

# Astrocytes Couple Synaptic Activity to Glucose Utilization in the Brain

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*Astrocytes have functional characteristics that make them particularly well suited to couple glutamate uptake from the synaptic cleft to Na<sup>+</sup>-K<sup>+</sup>-ATPase activation and glucose utilization. The changes in glucose metabolism associated with these processes may provide signals detected by positron emission tomography.*

Two fundamental principles of brain physiology, postulated over one hundred years ago, are finally finding experimental support. Thus, in a seminal article published in 1890, Charles Sherrington suggested that the brain possesses intrinsic mechanisms to regulate the availability of energy substrates in correspondence with local variations of functional activity (see Ref. 15). Although the article focused primarily on blood flow, the notion of a tight coupling between neuronal activity and energy metabolism was clearly formulated. This notion represents the basis of all functional imaging techniques whereby local changes in brain activity can be visualized by monitoring the changes in blood flow, glucose utilization, or oxygen consumption associated with activity of specific neuronal circuits. The second principle was the suggestion by Camillo Golgi and his associates that, in view of their cytological characteristics, astrocytes should play a central role in the distribution of energy substrates from the circulation to neurons (see Ref. 15). Further structural and functional arguments for such a role of astrocytes have accumulated in recent years. Thus, whereas the ratio between neurons and nonneuronal cells depends on species, brain areas, or developmental ages, it is a well-established fact that neurons contribute, at most, 50% of cerebral cortical volume (15). An astrocyte-to-neuron ratio of 10:1 is a feature of most brain regions (15). In addition, particular astrocytic profiles, the end feet, surround intraparenchymal capillaries, which are the source of glucose. Glucose transporters of the GLUT-1 type are expressed on astrocytic end feet. In fact, the entire surface of intraparenchymal capillaries

is covered by astrocytic end feet (15). This cytoarchitectural arrangement implies that astrocytes form the first cellular barrier encountered by glucose entering the brain parenchyma, and it makes them a likely site of prevalent glucose uptake (Fig. 1). Other astrocyte processes are wrapped around synaptic contacts that possess receptors and reuptake sites for neurotransmitters. In fact, evidence obtained from quantitative morphometric studies supports the notion that astrocytes are indeed polarized cells, with one process contacting a cell of mesodermal origin, most frequently an endothelial cell of the capillary, and a multitude of processes that are intertwined within the neuropil ensheathing synaptic contacts. These features imply that astrocytes are ideally positioned to sense increases in synaptic activity and to couple them with energy metabolism.

## Glutamate-mediated neurotransmission is detected by astrocytes

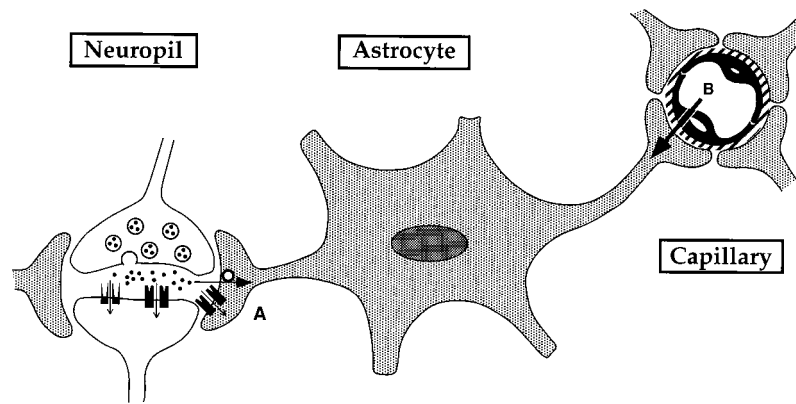
One of the well-established functions of astrocytes is the uptake of glutamate, which is released into the extracellular space during synaptic activation. Glutamate is the main excitatory neurotransmitter in the brain. In the cerebral cortex, activation of afferent pathways by specific modalities (e.g., somatosensory, visual, auditory) or of cortico-cortical association circuits results in a spatially and temporally defined local release of glutamate from the activated synaptic terminals. The released glutamate exerts profound effects on the excitability of target neurons, which are mediated by specific subtypes of glutamate receptors. The action of glutamate on postsynaptic neurons is rapidly terminated by an avid reuptake system present on astrocyte processes, which ensheath synaptic contacts (11). This removal of glutamate

