Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2015 | 134 | 982–1007 doi: 10.1111/jnc.13200

REVIEW

Current approaches to enhance glutamate transporter function and expression

Andréia C. K. Fontana

Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA

Abstract

L-glutamate is the predominant excitatory neurotransmitter in the CNS and has a central role in a variety of brain functions. The termination of glutamate neurotransmission by excitatory amino acid transporters (EAATs) is essential to maintain glutamate concentration low in extracellular space and avoid excitotoxicity. EAAT2/GLT-1, being the most abundant subtype of glutamate transporter in the CNS, plays a key role in regulation of glutamate transmission. Dysfunction of EAAT2 has been correlated with various pathologies such as traumatic brain injury, stroke, amyotrophic lateral sclerosis, Alzheimer's disease, among others. Therefore, activators of the function or enhancers of the expression of EAAT2/GLT-1 could serve as a potential therapy for these conditions. Translational activators of EAAT2/GLT-1, such as ceftriaxone and LDN/OSU-0212320, have been described to have significant protective effects in animal models of amyotrophic lateral sclerosis and epilepsy. In addition, pharmacological activators of the activity of EAAT2/GLT-1 have been explored for decades and are currently emerging as promising tools for neuroprotection, having potential advantages over expression activators. This review describes the current status of the search for EAAT2/GLT-1 activators and addresses challenges and limitations that this approach might encounter.

Keywords: allosteric modulation, ceftriaxone, EAAT2 activator, excitotoxicity, glutamate transporter, Parawixin1.

J. Neurochem. (2015) **134**, 982–1007.

Glutamate is the predominant excitatory amino acid neurotransmitter in the mammalian CNS and is essential for many aspects of normal brain function including cognition, memory, learning, developmental plasticity, and long-term potentiation (McEntee & Crook 1993; Weiler *et al.* 1995; Lopez-Bayghen & Ortega 2011; Peng *et al.* 2011).

The termination of glutamate neurotransmission is achieved by rapid uptake of the released glutamate by presynaptic and astrocytic high-affinity sodium-dependent transporters (Danbolt 2001; Beart & O'Shea 2007). Five structurally distinct subtypes of glutamate transporters have been cloned from animal and human tissue. Glutamate and aspartate transporter (GLAST) or EAAT1 (Storck *et al.* 1992) is localized in astroglial cells (Lehre *et al.* 1995).

Received January 31, 2015; revised manuscript received May 19, 2015; accepted May 20, 2015.

Address correspondence and reprint requests to Dr Andréia C. K. Fontana, Drexel University College of Medicine, Department of Pharmacology and Physiology, NCB, MS 488, 245 N. 15th Street, Philadelphia, PA 19012, USA. E-mail: andreia.mortensen@drexelmed.edu

Abbreviations used: 6-OHDA, 6-hydroxydopamine; AAV, adeno-associated viral; AD, Alzheimer's disease; Akt, protein kinase B; ALS, amyotrophic lateral sclerosis; Aβ, amyloid β; AβPP, amyloid-β precursor protein; CREB, cAMP response element-binding protein; EAAC1, excitatory amino acid carrier 1; EAAT1-5, human excitatory amino acid transporter subtypes 1-5; EAAT2, human glutamate transporter 2; EAATs, excitatory amino acid transporters; GABA, gamma-aminobutyric acid; GLAST, glutamate and aspartate transporter; GLT-1, rat glutamate transporter 1; GltPh, glutamate transporter homolog from *Pyrococcus horikoshii*; GSK3β, glycogen synthase kinase 3 beta; HAND, HIV-associated neurocognitive disorder; HD, Huntington's disease; HEK, human embryonic kidney; HP, hairpin loop; HSB, hybrid structure-based; JAK2, Janus-activated kinase-2; L-DOPA, L-3,4-dihydroxyphenylalanine; MCAO, middle cerebral artery occlusion; MS-153, [R]-[-]-5-methyl-1-nicotinoyl-2-pyrazoline; mTLE, mesial temporal lobo epilepsy; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate receptor; N-myc, N-myc proto-oncogene protein; OGD, oxygen and glucose deprivation; PACAP, pituitary adenylate cyclase-activating peptide; PDC, L-trans-pyrrolidine-2,4-dicarboxylate; PD, human idiopathic Parkinson's disease; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PS1, presenilin-1; SOD1, superoxide dismutase 1; TBI, traumatic brain injury; TBOA, threo-beta-benzyloxyaspartate; TM, transmembrane; tPA, tissue plasminogen activator; WT, wild type.

GLT-1 or EAAT2 (Pines *et al*. 1992) is also primarily expressed in astroglial cells (Lehre *et al*. 1995), but at lower levels also in several neuronal populations (Berger & Hediger 1998; Chen *et al.* 2004; Furness *et al.* 2008; Petr *et al.* 2015). Excitatory amino acid carrier 1 or EAAT3 (Kanai & Hediger 1992), was initially localized to neuronal cell bodies and dendrites avoiding both axon-terminals and astrocytes (Rothstein *et al.* 1994). This finding has been somewhat controversial, but has recently been shown to be correct (Holmseth *et al.* 2012) and in agreement with a cell culture study suggesting that the C-terminus contains a sorting motif directing it to dendrites rather than terminals (Cheng *et al.* 2002). EAAT4 (Fairman *et al.* 1995) is a glutamate transporter subtype that was demonstrated to be predominantly expressed in cerebellar Purkinje cells by traditional antibody-based experiments (Dehnes *et al.* 1998) and by the use of bacterial artificial chromosome-transgenic mice (Gincel *et al.* 2007). EAAT5 is a transporter subtype that is expressed in vertebrate retina (Arriza *et al*. 1997). Interestingly, a report has shown that EAAT5 transporter-associated anion current hyperpolarizes the presynaptic terminal and thereby inhibits synaptic transmission by suppressing transmitter release. This demonstrates that EAAT5 does not seem to be important for removing glutamate, but rather acts like an inhibitory receptor, while EAAT1 in Müller glial cells mediates the bulk of glutamate uptake (Veruki *et al.* 2006).

Other transporters of glutamate include the glutamate–cysteine exchangers, present in neurons and glia (Sato *et al.* 2002; Sato *et al.* 2005; De Bundel *et al.* 2011) and the vesicular glutamate transporters (vGLUTs), a class of intracellular transporters that is only present in neurons (Li *et al.* 2013).

The glutamate transporter subtype EAAT2/GLT-1 (human/rat homolog, nomenclature that will be used interchangeably in this manuscript) is expressed throughout the brain, in the spinal cord, primarily in astrocytes, and also in neurons and oligodendrocytes. GLT-1 contributes 95% of the total glutamate transport activity and 1% of total brain protein in the CNS (Haugeto *et al.* 1996; Tanaka *et al.* 1997; Lehre & Danbolt 1998; Suchak *et al.* 2003; Scofield & Kalivas 2014). This important observation has been confirmed by several electrophysiology studies (Bergles & Jahr 1997; Diamond & Jahr 1997; Otis & Kavanaugh 2000; Herman & Jahr 2007). Hence, EAAT2 plays a central role in maintenance of extracellular glutamate homeostasis (Maragakis *et al.* 2004; Lauriat & McInnes 2007; Sheldon & Robinson 2007; Kim *et al.* 2011).

Molecular functioning and mechanisms of glutamate transporters

The transport of glutamate across the plasma membrane is coupled to the movement of cations (Na^+, K^+, and H^+) (Kanner 1983; Kanner & Schuldiner 1987; Kanner 1989), that are necessary for glutamate uptake and transporter cycling as well as anions that are uncoupled from the flux of glutamate (Szatkowski & Attwell 1994; Zerangue & Kavanaugh 1996). Electrophysiological studies in *Xenopus* oocytes have suggested that glutamate diffusion and binding to transporters, rather than uptake, are likely to dominate the synaptic concentration decay kinetics (Wadiche *et al.* 1995a; Wadiche *et al.* 1995b). Later studies aimed to define the kinetic relationship between the two major access states that alternatively expose glutamate-binding sites to the extracellular or to the intracellular solution have proposed that transporter-associated anion currents were approximately twice as slow to rise and decay as stoichiometric transport currents, but the presence of permeant anions did not slow transporter cycling (Bergles *et al.* 2002). It is important to state that EAATs are not only secondary-active glutamate transporters, but also anion-selective channels and thus represent prototypical dual function membrane transport proteins. Recent studies combining molecular dynamics simulations and fluorescence spectroscopy of GltPh and patch-clamp recordings of mammalian EAATs have been able to determine that the transporters conduct anions through a lateral movement of the glutamate transport domain during intermediate transporter conformations that result in the formation of an anion-selective conduction pathway (Jensen *et al.* 2015; Machtens *et al.* 2015).

Additionally, voltage-clamped fluorometry assays measuring conformational changes in EAAT3 have shown that there are significant Na^+-dependent conformational changes preceding glutamate binding, and that Na^+ and H^+ are cotransported with glutamate in the forward part of the transport cycle. The data further suggest that an increase in proton concentrations slows the reverse transport of glutamate, which may play a neuroprotective role during ischemia (Larsson *et al.* 2004). Recently, a reconstituted system and simulations suggest that the relative rates of net uptake and heteroexchange are comparable in EAAT2 (Zhou *et al.* 2014b).

Importantly, the identification of several crystal structures of a bacterial homolog of glutamate transporters GltPh has greatly facilitated our understanding of mechanistic aspects of the transport cycle (Yernool *et al.* 2004; Boudker *et al.* 2007; Reyes *et al.* 2009; Verdon & Boudker 2012; Reyes *et al.* 2013), setting the stage for structure-based drug discovery. GltPh consists of three identical protomers that associate within the membrane to form a bowl-like structure (Gether *et al.* 2006). Each protomer has eight transmembranes (TMs) and two helix-turn-helix motifs termed hairpin 1 (HP1) and HP2. Specifically, HP2 serves as the extracellular gate of the transporter (Qu & Kanner 2008). The individual protomers in the trimeric complex are held together by a central core; this core is made of TMs 1, 2, 4, and 5 from each protomer that will associate together,

while TMs 3, 6, 7, and 8, along with HP1 and HP2, form a transport domain (Vandenberg & Ryan 2013). Fluorescence spectroscopy studies have demonstrated that substrate binding to the transporter is coupled to the binding of all three Na^+ in a highly cooperative manner at both outward-facing and inward-facing conformations (Reyes *et al*. 2013). It has been suggested that transport domains translocate substrates by moving across the membrane within a central trimerization scaffold in an 'elevator-like' transport domain motion. Recently it was shown that two 'humanizing' mutations introduced in GltPh result in markedly increased transport domain dynamics, suggesting that these mutations favor structurally 'unlocked' intermediate states in the transport cycle at the interface between the transport domain and the trimeric scaffold (Akyuz *et al.* 2015).

Glutamate excitotoxicity and pathologies

Glutamate excitotoxicity is the pathological process of failure of the proper removal of glutamate by astrocytes from the synapses. This results in sustained elevation of extracellular glutamate levels and excessive activation of post-synaptic glutamate receptors resulting in Ca^{2+} influx (Nilsson *et al.* 1990a) and activation of a cascade of phospholipases, endonucleases, and proteases such as calpain that can lead to apoptotic or necrotic cell death (Raghupathi 2004). In fact, ischemic events in humans and in animals lead to an acute and sustained increase in extracellular glutamate concentrations (Benveniste *et al.* 1984; Faden *et al.* 1989; Brown *et al.* 1998; Vespa *et al.* 1998), indicating a lack of timely clearance by glutamate transporters. In excitotoxic states, the extracellular concentrations of glutamate may reach a millimolar range, causing degeneration of neurons through excessive stimulation of glutamate receptors (Meldrum & Garthwaite 1990; Clements *et al.* 1992; Rosenberg *et al.* 1992; Zhou & Danbolt 2014).

The speed of clearance of transmitter from the cleft influences many aspects of synaptic function, including the time course of the post-synaptic response and the peak post-synaptic receptor occupancy. Estimates of the time course of transmitter clearance, either by detailed theoretical modeling, or from the attenuation of synaptic transmission produced by a low-affinity competitive antagonist, have produced results that are in agreement, suggesting an average concentration of transmitter peaks in the range 1–5 mM, and a biphasic clearance, with time constants of approximately 100 μs and 2 ms (Clements 1996).

A study investigated the synaptic glutamate concentration using NMDARs (NMDA receptors) expressed by CA1 pyramidal cells in acute hippocampal slices, and found that its baseline concentration is near 25 nM (Herman & Jahr 2007). Such low resting concentrations will only be possible if the expression levels of GLT-1 are extremely high, as discussed in (Zhou & Danbolt 2013), therefore supporting the notion that expression levels of GLT-1 are high, and ambient glutamate is, in physiological conditions, very low.

Furthermore, it is important to discuss how EAAT2 modulation could possibly affect the process of transmitter diffusion. Studies have demonstrated that rapid communication in the brain between glia and neurons relies on the release and diffusion of transmitter molecules across the synaptic cleft (Rusakov *et al*. 2011). By focusing on the interplay between extracellular and intramembrane diffusion processes, a recent study illustrates the remarkable versatility of signal formation in synapses. An integrative measure of three-dimensional glial coverage confirms that thin spine post-synaptic densities are more tightly surrounded by glia. This distinction suggests that diffusion-dependent synapse–glia communication and astrocytic glutamate uptake near 'learning' synapses (associated with thin spines) could be stronger than that near 'memory' synapses (associated with larger spines) (Medvedev *et al.* 2014).

Recent evidence shows that EAAT2 on rat astrocytes appears to be concentrated near synapses, where it acts acutely in a very dynamic manner to shape synaptic transmission by binding released glutamate and, through a process that involved movements of the transporters in the cell membrane. This movement was dependent on neuronal and glial activities and transporter concentration was strongly reduced in the vicinity of active glutamatergic synapses. Notably, glutamate uncaging at synaptic sites increased GLT-1 diffusion, displacing transporters away from this compartment and impairment of GLT-1 membrane diffusion through cross-linking slowed the kinetics of excitatory post-synaptic currents, resulting in a prolonged time course of synaptic glutamate. These data provide evidence for a physiological and highly dynamic role of GLT-1 in shaping synaptic transmission through surface diffusion (Edwards 2015; Murphy-Royal *et al.* 2015).

Therefore, one might suggest that the tight regulation of the glutamate signal by GLT-1 is of great importance for normal glutamate neurotransmission and when diminished in injured states activators of glutamate transport can prevent aberrant glutamate signaling.

Mechanisms involved in glutamate-mediated excitotoxicity include down regulation of glutamate transporters or glutamate efflux via transporter reversal, as further discussed below. See Fig. 1 for a simplified depiction of the role of EAAT2 at glutamatergic synapses at physiological (Fig. 1a) and pathological (excitotoxicity, Fig. 1b) states.

Glutamate transporter down-regulation

Reduced expression and function of EAAT2 has been reported in numerous neurological disorders, as will be further discussed below. The first report was on cerebral ischemia (Levy *et al*. 1995), with many other examples including amyotrophic lateral sclerosis (ALS) (Rothstein *et al.* 1995; Trotti *et al.* 2001; Wilson *et al.* 2003) and

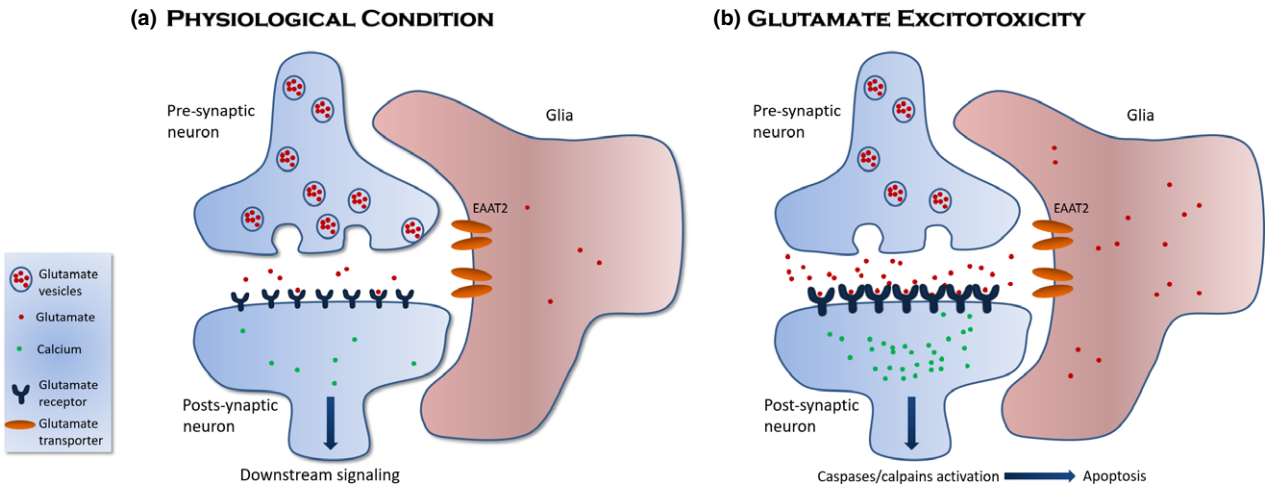

Fig. 1 Glutamatergic signaling under physiological (a) and excitotoxic (b) states. L-glutamate is stored in synaptic vesicles at presynaptic terminals. Glutamate (red dots) is released into the synaptic cleft, activating post-synaptic glutamate receptors. EAAT2 takes up released glutamate, removing it from the synaptic cleft to maintain the extracellular glutamate concentration below neurotoxic levels. In excitotoxicity glutamate is released in higher concentration and glutamate clearance is decreased, causing excessive activation of glutamate receptors resulting in Ca^{2+} influx (green dots) and activation of a cascade of phospholipases, endonucleases, and proteases such as calpain that can lead to apoptotic or necrotic cell death.

Alzheimer's disease (AD) (Li *et al.* 1997) among others. Additionally, decreased GLT-1 expression was shown in an animal model of brain trauma (Rao *et al.* 1998) and hippocampal neuronal damage was exacerbated after brain injury in an antisense knockdown model of GLT-1 (Rao *et al.* 2001). These results are consistent with the decreased EAAT2 activity observed in patients of traumatic brain injury (TBI) and stroke (Ikematsu *et al.* 2002; van Landeghem *et al.* 2006; Yi & Hazell 2006).

Moreover, a study on antisense knockdown revealed that loss of glutamate transporters GLAST or GLT-1 produced elevated extracellular glutamate levels, neurodegeneration characteristic of excitotoxicity, and a progressive paralysis, while loss of the neuronal glutamate transporter excitatory amino acid carrier 1 did not elevate extracellular glutamate in the striatum but did produce mild neurotoxicity and resulted in epilepsy (Rothstein *et al.* 1996).

Furthermore, a very important work by (Tanaka *et al.* 1997) has shown that knockout of the EAAT2 gene resulted in exacerbated damage, with lethal spontaneous seizures and increased susceptibility to acute cortical injury, compared to their wild-type counterparts, following cerebral injury in mice. This was the first time a GLT-1 knockout mice model was described. Later studies confirmed severe disturbances in mice lacking GLT-1 glutamate transporter (Hakuba *et al.* 2000; Katagiri *et al.* 2001; Mitani & Tanaka 2003). Another study showed that the brains of mice lacking GLAST/GLT-1 developed normally but that GLAST/GLT-1 double-knockout mice died around embryonic days 17–18 and exhibited cortical, hippocampal, olfactory bulb disorganization, and impairment of several essential aspects of neuronal development (Matsugami *et al.* 2006). Recently, a study with conditional knockout mice confirmed that GLT-1 plays main roles in the brain (Zhou *et al.* 2014a).

