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REVIEW ARTICLE

Microglial self-defence mediated through GLT-1 and glutathione

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Abstract Glutamate is stored in synaptic vesicles in presynaptic neurons. It is released into the synaptic cleft to provide signalling to postsynaptic neurons. Normally, the astroglial glutamate transporters GLT-1 and GLAST take up glutamate to mediate a high signal-to-noise ratio in the synaptic signalling, and also to prevent excitotoxic effects by glutamate. In astrocytes, glutamate is transformed into glutamine, which is safely transported back to neurons. However, in pathological conditions, such as an ischemia or virus infection, astroglial transporters are down-regulated which could lead to excitotoxicity. Lately, it was shown that even microglia can express glutamate transporters during pathological events. Microglia have two systems for glutamate transport: GLT-1 for transport into the cells and the x_c^- system for transport out of the cells. We here review results from our work and others, which demonstrate that microglia in culture express GLT-1, but not GLAST, and transport glutamate from the extracellular space. We also show that TNF-α can induce increased microglial GLT-1 expression, possibly associating the expression with inflammatory systems. Furthermore, glutamate taken up through GLT-1 may be used for direct incorporation into glutathione and to fuel the intracellular glutamate pool to allow cystine uptake through the x_c^- system. This can lead to a defence against oxidative stress and have an antiviral function.

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On microglia

Microglia are considered as the immunocompetent cells of the central nervous system (CNS), forming the interface between the brain and the immune system. In the mature brain, microglia make up 5–20% of the glial population and they are more numerous in grey matter than white matter (Lawson et al. 1990). They are present throughout the CNS and form a network of cells with the capacity of immune surveillance and control, and can be described as sensors for pathological events (Kreutzberg 1996). They are mediators of the CNS homeostasis by expressing ion channels, neurotransmitter and pathogen receptors which make them able to sense neuronal activity, pH shifts and other physiological changes (reviewed by Farber and Kettenmann 2005). Microglia are extraordinarily sensitive to changes in their microenvironment and can rapidly be prepared to deal with infections, physical injuries and physiological changes by becoming activated (Barron 1995). It has been shown that the fine microglial branches are constantly moving and sensing the environment (Raivich 2005). Upon activation, the microglia transform to an amoeboid morphology and up-regulate their phenotype by expressing cell surface markers, cytokines, chemokines, reactive oxygen species (ROS) and membrane proteins such as receptors and channels (Gebicke-Haerter et al. 1996; Streit et al. 1999). They are also able to proliferate and migrate to the injured area and to remove invading or dying cells by phagocytosis (Streit et al. 1999). Several factors for microglial activation have been identified including bacterial cell wall components such as

lipopolysaccharide (LPS), cytokines, plaque-related molecules such as the β-amyloid peptide and prion proteins, serum factors and several other stimuli (for review see, Nakamura 2002).

Due to the microglial characteristics and the fact that the blood brain barrier (BBB) shields the CNS from access by blood-borne immune cells and various pathogens (Abbott et al. 2006), making it an immunoprivileged organ, microglia are thought of as the immunocompetent cells of the CNS (Aloisi 2001; Gehrmann et al. 1995). As the first line defence cells, microglia are able to participate in the regulation of both non-specific inflammation and adaptive immune responses (Aloisi 2001). Activated microglia can express major histocompatability complex (MHC) I and II (Gehrmann et al. 1995), making them antigen presenting cells and able to control T cell responses (for review, see Aloisi 2001). Furthermore, they express surface receptors for immune system components such as immunoglobulins, complement, and apoptotic cell markers (Farber and Kettenmann 2005; Raivich 2005; Streit et al. 1999).

Glutamate and glutamate receptors

The amino acid L-glutamate is considered as the main excitatory neurotransmitter in the mammalian nervous system (Fonnum 1984). It is involved in normal brain function, including cognition and memory (Nakanishi et al. 1998). Glutamate exists in all cells where it may have important metabolic functions, and is not used as a signal transmitter by most cells but neurons and possibly also astrocytes (see Had-Aissouni 2011 and references therein). Glutamate, released mainly from the presynaptic terminal in neurons where it is stored in synaptic vesicles, can activate different subtypes of glutamate receptors located on postsynaptic membranes and cells in the vicinity (Danbolt 2001). However, although glutamate is essential, it can also be toxic. The brain contains large amounts of glutamate, about 5–15 mmol/kg wet weight, but only a small fraction is present extracellularly (Schousboe 1981). There is a several thousand fold concentration gradient of glutamate across the plasma membrane of cells, with glutamate concentrations of only 2–4 μM in the extracellular fluid and approximately 10 μM in the cerebrospinal fluid (Benveniste et al. 1984; Danbolt 2001), while there is 1–10 mM cytosolic glutamate (Erecinska and Silver 1990). This is in order to avoid over-activation of glutamate receptors which otherwise can lead to cell death, a process termed excitotoxicity by Olney (1969). Excitotoxicity is considered a major mechanism in many human disease states such as cerebral ischaemia, nervous system trauma, epilepsy, and neurodegenerative disorders (Dodd 2002; O'Shea 2002), though the mechanisms behind it remains to be fully explored. Although the molecular basis for the excitotoxicity is still uncertain, it is believed that it is due to a Ca^{2+} overload that ultimately triggers intracellular Ca^{2+}-dependent signalling cascades that eventually lead to neuronal cell death (Sattler and Tymianski 2000). Since it is glutamate, or glutamate analogues, that make the glutamate receptors overactive, it is of vital importance to keep the extracellular glutamate concentrations low.

Glutamate transporters

The extracellular concentration of glutamate must be kept below the activating threshold to avoid over-activation of glutamate receptors which could potentially cause excitotoxicity. Unlike most neurotransmitters, glutamate does not appear to have any degrading enzyme in the extracellular space. Consequently, the glutamate must be rapidly removed by glutamate transporters of the SLC1A family of solute carriers, which can transport it across the plasma membrane and against the steep concentration gradient. In addition to preventing excitotoxicity, rapid clearance of glutamate will also ensure a high signal to noise ratio in the glutamate signalling (Hansson et al. 2000; Riedel 1996; Ronnback and Hansson 2004). In the normal brain, astrocytes are thought of as the main glutamate scavengers (Schousboe 1981). In fact, neuronal vulnerability to glutamate is a hundred-fold greater in astrocyte-poor cultures than in cultures abundant with astrocytes (Rosenberg et al. 1992). Glutamate taken up by astrocytes is converted to glutamine by glutamine synthetase (GS, EC 6.3.1.2) which can be transported back to neurons since it does not have any known synaptic activity (reviewed by Sonnewald et al. 1997; Hertz et al. 1999).

Not much is known about how the glutamate transporters are expressed and regulated in microglial cells, and, consequently, most data referred to will be on astroglial cells and the glial-specific transporters. A schematic figure of how glutamate is utilized and transported in the CNS can be seen in Fig. 1.

High affinity Na^+-dependent glutamate transport

To date, not counting various splice variants, five high affinity Na^+-dependent glutamate transporters in the CNS, called excitatory amino acid transporters (EAATs), have been cloned: EAAT1 (Storck et al. 1992), EAAT2 (Pines et al. 1992), EAAT3 (Kanai and Hediger 1992), EAAT4 (Fairman et al. 1995), and EAAT5 (Arriza et al. 1997). EAAT1 and EAAT2, the human homologues to the rodent glutamate and aspartate transporter (GLAST) and glutamate transporter 1 (GLT-1), respectively, are predominantly

