Altered Glutamate Reuptake in Relapsing-Remitting and Secondary Progressive Multiple Sclerosis Cortex: Correlation With Microglia Infiltration, Demyelination, and Neuronal and Synaptic Damage

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Abstract
Cortical involvement in multiple sclerosis (MS) is emerging as an important determinant of disease progression. The mechanisms responsible for MS cortical pathology are not fully characterized. The objective of this study was to assess the role of excitotoxicity in MS cortex, evaluating excitatory amino acid transporter (EAAT) expression and its relationship with demyelination, inflammation, gliosis, and neuronal and synaptic pathology. EAATs are essential in maintaining low extracellular glutamate concentrations and preventing excitotoxicity. Ten MS brains (3 relapsing-remitting MS cases and 7 secondary progressive MS cases) were evaluated by immunohistochemistry for myelin basic protein, CD68, HLA-DR, EAAT1, EAAT2, glial fibrillary acidic protein, phosphorylated c-Jun N-terminal kinase (pJNK), synaptophysin, and neurofilaments. Cortical lesions were frequently observed in MS brains in variable numbers and extensions. In cortical lesions, activated microglia infiltration correlated with focal loss of EAAT1, EAAT2, and synaptophysin immunostaining, and with neuronal immunostaining for pJNK, a protein involved in response to excitotoxic injury. No reduction of EAATs or synaptophysin immunostaining was observed in demyelinated cortex in the absence of activated microglia. Alterations of the mechanisms of glutamate reuptake are found in cortical MS lesions in the presence of activated microglia and are associated with signs of neuronal and synaptic damage suggestive of excitotoxicity. Excitotoxicity may be involved in the pathogenesis of demyelination and of neuronal and synaptic damage in MS cortex.

Key Words: Cortical lesions, Excitotoxicity, Glutamate, Microglia, Multiple sclerosis, Neuron, Synapse.

INTRODUCTION
The importance of grey matter involvement in multiple sclerosis (MS) has been highlighted in several recent neuro-pathologic (1–5) and magnetic resonance imaging studies (6). Demyelination in the cerebral cortex has been found to be widespread and extensive in MS brains, especially in progressive MS (2–4). Axonal and neuronal loss has been described in MS cortex (1, 3, 5). In magnetic resonance imaging studies, cortical damage correlates with the severity of cognitive impairment and of clinical disability in patients with MS (6). The pathogenesis of cortical demyelination and of neuronal and axonal damage in MS cortex is far less known than that of white matter lesions. Cortical lesions (CLs) show little lymphocyte infiltration and lack deposition of complement and immunoglobulins (7, 8).

Glutamate excitotoxicity has been proposed as a putative pathogenetic factor in MS. Excitotoxicity is a common pathologic mechanism in several neurologic diseases, occurring from accumulation of excess extracellular glutamate and consequent overstimulation of glutamatergic receptors. Oligodendrocytes and neurons are highly vulnerable to excitotoxic damage, both in vivo and in vitro (9, 10). Blockade of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate glutamatergic receptors has been shown to ameliorate experimental allergic encephalomyelitis (EAE) (11, 12). Glutamate levels are increased in acute white matter MS lesions and in normal-appearing white matter (13). Agents that modulate glutamatergic transmission, such as memantine and riluzole, have shown promising results in EAE and MS (14, 15).

Removal of excess glutamate from the cortex is largely dependent on glutamate reuptake by the excitatory amino acid transporters (EAATs). EAATs are crucial in maintaining extracellular glutamate concentrations below excitotoxic levels and clearing glutamate released during neurotransmission (9). In the cerebral cortex the astrogial glutamate transporters EAAT1 (GLAST) and EAAT2 (GLT-1) are the primary regulators of extracellular glutamate concentrations; the role of the neuronal glutamate transporter EAAT3 (EAAC1) in glutamate removal is far less important (9). EAAT2 is the quantitatively dominating glutamate transporter.
in the forebrain, whereas EAAT1 predominates in the cerebellum (9). Blockade of glutamate reuptake makes neurons and oligodendrocytes highly vulnerable to even minimal quantities of glutamate (9). Inhibition of EAAT1 or EAAT2 functioning in animal models leads to neurodegeneration (16), oligodendroglial loss, and demyelination (17).