On the other hand, a study on GLT-1-deficient mice, GLT-1 homozygous (−/−), and heterozygous (+/−), has shown that a 50% reduction in GLT-1 levels had minor effects (Kiryk *et al.* 2008). The GLT-1 (−/−) mice had lower body and brain weight, mild neuronal loss in CA1 hippocampal region, focal gliosis, severe focal neuronal paucity in layer II of the neocortex, and short life-span. In contrast, GLT-1 (+/−) mice exhibited a 59% decrease in GLT-1 immunoreactivity in their brain tissue, with surprisingly no apparent morphological brain abnormalities, and their life-span and behavior not markedly different from controls, suggesting that GLT-1 (+/−) mice may serve as a potentially useful model to study neurodegenerative disease conditions with mild hyperglutamatergic activity.

Although the mechanism of down-regulation of EAAT2 in these conditions/diseases has yet to be fully established, it is clear that it results in impairment of their functions, which plays an important role in the etiology of neurological diseases. Consequently, there has been an extensive effort to identify molecular targets for enhancement of EAAT2 expression as a potential therapeutic approach.

Glutamate transporter reversal

As explained above, glutamate translocation is a multi-step process that utilizes energy stored in the Na^+/K^+ electrochemical gradient to move glutamate against its concentration. Under physiological conditions, the conventional transport direction is inward, however, in excitotoxic

conditions when the extracellular [Na^+]/intracellular [K^+] ratio decrease and/or intracellular [Na^+]/extracellular [K^+] ratio increase, glutamate is transported in the outward direction (Szatkowski *et al.* 1990; Billups & Attwell 1996; Jabaudon *et al.* 2000). This has been demonstrated in a study where raising extracellular potassium concentration evoked a reversed uptake current (Levy *et al.* 1998). Furthermore, massive increases in extracellular potassium and indiscriminate release of glutamate have been reported following concussive brain injury (Katayama *et al.* 1990) in addition to studies demonstrating a decreased sodium concentration after lateral fluid percussion (Soares *et al.* 1992). Also, GLT-1 reversal was shown in ischemic glutamate release (Phillis & O'Regan 1996; Grewer *et al.* 2008). Furthermore, the Na^+/K^+-ATPase that maintains these ion gradients are compromised in the injured brain (Werner & Engelhard 2007; Lima *et al.* 2008). Additionally, GLT-1 can be rendered inactive due to oxidative stress, as demonstrated by reduction in GLT-1/EAAT2 function in an ALS-causing superoxide dismutase 1 (SOD1) mutation in mice and rats, in both *in vitro* and *in vivo* studies (Volterra *et al.* 1994; Trotti *et al.* 1999a).

In this context, it remains to be clarified whether EAAT2 activators will facilitate glutamate clearance under excitotoxic conditions, or will rather intensify reverse transport. A study showed that *in vitro* ischemia led to neuronal damage, however, the ischemia-induced reversal of GLT-1 contributed to the survival of astrocytes themselves, demonstrating that reversal of glutamate transporter activity, while damaging to neurons, is also important for the survival of astrocytes under conditions of ischemia and might contribute to later survival of neurons (Kosugi & Kawahara 2006). Also, several strategies involving the application of pharmacological agents such as ceftriaxone, riluzole, and others, as reviewed in (Krzyzanowska *et al.* 2014) have shown significant protection of nervous tissues against ischemia.

Collectively, these are indications that modulation of glutamate transporters as a therapeutic concept is relevant. However, this concept faces the same inherent obstacle as other drugs acting through glutamate-mediated mechanisms, such as a significant risk of inducing adverse effects, as glutamatergic signaling is involved in several functions including brain development, cell survival, and synapsis differentiation.

Next, we will briefly discuss the current understanding of the glutamate-mediated excitotoxicity and the role of GLT-1 in several acute and chronic CNS pathologies. For extensive reviews on glutamate excitotoxicity and pathologies, see (Rothstein 1995b; Mark *et al.* 2001; Hazell 2007; Kim *et al.* 2011; Mehta *et al.* 2013).

Stroke

The third leading cause of death in industrialized countries and the most frequent cause of permanent disability in adults worldwide (Beresford *et al.* 2003), is primarily either hemorrhagic or ischemic, with almost 80% of the stroke being ischemic (Banerjee *et al.* 2011). Glutamate released during ischemia was shown to be responsible for cell death in models of retinal ischemia (Sisk & Kuwabara 1985; Louzada-Junior *et al.* 1992) and of cerebral ischemia. A pivotal study on transient cerebral ischemia monitored by intracerebral microdialysis revealed elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus, respectively, eight- and threefold during the ischemic period. These results suggest that the large increase in the content of extracellular glutamate and aspartate in the hippocampus induced by the ischemia may be one of the causal factors in the damage to certain neurons observed after ischemia (Benveniste *et al*. 1984), a result later confirmed in other studies (Taoufik & Probert 2008; Chao *et al.* 2010). A study showed that release of glutamate occurs within minutes of ischemic onset, with a tenfold higher concentration of glutamate within the cells resulting in failure of the energy and ion gradient (Nishizawa 2001). These studies indicate that abnormal function of glutamate transport plays an essential role in the excitotoxic neurodegeneration that occurs in the models.

Interestingly, a study supports the notion that glutamate transporters work in reverse during ischemic events, thus contributing to the increased extracellular glutamate by releasing glutamate that triggers neuronal death. Hence, to use GLT-1 modulators may in such situations potentiate neuronal death and complexity of the disease (Rossi *et al.* 2000).

On the other hand, other studies suggest that modulation of EAAT2 to inhibit glutamate-induced excitotoxicity may be a potential therapeutic target for the treatment of stroke and this hypothesis has been explored in many ways. First, decreased glutamate transporter expression was observed in ischemic neuronal death, and this may have disrupted the normal clearance of the synaptically released glutamate that contributed to the death (Rao *et al*. 2001). Then, studies using pharmacological manipulation of EAAT2 levels (Chu *et al.* 2007; Lipski *et al.* 2007) and increased expression of GLT-1 by using an adeno-associated viral vector expressing GLT-1 cDNA were shown to reduce the damage caused by brain ischemia, with decrease in the size of lesion and improvement of behavioral recovery (Harvey *et al.* 2011). Additionally, ceftriaxone, an up-regulator of EAAT2 protein expression and activity, confers neuroprotection in cerebral ischemia/reperfusion injury (Verma *et al.* 2010).

Since treatments for stroke are, to date, restricted to the administration of plasminogen activator (tPA) to break up blood clots in the arteries of the brain, and no treatments available to halt the progression of secondary damages, we expect that future research may further explore the potential role of EAAT2 as target for neuroprotection after ischemic stroke.

Epilepsy

A group of disorders characterized by recurrent spontaneous seizures that apparently result from complex processes involving several neurotransmitter systems including glutamate (Jabs *et al.* 2008; Wang & Qin 2010; Werner & Covenas 2015), that produce an imbalance between neuronal excitatory–inhibitory activities that has been suspected to occur during seizures and to underlie epileptogenesis (Coutinho-Netto *et al.* 1981; Kaila *et al.* 2014).

Increased extracellular glutamate levels were found in the hippocampi of patients with mesial temporal lobo epilepsy (mTLE) (During & Spencer 1993; Cavus *et al.* 2005; van der Hel *et al.* 2005). Evidence that GLT-1 is involved in epilepsy come from several studies, however, GLT-1 levels reported in animal models and human patients are not consistent, as discussed below.

Studies with mice lacking GLT-1 revealed that they are prone to exhibiting seizures (Tanaka *et al.* 1997). On the other hand, another study with GLT-1 knockdown in rats resulted in increased extracellular glutamate, but not seizures (Rothstein *et al.* 1996). These findings raise the question of whether GLT-1 is really involved in epilepsy.

Patients with mTLE have been reported with decreased levels of EAAT1 and EAAT2 as well as increased extracellular glutamate levels in the epileptogenic hippocampus both during and after clinical seizures (Mathern *et al.* 1999; Proper *et al.* 2002). On the other hand, (Tessler *et al.* 1999) proposed that major changes in the level of expression of the glutamate transporters do not play an important role in the development of human mTLE, but may be implicated in the etiology of other types of epilepsy. In the same line, another study found no significant change in the amount of EAAT2 in the human epileptogenic hippocampus, which led them to suggest a deficiency in glutamine synthetase in astrocytes as a molecular basis for extracellular glutamate accumulation and seizure generation (Eid *et al.* 2004). Later, a study with human epilepsy patients also claimed that EAAT2 is not altered (Bjornsen *et al.* 2007).

Regarding animal models of epilepsy, several studies claim that the levels of GLT-1 are unchanged, as reported in kindled rats (Akbar *et al.* 1997; Miller *et al.* 1997; Simantov *et al.* 1999), in rats fed with an anticonvulsant ketogenic diet (Bough *et al.* 2007), and in spontaneously epileptic rats (Guo *et al.* 2010).

On the other hand, decreased levels of GLT-1 were observed in pilocarpine (Lopes *et al.* 2013) and albumin models (David *et al.* 2009), as well as in tuberous sclerosis (Wong *et al.* 2003), in the $FeCl_3$- induced limbic epilepsy model (Ueda *et al.* 2007), and in the chest compression-induced audiogenic epilepsy model (Lu *et al.* 2008).

Interestingly, increasing GLT-1 expression protected mice against status epilepticus-induced death, neuropathological changes, and chronic seizure development (Kong *et al.* 2012).

Recently, it was demonstrated that conditional deletion of GLT-1 protected against fatal epilepsy (Petr *et al.* 2015). This study demonstrated that astrocytic GLT-1 is indeed involved in critical functions required for normal weight gain, resistance to epilepsy, and survival.

Together, these studies indicate that astrocytic glutamate uptake plays a crucial role in protecting neurons from hyperexcitability. However, how exactly this mechanism is disturbed in epilepsy is still under discussion. Furthermore, it is still unclear whether changes in transporters represent causative or compensatory changes during epileptogenesis. Finally, on the basis of some of the previous reports, the notion that enhancing GLT-1/EAAT2 protein expression is a potential therapeutic approach to treat epilepsy cannot be ruled out.

For more information on astrocytic targets (including GLT-1) and new avenues for therapeutic treatment of epilepsy, please see the reviews (Wetherington *et al.* 2008; Coulter & Eid 2012; Crunelli *et al.* 2015).

Autism

Autism is a disorder of unknown etiology, and co-occurrence with epilepsy being common. It is thought that reduced density of cerebellar Purkinje neurons is a key neuropathological component in autism (Courchesne 1997; Palmen *et al.* 2004), which results in excitotoxicity, mitochondrial dysfunction, and degeneration (Khodorov 2000). A recent report by the Autism Genome Project Consortium identified a new linkage peak for autism in the region of chromosome 11 where the gene for EAAT2 is located (Szatmari *et al.* 2007). Recent considerations revealed signs of astroglial, oligodendroglial, and microglial dysfunction in the autistic brain, suggesting that all these cellular processes may represent presumptive targets for novel therapeutic strategies (Zeidan-Chulia *et al.* 2014). Recently, a study investigating the effects of ceftriaxone and cefixime, activators of GLT-1, demonstrated that these drugs improved some symptoms of autism and decreased epilepsy seizures (Ghanizadeh & Berk 2015).

Traumatic brain injury

TBI is a complex pathology of many etiologies (Matute *et al.* 2006; Guerriero *et al.* 2015). TBI has been studied with multiple different animal models, including non-penetrating and penetrating impact, lesion studies, fluid percussion, shock waves (explosion), among others. For reviews on animal models of TBI, see (Finnie 2001; de Lanerolle *et al.* 2015).

Studies on TBI in humans and in animals have demonstrated an acute increase in tissue glutamate concentrations that remain high for up to 5 days in humans, suggesting a lack of timely clearance by glutamate transporters (Nilsson *et al.* 1990b; Baker *et al.* 1993; Palmer *et al.* 1993; Brown *et al.* 1998; Vespa *et al.* 1998; Yamamoto *et al.* 1999). Clinical and experimental studies have determined that the resulting glutamate-mediated excitotoxicity is a significant

contributor to acute post-injury neurodegenerative events (Faden *et al*. 1989; Yi & Hazell 2006). The failure of proper glutamate removal results in sustained elevation of extracellular glutamate levels and overstimulation of the NMDA glutamate receptor that results in overloading of neurons with Ca^{2+} and Na^{+} (Rothman & Olney 1995), leading to the activation of phospholipases, endonucleases, and proteases such as calpain resulting in the apoptotic and/or necrotic cell death (reviewed in (Raghupathi 2004).

Previous studies in animal models of lateral fluid percussion injury reported decreased GLT-1 expression between 6 and 72 h in the rat (Rao *et al*. 1998), other studies have shown differences in EAAT2 expression in the cerebral cortex relative to the survival time and severity of cerebral contusion after TBI in humans (Ikematsu *et al*. 2002; van Landeghem *et al*. 2006). In addition, antisense knockdown of GLT-1 in rat exacerbates neuronal damage following controlled cortical impact (Rao *et al*. 2001). Collectively, these studies suggest that targeting GLT-1 could be a strategy to find a therapy for the treatment of TBI.

Importantly, the pathophysiology of TBI differs between lesional and perilesional tissue and the degree of injury severity; cell injury proceeds more slowly in the brain tissue that is immediately proximal to the most severe injury and may be reversible in this penumbra. Similarly, following concussion the majority of brain injury is reversible, given the typical course of symptom resolution (Collins *et al.* 2006; Zlotnik *et al.* 2012; Eisenberg *et al.* 2013; Eisenberg *et al.* 2014; Burda *et al.* 2015). Hence, in brain areas with reversible damage, the opportunity to decrease acute glutamate excitotoxicity holds promise for neuroprotection, while other pharmacological approaches might be needed for other types of TBI.

Amyotrophic lateral sclerosis

ALS is a debilitating disease characterized by progressive loss of voluntary motor neurons leading to muscle atrophy, weight loss, and respiratory failure. The pathogenesis of ALS involves inflammation, oxidant stress, apoptosis, mitochondrial dysfunction, SOD1 protein aggregation, and astroglial dysfunction, including dramatic loss of EAAT2/GLT-1 in both in the motor cortex and in the spinal cord (Rothstein *et al.* 1992; Rothstein *et al*. 1995; Rothstein 1995a; Wilson *et al*. 2003). Additionally, mouse models of ALS including lines G85R, A4V, and I113T SOD1-mutants all have a marked loss or inactivation of glutamate transporters (Bruijn *et al.* 1997; Trotti *et al.* 1999b; Bendotti *et al.* 2001). Moreover, selective loss of EAAT2 has also been demonstrated in both sporadic and familial cases of ALS (Van Den Bosch *et al.* 2006).

Although several lines of evidence point to the possibility that glutamate transport defects and the related excitotoxicity may play a role in the late stage of ALS disease progression, there are no consistent data to support defects in EAAT2 as having a primary role in the induction or the selectivity of motor neuron degeneration taking place in ALS. In this sense, a study showed that GLT-1 over-expression in SOD1G93A mouse cervical spinal cord did not protect motor neurons, preserve diaphragm function, or prolong animal survival, suggesting that focal restoration of GLT-1 expression in cervical spinal cord astrocytes was not an effective therapy for ALS (Li *et al.* 2015). Moreover, it is possible that multiple therapies aimed at simultaneously diminishing the overwhelming Ca^{2+} overload, endoplasmic reticulum stress, and mitochondrial dysfunctional pathways would be effective on halting ALS progression, as proposed by (Verche *et al.* 2011).

Extensive studies using ceftriaxone and other GLT-1 expression activators have been performed with hopes that it would restore GLT-1 expression to normal levels and slow, or even possibly, stop ALS progression in patients. These studies will be further discussed below.

Huntington's disease

Huntington's disease (HD) is a devastating progressive autosomal dominant neurodegenerative disorder in which multiple areas of the brain degenerate, mainly involving the dopamine, glutamate, and GABA. It is caused by a mutated form of the huntingtin gene, where excessive CAG repeats result in accumulation of the mutant protein as cytoplasmic and nuclear aggregate inclusions (Bates 2003; Walker 2007), resulting in oxidative stress and mitochondrial dysfunction (Browne & Beal 2006).

Moreover, excitotoxicity is thought to be important in the pathogenesis of HD (Massieu & Garcia 1998; Shin *et al.* 2005; Estrada-Sanchez & Rebec 2012). In this regard, it has also been shown that the EAAT2 mRNAs of post-mortem brains of HD patients are decreased in correlation to disease severity (Arzberger *et al.* 1997; Faideau *et al.* 2010).

Additionally, studies have shown that increase in the functional expression of GLT-1 can improve the behavioral phenotype of the mouse model of HD (Estrada-Sanchez *et al.* 2009; Miller *et al.* 2012a; Petr *et al.* 2013). Furthermore, ascorbate release and GLT-1 function are impaired in the striatum of transgenic mouse models of HD (Rebec 2013). These studies suggest that changes in GLT-1/EAAT-2 expression or function can potentiate or ameliorate the progression of HD, however, effective therapies are yet to be discovered and developed. However, in a R6/2 transgenic mouse model that is heterozygous for the null allele of GLT-1/EAAT-2 and carries the double mutation, weight loss, accelerating rotarod, climbing, and paw-clasping were not exacerbated, suggesting that decreased expression of GLT-1 in this model does not worsen disease progression.

Neuropathic pain

Neurons responsible for sensing noxious stimuli and conducting pain signals from periphery to the spinal cord are

predominantly glutamatergic (Tao *et al.* 2005). Decreases in glutamate uptake activity and expression of spinal glutamate transporters have been reported in animal models of pathological pain, with pharmacological inhibition or antisense down-regulation of spinal transporters being reported to induce/aggravate pain behaviors (Gegelashvili & Bjerrum 2014).

On the other hand, over-expression of GLT-1 in astrocytes in the spinal cord attenuated the induction, but not maintenance, of inflammatory and neuropathic pain, suggesting that up-regulation of spinal GLT-1 could be a novel strategy for the prevention of pathological pain (Maeda *et al.* 2008).

Additionally, emerging data point at key roles of glutamate transporters in molecular mechanisms of chronic pain and analgesia, including the development of opioid tolerance. However, precise pharmacological targeting of the glutamate transport system requires detailed elucidation of molecular factors and signaling pathways underlying expression and activity of individual glutamate transporters subtypes, including their splice variants, and molecular behavior on the pathological states of neuropathic pain (reviewed in (Gegelashvili & Bjerrum 2014).

HIV-associated neurocognitive disorder

A common neuropathology found in the brains of HIV infected individuals is astrocytes apoptosis. Excess glutamate is released upon HIV infection of macrophage/microglial cells, which has been associated with neurotoxicity mediated by gp120, transactivator of transcription and other HIV proteins (Sabri *et al.* 2003).

To prevent glutamate excitotoxicity in HIV-associated neurocognitive disorder (HAND), antagonists of NMDA receptors have been explored, however, this approach has failed as a result of side effects, and therefore alternatives are being sought. Another approach has been to regulate the glutamate transporters, and there is preliminary experimental evidence that these approaches have potential therapeutic utility for the treatment of HAND. These efforts, however, are at an early stage where the next steps are dependent on the identification of drug-like inhibitors as well as the development of predictive neuroAIDS animal models (Potter *et al.* 2013). Recently, a study showed that methamphetamine and HIV treatment activated trace amine associated receptor 1, leading to intracellular cAMP in human astrocytes and decreased EAAT-2 mRNA, significantly decreasing glutamate clearance. Furthermore, molecular alterations in astrocyte TAAR1 levels correspond to changes in astrocyte EAAT2 levels and function, confirming the involvement of EAAT2 in the context of HAND (Cisneros & Ghorpade 2014).

However, our precise understanding of host factors that mediate the neurotoxic insult launched by exposure of CNS to HIV-1 is still developing. We need to better understand the regional genetic variations in viral genes and further identify opportunities to specifically block the neurotoxicity of viral and host proteins as well as identify potential neuroprotective factors that could be augmented to help alleviate the negative impact of HIV-1 on the brain (Rao *et al.* 2014).