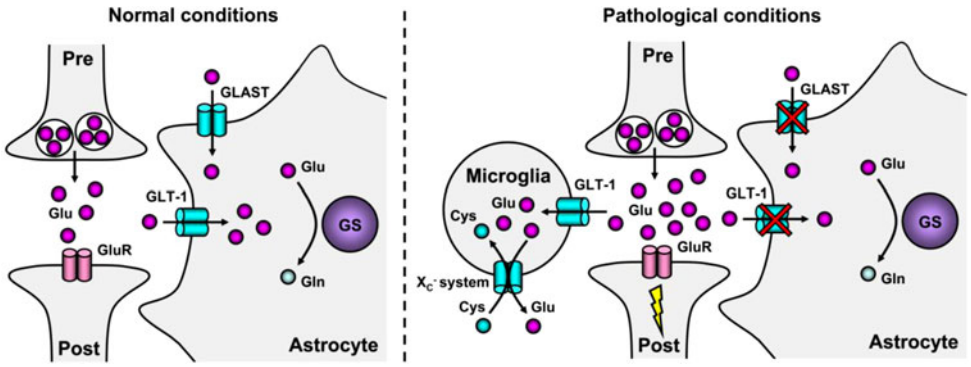

Fig. 1 Schematic drawing of the synaptic cleft and surrounding cells. Glutamate (*Glu*) is stored in synaptic vesicles in presynaptic nerve terminals (*Pre*) and can be released into the synaptic cleft. Glutamate can activate glutamate receptors (*GluRs*) on postsynaptic cells such as postsynaptic neurons (*Post*) and transmit signals. During physiological conditions (*left*), the glutamate is sequestered from the synaptic cleft mainly by the astroglial glutamate transporters GLT-1 and GLAST to prevent excitotoxicity due to prolonged activation of glutamate receptors. Astrocytes metabolize glutamate into glutamine (*Gln*) using glutamine synthetase (*GS*). Glutamine can then be safely transported back to neurons. During pathological conditions (*right*), the astroglial glutamate transporters are down-regulated and microglial transporters are induced. Consequently, there will be an accumulation of glutamate in the extracellular space which may lead to excitotoxicity due to excessive activation of glutamate receptors. Microglia may transport glutamate from the extracellular space and exchange it for cystine (*Cys*) using the x_c^- system (from Persson 2007)

expressed by astrocytes in the normal brain, while EAAT3, also known in rodent as excitatory amino acid carrier 1 (EAAC1), is expressed by neurons (for review, see Gegelashvili and Schousboe 1998). EAAT4 is expressed by Purkinje neurons in the cerebellum (Yamada et al. 1996) and EAAT5 is expressed by neurons in the retina (Arriza et al. 1997). For further details on transport mechanism see the review by Had-Aissouni (2011) and references therein.

Although messenger RNA for both GLAST and GLT-1 has been found in microglia (Kondo et al. 1995), they do not express any EAATs under physiological conditions in vivo. However, during pathological situations, in response to harmful conditions or stress, microglia have been shown to be able to express EAATs both in vivo and in vitro. It has been shown that activated microglia surrounding motoneurons express GLT-1 in the facial nerve axotomy paradigm (Lopez-Redondo et al. 2000). Furthermore, microglia can express glutamate transporters after controlled cortical impact (van Landeghem et al. 2001) and in connection with infectious diseases (Persson et al. 2007) and neurodegenerative diseases such as prion diseases (Chretien et al. 2004; Gras et al. 2006). However, there are indications that the expression of microglial EAATs is species-specific. Murine microglia have only been shown to express GLT-1 (Nakajima et al. 2001). Microglia from macaque monkeys infected with simian immunodeficiency virus express GLT-1 (Chretien et al. 2002), while microglia from patients infected with HIV express GLAST (Vallat-Decouvelaere et al. 2003).

Alterations in EAAT expression, or EAAT function, have been implied in several disease states, including Alzheimer's disease, cerebral ischaemia, epilepsy, traumatic brain injury and amyotrophic lateral sclerosis (for reviews, see Danbolt 2001; O'Shea 2002). The regulation and expression patterns of the EAATs are highly complex and the focus of many studies. Numerous substances have been found that down-regulate the transporters, but only a few that up-regulate them in astrocytes. For instance, dibutyryl cyclic adenosine monophosphate (Schlag et al. 1998), epidermal growth factor (Suzuki et al. 2001), pituitary adenylate cyclase activating peptide (Figiel and Engele 2000) and β-lactams (Rothstein et al. 2005) have been used to increase astroglial GLT-1 expression. The relative lack of substances able to increase the EAAT expression has made it exceedingly difficult to counteract the neurodegenerative diseases with excitotoxic characteristics. Instead, research focus has been on factors that down-regulate astroglial EAAT expression or glutamate uptake like acidosis (Swanson et al. 1995), hypoxia (Swanson 1992), endothelin-1 (Leonova et al. 2001), cytokines (Fine et al. 1996; Liao and Chen 2001) and oxidative stress (Pogun et al. 1994; Trotti et al. 1996; Volterra et al. 1994). Interestingly, in conditions where the astroglial glutamate transporters are down-regulated, such as after controlled cortical impact, their microglial counterparts are being induced or up-regulated.

Na^+-independent glutamate transport

The Na^+-independent glutamate transport system is typically a Cl^--dependent glutamate/cystine antiporter, which exchanges internal glutamate for cystine, the oxidized form of cysteine, across the plasma membrane (Bannai 1986). This transport system has been termed system x_c^- by opposition to the sodium-dependant transporters that

transport both glutamate and aspartate, termed X_{AG}^-. Such a system has been cloned from macrophages (Sato et al. 1999). System X_{AG}^- and system x_c^- have similar affinity for glutamate, but system x_c^- has lower transport velocity, suggesting that it may have a limited role in the physiological brain for glutamate transport. Instead, it has been proposed that the antiporter has a primary role in cystine uptake and in maintenance of glutathione levels in astrocytes (Cho and Bannai 1990) and in microglia (Rimaniol et al. 2001). Using internal glutamate as a driving force, it is no surprise that the cystine uptake is inhibited by extracellular glutamate (Murphy et al. 1989). If the extracellular glutamate concentrations are sufficiently high, system x_c^- may even release cystine which consequently leads to cell death due to oxidative stress (Cho and Bannai 1990; Murphy et al. 1989; Murphy et al. 1990) since cystine is used for synthesis of the antioxidant glutathione (Dringen and Hirrlinger 2003). For further details on system x_c^- cloning, regulation and functions see the reviews by Lewerenz et al. (2011) and Conrad and Sato (2011).

Neuroinflammation

Neuroinflammation is often defined as the presence of activated microglia, reactive astrocytes and inflammatory mediators (Minghetti 2005). Inflammation is a self-defensive reaction aimed to eliminate or neutralize injurious stimuli, and restoring tissue integrity (Minghetti 2005). Activated microglia are able to secrete proinflammatory cytokines and chemokines in addition to more non-specific inflammatory mediatiors such as ROS and nitric oxide (Streit et al. 1999), and are thus major contributors to neuroinflammation. In comparison to microglia, astrocytes have a delayed response but can become reactive, express MHC class II, and form glial scars to isolate a damaged area (for review, see Dong and Benveniste 2001; Fawcett and Asher 1999; Pekny and Nilsson 2005). Neurons have classically been considered as passive bystanders, only regulating immune reactions, but are now known to be able to express MHC class I and produce several cytokines (for review, see Piehl and Lidman 2001).

Neuroinflammation occurs during almost any pathological event in the CNS such as brain trauma, ischaemia, infections and chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Creutzfeldt-Jacob's disease (Bradl and Hohlfeld 2003; Minghetti 2005; Piehl and Lidman 2001). In the laboratory environment, a neuroinflammatory state is often induced using LPS as a model substance both in vitro and in vivo. LPS is a cell wall component of Gram-negative bacteria and a very potent inducer of inflammation (Nakamura 2002). Regardless of which stimuli it is that initiates it, neuroinflammation can be both beneficial and detrimental (for review, see Minghetti 2005).

Glutathione and oxidative stress

Glutathione (GSH) is a tripeptide (γ-glutamylcysteinylglycine) and is synthesized from cysteine, glutamate and glycine. It is synthesized by the consecutive actions of two enzymes in an ATP consuming process (for review, see Dringen 2000). First, the dipeptide γ-glutamylcysteine is formed from glutamate and cysteine by γ-glutamylcysteine synthetase (EC 6.3.2.2; also named glutamate cysteine ligase). The dipeptide is then further synthesized to GSH with the addition of glycine in a reaction catalysed by glutathione synthetase (EC 6.3.2.3). The GSH synthesis is balanced by a feedback inhibition of γ-glutamylcysteine synthetase by the end product GSH (Richman and Meister 1975).

Being the most abundant thiol in mammalian cells, with concentrations up to 12 mM (Cooper et al. 1997), GSH is a major antioxidant and protects cells against oxidative stress by detoxifying ROS. The imbalance between the production of ROS, or other free radicals, and the inability of cells to defend against them is termed oxidative stress (Gilgun-Sherki et al. 2002). Oxidative stress can thus occur when there is an increase in ROS or other free radicals (Simonian and Coyle 1996). Compared to other organs, the brain appears to be especially endangered when it comes to generation and detoxification of ROS. The human brain comprises about 2% of the body weight but uses 20% of the total oxygen consumption (Clarke et al. 1999). Free radicals are constantly generated by the mitochondria when it uses oxygen to supply the energy needs of the CNS. Several mechanisms are active in the formation of ROS, including some enzyme activities, i.e. activity of monoamine oxidase (EC 1.4.3.4) or tyrosine hydroxylase (EC 1.14.16.2), and even metabolism of glutamate (for review, see Gilgun-Sherki et al. 2002). Oxidative stress can produce functional and detrimental alterations in lipids, proteins and deoxyribonucleic acid (DNA) to name a few, and have been implied in most pathological states in the CNS, including neurodegenerative diseases and acute CNS injuries (Gilgun-Sherki et al. 2002; Simonian and Coyle 1996).

