Review

Glial Glutamate Transporter-Mediated Plasticity: System x_c^-/xCT/SLC7A11 and EAAT1/2 in Brain Diseases

Marc Dahlmanns[1,†], Jana Katharina Dahlmanns[1,†], Nicolai Savaskan[2,3], Hans-Herbert Steiner[4], Eduard Yakubov[2,4,*]

[1]Institute for Physiology and Pathophysiology, Friedrich-Alexander-University Erlangen-Nuremberg (FAU), 91054 Erlangen, Germany
[2]Department of Neurosurgery, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nuremberg (FAU), 91054 Erlangen, Germany
[3]Department of Public Health Neukölln, District Office Neukölln of Berlin Neukölln, 12359 Berlin, Germany
[4]Department of Neurosurgery, Paracelsus Medical University, 90471 Nuremberg, Germany
*Correspondence: edmond-ariel@hotmail.de (Eduard Yakubov)
[†]These authors contributed equally.

Abstract

Glial cells play an essential role in the complex function of the nervous system. In particular, astrocytes provide nutritive support for neuronal cells and are involved in regulating synaptic transmission. Oligodendrocytes ensheath axons and support information transfer over long distances. Microglial cells constitute part of the innate immune system in the brain. Glial cells are equipped with the glutamate-cystine-exchanger xCT (SLC7A11), the catalytic subunit of system x_c^-, and the excitatory amino acid transporter 1 (EAAT1, GLAST) and EAAT2 (GLT-1). Thereby, glial cells maintain balanced extracellular glutamate levels that enable synaptic transmission and prevent excitotoxic states. Expression levels of these transporters, however, are not fixed. Instead, expression of glial glutamate transporters are highly regulated in reaction to the external situations. Interestingly, such regulation and homeostasis is lost in diseases such as glioma, (tumor-associated) epilepsy, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis or multiple sclerosis. Upregulation of system x_c^- (xCT or SLC7A11) increases glutamate export from the cell, while a downregulation of EAATs decreases intracellular glutamate import. Occurring simultaneously, these reactions entail excitotoxicity and thus harm neuronal function. The release of glutamate via the antiporter system x_c^- is accompanied by the import of cystine—an amino acid essential in the antioxidant glutathione. This homeostasis between excitotoxicity and intracellular antioxidant response is plastic and off-balance in central nervous system (CNS) diseases. System x_c^- is highly expressed on glioma cells and sensitizes them to ferroptotic cell death. Hence, system x_c^- is a potential target for chemotherapeutic add-on therapy. Recent research reveals a pivotal role of system x_c^- and EAAT1/2 in tumor-associated and other types of epilepsy. Numerous studies show that in Alzheimer's disease, amyotrophic lateral sclerosis and Parkinson's disease, these glutamate transporters are dysregulated—and disease mechanisms could be interposed by targeting system x_c^- and EAAT1/2. Interestingly, in neuroinflammatory diseases such as multiple sclerosis, there is growing evidence for glutamate transporter involvement. Here, we propose that the current knowledge strongly suggest a benefit from rebalancing glial transporters during treatment.

Keywords: SLC7A11; GLT-1; GLAST; glioma; Alzheimer's disease; amyotrophic lateral sclerosis; Parkinson's disease; neuroinflammation

1. Introduction

Glial cells play a major role in the central nervous system (CNS). Comprising different cell types, glial cells regulate many physiological processes: Astrocytes, participating in the tripartite synapse, remove excess levels of glutamate, thereby enabling proper glutamatergic neurotransmission and preventing extrasynaptic N-methyl-D-aspartate (NMDA) receptor-induced excitotoxicity [1,2]. Microglia are CNS resident immune cells that regulate the immune response to threatening stimuli [3]. Oligodendrocytes ensheath axons to protect and support them and to enable rapid information transfer inside neuronal networks [4].

The excitatory amino acid glutamate plays an important role in the homeostasis of the brain [5], thus its regulation by glial transporters is particularly relevant in diseases. In the CNS, neuronal damage can occur through increased extracellular glutamate, which then triggers cell death-inducing cascade by the activation of extrasynaptic NMDA receptors, giving rise to the so-called mechanism of excitotoxicity [6,7]. This has strong implications for various diseases of the CNS such as Alzheimer's disease or Huntington disease [8]. To regulate glutamate homeostasis, astroglial cell express glutamate exporters and glutamate importers. Through this interplay the level of extracellular glutamate can be regulated. The major glutamate exporter in the CNS is the system x_c^-. It is mainly expressed in the astroglia [9], and during the development of the rat, the expressions of the system x_c^- subunits 'xCT' or 'SLC7A11' (light chain) and '4F2hc' (heavy chain) increase until at least 3 months of age [10]. With aging, sys-

tem x_c^- is overexpressed in rats [11]. These findings suggest that with the growing nervous systems, the need to balance oxidative stress and glutamate homeostastis remains an important task that is fulfilled by system x_c^-.

Astroglial glutamate importers such as the excitatory amino acid transporters 1 and 2 (EAAT1/2) counteract the extrusion of glutamate. EAATs are sodium-dependent glutamate transporters that are responsible for the removal of extracellular glutamate by importing glutamate into the astrocytes' cytosol [12]. The examination of both glutamate-exporting and -importing transporters provides important insights into the overall astroglial contribution to glutamate homeostasis and its pathophysiological relevance. The detailed molecular aspects and the physiology of glutamate transporters are beyond the scope of this plasticity-focused review. We refer the reader to excellent reviews that comprehend this knowledge [13–16].

Oxidative stress can strongly interfere with physiologic function. System x_c^- imports cystine to fuel the antioxidant response of the cells [17] in exchange for glutamate, which is exported to the extracellular fluid. System x_c^- activity in astrocytes is not only regulated by glutamate but also by its second substrate, cystine, since the intracellular glutathione (GSH) levels can regulate the expression of system x_c^- [18]. However, while system x_c^- contributes to the antioxidative response [17], its deficiency in system x_c^- does not induce oxidative stress by itself, but may disrupt glutamate homeostasis [19].

Neurons in the CNS are susceptible to oxidative stress due to their high oxygen consumption during metabolism and the high abundance of lipids, which represent targets for oxygen radicals [20]. As some neurons are especially vulnerable to reactive oxygen species, it is important for the brain to maintain an antioxidative response [21]. The glutamate-cystine antiporter system x_c^- is involved in the antioxidative response, which has been also strongly investigated in the context of cancer biology [22]. On a molecular level, the import of cystine through system x_c^- fuels the formation of antioxidant glutathione. Inhibiting cystine import through system x_c^- inhibitors such as erastin promotes ferroptosis, an iron-dependent form of cell death triggered by missing antioxidants [23]. In the field of neurooncology, system x_c^- thus represents a potential therapeutic target, and tumor cell death during chemotherapy could potentially be boosted by add-on therapy with system x_c^- inhibitors [24,25].

In this review, we will focus on the issue of whether alterations in glutamate transporter activity are a consequence of the disease pathology or are part of the pathogenesis in glioma, tumor-associated epilepsy, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, or multiple sclerosis. We further address the benefits of boosting/inhibiting these proteins and we are going to approach the question of how to selectively target glial cells, because of their various responsabilities in the nervous system.

2. Overview of the Function of Glial Glutamate Transporters System x_c^- and EAAT1/2

There is no final consensus yet on the expression levels of the cystine-glutamate antiporter system x_c^- in different CNS cell types. Soria *et al.* [26] reported co-expression of system x_c^- with markers for neurons, as it was shown in other studies [10,27], and for oligodendrocytes. Recent findings questioned these data by claiming that system x_c^- is expressed mainly in astrocytes, where it is coexpressed with the sodium-dependent glutamate transporter EAAT1 [9]. System x_c^- expression was not found in neurons, oligodendrocytes, or microglia. In line with this, Mesci *et al.* [28], could not detect system x_c^- in motor neurons. In a systematic approach to evaluate antibody specificity and technical specifications, it became evident that the antibody production process and the staining protocols can heavily impact if system x_c^- can be detected [29]. Further studies are required to confirm in which cell system x_c^- may be expressed. While we wanted readers to bear in mind this uncertainty when evaluating study results, system x_c^- modulation affects every cell type that is in contact with system x_c^- modulated extracellular glutamate level regardless of their individual expression.

Although system x_c^- expression in neurons is uncertain, its effect on neuronal activity is undebated. Around the time when Sato *et al.* [30] used molecular-biological techniques to clone human system x_c^- and to describe system x_c^- expression across the brain with *in situ* hybridization [31], the first electrophysiological evidence for system x_c^-'s impact on vesicular release was obtained: in acute brain slices containing the medial prefrontal cortex, bath application of cystine reduced the frequency of miniature excitatory postsynaptic currents (mEPSCs) that are the electrophysiological expression of the readily releasable pool (RRP) of synaptic vesicles [32]. The effect was suppressed by system x_c^- inhibitor (S)-4-carboxyphenylglycine [33]. (S)-4-carboxyphenylglycine is a rather unspecific blocker for system x_c^- and also acts as an antagonist for the group 1 metabotropic glutamate receptors [34]. This weak receptor specificity of (S)-4-carboxyphenylglycine could interfere with the inhibition of system x_c^-. There is no indication that the impact of (S)-4-carboxyphenylglycine on system x_c^- than its modulation of metabotropic glutamate receptors, since data reveal no direct connection between the RRP size and group I mGluR activity, at least in the functional context of synaptic long-term depression [35].