Alzheimer's disease

AD is a progressive age-related neurodegenerative disorder. The pathophysiological characteristic of AD is abnormal deposition of fibrillar amyloid β (Aβ) protein, intracellular neurofibrillary tangles, oxidative damage, and neuronal death (Kumar *et al.* 2015). Aberrant glutamate stimulation and resulting synaptic dysfunction have been proposed as one of several mechanisms by which synapses are damaged in AD (Mattson & Chan 2003; Hynd *et al.* 2004; Talantova *et al.* 2013).

Moreover, a number of studies have found that GLT-1/EAAT-2 is significantly reduced or damaged in AD (Hardy *et al.* 1987; Li *et al*. 1997; Lauderback *et al.* 2001; Woltjer *et al.* 2010; Scott *et al.* 2011). Additionally, a report showed that deficits in GLT-1 function compound the effects of familial AD amyloid-β precursor protein/presenilin-1 mutant transgenes in younger animals, suggesting that this dysfunction may contribute to early occurring pathogenic processes associated with AD (Mookherjee *et al.* 2011), suggesting that GLT-1/EAAT-2 loss is capable of driving cognitive impairment in the context of Aβ-related neuropathology (Schallier *et al.* 2011). Also, a study showed that Aβ1–42 induces rapid GLT-1/EAAT-2 mislocalization and internalization in astrocytes, which leads to a marked reduction in the rate of glutamate clearance from the extracellular space (Scimemi *et al.* 2013).

Recently, a study revealed that GLT-1 loss also caused an apparent compensatory increase in insulin-degrading enzyme activity in the liver, an organ that has been shown to regulate peripheral aβ levels and expresses GLT-1, suggesting that partial GLT-1 loss can cause insulin/Akt (protein kinase B) signaling abnormalities observed in AD (Meeker *et al.* 2015).

Nonetheless, it is not yet entirely clear whether GLT-1/EAAT-2 dysfunction plays a pathogenic role in AD, and whether Aβ-related pathogenic processes have a functional impact on the rate at which astrocytes remove endogenous, synaptically released glutamate is unknown (Scimemi *et al*. 2013). Furthermore, a study suggested that GLT-1 is not involved in AD, as the authors reported decreased expression of glutamine synthetase but no changes in GLT-1 expression in the prefrontal cortex in a mouse model of AD (Kulijewicz-Nawrot *et al.* 2013). Therefore, the question whether GLT-1/EAAT-2 may be used as target for neuroprotective functions in AD pathogenesis remains to be further explored.

Currently available treatments for AD include acetylcholinesterase inhibitors (Hogan 2014) and NMDA receptor antagonist memantine (Zimmer *et al.* 2012; Kumar *et al*. 2015), however, they provide symptomatic relief and target

late aspects of the disease, and therefore a definite treatment for this disease is yet to be identified.

Human idiopathic Parkinson's disease

Human idiopathic Parkinson's disease (PD) is a progressive neurodegenerative movement disorder that is primarily characterized by degeneration of the dopaminergic neurons at the nigrostriatal pathway (Deumens *et al.* 2002). This degeneration is believed to lead to an overactivation of the subthalamic nucleus (Rodriguez *et al.* 1998), increasing the firing rate of the glutamatergic excitatory projections to the substantia nigra (Bamford *et al.* 2004; Ambrosi *et al.* 2014) causing a sustained exposure to glutamate that could accelerate the degeneration of dopaminergic neurons (Oster *et al.* 2014). Therefore, glutamate-mediated excitotoxicity may be involved in a lethal vicious cycle, which critically contributes to the exacerbation of nigrostriatal degeneration in PD.

The role of glutamate transporters in the pathogenesis of PD is not entirely clear, however, some studies clearly demonstrate a link between disturbed glutamatergic neurotransmission and glutamate transporter functioning in the striatum of an unilateral 6-hydroxydopamine animal model for PD (Massie *et al.* 2010). Another study using the same animal model showed a time-dependent bilateral effect of lesioning on the expression and the activity of GLT-1 (Carbone *et al.* 2012b). Additionally, acutely induced dysfunction of EAATs in the rat by single unilateral injection of their substrate inhibitor L-trans-pyrrolidine-2,4-dicarboxylate (PDC), triggers a neurodegenerative process mimicking several PD features (Assous *et al.* 2014). Moreover, it was reported that ceftriaxone, a drug that increases expression of GLT-1, ameliorated locomotor impairments in this animal model of PD (Chotibut *et al.* 2014; Kelsey & Neville 2014).

The major treatment for PD is L-3,4-dihydroxyphenylalanine (L-DOPA), to replace the progressive loss of dopamine; however repeated L-DOPA treatment leads to motor complications, such as dyskinesias associated with increased extracellular glutamate levels in the basal ganglia (Robelet *et al.* 2004), therefore alternative treatments are needed. Based on previous studies and data, GLT-1/EAAT-2 may be a potential target for PD (Sheldon & Robinson 2007).

EAAT2 as target for neuroprotection

Several classes of compounds that target glutamate-mediated excitotoxicity, such as NMDAR antagonists (McIntosh *et al.* 1990; Baker *et al.* 1993; Okiyama *et al.* 1997; Okiyama *et al.* 1998; Ikonomidou & Turski 2002; Ginsberg 2008) and compounds that target calcium influx (Okiyama *et al.* 1992; Cheney *et al.* 2000) have been shown to alleviate cellular damage and neurologic deficits. However, they were shown to exhibit substantial side effects, making them less attractive for translation to the clinic.

Interestingly, a substantial proportion of extrasynaptic NMDARs are located adjacent to glia, which express EAAT2 (Hardingham & Bading 2010). EAAT2 might be a more attractive therapeutic target since it is positioned to take up glutamate near neurotoxic extrasynaptic NMDARs.

Historically, modulation of that GLT-1/EAAT2 expression and/or activity has received surprisingly little attention as putative drug targets, considering the immense therapeutic potential in pharmacological intervention into glutamatergic neurotransmission and the fact that other neurotransmitter transporters are targeted by clinically administered drugs against epilepsy, depression, and attention-deficit hyperactivity disorder (Kristensen *et al.* 2011).

Nonetheless, finding alternative strategies to treat excitotoxic conditions without producing considerable side effects has become a very significant endeavor in the last decade. In this way, pharmacological manipulation of transporter expression or function is of high interest from a therapeutic point of view, and specially EAAT2, accounting for most of the glutamate clearance in the CNS, has become a high priority target for the development of therapeutic agents to prevent excitotoxicity (Bunch *et al*. 2009).

Albeit this review is focused on positive modulators of GLT-1 activity/expression, it is important to reflect that the drug development using EAAT2 as target started with the discovery of pharmacological agents that were developed to study the intrinsic properties and function of the EAATs, as reviewed in (Bridges *et al*. 1999). In this regard, a very important and pivotal series of medicinal chemistry work is illustrated by the development of the potent EAAT2 inhibitor TBOA (threo-beta-benzyloxyaspartate) and analogs (Lebrun *et al.* 1997; Shimamoto *et al.* 1998; Shigeri *et al.* 2004; Shimamoto 2008). These series of work revolutionized the field, as TBOA was the first non-transportable blocker for all subtypes of EAATs ever identified. Indeed, blockade of EAAT2 by TBOA results in marked neurotoxicity through the activation of NMDARs because of an increased extracellular glutamate level (Izumi *et al.* 2002; O'Shea *et al.* 2002; Bonde *et al.* 2005). Additionally, this discovery helped elucidate several functions of the EAATs; and it was also an encouraging step in the search for other modulators of the function of EAATs, as seen subsequently with the identification of positive ones, as will be discussed below.

Furthermore, it is important to consider developmental changes of expression when designing therapies based on GLT-1 activators. It has been demonstrated that the development of the total glutamate uptake activity in the forebrain corresponds to that of GLT-1, in agreement with the observation that GLT-1 is the predominant transporter in the adult brain (Ullensvang *et al.* 1997). Moreover, it has been shown that GLT-1 was present in fetal brain and spinal cord, with expression progressively increasing to adult levels throughout the neuraxis by postnatal day 26 (Furuta *et al.*

1997). Another study suggests a postnatal development- and age-dependent differential interaction of transcription factors NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and N-myc (N-myc proto-oncogene protein) to their respective sequences and they act as positive and negative regulator, respectively, of GLT-1 gene expression in the brain during early developmental period in both cerebral and cerebellar cortices, which might be different in aging mice (Gupta & Prasad 2014).

In this regard, a very well-studied pathology that illustrates striking differences according to the stage of development is TBI, as observed by the differences between adult and pediatric TBI (Giza *et al.* 2007). Important distinctions of the younger brain after TBI include an increased propensity for apoptosis, age-dependent parameters for cerebral blood flow and metabolism, development-specific biomarkers, increased likelihood of early post-traumatic seizures, differential sensitivity to commonly used neuroactive medications, and anesthesia and altered neuroplasticity during recovery from injury (Huh & Raghupathi 2009). Questions that remain to be answered, among others, include whether, following pediatric injury, there is persistent decrease in GLT-1 and other proteins, leading to neuronal hyperexcitability, whether loss of these proteins is a direct result of epigenetic modulation of gene transcription, whether manipulation of DNA methylation reverses these losses, and, finally, whether therapies based on GLT-1 enhancement would be beneficial.

Many studies suggest that targeting GLT-1/EAAT2 provides a novel approach for the treatment of conditions that involve glutamate excitotoxicity. This review attempts to review the current state of research on the development of compounds with the ability to increase levels of EAAT2 protein expression (summarized in Table 1) or compounds that interact directly or indirectly with EAAT2 enhancing its catalytic activity (summarized in Table 2).

Transcription/translational modulators

GLT-1 expression can be regulated in several ways, at both the transcriptional and translational level (Anderson & Swanson 2000; Sheldon & Robinson 2007). Even though GLT-1 protein is already highly expressed, several studies show evidence that GLT-1 can be up-regulated. In this manuscript, we will focus on approaches demonstrated to increase glutamate transporter expression with a therapeutic goal, and we will present the development of these regulators in a chronological sequence below and in Table 1 [with regulators classified as proteins (a), endogenous molecules (b) or synthetic molecules (c)]. For a review on the mechanisms of regulation of glutamate transporter function discussing transcriptional regulation and post-transcriptional regulation and modifications, see (Grewer *et al.* 2014).

Early studies showed increased expression of GLT-1 protein in astroglial cultures by the epidermal growth factor and this was thought to be mediated through intracellular signaling involving cAMP as dibutyryl-cAMP incubation had a similar effect (Zelenaia *et al.* 2000). Pituitary adenylate cyclase-activating peptide was also found to stimulate GLT-1 expression (Figiel & Engele 2000).

Dexamethasone, a glucocorticoid, increases EAAT2 mRNA levels and subsequently up-regulates EAAT2 protein expression and activity (Wen *et al.* 2005; Zschocke *et al.* 2005). A recent study shows that dexamethasone induces neuroprotection in hypoxic-ischemic brain injury in newborn rats, partly mediated via Akt activation (Feng *et al.* 2014b).

Importantly, studies concluded that Akt induces the expression of GLT-1 through increased transcription and that Akt can regulate GLT-1 expression without increasing GLAST expression in astrocytes (Li *et al.* 2006).

Corticosterone and retinol are both able to increase the translation of EAAT2 transcripts and, moreover, many disease-associated insults affected the efficiency of the induced translation of the EAAT2 transcript by these compounds (Tian *et al.* 2007).

GPI-1046, a synthetic neuroimmunophilin ligand that selectively increases GLT-1 expression and activity, was shown to protect motor neurons against excitotoxicity *in vitro* and increases the survival rate of transgenic ALS mice (Ganel *et al.* 2006) and more recently was shown to have a role in reduction of ethanol consumption (Sari & Sreemantula 2012).

The antiepileptic drug sodium valproate (valproic acid) was shown to up-regulate hippocampal glutamate transport following chronic treatment (Hassel *et al.* 2001). Because the mechanism of action of valproate involves modulation of multiple mechanisms its beneficial effects cannot be contributed to only EAAT2 regulations. These other mechanisms include the regulation of GABA and glutamate neurotransmissions, activation of pro-survival protein kinases, and inhibition of histone deacetylase. However, evidence for its neuroprotective properties is emerging. Recently, it was suggested that valproate reduces the development of chronic pain after nerve injury, in part by preventing down-regulation of GLT-1 (Yoshizumi *et al.* 2013) and to prevent retinal degeneration in a murine model of normal tension glaucoma (Kimura *et al.* 2015).

Minocycline, a broad-spectrum tetracycline antibiotic, was shown in neuropathic rats to maintain the expression of GLT-1 in the spinal dorsal horn and this was suggested to be involved in the attenuation of behavioral hypersensitivity (Nie *et al.* 2010). This study supports the idea that GLT-1 may be a potential target for the development of analgesics.

The tricyclic antidepressant amitriptyline was shown to reverse the down-regulation of EAAT1 and EAAT2 and to induce up-regulation of EAATs in an animal model of spared nerve injured (Mao & Yang 2010), suggesting that this up-regulation may be one of the therapeutic mechanisms of amitriptyline in the treatment of neuropathic pain.

Table 1 Neuroprotective activators of EAAT2 expression

Compound/protein	Mechanisms	Key findings	References
EGF[b], dibutyryl-cAMP[b], PACAP[b]	Endogenous hormones or second messengers, inducers of GLT-1 expression	Early studies showed increased expression of GLT-1 protein by EGF, cAMP, and PACAP, setting the stage for studies aimed to further understand the regulation of GLT-1	Figiel & Engele (2000) and Zelenaia *et al.* (2000)
Dexamethasone[c]	Synthetic glucocorticoid, inducer of EAAT2 protein expression	Neuroprotection in hypoxic-ischemic newborn rat brain injury	Wen *et al.* (2005), Zschocke *et al.* (2005), and Feng *et al.* (2014b)
Akt[a]	Protein kinase B, inducer of GLT-1 expression	Basic research that identified Akt as a control signal for GLT-1 expression *in vivo*	Li *et al.* (2006)
Corticosterone[b] and retinol[b]	Steroid hormone and metabolite of vitamin A, modulators of EAAT2 mRNA translation	Identification of glucocorticoids as EAAT2 protein expression inducers	Tian *et al.* (2007)
GPI-1046[c]	Neuroimmunophilin, inducer of GLT-1 expression	Attenuates ethanol intake in part through GLT-1 up-regulation	Ganel *et al.* (2006) and Sari & Sreemantula (2012)
Valproate[c]	Multiple mechanisms through regulation of GABA and glutamate neurotransmissions, activation of prosurvival protein kinases, inhibition of histone deacetylase	Reduces chronic pain after nerve injury and prevents retinal degeneration in glaucoma model	Hassel *et al.* (2001), Yoshizumi *et al.* (2013), and Kimura *et al.* (2015)
Minocycline[c]	Broad-spectrum tetracycline antibiotic, ameliorates down-regulation of GLT-1 expression in neuropathy model	Attenuates behavioral hypersensitivity in neuropathic rats	Nie *et al.* (2010)
Amitriptyline[c]	Tricyclic antidepressant, inducer of EAAT2 expression	Attenuates mechanical allodynia, used in the treatment of neuropathic pain	Mao & Yang (2010)
mTOR[a]	Serine-threonine protein kinase mammalian target of rapamycin, induces EAAT2 over-expression	Up-regulation of GLT-1 via mTOR- NF-κB signaling cascade in OGD may promote glutamate uptake in brain ischemia and neurodegenerative diseases	Wu *et al.* (2010), Pignataro *et al.* (2011), and Ji *et al.* (2013))
Pyridazine derivatives[c]	Small-molecule activators of EAAT2 expression	A cell-based assay identified 61 compounds as modulators of EAAT2 translation, thiopyridazine derivatives further explored.	Colton *et al.* (2010) and Xing *et al.* (2011)
JAK2[a]	Non-receptor tyrosine kinase, inducer of EAAT2 expression	JAK2 is proposed to participate in protection against excitotoxicity	Hosseinzadeh *et al.* (2011)
Tamoxifen[c]	Antagonist of the estrogen receptor, increases TGF-α and GLT-1 expression	Regulates GLT-1 via the CREB and NF-κB pathways	Karki *et al.* (2013) and Lee *et al.* (2013)
Estradiol[b]	Estrogen hormone, enhancer of GLT-1 function	Neuroprotective in clinical and experimental models of neuronal injury.	Karki *et al.* (2014)
GSK3β[a]	Kinase, stimulates the expression of GLT-1	Constitutively active GSK3β is important in controlling the expression of functional glutamate transporters on the plasma membrane.	Jimenez *et al.* (2014)
Histamine[b]	Neuromodulator, selectively increases GLT-1 expression	Neuroprotective after OGD and MCAO	Fang *et al.* (2014)

(continued)

Table 1. (continued)

Compound/protein	Mechanisms	Key findings	References
Harmine[c]	Beta-carboline alkaloid, increases GLT-1 expression	Neuroprotective in animal of ALS and cerebral ischemia	Li *et al*. (2011) and Sun *et al*. (2014)
Sulbactam[c]	β-lactamase inhibitor, GLT-1 expression up-regulator	Protects pyramidal neurons against brain ischemia.	Cui *et al*. (2015)
Ceftriaxone[c]	Transcriptional activator of GLT-1 expression	Neuroprotective in several animal models of brain trauma and neurodegeneration, failed clinical trials on ALS patients	Rothstein *et al*. (2005), Beghi *et al*. (2006), Chu *et al*. (2007), Lipski *et al*. (2007), Hota *et al*. (2008), Lee *et al*. (2008), Melzer *et al*. (2008), Miller *et al*. (2008), Thone-Reineke *et al*. (2008), Verma *et al*. (2010), Pan *et al*. (2011), Leung *et al*. (2012), Wei *et al*. (2012), Berry *et al*. (2013), Cudkowicz *et al*. (2013), Rebec (2013), Cui *et al*. (2014), Feng *et al*. (2014a), Hu *et al*. (2015), and Kelsey & Neville (2014)
LDN/OSU-0212320[c]	Pyridazine derivative, translational activator of GLT-1 expression	Protects cultured neurons from excitotoxicity and delays motor function decline and extend life span in animal model of ALS	Kong *et al*. (2014)

This class of regulators can be divided into three major subcategories: [a]proteins, [b]endogenous molecules, and [c]synthetic molecules.

Table 2 Neuroprotective activators of EAAT2 activity

Compound	Mechanisms	Key findings	References
Riluzole	Sodium channel blocker, activator of glutamate uptake	Neuroprotective *in vitro*, approved by FDA to ALS patients, efficacious in preclinical models of spinal cord injury	McIntosh *et al*. (1996), Zhang *et al*. (1998), Miller *et al*. (2000), Frizzo *et al*. (2004), Traynor *et al*. (2006), Dagci *et al*. (2007), Fumagalli *et al*. (2008), Banasr *et al*. (2010), Carbone *et al*. (2012a), Miller *et al*. (2012b), Mu *et al*. (2000), and Wilson & Fehlings (2014)
Guanosine	Guanine-based purine, activator of glutamate uptake	Currently examined for treatment of ischemic brain injury	Frizzo *et al*. (2001), Frizzo *et al*. (2005), Moretto *et al*. (2005), and Hansel *et al*. (2014)
Nicergoline	Ergot derivative activator of glutamate uptake	Currently examined for cerebrovascular disease and dementia	(Fioravanti & Flicker (2001), Nishida *et al*. (2004), and Fioravanti *et al*. (2014)
MS-153	R]-[-]-5-methyl-1-nicotinoyl-2-pyrazoline, activator of GLT-1 activity, inhibitor of glutamate release and calcium channels	Neuroprotective in MCAO, focal ischemia and lateral fluid percussion brain injury in rats	(Umemura *et al*. (1996), Kawazura *et al*. (1997), Shimada *et al*. (1999), Uenishi *et al*. (1999), Nakagawa *et al*. (2001), Abekawa *et al*. (2002b), Li *et al*. (2004), Nakagawa *et al*. 2005, Abekawa *et al*. (2002a), Alhaddad *et al*. (2014), and Fontana *et al*. (2015)
Parawixin1	Specific activator of EAAT2 activity	Neuroprotective in animal model of retinal ischemia	Fontana *et al*. (2003) and Fontana *et al*. (2007)
GT949 and GT951	Direct activators of EAAT2 activity, no effect on NMDA-mediated currents	Ongoing research	Unpublished

mTOR (a family of serine-threonine protein kinase mammalian target of rapamycin) is involved in the control of a wide variety of cellular processes, and there is evidence for the involvement of PI3K(phosphatidylinositol 3-kinase)/Akt/mTOR signaling regulation of GLT-1 in astrocytes (Pignataro *et al*. 2011). A study showed that treatment with PI3K or Akt inhibitors suppresses the phosphorylation of Akt and mTOR and decreased GLT-1 up-regulation in astrocytes.