In this study we evaluated the role of excitotoxicity in MS cortical pathology by assessing the pattern of expression of glutamate transporters in MS cortex and its relationship with demyelination, inflammation, gliosis, and neuronal and synaptic pathology.

MATERIALS AND METHODS

This study was performed on formalin-fixed, paraffin-embedded archival material of 10 autopsied MS brains (3 relapsing-remitting MS and 7 secondary progressive MS) and 3 control brains from patients without neurologic diseases. The brains were obtained from the University of Turin and the University of Genoa.

Whole hemicoronal sections (1 hemicoronal section for each case), including cingulate cortex, prefrontal cortex, insula, and temporal cortex, were used, in order to obtain an extensive evaluation of each case. The postmortem interval was less than 36 hours in all cases. Specimens were routinely fixed in 4% buffered formalin and then embedded in paraffin.

The clinical details of the MS cases are summarized in Table 1. Mean age of death in MS cases was 53.4 years (range 27–66 years). Mean duration of disease course was 17.44 years (range 8–30 years). Mean age of death in control cases was 50.33 years (range 44–59 years). The cases were retrospectively defined as having relapsing-remitting MS or secondary progressive MS from hospital records.

Histology and Immunohistochemistry

Each hemicoronal section was dissected into smaller blocks for processing. Standard hematoxylin-eosin and Luxol stainings were obtained for each section. Immunohistochemistry was performed on 5-μm consecutive sections, using an avidin-biotin method. After deparaffinization, sections were treated with 3% H₂O₂ for 10 minutes and then processed for antigen retrieval; details on antigen retrieval are provided in Table 2. The sections were incubated with 10% normal serum for 30 minutes and later incubated with the primary antibodies listed in Table 2. After washing with Tris-buffered saline, the sections were incubated at room temperature for 30 minutes with peroxidase-conjugated secondary antibodies (Dako, Glostrup, Denmark), followed by incubation with ABC complex (Dako) for 30 minutes. Peroxidase labeling was visualized with 10% 3, 3-diaminobenzidine. Sections were counterstained with hematoxylin.

Anti-myelin basic protein (MBP) antibody was used to identify CLs. Antibodies to CD68 and HLA-DR were used to stain macrophages and activated microglia. Anti-glial fibrillary acid protein (GFAP) antibody was used to evaluate presence of gliosis. Synaptic loss was estimated using anti-synaptophysin antibody. Glutamate reuptake was investigated using antibodies to EAAT1 and EAAT2. Anti-phosphorylated c-Jun N-terminal kinase (pJNK) antibody (identifying phosphorylated JNK1, JNK2, and JNK3) was used to assess neuronal damage. Relative preservation of axons in CLs was evaluated using an antibody to neurofilaments.

Image Acquisition

The sections were examined using either a Zeiss Axiohot microscope or a Zeiss Axio Imager.A1 microscope (Carl Zeiss Microlmaging, Göttingen, Germany). Images were acquired using a Nikon Digital Sight DS-DM camera (Nikon Corporation, Tokyo, Japan) or a Zeiss Axiocam MRc5 camera (Carl Zeiss Microlmaging).

Lesion Quantification and Classification

The area of cortical demyelination and the total area of the cortex in each MBP-stained section were measured on the digital image with Eclipse.Net software (version 1.16.6; Laboratory Imaging, Prague, Czech Republic). Values were compounded, and the percentage of demyelinated cortex was calculated for each case. CLs were classified according to Bo et al (2) as leucocortical lesions (type I), intracortical lesions (type II), subpial lesions (type III), or full-width lesions (type IV).