In electrophysiological recordings, mEPSCs can only be detected when action potential-driven release is blocked, e.g., with the voltage-gated sodium channel blocker tetrodotoxin (TTX). Spontaneous EPSCs (measured in the absence of TTX) are not decreased by cystine [33]. Cystine's dampening effect on mEPSCs is mediated through group II metabotropic glutamate receptors that balance glutamate release by suppressing synaptic firing upon above-

threshold extracellular glutamate detection [36]. The increase in extracellular cystine could elevate extracellular glutamate concentrations, which triggers metabotropic glutamate receptors, which in turn suppress neuronal vesicle release [37,38]. When evoked EPSCs (eEPSC) from cortical layer 2/3 neurons were recorded, system x_c^- inhibitor sulfasalazine could decrease these eEPSCs [39]. In mixed primary hippocampus cultures with both astrocytes and neurons, treatment with system x_c^- inhibitor erastin led to a reduction of the RRP size without affecting the recycling pool size [40]. In these cultures, treatment with system x_c^- inhibitors erastin [23] and sorafenib [41] resulted in increased extracellular glutamate levels. In addition to acting on system x_c^-, sorafenib inhibits several targets, such as the RAF pathway and the vascular endothelial growth factor receptor family (VEGFR), which is of particular importance since VEGF has direct synaptic effects [42]. The broad range of targets on which sorafenib acts makes it difficult to determine which target is particularly relevant in this context. Thus, there is a need for more specific inhibitors to narrow down the targets involved when 'dirty drugs' are used.

The action of system x_c^- is not restricted to the presynaptic site. A study in *Drosophila* revealed that a reduction in system x_c^- reduced extracellular glutamate levels [43]. Specifically, in *Drosophila* mutants with genetic elimination of the so-called xCT gene "genderblind", extracellular glutamate falls off by 50% compared to wild-type-level [43]. In response to this reduced glutamate level, the postsynaptic receptors become more expressed [43]. This elevated postsynaptic receptor expression could be reversed by exposing postsynaptic glutamate receptors to normal levels of glutamate [43]. System x_c^-'s regulation of extracellular glutamate hence has a direct effect on synaptic transmission. This finding was further substantiated by recordings from the Schaffer collaterals, the CA3-to-CA1 synapse in the hippocampus, showing that a knockout of system x_c^- leads to more α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors being expressed at the membrane, and to a strengthened synaptic transmission [44]. Furthermore, in system x_c^- deficient *sut* mice, long-term potentiation and long-term memory were decreased, while the basic synaptic transmission was unaltered [45]. In line with that normal synaptic transmission, the assessment of mouse behavior in terms of spontaneous alternation, rotarod, and open field behavior, revealed that a genetic knockout of system x_c^- does not influence these basic kinds of behavior [46].

Extracellular glutamate may bind to a variety of receptors, amongst them AMPA receptors, N-methyl-D-aspartate (NMDA) receptors, or metabotropic glutamate receptors (mGluRs), and also to glutamate transporters such as EAAT1/2. While mEPSCs abate upon an excess extracellular glutamate, mGluR1-mediated EPSCs swell [47]. This indicates competition for the extracellular glutamate between neuronal receptors and glial transporter. Extracellular glutamate levels cannot only be increased by system x_c^- activation. Also the inhibition of EAAT1 (GLAST) and of EAAT2 (GLT1) has similar effects [47]. The interplay between all different glutamate transporters is complex and essential in determining how the synaptic transmission is affected by the manipulation of one of them. This is illustrated for example by the finding that system x_c^- deletion led to increased expression of EAAT2 [48], which does not represent a compensatory, but rather a reinforcing mechanism of decreasing extracellular glutamate. The extent to which glial glutamate transporter-mediated glutamate uptake (via EAAT1/2) affects neurotransmission is even brain region-specific: compared with the neonatal rat hippocampus, the expression of EAAT1 and EAAT2 is lower, and the entry of glutamate into neonatal rat cortical astrocytes is slower [49].

When pharmacological action on the glutamate transporters is planned, it is important to assess how this broad inhibition would impact the glutamate transporters on the diverse glial cells. One promising approach to tackle this question is the investigation of cell-type specific knockouts, e.g., of EAAT2. When EAAT-2 was genetically knocked out using neuron- or astrocyte-specific Cre-loxP systems, it turned out that the astrocytic EAAT2-deletion had more severe effects than the neuronal deletion of EAAT2 [50]. When astrocytes had lost their glutamate-importing capacities, mortality and seizure susceptibility increased [50]. Interestingly, during aging an astrocyte-specific EAAT2 knock-out differs from a neuron-specific knock-out on behavioral and transcriptional levels [51], indicating that the different contributions of neuron- and astroglial-mediated glutamate imports are directly linked to behavioral states. These findings stress the fact that it is important to precisely investigate each transporter and its role in physiological and pathophysiological settings.

Altogether, glial glutamate transporters control important aspects of the physiologic neuronal activity that gives rise to behavior. In the following paragraph, we will discuss how these transporters shape astrocytes' pathophysiological role in the context of different CNS diseases.

3. System x_c^- and EAAT1/2 in Peritumoral Astrocytes and Their Impact on Epileptic Activity

The knowledge that astrocytes are associated with human disease is growing rapidly [52]. One of the most devastating diseases of the human CNS represents malignant glioma. Resections—if even possible at all—are incomplete at best. The tumors are often treatment resistant and can progress to higher CNS WHO grades even after early detection, up to glioma of WHO grade 4 (mostly glioblastoma) [53,54]. The expression levels of system x_c^- in such highly malignant human glioblastoma cells are positively correlated with tumor invasion, and negatively correlated

with patient survival [55,56]. In contrast to the high expression of system x_c^- in tumor cells [57,58], the glutamate importers EAAT1 and EAAT2 are abnormally low expressed in glioma cell lines [59], animal models [60] and human glioblastoma [61]. It has even been shown that increased EAAT2 expression inhibits tumor growth [62], further underscoring the idea of glutamate as a proponent of malignant growth [63].

In addition to the harms caused by the tumor itself, healthy CNS function is often affected in the peritumoral area as well. In a very recent study, the transcriptome of human astrocytes that stem from tissue surrounding the tumor site was analyzed [64]. The authors found that peritumoral astrocytes downregulated genes related to reacting to their microenvironment and synaptic function. Interestingly, amongst those genes were the EAAT2-encoding gene SLC1A2 and the EAAT1-encoding gene SLC1A3 [64]. In the peritumoral area of human tumor samples, system x_c^- expression was not elevated [39], suggesting that solely glutamate importers were downregulated and thereby could contribute to increase extracellular glutamate levels. In a tumor-transplant model performed in mice, it was found that primary CNS tumors release glutamate via system x_c^-, which evokes epileptic activity in the peritumoral area [65]. High expression of system x_c^- and low expression of EAAT are biomarkers for glioma-associated seizures [66,67]. While the primary tumor enhances its glutamate-releasing capabilities (high system x_c^- expression), astrocytes in the peritumoral area decrease their glutamate-importing capacities (low EAAT1/2 expression). This leads to a higher glutamatergic tone in the peritumoral area. As a result, glioma often coincides with epilepsy [68].

Glutamate transporters are involved in the pathomechanisms of epileptic seizures also without glioma as the underlying cause. In biopsies from patients with pharmacoresistant temporal lobe epilepsy, qPCR analysis revealed an upregulation of xCT mRNA [69]. In humans, EAAT1/2 is also involved in epilepsy. Astroglial glutamate transporters EAAT1/2 are investigated in the context of seizures since almost thirty years ago, in mice, the *EAAT2* gene was located in a chromosomal region known to modulate neuroexcitability and seizure frequencies [70]. In patients suffering from pharmacoresistant temporal lobe epilepsy but without hippocampal sclerosis (i.e., without significant cell loss), EAAT2 was upregulated in hippocampal subregions, as confirmed by immunohistochemical stainings and in situ hybridization from post-mortem tissue [71]. In human epileptic foci in the neocortex, EAAT2 protein expression was decreased [72], similar to other cohorts of patients with intractable epilepsy in which EAAT1 and EAAT2 were decreased [73,74]. Taken together, it appears that in human patients suffering from epilepsy, the glutamate homeostasis is shifted towards an increase in the extracellular glutamate level. However, human studies on measurable levels of glutamate in epilepsy have yielded mixed results and fur-

ther studies using standardized experimental paradigms are needed to elucidate this topic [75].