Moreover, treatment with the mTOR inhibitor rapamycin decreased GLT-1 protein and mRNA levels and did not affect Akt phosphorylation, suggesting that mTOR is a downstream target of the PI3K/Akt pathway regulating GLT-1 expression (Wu *et al.* 2010). Another study demonstrated up-regulation of GLT-1 via mTOR-Akt- NF-κB (nuclear factor-κB) signaling cascade in a model of oxygen and glucose deprivation, suggesting that this signaling cascade may work to promote glutamate uptake in brain ischemia and neurodegenerative diseases (Ji *et al.* 2013).

However, even though mTOR inhibitors, such as rapamycin and its analogs may represent novel, rational therapies for a variety of neurological disorders, they have not been tested yet in patients with epilepsy, Alzheimer's, Huntington's, and Parkinson's disease. With continuing progress in basic and clinical research, there are reasons to be optimistic that the clinical indications of mTOR inhibitors for neurological disease will continue to expand in future (Wong 2013).

An approach to identify translational activators using a cell-based enzyme-linked immunosorbent assay identified 61 compounds that showed a dose-dependent increase in EAAT2 protein levels (Colton *et al.* 2010). In addition, the same group developed thiopyridazine and pyridazine derivatives that increase EAAT2 expression, although its neuroprotective properties remain to be evaluated (Xing *et al.* 2011).

Targeting the Janus-activated kinase-2 (JAK2) has also been demonstrated to be a potential therapeutic avenue for regulating glutamate transporter function as it was described to be a powerful regulator of glutamate transporters, by showing that co-expression of JAK2 with EAAT2 in *Xenopus* oocytes increased glutamate-induced current by 67%, and thus JAK2 was proposed to participate in the protection against excitotoxicity (Hosseinzadeh *et al.* 2011). However, thus far EAAT2 has not been validated as target for JAK2 and its activation has not been investigated in animal models of excitotoxicity.

Tamoxifen, a selective estrogen receptor modulator that is used to treat breast cancer, was shown to enhance the expression and function of GLT-1/EAAT2 in rat astrocytes (Lee *et al.* 2013). Tamoxifen regulates GLT-1/EAAT-2 via the cAMP response element-binding protein and NF-κB pathways (Karki *et al.* 2013).

Estradiol (17β-estradiol), one of the most active estrogen hormones possessing neuroprotective effects in both *in vivo* and *in vitro* models, has been shown to increase expression of both GLAST and GLT-1 mRNA and protein and glutamate uptake in astrocytes (Lee *et al*. 2013), an effect that seems to be mediated by growth factors such as transforming growth factor-α. However, the adverse effects associated with long-term use of estradiol have hampered its clinical utility (Karki *et al.* 2014).

Another promising therapeutic target for regulating GLT-1 expression is GSK3β (glycogen synthase kinase 3 beta), a kinase involved in multiple cellular processes including neuronal development and synaptic plasticity, that was found through both co-expression and pharmacological studies to stimulate the expression of GLT-1 (while, interestingly, reduced that of GLAST) in COS-7 cells and *Xenopus laevis* oocytes. The authors suggest that this differential modulation may be particularly relevant in pathological conditions such as AD or ischemia in which glutamate transporters and GSK3β are involved (Jimenez *et al.* 2014). Recent evidence suggests that GSK3β regulation of GLT-1 expression in the spinal dorsal horn plays a role with neuropathic pain (Weng *et al.* 2014).

A recent study showed that histamine increased GLT-1 expression in pure cultured astrocytes, decreased the extracellular glutamate content and alleviated neuronal cell death induced by exogenous glutamate. Importantly, histamine was neuroprotective in brain slices after oxygen and glucose deprivation and against ischemic injury in animal model of middle cerebral artery occlusion (Fang *et al.* 2014).

Harmine, a beta-carboline alkaloid, was identified through a screening of a library of 1040 FDA-approved compounds and natural products in fetal-derived human-immortalized astroglial cells stably expressing a firefly luciferase reporter under the control of EAAT2 promoter. Harmine effectively increased GLT-1 expression and transporter activity *in vivo* and presented neuroprotection effects in a rat model of ALS, with the beneficial effects specifically because of up-regulation of GLT-1 (Li *et al.* 2011). A recent study shows that harmine also provides neuroprotection in a global cerebral ischemia model (Sun *et al.* 2014).

Treatment with sulbactam, a β-lactamase inhibitor, was shown to protect pyramidal neurons against brain ischemia. A study showed that both antisense knockdown of GLT-1 expression or inhibition of the GLT-1 uptake activity with dihydrokainate, a selective inhibitor of GLT-1, significantly blocked the neuronal protective effect of sulbactam, indicating that sulbactam has a neuronal protective effect though up-regulation of GLT-1 (Cui *et al.* 2015).

Although β-lactams have been historically used as antimicrobials, a notable ancillary effect in the host was identified by (Rothstein *et al.* 2005), with a blind screen of over 1000 FDA-approved drugs that discovered ceftriaxone enhances the expression of EAAT2. This work was the foundation for the search of therapies using ceftriaxone as up-regulator of GLT-1 expression, and currently there are over 100 reports of the use of ceftriaxone as a neuroprotective drug that increases GLT-1 in many animal models of excitotoxic diseases.

Some of these studies include: ceftriaxone treatment delayed neuronal death and muscle strength loss and increases survival in a mouse model of ALS (Melzer *et al.* 2008). Ceftriaxone was also reported to reduce neurodegeneration and motor deficits in rodent models of stroke (Lipski *et al*. 2007; Thone-Reineke *et al.* 2008) and to attenuate the

damage observed in models of both acute and chronic neurodegenerative disorders (Hota *et al.* 2008; Lee *et al.* 2008, Verma *et al*. 2010). In addition, ceftriaxone treatment before transient focal ischemia up-regulates GLT-1 mRNA and protein and reduces infarct volume in rats (Chu *et al*. 2007). Ceftriaxone was shown to have antinociceptive role in chronic neuropathic pain in rats (Hu *et al.* 2010). Ceftriaxone was shown to reduce the level of brain glutamate, brain edema, and neuronal death following lateral cortical impact injury in rats (Pan *et al.* 2011; Wei *et al.* 2012; Cui *et al.* 2014). There is also evidence that ceftriaxone, by removing glutamate from the synapse more efficiently through up-regulation of GLT-1, resulted in a decrease in post-traumatic seizures after TBI (Goodrich *et al.* 2013). Additionally, in the 6-hydroxydopamine-lesioned model of PD, ceftriaxone has been shown to increase striatal GLT-1 expression and attenuate the normally observed motor symptoms (Leung *et al.* 2012). In the same model, the therapeutic effects of ceftriaxone were equal to L-DOPA, the current gold standard treatment for PD; however, unlike L-DOPA, ceftriaxone did not result in dyskinesia as a side effect (Kelsey & Neville 2014). Additionally, ceftriaxone was shown to have neuroprotective effects in HD (Miller *et al.* 2008; Rebec 2013). Ceftriaxone was also reported to alleviate early brain injury after subarachnoid hemorrhage by increasing EAAT2 expression via the PI3K/Akt/NF-kappaB (Feng *et al.* 2014a). Recently ceftriaxone was shown to protect neurons against global brain ischemia via up-regulation of GLT-1 expression and glutamate uptake (Hu *et al.* 2015) and to preserve glutamate transporters and prevent intermittent hypoxia-induced vulnerability to hypoxia in an *in vitro* on model of chronic intermittent hypoxia (Jagadapillai *et al.* 2014).

Regarding translational studies, an open trial of 108 ALS patients showed that ceftriaxone did not substantially improve muscle strength and disability scores (Beghi *et al.* 2006). Ceftriaxone has failed to show significant efficacy in clinical trials for stroke (Nederkoorn *et al.* 2011). Nevertheless, a clinical trial of ceftriaxone treatment for ALS patients was conducted, which reported to be a safe and tolerable drug for humans, in stages 1 and 2 (Berry *et al.* 2013). Unfortunately, stage 3 was discontinued because of lack of efficacy in increasing length of patient survival or preventing a decline in function (Cudkowicz *et al.* 2013).

Recently, the effects of β-lactam antibiotics ampicillin, cefazolin, and cefoperazone were investigated in a model of ethanol intake in alcohol-preferring rats. Chronic ethanol consumption is known to down-regulate expression of GLT-1, which increases extracellular glutamate levels in subregions of the mesocorticolimbic reward pathway. The compounds significantly reduced ethanol intake and significantly up-regulated both GLT-1 and pAKT expressions in the nucleus accumbens and prefrontal cortex, demonstrating that these compounds appear to be potential therapeutic compounds for treating alcohol abuse and/or dependence (Rao *et al.* 2015).

In contrast to studies demonstrating positive effects of beta-lactams on glutamate expression, other studies have shown inconsistent results. A study found that ceftriaxone did not increase GLT-1 promoter activity in rat hippocampal slices, although a neuroprotective effect was shown, suggesting that the neuroprotection was not associated to the level of GLT-1 protein expression (Lipski *et al*. 2007). Another study showed that chronic administration of ceftriaxone in rats failed to increase GLT-1 mRNA and protein levels in different brain regions, however, intriguingly, it increased GLT-1 activity and conferred neuroprotection in a stroke model (Thone-Reineke *et al*. 2008). Additionally, no effect of ceftriaxone has been detected in various CNS regions in WT C57BL/6 mice, including the spinal cord, however, the drug attenuated disease course and severity in a model of autoimmune CNS inflammation (Melzer *et al*. 2008). Moreover, ceftriaxone slightly reduced GLT-1 levels and significantly decreased glutamate uptake activity in mouse striatal astrocytes (Carbone *et al.* 2012a). Thus, it seems that some yet unidentified factors characteristic to different model systems can prevent the beta-lactam-dependent up-regulation of GLT-1. Therefore, ceftriaxone may not always exhibit antinociceptive or neuroprotective efficacies.

There are many possible explanations for these discrepant results on GLT-1 regulation by beta-lactam antibiotics. First, there seems to be indications that the protective effects of beta-lactam antibiotics are highly dependent on the experimental model, as suggested by the studies presented above. Second, the many ways that GLT-1 expression is measured can have different results. One way is indirect, by measuring survival or amelioration of behavior defects; however, this is not a reliable approach as the drugs might have indirect effects on other targets. Other approaches include to measure transporter expression using immunoblots or measuring mRNA levels, or to measure uptake activity in synaptosome assays. There is some skepticism that these measures are comparable, this is illustrated by one example and challenging idea that synaptosomal assays primarily detect the 5–10% of GLT-1 present in neurons rather than the 90–95% present in astrocytes (Furness *et al*. 2008). If this is correct, one might expect that the uptake activity in synaptosomes could go up dramatically with minor changes to the total brain activity of GLT-1. This could have an impact on the interpretation of a number of studies, and this could also explain why some studies on EAAT2 activators demonstrate lack of up-regulation of expression, despite the fact that neuroprotection is observed. Third, the preservation of tissue used in the assays should be considered, e.g., from human autopsy versus freshly obtained from animals, as these approaches can yield significant different levels of transport (Li *et al.* 2012).

Further studies on ceftriaxone, other beta-lactam antibiotics and derivatives are necessary to determine whether an

analog compound with good brain penetrance, pharmacokinetic properties, and with less risk of side effects and undesired toxicity can be developed into a therapeutic drug.

Recently, a pyridazine derivative (LDN/OSU-0212320) that increases EAAT2 expression through translational activation was shown to protect cultured neurons from glutamate-mediated excitotoxic injury and to delay motor function decline and extend lifespan in an animal model of ALS (Kong *et al.* 2014).

Final remarks on EAAT2 expression enhancers

Reported transcriptional activators of GLT-1, such as ceftriaxone, have been tested in many disease models and are capable of providing neuronal protection. This is also illustrated the newly identified LDN-212320, that was developed to identify more GLT-1/EAAT-2 modulators with improved pharmacological and bioactivity properties.

Moreover, transgenic approaches for EAAT2 over-expression confirm its neuroprotective effects, as shown in a study with double transgenic mice created from crossing the ALS mouse model to a mouse model over-expressing EAAT2, that resulted in an animal that displays delayed grip strength decline and motor neuron loss and increased life expectancy (Guo *et al.* 2003). In addition, HEK (Human Embryonic Kidney) cells engineered to over-express EAAT2 protect cultured motor neurons exposed to glutamate toxicity (Wisman *et al.* 2003).

However, transition from preclinical animal studies to human clinical trials has been challenging, as successful preclinical studies often fail in subsequent clinical trials. Furthermore, increasing the *in vivo* expression levels of EAAT2 could have profound impact on glutamatergic neurotransmission throughout the brain with the intrinsic risk of inducing adverse effects (as exemplified in (Matos-Ocasio *et al.* 2014). Another potential issue is that many of the mechanisms targeted underlie the expression of numerous other genes, and thus transcriptional/translational modulators could potentially exert off-target effects outside the glutamatergic system. Interestingly, the kinetics and duration of the modulation exerted by at least some of these modulators are likely to be significantly different from the properties of those targeting the EAAT protein, which could constitute an advantage or a problem depending on the specific use. Additional issues for EAAT2 translation activators lay in the effect that, although having neuroprotective properties, this class of compounds must be administered prophylactically to be neuroprotective; therefore they have low clinical relevance for acute conditions.

Compounds that increase transporter activity

It has been known for many years that the activity of transporters can be regulated by many means, such as phosphorylation, sulfhydryl oxidation, arachidonic acid, among others (Conradt & Stoffel 1997; Gegelashvili *et al.* 2000).

On the other hand, positive allosteric modulation of GLT-1/EAAT2 with pharmacological compounds represents a relatively novel approach to the treatment of conditions/diseases involving glutamate excitotoxicity. Table 2 summarizes known compounds that interact directly or indirectly with EAAT2 augmenting its catalytic activity.

Riluzole, a highly studied synthetic compound modulator of EAAT2, is a sodium channel-blocking benzothiazole anticonvulsant drug that was demonstrated to decrease excitotoxicity and neurodegeneration through many different mechanisms, including increased glutamate uptake (McIntosh *et al.* 1996; Zhang *et al.* 1998; Azbill *et al.* 2000; Mu *et al.* 2000; Brothers *et al.* 2013). Riluzole significantly increased GLT-1-mediated uptake in transfected HEK293 (Fumagalli *et al.* 2008), rat cortical astrocyte cultures (Frizzo *et al.* 2004), and within striatal astrocytes (Carbone *et al.* 2012a).

Moreover, riluzole was shown to have neuroprotective effects on rat glial cells subjected to glutamate excitotoxicity (Dagci *et al.* 2007), to attenuate the behavioral effects of chronic unpredictable stress, a rodent model of depression (Banasr *et al.* 2010) and to delay the onset of muscle weakness and extends life span by 4–6 weeks in animal models of ALS (Gurney *et al.* 1996; Waibel *et al.* 2004; Del Signore *et al.* 2009).

Currently riluzole is approved by the U.S. Food and Drug Administration for the treatment of ALS (Traynor *et al.* 2006), albeit it prolongs patient life by only a few months (Miller *et al.* 2012b). Additionally, riluzole showed efficacy in preclinical models of spinal cord injury in reducing the extent of sodium and glutamate-mediated secondary injury (Wilson & Fehlings 2014).

Guanosine is a guanine-based purine that activates glutamate uptake and exerts neurotrophic and neuroprotective effects in several models of CNS trauma (Frizzo *et al.* 2001; Frizzo *et al.* 2005), including a model of neonatal hypoxia-ischemia (Moretto *et al.* 2005), with recent evidence suggesting that guanosine is a promising therapeutic agent for the treatment of ischemic brain injury (Hansel *et al.* 2014).

Nicergoline, an ergot derivative marketed under the trade name Sermion for the treatment of cognitive, affective, and behavioral disorders of older people (Fioravanti & Flicker 2001) has been shown to have neuroprotective actions through increase of glutamate transport in rat cortical synaptosomes and cloned glutamate transporters (Nishida *et al.* 2004). This compound was recently subject of a systematic review and meta-analysis that suggested a good safety profile for cerebrovascular disease and dementia (Fioravanti *et al.* 2014).

Additionally, MS-153 ([R]-[-]-5-methyl-1-nicotinoyl-2-pyrazoline), characterized to be a glutamate uptake enhancer acting through GLT-1 (Shimada *et al.* 1999), was first

demonstrated to inhibit elevated brain glutamate levels induced by rat middle cerebral artery occlusion (Umemura *et al.* 1996), then later characterized as neuroprotective in a model of focal ischemia in rats (Kawazura *et al.* 1997). Further studies showed that MS-153 inhibited high voltage-gated calcium channels through interactions with protein kinase C, preventing massive release of glutamate from nerve terminals in ischemic conditions (Uenishi *et al.* 1999). MS-153 has also been shown to affect other conditions that are believed to involve excessive glutamate signaling, including the inhibition of morphine tolerance and dependence (Nakagawa *et al.* 2001), the attenuation of behavioral sensitization development to phencyclidine (Abekawa *et al.* 2002a; Abekawa *et al.* 2002b), an anxiolytic effect in fear conditioning (Li *et al.* 2004), and attenuation of the conditioned rewarding effects of morphine, methamphetamine and cocaine (Nakagawa *et al.* 2005). MS-153 was also recently suggested to have potential as a therapeutic drug for the treatment of alcohol dependence (Alhaddad *et al.* 2014).

Our laboratory has studied the actions of MS-153 in the lateral fluid percussion model of TBI. We found that administration of MS-153 in the acute post-traumatic period provides acute and long-term neuroprotection for TBI, and that the neuroprotective actions of MS-153 can be explained on the basis of inhibition of calcium channels and interactions with PKC (protein Kinase C), rather than activation of glutamate transport. We conclude that MS-153 displays promising potential for developing a much needed therapy for treatment of TBI (Fontana *et al.* 2015).

Our approach to identify compounds that modulate glutamate transport was through the characterization of spider venoms, a valuable source of compounds bioactive on synaptic transmission (Fontana *et al.* 2003; de O Beleboni *et al*. 2004; Estrada *et al.* 2007; Fontana *et al.* 2007). Our group has purified a compound from *Parawixia bistriata* spider venom, referred to as Parawixin1, that was shown to stimulate glutamate uptake in rat synaptosomes and to protect retinal tissue from ischemic damage (Fontana *et al*. 2003) and further characterized to act directly and selectively on the glutamate transporter EAAT2, specifically by facilitating conformational transitions involved in substrate translocation (Fontana *et al*. 2007).

This spider venom provides proof of principle for the development of drugs that act through a novel mechanism and also as a useful tool to further understand the mechanisms that may be involved in augmentation of the transporter function.