Several cellular defence systems, including superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6) and antioxidants such as GSH, exists in the brain (Simonian and Coyle 1996). GSH can react directly in a non-enzymatic way with radicals, or it can act as an electron donor in the reduction of peroxides by glutathione peroxide (GPx, EC 1.11.1.9; Chance et al. 1979). The product of the reaction is glutathione disulphide (GSSG), the oxidized form of GSH.

GSSG can be recycled to GSH by the enzyme glutathione reductase (GRed, EC 1.8.1.7; Dringen 2000). Additionally, GSH is also antiviral (Garaci et al. 1992; Palamara et al. 1995, 1996b), exerting its effect by a mechanism that is connected to the redox state of the cells (Ciriolo et al. 1997; Palamara et al. 1996a).

Microglia posses a prominent glutathione system with a glutathione content significantly higher than in neurons or astrocytes (Hirrlinger et al. 2000). Furthermore, microglia have been shown to express the highest immunoreactivity for GPx (Lindenau et al. 1998), and high levels of GRed in microglia have been reported (Gutterer et al. 1999).

Herpes simplex virus infections in the CNS

Herpes viridae is a large family of viruses of which herpes simplex virus 1 and herpes simplex virus 2 (HSV-1 and HSV-2) are the most serious human pathogens (Whitley and Roizman 2001). They are double-stranded DNA viruses and the virus particles are composed of at least 84 different polypeptides and the virus genome, encapsulated by a membrane envelope called a capsid (Homa and Brown 1997). The viral genome encodes for viral glycoproteins that are necessary for viral attachment and penetration as well as polypeptides with many diverse functions such as viral host shut-off proteins which enables the virus to take over the invaded cell and allow viral replication (Matis and Kudelova 2001; Mossman et al. 2001; Whitley and Roizman 2001).

The subtypes of HSV have different, although clinically somewhat overlapping, pathogenesis. HSV-1 leads normally to orolabial herpes vesicles and blistering, and HSV-2 often causes genital herpes vesicles and blistering (Whitley and Roizman 2001). HSV can gain access and enter the CNS by lytic infection of epithelial or mucosal surfaces which allows the virus to enter axons of sensory neurons in which the virus can be axonally transported to the neuronal nuclei in either the dorsal root ganglia or the trigeminal ganglion and spread to neighbouring cells (for review, see Frampton et al. 2005). The effect of the virus in the CNS is then determined by its neuroinvasiveness, neurotoxicity and latency. HSV-1 typically causes encephalitis while meningitis is most commonly caused by HSV-2 (for review, see Schmutzhard 2001; Tyler 2004).

Microglia have been shown to be able to recognize HSV by Toll-like receptors (Aravalli et al. 2005; Finberg et al. 2005), and subsequently initiate an immunological response by secreting cytokines (Lokensgard et al. 2001, 2002). In fact, neuroinflammation with circulating cytokines are one of the hallmarks of HSV infections in the brain (Skoldenberg 1996). It has been theorized that glial cells, with microglia as key players, can orchestrate a defence against HSV in the CNS by evoking an inflammatory and immunological response (Lokensgard et al. 2002).

Increased microglial GLT-1 expression mediated by TNF-α (see Figs. 2, 3)

Microglia are able to express Na^+-dependent high affinity glutamate transporters in vivo during pathological conditions such as controlled cortical impact (van Landeghem et al. 2001), facial nerve axotomy (Lopez-Redondo et al. 2000), viral infections (Chretien et al. 2002; Porcheray et al. 2006a; Vallat-Decouvelaere et al. 2003) and prion diseases (Chretien et al. 2004). These are situations where the astroglial glutamate transporters are down-regulated, which could contribute to neurodegeneration (O'Shea 2002). Microglia have also been shown to express glutamate transporters in vitro and have a capacity for glutamate uptake (Nakajima et al. 2001; Rimaniol et al. 2000). However, not much is known about how the glutamate transporters are regulated in microglia.

With the knowledge that microglia are activated in a number of CNS diseases, it is reasonable to think that glutamate transporter expression is activation-dependent and possibly linked to inflammatory events. Examining the effects of several different stimuli known to activate microglia and to be associated with a down-regulated astroglial glutamate transport, it was found that LPS, the only stimulus in the study to induce release of cytokines, increased expression of GLT-1 (Persson et al. 2005). The

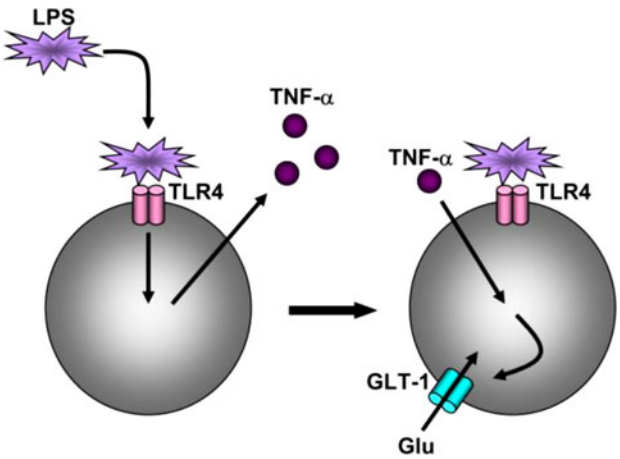

Fig. 2 The inflammatory stimulus LPS is detected by microglia via the Toll-like receptor 4 (TLR4), causing a prominent release of the cytokine TNF-α into the extracellular space. TNF-α can then activate the microglia in an autocrine manner and induce/increase the microglial expression of the glutamate transporter GLT-1, making the cells able to transport glutamate from the extracellular space (from Persson 2007)

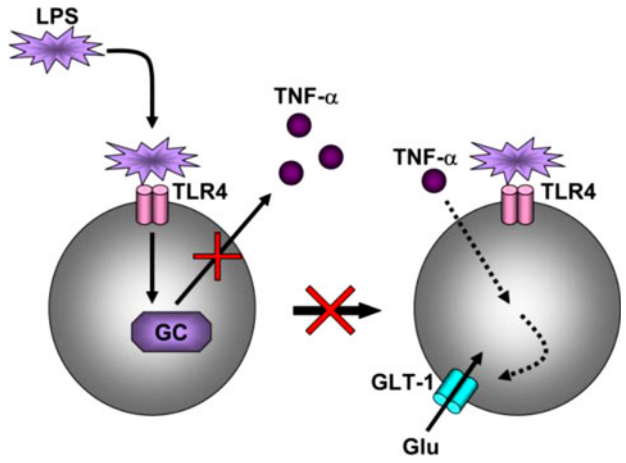

Fig. 3 The inflammatory stimulus LPS is detected by microglia via the Toll-like receptor 4 (TLR4) which normally causes a prominent release of TNF-α into the extracellular space. However, antinflammatory glucocorticoids (GCs), like corticosterone, inhibit the synthesis and thereby the release of TNF-α. Consequently, there is an inhibition of the TNF-α-dependent expression of GLT-1 in microglia. As depicted by the *dotted arrows*, the microglial GLT-1 expression can be rescued by exogenously added TNF-α (from Persson 2007)

fact that LPS induces/up-regulates GLT-1 in microglia has now been confirmed in a study by O'Shea et al. (2006) using microglia in culture and for human monocyte-derived macrophages by Porcheray et al. (2006a). In our study, it was also found that the pro-inflammatory cytokine TNF-α was able to mimic the LPS induced GLT-1 increase and that the TNF-α synthesis inhibitor thalidomide (Sampaio et al. 1991), or neutralization with TNF-α antibodies, was able to inhibit the increase in GLT-1 expression. This suggests that microglial GLT-1 expression could be coupled to inflammatory events and that TNF-α can act as an inducer of the expression. However, it is not known if TNF-α per se induces the expression or if it is some other factor that in turn is induced by the cytokine. One such factor could be interferon-γ (IFN-γ) which has been shown to increase microglial glutamate uptake (Shaked et al. 2005). Nonetheless, it is plausible that pathological conditions that lead to release of TNF-α can also lead to an induced expression of microglial glutamate transporters.