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*"...astrocytes ... sense increases in synaptic activity..."*

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**FIGURE 1.** Cytological relationships among astrocytes, neurons, and blood vessels. Astrocytes form the first cellular barrier encountered by glucose entering the brain parenchyma and are likely a prevalent uptake site (B). They are also ideally located to sense synaptic activity (A) because one of their main functions is to eliminate glutamate and potassium accumulating in the extracellular space after neuronal activation. Finally, they can provide metabolic intermediates to neurons such as lactate that can be used by them as fuel. From Ref. 15 with permission.

from the synaptic cleft is operated through specific glutamate transporters, two of which are predominantly, if not exclusively, glia specific. These are GLAST (or EAAT1) and GLT1 (or EAAT2) (11). The third glutamate transporter subtype, EAAC-1 (or EAAT3), is exclusively localized in neurons but does not appear to be involved in the clearance of synaptically released glutamate (11). Glutamate uptake into astrocytes is driven by the electrochemical gradient of sodium, implying that it is a sodium-dependent mechanism with a stoichiometry of three sodium ions cotransported with one glutamate (Fig. 2). The coupling between synaptic glutamate release and its reuptake into astrocytes is so tight that determination of the  $\text{Na}^+$  current generated in astrocytes by the cotransport of glutamate and  $\text{Na}^+$  through the glutamate transporter provides an accurate reflection of glutamate release from the synapse (1). Once in astrocytes, glutamate is predominantly converted to glutamine through an ATP-requiring reaction catalyzed by the astrocyte-specific enzyme glutamine synthase. Glutamine is then released by astrocytes and taken up by neurons to replenish the neurotransmitter pool of glutamate (Fig. 2).

### Stimulation of the astrocytic glutamate transporter is a signaling mechanism for activity-dependent glucose utilization

Experimental and clinical evidence has indicated the existence of a tight coupling between cortical activation and localized increases in glucose utilization (see Ref. 5). The 2-deoxyglucose (2-DG) technique developed by Louis Sokoloff (5) for laboratory animals and its adaptation to positron emission tomography (PET) for humans have provided a direct demonstration of the coupling between neuronal activation and glucose utilization (5). Despite this compelling evidence,

the cellular and molecular mechanisms that underlie such a coupling are still under scrutiny.

In 1994, we described (7) a mechanism by which glutamate transport into astrocytes could trigger aerobic glycolysis in these cells, i.e., glucose utilization and lactate production. This metabolic effect of glutamate is expressed with a concentration giving half-maximal response ( $\text{EC}_{50}$ ) of  $\sim 80 \mu\text{M}$ . Pharmacological evidence that this effect of glutamate is not mediated by specific glutamate receptors was provided by the absence of effect by agonists specific for each glutamate receptor subtype. Consistent with this finding, receptor antagonists do not inhibit the effect of glutamate (7). Similar conclusions on the role of glutamate transporters in triggering glucose utilization by astrocytes were later confirmed by Sokoloff and colleagues (14).

Further confirming the metabolic signaling role of glutamate transporters is the observation that the glutamate-stimulated increase in glucose uptake and phosphorylation into astrocytes is abolished in the absence of sodium in the extracellular medium (7), consistent with the necessity of an electrochemical gradient for the ion to drive glutamate uptake. In addition, L- and D-aspartate, but not D-glutamate, mimic the effect of L-glutamate, the physiological agonist. Such a specific pharmacological profile provided the signature for a phenomenon mediated by the glutamate transporter. Finally, the specific glutamate transporter inhibitor *threo*- $\beta$ -hydroxyaspartate (THA) also inhibits the glutamate-stimulated glucose utilization (7).