The exogenous EAAT2 activators compounds reported herein have, in their majority, non-specific effects that are associated with side effects that limit their utility or exhibit rather promiscuous pharmacological profiles (Table 2). With a goal to identify direct, selective, and potent EAAT2 activators, our laboratory has performed mutagenesis studies that identified a structural region within EAAT2 that is important for the transporter-enhancing activity, comprised of TM domains 2, 5, and 8 (Mortensen *et al.* 2015). Subsequently, this unique structural information was employed in hybrid structure-based virtual screening of a large library to identify novel EAAT2 activating compounds. This approach successfully identified hit compounds (GT949 and GT951) that enhance the activity of EAAT2 in cultured cells and that have neuroprotective properties in glia-neuronal cultures subjected to glutamate excitotoxicity (Fontana, A.C.K. *et al*., unpublished data). Further characterization of these novel allosteric modulators of EAAT2 using medicinal chemistry approaches and *in vivo* pharmacokinetic advanced profiling is currently being performed.

Concluding remarks and prospects

L-glutamate-mediated excitotoxicity is involved in a wide range of acute and chronic pathologies. Over the last decade the scientific community has concluded that drugs targeting glutamate receptors are limited and inefficient, as well as other drugs designed to target the secondary damages of glutamate-mediated excitotoxicity, due to unwanted side effects. Currently, there are no safe and effective drugs for the prevention or treatment of glutamate-mediated excitotoxicity diseases/conditions, thus, there is a need for better therapeutics.

The concept of pursuing GLT-1/EAAT-2 to prevent excitotoxicity has made great progress in recent years; it is now accepted that GLT-1/EAAT-2 transporters are a major target to combat neurotoxicity and provide novel potential therapeutic opportunities for the treatment of neurological diseases. However, the true potential of EAAT2 as target for therapeutics still remains to be understood, appreciated, and explored. Also, as reviewed in this article, the molecular behavior of glutamate transporters in excitotoxicity models is more complex than just general down-regulation or reversal of GLT-1/EAAT2. There is still much to further understand regarding when and how to manipulate the glutamate transport system as a target for excitotoxicity therapies, and also to further elucidate the intracellular signaling pathways that accompany the changes on disease states.

Nevertheless, small molecules designed to up-regulate the activity of GLT-1/EAAT2 are still being developed, and these efforts may reveal intriguing and promising therapeutic avenues. Moreover, efforts toward identification of direct EAAT2 allosteric activators seem to have gained more attention recently as an approach to target these proteins. Additionally, positive EAAT2 allosteric modulators can be administered acutely and be advantageous over the expression enhancers that generally require to be administered prophylactically. It remains to be determined whether small molecules allosteric activators of EAAT2, as for many biologics, lend themselves to conventional pharmacokinetic analysis and are efficacious in animal models of CNS injury.

Perhaps the future will reveal that such a class of compounds is effective in chronic conditions and in combination therapies.

We expect to see, in the upcoming years, the identification of an arsenal of selective pharmacological compounds that target these fascinating transporters and the investigation of their translational possibilities.

Acknowledgments and conflict of interest disclosure

The author thanks Ole V. Mortensen, Argie Zoubroulis, and Ryan Wyrofsky (Drexel University College of Medicine) for helpful discussions. The author also acknowledges Shei Fen (Drexel University College of Medicine) for the art of Fig. 1 (with permission). This work has been supported by startup funds to A.C.K.F. (Drexel University College of Medicine). The author declares none.

All experiments were conducted in compliance with the ARRIVE guidelines.

References

Abekawa T., Honda M., Ito K., Inoue T. and Koyama T. (2002a) Effect of MS-153 on the development of behavioral sensitization to locomotion- and ataxia-inducing effects of phencyclidine. *Psychopharmacology* **160**, 122–131.

Abekawa T., Honda M., Ito K., Inoue T. and Koyama T. (2002b) Effect of MS-153 on the development of behavioral sensitization to stereotypy-inducing effect of phencyclidine. *Brain Res.* **926**, 176–180.

Akbar M. T., Torp R., Danbolt N. C., Levy L. M., Meldrum B. S. and Ottersen O. P. (1997) Expression of glial glutamate transporters GLT-1 and GLAST is unchanged in the hippocampus in fully kindled rats. *Neuroscience* **78**, 351–359.

Akyuz N., Georgieva E. R., Zhou Z. *et al.* (2015) Transport domain unlocking sets the uptake rate of an aspartate transporter. *Nature* **518**, 68–73.

Alhaddad H., Kim N. T., Aal-Aaboda M., Althobaiti Y. S., Leighton J., Boddu S. H., Wei Y. and Sari Y. (2014) Effects of MS-153 on chronic ethanol consumption and GLT1 modulation of glutamate levels in male alcohol-preferring rats. *Front. Behav. Neurosci.* **8**, 366.

Ambrosi G., Cerri S. and Blandini F. (2014) A further update on the role of excitotoxicity in the pathogenesis of Parkinson's disease. *J. Neural. Transm.* **121**, 849–859.

Anderson C. M. and Swanson R. A. (2000) Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* **32**, 1–14.

Arriza J. L., Eliasof S., Kavanaugh M. P. and Amara S. G. (1997) Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proc. Natl Acad. Sci. USA* **94**, 4155–4160.

Arzberger T., Krampfl K., Leimgruber S. and Weindl A. (1997) Changes of NMDA receptor subunit (NR1, NR2B) and glutamate transporter (GLT1) mRNA expression in Huntington's disease–an in situ hybridization study. *J. Neuropathol. Exp. Neurol.* **56**, 440–454.

Assous M., Had-Aissouni L., Gubellini P., Melon C., Nafia I., Salin P., Kerkerian-Le-Goff L. and Kachidian P. (2014) Progressive Parkinsonism by acute dysfunction of excitatory amino acid transporters in the rat substantia nigra. *Neurobiol. Dis.* **65**, 69–81.

Azbill R. D., Mu X. and Springer J. E. (2000) Riluzole increases high-affinity glutamate uptake in rat spinal cord synaptosomes. *Brain Res.* **871**, 175–180.

Baker A. J., Moulton R. J., MacMillan V. H. and Shedden P. M. (1993) Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans. *J. Neurosurg.* **79**, 369–372.

Bamford N. S., Zhang H., Schmitz Y. *et al.* (2004) Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. *Neuron* **42**, 653–663.

Banasr M., Chowdhury G. M., Terwilliger R., Newton S. S., Duman R. S., Behar K. L. and Sanacora G. (2010) Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Mol. Psychiatry* **15**, 501–511.

Banerjee A., Silver L. E., Heneghan C., Welch S. J., Mehta Z., Banning A. P. and Rothwell P. M. (2011) Relative familial clustering of cerebral versus coronary ischemic events. *Circ. Cardiovasc. Genet.* **4**, 390–396.

Bates G. (2003) Huntingtin aggregation and toxicity in Huntington's disease. *Lancet* **361**, 1642–1644.

Beart P. M. and O'Shea R. D. (2007) Transporters for L-glutamate: an update on their molecular pharmacology and pathological involvement. *Br. J. Pharmacol.* **150**, 5–17.

Beghi E., Bendotti C. and Mennini T. (2006) New ideas for therapy in ALS: critical considerations. *Amyotroph. Lateral Scler.* **7**, 126–127; discussion 127.

Bendotti C., Tortarolo M., Suchak S. K., Calvaresi N., Carvelli L., Bastone A., Rizzi M., Rattray M. and Mennini T. (2001) Transgenic SOD1 G93A mice develop reduced GLT-1 in spinal cord without alterations in cerebrospinal fluid glutamate levels. *J. Neurochem.* **79**, 737–746.

Benveniste H., Drejer J., Schousboe A. and Diemer N. H. (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J. Neurochem.* **43**, 1369–1374.

Beresford I. J., Parsons A. A. and Hunter A. J. (2003) Treatments for stroke. *Expert Opin. Emerg. Drugs* **8**, 103–122.

Berger U. V. and Hediger M. A. (1998) Comparative analysis of glutamate transporter expression in rat brain using differential double in situ hybridization. *Anat. Embryol.* **198**, 13–30.

Bergles D. E. and Jahr C. E. (1997) Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* **19**, 1297–1308.

Bergles D. E., Tzingounis A. V. and Jahr C. E. (2002) Comparison of coupled and uncoupled currents during glutamate uptake by GLT-1 transporters. *J. Neurosci.* **22**, 10153–10162.

Berry J. D., Shefner J. M., Conwit R. *et al.* (2013) Design and initial results of a multi-phase randomized trial of ceftriaxone in amyotrophic lateral sclerosis. *PLoS ONE* **8**, e61177.

Billups B. and Attwell D. (1996) Modulation of non-vesicular glutamate release by pH. *Nature* **379**, 171–174.

Bjornsen L. P., Eid T., Holmseth S., Danbolt N. C., Spencer D. D. and de Lanerolle N. C. (2007) Changes in glial glutamate transporters in human epileptogenic hippocampus: inadequate explanation for high extracellular glutamate during seizures. *Neurobiol. Dis.* **25**, 319–330.

Bonde C., Noraberg J., Noer H. and Zimmer J. (2005) Ionotropic glutamate receptors and glutamate transporters are involved in necrotic neuronal cell death induced by oxygen-glucose deprivation of hippocampal slice cultures. *Neuroscience* **136**, 779–794.

Boudker O., Ryan R. M., Yernool D., Shimamoto K. and Gouaux E. (2007) Coupling substrate and ion binding to extracellular gate of a sodium-dependent aspartate transporter. *Nature* **445**, 387–393.

Bough K. J., Paquet M., Pare J. F., Hassel B., Smith Y., Hall R. A. and Dingledine R. (2007) Evidence against enhanced glutamate transport in the anticonvulsant mechanism of the ketogenic diet. *Epilepsy Res.* **74**, 232–236.

Bridges R. J., Kavanaugh M. P. and Chamberlin A. R. (1999) A pharmacological review of competitive inhibitors and substrates of high-affinity, sodium-dependent glutamate transport in the central nervous system. *Curr. Pharm. Des.* **5**, 363–379.

Brothers H. M., Bardou I., Hopp S. C., Kaercher R. M., Corona A. W., Fenn A. M., Godbout J. P. and Wenk G. L. (2013) Riluzole partially rescues age-associated, but not LPS-induced, loss of glutamate transporters and spatial memory. *J. Neuroimmune Pharmacol.* **8**, 1098–1105.

Brown J. I., Baker A. J., Konasiewicz S. J. and Moulton R. J. (1998) Clinical significance of CSF glutamate concentrations following severe traumatic brain injury in humans. *J. Neurotrauma* **15**, 253–263.

Browne S. E. and Beal M. F. (2006) Oxidative damage in Huntington's disease pathogenesis. *Antioxid. Redox Signal.* **8**, 2061–2073.

Bruijn L. I., Becher M. W., Lee M. K. *et al.* (1997) ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* **18**, 327–338.

Bunch L., Erichsen M. N. and Jensen A. A. (2009) Excitatory amino acid transporters as potential drug targets. *Expert Opin. Ther. Targets* **13**, 719–731.

Burda J. E., Bernstein A. M. and Sofroniew M. V. (2015) Astrocyte roles in traumatic brain injury. *Exp. Neurol.* pii: S0014-4886(15)00089-8. doi: 10.1016/j.expneurol.2015.03.020. [Epub ahead of print] PubMed PMID: 25828533.

Carbone M., Duty S. and Rattray M. (2012a) Riluzole elevates GLT-1 activity and levels in striatal astrocytes. *Neurochem. Int.* **60**, 31–38.

Carbone M., Duty S. and Rattray M. (2012b) Riluzole neuroprotection in a Parkinson's disease model involves suppression of reactive astrocytosis but not GLT-1 regulation. *BMC Neurosci.* **13**, 38.

Cavus I., Kasoff W. S., Cassaday M. P., Jacob R., Gueorguieva R., Sherwin R. S., Krystal J. H., Spencer D. D. and Abi-Saab W. M. (2005) Extracellular metabolites in the cortex and hippocampus of epileptic patients. *Ann. Neurol.* **57**, 226–235.

Chao X. D., Fei F. and Fei Z. (2010) The role of excitatory amino acid transporters in cerebral ischemia. *Neurochem. Res.* **35**, 1224–1230.

Chen W., Mahadomrongkul V., Berger U. V., Bassan M., DeSilva T., Tanaka K., Irwin N., Aoki C. and Rosenberg P. A. (2004) The glutamate transporter GLT1a is expressed in excitatory axon terminals of mature hippocampal neurons. *J. Neurosci.* **24**, 1136–1148.

Cheney J. A., Brown A. L., Bareyre F. M., Russ A. B., Weisser J. D., Ensinger H. A., Leusch A., Raghupathi R. and Saatman K. E. (2000) The novel compound LOE 908 attenuates acute neuromotor dysfunction but not cognitive impairment or cortical tissue loss following traumatic brain injury in rats. *J. Neurotrauma* **17**, 83–91.

Cheng C., Glover G., Banker G. and Amara S. G. (2002) A novel sorting motif in the glutamate transporter excitatory amino acid transporter 3 directs its targeting in Madin-Darby canine kidney cells and hippocampal neurons. *J. Neurosci.* **22**, 10643–10652.

Chotibut T., Davis R. W., Arnold J. C., Frenchek Z., Gurwara S., Bondada V., Geddes J. W. and Salvatore M. F. (2014) Ceftriaxone increases glutamate uptake and reduces striatal tyrosine hydroxylase loss in 6-OHDA Parkinson's model. *Mol. Neurobiol.* **49**, 1282–1292.

Chu K., Lee S. T., Sinn D. I. *et al.* (2007) Pharmacological induction of ischemic tolerance by glutamate transporter-1 (EAAT2) upregulation. *Stroke* **38**, 177–182.

Cisneros I. E. and Ghorpade A. (2014) Methamphetamine and HIV-1-induced neurotoxicity: role of trace amine associated receptor 1 cAMP signaling in astrocytes. *Neuropharmacology* **85**, 499–507.

Clements J. D. (1996) Transmitter timecourse in the synaptic cleft: its role in central synaptic function. *Trends Neurosci.* **19**, 163–171.

Clements J., Lester R., Tong G., Jahr C. and Westbrook G. (1992) The time course of glutamate in the synaptic cleft. *Science* **258**, 1498–1501.

Collins M., Lovell M. R., Iverson G. L., Ide T. and Maroon J. (2006) Examining concussion rates and return to play in high school football players wearing newer helmet technology: a three-year prospective cohort study. *Neurosurgery* **58**, 275–286; discussion 275-286.

Colton C. K., Kong Q., Lai L., Zhu M. X., Seyb K. I., Cuny G. D., Xian J., Glicksman M. A. and Lin C. L. (2010) Identification of translational activators of glial glutamate transporter EAAT2 through cell-based high-throughput screening: an approach to prevent excitotoxicity. *J. Biomol. Screen.* **15**, 653–662.

Conradt M. and Stoffel W. (1997) Inhibition of the high-affinity brain glutamate transporter GLAST-1 via direct phosphorylation. *J. Neurochem.* **68**, 1244–1251.

Coulter D. A. and Eid T. (2012) Astrocytic regulation of glutamate homeostasis in epilepsy. *Glia* **60**, 1215–1226.

Courchesne E. (1997) Brainstem, cerebellar and limbic neuroanatomical abnormalities in autism. *Curr. Opin. Neurobiol.* **7**, 269–278.

Coutinho-Netto J., Abdul-Ghani A. S., Collins J. F. and Bradford H. F. (1981) Is glutamate a trigger factor in epileptic hyperactivity? *Epilepsia* **22**, 289–296.

Crunelli V., Carmignoto G. and Steinhäuser C. (2015) Astrocytic targets provide new avenues for the therapeutic treatment of epilepsy. *Neuroscientist* **21**, 62–83.

Cudkowicz M., Shefner J. and Consortium N. (2013) STAGE 3 clinical trial of ceftriaxone in subjects with ALS (S36.001). *Neurology* **80** (Meeting Abstracts 1): S36.001.

Cui C., Cui Y., Gao J. *et al.* (2014) Neuroprotective effect of ceftriaxone in a rat model of traumatic brain injury. *Neurol. Sci.* **35**, 695–700.

Cui X., Li L., Hu Y. Y., Ren S., Zhang M. and Li W. B. (2015) Sulbactam plays neuronal protective effect against brain ischemia via upregulating GLT1 in rats. *Mol. Neurobiol.* **51**, 1322–1333.

Dagci T., Yilmaz O., Taskiran D. and Peker G. (2007) Neuroprotective agents: is effective on toxicity in glial cells? *Cell. Mol. Neurobiol.* **27**, 171–177.

Danbolt N. C. (2001) Glutamate uptake. *Prog. Neurobiol.* **65**, 1–105.

David Y., Cacheaux L. P., Ivens S., Lapilover E., Heinemann U., Kaufer D. and Friedman A. (2009) Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J. Neurosci.* **29**, 10588–10599.

De Bundel D., Schallier A., Loyens E. *et al.* (2011) 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. *J. Neurosci.* **31**, 5792–5803.

Dehnes Y., Chaudhry F. A., Ullensvang K., Lehre K. P., Storm-Mathisen J. and Danbolt N. C. (1998) The glutamate transporter EAAT4 in rat cerebellar Purkinje cells: a glutamate-gated chloride channel concentrated near the synapse in parts of the dendritic membrane facing astroglia. *J. Neurosci.* **18**, 3606–3619.

Del Signore S. J., Amante D. J., Kim J., Stack E. C., Goodrich S., Cormier K., Smith K., Cudkowicz M. E. and Ferrante R. J. (2009) Combined riluzole and sodium phenylbutyrate therapy in transgenic amyotrophic lateral sclerosis mice. *Amyotroph. Lateral Scler.* **10**, 85–94.

Deumens R., Blokland A. and Prickaerts J. (2002) Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. *Exp. Neurol.* **175**, 303–317.

Diamond J. S. and Jahr C. E. (1997) Transporters buffer synaptically released glutamate on a submillisecond time scale. *J. Neurosci.* **17**, 4672–4687.

During M. J. and Spencer D. D. (1993) Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet* **341**, 1607–1610.

Edwards R. H. (2015) Mobile binding sites regulate glutamate clearance. *Nat. Neurosci.* **18**, 166–168.

Eid T., Thomas M. J., Spencer D. D. *et al.* (2004) Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet* **363**, 28–37.

Eisenberg M. A., Andrea J., Meehan W. and Mannix R. (2013) Time interval between concussions and symptom duration. *Pediatrics* **132**, 8–17.

Eisenberg M. A., Meehan W. P., 3rd and Mannix R. (2014) Duration and course of post-concussive symptoms. *Pediatrics* **133**, 999–1006.

Estrada G., Villegas E. and Corzo G. (2007) Spider venoms: a rich source of acylpolyamines and peptides as new leads for CNS drugs. *Nat. Prod. Rep.* **24**, 145–161.

Estrada-Sanchez A. M. and Rebec G. V. (2012) Corticostriatal dysfunction and glutamate transporter 1 (GLT1) in Huntington's disease: interactions between neurons and astrocytes. *Basal Ganglia* **2**, 57–66.

Estrada-Sanchez A. M., Montiel T., Segovia J. and Massieu L. (2009) Glutamate toxicity in the striatum of the R6/2 Huntington's disease transgenic mice is age-dependent and correlates with decreased levels of glutamate transporters. *Neurobiol. Dis.* **34**, 78–86.

Faden A. I., Demediuk P., Panter S. S. and Vink R. (1989) The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science* **244**, 798–800.

Faideau M., Kim J., Cormier K. *et al.* (2010) In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. *Hum. Mol. Genet.* **19**, 3053–3067.

Fairman W. A., Vandenberg R. J., Arriza J. L., Kavanaugh M. P. and Amara S. G. (1995) An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature* **375**, 599–603.

Fang Q., Hu W. W., Wang X. F. *et al.* (2014) Histamine up-regulates astrocytic glutamate transporter 1 and protects neurons against ischemic injury. *Neuropharmacology* **77**, 156–166.