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Gender</th>
<th>Age at Death (years)</th>
<th>Duration of Disease (years)</th>
<th>Disease Course</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>39</td>
<td>13</td>
<td>RR</td>
<td>Status epilepticus (acute MS relapse)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>44</td>
<td>8</td>
<td>RR</td>
<td>Spinal glioma (in cervical spinal cord, with tetraparesis and respiratory failure)</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>27</td>
<td>8</td>
<td>RR</td>
<td>Bone marrow failure after cyclophosphamide therapy</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>60</td>
<td>30</td>
<td>SP</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>65</td>
<td>29</td>
<td>SP</td>
<td>Decubitus infection</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>59</td>
<td>NA</td>
<td>SP</td>
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<td>10</td>
<td>F</td>
<td>58</td>
<td>25</td>
<td>SP</td>
<td>Pneumonia</td>
</tr>
</tbody>
</table>

MS, multiple sclerosis; RR, relapsing-remitting MS; SP, secondary progressive MS. NA, not applicable.
Inflammation in MS cortex was quantified through immunostaining for HLA-DR and CD68 according to methods used in previous reports (1, 7). Lesions were classified as active, chronic active, and chronic inactive on the basis of the density of HLA-DR-positive cells in the lesion and at the lesion border (1).

Excitatory Amino Acid Transporter Immunohistochemistry Quantification

EAAT1 and EAAT2 immunostaining was quantified in 10× microscopic fields using a 10 × 10 500 μm² grid. A score was obtained as the percent number of squares in the grid occupied by EAAT1- and EAAT2-stained cells.

Ten microscopic fields were photographed in the demyelinated cortex and in the adjacent nondemyelinated cortex in each cortical area (cingulate cortex, frontal cortex, insula, and temporal cortex) in the same cortical layers. All images of EAAT-stained sections were then evaluated on the video screen unit by 2 independent observers blinded to demyelinated/nondemyelinated cortex status. In each case the scores for EAAT1 and EAAT2 immunostaining in the demyelinated cortex were compared with the scores observed in the adjacent nondemyelinated cortex.

Evaluation of Synaptic Density

Synaptic density was evaluated by measuring optical density of synaptophysin staining according to a method used on a subset of the present cases in a previous study (3). The analysis was performed using Scion Image software (version 4.0.2, National Institutes of Health; Scion Corporation, Frederick, MD). Ten microscopic fields were assessed in the demyelinated cortex and in the adjacent nondemyelinated cortex in each cortical area (cingulate cortex, frontal cortex, insula, and temporal cortex) in the same cortical layers. In each case, the values of optical density in the demyelinated cortex were compared to the values of optical density observed in the nondemyelinated cortex.

Glial Fibrillary Acidic Protein Immunohistochemistry Quantification

Glial fibrillary acidic protein immunostaining was quantified by counting the number of stained cells in 20× microscopic fields. Ten microscopic fields were assessed in the demyelinated cortex and in the adjacent nondemyelinated cortex in each cortical area (cingulate cortex, frontal cortex, insula, and temporal cortex) in each case, and the density of GFAP-positive astrocytes in the demyelinated cortex was compared with the density of GFAP-positive astrocytes in nondemyelinated cortex.

Statistical Analysis

Two-tailed Student t-tests were used to compare EAAT1 and EAAT2 immunostaining scores, the optical density of synaptophysin immunostaining, and the density of GFAP-positive astrocytes between the demyelinated cortex and the adjacent nondemyelinated cortex in each case. A p < 0.05 was considered significant. SPSS software (version 12.0; SPSS Inc., Chicago, IL) was used for statistical analysis.

RESULTS

Identification and Classification of Cortical Lesions

Using MBP immunostaining, CLs were observed in the cerebral cortex in all MS cases. CLs were identified as areas of complete and sharply delimited loss of MBP immunostaining, with relative preservation of axons in neurofilament immunostaining (1–5). Two cases of secondary progressive MS (cases 5 and 7) showed a pattern of generalized subpial demyelination. In the remaining cases,
several focal CLs were observed, in variable amount and extension. The extent of cortical demyelination ranged from 2% to 48% of the total area of the cortex in the hemicoronal section (mean 11.55%; median 4.75%). The majority (~80%) of CLs were subpial lesions (type III). Frequent intracortical lesions (type II) (Fig. 1A) were also observed, as well as full-width lesions (type IV). Only rare leucocortical lesions (type I) were observed. No demyelinating lesions were observed in control brains.

**Evaluation of Inflammation in Multiple Sclerosis Cortex**

The density of HLA-DR-positive microglia was low in most demyelinated cortex (mean 19.7/mm²; range 0–37/mm²) and comparable to the density observed in the nondemyelinated cortex (mean 18.6/mm²; range 0–35/mm²).