### Activation of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ couples glutamate transport into astrocytes with glucose utilization

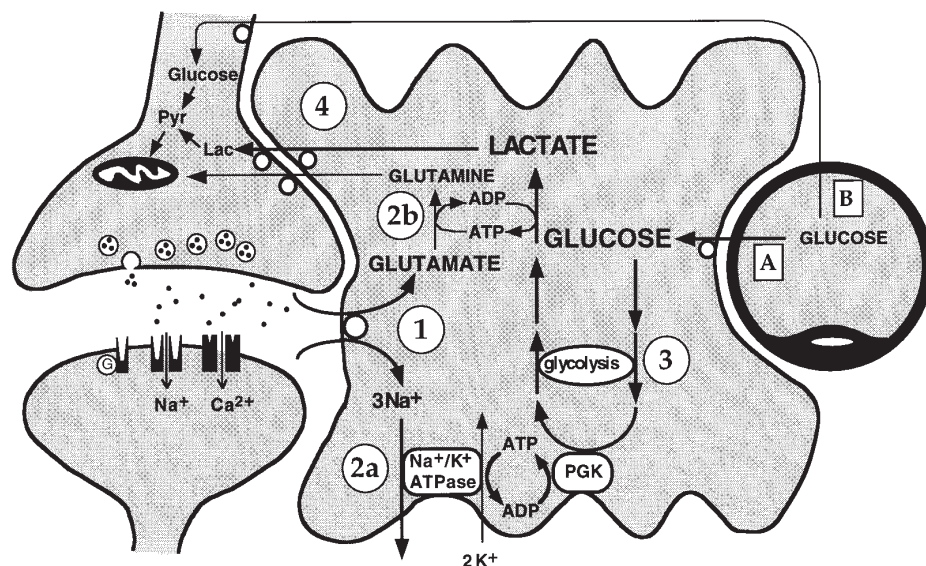
The intracellular molecular mechanism that couples glutamate uptake into astrocytes with

“...the metabolic signaling role of glutamate transporters...”

# Glutamatergic Synapse

# Astrocyte

# Capillary



**FIGURE 2.** Model for coupling of synaptic activity with glucose utilization. At glutamatergic synapses, the action of glutamate is terminated by an efficient glutamate uptake system located in astrocytes (1). Glutamate is cotransported with  $\text{Na}^+$ , resulting in an increase in the intracellular concentration of  $\text{Na}^+$ , leading to the activation of the  $\text{Na}^+$ - $\text{K}^+$ -ATPase (2a). Glutamate is converted to glutamine by glutamine synthase (2b). Activation of the  $\text{Na}^+$ - $\text{K}^+$ -ATPase triggers aerobic glycolysis (3). Lactate produced by the glutamate-stimulated glycolysis is released from astrocytes (4). A, synaptic activation; B, direct glucose uptake into neurons under basal conditions. Pyr, pyruvate; Lac, lactate; Gln, glutamine; G, G protein; PGK, phosphoglycerate kinase. From Ref. 7 with permission. Copyright (1994) National Academy of Sciences, U.S.A.

glucose utilization involves the  $\text{Na}^+$ - $\text{K}^+$ -ATPase, as indicated by the fact that ouabain completely inhibits the glutamate-evoked 2-DG uptake by astrocytes (7). The astrocytic  $\text{Na}^+$ - $\text{K}^+$ -ATPase responds predominantly to increases in intracellular  $\text{Na}^+$  concentration ( $[\text{Na}^+]_i$ ), for which it shows a Michaelis constant ( $K_m$ ) of  $\sim 10$  mM. Because in cultured astrocytes,  $[\text{Na}^+]_i$  ranges between 10 and 20 mM,  $\text{Na}^+$ - $\text{K}^+$ -ATPase is set to be readily activated when  $[\text{Na}^+]_i$  rises concomitantly with glutamate uptake. Indeed, using rubidium-86 uptake to directly monitor the activity of the pump, we have shown that glutamate activates the  $\text{Na}^+$ - $\text{K}^+$ -ATPase (8). For this effect also, the glutamate transporter acts as the signaling mechanism, as confirmed by the fact that activation of  $\text{Na}^+$ - $\text{K}^+$ -ATPase is also triggered by D-aspartate (Ref. 8; Fig. 3A). The very high sensitivity to ouabain of the glutamate-stimulated pump activity, which is already apparent at nanomolar concentrations of the cardiac glycoside, argues in favor of a key role of the  $\alpha_2$ -subunit in coupling glutamate uptake to activation of glycolysis (Fig. 3B). Indeed, in a variety of cell types, including smooth muscle cells and erythrocytes, increases in the activity of the  $\text{Na}^+$ - $\text{K}^+$ -ATPase stimulate glucose uptake and glycolysis. Consistent with this view, glutamate stimulates