Feng D., Wang W., Dong Y. *et al.* (2014a) Ceftriaxone alleviates early brain injury after subarachnoid hemorrhage by increasing excitatory amino acid transporter 2 expression via the PI3K/Akt/NF-kappaB signaling pathway. *Neuroscience* **268**, 21–32.

Feng Y., Lu S., Wang J., Kumar P., Zhang L. and Bhatt A. J. (2014b) Dexamethasone-induced neuroprotection in hypoxic-ischemic brain injury in newborn rats is partly mediated via Akt activation. *Brain Res.* **1589c**, 68–77.

Figiel M. and Engele J. (2000) Pituitary adenylate cyclase-activating polypeptide (PACAP), a neuron-derived peptide regulating glial glutamate transport and metabolism. *J. Neurosci.* **20**, 3596–3605.

Finnie J. (2001) Animal models of traumatic brain injury: a review. *Aust. Vet. J.* **79**, 628–633.

Fioravanti M. and Flicker L. (2001) Efficacy of nicergoline in dementia and other age associated forms of cognitive impairment. *Cochrane Database Syst. Rev.* **4**, Cd003159.

Fioravanti M., Nakashima T., Xu J. and Garg A. (2014) A systematic review and meta-analysis assessing adverse event profile and tolerability of nicergoline. *BMJ open* **4**, e005090.

Fontana A. C., Guizzo R., de Oliveira Beleboni R., Meirelles E. S. A. R., Coimbra N. C., Amara S. G., dos Santos W. F. and Coutinho-Netto J. (2003) Purification of a neuroprotective component of Parawixia bistriata spider venom that enhances glutamate uptake. *Br. J. Pharmacol.* **139**, 1297–1309.

Fontana A. C., de Oliveira Beleboni R., Wojewodzic M. W., Ferreira Dos Santos W., Coutinho-Netto J., Grutle N. J., Watts S. D., Danbolt N. C. and Amara S. G. (2007) Enhancing glutamate transport: mechanism of action of Parawixin1, a neuroprotective compound from Parawixia bistriata spider venom. *Mol. Pharmacol.* **72**, 1228–1237.

Fontana A. C., Fox D. P., Zoubroulis A., Mortensen O. V. and Raghupathi R. (2015) Neuroprotective effects of the glutamate transporter activator, MS-153, following traumatic brain injury in the adult rat. *J. Neurotrauma* doi: 10.1089/neu.2015.4079 [Epub ahead of print].

Frizzo M. E., Lara D. R., Dahm K. C., Prokopiuk A. S., Swanson R. A. and Souza D. O. (2001) Activation of glutamate uptake by guanosine in primary astrocyte cultures. *NeuroReport* **12**, 879–881.

Frizzo M. E., Dall'Onder L. P., Dalcin K. B. and Souza D. O. (2004) Riluzole enhances glutamate uptake in rat astrocyte cultures. *Cell. Mol. Neurobiol.* **24**, 123–128.

Frizzo M. E., Schwalm F. D., Frizzo J. K., Soares F. A. and Souza D. O. (2005) Guanosine enhances glutamate transport capacity in brain cortical slices. *Cell. Mol. Neurobiol.* **25**, 913–921.

Fumagalli E., Funicello M., Rauen T., Gobbi M. and Mennini T. (2008) Riluzole enhances the activity of glutamate transporters GLAST, GLT1 and EAAC1. *Eur. J. Pharmacol.* **578**, 171–176.

Furness D. N., Dehnes Y., Akhtar A. Q. *et al.* (2008) A quantitative assessment of glutamate uptake into hippocampal synaptic terminals and astrocytes: new insights into a neuronal role for excitatory amino acid transporter 2 (EAAT2). *Neuroscience* **157**, 80–94.

Furuta A., Rothstein J. D. and Martin L. J. (1997) Glutamate transporter protein subtypes are expressed differentially during rat CNS development. *J. Neurosci.* **17**, 8363–8375.

Ganel R., Ho T., Maragakis N. J., Jackson M., Steiner J. P. and Rothstein J. D. (2006) Selective up-regulation of the glial Na+-dependent glutamate transporter GLT1 by a neuroimmunophilin ligand results in neuroprotection. *Neurobiol. Dis.* **21**, 556–567.

Gegelashvili G. and Bjerrum O. J. (2014) High-affinity glutamate transporters in chronic pain: an emerging therapeutic target. *J. Neurochem.* **131**, 712–730.

Gegelashvili G., Dehnes Y., Danbolt N. C. and Schousboe A. (2000) The high-affinity glutamate transporters GLT1, GLAST, and EAAT4 are regulated via different signalling mechanisms. *Neurochem. Int.* **37**, 163–170.

Gether U., Andersen P. H., Larsson O. M. and Schousboe A. (2006) Neurotransmitter transporters: molecular function of important drug targets. *Trends Pharmacol. Sci.* **27**, 375–383.

Ghanizadeh A. and Berk M. (2015) Beta-lactam antibiotics as a possible novel therapy for managing epilepsy and autism, a case report and review of literature. *Iran. J. Child Neurol.* **9**, 99–102.

Gincel D., Regan M. R., Jin L., Watkins A. M., Bergles D. E. and Rothstein J. D. (2007) Analysis of cerebellar Purkinje cells using EAAT4 glutamate transporter promoter reporter in mice generated via bacterial artificial chromosome-mediated transgenesis. *Exp. Neurol.* **203**, 205–212.

Ginsberg M. D. (2008) Neuroprotection for ischemic stroke: past, present and future. *Neuropharmacology* **55**, 363–389.

Giza C. C., Mink R. B. and Madikians A. (2007) Pediatric traumatic brain injury: not just little adults. *Curr. Opin. Crit. Care* **13**, 143–152.

Goodrich G. S., Kabakov A. Y., Hameed M. Q., Dhamne S. C., Rosenberg P. A. and Rotenberg A. (2013) Ceftriaxone treatment after traumatic brain injury restores expression of the glutamate transporter, GLT-1, reduces regional gliosis, and reduces post-traumatic seizures in the rat. *J. Neurotrauma* **30**, 1434–1441.

Grewer C., Gameiro A., Zhang Z., Tao Z., Braams S. and Rauen T. (2008) Glutamate forward and reverse transport: from molecular mechanism to transporter-mediated release after ischemia. *IUBMB Life* **60**, 609–619.

Grewer C., Gameiro A. and Rauen T. (2014) SLC1 glutamate transporters. *Pflugers Arch.* **466**, 3–24.

Guerriero R. M., Giza C. C. and Rotenberg A. (2015) Glutamate and GABA imbalance following traumatic brain injury. *Curr. Neurol. Neurosci. Rep.* **15**, 545.

Guo H., Lai L., Butchbach M. E., Stockinger M. P., Shan X., Bishop G. A. and Lin C. L. (2003) Increased expression of the glial glutamate transporter EAAT2 modulates excitotoxicity and delays the onset but not the outcome of ALS in mice. *Hum. Mol. Genet.* **12**, 2519–2532.

Guo F., Sun F., Yu J. L. *et al.* (2010) Abnormal expressions of glutamate transporters and metabotropic glutamate receptor 1 in the spontaneously epileptic rat hippocampus. *Brain Res. Bull.* **81**, 510–516.

Gupta R. K. and Prasad S. (2014) Differential regulation of GLT-1/EAAT2 gene expression by NF-kappaB and N-myc in male mouse brain during postnatal development. *Neurochem. Res.* **39**, 150–160.

Gurney M. E., Cutting F. B., Zhai P., Doble A., Taylor C. P., Andrus P. K. and Hall E. D. (1996) Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. *Ann. Neurol.* **39**, 147–157.

Hakuba N., Koga K., Gyo K., Usami S. I. and Tanaka K. (2000) Exacerbation of noise-induced hearing loss in mice lacking the glutamate transporter GLAST. *J. Neurosci.* **20**, 8750–8753.

Hansel G., Tonon A. C., Guella F. L., Pettenuzzo L. F., Duarte T., Duarte M. M., Oses J. P., Achaval M. and Souza D. O. (2014) Guanosine protects against cortical focal ischemia. Involvement of inflammatory response. *Mol. Neurobiol.* PMID: 25394382.

Hardingham G. E. and Bading H. (2010) Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat. Rev. Neurosci.* **11**, 682–696.

Hardy J., Cowburn R., Barton A., Reynolds G., Lofdahl E., O'Carroll A. M., Wester P. and Winblad B. (1987) Region-specific loss of glutamate innervation in Alzheimer's disease. *Neurosci. Lett.* **73**, 77–80.

Harvey B. K., Airavaara M., Hinzman J. *et al.* (2011) Targeted over-expression of glutamate transporter 1 (GLT-1) reduces ischemic brain injury in a rat model of stroke. *PLoS ONE* **6**, e22135.

Hassel B., Iversen E. G., Gjerstad L. and Tauboll E. (2001) Up-regulation of hippocampal glutamate transport during chronic treatment with sodium valproate. *J. Neurochem.* **77**, 1285–1292.

Haugeto O., Ullensvang K., Levy L. M., Chaudhry F. A., Honore T., Nielsen M., Lehre K. P. and Danbolt N. C. (1996) Brain glutamate transporter proteins form homomultimers. *J. Biol. Chem.* **271**, 27715–27722.

Hazell A. S. (2007) Excitotoxic mechanisms in stroke: an update of concepts and treatment strategies. *Neurochem. Int.* **50**, 941–953.

van der Hel W. S., Notenboom R. G., Bos I. W., van Rijen P. C., van Veelen C. W. and de Graan P. N. (2005) Reduced glutamine synthetase in hippocampal areas with neuron loss in temporal lobe epilepsy. *Neurology* **64**, 326–333.

Herman M. A. and Jahr C. E. (2007) Extracellular glutamate concentration in hippocampal slice. *J. Neurosci.* **27**, 9736–9741.

Hogan D. B. (2014) Long-term efficacy and toxicity of cholinesterase inhibitors in the treatment of Alzheimer disease. *Can. J. Psychiatry* **59**, 618–623.

Holmseth S., Dehnes Y., Huang Y. H. *et al.* (2012) The density of EAAC1 (EAAT3) glutamate transporters expressed by neurons in the mammalian CNS. *J. Neurosci.* **32**, 6000–6013.

Hosseinzadeh Z., Bhavsar S. K., Sopjani M., Alesutan I., Saxena A., Dermaku-Sopjani M. and Lang F. (2011) Regulation of the glutamate transporters by JAK2. *Cell. Physiol. Biochem.* **28**, 693–702.

Hota S. K., Barhwal K., Ray K., Singh S. B. and Ilavazhagan G. (2008) Ceftriaxone rescues hippocampal neurons from excitotoxicity and enhances memory retrieval in chronic hypobaric hypoxia. *Neurobiol. Learn. Mem.* **89**, 522–532.

Hu Y., Li W., Lu L., Cai J., Xian X., Zhang M., Li Q. and Li L. (2010) An anti-nociceptive role for ceftriaxone in chronic neuropathic pain in rats. *Pain* **148**, 284–301.

Hu Y. Y., Xu J., Zhang M., Wang D., Li L. and Li W. B. (2015) Ceftriaxone modulates uptake activity of glial glutamate transporter-1 against global brain ischemia in rats. *J. Neurochem.* **132**, 194–205.

Huh J. W. and Raghupathi R. (2009) New concepts in treatment of pediatric traumatic brain injury. *Anesthesiol. Clin.* **27**, 213–240.

Hynd M. R., Scott H. L. and Dodd P. R. (2004) Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochem. Int.* **45**, 583–595.

Ikematsu K., Tsuda R., Kondo T. and Nakasono I. (2002) The expression of excitatory amino acid transporter 2 in traumatic brain injury. *Forensic Sci. Int.* **130**, 83–89.

Ikonomidou C. and Turski L. (2002) Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? *Lancet Neurol.* **1**, 383–386.

Izumi Y., Shimamoto K., Benz A. M., Hammerman S. B., Olney J. W. and Zorumski C. F. (2002) Glutamate transporters and retinal excitotoxicity. *Glia* **39**, 58–68.

Jabaudon D., Scanziani M., Gahwiler B. H. and Gerber U. (2000) Acute decrease in net glutamate uptake during energy deprivation. *Proc. Natl Acad. Sci. USA* **97**, 5610–5615.

Jabs R., Seifert G. and Steinhauser C. (2008) Astrocytic function and its alteration in the epileptic brain. *Epilepsia* **49**(Suppl 2), 3–12.

Jagadapillai R., Mellen N. M., Sachleben L. R., Jr and Gozal E. (2014) Ceftriaxone preserves glutamate transporters and prevents intermittent hypoxia-induced vulnerability to brain excitotoxic injury. *PLoS ONE* **9**, e100230.

Jensen A. A., Fahlke C., Bjorn-Yoshimoto W. E. and Bunch L. (2015) Excitatory amino acid transporters: recent insights into molecular mechanisms, novel modes of modulation and new therapeutic possibilities. *Curr. Opin. Pharmacol.* **20**, 116–123.

Ji Y. F., Zhou L., Xie Y. J. *et al.* (2013) Upregulation of glutamate transporter GLT-1 by mTOR-Akt-NF-small ka, CyrillicB cascade in astrocytic oxygen-glucose deprivation. *Glia* **61**, 1959–1975.

Jimenez E., Nunez E., Ibanez I., Draffin J. E., Zafra F. and Gimenez C. (2014) Differential regulation of the glutamate transporters GLT-1 and GLAST by GSK3beta. *Neurochem. Int.* **79**, 33–43.

Kaila K., Ruusuvuori E., Seja P., Voipio J. and Puskarjov M. (2014) GABA actions and ionic plasticity in epilepsy. *Curr. Opin. Neurobiol.* **26**, 34–41.

Kanai Y. and Hediger M. A. (1992) Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature* **360**, 467–471.

Kanner B. I. (1983) Bioenergetics of neurotransmitter transport. *Biochim. Biophys. Acta* **726**, 293–316.

Kanner B. I. (1989) Ion-coupled neurotransmitter transport. *Curr. Opin. Cell Biol.* **1**, 735–738.

Kanner B. I. and Schuldiner S. (1987) Mechanism of transport and storage of neurotransmitters. *CRC Crit. Rev. Biochem.* **22**, 1–38.

Karki P., Webb A., Smith K., Lee K., Son D. S., Aschner M. and Lee E. (2013) cAMP response element-binding protein (CREB) and nuclear factor kappaB mediate the tamoxifen-induced up-

regulation of glutamate transporter 1 (GLT-1) in rat astrocytes. *J. Biol. Chem.* **288**, 28975–28986.

Karki P., Smith K., Johnson J., Jr and Lee E. (2014) Astrocyte-derived growth factors and estrogen neuroprotection: role of transforming growth factor-alpha in estrogen-induced upregulation of glutamate transporters in astrocytes. *Mol. Cell. Endocrinol.* **389**, 58–64.

Katagiri H., Tanaka K. and Manabe T. (2001) Requirement of appropriate glutamate concentrations in the synaptic cleft for hippocampal LTP induction. *Eur. J. Neuorsci.* **14**, 547–553.

Katayama Y., Becker D. P., Tamura T. and Hovda D. A. (1990) Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. *J. Neurosurg.* **73**, 889–900.

Kawazura H., Takahashi Y., Shiga Y., Shimada F., Ohto N. and Tamura A. (1997) Cerebroprotective effects of a novel pyrazoline derivative, MS-153, on focal ischemia in rats. *Jpn. J. Pharmacol.* **73**, 317–324.

Kelsey J. E. and Neville C. (2014) The effects of the beta-lactam antibiotic, ceftriaxone, on forepaw stepping and L-DOPA-induced dyskinesia in a rodent model of Parkinson's disease. *Psychopharmacology* **231**, 2405–2415.

Khodorov B. I. (2000) Mechanisms of destabilization of Ca^{2+}-homeostasis of brain neurons caused by toxic glutamate challenge. *Membr. Cell Biol.* **14**, 149–162.

Kim K., Lee S. G., Kegelman T. P. *et al.* (2011) Role of excitatory amino acid transporter-2 (EAAT2) and glutamate in neurodegeneration: opportunities for developing novel therapeutics. *J. Cell. Physiol.* **226**, 2484–2493.

Kimura A., Guo X., Noro T., Harada C., Tanaka K., Namekata K. and Harada T. (2015) Valproic acid prevents retinal degeneration in a murine model of normal tension glaucoma. *Neurosci. Lett.* **588**, 108–113.

Kiryk A., Aida T., Tanaka K. *et al.* (2008) Behavioral characterization of GLT1 (+/−) mice as a model of mild glutamatergic hyperfunction. *Neurotox. Res.* **13**, 19–30.

Kong Q., Takahashi K., Schulte D., Stouffer N., Lin Y. and Lin C. L. (2012) Increased glial glutamate transporter EAAT2 expression reduces epileptogenic processes following pilocarpine-induced status epilepticus. *Neurobiol. Dis.* **47**, 145–154.

Kong Q., Chang L. C., Takahashi K. *et al.* (2014) Small-molecule activator of glutamate transporter EAAT2 translation provides neuroprotection. *J. Clin. Investig.* **124**, 1255–1267.

Kosugi T. and Kawahara K. (2006) Reversed actrocytic GLT-1 during ischemia is crucial to excitotoxic death of neurons, but contributes to the survival of astrocytes themselves. *Neurochem. Res.* **31**, 933–943.

Kristensen A. S., Andersen J., Jorgensen T. N., Sorensen L., Eriksen J., Loland C. J., Stromgaard K. and Gether U. (2011) SLC6 neurotransmitter transporters: structure, function, and regulation. *Pharmacol. Rev.* **63**, 585–640.

Krzyzanowska W., Pomierny B., Filip M. and Pera J. (2014) Glutamate transporters in brain ischemia: to modulate or not? *Acta Pharmacol. Sin.* **35**, 444–462.

Kulijewicz-Nawrot M., Sykova E., Chvatal A., Verkhratsky A. and Rodriguez J. J. (2013) Astrocytes and glutamate homoeostasis in Alzheimer's disease: a decrease in glutamine synthetase, but not in glutamate transporter-1, in the prefrontal cortex. *ASN Neuro.* **5**, 273–282.

Kumar A., Singh A. and Ekavali (2015) A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol. Rep.* **67**, 195–203.

van Landeghem F. K., Weiss T., Oehmichen M. and von Deimling A. (2006) Decreased expression of glutamate transporters in astrocytes after human traumatic brain injury. *J. Neurotrauma* **23**, 1518–1528.

de Lanerolle N. C., Kim J. H. and Bandak F. A. (2015) Neuropathology of traumatic brain injury: comparison of penetrating, nonpenetrating direct impact and explosive blast etiologies. *Semin. Neurol.* **35**, 012–019.

Larsson H. P., Tzingounis A. V., Koch H. P. and Kavanaugh M. P. (2004) Fluorometric measurements of conformational changes in glutamate transporters. *Proc. Natl Acad. Sci. USA* **101**, 3951–3956.

Lauderback C. M., Hackett J. M., Huang F. F., Keller J. N., Szweda L. I., Markesbery W. R. and Butterfield D. A. (2001) The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of Abeta1-42. *J. Neurochem.* **78**, 413–416.

Lauriat T. L. and McInnes L. A. (2007) EAAT2 regulation and splicing: relevance to psychiatric and neurological disorders. *Mol. Psychiatry* **12**, 1065–1078.

Lebrun B., Sakaitani M., Shimamoto K., Yasuda-Kamatani Y. and Nakajima T. (1997) New beta-hydroxyaspartate derivatives are competitive blockers for the bovine glutamate/aspartate transporter. *J. Biol. Chem.* **272**, 20336–20339.

Lee S. G., Su Z. Z., Emdad L., Gupta P., Sarkar D., Borjabad A., Volsky D. J. and Fisher P. B. (2008) Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. *J. Biol. Chem.* **283**, 13116–13123.

