

Astrocytic control of glutamatergic activity: astrocytes as stars of the show

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It is a major recent finding that astrocytes can influence synaptic activity by release of glutamate, but many other glutamate-mediated activities are also controlled by astrocytes. Even the most obvious neuronal function of glutamate – its release as a transmitter – is regulated by astrocytes; these cells are needed for formation of precursors for glutamate synthesis, for reuptake of released transmitter, and for disposal of excess glutamate. Without astrocytic involvement, normal function of glutamatergic neurons is not possible, as exemplified by almost instantaneous abrogation of normal vision and learning upon inhibition of astrocyte-specific metabolic pathways. In addition, astrocytes are essential for production of the neuroprotectant glutathione, yet they can also contribute to neuronal death during ischemia by maintaining glutamine synthesis, enabling neuronal formation of neurotoxic glutamate.

It is an exciting recent observation that Ca^{2+} -dependent vesicular release of glutamate from astrocytes influences synaptic activity [1–3] (Box 1). Because free cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in astrocytes responds to glutamate-mediated neuronal activity via astrocytic glutamate receptors [4], and because elevation of $[\text{Ca}^{2+}]_i$ mediated by inositol (1,4,5)-trisphosphate [IP_3 or $\text{Ins}(1,4,5)\text{P}_3$] can propagate through an astrocytic network [5,6], this provides the potential for astrocyte-mediated signaling in the CNS. Mathematic modeling has demonstrated that if the astrocytic density of metabotropic glutamate receptors is large, time-limited stimulation of a neuron can evoke self-perpetuating neuronal oscillations (Figure 1), a mechanism that could play a role in epilepsy [6]. However, release of glutamate is far from the only manner in which astrocytes play a crucial role in glutamate-mediated brain function. Besides being the most important excitatory transmitter, glutamate is an important metabolic fuel, which is oxidatively degraded to carbon dioxide and water in the brain *in vivo* and in cultured astrocytes [7,8]. Because glutamate does not readily cross the blood–brain barrier, glutamate degradation must be compensated for by net synthesis of glutamate from glucose (so-called *de novo* synthesis). Glucose is the only glutamate precursor that (i) normally enters the CNS in large amounts and (ii) is able to generate the carbon skeleton

of glutamate [9]. Neurons lack the enzyme pyruvate carboxylase and therefore cannot perform net synthesis of glutamate from glucose [10–14]. This enzyme mediates effective synthesis of tricarboxylic acid (TCA) cycle constituents needed as precursors for glutamate synthesis (Figure 2), whereas the potential contribution by neuronal malic enzyme can be calculated as minimal ($<1 \text{ nmol mg}^{-1}$ protein after 60 min in glutamatergic cerebellar granule neurons [15], compared with K^+ -mediated glutamate release from these cells of $10 \text{ nmol min}^{-1} \text{ mg}^{-1}$ protein [16]). Neurons therefore depend on production of transmitter glutamate by their astrocytic neighbors.

Neuronal reuptake of previously released transmitter glutamate is also deficient, leading to uptake by astrocytes of most neuronally released transmitter glutamate by efficient astrocytic glutamate transporters, securing submicromolar synaptic glutamate concentrations under resting conditions [17]. Neurons therefore depend on astrocytes for return and reutilization of previously released transmitter glutamate. Moreover, the localization of astrocytic glutamate transporters [18] provides anatomical support for the hypothesis that these transporters help to shape fast point-to-point excitatory synaptic transmission.

Box 1. Astrocytic influences on glutamate function in neurons

- (i) Release of glutamate by astrocytes influences synaptic function [1–3,6]
- (ii) Sufficiently high density of metabotropic glutamate receptors on astrocytes can cause self-perpetuating neuronal oscillation [6]
- (iii) Net synthesis of transmitter glutamate requires astrocytic pyruvate carboxylase activity and tricarboxylic acid (TCA) cycle activity [9–14,43]
- (iv) Highly efficient astrocytic glutamate transporters accumulate released transmitter glutamate and help shape excitatory synaptic transmission [17,18,20]
- (v) Return to neurons of released transmitter glutamate (in the form of glutamine) and continued release of transmitter glutamate depend on astrocytic glutamine synthetase activity [21–26,29,30,35]
- (vi) Inhibition of the astrocytic TCA cycle impairs release of transmitter glutamate [32,33]
- (vii) Glutamate-dependent learning in day-old chicks is inhibited within 5 min by inhibition of the astrocytic TCA cycle [34]
- (viii) A retinal response to light is inhibited within 2 min after inhibition of astrocytic glutamine synthetase activity [29]