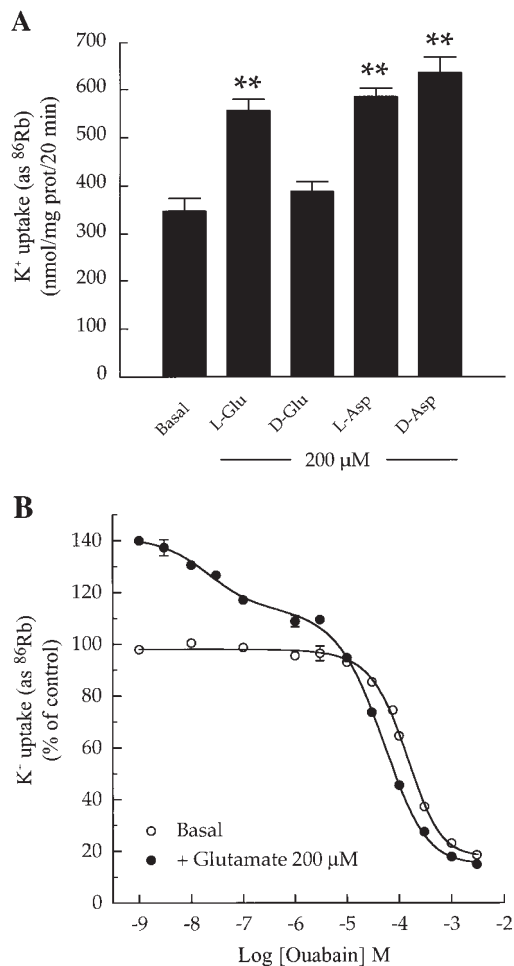
the glycolytic processing of glucose in astrocytes, as indicated by the increase in lactate release (7).

To summarize, glutamate release from activated synaptic terminals is the key signal for coupling neuronal activity to glucose utilization. The cell type that mediates this signaling is astrocytes into which synaptically released glutamate is taken up and stimulates aerobic glycolysis (i.e., the transformation of glucose into lactate in the presence of sufficient oxygen) by a mechanism involving an activation of the  $\alpha_2$ -subunit of the  $\text{Na}^+$ - $\text{K}^+$ -ATPase. In this context, it is important to note that in vivo, the main mechanism that accounts for the activation-induced 2-DG uptake is represented by the activity of the  $\text{Na}^+$ - $\text{K}^+$ -ATPase (15).

## Synaptically released glutamate and glucose utilization are stoichiometrically linked

The overall stoichiometry of the molecular steps involved in the coupling between glutamate uptake and glucose utilization is the following. One glutamate is taken up with three sodium ions while one glucose consumed through glycolysis produces two ATPs. One ATP is used by the  $\text{Na}^+$ - $\text{K}^+$ -ATPase for the extrusion of

“...glutamate release ... is the key signal for coupling neuronal activity...”