Lee E., Sidoryk-Wegrzynowicz M., Farina M., Rocha J. B. and Aschner M. (2013) Estrogen attenuates manganese-induced glutamate transporter impairment in rat primary astrocytes. *Neurotox. Res.* **23**, 124–130.

Lehre K. P. and Danbolt N. C. (1998) The number of glutamate transporter subtype molecules at glutamatergic synapses: chemical and stereological quantification in young adult rat brain. *J. Neurosci.* **18**, 8751–8757.

Lehre K. P., Levy L. M., Ottersen O. P., Storm-Mathisen J. and Danbolt N. C. (1995) Differential expression of two glial glutamate transporters in the rat brain: quantitative and immunocytochemical observations. *J. Neurosci.* **15**, 1835–1853.

Leung T. C., Lui C. N., Chen L. W., Yung W. H., Chan Y. S. and Yung K. K. (2012) Ceftriaxone ameliorates motor deficits and protects dopaminergic neurons in 6-hydroxydopamine-lesioned rats. *ACS Chem. Neurosci.* **3**, 22–30.

Levy L. M., Lehre K. P., Walaas S. I., Storm-Mathisen J. and Danbolt N. C. (1995) Down-regulation of glial glutamate transporters after glutamatergic denervation in the rat brain. *Eur. J. Neuorsci.* **7**, 2036–2041.

Levy L. M., Warr O. and Attwell D. (1998) Stoichiometry of the glial glutamate transporter GLT-1 expressed inducibly in a Chinese hamster ovary cell line selected for low endogenous Na+-dependent glutamate uptake. *J. Neurosci.* **18**, 9620–9628.

Li S., Mallory M., Alford M., Tanaka S. and Masliah E. (1997) Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. *J. Neuropathol. Exp. Neurol.* **56**, 901–911.

Li X., Inouei T., Abekawai T., YiRui F. and Koyama T. (2004) Effect of MS-153 on the acquisition and expression of conditioned fear in rats. *Eur. J. Pharmacol.* **505**, 145–149.

Li L. B., Toan S. V., Zelenaia O., Watson D. J., Wolfe J. H., Rothstein J. D. and Robinson M. B. (2006) Regulation of astrocytic glutamate transporter expression by Akt: evidence for a selective transcriptional effect on the GLT-1/EAAT2 subtype. *J. Neurochem.* **97**, 759–771.

Li Y., Sattler R., Yang E. J., Nunes A., Ayukawa Y., Akhtar S., Ji G., Zhang P. W. and Rothstein J. D. (2011) Harmine, a natural beta-carboline alkaloid, upregulates astroglial glutamate transporter expression. *Neuropharmacology* **60**, 1168–1175.

Li Y., Zhou Y. and Danbolt N. C. (2012) The rates of postmortem proteolysis of glutamate transporters differ dramatically between cells and between transporter subtypes. *J. Histochem. Cytochem.* **60**, 811–821.

Li D., Herault K., Silm K., Evrard A., Wojcik S., Oheim M., Herzog E. and Ropert N. (2013) Lack of evidence for vesicular glutamate transporter expression in mouse astrocytes. *J. Neurosci.* **33**, 4434–4455.

Li K., Hala T. J., Seetharam S., Poulsen D. J., Wright M. C. and Lepore A. C. (2015) GLT1 overexpression in SOD1 (G93A) mouse cervical spinal cord does not preserve diaphragm function or extend disease. *Neurobiol. Dis.* **78**, 12–23.

Lima F. D., Souza M. A., Furian A. F. *et al.* (2008) Na+, K+-ATPase activity impairment after experimental traumatic brain injury: relationship to spatial learning deficits and oxidative stress. *Behav. Brain Res.* **193**, 306–310.

Lipski J., Wan C. K., Bai J. Z., Pi R., Li D. and Donnelly D. (2007) Neuroprotective potential of ceftriaxone in in vitro models of stroke. *Neuroscience* **146**, 617–629.

Lopes M. W., Soares F. M., de Mello N. *et al.* (2013) Time-dependent modulation of AMPA receptor phosphorylation and mRNA expression of NMDA receptors and glial glutamate transporters in the rat hippocampus and cerebral cortex in a pilocarpine model of epilepsy. *Exp. Brain Res.* **226**, 153–163.

Lopez-Bayghen E. and Ortega A. (2011) Glial glutamate transporters: new actors in brain signaling. *IUBMB Life* **63**, 816–823.

Louzada-Junior P., Dias J. J., Santos W. F., Lachat J. J., Bradford H. F. and Coutinho-Netto J. (1992) Glutamate release in experimental ischaemia of the retina: an approach using microdialysis. *J. Neurochem.* **59**, 358–363.

Lu Z., Zhang W., Zhang N., Jiang J., Luo Q. and Qiu Y. (2008) The expression of glutamate transporters in chest compression-induced audiogenic epilepsy: a comparative study. *Neurol. Res.* **30**, 915–919.

Machtens J. P., Kortzak D., Lansche C. *et al.* (2015) Mechanisms of anion conduction by coupled glutamate transporters. *Cell* **160**, 542–553.

Maeda S., Kawamoto A., Yatani Y., Shirakawa H., Nakagawa T. and Kaneko S. (2008) Gene transfer of GLT-1, a glial glutamate transporter, into the spinal cord by recombinant adenovirus attenuates inflammatory and neuropathic pain in rats. *Mol. Pain.* **4**, 65.

Mao Q. X. and Yang T. D. (2010) Amitriptyline upregulates EAAT1 and EAAT2 in neuropathic pain rats. *Brain Res. Bull.* **81**, 424–427.

Maragakis N. J., Dykes-Hoberg M. and Rothstein J. D. (2004) Altered expression of the glutamate transporter EAAT2b in neurological disease. *Ann. Neurol.* **55**, 469–477.

Mark L. P., Prost R. W., Ulmer J. L., Smith M. M., Daniels D. L., Strottmann J. M., Brown W. D. and Hacein-Bey L. (2001) Pictorial review of glutamate excitotoxicity: fundamental concepts for neuroimaging. *AJNR Am. J. Neuroradiol.* **22**, 1813–1824.

Massie A., Goursaud S., Schallier A., Vermoesen K., Meshul C. K., Hermans E. and Michotte Y. (2010) Time-dependent changes in GLT-1 functioning in striatum of hemi-Parkinson rats. *Neurochem. Int.* **57**, 572–578.

Massieu L. and Garcia O. (1998) The role of excitotoxicity and metabolic failure in the pathogenesis of neurological disorders. *Neurobiology (Bp)* **6**, 99–108.

Mathern G. W., Mendoza D., Lozada A. *et al.* (1999) Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy. *Neurology* **52**, 453–472.

Matos-Ocasio F., Hernandez-Lopez A. and Thompson K. J. (2014) Ceftriaxone, a GLT-1 transporter activator, disrupts hippocampal learning in rats. *Pharmacol. Biochem. Behav.* **122**, 118–121.

Matsugami T. R., Tanemura K., Mieda M. *et al.* (2006) From the cover: indispensability of the glutamate transporters GLAST and GLT1 to brain development. *Proc. Natl Acad. Sci. USA* **103**, 12161–12166.

Mattson M. P. and Chan S. L. (2003) Neuronal and glial calcium signaling in Alzheimer's disease. *Cell Calcium* **34**, 385–397.

Matute C., Domercq M. and Sanchez-Gomez M. V. (2006) Glutamate-mediated glial injury: mechanisms and clinical importance. *Glia* **53**, 212–224.

McEntee W. J. and Crook T. H. (1993) Glutamate: its role in learning, memory, and the aging brain. *Psychopharmacology* **111**, 391–401.

McIntosh T. K., Vink R., Soares H., Hayes R. and Simon R. (1990) Effect of noncompetitive blockade of N-methyl-D-aspartate receptors on the neurochemical sequelae of experimental brain injury. *J. Neurochem.* **55**, 1170–1179.

McIntosh T. K., Smith D. H., Voddi M., Perri B. R. and Stutzmann J. M. (1996) Riluzole, a novel neuroprotective agent, attenuates both neurologic motor and cognitive dysfunction following experimental brain injury in the rat. *J. Neurotrauma* **13**, 767–780.

Medvedev N., Popov V., Henneberger C., Kraev I., Rusakov D. A. and Stewart M. G. (2014) Glia selectively approach synapses on thin dendritic spines. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **369**, 20140047.

Meeker K. D., Meabon J. S. and Cook D. G. (2015) Partial loss of the glutamate transporter GLT-1 alters brain Akt and insulin signaling in a mouse model of Alzheimer's disease. *J. Alzheimers Dis.* **45**, 509–520.

Mehta A., Prabhakar M., Kumar P., Deshmukh R. and Sharma P. L. (2013) Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *Eur. J. Pharmacol.* **698**, 6–18.

Meldrum B. and Garthwaite J. (1990) Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol. Sci.* **11**, 379–387.

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

Miller H. P., Levey A. I., Rothstein J. D., Tzingounis A. V. and Conn P. J. (1997) Alterations in glutamate transporter protein levels in kindling-induced epilepsy. *J. Neurochem.* **68**, 1564–1570.

Miller R. G., Mitchell J. D. and Moore D. H. (2000) Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst. Rev.* **2**, CD001447.

Miller B. R., Dorner J. L., Shou M., Sari Y., Barton S. J., Sengelaub D. R., Kennedy R. T. and Rebec G. V. (2008) Up-regulation of GLT1 expression increases glutamate uptake and attenuates the Huntington's disease phenotype in the R6/2 mouse. *Neuroscience* **153**, 329–337.

Miller B. R., Dorner J. L., Bunner K. D., Gaither T. W., Klein E. L., Barton S. J. and Rebec G. V. (2012a) Up-regulation of GLT1 reverses the deficit in cortically evoked striatal ascorbate efflux in the R6/2 mouse model of Huntington's disease. *J. Neurochem.* **121**, 629–638.

Miller R. G., Mitchell J. D. and Moore D. H. (2012b) Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst. Rev.* **3**, CD001447.

Mitani A. and Tanaka K. (2003) Functional changes of glial glutamate transporter GLT-1 during ischemia: an in vivo study in the hippocampal CA1 of normal mice and mutant mice lacking GLT-1. *J. Neurosci.* **23**, 7176–7182.

Mookherjee P., Green P. S., Watson G. S. *et al.* (2011) GLT-1 loss accelerates cognitive deficit onset in an Alzheimer's disease animal model. *J. Alzheimers Dis.* **26**, 447–455.

Moretto M. B., Arteni N. S., Lavinsky D., Netto C. A., Rocha J. B., Souza D. O. and Wofchuk S. (2005) Hypoxic-ischemic insult

decreases glutamate uptake by hippocampal slices from neonatal rats: prevention by guanosine. *Exp. Neurol.* **195**, 400–406.

Mortensen O. V., Liberato J. L., Coutinho-Netto J., Dos Santos W. F. and Fontana A. C. (2015) Molecular determinants of transport stimulation of EAAT2 are located at interface between the trimerization and substrate transport domains. *J. Neurochem.* **133**, 199–210.

Mu X., Azbill R. D. and Springer J. E. (2000) Riluzole and methylprednisolone combined treatment improves functional recovery in traumatic spinal cord injury. *J. Neurotrauma* **17**, 773–780.

Murphy-Royal C., Dupuis J. P., Varela J. A., Panatier A., Pinson B., Baufreton J., Groc L. and Oliet S. H. (2015) Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. *Nat. Neurosci.* **18**, 219–226.

Nakagawa T., Ozawa T., Shige K., Yamamoto R., Minami M. and Satoh M. (2001) Inhibition of morphine tolerance and dependence by MS-153, a glutamate transporter activator. *Eur. J. Pharmacol.* **419**, 39–45.

Nakagawa T., Fujio M., Ozawa T., Minami M. and Satoh M. (2005) Effect of MS-153, a glutamate transporter activator, on the conditioned rewarding effects of morphine, methamphetamine and cocaine in mice. *Behav. Brain Res.* **156**, 233–239.

Nederkoorn P. J., Westendorp W. F., Hooijenga I. J. *et al.* (2011) Preventive antibiotics in stroke study: rationale and protocol for a randomised trial. *Int. J. Stroke* **6**, 159–163.

Nie H., Zhang H. and Weng H. R. (2010) Minocycline prevents impaired glial glutamate uptake in the spinal sensory synapses of neuropathic rats. *Neuroscience* **170**, 901–912.

Nilsson E., Alafuzoff I., Blennow K. *et al.* (1990a) Calpain and calpastatin in normal and Alzheimer-degenerated human brain tissue. *Neurobiol. Aging* **11**, 425–431.

Nilsson P., Hillered L., Ponten U. and Ungerstedt U. (1990b) Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. *J. Cereb. Blood Flow Metab.* **10**, 631–637.

Nishida A., Iwata H., Kudo Y., Kobayashi T., Matsuoka Y., Kanai Y. and Endou H. (2004) Nicergoline enhances glutamate uptake via glutamate transporters in rat cortical synaptosomes. *Biol. Pharm. Bull.* **27**, 817–820.

Nishizawa Y. (2001) Glutamate release and neuronal damage in ischemia. *Life Sci.* **69**, 369–381.

de O Beleboni R., Pizzo A. B., Fontana A. C., Carolino R. O., Coutinho-Netto J. and Dos Santos W. F. (2004) Spider and wasp neurotoxins: pharmacological and biochemical aspects. *Eur. J. Pharmacol.* **493**, 1–17.

Okiyama K., Smith D. H., Thomas M. J. and McIntosh T. K. (1992) Evaluation of a novel calcium channel blocker, (S)-emopamil, on regional cerebral edema and neurobehavioral function after experimental brain injury. *J. Neurosurg.* **77**, 607–615.

Okiyama K., Smith D. H., White W. F., Richter K. and McIntosh T. K. (1997) Effects of the novel NMDA antagonists CP-98,113, CP-101,581 and CP-101,606 on cognitive function and regional cerebral edema following experimental brain injury in the rat. *J. Neurotrauma* **14**, 211–222.

Okiyama K., Smith D. H., White W. F. and McIntosh T. K. (1998) Effects of the NMDA antagonist CP-98,113 on regional cerebral edema and cardiovascular, cognitive, and neurobehavioral function following experimental brain injury in the rat. *Brain Res.* **792**, 291–298.

O'Shea R. D., Fodera M. V., Aprico K., Dehnes Y., Danbolt N. C., Crawford D. and Beart P. M. (2002) Evaluation of drugs acting at glutamate transporters in organotypic hippocampal cultures: new evidence on substrates and blockers in excitotoxicity. *Neurochem. Res.* **27**, 5–13.

Oster S., Radad K., Scheller D., Hesse M., Balanzew W., Reichmann H. and Gille G. (2014) Rotigotine protects against glutamate toxicity in primary dopaminergic cell culture. *Eur. J. Pharmacol.* **724**, 31–42.

Otis T. S. and Kavanaugh M. P. (2000) Isolation of current components and partial reaction cycles in the glial glutamate transporter EAAT2. *J. Neurosci.* **20**, 2749–2757.

Palmen S. J., van Engeland H., Hof P. R. and Schmitz C. (2004) Neuropathological findings in autism. *Brain* **127**, 2572–2583.

Palmer A. M., Marion D. W., Botscheller M. L., Swedlow P. E., Styren S. D. and DeKosky S. T. (1993) Traumatic brain injury-induced excitotoxicity assessed in a controlled cortical impact model. *J. Neurochem.* **61**, 2015–2024.

Pan X. D., Wei J. and Xiao G. M. (2011) [Effects of beta-lactam antibiotics ceftriaxone on expression of glutamate in hippocampus after traumatic brain injury in rats. *Zhejiang Da Xue Xue Bao Yi Xue Ban* **40**, 522–526.

Peng S., Zhang Y., Zhang J., Wang H. and Ren B. (2011) Glutamate receptors and signal transduction in learning and memory. *Mol. Biol. Rep.* **38**, 453–460.

Petr G. T., Schultheis L. A., Hussey K. C., Sun Y., Dubinsky J. M., Aoki C. and Rosenberg P. A. (2013) Decreased expression of GLT-1 in the R6/2 model of Huntington's disease does not worsen disease progression. *Eur. J. Neuorsci.* **38**, 2477–2490.

Petr G. T., Sun Y., Frederick N. M. *et al.* (2015) 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. *J. Neurosci.* **35**, 5187–5201.

Phillis J. W. and O'Regan M. H. (1996) Mechanisms of glutamate and aspartate release in the ischemic rat cerebral cortex. *Brain Res.* **730**, 150–164.

Pignataro G., Capone D., Polichetti G., Vinciguerra A., Gentile A., Di Renzo G. and Annunziato L. (2011) Neuroprotective, immunosuppressant and antineoplastic properties of mTOR inhibitors: current and emerging therapeutic options. *Curr. Opin. Pharmacol.* **11**, 378–394.

Pines G., Danbolt N. C., Bjoras M. *et al.* (1992) Cloning and expression of a rat brain L-glutamate transporter. *Nature* **360**, 464–467.

Potter M. C., Figuera-Losada M., Rojas C. and Slusher B. S. (2013) Targeting the glutamatergic system for the treatment of HIV-associated neurocognitive disorders. *J. Neuroimmune Pharmacol.* **8**, 594–607.

Proper E. A., Hoogland G., Kappen S. M. *et al.* (2002) Distribution of glutamate transporters in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. *Brain* **125**, 32–43.

Qu S. and Kanner B. I. (2008) Substrates and non-transportable analogues induce structural rearrangements at the extracellular entrance of the glial glutamate transporter GLT-1/EAAT2. *J. Biol. Chem.* **283**, 26391–26400.

Raghupathi R. (2004) Cell death mechanisms following traumatic brain injury. *Brain Pathol.* **14**, 215–222.

Rao V. L., Baskaya M. K., Dogan A., Rothstein J. D. and Dempsey R. J. (1998) Traumatic brain injury down-regulates glial glutamate transporter (GLT-1 and GLAST) proteins in rat brain. *J. Neurochem.* **70**, 2020–2027.

Rao V. L., Dogan A., Bowen K. K., Todd K. G. and Dempsey R. J. (2001) Antisense knockdown of the glial glutamate transporter GLT-1 exacerbates hippocampal neuronal damage following traumatic injury to rat brain. *Eur. J. Neuorsci.* **13**, 119–128.

Rao V. R., Ruiz A. P. and Prasad V. R. (2014) Viral and cellular factors underlying neuropathogenesis in HIV associated neurocognitive disorders (HAND). *AIDS Res. Ther.* **11**, 13.

Rao P. S., Goodwani S., Bell R. L., Wei Y., Boddu S. H. and Sari Y. (2015) Effects of ampicillin, cefazolin and cefoperazone treatments

on GLT1 expressions in the mesocorticolimbic system and ethanol intake in alcohol-preferring rats. *Neuroscience* **295**, 164–174.

Rebec G. V. (2013) Dysregulation of corticostriatal ascorbate release and glutamate uptake in transgenic models of Huntington's disease. *Antioxid. Redox Signal.* **19**, 2115–2128.

Reyes N., Ginter C. and Boudker O. (2009) Transport mechanism of a bacterial homologue of glutamate transporters. *Nature* **462**, 880–885.

Reyes N., Oh S. and Boudker O. (2013) Binding thermodynamics of a glutamate transporter homolog. *Nat. Struct. Mol. Biol.* **20**, 634–640.

Robelet S., Melon C., Guillet B., Salin P. and Kerkerian-Le Goff L. (2004) Chronic L-DOPA treatment increases extracellular glutamate levels and GLT1 expression in the basal ganglia in a rat model of Parkinson's disease. *Eur. J. Neuorsci.* **20**, 1255–1266.

Rodriguez M. C., Obeso J. A. and Olanow C. W. (1998) Subthalamic nucleus-mediated excitotoxicity in Parkinson's disease: a target for neuroprotection. *Ann. Neurol.* **44**, S175–S188.

