

ASTROCYTES

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The astrocyte is a ubiquitous type of glial cell that is defined in part by what it lacks: axons, action potentials, and synaptic potentials. Astrocytes greatly outnumber neurons, often 10:1 and occupy 25% to 50% of brain volume (1–3). Although these cells are anatomically obvious, their functions have been difficult to determine. Discoveries in the last 25 years, however, have revealed some of their functions and established the essential nature of interactions between neurons and astrocytes for normal brain function. We briefly review several basic facts about astrocytes and then selectively survey some of their functions, particularly emphasizing recent findings about metabolic interactions between astrocytes and neurons. We also discuss features of astrocyte function as they relate to synaptic plasticity and emerging concepts in the pathophysiology of psychiatric disorders.

STRUCTURAL AND PHYSIOLOGIC PROPERTIES

The form of astrocytes is important in thinking about their functions. Astrocytes are stellate cells (hence their name) with multiple fine processes. Astrocytes in white matter are complex cells with 50 to 60 long branching processes that radiate from the cell body and terminate in end-feet at the pial surface, on blood vessels, or freely among axons; white matter astrocytes are usually called fibrous astrocytes (4). Astrocytes in gray matter, called protoplasmic astrocytes, have profuse, short stubby processes that contact blood vessels and the pial surface, and surround neurons. Astrocytic end-feet cover the entire surface of intraparenchymal capillaries (5). These end-feet express glucose transporters of the GluT 1 type (6) and are a likely site of glucose uptake. In gray matter, astrocytic processes ensheath virtually every synapse; the ensheathing membranes constitute about 80% of total membrane surface and are devoid of organelles (7).

Thus, astrocytes are polarized cells with some processes contacting cells of mesodermal origin (i.e., endothelial cells of the capillary or fibroblasts of the pia mater), whereas other processes are intimately intertwined with neuronal processes and synapses (4,7).

Astrocytes are the only cells in the brain that contain the energy storage molecule glycogen (8). The importance of this is discussed elsewhere in this chapter. They also contain distinctive 9-nm intermediate filaments composed of a unique protein called glial fibrillary acidic protein (GFAP). Fibrous astrocytes contain more of these filaments than protoplasmic astrocytes. Recent work has assessed the functional significance of this defining astrocytic protein using genetic knockout experiments (9–11). Astrocytes in GFAP knockout animals have disturbed neuronal plasticity manifest as a loss of long-term depression (10), late-onset dysmyelination (9), and increased susceptibility to ischemia (12). It is not known how GFAP deficiency causes these changes.

Astrocytes are strongly coupled to one another by gap junctions (13), aqueous pores that are permeable to ions and other molecules with a molecular weight less than 1,000. A broad range of biologically important molecules, including nucleotides, sugars, amino acids, small peptides, cAMP, Ca^{2+} and inositol triphosphate (IP_3) have access to this pathway. Such intercellular communication is believed to mediate the coordinated action of adjacent but individual cells in terms of electrical and biochemical activity (13), and equalizes their intracellular ion concentrations (14). Gap junction permeability is strongly reduced by intracellular acidification or large increases in intracellular $[\text{Ca}^{2+}]$.

The membrane potential (V_m) of astrocytes is more negative than that of neurons. For example, astrocytes have a V_m of about -85 mV, whereas neuronal membrane potential is about -65 mV. Although glial cells express a variety of K^+ channels, inwardly rectifying K^+ channels seem to be important in setting the resting potential (15). These channels are voltage sensitive and are open at membrane potentials more negative than about -80 mV, close to the observed resting potential of astrocytes. Astrocytes express many other voltage-activated ion channels, previously thought to be restricted to neurons (15). The significance

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of voltage-activated Na^+ and Ca^{2+} channels in glial cells is unknown. Because the ratio of Na^+ to K^+ channels is low in adult astrocytes, these cells are not capable of regenerative electrical responses like the action potential.