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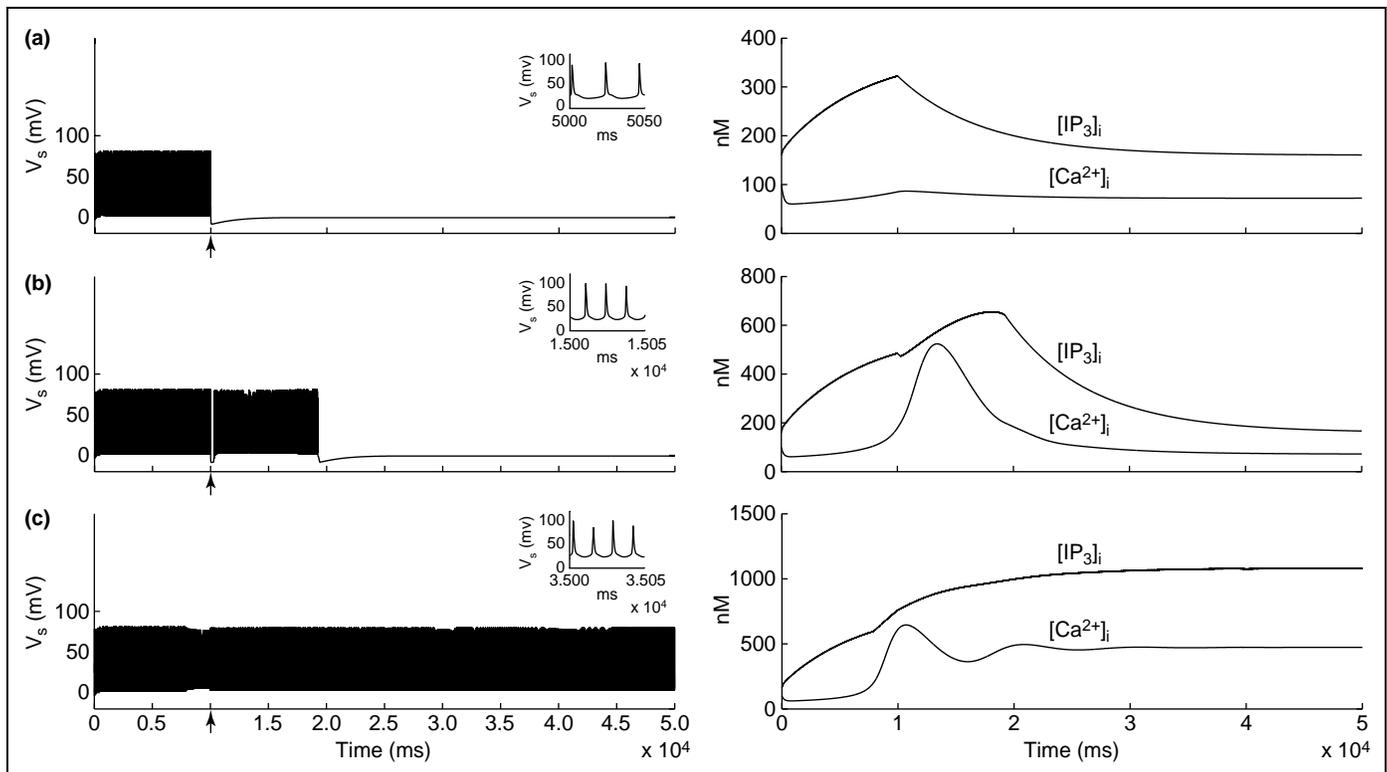


Figure 1. Modeling of effect of stimulation with DC current (0–10 s) of a neuron assumed to be surrounded by astrocytes that express metabotropic glutamate receptors allowing inositol (1,4,5)-triphosphate [IP_3 or $\text{Ins}(1,4,5)\text{P}_3$] production rates of 0.5 (a), 1.0 (b) and 1.5 (c) $\mu\text{mol l}^{-1} \text{s}^{-1}$. Neuronal membrane potentials (V_s) are shown on the left, and astrocytic concentrations of IP_3 ($[\text{IP}_3]_i$) and free cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) as functions of time are shown on the right. Arrows at 1×10^4 ms indicate the end of stimulation, which began at zero time. Inserts show neuronal spiking during a 50 ms period on a faster timescale at different times during the stimulation (a) or the continued spiking evoked by astrocytic Ca^{2+} signaling (b and c). In (a), astrocytic $[\text{IP}_3]_i$ builds up and decays (owing to ongoing degradation) as the neuron stops firing; astrocytic $[\text{Ca}^{2+}]_i$ increases only slightly. In (b), $[\text{IP}_3]_i$ builds up faster and to a higher level (note the different scales on the ordinates). Because $[\text{Ca}^{2+}]_i$ dynamics are much slower than neuronal dynamics, $[\text{Ca}^{2+}]_i$ spikes after stimulation of the neurons is terminated; the increased $[\text{Ca}^{2+}]_i$ triggers astrocytic release of glutamate, which creates slow neuronal inward currents that keep the neuron firing after stimulus termination, further increasing $[\text{IP}_3]_i$ in the astrocytes. Thus, astrocytic feedback keeps the neuron firing beyond the electrical stimulation. When IP_3 degradation eventually exceeds IP_3 production, spiking stops. In (c), the larger IP_3 production rate prevents decline of $[\text{Ca}^{2+}]_i$ below the level where it ceases to feedback to the neuron, and neuronal spiking continues. Reproduced, with permission, from Ref. [6], to which the reader is referred for further details. © (2004) Institute of Physics Publishing.

Early observations: metabolically handicapped neurons rely on astrocytes

A glutamate–glutamine cycle (Figure 2) carrying glutamine from astrocytes to neurons and glutamate in the opposite direction was first suggested by Benjamin and Quastel [19]. Avid accumulation of glutamate by astrocytes in primary cultures [20], together with the histochemical demonstration that glutamine synthetase is expressed in astrocytes but absent in neurons [21], corroborated the emerging concept that glutamate released from neurons is accumulated by astrocytes and converted to glutamine, which is returned to neurons and reconverted to glutamate. However, astrocytes also degrade glutamate oxidatively [8,22,23], indicating that accumulated glutamate is not returned quantitatively to neurons by the glutamate–glutamine cycle, and necessitating net formation of glutamate from glucose to maintain glutamate activity. This process requires activity of pyruvate carboxylase, the major enzyme allowing net synthesis of a TCA cycle constituent (oxaloacetate) from glucose. Pyruvate carboxylase is active in astrocytes but absent from neurons [10–14]. Pyruvate carboxylation generates oxaloacetate to replenish the TCA cycle, the constituents of which are continually depleted by anaplerotic reactions, especially for glutamate production in brain. Newly formed oxaloacetate condenses with acetyl

coenzyme A (acetyl CoA), which is formed by dehydrogenation of another molecule of pyruvate to form citrate. Citrate is metabolized in the astrocytic TCA cycle to form α -ketoglutarate, a direct precursor of glutamate (Figure 2), which is transferred to neurons in the glutamate–glutamine cycle. In addition to this net synthesis of glutamate, there is a transamination-mediated, bidirectional exchange between α -ketoglutarate and glutamate, which mainly occurs in neurons. After administration of a labeled substrate (e.g. $[1-^{13}\text{C}]$ glucose), this labeling can be used to determine rate of oxidative metabolism in nuclear magnetic resonance (NMR) imaging. However, this labeling must not be confused with net synthesis of glutamate from glucose [9]. Conclusions from these observations, and questions arising from them that are dealt with later in this review, are summarized in Box 2.