The hypothesis that glutamate excess is contributing to epileptic seizures [76] was tested in several animal models with blocked system x_c^- function: at first, it was described that xCT$^{-/-}$ mice have an elevated threshold for seizures induced by pilocarpine or kainic acid [19]. Thus, fewer seizures occur in the absence of system x_c^-. In these experiments, pilocarpine applications induce seizures in the temporal lobe via its action on the M1 receptor and represent a widely used experimental model to study epileptic seizures [77]. Seizures are induced by kainic acid because of its analogism with glutamate and the agonistic action on ionotropic glutamate receptors [78]. N-acetylcysteine as an activator of system x_c^- could not exert its usually proconvulsive effects in xCT$^{-/-}$ mice – ascribing a seizure-promoting role to system x_c^- through its glutamate-extruding activity [19]. In hippocampal rat brain slices, convulsive agents such as kainic acid, pilocarpine, and veratridine reduced the EAATs activity and could therefore be rescued by the application of a mGluR III agonist [79]. In this study, the injection of an EAAT inhibitor into rats exacerbated kainic acid-induced seizures. Thus, more seizures occured in the absence of EAATs because blockage of glutamate transporters increased their extracellular concentrations. Involving mGluRs, EAAT inhibition in hippocampal pyramidal cells activated mGluR group I and II receptors and led to epileptic-like activity [80]. Even in zebrafish, EAAT2 deficiency led to increased glutamate levels as well as light-induced seizures in neurons and glial cells [81].

In a model of self-sustained status epilepticus—where amygdala-implanted electrodes were used for stimulation that results in ongoing seizure events—genetic system x_c^- inactivation had anticonvulsive and antiepileptogenic effects, weighing also towards the seizure-promoting role of system x_c^- [82]. In their model, pharmacological system x_c^- inhibition (by sulfasalazine treatment) also prevented seizures. In line with this, system x_c^- null mice (xCT$^{sut/sut}$) displayed a reduced occurrence of seizures in a pentylenetetrazole kindling model, a model in which inhibition of GABA$_A$ receptors increases synaptic stimulation and excitation [83,84]. Although the same authors, however, later reported lower seizure thresholds in system x_c^- null mice after kainic acid or pentylenetetrazole and more severe seizures [85]—ascribing a seizure-preventing role to system x_c^-—it generally appears that targeting glial system x_c^- may support therapy of seizures. In a very recent study, the system x_c^- inhibitor sulfasalazine could ameliorate seizures that were elicited by astrogliosis [86], ruling out pharmacological manipulations that potentially may occur during seizure induction.

In summary, increased expression of system x_c^- and reduced expression of EAATs promote epileptic activity and seizures in peritumoral astrocytes and in temporal lobe

epilepsy. From zebrafish to humans, a decrease in astroglial EAAT activity promotes seizures. This raises the hypothesis that a system x_c^--driven increase in glutamate promotes seizures, while the EAAT-driven decrease in extracellular glutamate prevents seizures. In conclusion, epilepsy treatment might benefit from selectively targeting astrocytic glutamate transporters in a manner that promotes the activity of EAATs but decreases system x_c^- activity.

4. The Roles of Glutamate Transporters in Neurodegenerative Diseases and Multiple Sclerosis

Neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, is heavily impacting human society. Despite years of research efforts, our understanding of the pathophysiology of neurodegeneration is still ill-defined [87]. Moreover, up to now there is no cure for diseased patients. Amongst many cellular and molecular suspects, astrocytes have been implicated in neurodegeneration and in neuroprotection [88,89]. In the following, we specifically discuss the roles of system x_c^- and EAAT1/2 in Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinsonian disease.

4.1 Alzheimer's Disease

It is well established that excitotoxicity can lead to severe neuronal cell death in the central nervous system. In particular, excitotoxicity has been suspected to be also involved in Alzheimer's disease (AD) pathology [90,91], and the reduction of extracellular glutamate by targeting glutamate transporters could then prove beneficial for neuronal survival.

A study in post-mortem tissue from AD patients revealed an increased expression of the light-chain subunit of system x_c^- compared to age-matched controls [92]. In line with this, AD model AβPP23 mice displayed a stronger system x_c^- expression with aging [93]. These transgenic mice carry a human amyloid-β precursor protein with the so-called Swedish mutation, a double mutation near the β-secretase site. Adding the Alzheimer's disease-related peptide amyloid-β_{1-40} to neuron-glia-co-cultures amplified the transcription of system x_c^- and induced neurotoxicity [94]. Similarly, the peptide amyloid-β_{25-35} could evoke system x_c^- upregulation in human astroglial cells. This entailed neuronal cell death, which could be prevented by adding the system x_c^- inhibitor sulfasalazine [95]. Taken together, these studies indicate that in the course of the disease, system x_c^- becomes upregulated. AD patients displayed a reduction of EAAT1 and EAAT2 in the hippocampus or the medial frontal lobe, brain regions being affected in early disease stages [96]. Application of Amyloid-β_{1-42}, which is central to AD pathology [97] as a neurotoxic agent, induced a decrease in the expression of EAAT1 and EAAT2 in cultured astrocytes [98]. Using an elegant approach of *in vivo* two-photon microscopical detection of glutamate with iGluSnFR [99], Hefendehl *et al.* [100] have found that glutamate fluctuates and EAAT2 is downregulated in the vicinity of Amyloid-β plaques. Increasing the glutamate transporter EAAT2 expression by gain-of-function gene targeting [101,102] ameliorated this phenotype [100]. These data display that glutamate levels are normalized due to its increased import. In the transgenic AβPP23 mice, hippocampal expression of EAAT1 and EAAT2 were also decreased [93]. In conclusion, it can be stated that AD is accompanied by a decrease in astrocytic glutamate import capabilities.

Therefore, the question appears how the extracellular glutamate level develops in human AD patients. While magnetic resonance spectroscopy in the bilateral posterior cingulate gyrus showed less glutamate content in AD patients [103], analysis of the cerebrospinal fluid from patients with probable AD indicated that glutamate is more prevalent than in control individuals [104]. Given the discrepancy between AD research performed in mice and what could be translated to humans in the past [105,106], it would be important to further examine glutamate levels in human patients to receive consistent results that are based on standardized experimental conditions, i.e., at which disease stage glutamate is measured, in which brain region or fluid it is measured, and by which method. However, it appears that glutamate transporter disturbances occur over the course of the disease—with increased system x_c^- activity and impeded EAAT activity—and could be potentially suited to ameliorate symptoms in patients by selectively targeting them in astrocytes to not interfere with intrinsic neuronal function.

4.2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of motor neurons with the final stage of paralysis, whose pathogenesis is still unclear and treatment options are strongly limited to symptomatic approaches [107].

Recently, it was found for sporadic ALS patients, that system x_c^- in their astrocytes is significantly stronger expressed than in those of healthy subjects [108]. In the SOD-1 mutation model of ALS, and also in human samples, spinal cord-residing microglia expressed more system x_c^- than healthy controls [28]. Data from SOD-1 mutated mice also suggest that increased system x_c^- activity is a major contributor to excitotoxicity-mediated disease activity at early time points in ALS [109]. Interestingly, a cystine-rich dietary supplement in the transgenic hSOD1(G93A) mice has been shown to delay the onset of ALS symptoms, and survival was even extended when riluzole, a medication for ALS patients targeting glutamate release [110], was added to the dietary supplement [111]. This finding demonstrates that targeting both the oxidative stress and the glutamate effects proves beneficial for therapy. In summary, system x_c^- activity is increased in ALS.

In post-mortem samples of the spinal cord and motor cortex of ALS patients, glutamate importer EAAT2 was dramatically decreased [112]. The loss of function of EAAT2 during ALS has also been demonstrated experimentally on different levels: in an ALS rat model with SOD-1 mutation, glutamate transport was impaired [113] and EAAT2 expression was diminished [114]. In several studies performed in SOD-1 mutated mice, a reduced expression of EAAT2 in the spinal ccord was observed [115–119]. EAAT1, however, was not changed over the course of the disease in the motor cortex from ALS patients or in transgenic rats expressing the human SOD-1 mutant G93A [112,119]. Providing a potential therapeutical approach to the downregulation of glutamate importers, activation of metabotropic glutamate receptors has proven beneficial [120]. Based on all this knowledge and technological advances, in a recent study, EAAT2-based gene therapy in an ALS mouse model with SOD-1 mutation improved motor function and survival [121]. A cellular model of ALS with SOD-1 mutation showed that EAAT2 undergoes enhanced internalization and degradation, which limits its function to import glutamate [122]. An early study suggested that defects during the processing of EAAT2 mRNA is the cause for the lack of protein expression found in the aforementioned studies [123]. In contrast to EAAT2, alterations in EAAT1 do not play a major role in ALS [112,124]. Taken together, ALS is characterized by an increased expression of system x_c^-, a reduced expression of EAAT2, and rather unaffected levels of EAAT1, indicating that enhanced system x_c^- driven glutamate release and impaired EAAT2-mediated glutamate uptake contribute to ALS pathology. Both effects appear to occur as a consequence of the disease and are most probably not the causal agent. Targeting glutamate homeostasis may represent a beneficial option to ameliorate ALS symptoms, because neurodegeneration occuring due to excitotoxicity could be slowed down. Future studies are required to judge this hypothesis and to shed light on the definite impact of glutamate-driven excitotoxicity on the ALS disease course.