One consequence of the high K^+ selectivity of astrocytes, compared to neurons, is that the membrane voltage of astrocytes is more sensitive to changes in extracellular $[\text{K}^+]_o$ ($[\text{K}^+]_o$). For example, when $[\text{K}^+]_o$ is raised from 4 to 20 mM, astrocytes depolarize by ~ 25 mV, compared to only ~ 5 mV for neurons (16). This relative insensitivity of neuronal resting potential to changes in $[\text{K}^+]_o$ in the “physiologic” range may have emerged as an adaptive feature that stabilizes the resting potential of neurons in the face of the transient increases in $[\text{K}^+]_o$ that accompany neuronal activity. In contrast, natural stimulation, such as viewing visual targets of different shapes or orientations, can cause depolarizations of up to 10 mV in astrocytes of the visual cortex (17). The accumulation of extracellular K^+ that is secondary to neural activity may serve as a signal to glial cells that is proportional to the extent of the activity. For example, small increases in $[\text{K}^+]_o$ cause breakdown of glycogen (18), perhaps providing fuel for nearby active neurons (see the following).

Neurons and glial cells do not make functional synaptic or gap junction contacts with one another; therefore, interactions between these cell types must occur via the narrow extracellular space (ECS) between them (16). There may be rare exceptions to this rule (19,20). In the mammalian central nervous system (CNS), the ECS is a uniform and very small compartment formed by adjacent cell membranes that are, on average, separated by approximately by 0.02 μm . Brain ECS is a dynamic compartment in terms of its ionic contents and even its dimensions (15,21). Because of the extreme narrowness of the ECS, molecules released from one cell diffuse almost instantly to adjacent cells. Glial cells interact with neurons by influencing the contents (e.g., ions, energy metabolites, neurotransmitters, etc.) of the ECS. It should be emphasized that nearly every neuron in the brain shares common ECS with adjacent astrocytes, and astrocytic processes entirely surround synapses. It has been surprising to discover that glial cells release and express receptors for a wide range of informational molecules, including neurotransmitters (22); this greatly expands the possibilities for glial–neuronal interactions. Indeed, astrocytes are in a position to sense and modulate synaptic transmission through the pervasive lamellar processes that surround synaptic contacts (7).

FUNCTIONS

Ion Homeostasis

One of the best-established functions of astrocytes is regulation of brain $[\text{K}^+]_o$. Astrocytes are also likely to participate in the regulation of extracellular pH, but this aspect of astro-

cyte function is still evolving and is not considered further here (23,24). Neural activity can rapidly increase $[\text{K}^+]_o$, which is tightly regulated to a resting level of about 3 mM (25). A single action potential increases the instantaneous $[\text{K}^+]_o$ by ~ 0.75 mM (26). The increase in $[\text{K}^+]_o$ is proportional to the intensity of neural activity but has a so-called “ceiling” level of accumulation of 10 to 12 mM (27,28), which is only exceeded under pathologic conditions (29). If diffusion alone were responsible for dissipating K^+ released from neurons, it is easily calculated that extracellular K^+ accumulation would exceed 10 mM during normal neural activity, whereas measured increases in $[\text{K}^+]_o$ are in the range of 1 to 3 mM indicating powerful control mechanisms (30). Homeostatic control of $[\text{K}^+]_o$ is needed because brain $[\text{K}^+]_o$ can influence transmitter release (31), cerebral blood flow (32), ECS volume (33,34), glucose metabolism (35), and neuronal activity (36). Unchecked increases in $[\text{K}^+]_o$ act as an unstable positive feedback loop increasing excitability.