Astrocytic glutamine synthesis is essential for maintaining neuronal glutamate release

Treatment with methionine sulfoximine (MSO), a relatively specific inhibitor of glutamine synthetase (Figure 2), resulted in $\sim 50\%$ decrease in release of glutamate from brain slices [24] and from intact neurons into the extracellular space in the brain *in situ* [25], showing that astrocytic glutamine synthetase activity is important for K^+ -induced, Ca^{2+} -dependent glutamate release in

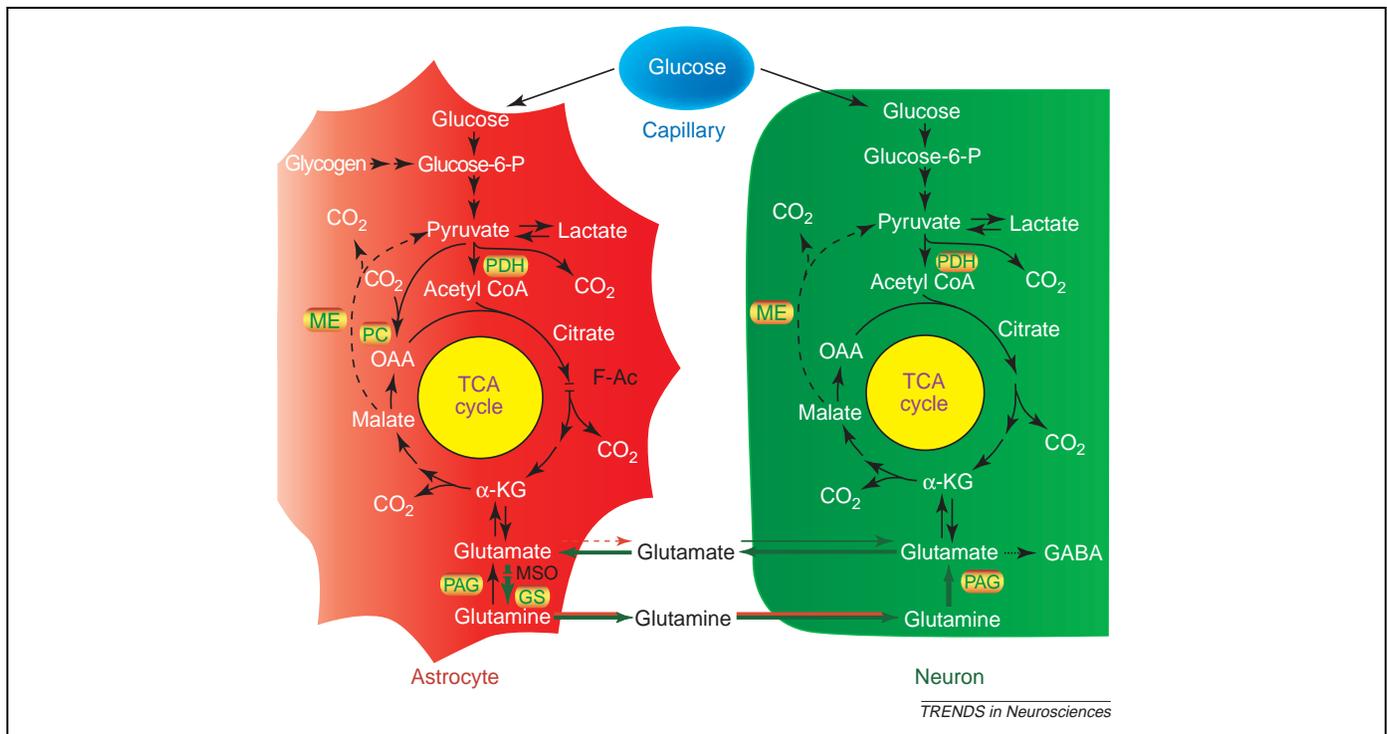


Figure 2. Compartmentalization of glucose metabolism (metabolic compartmentation) between astrocytes and neurons (not all intermediates are shown). One molecule of glucose can be glycolytically converted into two molecules of pyruvate in both neurons and astrocytes. In astrocytes, pyruvate is also formed from glycogen by glycogenolysis. In both cases, pyruvate production is via intermediates such as glucose-6-phosphate (glucose-6-P). Under anaerobic conditions, pyruvate is quantitatively converted to lactate, and in the brain some lactate is formed even under aerobic conditions. The main metabolic fate of pyruvate is entry into the tricarboxylic acid (TCA) cycle. In neurons, pyruvate enters the TCA cycle exclusively via acetyl coenzyme A (acetyl CoA), formed by oxidative decarboxylation, which is catalyzed by the pyruvate dehydrogenase complex (PDH). Acetyl CoA is metabolized in the TCA cycle after condensation with oxaloacetate (OAA) to form citrate, which is subsequently converted to oxaloacetate during one turn of the cycle generating a substantial amount of ATP without net formation of any TCA cycle intermediate. This does not mean that this process cannot give rise to labeling of glutamate if, for example, $[1-^{14}\text{C}]$ glucose is used as the substrate. However, such labeling is due to bidirectional transamination between α -ketoglutarate (α -KG) and glutamate, which does not change the content of either metabolite. In astrocytes, pyruvate can enter the TCA cycle both via acetyl CoA and via carboxylation mediated by pyruvate carboxylase (PC). The latter reaction generates a new molecule of oxaloacetate, which condenses with acetyl CoA to produce a new molecule of citrate, from which α -ketoglutarate, the immediate precursor of glutamate (and thus also a precursor of GABA), is formed. Although α -ketoglutarate as such might travel from astrocytes to neurons, in the mammalian brain it appears to be mainly or exclusively converted in astrocytes via glutamate to glutamine. This is transported in the glutamate–glutamine cycle (lower rectangle) to neurons, where it is hydrolyzed to form transmitter glutamate by phosphate-activated glutaminase (PAG). Released transmitter glutamate is also predominantly accumulated in astrocytes, where approximately two-thirds are converted by glutamine synthetase (GS) into glutamine, which after return to neurons in the glutamate–glutamine cycle can be reused as a precursor for transmitter glutamate; the remaining one-third is reconverted to α -ketoglutarate, metabolized in the TCA cycle to malate and decarboxylated by malic enzyme (ME) to pyruvate, predominantly although probably not exclusively in astrocytes (indicated by broken arrows in both cell types). Inhibition of astrocyte-specific processes by methionine sulfoximine (MSO; a relatively specific inhibitor of glutamine synthetase) or by fluoroacetate or fluorocitrate (F-Ac; specific inhibitors of the astrocytic TCA cycle) is indicated by breaks in the arrows.

intact CNS tissue. An elegant immunohistochemical study confirmed that K^+ -induced depolarization of hippocampal brain slices depletes glutamate from nerve endings, and that this effect is prevented in the presence of glutamine but enhanced in the presence of MSO [26]. It also showed that depolarization in intact CNS tissue increases levels of glutamate in glia. Normally the content of glutamate is low in glia [27], reflecting conversion of glutamate to glutamine [28–29], but it increases when glutamine synthesis is inhibited (Figure 3). In organotypic cultures prepared from hippocampal slices, inhibition of glutamine synthetase with MSO similarly causes $\sim 50\%$ reduction of glutamate-like immunoreactivity in nerve terminals, whereas it leads to a nearly fourfold increase in glia [30]. Glutamate in the terminals was maintained at control levels if the treatment with MSO was combined with administration of exogenous glutamine, but the level of glutamine in glia was reduced by two-thirds following MSO administration, even in the presence of glutamine. This indicates that exogenous glutamine, in contrast to exogenous glutamate, is mainly accumulated in neurons.