**FIGURE 3.** A: stereospecificity of excitatory amino acid-induced increase in Na<sup>+</sup>-K<sup>+</sup>-ATPase activity. Primary cultures of astrocytes were exposed to each stereoisomer at a concentration of 200 μM for a 20-min incubation period during which <sup>86</sup>Rb uptake was determined. Results are means ±SE of 3 separate determinations from 1 experiment repeated twice with similar results. Data were statistically analyzed with ANOVA followed by Dunnett's test. \*\* <sup>86</sup>Rb uptake level significantly different from basal uptake (*P* < 0.01). B: ouabain concentration-response curves for <sup>86</sup>Rb uptake inhibition into astrocytes in the presence or absence of glutamate. Accumulation of <sup>86</sup>Rb into primary cultures of astrocytes was measured during a 20-min incubation period in the presence of increasing concentrations of ouabain and the presence or absence of glutamate at a concentration of 200 μM. From Ref. 8 with permission. Copyright Intl. Society for Neurochemistry Ltd.

three sodium ions; the other ATP is used for the synthesis of glutamine from glutamate by the glutamine synthase. The glycolytic processing of glucose results in approximately two lactate molecules produced per one glucose molecule, i.e., a stoichiometric relationship between glucose and lactate, as expected (Fig. 2).

Recent data obtained in vivo by magnetic resonance spectroscopy (MRS) provide strong support for a tight coupling between glutamate-mediated synaptic activity and glucose utilization. Thus the simultaneous measurements over a

range of synaptic activity of the tricarboxylic acid (TCA) cycle and the cycling of glutamate to glutamine (a process that occurs exclusively in astrocytes) using <sup>13</sup>C MRS have revealed a striking stoichiometric relationship of 1:1 between glutamate cycling (a reflection of synaptic activity) and glucose utilization (13). According to these data, for each glutamate released from active terminals and taken up by astrocytes, one glucose would be oxidized—a result fully consistent with the stoichiometry proposed from data obtained in vitro (8).

### Glutamate-stimulated lactate production by astrocytes provides an energy source for neurons

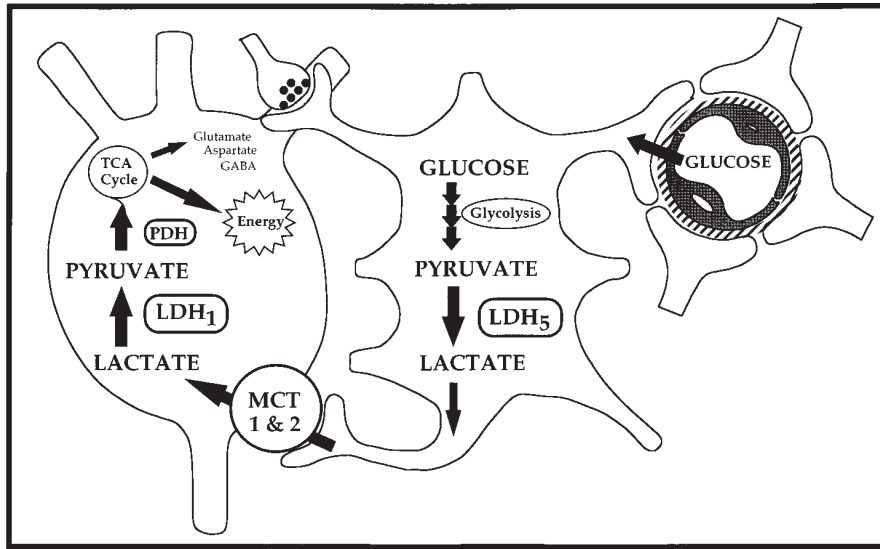
According to this model, lactate is the main energy substrate for neurons during activation. In in vitro preparations, such as isolated brain slices, lactate maintains synaptic activity in the absence of glucose (12). In vivo, lactate is not an adequate substrate because it only marginally crosses the blood-brain barrier; however, if formed within the brain parenchyma through the mechanism described above (Fig. 2), or if applied to in vitro preparations, lactate appears to be consumed preferentially to glucose, particularly during periods of intense activity (12).