Astrocytes expedite the removal of evoked increases in $[\text{K}^+]_o$ and limit its accumulation to a maximum level of 10 to 12 mM, the ceiling level seen with intense activity such as epileptic discharge (37,38). Neurons, and perhaps blood vessels, also participate in $[\text{K}^+]_o$ regulation, but glial mechanisms are probably most important. Two general mechanisms of astrocyte K^+ removal have been proposed (39): 1) net K^+ uptake into astrocytes (by transport mechanisms and/or Donnan forces) and 2) K^+ redistribution through astrocytes, which is known as K^+ spatial buffering. The relative importance of these two mechanisms of $[\text{K}^+]_o$ regulation remains an open question and may depend on the nature of the $[\text{K}^+]_o$ increase as well as brain region (38).

If glial cells take up K^+ during neural activity and release it thereafter, a transient increase in glial $[\text{K}^+]_i$ should result. Astrocyte $[\text{K}^+]_i$ does transiently increase during neural activity and has a similar time course to the K^+ lost from active neurons and the increase in $[\text{K}^+]_o$, indicating that the K^+ released from neurons is passing by way of the ECS into glial cells (40–42). Uptake of K^+ into glial cells depends on the glial Na^+ pump (38,42–44), an anion transporter that cotransports K^+ and Na^+ with Cl^- (43) and Donnan forces that propel KCl into glial cells in the face of elevated $[\text{K}^+]_o$ (42) (Fig 10.1). It has not been determined with certainty which of these mechanisms is quantitatively most important for K^+ uptake. The astrocyte Na^+ pump, however, is exquisitely sensitive to elevations of $[\text{K}^+]_o$. Even a 1 mM increase in $[\text{K}^+]_o$ activates the Na^+ pump in these cells indicating, perhaps, that this is the major mechanism of K^+ sequestration (44). Neurons, of course, must eventually reaccumulate K^+ lost during activity using their Na^+ pump, but only glial cells show net accumulation of K^+ (Fig. 10.1). It is interesting to note that the neuronal Na^+ pump is not sensitive to small increases in $[\text{K}^+]_o$ and is probably activated mainly by increases in intracellular $[\text{Na}^+]$ (45).

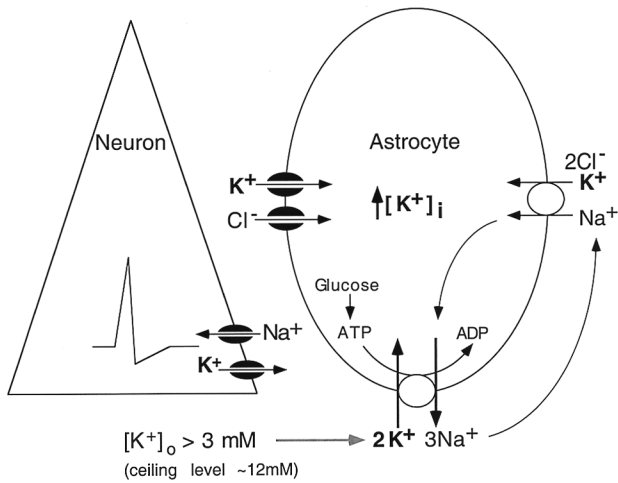


FIGURE 10.1. Schematic representation of mechanisms of K^+ uptake in astrocytes. K^+ released by firing neurons is actively accumulated by astrocytes in three ways. The sodium pump and an anion transporter both take up K^+ ; the sodium pump relies directly on the availability of ATP, whereas the anion transporter is indirectly powered by the energy stored in the transmembrane Na^+ gradient. The presence of channels for Cl^- and K^+ , allow Donnan forces to produce KCl influx. These mechanisms along with K^+ spatial buffering (see text), prevent $[K^+]_o$ from exceeding ~ 12 mM. Increases in $[K^+]_i$ are seen during neural activity as $[K^+]_o$ increases.