Maintenance of neuronal glutamate requires synthesis from glucose

The astrocytic TCA cycle can be selectively inhibited by the toxins fluoroacetate or fluorocitrate. At appropriate concentrations, these toxins are accumulated in astrocytes but not in neurons, such that they inhibit conversion of citrate to isocitrate by aconitase only in astrocytes [31]. Inhibition of the astrocytic TCA cycle impairs not only the synthesis of glutamate precursor but also the generation of oxidatively derived energy, which could reduce glutamate uptake. This explains the finding that, although addition of fluoroacetate to glutamine-free superfusion fluid reduces the release and content of glutamine during electrical field stimulation of rat hippocampal slices, it slightly increases the overflow of glutamate from the tissue [32]. However, overflow of glutamate is increased greatly if both fluoroacetate and glutamine are added to the superfusion fluid to compensate for the reduced astrocytic production of glutamine. Moreover, in analogous experiments in which the effect of perforant path stimulation was recorded in the granule cell layer of the *fascia*

Box 2. Metabolic dependence of neurons on astrocytes: early conclusions and subsequent developments

Conclusions from early observations, mainly in cell cultures

- (i) Glucose is used as an energy substrate by both neurons and astrocytes but can only provide significant net synthesis of glutamate in astrocytes [10–14]
- (ii) Released transmitter glutamate is accumulated mainly by astrocytes and partly returned to neurons in the glutamate–glutamine cycle [17,19,20]
- (iii) Substantial amounts of glutamate are oxidatively degraded, necessitating net synthesis of glutamate from glucose in astrocytes [7,22,23]

Later developments

- (i) Confirmation that glutamate–glutamine cycling and net formation of glutamate from glucose are important for glutamatergic neuronal signaling in intact CNS (Box 1) [24–26,29,30,32,33]
- (ii) Information about rates of actual metabolic fluxes in astrocytes that provide neurons in the intact brain with transmitter glutamate [39,40,42,43]
- (iii) Detailed information about metabolic pathway(s) during complete oxidative degradation of glutamate, and about the cellular localization of these processes [8,9,44]
- (iv) Information about vesicular release of glutamate from astrocytes and its importance for neuronal signaling [1–3,6]
- (v) Information about astrocytic contribution to increases in extracellular glutamate concentrations by non-vesicular mechanisms [51,52,61–63,66,67,69]

dentata, microdialysis with fluoroacetate consistently decreased the efflux of both glutamine and glutamate [33], possibly reflecting a smaller release of glutamate, requiring less uptake capacity.

Rapid functional effects of interference with glutamate supply

It is often assumed that neurons have sufficient metabolic reserves of glutamate to function independently of glia for extended periods. However, two sets of findings indicate that this is not the case: (i) intraventricular administration of fluoroacetate into the chick brain 0–5 min after one-trial aversive learning (simultaneous exposure to a tainted bead of one color and a non-tainted bead of different color) prevents the establishment of memory for the distinction between the two colors, an inhibition which becomes manifest 10 min post-training [34]; and (ii) intraocular injection of MSO in rats reduces within 2 min the b-wave of the electroretinogram, a retinal response to light (Figure 3). This effect is accompanied by abolition of immunoreactivity for glutamate in retinal ganglion and bipolar nerve cells [29,35]. Both effects of MSO, as well as prevention of learning by fluoroacetate, can be counteracted by injection of glutamine.