4.3 Parkinson's Disease

Parkinson's disease (PD) is a disease of the basal ganglia with strong motor signs that are the result of neurodegeneration [125]. In mice, PD can be pharmacologically modeled using substances such as substance 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), or the direct application of alpha-synuclein, which enables more detailed investigations of the underlying mechanism of neurodegenerative changes in PD [126].

After MPTP application to induce parkinsonism in mice, it was found that xCT expression was increased in the striatum, but reduced in the substantia nigra, however, MPTP could induce similar phenotypes in wild-type mice and mice with a system x_c^- deletion [127], indicating that

system x_c^- responds to neurodegeneration but does not affect its induction, at least in this case. Following acute MPTP application in mice, EAAT2 immunolabeling in the dorsolateral striatum decreased [128].

In 6-OHDA-lesioned parkinsonian mice, treatment with levetiracetam—upregulating system x_c^-—had neuroprotective effects in nigrostriatal dopaminergic neurons [129]. In another study, 6-OHDA treatment induced less neurodegeneration: a comparison of the substantia nigra pars compacta of $xCT^{-/-}$ mice with their wild-type littermates suggests that deletion of the glutamate extrusion system x_c^- provides protection against this form of neurodegeneration [130]. When 6-OHDA was applied to the bilateral substantia nigra pars compacta of rats, after two weeks EAAT1 expression decreased [131]. To counteract this decrease in the expression of glutamate importers, ceftriaxone was able to increase EAAT2 expression in the 6-OHDA model of Parkinsonian disease [132], similar to what was found in the MPTP model [133]. Furthermore, EAAT2 expression was increased inside the basal ganglia following the clinically relevant L-DOPA application [134].

When nigrostriatal lesions in mice were induced through the application of the proteasome inhibitor lactacystin, treatment with the anticonvulsant zonisamide ameliorated this phenotype without any modulation of system x_c^- [135], implicating that system x_c^- is neglectable in some cases of anti-neurodegenerative therapy. With age, system x_c^- deficient mice were less prone to this lactacystin-induced nigrostriatal degeneration [136].

After the application of alpha-synuclein onto astrocytes in cultures, or in a synucleinopathy mouse model, the expression levels of EAAT1 and EAAT2 were increased [137]. These findings indicate that the glutamate transporter system is responsive to the induction of the disease as well as the subsequent therapy, making glutamate homeostasis a promising target for the modulation of PD symptoms.

In an interesting and novel approach, PD was modeled in mice by directly targeting the EAATs. When EAAT2 expression was genetically abrogated in the substantia nigra pars compacta, mice developed PD-related phenotypes including cell death. These observations were associated with aberrant calcium signaling [138].

In summary, astroglial glutamate transporters—such as EAAT1/2 and system x_c^-—are dysregulated in diverse neurodegenerative diseases. They are implicated in the pathophysiology underlying those diseases, and they are responsive to treatment approaches. This underlines the importance of the sensitive regulation of extracellular glutamate for proper CNS function.

4.4 Multiple Slcerosis

In the previous paragraphs, we have highlighted the contribution of the glial glutamate transporters to CNS neurodegenerative diseases. Neurodegeneration is often

accompanied by neuroinflammatory components [139], which can be cell-mediated by microglia, the CNS resident immune cells [140], or also T cells [141]. As previously mentioned, microglia might express system x_c^- and thereby might contribute to pathological states [28,142]. We discuss the contribution of glial glutamate transporters system x_c^- and EAAT1/2 to neuroinflammation in more detail using multiple sclerosis (MS) as an example.

In the cerebrospinal fluid of MS patients, the glutamate level is increased [143], which raises the question of the underlying mechanism. To investigate MS in animal models, experimental autoimmune encephalomyelitis (EAE) can be induced to bring key features of MS into the model animal [144]. Investigations of human monocytes and EAE revealed an upregulation of system x_c^- compared to controls, potentially leading to excitotoxic insults [145]. When system x_c^- was inhibited by introducing a mutation in xCT, mice became resistant to the induction of EAE, indicating a strong role of system x_c^- in mediating disease in activity by acting on immune cells [146]. In another study, $xCT^{-/-}$ mice were as susceptible to EAE as control mice, but mice after bone marrow transplantation from $xCT^{-/-}$ mice—with therefore xCT-deficient immune cells—displayed attenuation of EAE [147]. Differences between both studies could have occurred through the usage of different antigens for immunization, however, the main finding of system x_c^- in EAE severity remains rather independent from the chosen protocol. The increased system x_c^- activity in EAE mice has been confirmed in ^{18}F-fluorodeoxyglucose PET scans [148]. In addition to solely investigating xCT, its molecular and cellular interplay is of particular importance. Interleukin 1β (IL1β) is an important factor in EAE and MS [149,150], which regulates system x_c^- mRNA and thereby may contribute to excitotoxicity [151]. The glutamate transporters system x_c^- and EAATs are involved in the microglial toxicity to oligodendrocytes [142], providing evidence for a complex interplay between different cell types that are involved in EAE/ MS.

In addition to the many studies that revealed increased system x_c^- activity in EAE, the protein expression of EAAT1/2 in MS patients was found to be decreased in the vicinity of cortical lesions [152]. EAE rats display an initial upregulation of EAAT1 and EAAT2 mRNAs, but respective proteins do not follow this pattern [153]. This has been confirmed in an independent EAE rat study [154]. In mouse spinal cord from EAE mice, EAAT2 protein is less expressed [155]. Glutamate transporters are not affected in all neuroinflammatory models, though: in contrast to results obtained from EAE, infection with Theiler's murine encephalomyelitis virus (TMEV) did not modulate system x_c^- expression [127].

EAAT1/2 is decreased, while system x_c^- is positively associated with MS, and system x_c^- inhibition has been proven to be beneficial for disease activity. These findings highlight the important role of astrocytes in the regulation of glutamate homeostasis through plasticity of transporter expressions during neuroinflammation.

5. Conclusions

In this review we brigded the experimental data with clinical data on glial glutamate transporters system x_c^- and EAAT1/2. We present evidence for the described plasticity as a consequence of the disease pathology as well as part of the pathogenic process. We took a closer look at (tumor-associated) epilepsy, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis.

Under physiological conditions, the astroglial glutamate transporters system x_c^- and EAAT1/2 control neuronal function through pre- and postsynaptic modulation of synaptic transmission.

For epileptic seizures induced by adjacent tumor tissue, an aberrant glutamate transporter activity appears as a causative. In contrast, the resulting glutamate levels in epileptic patients with non-tumor related epileptic disorders are yet unclear. It would be interesting to take a further look at the many different pharmacological models that are used to induce epileptic seizures with many underlying signaling cascades.

The evidence presented in Alzheimer's disease showed that glial glutamate transporter plasticity can be directly induced via Amyloid-β-induced changes.

In ALS, studies showed how restoring glutamate transporter works, strongly indicates their pathogenetic role since those interventions proved beneficial to ameliorate symptoms and prolong survival. Similarly, the role of glutamate transporters in Parkinson's disease may be causative for the disease since treatment approaches directly to these transporters were beneficial for the disease outcome. Since multiple sclerosis is the interplay of many cell types and molecular events that are not fully understood yet, it is more difficult to categorize changes in glutamate transporters as causative or consequential.

The role of glial glutamate transporter-mediated plasticity is still ambiguous. The relevance of these proteins in some brain diseases is increasing and worth for further studies. Approaches addressing the selectivity of inducers or inhibitors to glutamate transporters could further deepen our knowledge of the pathophysiological processes of these brain disorders in order to improve treatment management.

Abbreviations

AβPP23, amyloid-protein precursor 23 gene; ALS, amyotrophic lateral sclerosis; CA, cornu ammonis; CN-Pase, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; CNS, central nervous system; EAAT1/2, excitatory amino acid transporters 1 and 2; EAE, experimental autoimmune encephalomyelitis; EPSC, excitatory postsynaptic currents; FGF-2, fibroblast growth factor 2; GLAST, glutamate aspartate transporter 1; GLT-1, glutamate transporter-1; GSH, glutathione; Iba-1, ionized calcium binding adap-

tor molecule 1; IL 1β, Interleukin-1 beta; L-DOPA, l-3,4-dihydroxyphenylalanine; mGluR1, metabotropic glutamate receptor 1; MPTP, 1 methyl 4 phenyl 1,2,3,6-tetrahydropyridine; mRNA, messenger ribonucleic acid; MS, multiple sclerosis; NMDA receptor, N-methyl-D-aspartate receptor; 6-OHDA, 6-hydroxydopamine; PET, positron emission tomography; qPCR, quantitative polymerase chain reaction; RRP, readily releasable pool; SLC1A2, solute carrier family 1 member 2; SLC1A3, solute carrier family 1 member 3; SLC7A11, solute carrier family 7 member 11; SOD-1, superoxide dismutase 1; TMEV, Theiler's murine encephalomyelitis virus; TTX, tetrodotoxin; xCT, cystine/glutamate antiporter; WHO, world health organization.

Author Contributions

EY, NS and MD designed the study. EY, JKD and MD collected and analyzed the literatures. EY, HHS, JKD, MD and NS wrote, read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Funding

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. Trends in Neurosciences. 2009; 32: 421–431.