The increase in transporter expression leads to increased functional glutamate uptake. However, the glutamate uptake capacity of microglia is only about 10% of that measured for astrocytes (Persson et al. 2005; Shaked et al. 2005). This could suggest different physiological functions for astroglial and microglial glutamate transporters although it should be mentioned that microglia does indeed clear added glutamate from medium in vitro (Rimaniol et al. 2000). Interestingly, TNF-α has been shown to inhibit glutamate uptake in astrocytes (Fine et al. 1996). This could suggest a reciprocal control of glutamate transporters between astrocytes and microglia. Indeed, microglia have been proposed to serve as a backup system for diminished astroglial glutamate transport (Gras et al. 2006; Rimaniol et al. 2000).

The role of TNF-α in pathological conditions has been debated. TNF-α has classically been considered as deleterious since it has the ability to induce inflammation and cell death, but it also has several other functions that are non-deleterious (for review see Hehlgans and Pfeffer 2005). In fact, TNF-α has been shown to be neuroprotective in some paradigms (for reviews, see Hallenbeck 2002; Shohami et al. 1999). This seems to be true for several cytokines since they can act as neuromodulators of the CNS (Vitkovic et al. 2000) and even protect against excitotoxic insults (Carlson et al. 1999). The concept of an immunological protection against glutamate excitotoxicity, coined protective autoimmunity, has been proposed (Schwartz et al. 2003).

It has been shown that TNF-α has deleterious effects during and after ischaemia (Nawashiro et al. 1997a), but that pre-treatment with the cytokine is protective against the insult (Marchetti et al. 2004; Nawashiro et al. 1997b; Romera et al. 2004). It has also been shown that pre-treatment with a single dose of LPS reduces ischaemic damage after middle cerebral artery occlusion with TNF-α as the likely mediator (Tasaki et al. 1997). Furthermore, Marchetti et al. (2004) have shown that signalling through TNFR1 aggravates neuronal loss while signalling through TNFR2 has the adverse effect. Taken together, these studies show that the timing, context and concentration of TNF-α is important for determining the effects of the cytokine. It is not known whether the neuroprotective abilities of cytokines, especially TNF-α, is mediated at least in part by microglial glutamate transporters, but it makes an interesting hypothesis and field of research.

Since the expression of microglial high affinity glutamate transporters seems to be regulated by inflammatory events, it is believable that anti-inflammatory molecules that are known to affect microglia and microglial production and/or release of TNF-α, might negatively regulate the expression. One such potential molecule is corticosterone, the species-specific glucocorticoid of rats.

A study performed in our group showed that corticosterone, at physiological stress response related levels, was indeed able to inhibit the microglial expression of GLT-1 (Jacobsson et al. 2006). Corticosterone was found to inhibit LPS-induced synthesis/release of TNF-α. The effect of corticosterone on microglial GLT-1 is probably due to the inhibition of TNF-α since this cytokine has previously been linked to glutamate transporter expression in both microglia (Persson et al. 2005) and in blood-borne monocytes (Rimaniol et al. 2000). The inhibitory effect of corticosterone could also be seen for otherwise unstimulated microglia. The low basal levels of TNF-α that are responsible for the basal

microglial GLT-1 expression seen in vitro are likely to be inhibited by corticosterone and subsequently, the basal microglial GLT-1 expression is lowered. It should be noted that dexamethasone, a synthetic glucocorticoid analogue, have been shown to be a potent inducer of EAAT1 in human monocyte-derived macrophages (Gras et al. 2006; Porcheray et al. 2006a). The difference between murine microglia and human monocyte-derived macrophages may be due to differences in TNF-α sensitivity since the macrophages do not secrete detectable TNF-α when differentiated (Gras et al. 2006). For a further detailed comparison between microglia and macrophages see Gras et al. (2011).

These results further highlight the connectivity between neuroinflammatory mediators, such as TNF-α, and microglial glutamate transporter expression. Anti-inflammatory substances like corticosterone are indeed able to inhibit microglial GLT-1 expression by inhibiting the release of TNF-α. They do not affect the mechanism for GLT-1 expression per se, since exogenously added TNF-α could induce microglial GLT-1 while the TNF-α synthesis/release was inhibited by corticosterone in our study.

Taken together, the results raise some interesting questions regarding the mechanisms behind microglial glutamate transporter expression. Microglia are capable of de novo expression of glutamate transporters after controlled cortical impact while their astroglial counterparts are down-regulated (van Landeghem et al. 2001). During traumatic brain injury there is a release of TNF-α (Stover et al. 2000) and glucocorticoids (McCullers et al. 2002). There will also be an accumulation of extracellular glutamate (McCullers et al. 2002), which in part can be due to inhibition of astroglial glutamate uptake. In fact, TNF-α has been shown to inhibit astroglial glutamate uptake (Fine et al. 1996). Corticosterone has been shown to inhibit glutamate uptake in hippocampal cultures (Brooke and Sapolsky 2003) by a mechanism that has been suggested to impair astroglial glutamate uptake during neurological crisis (Virgin et al. 1991) although it increases astroglial GLT-1 expression and glutamate uptake in cortical cultures (Zschocke et al. 2005). On the other hand, our studies have shown that TNF-α and corticosterone have differential effects on microglial GLT-1 expression and glutamate uptake when compared to astrocytes derived from cortex. This further highlights that microglial and astroglial GLT-1 may be regulated reciprocally although differences may occur in different brain regions.

The timescale of TNF-α and glucocorticoid presence may be of importance for the microglial glutamate transporter expression. Glucocorticoid levels are elevated early and until 6 h after the insult (McCullers et al. 2002), while TNF-α has its peak 8–48 h after the insult (Stover et al. 2000), indicating that the microglial transporters may be induced with some delay. In van Landeghem et al.'s (2001) controlled cortical impact model, they detected microglial glutamate transport expression as early as 4 h after the impact which reached stable levels after 48 h. This at least allows for a time window where the microglia are affected by both TNF-α and corticosterone. Therefore, it is plausible that the microglial GLT-1 expression is regulated during pathological events by a balance of inflammatory and anti-inflammatory substances.

Certainly, more research is needed to understand the expression pattern of microglial glutamate transporters during pathophysiology and the physiological meaning of the expression.

Microglial glutamate uptake is coupled to glutathione synthesis and glutamate release (see Fig. 4)

The physiological function of glutamate uptake through microglial glutamate transporters has been questioned. At least three major hypotheses exist. The first two theories concern the actual transport of glutamate in microglia. According to one theory, the microglial glutamate uptake serves as a back up system for the diminished astroglial glutamate uptake (Gras et al. 2006; Nakajima et al. 2001; Schwartz et al. 2003), but this has been debated since the microglial glutamate uptake capacity is only 10% of that measured for astrocytes (Persson et al. 2005; Shaked et al. 2005). In any case, during virus infection, microglia have been shown to express GS (Chretien et al. 2002; Vallat-Decouvelaere et al. 2003), like astrocytes do, which could make them capable of metabolising glutamate into glutamine to aid neurons (Gras et al. 2006; Hertz et al. 1999; Sonnewald et al. 1997). Another plausible theory is that the glutamate uptake is used for glutamate release (Noda et al. 1999). The third theory, presented by Rimaniol et al. (2001), proposes that the glutamate uptake is used for cystine uptake and for a direct incorporation into glutathione.