NMR spectroscopy shows high rates of glutamate synthesis and turnover

Introduction of NMR spectroscopy for studies of metabolic fluxes in brain [36,37] opened the door for determination of actual metabolic rates for both the glutamate–glutamine cycle and net formation of glutamate from glucose. This technique allows determination of the exact position of one or more labeled atom(s) within a given molecule after administration of [1-¹³C]-glucose. Because pyruvate

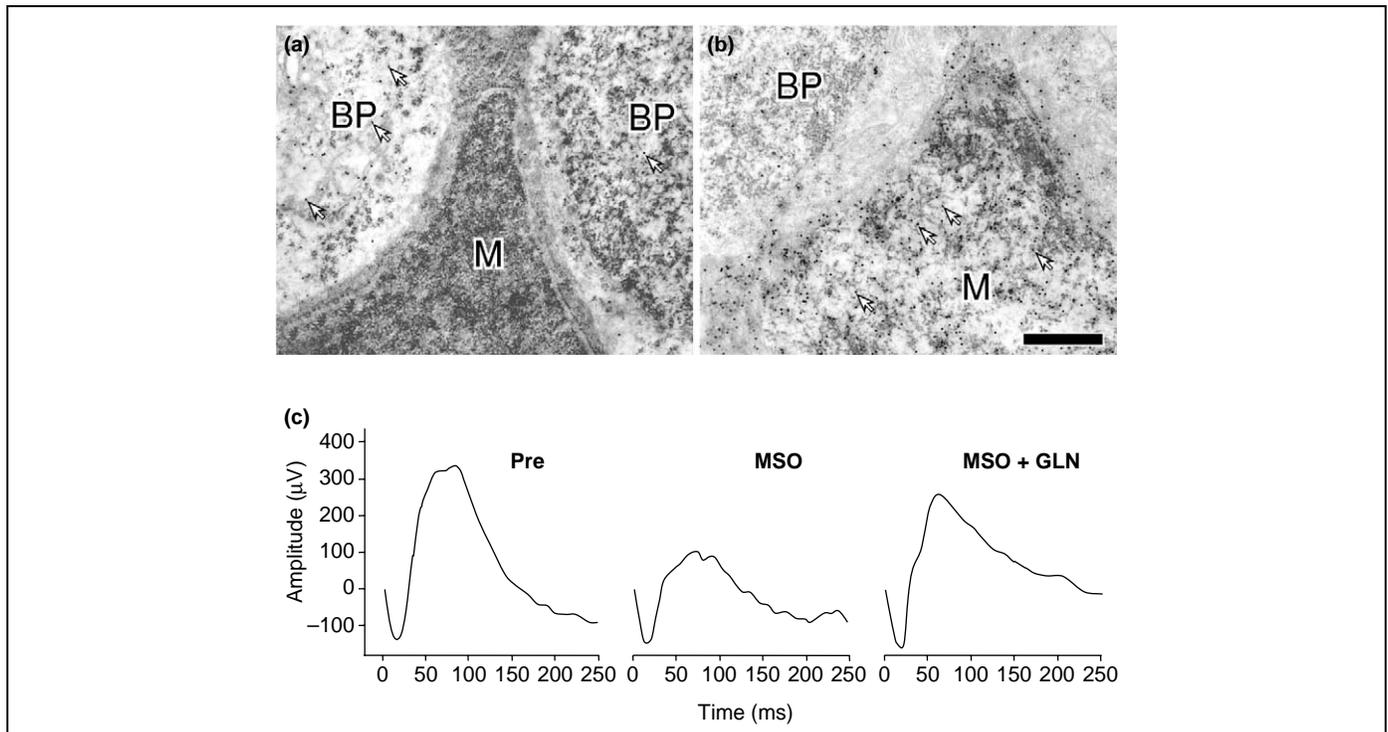


Figure 3. Effect of the glutamine synthetase inhibitor methionine sulfoximine on cellular distribution of glutamate in retina and on the b-wave of the retinogram. (a) Under control conditions, bipolar neurons (BP) in the retina stain heavily for glutamate (arrows), as indicated by immunogold labeling and electron microscopy, whereas Müller cells (M; an astrocyte-like cell type) are virtually devoid of glutamate immunostaining. (b) Two hours after intraocular injection of methionine sulfoximine (MSO; a relatively specific inhibitor of the astrocytic enzyme glutamine synthetase), Müller cells show immunogold staining for glutamate (arrows), whereas bipolar cells are almost devoid of staining. (c) Electroretinograms recorded from a rat before drug administration ('Pre'), 30 min after intraocular injection of 40 mM MSO ('MSO'), and 15 min after an additional intraocular injection with 4 mM glutamine ('MSO + GLN'). The b-wave (the positive component of the wave) is exquisitely sensitive to inhibition of glutamine supply, whereas the a-wave (the negative component) is insensitive. Scale bar in (b), 0.5 µm. Reproduced, with permission, from Ref. [29] © (2000) Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.