[2] Mahmoud S, Gharagozloo M, Simard C, Gris D. Astrocytes Maintain Glutamate Homeostasis in the CNS by Controlling the Balance between Glutamate Uptake and Release. Cells. 2019; 8: 184.

[3] Kraft AD, Harry GJ. Features of microglia and neuroinflammation relevant to environmental exposure and neurotoxicity. International Journal of Environmental Research and Public Health. 2011; 8: 2980–3018.

[4] Simons M, Nave KA. Oligodendrocytes: Myelination and Axonal Support. Cold Spring Harbor Perspectives in Biology. 2015; 8: a020479.

[5] Zhou Y, Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. Journal of Neural Transmission. 2014; 121: 799–817.

[6] Lau D, Bengtson CP, Buchthal B, Bading H. BDNF Reduces Toxic Extrasynaptic NMDA Receptor Signaling via Synaptic NMDA Receptors and Nuclear-Calcium-Induced Transcription of inhba/Activin A. Cell Reports. 2015; 12: 1353–1366.

[7] Zhou X, Chen Z, Yun W, Ren J, Li C, Wang H. Extrasynaptic NMDA Receptor in Excitotoxicity: Function Revisited. The Neuroscientist. 2015; 21: 337–344.

[8] Parsons MP, Raymond LA. Extrasynaptic NMDA receptor involvement in central nervous system disorders. Neuron. 2014; 82: 279–293.

[9] Ottestad-Hansen S, Hu QX, Follin-Arbelet VV, Bentea E, Sato H, Massie A, *et al*. The cystine-glutamate exchanger (xCT, Slc7a11) is expressed in significant concentrations in a subpopulation of astrocytes in the mouse brain. Glia. 2018; 66: 951–970.

[10] La Bella V, Valentino F, Piccoli T, Piccoli F. Expression and developmental regulation of the cystine/glutamate exchanger (xc-) in the rat. Neurochemical Research. 2007; 32: 1081–1090.

[11] Souza DG, Bellaver B, Raupp GS, Souza DO, Quincozes-Santos A. Astrocytes from adult Wistar rats aged *in vitro* show changes in glial functions. Neurochemistry International. 2015; 90: 93–97.

[12] Vandenberg RJ, Ryan RM. Mechanisms of glutamate transport. Physiological Reviews. 2013; 93: 1621–1657.

[13] Jensen AA, Fahlke C, Bjørn-Yoshimoto WE, Bunch L. Excitatory amino acid transporters: recent insights into molecular mechanisms, novel modes of modulation and new therapeutic possibilities. Current Opinion in Pharmacology. 2015; 20: 116–123.

[14] Beart PM, O'Shea RD. Transporters for L-glutamate: an update on their molecular pharmacology and pathological involvement. British Journal of Pharmacology. 2007; 150: 5–17.

[15] Lewerenz J, Maher P, Methner A. Regulation of xCT expression and system x (c) (-) function in neuronal cells. Amino Acids. 2012; 42: 171–179.

[16] Bridges RJ, Natale NR, Patel SA. System xc⁻ cystine/glutamate antiporter: an update on molecular pharmacology and roles within the CNS. British Journal of Pharmacology. 2012; 165: 20–34.

[17] Shih AY, Erb H, Sun X, Toda S, Kalivas PW, Murphy TH. Cystine/glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation. The Journal of Neuroscience. 2006; 26: 10514–10523.

[18] Seib TM, Patel SA, Bridges RJ. Regulation of the system x(C)-cystine/glutamate exchanger by intracellular glutathione levels in rat astrocyte primary cultures. Glia. 2011; 59: 1387–1401.

[19] De Bundel D, Schallier A, Loyens E, Fernando R, Miyashita H, Van Liefferinge J, *et al*. Loss of system x(c)- does not induce oxidative stress but decreases extracellular glutamate in hippocampus and influences spatial working memory and limbic seizure susceptibility. The Journal of Neuroscience. 2011; 31: 5792–5803.

[20] Salim S. Oxidative Stress and the Central Nervous System. The Journal of Pharmacology and Experimental Therapeutics. 2017; 360: 201–205.

[21] Wang X, Michaelis EK. Selective neuronal vulnerability to oxidative stress in the brain. Frontiers in Aging Neuroscience. 2010; 2: 12.

[22] Liu J, Xia X, Huang P. xCT: A Critical Molecule That Links Cancer Metabolism to Redox Signaling. Molecular Therapy. 2020; 28: 2358–2366.

[23] Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, *et al*. Ferroptosis: an iron-dependent form of non-apoptotic cell death. Cell. 2012; 149: 1060–1072.

[24] Dahlmanns M, Yakubov E, Dahlmanns JK. Genetic Profiles of Ferroptosis in Malignant Brain Tumors and Off-Target Effects of Ferroptosis Induction. Frontiers in Oncology. 2021; 11: 783067.

[25] Chen L, Li X, Liu L, Yu B, Xue Y, Liu Y. Erastin sensitizes glioblastoma cells to temozolomide by restraining xCT and

cystathionine-γ-lyase function. Oncology Reports. 2015; 33: 1465–1474.

[26] Soria FN, Zabala A, Pampliega O, Palomino A, Miguelez C, Ugedo L, *et al*. Cystine/glutamate antiporter blockage induces myelin degeneration. Glia. 2016; 64: 1381–1395.

[27] Burdo J, Dargusch R, Schubert D. Distribution of the cystine/glutamate antiporter system xc- in the brain, kidney, and duodenum. The Journal of Histochemistry and Cytochemistry. 2006; 54: 549–557.

[28] Mesci P, Zaïdi S, Lobsiger CS, Millecamps S, Escartin C, Seilhean D, *et al*. System xC- is a mediator of microglial function and its deletion slows symptoms in amyotrophic lateral sclerosis mice. Brain. 2015; 138: 53–68.

[29] Van Liefferinge J, Bentea E, Demuyser T, Albertini G, Follin-Arbelet V, Holmseth S, *et al*. Comparative analysis of antibodies to xCT (Slc7a11): Forewarned is forearmed. The Journal of Comparative Neurology. 2016; 524: 1015–1032.

[30] Sato H, Tamba M, Kuriyama-Matsumura K, Okuno S, Bannai S. Molecular cloning and expression of human xCT, the light chain of amino acid transport system xc-. Antioxidants & Redox Signaling. 2000; 2: 665–671.

[31] Sato H, Tamba M, Okuno S, Sato K, Keino-Masu K, Masu M, *et al*. Distribution of cystine/glutamate exchange transporter, system x(c)-, in the mouse brain. The Journal of Neuroscience. 2002; 22: 8028–8033.

[32] Rosenmund C, Stevens CF. Definition of the readily releasable pool of vesicles at hippocampal synapses. Neuron. 1996; 16: 1197–1207.

[33] Moran MM, Melendez R, Baker D, Kalivas PW, Seamans JK. Cystine/glutamate antiporter regulation of vesicular glutamate release. Annals of the New York Academy of Sciences. 2003; 1003: 445–447.

[34] Thomsen C, Boel E, Suzdak PD. Actions of phenylglycine analogs at subtypes of the metabotropic glutamate receptor family. European Journal of Pharmacology. 1994; 267: 77–84.

[35] Zhang XL, Zhou ZY, Winterer J, Müller W, Stanton PK. NMDA-dependent, but not group I metabotropic glutamate receptor-dependent, long-term depression at Schaffer collateral-CA1 synapses is associated with long-term reduction of release from the rapidly recycling presynaptic vesicle pool. The Journal of Neuroscience. 2006; 26: 10270–10280.

[36] Crupi R, Impellizzeri D, Cuzzocrea S. Role of Metabotropic Glutamate Receptors in Neurological Disorders. Frontiers in Molecular Neuroscience. 2019; 12: 20.

[37] Zakharenko SS, Zablow L, Siegelbaum SA. Altered presynaptic vesicle release and cycling during mGluR-dependent LTD. Neuron. 2002; 35: 1099–1110.

[38] Upreti C, Zhang XL, Alford S, Stanton PK. Role of presynaptic metabotropic glutamate receptors in the induction of long-term synaptic plasticity of vesicular release. Neuropharmacology. 2013; 66: 31–39.

[39] Alcoreza O, Tewari BP, Bouslog A, Savoia A, Sontheimer H, Campbell SL. Sulfasalazine decreases mouse cortical hyperexcitability. Epilepsia. 2019; 60: 1365–1377.

[40] Dahlmanns M, Yakubov E, Chen D, Sehm T, Rauh M, Savaskan N, *et al*. Chemotherapeutic xCT inhibitors sorafenib and erastin unraveled with the synaptic optogenetic function analysis tool. Cell Death Discovery. 2017; 3: 17030.

[41] Dixon SJ, Patel DN, Welsch M, Skouta R, Lee ED, Hayano M, *et al*. Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. eLife. 2014; 3: e02523.

[42] McCloskey DP, Croll SD, Scharfman HE. Depression of synaptic transmission by vascular endothelial growth factor in adult rat hippocampus and evidence for increased efficacy after chronic seizures. The Journal of Neuroscience. 2005; 25: 8889–8897.