Rosenberg P. A., Amin S. and Leitner M. (1992) Glutamate uptake disguises neurotoxic potency of glutamate agonists in cerebral cortex in dissociated cell culture. *J. Neurosci.* **12**, 56–61.

Rossi D. J., Oshima T. and Attwell D. (2000) Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature* **403**, 316–321.

Rothman S. M. and Olney J. W. (1995) Excitotoxicity and the NMDA receptor–still lethal after eight years. *Trends Neurosci.* **18**, 57–58.

Rothstein J. D. (1995a) Excitotoxic mechanisms in the pathogenesis of amyotrophic lateral sclerosis. *Adv. Neurol.* **68**, 7–20; discussion 21-27.

Rothstein J. D. (1995b) Excitotoxicity and neurodegeneration in amyotrophic lateral sclerosis. *Clin. Neurosci.* **3**, 348–359.

Rothstein J. D., Martin L. J. and Kuncl R. W. (1992) Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N. Engl. J. Med.* **326**, 1464–1468.

Rothstein J. D., Martin L., Levey A. I., Dykes-Hoberg M., Jin L., Wu D., Nash N. and Kuncl R. W. (1994) Localization of neuronal and glial glutamate transporters. *Neuron* **13**, 713–725.

Rothstein J. D., Van Kammen M., Levey A. I., Martin L. J. and Kuncl R. W. (1995) Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann. Neurol.* **38**, 73–84.

Rothstein J. D., Dykes-Hoberg M., Pardo C. A. *et al.* (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* **16**, 675–686.

Rothstein J. D., Patel S., Regan M. R. *et al.* (2005) Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* **433**, 73–77.

Rusakov D. A., Savtchenko L. P., Zheng K. and Henley J. M. (2011) Shaping the synaptic signal: molecular mobility inside and outside the cleft. *Trends Neurosci.* **34**, 359–369.

Sabri F., Titanji K., De Milito A. and Chiodi F. (2003) Astrocyte activation and apoptosis: their roles in the neuropathology of HIV infection. *Brain Pathol.* **13**, 84–94.

Sari Y. and Sreemantula S. N. (2012) Neuroimmunophilin GPI-1046 reduces ethanol consumption in part through activation of GLT1 in alcohol-preferring rats. *Neuroscience* **227**, 327–335.

Sato H., Tamba M., Okuno S., Sato K., Keino-Masu K., Masu M. and Bannai S. (2002) Distribution of cystine/glutamate exchange transporter, system x(c)-, in the mouse brain. *J. Neurosci.* **22**, 8028–8033.

Sato H., Shiiya A., Kimata M. *et al.* (2005) Redox imbalance in cystine/glutamate transporter-deficient mice. *J. Biol. Chem.* **280**, 37423–37429.

Schallier A., Smolders I., Van Dam D., Loyens E., De Deyn P. P., Michotte A., Michotte Y. and Massie A. (2011) Region- and age-specific changes in glutamate transport in the AbetaPP23 mouse model for Alzheimer's disease. *J. Alzheimers Dis.* **24**, 287–300.

Scimemi A., Meabon J. S., Woltjer R. L., Sullivan J. M., Diamond J. S. and Cook D. G. (2013) Amyloid-beta1-42 slows clearance of synaptically released glutamate by mislocalizing astrocytic GLT-1. *J. Neurosci.* **33**, 5312–5318.

Scofield M. D. and Kalivas P. W. (2014) Astrocytic dysfunction and addiction: consequences of impaired glutamate homeostasis. *Neuroscientist* **20**, 610–622.

Scott H. A., Gebhardt F. M., Mitrovic A. D., Vandenberg R. J. and Dodd P. R. (2011) Glutamate transporter variants reduce glutamate uptake in Alzheimer's disease. *Neurobiol. Aging* **32**, 553.e551–511.

Sheldon A. L. and Robinson M. B. (2007) The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. *Neurochem. Int.* **51**, 333–355.

Shigeri Y., Seal R. P. and Shimamoto K. (2004) Molecular pharmacology of glutamate transporters, EAATs and VGLUTs. *Brain Res. Brain Res. Rev.* **45**, 250–265.

Shimada F., Shiga Y., Morikawa M., Kawazura H., Morikawa O., Matsuoka T., Nishizaki T. and Saito N. (1999) The neuroprotective agent MS-153 stimulates glutamate uptake. *Eur. J. Pharmacol.* **386**, 263–270.

Shimamoto K. (2008) Glutamate transporter blockers for elucidation of the function of excitatory neurotransmission systems. *Chem. Rec.* **8**, 182–199.

Shimamoto K., Lebrun B., Yasuda-Kamatani Y., Sakaitani M., Shigeri Y., Yumoto N. and Nakajima T. (1998) DL-threo-beta-benzyloxyaspartate, a potent blocker of excitatory amino acid transporters. *Mol. Pharmacol.* **53**, 195–201.

Shin J. Y., Fang Z. H., Yu Z. X., Wang C. E., Li S. H. and Li X. J. (2005) Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. *J. Cell. Biol.* **171**, 1001–1012.

Simantov R., Crispino M., Hoe W., Broutman G., Tocco G., Rothstein J. D. and Baudry M. (1999) Changes in expression of neuronal and glial glutamate transporters in rat hippocampus following kainate-induced seizure activity. *Brain Res. Mol. Brain Res.* **65**, 112–123.

Sisk D. R. and Kuwabara T. (1985) Histologic changes in the inner retina of albino rats following intravitreal injection of monosodium L-glutamate. *Graefes Arch. Clin. Exp. Ophthalmol.* **223**, 250–258.

Soares H. D., Thomas M., Cloherty K. and McIntosh T. K. (1992) Development of prolonged focal cerebral edema and regional cation changes following experimental brain injury in the rat. *J. Neurochem.* **58**, 1845–1852.

Storck T., Schulte S., Hofmann K. and Stoffel W. (1992) Structure, expression, and functional analysis of a Na(+)-dependent glutamate/aspartate transporter from rat brain. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 10955–10959.

Suchak S. K., Baloyianni N. V., Perkinton M. S., Williams R. J., Meldrum B. S. and Rattray M. (2003) The 'glial' glutamate transporter, EAAT2 (Glt-1) accounts for high affinity glutamate uptake into adult rodent nerve endings. *J. Neurochem.* **84**, 522–532.

Sun P., Zhang S., Li Y. and Wang L. (2014) Harmine mediated neuroprotection via evaluation of glutamate transporter 1 in a rat model of global cerebral ischemia. *Neurosci. Lett.* **583c**, 32–36.

Szatkowski M. and Attwell D. (1994) Triggering and execution of neuronal death in brain ischaemia: two phases of glutamate release by different mechanisms. *Trends Neurosci.* **17**, 359–365.

Szatkowski M., Barbour B. and Attwell D. (1990) Non-vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake. *Nature* **348**, 443–446.

Szatmari P., Paterson A. D., Zwaigenbaum L. (2007) Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nat. Genet.* **39**, 319–328.

Talantova M., Sanz-Blasco S., Zhang X. *et al.* (2013) Abeta induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. *Proc. Natl Acad. Sci. USA* **110**, E2518–E2527.

Tanaka K., Watase K., Manabe T. *et al.* (1997) Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science* **276**, 1699–1702.

Tao Y. X., Gu J. and Stephens R. L., Jr (2005) Role of spinal cord glutamate transporter during normal sensory transmission and pathological pain states. *Mol. Pain.* **1**, 30.

Taoufik E. and Probert L. (2008) Ischemic neuronal damage. *Curr. Pharm. Des.* **14**, 3565–3573.

Tessler S., Danbolt N. C., Faull R. L., Storm-Mathisen J. and Emson P. C. (1999) Expression of the glutamate transporters in human temporal lobe epilepsy. *Neuroscience* **88**, 1083–1091.

Thone-Reineke C., Neumann C., Namsolleck P. *et al.* (2008) The beta-lactam antibiotic, ceftriaxone, dramatically improves survival, increases glutamate uptake and induces neurotrophins in stroke. *J. Hypertens.* **26**, 2426–2435.

Tian G., Lai L., Guo H., Lin Y., Butchbach M. E., Chang Y. and Lin C. L. (2007) Translational control of glial glutamate transporter EAAT2 expression. *J. Biol. Chem.* **282**, 1727–1737.

Traynor B. J., Bruijn L., Conwit R., Beal F., O'Neill G., Fagan S. C. and Cudkowicz M. E. (2006) Neuroprotective agents for clinical trials in ALS: a systematic assessment. *Neurology* **67**, 20–27.

Trotti D., Rolfs A., Danbolt N. C., Brown R. H., Jr and Hediger M. A. (1999a) SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. *Nat. Neurosci.* **2**, 848.

Trotti D., Rolfs A., Danbolt N. C., Brown R. H., Jr and Hediger M. A. (1999b) SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. *Nat. Neurosci.* **2**, 427–433.

Trotti D., Aoki M., Pasinelli P., Berger U. V., Danbolt N. C., Brown R. H., Jr and Hediger M. A. (2001) Amyotrophic lateral sclerosis-linked glutamate transporter mutant has impaired glutamate clearance capacity. *J. Biol. Chem.* **276**, 576–582.

Ueda Y., Doi T., Nagatomo K., Willmore L. J. and Nakajima A. (2007) Functional role for redox in the epileptogenesis: molecular regulation of glutamate in the hippocampus of FeCl3-induced limbic epilepsy model. *Exp. Brain Res.* **181**, 571–577.

Uenishi H., Huang C. S., Song J. H., Marszalec W. and Narahashi T. (1999) Ion channel modulation as the basis for neuroprotective action of MS-153. *Ann. N. Y. Acad. Sci.* **890**, 385–399.

Ullensvang K., Lehre K. P., Storm-Mathisen J. and Danbolt N. C. (1997) Differential Developmental Expression of the Two Rat Brain Glutamate Transporter Proteins GLAST and GLT. *Eur. J. Neurosci.* **9**, 1646–1655.

Umemura K., Gemba T., Mizuno A. and Nakashima M. (1996) Inhibitory effect of MS-153 on elevated brain glutamate level induced by rat middle cerebral artery occlusion. *Stroke* **27**, 1624–1628.

Van Den Bosch L., Van Damme P., Bogaert E. and Robberecht W. (2006) The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochim. Biophys. Acta* **1762**, 1068–1082.

Vandenberg R. J. and Ryan R. M. (2013) Mechanisms of glutamate transport. *Physiol. Rev.* **93**, 1621–1657.

Verche V. L., Ikiz B., Jacquier A., Przedborski S. and Re D. B. (2011) Glutamate pathway implication in amyotrophic lateral sclerosis: what is the signal in the noise? *J. Recept. Ligang Channel Res.* **4**, 1–22.

Verdon G. and Boudker O. (2012) Crystal structure of an asymmetric trimer of a bacterial glutamate transporter homolog. *Nat. Struct. Mol. Biol.* **19**, 355–357.

Verma R., Mishra V., Sasmal D. and Raghubir R. (2010) Pharmacological evaluation of glutamate transporter 1 (GLT-1) mediated neuroprotection following cerebral ischemia/reperfusion injury. *Eur. J. Pharmacol.* **638**, 65–71.

Veruki M. L., Morkve S. H. and Hartveit E. (2006) Activation of a presynaptic glutamate transporter regulates synaptic transmission through electrical signaling. *Nat. Neurosci.* **9**, 1388–1396.

Vespa P., Prins M., Ronne-Engstrom E., Caron M., Shalmon E., Hovda D. A., Martin N. A. and Becker D. P. (1998) Increase in extracellular glutamate caused by reduced cerebral perfusion pressure and seizures after human traumatic brain injury: a microdialysis study. *J. Neurosurg.* **89**, 971–982.

Volterra A., Trotti D., Tromba C., Floridi S. and Racagni G. (1994) Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. *J. Neurosci.* **14**, 2924–2932.

Wadiche J. I., Amara S. G. and Kavanaugh M. P. (1995a) Ion fluxes associated with excitatory amino acid transport. *Neuron* **15**, 721–728.

Wadiche J. I., Arriza J. L., Amara S. G. and Kavanaugh M. P. (1995b) Kinetics of a human glutamate transporter. *Neuron* **14**, 1019–1027.

Waibel S., Reuter A., Malessa S., Blaugrund E. and Ludolph A. C. (2004) Rasagiline alone and in combination with riluzole prolongs survival in an ALS mouse model. *J. Neurol.* **251**, 1080–1084.

Walker F. O. (2007) Huntington's disease. *Lancet* **369**, 218–228.

Wang Y. and Qin Z. H. (2010) Molecular and cellular mechanisms of excitotoxic neuronal death. *Apoptosis* **15**, 1382–1402.

Wei J., Pan X., Pei Z., Wang W., Qiu W., Shi Z. and Xiao G. (2012) The beta-lactam antibiotic, ceftriaxone, provides neuroprotective potential via anti-excitotoxicity and anti-inflammation response in a rat model of traumatic brain injury. *J. Trauma Acute Care Surg.* **73**, 654–660.

Weiler I. J., Hawrylak N. and Greenough W. T. (1995) Morphogenesis in memory formation: synaptic and cellular mechanisms. *Behav. Brain Res.* **66**, 1–6.

Wen Z. H., Wu G. J., Chang Y. C., Wang J. J. and Wong C. S. (2005) Dexamethasone modulates the development of morphine tolerance and expression of glutamate transporters in rats. *Neuroscience* **133**, 807–817.

Weng H. R., Gao M. and Maixner D. W. (2014) Glycogen synthase kinase 3 beta regulates glial glutamate transporter protein expression in the spinal dorsal horn in rats with neuropathic pain. *Exp. Neurol.* **252**, 18–27.

Werner F. M. and Covenas R. (2015) Review: classical neurotransmitters and neuropeptides involved in generalized epilepsy in a multi-neurotransmitter system: how to improve the antiepileptic effect? *Epilepsy Behav.* pii: S1525-5050(15)00045-1. doi: 10.1016/j.yebeh.2015.01.038.

Werner C. and Engelhard K. (2007) Pathophysiology of traumatic brain injury. *Br. J. Anaesth.* **99**, 4–9.

Wetherington J., Serrano G. and Dingledine R. (2008) Astrocytes in the Epileptic Brain. *Neuron* **58**, 168–178.

Wilson J. R. and Fehlings M. G. (2014) Riluzole for acute traumatic spinal cord injury: a promising neuroprotective treatment strategy. *World Neurosurg.* **81**, 825–829.

Wilson J. M., Khabazian I., Pow D. V., Craig U. K. and Shaw C. A. (2003) Decrease in glial glutamate transporter variants and excitatory amino acid receptor down-regulation in a murine model of ALS-PDC. *NeuroMol. Med.* **3**, 105–118.

Wisman L. A., van Muiswinkel F. L., de Graan P. N., Hol E. M. and Bar P. R. (2003) Cells over-expressing EAAT2 protect motoneurons from excitotoxic death in vitro. *NeuroReport* **14**, 1967–1970.

Woltjer R. L., Duerson K., Fullmer J. M. *et al.* (2010) Aberrant detergent-insoluble excitatory amino acid transporter 2

accumulates in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **69**, 667–676.

Wong M. (2013) Mammalian target of rapamycin (mTOR) pathways in neurological diseases. *Biomed. J.* **36**, doi:10.4103/2319-4170.110365.

Wong M., Ess K. C., Uhlmann E. J., Jansen L. A., Li W., Crino P. B., Mennerick S., Yamada K. A. and Gutmann D. H. (2003) Impaired glial glutamate transport in a mouse tuberous sclerosis epilepsy model. *Ann. Neurol.* **54**, 251–256.

Wu X., Kihara T., Akaike A., Niidome T. and Sugimoto H. (2010) PI3K/Akt/mTOR signaling regulates glutamate transporter 1 in astrocytes. *Biochem. Biophys. Res. Commun.* **393**, 514–518.

Xing X., Chang L. C., Kong Q., Colton C. K., Lai L., Glicksman M. A., Lin C. L. and Cuny G. D. (2011) Structure-activity relationship study of pyridazine derivatives as glutamate transporter EAAT2 activators. *Bioorg. Med. Chem. Lett.* **21**, 5774–5777.

Yamamoto T., Rossi S., Stiefel M., Doppenberg E., Zauner A., Bullock R. and Marmarou A. (1999) CSF and ECF glutamate concentrations in head injured patients. *Acta Neurochir. Suppl.* **75**, 17–19.

Yernool D., Boudker O., Jin Y. and Gouaux E. (2004) Structure of a glutamate transporter homologue from Pyrococcus horikoshii. *Nature* **431**, 811–818.

Yi J. H. and Hazell A. S. (2006) Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. *Neurochem. Int.* **48**, 394–403.

Yoshizumi M., Eisenach J. C. and Hayashida K. (2013) Valproate prevents dysregulation of spinal glutamate and reduces the development of hypersensitivity in rats after peripheral nerve injury. *J. Pain.* **14**, 1485–1491.

Zeidan-Chulia F., Salmina A. B., Malinovskaya N. A., Noda M., Verkhratsky A. and Moreira J. C. (2014) The glial perspective of autism spectrum disorders. *Neurosci. Biobehav. Rev.* **38**, 160–172.

Zelenaia O., Schlag B. D., Gochenauer G. E., Ganel R., Song W., Beesley J. S., Grinspan J. B., Rothstein J. D. and Robinson M. B. (2000) Epidermal growth factor receptor agonists increase expression of glutamate transporter GLT-1 in astrocytes through pathways dependent on phosphatidylinositol 3-kinase and transcription factor NF-kappaB. *Mol. Pharmacol.* **57**, 667–678.

Zerangue N. and Kavanaugh M. P. (1996) Flux coupling in a neuronal glutamate transporter. *Nature* **383**, 634–637.

Zhang C., Raghupathi R., Saatman K. E., Smith D. H., Stutzmann J. M., Wahl F. and McIntosh T. K. (1998) Riluzole attenuates cortical lesion size, but not hippocampal neuronal loss, following traumatic brain injury in the rat. *J. Neurosci. Res.* **52**, 342–349.

Zhou Y. and Danbolt N. C. (2013) GABA and glutamate transporters in brain. *Front. Endocrinol.* **4**, 165.

Zhou Y. and Danbolt N. C. (2014) Glutamate as a neurotransmitter in the healthy brain. *J. Neural. Transm.* **121**, 799–817.

Zhou Y., Waanders L. F., Holmseth S., Guo C., Berger U. V., Li Y., Lehre A. C., Lehre K. P. and Danbolt N. C. (2014a) Proteome analysis and conditional deletion of the EAAT2 glutamate transporter provide evidence against a role of EAAT2 in pancreatic insulin secretion in mice. *J. Biol. Chem.* **289**, 1329–1344.

Zhou Y., Wang X., Tzingounis A. V., Danbolt N. C. and Larsson H. P. (2014b) EAAT2 (GLT-1; slc1a2) glutamate transporters reconstituted in liposomes argues against heteroexchange being substantially faster than net uptake. *J. Neurosci.* **34**, 13472–13485.

Zimmer E. R., Kalinine E., Haas C. B., Torrez V. R., Souza D. O., Muller A. P. and Portela L. V. (2012) Pretreatment with memantine prevents Alzheimer-like alterations induced by intrahippocampal okadaic acid administration in rats. *Curr. Alzheimer Res.* **9**, 1182–1190.

Zlotnik A., Sinelnikov I., Gruenbaum B. F. *et al.* (2012) Effect of glutamate and blood glutamate scavengers oxaloacetate and pyruvate on neurological outcome and pathohistology of the hippocampus after traumatic brain injury in rats. *Anesthesiology* **116**, 73–83.

Zschocke J., Bayatti N., Clement A. M., Witan H., Figiel M., Engele J. and Behl C. (2005) Differential promotion of glutamate transporter expression and function by glucocorticoids in astrocytes from various brain regions. *J. Biol. Chem.* **280**, 34924–34932.