carboxylation labels a different carbon atom in glutamate, glutamine and GABA from that labeled during oxidative degradation via acetyl CoA, the rate of pyruvate carboxylation, which in many cases can equal the rate of net synthesis of glutamate, can also be measured. Moreover, the rate of incorporation of label into glutamine provides a direct measure of the rate of glutamine synthesis [38,39], and rates of glucose oxidation via acetyl CoA in astrocytes and neurons in human brain can be deduced as described by Gruetter *et al.* [40] (Table 1). In addition [^{13}C]acetate, which is degraded oxidatively in astrocytes but not in neurons, is used to determine metabolism via acetyl CoA in astrocytes [27,41]. The total rate of glucose oxidation (CMR_{glc}) shown in Table 1 is consistent with results obtained by different methods and, because one molecule of glucose is metabolized into two molecules of pyruvate, it corresponds to a total pyruvate metabolism of $0.82 \mu\text{mol min}^{-1} \text{g}^{-1}$ wet weight. Seventy percent of this occurs in neurons, almost 20% is metabolized via acetyl CoA in glia, and $>10\%$ is carboxylated in glia. Metabolism of acetate via acetyl CoA [27,41] is identical to that calculated from the metabolic fate of labeled glucose (Table 1). Because net synthesis of a TCA cycle constituent and its interacting amino acid requires joint pyruvate carboxylase and pyruvate dehydrogenase activity, the synthesis of glutamate as indicated by the rate of pyruvate carboxylation accounts for more than half of the pyruvate metabolism via acetyl CoA in astrocytes. In human brain, the rate of glutamine production can amount to $\sim 40\%$ of the total rate of oxidative metabolism or $\sim 50\%$ of the metabolic rate in neurons [40], although higher values have also been reported [39]. These values represent both cycling in the glutamate–glutamine cycle and transfer of recently synthesized glutamate, with a ratio between the two of $\sim 2:1$, as shown in Table 1 ($\text{Cycle}_{\text{return}}/\text{Cycle}_{\text{glc.der.}}$). A similar ratio has been determined in rat brain [42,43]. The finding that one-third of the trafficking in the glutamate–glutamine cycle represents transfer of glutamate newly synthesized from glucose in astrocytes confirms the conclusion drawn from demonstration of glutamate oxidation in cultured astrocytes, that the glutamate–glutamine cycle does not provide quantitative recovery in neurons of transmitter glutamate after its accumulation in these cells. Degradation of glutamate requires conversion to α -ketoglutarate, TCA cycle metabolism to malate, formation of pyruvate plus carbon dioxide (catalyzed by malic enzyme), and re-entry of the pyruvate into the TCA cycle (Figure 2). This process ('pyruvate recycling') occurs readily in astrocytes [44] and is sufficiently active to allow complete oxidative metabolism of glutamate [8].

Cellular glutamate and glutamine levels increase during glutamate-mediated activity

Formation of glutamate from glucose and oxidative degradation of glutamate are not necessarily simultaneous, and enhanced net formation and accumulation of glutamate from glucose can occur during brain activation. Recent studies by LaNoue have indicated a large increase in pyruvate carboxylation during an increase in glutamate-mediated activity in the retina (K.F. LaNoue, pers. commun.). Also, an increase of both glutamate and glutamine pool sizes has been demonstrated in day-old chicks during one-trial passive avoidance training [45], a process that depends on activity of glutamatergic neurons [46]. There was a concomitant, short-lasting decrease in glycogen content [45], suggesting that glycogen, besides providing a substrate for rapid production of energy, might be a major precursor for glutamate and glutamine.

Non-vesicular release of intracellular astrocytic glutamate

In addition to Ca^{2+} -dependent vesicular release of glutamate from neurons and astrocytes, glutamate is released in a non-vesicular fashion. A cystine–glutamate exchanger could account for up to 60% of non-synaptic glutamate released in the rat striatum [47]. This transporter is present in astrocytes but not in neurons [48]. Its most obvious function is to accumulate cystine, which is reduced to cysteine and used in the production of glutathione (Figure 4). Glutathione synthesis is a mainly astrocytic process [49], and astroglial glutathione export to the extracellular space is essential for providing neurons with the glutathione precursor L-cysteinylglycine, which is formed from glutathione by the ectoenzyme γ -glutamyl transpeptidase [50,51]. Cystine–glutamate exchange has been proposed to play a major role in the rapid increase in glutamate concentration in the extracellular space of rat organotypic hippocampal slice cultures; this increase, which becomes unmasked when glutamate uptake is abolished, is independent of vesicular release or glutamate exit through stress-activated anion channels [52]. That astrocytes are the major source of this increase is indicated by the observation that extracellular glutamate levels are elevated when glutamate content in astrocytes is increased by glutamine synthesis inhibition. Regulation of glutamate uptake might also contribute to fluctuations in its extracellular levels, because astrocytic glutamate transporter activity is influenced by stimulation of α_1 -adrenoceptors [53,54], by intracellular pH [55], by redox potential [56] and by free oxygen radicals [57].

Table 1. Metabolic fluxes in human brain measured by nuclear magnetic resonance spectroscopy^a

CMR_{glc}	$\text{PDH}_{\text{ac.CoA-N}}$	PC	$\text{PDH}_{\text{ac.CoA-A}}$	Cycle	$\text{Cycle}_{\text{glc.der.}}$	$\text{Cycle}_{\text{return}}$	Refs
0.41	0.57	0.09	0.15	0.26	0.09	0.17	[40]
	0.70		0.13				[41]
	0.80		0.14	0.32			[27]

^aMetabolic fluxes are given in $\mu\text{mol min}^{-1} \text{g}^{-1}$ wet weight. CMR_{glc} is the rate of glucose utilization; $\text{PDH}_{\text{ac.CoA-N}}$ is the pyruvate flux via acetyl coenzyme A in neurons (pyruvate dehydrogenase-mediated); PC is the pyruvate carboxylase-mediated flux, which is astrocyte-specific and is probably under many conditions equal to rate of net synthesis of glutamate from glucose. $\text{PDH}_{\text{ac.CoA-A}}$ is the pyruvate flux via acetyl coenzyme A in astrocytes (pyruvate dehydrogenase-mediated), part of which ($0.09 \mu\text{mol min}^{-1} \text{g}^{-1}$ wet weight) is required for glutamate synthesis. 'Cycle' values are of flux via the glutamate–glutamine cycle, which includes transfer of glucose-derived (glc.der.) newly synthesized glutamate by pyruvate carboxylase, for which reason return of previously released transmitter glutamate from neurons to astrocytes ($\text{Cycle}_{\text{return}}$) equals 'Cycle' flux minus 'PC'. For calculation of fluxes, see references.