Using LPS and TNF-α as molecular tools to modulate microglial GLT-1 expression together with radioactively labelled glutamate, it was investigated how the transported glutamate is metabolized (Persson et al. 2006). It was found that both TNF-α and LPS, even when TNF-α synthesis was inhibited by thalidomide, increased microglial glutathione levels. This shows that although TNF-α can induce GSH synthesis by itself, LPS induced GSH synthesis is TNF-α-independent. With the metabolic labelling, it was shown that most of the ^{3}H-glutamate ended up in the large intracellular pool of glutamate although there was seemingly only an increase in labelled ^{3}H-glutamate after TNF-α treatment. One might expect an increase after LPS

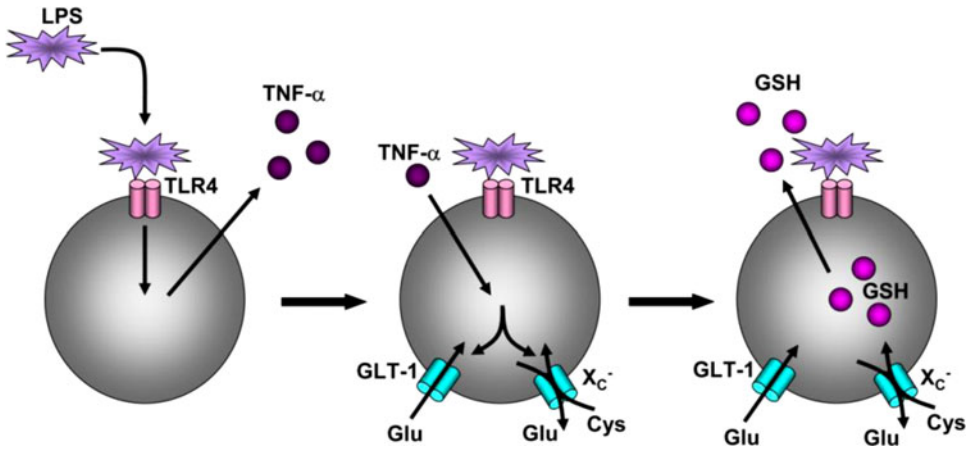

Fig. 4 The inflammatory stimulus LPS is detected by microglia via the Toll-like receptor 4 (TLR4), causing a prominent release of the cytokine TNF-α into the extracellular space. TNF-α can then activate the microglia in an autocrine manner and induce/increase the microglial expression of the glutamate transporter GLT-1, making the cells able to transport glutamate from the extracellular space. The sequestered glutamate can then either be stored in the intracellular glutamate pool, used to fuel the x_c^- system for cystine uptake, or be used directly for synthesis of the antioxidant glutathione (*GSH*). Increased levels of glutathione provide the microglia with a self-defence against oxidative stress (from Persson 2007)

treatment as well, but this stimulus also led to the largest release of labelled compounds in our model system which likely explains the result. Interestingly, there was ^{3}H-labelled contents in the glutathione fraction, with more labelling after LPS and TNF-α treatment. This is in line with the model proposed by Rimaniol et al. (2001) where microglia use the glutamate uptake through EAATs to fuel the x_c^- system for cystine uptake and for direct incorporation of glutamate into glutathione.

Glutathione is released from brain cells, reported mainly for astrocytes (Sagara et al. 1996), through multi-drug resistance protein (MRP) 1 and 2 (Leier et al. 1996; Paulusma et al. 1999; Rebbeor et al. 2002). MRP1 has been reported to be expressed functionally in microglia (Dallas et al. 2003). This leads to the conclusion that microglia may use the GSH system, and subsequently the EAAT system, to protect, at least themselves but also possibly other cell types, from oxidative stress (Persson et al. 2006). In fact, in cell cultures, microglia have a more prominent GSH system than neurons or astrocytes (Hirrlinger et al. 2000), and stain intensively for both GSH and GRed (Chatterjee et al. 1999; Gutterer et al. 1999). This prominent antioxidant system has been proposed to reflect the need of microglia to protect themselves from ROS that they can release upon activation (Hirrlinger et al. 2000). The question still remains whether the GSH system may protect other vulnerable cells like neurons as well. This would give the microglia some neuroprotective capabilities as previously suggested (Rimaniol et al. 2001; Vallat-Decouvelaere et al. 2003). In fact, microglial conditioned media has been shown to have neuroprotective effects (Watanabe et al. 2000).

Microglial GLT-1 is up-regulated in response to herpes simplex virus infection to provide an antiviral defence via glutathione (see Fig. 5)

HSV infections in the CNS are relatively uncommon in the population since HSV normally have higher affinity and easier access to other non-neuronal tissue. Nonetheless, highly neurovirulent and neurotropic HSV strains exist (Broberg and Hukkanen 2005; Frampton et al. 2005; Kimberlin 2004) and HSV infections normally have devastating effects, causing encephalitis or meningitis (Tyler 2004). The previously described research was mainly focused on examining the effect of the inflammatory environment that occurs due to brain trauma, stroke or inflammation, or the inflammatory environment that is connected to neurodegeneration, with regard to microglial glutamate transporter expression and regulation. HSV infections in the CNS share several hallmarks with these conditions, including high levels of TNF-α (Lokensgard et al. 2002; Sköldenberg 1996), and are therefore very suitable to study under experimental conditions the physiological functions of the microglial glutamate transporters.

In our experimental paradigm, it was found that HSV indeed caused an inflammatory response by microglia (Persson et al. 2007). In line with the previous findings, increased levels of TNF-α led to increased expression of microglial GLT-1 and, presumably, increased microglial glutamate uptake. The up-regulation of microglial GLT-1 is remarkable since most proteins are down-regulated during infection due to viral host shut-off functions (Matis and Kudelova 2001; Mossman et al. 2001). An induced/increased microglial GLT-1 expression can therefore be

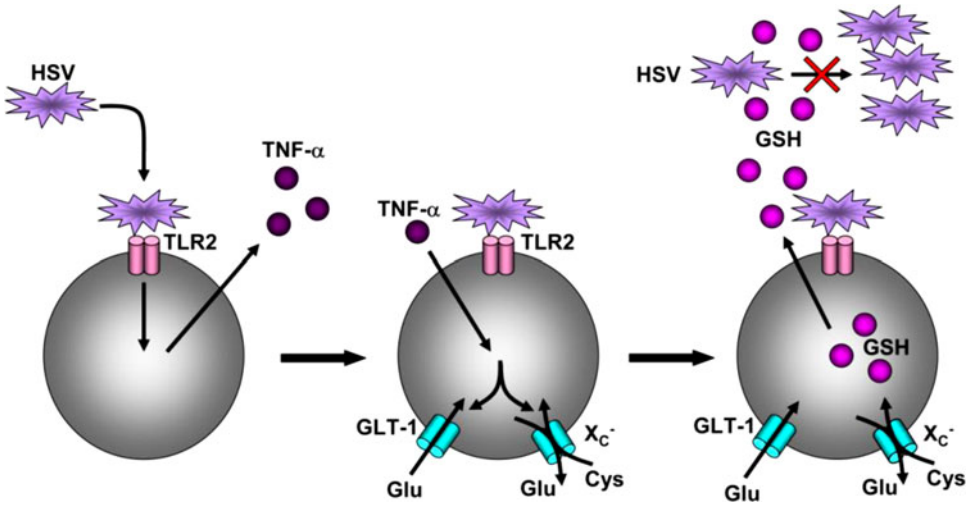

Fig. 5 Herpes simplex virus (HSV) can be detected by microglia, possibly through the Toll-like receptor 2 (TLR2), and causes a prominent TNF-α release into the extracellular space. TNF-α can then activate the microglia in an autocrine manner and induce/increase the microglial expression of the glutamate transporter GLT-1, making the cells able to transport glutamate from the extracellular space. The sequestered glutamate can then either be stored in the intracellular glutamate pool, used to fuel the x_c^- system for cystine uptake, or be used directly for synthesis of the antioxidant glutathione (GSH). Increased levels of glutathione provide microglia with a viral defence since glutathione has prominent antiviral properties, making the microglia more resistant to herpes simplex virus infections than astrocytes or neuron (from Persson 2007)

theorized to be part of an antiviral defence for microglia (Persson et al. 2007). Such a defence can be provided by GSH that is normally increased by increased glutamate uptake (Persson et al. 2006; Rimaniol et al. 2001) and have great antiviral properties (Palamara et al. 1995, 1996a) by counteracting the oxidative state that is crucial for viral replication (Ciriolo et al. 1997). Indeed, our results show that the microglial GSH levels are neither decreased in response to HSV nor increased as predicted by the increased levels of TNF-α (Persson et al. 2007). This can be interpreted as if the microglial GLT-1 provides enough glutamate for the microglia to allow enough synthesis of GSH to counteract the effect of the virus, thus inhibiting viral entry and viral replication. This is reflected by the fact that microglia have increased resistance to HSV infections compared to neurons or astrocytes, as shown by us and others (Lokensgard et al. 2001; Persson et al. 2007), and the fact that microglia have the highest levels of GSH among the cells in the CNS (Hirrlinger et al. 2000). Additionally, it is known that cells with high GSH levels have higher resistance to viral infections than cells with lower GSH levels (Macchia et al. 1999). Although increased GSH production via glutamate uptake may not be the only factor for the increased resistance shown by microglia, it nonetheless shows a mechanism that can provide an antiviral defence. Indeed, by pharmacological inhibition of the microglial glutamate transporters, it was shown that they are crucial for the microglia's viral resistance (Persson et al. 2007).