[43] Augustin H, Grosjean Y, Chen K, Sheng Q, Featherstone DE. Nonvesicular release of glutamate by glial xCT transporters suppresses glutamate receptor clustering *in vivo*. The Journal of Neuroscience. 2007; 27: 111–123.

[44] Williams LE, Featherstone DE. Regulation of hippocampal synaptic strength by glial xCT. The Journal of Neuroscience. 2014; 34: 16093–16102.

[45] Li Y, Tan Z, Li Z, Sun Z, Duan S, Li W. Impaired long-term potentiation and long-term memory deficits in xCT-deficient sut mice. Bioscience Reports. 2012; 32: 315–321.

[46] McCullagh EA, Featherstone DE. Behavioral characterization of system xc- mutant mice. Behavioural Brain Research. 2014; 265: 1–11.

[47] Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE. Astrocyte glutamate transporters regulate metabotropic glutamate receptor-mediated excitation of hippocampal interneurons. The Journal of Neuroscience. 2004; 24: 4551–4559.

[48] Loewen JL, Albertini G, Dahle EJ, Sato H, Smolders IJ, Massie A, *et al*. Genetic and pharmacological manipulation of glial glutamate transporters does not alter infection-induced seizure activity. Experimental Neurology. 2019; 318: 50–60.

[49] Hanson E, Armbruster M, Cantu D, Andresen L, Taylor A, Danbolt NC, *et al*. Astrocytic glutamate uptake is slow and does not limit neuronal NMDA receptor activation in the neonatal neocortex. Glia. 2015; 63: 1784–1796.

[50] Petr GT, Sun Y, Frederick NM, Zhou Y, Dhamne SC, Hameed MQ, *et al*. Conditional deletion of the glutamate transporter GLT-1 reveals that astrocytic GLT-1 protects against fatal epilepsy while neuronal GLT-1 contributes significantly to glutamate uptake into synaptosomes. The Journal of Neuroscience. 2015; 35: 5187–5201.

[51] Sharma A, Kazim SF, Larson CS, Ramakrishnan A, Gray JD, McEwen BS, *et al*. Divergent roles of astrocytic versus neuronal EAAT2 deficiency on cognition and overlap with aging and Alzheimer's molecular signatures. Proceedings of the National Academy of Sciences of the United States of America. 2019; 116: 21800–21811.

[52] Dossi E, Vasile F, Rouach N. Human astrocytes in the diseased brain. Brain Research Bulletin. 2018; 136: 139–156.

[53] Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee SU. Glioblastoma Multiforme: A Review of its Epidemiology and Pathogenesis through Clinical Presentation and Treatment. Asian Pacific Journal of Cancer Prevention. 2017; 18: 3–9.

[54] Yakubov E, Eibl T, Hammer A, Holtmannspötter M, Savaskan N, Steiner HH. Therapeutic Potential of Selenium in Glioblastoma. Frontiers in Neuroscience. 2021; 15: 666679.

[55] Takeuchi S, Wada K, Toyooka T, Shinomiya N, Shimazaki H, Nakanishi K, *et al*. Increased xCT expression correlates with tumor invasion and outcome in patients with glioblastomas. Neurosurgery. 2013; 72: 33–41; discussion 41.

[56] Robert SM, Buckingham SC, Campbell SL, Robel S, Holt KT, Ogunrinu-Babarinde T, *et al*. SLC7A11 expression is associated with seizures and predicts poor survival in patients with malignant glioma. Science Translational Medicine. 2015; 7: 289ra86.

[57] Savaskan NE, Heckel A, Hahnen E, Engelhorn T, Doerfler A, Ganslandt O, *et al*. Small interfering RNA-mediated xCT silencing in gliomas inhibits neurodegeneration and alleviates brain edema. Nature Medicine. 2008; 14: 629–632.

[58] Savaskan NE, Eyüpoglu IY. xCT modulation in gliomas: relevance to energy metabolism and tumor microenvironment normalization. Annals of Anatomy. 2010; 192: 309–313.

[59] Ye ZC, Rothstein JD, Sontheimer H. Compromised glutamate transport in human glioma cells: reduction-mislocalization of sodium-dependent glutamate transporters and enhanced activity of cystine-glutamate exchange. The Journal of Neuroscience. 1999; 19: 10767–10777.

[60] Sattler R, Tyler B, Hoover B, Coddington LT, Recinos V, Hwang L, et al. Increased expression of glutamate transporter GLT-1 in peritumoral tissue associated with prolonged survival and decreases in tumor growth in a rat model of experimental malignant glioma. Journal of Neurosurgery. 2013; 119: 878–886.

[61] Donmez Yalcin G, Oktay E, Yalcin A, Diniz G, Kahraman DS, Senoglu M. Glutamate Transporter 1 expression in human glioblastomas. Journal of Balkan Union of Oncology. 2020; 25: 2051–2058.

[62] de Groot JF, Liu TJ, Fuller G, Yung WKA. The excitatory amino acid transporter-2 induces apoptosis and decreases glioma growth in vitro and in vivo. Cancer Research. 2005; 65: 1934–1940.

[63] Yi H, Talmon G, Wang J. Glutamate in cancers: from metabolism to signaling. Journal of Biomedical Research. 2019; 34: 260–270.

[64] Krawczyk MC, Haney JR, Pan L, Caneda C, Khankan RR, Reyes SD, et al. Human Astrocytes Exhibit Tumor Microenvironment-, Age-, and Sex-Related Transcriptomic Signatures. The Journal of Neuroscience. 2022; 42: 1587–1603.

[65] Buckingham SC, Campbell SL, Haas BR, Montana V, Robel S, Ogunrinu T, et al. Glutamate release by primary brain tumors induces epileptic activity. Nature Medicine. 2011; 17: 1269–1274.

[66] Zhou XW, Wang X, Yang Y, Luo JW, Dong H, Liu YH, et al. Biomarkers related with seizure risk in glioma patients: A systematic review. Clinical Neurology and Neurosurgery. 2016; 151: 113–119.

[67] Sørensen MF, Heimisdóttir SB, Sørensen MD, Mellegaard CS, Wohlleben H, Kristensen BW, et al. High expression of cystine-glutamate antiporter xCT (SLC7A11) is an independent biomarker for epileptic seizures at diagnosis in glioma. Journal of Neuro-Oncology. 2018; 138: 49–53.

[68] Huberfeld G, Vecht CJ. Seizures and gliomas–towards a single therapeutic approach. Nature Reviews. Neurology. 2016; 12: 204–216.

[69] Castro-Torres RD, Ureña-Guerrero ME, Morales-Chacón LM, Lorigados-Pedre L, Estupiñan-Díaz B, Rocha L, et al. New Aspects of VEGF, GABA, and Glutamate Signaling in the Neocortex of Human Temporal Lobe Pharmacoresistant Epilepsy Revealed by RT-qPCR Arrays. Journal of Molecular Neuroscience. 2020; 70: 916–929.

[70] Kirschner MA, Copeland NG, Gilbert DJ, Jenkins NA, Amara SG. Mouse excitatory amino acid transporter EAAT2: isolation, characterization, and proximity to neuroexcitability loci on mouse chromosome 2. Genomics. 1994; 24: 218–224.

[71] Proper EA, Hoogland G, Kappen SM, Jansen GH, Rensen MGA, Schrama LH, et al. Distribution of glutamate transporters in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. Brain. 2002; 125: 32–43.

[72] Rakhade SN, Loeb JA. Focal reduction of neuronal glutamate transporters in human neocortical epilepsy. Epilepsia. 2008; 49: 226–236.

[73] Sarac S, Afzal S, Broholm H, Madsen FF, Ploug T, Laursen H. Excitatory amino acid transporters EAAT-1 and EAAT-2 in temporal lobe and hippocampus in intractable temporal lobe epilepsy. Acta Pathologica, Microbiologica, et Immunologica Scandinavica. 2009; 117: 291–301.

[74] Ulu MO, Tanriverdi T, Oz B, Biceroglu H, Isler C, Eraslan BS, et al. The expression of astroglial glutamate transporters in patients with focal cortical dysplasia: an immunohistochemical study. Acta Neurochirurgica. 2010; 152: 845–853.

[75] Sarlo GL, Holton KF. Brain concentrations of glutamate and GABA in human epilepsy: A review. Seizure. 2021; 91: 213–227.

[76] Barker-Haliski M, White HS. Glutamatergic Mechanisms Associated with Seizures and Epilepsy. Cold Spring Harbor Perspec-

[77] Curia G, Longo D, Biagini G, Jones RSG, Avoli M. The pilocarpine model of temporal lobe epilepsy. Journal of Neuroscience Methods. 2008; 172: 143–157.

[78] Lévesque M, Avoli M. The kainic acid model of temporal lobe epilepsy. Neuroscience and Biobehavioral Reviews. 2013; 37: 2887–2899.

[79] Claudio OI, Ferchmin P, Velísek L, Sperber EF, Moshé SL, Ortiz JG. Plasticity of excitatory amino acid transporters in experimental epilepsy. Epilepsia. 2000; 41: S104–S110.