A high density of HLA-DR-positive microglia (mean 161.1/mm²; range 108–252/mm²) was observed in some CLs (~5% of the total area of demyelinated cortex in the coronal

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**FIGURE 1.** Intracortical lesion (A, myelin basic protein immunostaining; original magnification: 10×; scale bar = 100 μm), showing a high density of activated microglia (B, HLA-DR immunostaining; original magnification: 10×; scale bar = 100 μm). A focal reduction of excitatory amino acid transporter (EAAT) 1 staining is observed in the lesion (C, EAAT1 immunostaining; original magnification: 10×; scale bar = 100 μm). A focal reduction of EAAT2 staining is observed in the lesion; in the adjacent nondemyelinated cortex a rim of EAAT2-stained astroglial cells surrounds the lesion (D, EAAT2 immunostaining; original magnification: 10×; scale bar = 100 μm). A focal loss of synaptophysin staining is observed in the lesion coinciding with the area of inflammation, demyelination, and EAATs loss (E, synaptophysin immunostaining; original magnification: 10×; scale bar = 100 μm). Phosphorylated c-Jun N-terminal kinase (pJNK) nuclear immunoreactivity is observed in neurons within the lesion (F, pJNK immunostaining; original magnification: 100×; scale bar = 10 μm).
section), mostly intracortical (Fig. 1B). These lesions were classified as active inflammatory CLs according to the classification used in previous studies (1, 7). The CD68 immunoreactivity pattern was similar to that for HLA-DR. No phagocytic, myelin-laden macrophages were observed in CLs, as already noted in previous studies (1, 18).

**Activated Microglia in Multiple Sclerosis Cortical Lesions Is Associated With Focal Loss of Excitatory Amino Acid Transporters 1 and 2 Within the Lesion and Increased Excitatory Amino Acid Transporter 2 Immunoreactivity in Surrounding Nondemyelinated Cortex**

The pattern of EAAT1 and EAAT2 immunostaining in MS and control cortex was similar to the pattern described in normal human cortex in previous studies (19, 20). EAAT1-stained astroglial cells were uniformly abundant through all the cortex, whereas EAAT2-stained astroglial cells showed a patchy pattern and were more frequent in the superficial layers (1, 2, and part of 3) and in the deeper layers (4 and 5) of the cortex (Fig. 2A, B).

A sharp and well-delimited focal loss of EAAT1 and EAAT2 immunostaining was observed in those CLs showing a high density of activated microglia, corresponding to the area of demyelination and of microglial infiltration (Fig. 1C, D).

The nondemyelinated cortex surrounding these CLs showed a high density of astroglial cells strongly stained for EAAT2, which formed a rim around the lesions; this pattern was not observed with EAAT1 (Fig. 1D).

**Activated Microglia in Multiple Sclerosis Cortical Lesions Is Associated With Focal Loss of Synaptophysin Immunostaining and With Phosphorylated c-Jun N-Terminal Kinase-Positive Neurons**

A well-delimited focal loss of synaptophysin immunoreactivity was observed in those CLs showing a high density of activated microglia, corresponding to the area of demyelination and of microglial infiltration and to the loss of EAAT1 and EAAT2 immunostaining (Fig. 1E). Nuclear pJNK immunostaining was observed in several neurons in these lesions (Fig. 1F) and also in glial cells and in microglia, similarly to that observed in white matter lesions in a previous study (21).

**No Reduction of Excitatory Amino Acid Transporter 1 or 2 Immunostaining Is Observed in Chronically Demyelinated Cortex**

No difference in EAAT1 or EAAT2 immunostaining was observed between demyelinated and adjacent nondemyelinated cortex, excluding those CLs showing a high density of microglial infiltration. The density of EAAT-stained astroglial