Interestingly, the antiviral mechanism provided by microglial GLT-1 and GSH up-regulation could be a general mechanism, holding true for several other types of viruses besides HSV. It has been shown that microglial glutamate transporters are involved in HIV infections of the CNS (Porcheray et al. 2006a; Vallat-Decouvelaere et al. 2003). HIV drives microglia towards a proinflammatory state (Porcheray et al. 2006b) and there is an induction of glutamate transporters in these cells (Porcheray et al. 2006a; Vallat-Decouvelaere et al. 2003). Furthermore, GSH has antiviral effects for HIV (Palamara et al. 1996b). Therefore, it seems plausible that the results obtained with HSV may hold true for HIV as well.

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References

Abbott NJ, Ronnback L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 7:41–53

Aloisi F (2001) Immune function of microglia. Glia 36:165–179

Aravalli RN, Hu S, Rowen TN, Palmquist JM, Lokensgard JR (2005) Cutting edge: TLR2-mediated proinflammatory cytokine and chemokine production by microglial cells in response to herpes simplex virus. J Immunol 175:4189–4193

Arriza JL, Eliasof S, Kavanaugh MP, Amara SG (1997) Excitatory amino acid transporter 5, a retinal glutamate transporter coupled

to a chloride conductance. Proc Natl Acad Sci USA 94: 4155–4160
Bannai S (1986) Exchange of cystine and glutamate across plasma membrane of human fibroblasts. J Biol Chem 261:2256–2263
Barron KD (1995) The microglial cell. A historical review. J Neurol Sci 134:57–68
Benveniste H, Drejer J, Schousboe A, Diemer NH (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
Bradl M, Hohlfeld R (2003) Molecular pathogenesis of neuroinflammation. J Neurol Neurosurg Psychiatry 74:1364–1370
Broberg EK, Hukkanen V (2005) Immune response to herpes simplex virus and gamma134.5 deleted HSV vectors. Curr Gene Ther 5:523–530
Brooke SM, Sapolsky RM (2003) Effects of glucocorticoids in the gp120-induced inhibition of glutamate uptake in hippocampal cultures. Brain Res 972:137–141
Carlson NG, Wieggel WA, Chen J, Bacchi A, Rogers SW, Gahring LC (1999) Inflammatory cytokines IL-1 alpha, IL-1 beta, IL-6, and TNF-alpha impart neuroprotection to an excitotoxin through distinct pathways. J Immunol 163:3963–3968
Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. Physiol Rev 59:527–605
Chatterjee S, Noack H, Possel H, Keilhoff G, Wolf G (1999) Glutathione levels in primary glial cultures: monochlorobimane provides evidence of cell type-specific distribution. Glia 27: 152–161
Cho Y, Bannai S (1990) Uptake of glutamate and cysteine in C-6 glioma cells and in cultured astrocytes. J Neurochem 55:2091–2097
Chretien F, Vallat-Decouvelaere AV, Bossuet C, Rimaniol AC, Le Grand R, Le Pavec G, Creminon C, Dormont D, Gray F, Gras G (2002) Expression of excitatory amino acid transporter-2 (EAAT-2) and glutamine synthetase (GS) in brain macrophages and microglia of SIVmac251-infected macaques. Neuropathol Appl Neurobiol 28:410–417
Chretien F, Le Pavec G, Vallat-Decouvelaere AV, Delisle MB, Uro-Coste E, Ironside JW, Gambetti P, Parchi P, Creminon C, Dormont D, Mikol J, Gray F, Gras G (2004) Expression of excitatory amino acid transporter-1 (EAAT-1) in brain macrophages and microglia of patients with prion diseases. J Neuropathol Exp Neurol 63:1058–1071
Ciriolo MR, Palamara AT, Incerpi S, Lafavia E, Bue MC, De Vito P, Garaci E, Rotilio G (1997) Loss of GSH, oxidative stress, and decrease of intracellular pH as sequential steps in viral infection. J Biol Chem 272:2700–2708
Clarke DD, Sokoloff L, Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD (1999) Circulation and energy metabolism of the brain. In: Basic neurochemistry: molecular, cellular and medical aspects. Lippincott-Raven, Philadelphia, pp 637–669
Conrad, Sato H (2011) The oxidative stress-inducible cystine/glutamate antiporter, system x_c^-: cystine supplier and beyond. Amino Acids (this issue)
Cooper AJL, Rosenberg RN, Prusiner SB, DiMauro S, Barchi RL, Kunk LM (1997) Glutathione in the brain: disorders of glutathione metabolism. In: The molecular and genetic basis of neurological disease. Butterworth-Heinemann, Boston, pp 1195–1230
Dallas S, Zhu X, Baruchel S, Schlichter L, Bendayan R (2003) Functional expression of the multidrug resistance protein 1 in microglia. J Pharmacol Exp Ther 307:282–290
Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65:1–105
Dodd PR (2002) Excited to death: different ways to lose your neurones. Biogerontology 3:51–56
Dong Y, Benveniste EN (2001) Immune function of astrocytes. Glia 36:180–190
Dringen R (2000) Metabolism and functions of glutathione in brain. Prog Neurobiol 62:649–671
Dringen R, Hirrlinger J (2003) Glutathione pathways in the brain. Biol Chem 384:505–516
Erecinska M, Silver IA (1990) Metabolism and role of glutamate in mammalian brain. Prog Neurobiol 35:245–296
Fairman WA, Vandenberg RJ, Arriza JL, Kavanaugh MP, Amara SG (1995) An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. Nature 375:599–603
Farber K, Kettenmann H (2005) Physiology of microglial cells. Brain Res Brain Res Rev 48:133–143
Fawcett JW, Asher RA (1999) The glial scar and central nervous system repair. Brain Res Bull 49:377–391
Figiel M, Engele J (2000) Pituitary adenylate cyclase-activating polypeptide (PACAP), a neuron-derived peptide regulating glial glutamate transport and metabolism. J Neurosci 20:3596–3605
Finberg RW, Knipe DM, Kurt-Jones EA (2005) Herpes simplex virus and toll-like receptors. Viral Immunol 18:457–465
Fine SM, Angel RA, Perry SW, Epstein LG, Rothstein JD, Dewhurst S, Gelbard HA (1996) Tumor necrosis factor alpha inhibits glutamate uptake by primary human astrocytes. Implications for pathogenesis of HIV-1 dementia. J Biol Chem 271:15303–15306
Fonnum F (1984) Glutamate: a neurotransmitter in mammalian brain. J Neurochem 42:1–11
Frampton AR Jr, Goins WF, Nakano K, Burton EA, Glorioso JC (2005) HSV trafficking and development of gene therapy vectors with applications in the nervous system. Gene Ther 12:891–901
Garaci E, Palamara AT, di Francesco P, Favalli C, Ciriolo MR, Rotilio G (1992) Glutathione inhibits replication and expression of viral proteins in cultured cells infected with Sendai virus. Biochem Biophys Res Commun 188:1090–1096
Gebicke-Haerter PJ, Van Calker D, Norenberg W, Illes P (1996) Molecular mechanisms of microglial activation. A. Implications for regeneration and neurodegenerative diseases. Neurochem Int 29:1–12
Gegelashvili G, Schousboe A (1998) Cellular distribution and kinetic properties of high-affinity glutamate transporters. Brain Res Bull 45:233–238
Gehrmann J, Matsumoto Y, Kreutzberg GW (1995) Microglia: intrinsic immuneffector cell of the brain. Brain Res Brain Res Rev 20:269–287
Gilgun-Sherki Y, Rosenbaum Z, Melamed E, Offen D (2002) Antioxidant therapy in acute central nervous system injury: current state. Pharmacol Rev 54:271–284
Gras G, Porcheray F, Samah B, Leone C (2006) The glutamate-glutamine cycle as an inducible, protective face of macrophage activation. J Leukoc Biol 80:1067–1075
Gras G, Samah B, Hubert A et al (2011) EEAT expression by macrophages and microglia: still more question than answers. Amino Acids (this issue)
Gutterer JM, Dringen R, Hirrlinger J, Hamprecht B (1999) Purification of glutathione reductase from bovine brain, generation of an antiserum, and immunocytochemical localization of the enzyme in neural cells. J Neurochem 73:1422–1430
Had-Aissouni L (2011) Toward a new role for plasma membrane sodium-dependent glutamate transporters of astrocytes: maintenance of antioxidant defenses beyond extracellular glutamate clearance. Amino Acids (this issue)
Hallenbeck JM (2002) The many faces of tumor necrosis factor in stroke. Nat Med 8:1363–1368
Hansson E, Muyderman H, Leonova J, Allansson L, Sinclair J, Blomstrand F, Thorlin T, Nilsson M, Ronnback L (2000) Astroglia and glutamate in physiology and pathology: aspects on glutamate transport, glutamate-induced cell swelling and gap-junction communication. Neurochem Int 37:317–329