[80] Molinari F, Cattani AA, Mdzomba JB, Aniksztejn L. Glutamate transporters control metabotropic glutamate receptors activation to prevent the genesis of paroxysmal burst in the developing hippocampus. Neuroscience. 2012; 207: 25–36.

[81] Hotz AL, Jamali A, Rieser NN, Niklaus S, Aydin E, Myren-Svelstad S, et al. Loss of glutamate transporter eaat2a leads to aberrant neuronal excitability, recurrent epileptic seizures, and basal hypoactivity. Glia. 2022; 70: 196–214.

[82] Leclercq K, Liefferinge JV, Albertini G, Neveux M, Dardenne S, Mairet-Coello G, et al. Anticonvulsant and antiepileptogenic effects of system xc- inactivation in chronic epilepsy models. Epilepsia. 2019; 60: 1412–1423.

[83] Sears SMS, Hewett JA, Hewett SJ. Decreased epileptogenesis in mice lacking the System $x_c{}^-$ transporter occurs in association with a reduction in AMPA receptor subunit GluA1. Epilepsia Open. 2019; 4: 133–143.

[84] Dhir A. Pentylenetetrazol (PTZ) kindling model of epilepsy. Current Protocols in Neuroscience. 2012; Chapter 9: Unit9.37.

[85] Sears SMS, Roberts SH, Hewett SJ. Hyperexcitability and brain morphological differences in mice lacking the cystine/glutamate antiporter, system x_c. Journal of Neuroscience Research. 2021; 99: 3339–3353.

[86] Alcoreza O, Jagarlamudi S, Savoia A, Campbell SL, Sontheimer H. Sulfasalazine decreases astrogliosis-mediated seizure burden. Epilepsia. 2022; 63: 844–854.

[87] Golde TE. The therapeutic importance of understanding mechanisms of neuronal cell death in neurodegenerative disease. Molecular Neurodegeneration. 2009; 4: 8.

[88] Maragakis NJ, Rothstein JD. Mechanisms of Disease: astrocytes in neurodegenerative disease. Nature Clinical Practice. Neurology. 2006; 2: 679–689.

[89] Phatnani H, Maniatis T. Astrocytes in neurodegenerative disease. Cold Spring Harbor Perspectives in Biology. 2015; 7: a020628.

[90] Hynd MR, Scott HL, Dodd PR. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. Neurochemistry International. 2004; 45: 583–595.

[91] Wang R, Reddy PH. Role of Glutamate and NMDA Receptors in Alzheimer's Disease. Journal of Alzheimer's Disease. 2017; 57: 1041–1048.

[92] Ashraf A, Jeandriens J, Parkes HG, So PW. Iron dyshomeostasis, lipid peroxidation and perturbed expression of cystine/glutamate antiporter in Alzheimer's disease: Evidence of ferroptosis. Redox Biology. 2020; 32: 101494.

[93] Schallier A, Smolders I, Van Dam D, Loyens E, De Deyn PP, Michotte A, et al. Region- and age-specific changes in glutamate transport in the AβPP23 mouse model for Alzheimer's disease. Journal of Alzheimer's Disease. 2011; 24: 287–300.

[94] Qin S, Colin C, Hinners I, Gervais A, Cheret C, Mallat M. System Xc- and apolipoprotein E expressed by microglia have opposite effects on the neurotoxicity of amyloid-beta peptide 1-40. The Journal of Neuroscience. 2006; 26: 3345–3356.

[95] D'Ezio V, Colasanti M, Persichini T. Amyloid-β 25-35 Induces Neurotoxicity through the Up-Regulation of Astrocytic System X$_c$. Antioxidants. 2021; 10: 1685.

[96] Jacob CP, Koutsilieri E, Bartl J, Neuen-Jacob E, Arzberger T,

Zander N, *et al*. Alterations in expression of glutamatergic transporters and receptors in sporadic Alzheimer's disease. Journal of Alzheimer's Disease. 2007; 11: 97–116.

[97] Butterfield DA. Amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer's disease brain. A review. Free Radical Research. 2002; 36: 1307–1313.

[98] Tong H, Zhang X, Meng X, Xu P, Zou X, Qu S. Amyloid-beta peptide decreases expression and function of glutamate transporters in nervous system cells. The International Journal of Biochemistry & Cell Biology. 2017; 85: 75–84.

[99] Dürst CD, Oertner TG. Imaging Synaptic Glutamate Release with Two-Photon Microscopy in Organotypic Slice Cultures. Methods in Molecular Biology. 2022; 2417: 205–219.

[100] Hefendehl JK, LeDue J, Ko RWY, Mahler J, Murphy TH, MacVicar BA. Mapping synaptic glutamate transporter dysfunction in vivo to regions surrounding Aβ plaques by iGluSnFR two-photon imaging. Nature Communications. 2016; 7: 13441.

[101] Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, *et al*. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature. 2005; 433: 73–77.

[102] Smaga I, Fierro D, Mesa J, Filip M, Knackstedt LA. Molecular changes evoked by the beta-lactam antibiotic ceftriaxone across rodent models of substance use disorder and neurological disease. Neuroscience and Biobehavioral Reviews. 2020; 115: 116–130.

[103] Fayed N, Modrego PJ, Rojas-Salinas G, Aguilar K. Brain glutamate levels are decreased in Alzheimer's disease: a magnetic resonance spectroscopy study. American Journal of Alzheimer's Disease and other Dementias. 2011; 26: 450–456.

[104] Madeira C, Vargas-Lopes C, Brandão CO, Reis T, Laks J, Panizzutti R, *et al*. Elevated Glutamate and Glutamine Levels in the Cerebrospinal Fluid of Patients With Probable Alzheimer's Disease and Depression. Frontiers in Psychiatry. 2018; 9: 561.

[105] Myers A, McGonigle P. Overview of Transgenic Mouse Models for Alzheimer's Disease. Current Protocols in Neuroscience. 2019; 89: e81.

[106] Drummond E, Wisniewski T. Alzheimer's disease: experimental models and reality. Acta Neuropathologica. 2017; 133: 155–175.

[107] Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, *et al*. Amyotrophic lateral sclerosis. Nature Reviews. Disease Primers. 2017; 3: 17071.

[108] Kazama M, Kato Y, Kakita A, Noguchi N, Urano Y, Masui K, *et al*. Astrocytes release glutamate via cystine/glutamate antiporter upregulated in response to increased oxidative stress related to sporadic amyotrophic lateral sclerosis. Neuropathology. 2020; 40: 587–598.

[109] Albano R, Liu X, Lobner D. Regulation of system x(c)- in the SOD1-G93A mouse model of ALS. Experimental Neurology. 2013; 250: 69–73.

[110] Kretschmer BD, Kratzer U, Schmidt WJ. Riluzole, a glutamate release inhibitor, and motor behavior. Naunyn-Schmiedeberg's Archives of Pharmacology. 1998; 358: 181–190.

[111] Ross EK, Winter AN, Wilkins HM, Sumner WA, Duval N, Patterson D, *et al*. A Cystine-Rich Whey Supplement (Immunocal(®)) Delays Disease Onset and Prevents Spinal Cord Glutathione Depletion in the hSOD1(G93A) Mouse Model of Amyotrophic Lateral Sclerosis. Antioxidants. 2014; 3: 843–865.

[112] Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Annals of Neurology. 1995; 38: 73–84.

[113] Dunlop J, Beal McIlvain H, She Y, Howland DS. Impaired spinal cord glutamate transport capacity and reduced sensitivity to riluzole in a transgenic superoxide dismutase mutant rat model of amyotrophic lateral sclerosis. The Journal of Neuroscience. 2003; 23: 1688–1696.

[114] Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, *et al*. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). Proceedings of the National Academy of Sciences of the United States of America. 2002; 99: 1604–1609.

[115] Yin HZ, Tang DT, Weiss JH. Intrathecal infusion of a Ca(2+)-permeable AMPA channel blocker slows loss of both motor neurons and of the astrocyte glutamate transporter, GLT-1 in a mutant SOD1 rat model of ALS. Experimental Neurology. 2007; 207: 177–185.

[116] Guo Y, Duan W, Li Z, Huang J, Yin Y, Zhang K, *et al*. Decreased GLT-1 and increased SOD1 and HO-1 expression in astrocytes contribute to lumbar spinal cord vulnerability of SOD1-G93A transgenic mice. FEBS Letters. 2010; 584: 1615–1622.

[117] Bendotti C, Tortarolo M, Suchak SK, Calvaresi N, Carvelli L, Bastone A, *et al*. Transgenic SOD1 G93A mice develop reduced GLT-1 in spinal cord without alterations in cerebrospinal fluid glutamate levels. Journal of Neurochemistry. 2001; 79: 737–746.

[118] Ohgomori T, Yamasaki R, Takeuchi H, Kadomatsu K, Kira JI, Jinno S. Differential involvement of vesicular and glial glutamate transporters around spinal α-motoneurons in the pathogenesis of SOD1^{G93A} mouse model of amyotrophic lateral sclerosis. Neuroscience. 2017; 356: 114–124.