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**FIGURE 2.** Pattern of excitatory amino acid transporter (EAAT) 2 immunostaining (A, EAAT2; original magnification: 2.5×; scale bar = 400 μm) and EAAT1 immunostaining (B, EAAT1; original magnification: 2.5×; scale bar = 400 μm) in multiple sclerosis (MS) cortex. The y-axis error bar graphs show the distribution of immunostaining scores (percent number of squares in the grid occupied by stained cells) for EAAT2 (C) and EAAT1 (D) in MS cortex in the 10 cases evaluated in this study, regardless of demyelination (mean, 95% confidence intervals, range).
cells in MS cortex was variable (Fig. 2C, D); in particular, EAAT2 was diffusely reduced in some MS cases compared with control cases, regardless of demyelination. We observed a trend, not reaching statistical significance toward lower scores of EAAT2 immunostaining in cases with longer duration of disease ($r = -0.515$), older age ($r = -0.514$), and higher extent of cortical demyelination ($r = -0.310$) (age, duration of disease, and extent of cortical demyelination were, in turn, positively correlated). No correlation was observed with postmortem interval ($r = -0.085$).

**No Reduction of Synaptic Density Is Observed in Chronically Demyelinated Cortex**

No difference in optical density of synaptophysin staining was observed between demyelinated and adjacent nondemyelinated cortex, excluding those CLs showing a high density of microglial infiltration. We can therefore confirm, on a larger series, the data already described in a previous study on a subset of our cases (3).

**Astrogliosis Is Not a Feature of Multiple Sclerosis Cortical Lesions**

No significant difference was found in the density of GFAP-positive astrocytes between demyelinated and adjacent nondemyelinated cortex; stained astrocytes were usually found close to the subpial rim (demyelinated cortex: mean 2.34 cells/field, range 0–12/field; nondemyelinated cortex: mean 2.01 cells/field, range 0–11/field).

**DISCUSSION**

Cortical demyelination is frequent and often extensive in MS brains. Increasing evidence suggests cortical pathology as an important determinant of disease progression (4) and disability in MS (6).

The immunopathogenesis of MS CLs presents substantial differences from that of white matter lesions: CLs lack many of the classic inflammatory features of white matter lesions, such as lymphocyte infiltration, complement activation, and myelin-laden macrophages (1, 7, 8, 18). The mechanisms leading to cortical demyelination are not yet fully understood. Inflammatory activity in CLs is expressed mainly by activated microglia infiltration and a scarce presence of T lymphocytes (1, 18). As observed in this study, no significant astrogliosis was present in CLs, whereas it is typically present and important in white matter lesions. Severe tissue damage, except for myelin loss, is usually not present in MS CLs, contrasting to that observed in white matter lesions.

In this study, alterations of glutamate reuptake mechanisms are found in MS cortex in the presence of activated microglia and are associated with demyelination and signs of neuronal and synaptic damage, suggesting glutamate excitotoxicity as a possible mechanism of MS cortical pathology.

**Loss of Excitatory Amino Acid Transporter Immunostaining Is Associated With Inflammation in Cortical Lesions**

In this study, a high density of activated microglia in CLs was correlated with a complete and sharply outlined loss of EAAT1 and EAAT2, corresponding to the area of inflammation and demyelination. This loss of EAAT immunostaining is probably due to a reduced expression rather than a loss of astroglial cells, which are usually well preserved even in the more destructive white matter demyelinating lesions. Moreover, no reduction of the number of GFAP-stained astroglial cells was observed in the demyelinated cortex. Peroxynitrites and oxygen radicals, produced by activated microglia, may downregulate the expression and function of EAATs (22). Microglia-derived inflammatory cytokines (23), such as tumor necrosis factor-α and interleukin-1β, can reduce glutamate reuptake by astrocytes in vitro (24). Similarly, a loss of EAAT1 and EAAT2 has been described in EAE spinal cord at the peak of inflammation (25) and in MS acute white matter lesions (26).

EAAT loss and activated microglia in CLs were associated with increased density of EAAT2-immunoreactive astroglial cells in the surrounding nondemyelinated cortex. Because EAAT2 is upregulated in the presence of increased extracellular glutamate (27), this pattern might be the result of increased concentrations of extracellular glutamate diffusing from CLs due to reduction of glutamate reuptake in the lesion and increased glutamate release. Activated microglial cells are an additional important source of glutamate release during inflammation (26, 28). This pattern of EAAT2 immunostaining is peculiar, having not been observed in white matter lesions (26).

The loss of glutamate transporters during inflammation in CLs may hamper the normal buffering of elevated glutamate by astrocytes, which is the main mechanism of glutamate removal from the extracellular environment. Thus, oligodendrocytes and neurons are exposed to high concentrations of glutamate with consequent excitotoxic damage (9, 10).

