.; Busceti, C.; Biciato, S.; Battaglia, G.; Bruno, V.; Puccetti, C.; Fioretti, M.C.; Nicoletti, F.; Grohmann, U.; Di Marco, R. Metabotropic glutamate receptor-4 modulates adaptive immunity and restrains neuroinflammation. *Nat Med*, **2010**, 16:897-902.
- [36] Vrenken, H.; Barkhof, F.; Uitdehaag, B.M.J.; Castelijns, J.A.; Polman, C.H.; Pouwels, P.J.W. NM Spectroscopic evidence for glial increase but not for neuro-axonal damage in MS normal-appearing white matter. *Magn Reson Med*, **2005**, 53 (2), 256-66.
- [37] Su, K.G.; Banker, G.; Bourdette, D.; Forte, M. Axonal degeneration in multiple sclerosis: the mitochondrial hypothesis. *Curr Neurol and Neurosci Rep*, **2009**, 9(5), 411-417.
- [38] Smith, T.; Groom, A.; Zhu, B.; Turski, L. Autoimmune encephalomyelitis ameliorated by AMPA antagonists. *Nature Medicine*, **2000**, 6(1):62-66.
- [39] Rose, J.W.; Hill, K.E.; Watt, H.E.; Carlson, N.G. Inflammatory cell expression of cyclooxygenase-2 in the multiple sclerosis lesion. *Journal of Neuroimmunology*, **2004**, 149(1-2):40-49.
- [40] Trotti, D.; Rossi, D.; Gjesdal, O.; Levy, L.M.; Racagni, G.; Danbolt, N.C.; Volterra, A. Peroxynitrite inhibits glutamate transporter subtypes. *The Journal of Biological Chemistry*, **1996**, 271(11):5976-5979.
- [41] Stys, P.K.; Waxman, S.G.; Ransom, B.R. Ionic mechanism of axonal injury in mammalian CNS white matter: role of Na⁺ channels and Na⁺-Ca²⁺ exchanger. *J Neurosci*, **1992**, 12(2):430-439.
- [42] Dutta, R.; McDonough, J.; Yin, X.; Peterson, J.; Chang, A.; Torres, T.; Gudiz, T.; Macklin, W.B.; Lewis, D.A.; Fox, R.J.; Rudick, R.; Mirnics, K.; Trapp, B.D. Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Ann Neurol*, **2006**, 59(3):478-89.
- [43] Carlson, N.G.; Rojas, M.A.; Redd, J.W.; Tang, P.; Wood, B.; Hill, K.E.; Rose, J.W. Cyclooxygenase-2 expression in oligodendrocytes increases sensitivity to excitotoxic death. *J of Neuroinflammation*, **2010**, 7:25.
- [44] Manabe, Y.; Anrather, J.; Kawano, T.; Niwa, K.; Zhou, P.; Ross, M.E.; Iadecola, C. Prostanoids, not reactive oxygen species, mediate COX-2-dependent neurotoxicity. *Ann Neurol*, **2004**, 55(5):668-675.
- [45] Carlson, N.G.: Neuroprotection of cultured cortical neurons mediated by the cyclooxygenase-2 inhibitor APHS can be reversed by a prostanoid. *J Neurosci Res*, **2003**, 71(1):79-88.
- [46] Hansson, E. and Rönnbäck, L. On the potential role of glutamate transport in mental fatigue. *J. of Neuroinflammation*, **2004**; 1:22.
- [47] Dutta, R.; Chang, A.; Doud, M.K.; Kidd, G.J.; Ribaldo, M.V.; Young, E.A.; Fox, R.J.; Staugaitis, S.M.; Trapp, B.D. Demyelination causes synaptic alterations in hippocampi from multiple sclerosis patients. *Ann Neurol*, **2011** Mar, 69(3):445-54.
- [48] Anderson, C.M. and Swanson, R.A. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia*, **2000**, 32(1):1-14.
- [49] Roelcke, U.; Kappos, L.; Lechner-Scott, J.; Brunnschweiler, H.; Huber, S.; Ammann, W.; Plohm, A.; Dellas, S.; Maguire, R.P.; Missimer, J.; Radu, E.W.; Steck, A.; Leenders, K.L. Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue: a 18F-fluorodeoxyglucose positron emission tomography study. *Neurology*, **1997**, 48(6):1566-1571.
- [50] Centonze, D.; Muzio, L.; Rossi, S.; Furlan, R.; Bernardi, G.; Martino, G. The link between inflammation, synaptic transmission and neurodegeneration in multiple sclerosis. *Cell Death Differ*, **2010**, 17(10), 1083-1091.