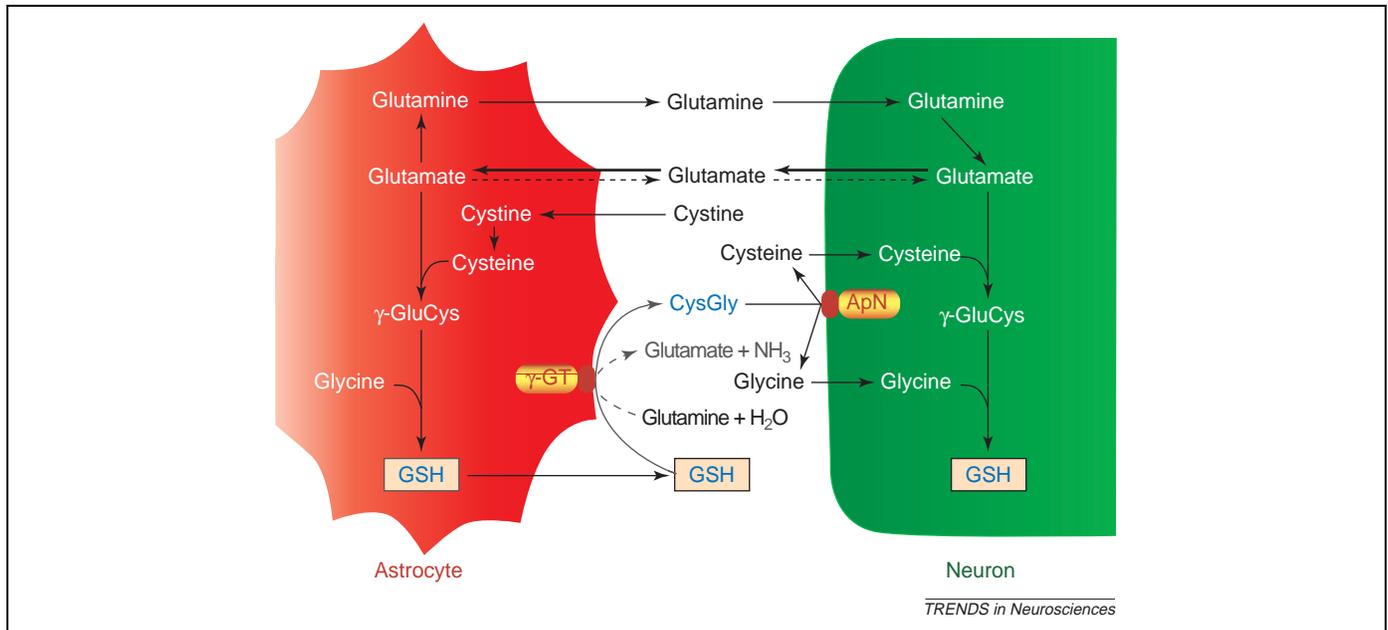


Figure 4. Interactions between astrocytes and neurons in production of glutathione (GSH). Astrocytes accumulate glycine and glutamate from the extracellular fluid (or generate net amounts of glutamate from glucose); they accumulate cystine by exchange with glutamate, an astrocyte-specific process that accounts for a large part of non-vesicular glutamate release from astrocytes [47]. Intracellularly, cystine is reduced to cysteine, which condenses with glutamate to form γ -glutamylcysteine (γ -GluCys). This dipeptide is converted to the tripeptide glutathione by further incorporation of glycine. Neurons cannot on their own form glutathione because they cannot reduce cystine to cysteine. They therefore utilize glutathione generated by astrocytes, which is released and converted by the ectopeptidase γ -glutamyl transpeptidase (γ -GT) to cysteinylglycine (CysGly) and glutamate. Cysteinylglycine is dissociated at the neuronal surface by another ectopeptidase, aminopeptidase N (ApN), to give glycine and cysteine, both of which are accumulated by the neuron. In the neurons, γ -glutamylcysteine is re-synthesized from cysteine and glutamate (that has been accumulated, or generated from accumulated glutamine). A further reaction with glycine yields glutathione.

During ischemic episodes, a marked elevation of extracellular glutamate levels occurs in the ischemic core [58–60]. Experiments in cultured cells and in the brain *in vivo* have shown that a considerable proportion of the glutamate accumulated in the core comes from astrocytes, which during exposure to elevated K^+ concentrations or ischemia release glutamate, through volume-sensitive channels and by reverse action of glutamate transporters [61–63]. However, during 30 min of severe hypoxia, cultured glutamatergic neurons (cerebellar granule cells) also produce and release large amounts of glutamate that is newly synthesized from glutamine, and the amount of released

glutamate depends on the glutamine concentration [64]. This glutamate must have been generated intracellularly, because its formation is inhibited by phenylsuccinate, which inhibits formation of glutamate from glutamine in glutamatergic neurons. *In vivo*, astrocytes in the penumbra continue to produce glutamine [65], providing neurons with precursors of transmitter glutamate that could contribute to expansion of the core region with time.