**Neuronal and Synaptic Damage Is Associated With Inflammation and Excitatory Amino Acid Transporter Loss in Cortical Lesions**

The presence of activated microglia and EAAT loss in CLs correlated with signs of neuronal injury represented by neuronal nuclear immunostaining for pJNK. Neuronal damage is known to induce JNK activation through a cascade of kinases, leading to phosphorylation in the nucleus of JNK (29, 30). Activation of the JNK pathway may be triggered by many different mechanisms, including excitotoxicity (31), inflammatory cytokines (e.g. tumor necrosis factor-α) (32), and axotomy close to the cell body (33). Excitotoxicity and inflammatory cytokines might be involved in JNK activation in CLs, as well as in intracortical axonal transection, as previously reported in CLs (1). pJNK in turn activates the transcription factor c-jun (30). Activated c-jun induces the expression of several genes involved in neuronal response to damage, resulting in survival/regeneration or apoptosis (29). Neuronal apoptosis, with caspase-3 activation, and neuronal loss have been previously described in MS CLs (1, 3, 5).

The presence of activated microglia and EAAT loss in CLs correlated with signs of synaptic damage represented by loss of the presynaptic protein synaptophysin. The loss of synaptophysin could be a consequence of excitotoxic damage to dendrites and synapses, as demonstrated by experimental...
models (34, 35) and as suggested in Alzheimer disease. In Alzheimer disease midfrontal cortex, synaptophysin loss has been shown to correlate with local reduction of glutamate reuptake (36). Alternatively, dendrites and synapses might also be a direct target of autoimmune inflammation in MS, as activated microglia in CLs has been described to selectively target synaptic terminals (1).

No loss of synaptophysin was observed in chronic CLs, as already described in a previous study on a subset of our cases (3). Synaptic damage in MS cortex could therefore be a transient feature associated with the presence of microglial activation. Accordingly, in EAE a severe decrease of synaptophysin immunoreactivity was described at the peak of inflammation, followed by subsequent complete recovery (35).

In CLs, neuronal and synaptic pathology and, to some extent, demyelination could result from excitotoxic damage, secondary to impairment of glutamate reuptake in the presence of activated microglia infiltration. Indeed, the inhibition of EAAT functioning in the rat optic nerve has been shown to cause oligodendroglial loss, demyelination, and axonal damage (17). These mechanisms probably also play a role in white matter lesions (26). In the cortex, which harbors a high number of glutamatergic synapses and a high content of glutamate, the importance of excitotoxicity resulting from altered glutamate reuptake is presumably much greater than that in the white matter. Although we investigated a possible pathogenetic mechanism of cortical damage in MS, further studies are needed to determine whether this mechanism is specific only to MS or is shared by other neuroinflammatory diseases with cortical involvement.

Findings in Chronically Demyelinated Cortex

Although no difference in EAAT immunostaining was observed between chronic CLs and nondemyelinated cortex, the EAAT immunostaining pattern in the cortex showed considerable variability between MS cases, regardless of demyelination. The patterns of EAAT1 and EAAT2 immunostaining in the cortex were quite different, with EAAT1 being more homogenously distributed and EAAT2 being more patchy and confined mostly to the superficial and deeper layers of the cortex. These patterns were similar to those already described in the normal human cortex in previous neuropathologic studies (19, 20); the heterogeneous distribution of EAAT2 has been attributed to a higher expression in the layers of the cortex with higher synaptic density (19). Overall, the expression of EAAT2 in the cortex appeared to be lower in MS cases compared with control cases, particularly in those MS patients who were older and had a longer disease duration. Further studies on larger series are needed to investigate a possible reduction of EAAT2 expression in the cortex in the later stages of MS.

In conclusion, EAAT pattern changes observed in MS cortex suggest a role for alterations of glutamate homeostasis in MS cortical pathology. Excitotoxic damage associated with inflammation could be involved in the formation and expansion of CLs and be a substrate of neuronal and synaptic pathology in MS cortex. Further studies are needed to determine whether modulation of glutamatergic transmission could be successful in MS therapy.

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