Consistent with a predominant lactate production by astrocytes and its utilization by neurons, a selective distribution of lactate dehydrogenase (LDH), the enzyme that catalyzes the interconversion of lactate and pyruvate, has been demonstrated (2). In the human brain, polyclonal antibodies against LDH5 (muscle type) and LDH1 (heart type) reveal that LDH5 (the form enriched in lactate-producing tissues) is restricted to astrocytes, whereas neurons are stained only by an antibody directed against LDH1 (the form enriched in lactate-consuming tissues). These data thus support the idea that astrocytes would preferentially process glucose glycolytically into lactate, which, once released, could be transformed by neurons into pyruvate and enter the TCA cycle to serve as an energy fuel. It should be stressed that one molecule of lactate entering the TCA cycle through the LDH-catalyzed reaction can yield, in normoxic conditions, 17 ATPs.

Further support for this notion of an "astrocyte-neuron lactate shuttle" in the brain (Fig. 4) has been provided by the recent identification of two lactate transporters, monocarboxylate transporter (MCT)-1 and MCT-2, in the central nervous system (9). In culture, MCT-1 is enriched in astrocytes whereas MCT-2 is predominantly expressed in neurons (3).

... a selective distribution of lactate dehydrogenase ..."

## ASTROCYTE-NEURON LACTATE SHUTTLE



**FIGURE 4.** Schematic representation of proposed astrocyte-neuron lactate shuttle. After neuronal activation and synaptic glutamate release, glutamate reuptake into astrocytes triggers increased glucose uptake from capillaries via activation of an isoform of the  $\text{Na}^+\text{-K}^+\text{-ATPase}$ , which is highly sensitive to ouabain, possibly the  $\alpha_2$ -isoform (7, 8). Glucose is then processed glycolytically to lactate by astrocytes that are enriched in the muscle form of LDH (LDH5). The exchange of lactate between astrocytes and neurons is operated by monocarboxylate transporters (MCT). Lactate is then converted to pyruvate because neurons contain the heart form of LDH (LDH1). Pyruvate, via the formation of acetyl CoA by pyruvate dehydrogenase (PDH), enters the tricarboxylic acid (TCA) cycle, thus generating 17 ATP/lactate. It is also conceivable that some of the carbons in the lactate molecule could enter into amino acid pools such as the neurotransmitters glutamate, aspartate, and  $\gamma$ -aminobutyric acid (GABA). From Pellerin, L., et al. Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. *Dev. Neurosci.* 20: 291–299, 1998. With permission from Karger, Basel.

### During activation, glucose is transiently processed through glycolysis

The model proposed on the basis of studies at the cellular level (Fig. 4) suggests an initial glycolytic processing of glucose occurring in astrocytes during neuronal activation, resulting in a transient lactate overproduction followed by a recoupling phase during which lactate would be oxidized by neurons.

Results obtained in a variety of in vivo paradigms, both in laboratory animals and in humans, support the existence of such a transient lactate production during activation. Thus microdialysis studies in rats indicate a marked increase in the concentration of lactate in the dialysate in striatum during physiological sensory stimulation (4). Interestingly, this activity-linked lactate peak is completely inhibited when the glutamate uptake inhibitor THA is present in the perfusate, thus providing further supporting evidence for the existence of glutamate-stimulated glycolysis during activation (4). MRS in humans has also revealed that during physiological activation of the visual system, a transient lactate peak is observed in primary visual cortex (10). Thus microdialysis and MRS data in vivo would support the notion of a transient glycolytic processing of glucose during activation.

The in vitro and in vivo data reviewed here that have been integrated into an operational model for the coupling between synaptic activity and glucose utilization (Figs. 2 and 4) are consistent with the notion that the signals detected during physiological activation in humans with  $^{18}\text{F}$ -2-DG PET and autoradiography in laboratory animals may reflect, predominantly, uptake of the tracer into astrocytes (6). This conclusion does not question the validity of the 2-DG-based techniques; rather, it provides a cellular and molecular basis for these functional brain imaging techniques.

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*“...a transient lactate peak is observed in primary visual cortex...”*

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