Extracellular formation of glutamate from glutamine

Nine hours of severe hypoxia or combined hypoxia and deprivation of substrates (glucose and glutamine) causes

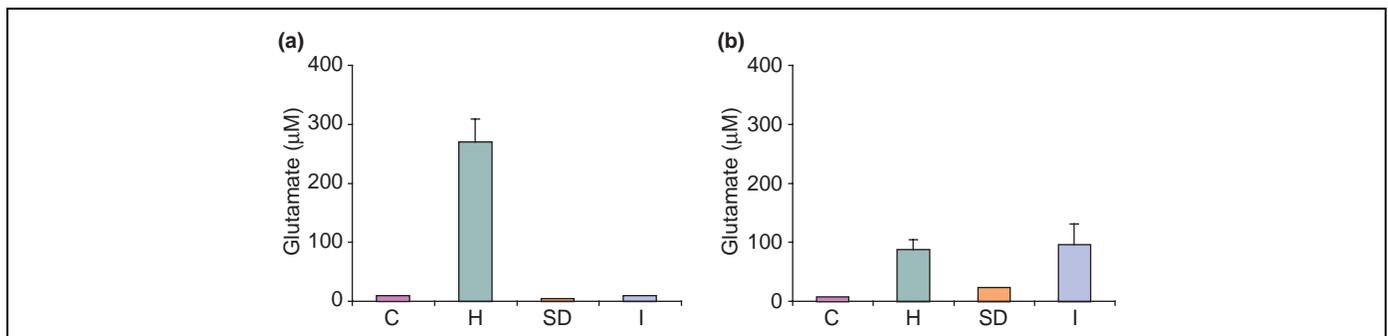


Figure 5. Glutamate accumulation in media of cultured cerebral cortical neurons during and after severe hypoxia, depletion of substrates (glucose and the glutamate precursor glutamine), and combined hypoxia and substrate deprivation. (a) Glutamate concentrations in tissue culture medium (1 ml) in which cultured cerebral cortical neurons (~0.5 mg protein per culture) were incubated for 9 h during control conditions (C), during severe hypoxia (H), during deprivation of the substrates glucose and glutamine (SD) and during simulated ischemia (I; i.e. combined severe hypoxia and substrate deprivation). Although cell death was pronounced during both hypoxia and simulated ischemia [64], there was a significant increase in glutamate concentration only during hypoxia, where 2 mM glutamine was present together with glucose, allowing glutamate production from glutamine. By contrast, the cell content of glutamate and glutamine at the start of the incubation was insufficient to cause a significant increase in the medium concentration of glutamate above that occurring even under control conditions (owing to medium change simultaneous with that of the other cultures). (b) After 9 h incubation under the specified condition, cells and cell debris remaining attached to the culture dishes were re-fed normal tissue culture medium, containing both glucose (7.5 mM) and glutamine (2 mM), and incubated for another 24 h under normoxic conditions. There was pronounced production of glutamate after both hypoxia and simulated ischemia, although only a few of the remaining cells were undamaged. Substrate deprivation did not lead to a significant increase in cell death [64] or glutamate release. Results are means \pm SEM of at least four individual experiments. Reproduced, with permission, from Ref. [64].

Table 2. Glutamate-related activities in astrocytes and neurons

Parameter	Astrocytes	Neurons	Refs
Vesicular glutamate release	Yes	Yes	[1–3,5]
Glutamate uptake	Very active	Limited	[17,20]
Glutamate formation from glucose	Yes	No	[9,10,13,38,39]
Glutamine synthesis	Yes	No	[9,21–23]
Glutathione synthesis	Yes	No ^a	[49–51]
Glutamate–cystine exchange	Yes	No	[48]
Non-vesicular glutamate release	Yes	Can be very pronounced ^b	[58–64]

^aRefers to synthesis directly from individual amino acids.

^bUnder abnormal conditions, when glutaminase activity is drastically increased and even displayed by glutaminase released from dead neurons or attached to cell debris.

widespread, but not complete, cell death in cultures of cerebral cortical neurons, which are GABAergic cells that withstand anoxia better than cerebellar granule cells [64]. During hypoxia, glutamate concentrations increase dramatically in the medium, whereas no such increase occurs in the absence of medium glutamine (Figure 5). During subsequent 'recovery' of the cultures in normal oxygenated medium (simulating reperfusion), there is considerable cell death and glutamate release, and it was suggested that the glutamate production occurred in the dead cell 'ghosts'. This hypothesis has been confirmed by direct demonstration of high activity of phosphate-activated glutaminase (PAG) associated with cellular fragments [66]. In the *in vivo* brain, significant glutaminase activity remains in the periphery of an ischemic lesion after 24 h of focal ischemia. At this time, the extracellular concentration of glutamate is increased tenfold relative to the contralateral side, and reverse microdialysis with 10 mM glutamine results in a 250% increase in glutamate. The glutamate release is further stimulated by inclusion of phosphate, an activator of PAG [67]. The importance of extracellular glutaminase is underscored by the observation of ~20-fold enhancement of glutaminase activity following cell breakage and release of intracellular feedback inhibition [68]. Support for direct synthesis of glutamate in the interstitial space has also been provided by the isolation of ¹⁴C-glutamate in the dialysate following reverse microdialysis with ¹⁴C-glutamine in the injured rat brain [69]. The expression of extracellular glutaminase activity adds yet another facet to the importance of astrocyte-specific glutamine synthesis. The question of whether glutamate is formed from glutamine in the interstitial space in undamaged brain tissue is unresolved. Mena *et al.* [69] proposed a potential role for both PAG and γ -glutamyl transpeptidase, the ectoenzyme involved in glutathione metabolism (Figure 4), which in the presence of maleate can catalyze conversion of glutamine to glutamate and function as maleate-activated glutaminase. Involvement of an ectoenzyme would alleviate the need for release of mitochondrial PAG. However, the presence of maleate-activated glutaminase in cultured brain cells and in the *in vivo* brain remains to be established [70].

Concluding remarks

The roles of glutamate in brain function are manifold and expanding. Although the most obvious function of glutamate, its release from glutamatergic neurons, is clearly neuronal, all functions of glutamate are regulated by astrocytes (Table 2). This applies not only to 'benevolent'

roles of glutamate (astrocytic production of glutamate precursors and glutathione, and capacity for vesicular release of glutamate) but also to its role in excitotoxicity and neuronal death, which ultimately depends on the ability of astrocytes to produce glutamate and glutamine.

Acknowledgements

The work of H.R.Z. was partly supported by NIH grant HD16596.

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