Hehlgans T, Pfeffer K (2005) The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. Immunology 115:1–20

Hertz L, Dringen R, Schousboe A, Robinson SR (1999) Astrocytes: glutamate producers for neurons. J Neurosci Res 57:417–428

Hirrlinger J, Gutterer JM, Kussmaul L, Hamprecht B, Dringen R (2000) Microglial cells in culture express a prominent glutathione system for the defense against reactive oxygen species. Dev Neurosci 22:384–392

Homa FL, Brown JC (1997) Capsid assembly and DNA packaging in herpes simplex virus. Rev Med Virol 7:107–122

Jacobsson J, Persson M, Hansson E, Ronnback L (2006) Corticosterone inhibits expression of the microglial glutamate transporter GLT-1 in vitro. Neuroscience 139:475–483

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

Kimberlin DW (2004) Neonatal herpes simplex infection. Clin Microbiol Rev 17:1–13

Kondo K, Hashimoto H, Kitanaka J, Sawada M, Suzumura A, Marunouchi T, Baba A (1995) Expression of glutamate transporters in cultured glial cells. Neurosci Lett 188:140–142

Kreutzberg GW (1996) Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19:312–318

Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39:151–170

Leier I, Jedlitschky G, Buchholz U, Center M, Cole SP, Deeley RG, Keppler D (1996) ATP-dependent glutathione disulphide transport mediated by the MRP gene-encoded conjugate export pump. Biochem J 314(Pt 2):433–437

Leonova J, Thorlin T, Aberg ND, Eriksson PS, Ronnback L, Hansson E (2001) Endothelin-1 decreases glutamate uptake in primary cultured rat astrocytes. Am J Physiol Cell Physiol 281:C1495–C1503

Lewerenz J, Maher P, Methner A (2011) Regulation of xCT expression and system x_c^- function in neuronal cells. Amino Acids (this issue)

Liao SL, Chen CJ (2001) Differential effects of cytokines and redox potential on glutamate uptake in rat cortical glial cultures. Neurosci Lett 299:113–116

Lindenau J, Noack H, Asayama K, Wolf G (1998) Enhanced cellular glutathione peroxidase immunoreactivity in activated astrocytes and in microglia during excitotoxin induced neurodegeneration. Glia 24:252–256

Lokensgard JR, Hu S, Sheng W, van Oijen M, Cox D, Cheeran MC, Peterson PK (2001) Robust expression of TNF-alpha, IL-1beta, RANTES, and IP-10 by human microglial cells during nonproductive infection with herpes simplex virus. J Neurovirol 7:208–219

Lokensgard JR, Cheeran MC, Hu S, Gekker G, Peterson PK (2002) Glial cell responses to herpesvirus infections: role in defense and immunopathogenesis. J Infect Dis 186(Suppl 2):S171–S179

Lopez-Redondo F, Nakajima K, Honda S, Kohsaka S (2000) Glutamate transporter GLT-1 is highly expressed in activated microglia following facial nerve axotomy. Brain Res Mol Brain Res 76:429–435

Macchia I, Palamara AT, Bue C, Savini P, Ciriolo M, Gaziano R, di Francesco P (1999) Increased replication of Sendai virus in morphine-treated epithelial cells: evidence for the involvement of the intracellular levels of glutathione. Int J Immunopharmacol 21:185–193

Marchetti L, Klein M, Schlett K, Pfizenmaier K, Eisel UL (2004) Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. J Biol Chem 279:32869–32881

Matis J, Kudelova M (2001) Early shutoff of host protein synthesis in cells infected with herpes simplex viruses. Acta Virol 45:269–277

McCullers DL, Sullivan PG, Scheff SW, Herman JP (2002) Traumatic brain injury regulates adrenocorticosteroid receptor mRNA levels in rat hippocampus. Brain Res 947:41–49

Minghetti L (2005) Role of inflammation in neurodegenerative diseases. Curr Opin Neurol 18:315–321

Mossman KL, Macgregor PF, Rozmus JJ, Goryachev AB, Edwards AM, Smiley JR (2001) Herpes simplex virus triggers and then disarms a host antiviral response. J Virol 75:750–758

Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT (1989) Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuron 2:1547–1558

Murphy TH, Schnaar RL, Coyle JT (1990) Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. FASEB J 4:1624–1633

Nakajima K, Tohyama Y, Kohsaka S, Kurihara T (2001) Ability of rat microglia to uptake extracellular glutamate. Neurosci Lett 307:171–174

Nakamura Y (2002) Regulating factors for microglial activation. Biol Pharm Bull 25:945–953

Nakanishi S, Nakajima Y, Masu M, Ueda Y, Nakahara K, Watanabe D, Yamaguchi S, Kawabata S, Okada M (1998) Glutamate receptors: brain function and signal transduction. Brain Res Brain Res Rev 26:230–235

Nawashiro H, Martin D, Hallenbeck JM (1997a) Inhibition of tumor necrosis factor and amelioration of brain infarction in mice. J Cereb Blood Flow Metab 17:229–232

Nawashiro H, Tasaki K, Ruetzler CA, Hallenbeck JM (1997b) TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. J Cereb Blood Flow Metab 17:483–490

Noda M, Nakanishi H, Akaike N (1999) Glutamate release from microglia via glutamate transporter is enhanced by amyloid-beta peptide. Neuroscience 92:1465–1474

O'Shea RD (2002) Roles and regulation of glutamate transporters in the central nervous system. Clin Exp Pharmacol Physiol 29:1018–1023

O'Shea RD, Lau CL, Farso MC, Diwakarla S, Zagami CJ, Svendsen BB, Feeney SJ, Callaway JK, Jones NM, Pow DV, Danbolt NC, Jarrott B, Beart PM (2006) Effects of lipopolysaccharide on glial phenotype and activity of glutamate transporters: evidence for delayed up-regulation and redistribution of GLT-1. Neurochem Int 48:604–610

Olney JW (1969) Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 164:719–721

Palamara AT, Perno CF, Ciriolo MR, Dini L, Balestra E, D'Agostini C, di Francesco P, Favalli C, Rotilio G, Garaci E (1995) Evidence for antiviral activity of glutathione: in vitro inhibition of herpes simplex virus type 1 replication. Antiviral Res 27:237–253

Palamara AT, Perno CF, Aquaro S, Bue MC, Dini L, Garaci E (1996a) Glutathione inhibits HIV replication by acting at late stages of the virus life cycle. AIDS Res Hum Retroviruses 12:1537–1541

Palamara AT, di Francesco P, Ciriolo MR, Bue C, Lafavia E, Rotilio G, Garaci E (1996b) Cocaine increases Sendai virus replication in cultured epithelial cells: critical role of the intracellular redox status. Biochem Biophys Res Commun 228:579–585

Paulusma CC, van Geer MA, Evers R, Heijn M, Ottenhoff R, Borst P, Oude Elferink RP (1999) Canalicular multispecific organic anion transporter/multidrug resistance protein 2 mediates low-affinity transport of reduced glutathione. Biochem J 338(Pt 2):393–401

Pekny M, Nilsson M (2005) Astrocyte activation and reactive gliosis. Glia 50:427–434

Persson M (2007) Microglial Glutamate Transporters—Regulation of Expression and Possible Physiological Functions. Thesis, Vasastadens Bokbinderi AB, Göteborg. ISBN 978-91-628-7110-9. http://dx.doi.org/2077/4419

Persson M, Brantefjord M, Hansson E, Ronnback L (2005) Lipopolysaccharide increases microglial GLT-1 expression and glutamate uptake capacity in vitro by a mechanism dependent on TNF-alpha. Glia 51:111–120

Persson M, Sandberg M, Hansson E, Ronnback L (2006) Microglial glutamate uptake is coupled to glutathione synthesis and glutamate release. Eur J Neurosci 24:1063–1070