[119] Dumont AO, Hermans E, Goursaud S. Differential regulation of the glutamate transporter variants GLT-1a and GLT-1b in the cortex and spinal cord of transgenic rats expressing hSOD1(G93A). Neurochemistry International. 2013; 63: 61–68.

[120] Battaglia G, Riozzi B, Bucci D, Di Menna L, Molinaro G, Pallottino S, *et al*. Activation of mGlu3 metabotropic glutamate receptors enhances GDNF and GLT-1 formation in the spinal cord and rescues motor neurons in the SOD-1 mouse model of amyotrophic lateral sclerosis. Neurobiology of Disease. 2015; 74: 126–136.

[121] Ohashi N, Terashima T, Katagi M, Nakae Y, Okano J, Suzuki Y, *et al*. GLT1 gene delivery based on bone marrow-derived cells ameliorates motor function and survival in a mouse model of ALS. Scientific Reports. 2021; 11: 12803.

[122] Vanoni C, Massari S, Losa M, Carrega P, Perego C, Conforti L, *et al*. Increased internalisation and degradation of GLT-1 glial glutamate transporter in a cell model for familial amyotrophic lateral sclerosis (ALS). Journal of Cell Science. 2004; 117: 5417–5426.

[123] Lin CL, Bristol LA, Jin L, Dykes-Hoberg M, Crawford T, Clawson L, *et al*. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. Neuron. 1998; 20: 589–602.

[124] Sasaki S, Komori T, Iwata M. Excitatory amino acid transporter 1 and 2 immunoreactivity in the spinal cord in amyotrophic lateral sclerosis. Acta Neuropathologica. 2000; 100: 138–144.

[125] Kalia LV, Lang AE. Parkinson's disease. Lancet. 2015; 386: 896–912.

[126] Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron. 2003; 39: 889–909.

[127] Merckx E, Demuyser T, Bentea E, Van Liefferinge J, Albertini G, Deneyer L, *et al*. Lack of effect of Theiler's murine encephalomyelitis virus infection on system xc⁻. Neuroscience Letters. 2015; 593: 124–128.

[128] Holmer HK, Keyghobadi M, Moore C, Meshul CK. l-dopa-induced reversal in striatal glutamate following partial deple-

tion of nigrostriatal dopamine with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Neuroscience. 2005; 136: 333–341.

[129] Miyazaki I, Murakami S, Torigoe N, Kitamura Y, Asanuma M. Neuroprotective effects of levetiracetam target xCT in astrocytes in parkinsonian mice. Journal of Neurochemistry. 2016; 136: 194–204.

[130] Massie A, Schallier A, Kim SW, Fernando R, Kobayashi S, Beck H, et al. Dopaminergic neurons of system x(c)⁻-deficient mice are highly protected against 6-hydroxydopamine-induced toxicity. FASEB Journal. 2011; 25: 1359–1369.

[131] El Arfani A, Albertini G, Bentea E, Demuyser T, Van Eeckhaut A, Smolders I, et al. Alterations in the motor cortical and striatal glutamatergic system and D-serine levels in the bilateral 6-hydroxydopamine rat model for Parkinson's disease. Neurochemistry International. 2015; 88: 88–96.

[132] Chotibut T, Davis RW, Arnold JC, Frenchek Z, Gurwara S, Bondada V, et al. Ceftriaxone increases glutamate uptake and reduces striatal tyrosine hydroxylase loss in 6-OHDA Parkinson's model. Molecular Neurobiology. 2014; 49: 1282–1292.

[133] Hsu CY, Hung CS, Chang HM, Liao WC, Ho SC, Ho YJ. Ceftriaxone prevents and reverses behavioral and neuronal deficits in an MPTP-induced animal model of Parkinson's disease dementia. Neuropharmacology. 2015; 91: 43–56.

[134] Robelet S, Melon C, Guillet B, Salin P, Kerkerian-Le Goff L. Chronic L-DOPA treatment increases extracellular glutamate levels and GLT1 expression in the basal ganglia in a rat model of Parkinson's disease. The European Journal of Neuroscience. 2004; 20: 1255–1266.

[135] Bentea E, Van Liefferinge J, Verbruggen L, Martens K, Kobayashi S, Deneyer L, et al. Zonisamide attenuates lactacystin-induced parkinsonism in mice without affecting system x_c. Experimental Neurology. 2017; 290: 15–28.

[136] Bentea E, De Pauw L, Verbruggen L, Winfrey LC, Deneyer L, Moore C, et al. Aged xCT-Deficient Mice Are Less Susceptible for Lactacystin-, but Not 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-, Induced Degeneration of the Nigrostriatal Pathway. Frontiers in Cellular Neuroscience. 2021; 15: 796635.

[137] Diniz LP, Araujo APB, Matias I, Garcia MN, Barros-Aragão FGQ, de Melo Reis RA, et al. Astrocyte glutamate transporters are increased in an early sporadic model of synucleinopathy. Neurochemistry International. 2020; 138: 104758.

[138] Zhang Y, Meng X, Jiao Z, Liu Y, Zhang X, Qu S. Generation of a Novel Mouse Model of Parkinson's Disease via Targeted Knockdown of Glutamate Transporter GLT-1 in the Substantia Nigra. ACS Chemical Neuroscience. 2020; 11: 406–417.

[139] Kempuraj D, Thangavel R, Natteru PA, Selvakumar GP, Saeed D, Zahoor H, et al. Neuroinflammation Induces Neurodegeneration. Journal of Neurology, Neurosurgery and Spine. 2016; 1: 1003.

[140] Streit WJ, Mrak RE, Griffin WST. Microglia and neuroinflammation: a pathological perspective. Journal of Neuroinflammation. 2004; 1: 14.

[141] Schetters STT, Gomez-Nicola D, Garcia-Vallejo JJ, Van Kooyk Y. Neuroinflammation: Microglia and T Cells Get Ready to Tango. Frontiers in Immunology. 2018; 8: 1905.

[142] Domercq M, Sánchez-Gómez MV, Sherwin C, Etxebarria E, Fern R, Matute C. System xc- and glutamate transporter inhibition mediates microglial toxicity to oligodendrocytes. Journal of Immunology. 2007; 178: 6549–6556.

[143] Sarchielli P, Greco L, Floridi A, Floridi A, Gallai V. Excitatory amino acids and multiple sclerosis: evidence from cerebrospinal fluid. Archives of Neurology. 2003; 60: 1082–1088.

[144] Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). British Journal of Pharmacology. 2011; 164: 1079–1106.

[145] Pampliega O, Domercq M, Soria FN, Villoslada P, Rodríguez-Antigüedad A, Matute C. Increased expression of cystine/glutamate antiporter in multiple sclerosis. Journal of Neuroinflammation. 2011; 8: 63.

[146] Evonuk KS, Baker BJ, Doyle RE, Moseley CE, Sestero CM, Johnston BP, et al. Inhibition of System Xc(-) Transporter Attenuates Autoimmune Inflammatory Demyelination. Journal of Immunology. 2015; 195: 450–463.

[147] Merckx E, Albertini G, Paterka M, Jensen C, Albrecht P, Dietrich M, et al. Absence of system x_c⁻ on immune cells invading the central nervous system alleviates experimental autoimmune encephalitis. Journal of Neuroinflammation. 2017; 14: 9.

[148] Hoehne A, James ML, Alam IS, Ronald JA, Schneider B, D'Souza A, et al. [(18)F]FSPG-PET reveals increased cystine/glutamate antiporter (xc-) activity in a mouse model of multiple sclerosis. Journal of Neuroinflammation. 2018; 15: 55.

[149] Lin CC, Edelson BT. New Insights into the Role of IL-1β in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis. Journal of Immunology. 2017; 198: 4553–4560.

[150] Burger D, Molnarfi N, Weber MS, Brandt KJ, Benkhoucha M, Gruaz L, et al. Glatiramer acetate increases IL-1 receptor antagonist but decreases T cell-induced IL-1beta in human monocytes and multiple sclerosis. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106: 4355–4359.

[151] Shi J, He Y, Hewett SJ, Hewett JA. Interleukin 1β Regulation of the System xc- Substrate-specific Subunit, xCT, in Primary Mouse Astrocytes Involves the RNA-binding Protein HuR. The Journal of Biological Chemistry. 2016; 291: 1643–1651.

[152] Todd AC, Hardingham GE. The Regulation of Astrocytic Glutamate Transporters in Health and Neurodegenerative Diseases. International Journal of Molecular Sciences. 2020; 21: 9607.

[153] Mitosek-Szewczyk K, Sulkowski G, Stelmasiak Z, Struzyńska L. Expression of glutamate transporters GLT-1 and GLAST in different regions of rat brain during the course of experimental autoimmune encephalomyelitis. Neuroscience. 2008; 155: 45–52.

[154] Sulkowski G, Dąbrowska-Bouta B, Salińska E, Strużyńska L. Modulation of glutamate transport and receptor binding by glutamate receptor antagonists in EAE rat brain. PLoS ONE. 2014; 9: e113954.

[155] Olechowski CJ, Tenorio G, Sauve Y, Kerr BJ. Changes in nociceptive sensitivity and object recognition in experimental autoimmune encephalomyelitis (EAE). Experimental Neurology. 2013; 241: 113–121.