Persson M, Brantefjord M, Liljeqvist JA, Bergstrom T, Hansson E, Ronnback L (2007) Microglial GLT-1 is upregulated in response to herpes simplex virus infection to provide an antiviral defence via glutathione. Glia 55:1449–1458

Piehl F, Lidman O (2001) Neuroinflammation in the rat—CNS cells and their role in the regulation of immune reactions. Immunol Rev 184:212–225

Pines G, Danbolt NC, Bjoras M, Zhang Y, Bendahan A, Eide L, Koepsell H, Storm-Mathisen J, Seeberg E, Kanner BI (1992) Cloning and expression of a rat brain L-glutamate transporter. Nature 360:464–467

Pogun S, Dawson V, Kuhar MJ (1994) Nitric oxide inhibits 3H-glutamate transport in synaptosomes. Synapse 18:21–26

Porcheray F, Samah B, Leone C, Dereuddre-Bosquet N, Gras G (2006a) Macrophage activation and human immunodeficiency virus infection: HIV replication directs macrophages towards a pro-inflammatory phenotype while previous activation modulates macrophage susceptibility to infection and viral production. Virology 349:112–120

Porcheray F, Leone C, Samah B, Rimaniol AC, Dereuddre-Bosquet N, Gras G (2006b) Glutamate metabolism in HIV-infected macrophages: implications for the CNS. Am J Physiol Cell Physiol 291:C618–C626

Raivich G (2005) Like cops on the beat: the active role of resting microglia. Trends Neurosci 28:571–573

Rebbeor JF, Connolly GC, Ballatori N (2002) Inhibition of Mrp2- and Ycf1p-mediated transport by reducing agents: evidence for GSH transport on rat Mrp2. Biochim Biophys Acta 1559:171–178

Richman PG, Meister A (1975) Regulation of gamma-glutamylcysteine synthetase by nonallosteric feedback inhibition by glutathione. J Biol Chem 250:1422–1426

Riedel G (1996) Function of metabotropic glutamate receptors in learning and memory. Trends Neurosci 19:219–224

Rimaniol AC, Haik S, Martin M, Le Grand R, Boussin FD, Dereuddre-Bosquet N, Gras G, Dormont D (2000) Na+-dependent high-affinity glutamate transport in macrophages. J Immunol 164:5430–5438

Rimaniol AC, Mialocq P, Clayette P, Dormont D, Gras G (2001) Role of glutamate transporters in the regulation of glutathione levels in human macrophages. Am J Physiol Cell Physiol 281:C1964–C1970

Romera C, Hurtado O, Botella SH, Lizasoain I, Cardenas A, Fernandez-Tome P, Leza JC, Lorenzo P, Moro MA (2004) In vitro ischemic tolerance involves upregulation of glutamate transport partly mediated by the TACE/ADAM17-tumor necrosis factor-alpha pathway. J Neurosci 24:1350–1357

Ronnback L, Hansson E (2004) On the potential role of glutamate transport in mental fatigue. J Neuroinflammation 1:22

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

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes HM, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB (2005) Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 433:73–77

Sagara J, Makino N, Bannai S (1996) Glutathione efflux from cultured astrocytes. J Neurochem 66:1876–1881

Sampaio EP, Sarno EN, Galilly R, Cohn ZA, Kaplan G (1991) Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes. J Exp Med 173:699–703

Sato H, Tamba M, Ishii T, Bannai S (1999) Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. J Biol Chem 274:11455–11458

Sattler R, Tymianski M (2000) Molecular mechanisms of calcium-dependent excitotoxicity. J Mol Med 78:3–13

Schlag BD, Vondrasek JR, Munir M, Kalandadze A, Zelenaia OA, Rothstein JD, Robinson MB (1998) Regulation of the glial Na+-dependent glutamate transporters by cyclic AMP analogs and neurons. Mol Pharmacol 53:355–369

Schmutzhard E (2001) Viral infections of the CNS with special emphasis on herpes simplex infections. J Neurol 248:469–477

Schousboe A (1981) Transport and metabolism of glutamate and GABA in neurons are glial cells. Int Rev Neurobiol 22:1–45

Schwartz M, Shaked I, Fisher J, Mizrahi T, Schori H (2003) Protective autoimmunity against the enemy within: fighting glutamate toxicity. Trends Neurosci 26:297–302

Shaked I, Tchoresh D, Gersner R, Meiri G, Mordechai S, Xiao X, Hart RP, Schwartz M (2005) Protective autoimmunity: interferon-gamma enables microglia to remove glutamate without evoking inflammatory mediators. J Neurochem 92:997–1009

Shohami E, Ginis I, Hallenbeck JM (1999) Dual role of tumor necrosis factor alpha in brain injury. Cytokine Growth Factor Rev 10:119–130

Simonian NA, Coyle JT (1996) Oxidative stress in neurodegenerative diseases. Annu Rev Pharmacol Toxicol 36:83–106

Skoldenberg B (1996) Herpes simplex encephalitis. Scand J Infect Dis Suppl 100:8–13

Sonnewald U, Westergaard N, Schousboe A (1997) Glutamate transport and metabolism in astrocytes. Glia 21:56–63

Storck T, Schulte S, Hofmann K, Stoffel W (1992) Structure, expression, and functional analysis of a Na(+)-dependent glutamate/aspartate transporter from rat brain. Proc Natl Acad Sci USA 89:10955–10959

Stover JF, Schoning B, Beyer TF, Woiciechowsky C, Unterberg AW (2000) Temporal profile of cerebrospinal fluid glutamate, interleukin-6, and tumor necrosis factor-alpha in relation to brain edema and contusion following controlled cortical impact injury in rats. Neurosci Lett 288:25–28

Streit WJ, Walter SA, Pennell NA (1999) Reactive microgliosis. Prog Neurobiol 57:563–581

Suzuki K, Ikegaya Y, Matsuura S, Kanai Y, Endou H, Matsuki N (2001) Transient upregulation of the glial glutamate transporter GLAST in response to fibroblast growth factor, insulin-like growth factor and epidermal growth factor in cultured astrocytes. J Cell Sci 114:3717–3725

Swanson RA (1992) Astrocyte glutamate uptake during chemical hypoxia in vitro. Neurosci Lett 147:143–146

Swanson RA, Farrell K, Simon RP (1995) Acidosis causes failure of astrocyte glutamate uptake during hypoxia. J Cereb Blood Flow Metab 15:417–424

Tasaki K, Ruetzler CA, Ohtsuki T, Martin D, Nawashiro H, Hallenbeck JM (1997) Lipopolysaccharide pre-treatment induces resistance against subsequent focal cerebral ischemic damage in spontaneously hypertensive rats. Brain Res 748:267–270

Trotti D, Rossi D, Gjesdal O, Levy LM, Racagni G, Danbolt NC, Volterra A (1996) Peroxynitrite inhibits glutamate transporter subtypes. J Biol Chem 271:5976–5979

Tyler KL (2004) Herpes simplex virus infections of the central nervous system: encephalitis and meningitis, including Mollaret's. Herpes 11(Suppl 2):57A–64A

Vallat-Decouvelaere AV, Chretien F, Gras G, Le Pavec G, Dormont D, Gray F (2003) Expression of excitatory amino acid transporter-1 in brain macrophages and microglia of HIV-infected patients. A neuroprotective role for activated microglia? J Neuropathol Exp Neurol 62:475–485

van Landeghem FK, Stover JF, Bechmann I, Bruck W, Unterberg A, Buhrer C, von Deimling A (2001) Early expression of glutamate transporter proteins in ramified microglia after controlled cortical impact injury in the rat. Glia 35:167–179

Virgin CE Jr, Ha TP, Packan DR, Tombaugh GC, Yang SH, Horner HC, Sapolsky RM (1991) Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: implications for glucocorticoid neurotoxicity. J Neurochem 57: 1422–1428

Vitkovic L, Bockaert J, Jacque C (2000) "Inflammatory" cytokines: neuromodulators in normal brain? J Neurochem 74:457–471

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

Watanabe H, Abe H, Takeuchi S, Tanaka R (2000) Protective effect of microglial conditioning medium on neuronal damage induced by glutamate. Neurosci Lett 289:53–56

Whitley RJ, Roizman B (2001) Herpes simplex virus infections. Lancet 357:1513–1518

Yamada K, Watanabe M, Shibata T, Tanaka K, Wada K, Inoue Y (1996) EAAT4 is a post-synaptic glutamate transporter at Purkinje cell synapses. Neuroreport 7:2013–2017

Zschocke J, Bayatti N, Clement AM, Witan H, Figiel M, Engele J, 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