The idea that focal increases in $[K^+]_o$ could be redistributed by glial cells was introduced by Kuffler and colleagues (46). They realized that the selective K^+ permeability of glia coupled with their low-resistance intercellular connections (mediated by gap junctions), would permit them to transport K^+ from focal areas of high $[K^+]_o$, where a portion of the glial network would be depolarized, to areas of normal $[K^+]_o$, where the glial network would have a near normal membrane potential (46). Experiments suggest that under conditions of focal increases in $[K^+]_o$, five times as much K^+ moves by way of glial cells as through the ECS, except where only very localized K^+ gradients are involved (25). A further specialization that contributes to spatial buffering is a nonuniform distribution of K^+ channels on a single cell. The density of K^+ channels on the cell membrane of retinal Müller cells, which are specialized astrocytes, is highest on the cell's end-foot. Because the end-foot of the Müller cell, which abuts the vitreous humor of the eye, has the highest density of K^+ channels, accumulated $[K^+]_o$ is preferentially transported to the vitreous, which acts as a disposal site. It is not known if nonuniform K^+ channel distribution is a general feature of astrocytes.

Anoxia/ischemia causes rapid increases in $[K^+]_o$ in both gray matter (to ~ 60 to 80 mM) and white matter (to ~ 12 to 15 mM *in vitro*) of the brain (27,47). The increases in $[K^+]_o$ result because energy-dependent ion gradients can no longer be maintained and K^+ entering the ECS can no longer be taken up by glial cells, which also depend on ATP

(48). In fact, under conditions of diminished energy supply, glial cells actually contribute K^+ to the ECS, rather than take it up (49).

Transmitter Synthesis

Glutamate is one of the most common amino acids in the brain, present at millimolar concentrations in brain tissue homogenate. It is also the predominant excitatory neurotransmitter (50). Only a small fraction of total brain glutamate is packaged for synaptic release and astrocytes are intimately involved in the synthesis of this crucial vesicular pool of glutamate.

Although glutamate can be derived from neuron glucose metabolism, carbon-labeling experiments reveal that astrocyte-derived glutamine is the principal precursor of synaptically released glutamate (51,52). The synthesis and release of glutamine by astrocytes is part of a biochemical shuttle mechanism called the glutamate-glutamine cycle (53) (Fig 10.2). After release from the presynaptic terminal, glutamate is taken up primarily by astrocytes (54,55). In the glial cell, glutamate is converted to glutamine through the ATP-dependent enzyme glutamine synthetase, located exclusively in astrocytes (56). In fact, glutamine synthetase is localized to astrocytic processes surrounding glutamatergic synapses

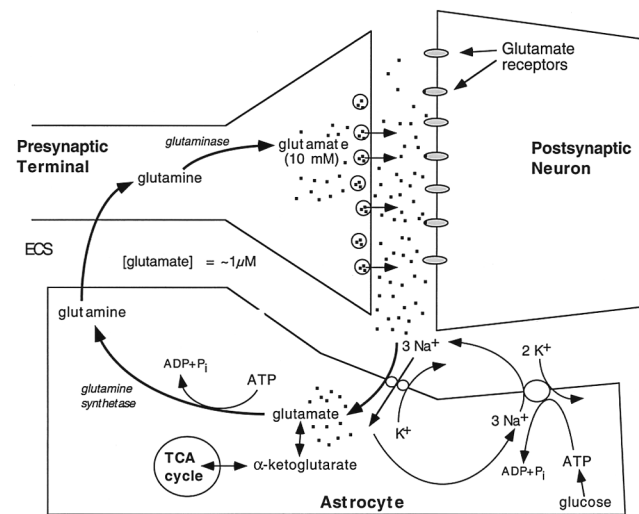


FIGURE 10.2. Scheme showing how astrocytes are involved in glutamate metabolism and uptake. Only astrocytes contain the enzyme glutamine synthetase, which converts glutamate to glutamine in an ATP-requiring reaction. Glutamine is transported to nearby presynaptic terminals where it is converted to glutamate for synaptic release. Finally, the released glutamate is recaptured by astrocytes via a high affinity glutamate uptake system. Although glutamate transporters are present in neurons, astrocytes are the most active in removing glutamate (see text). In the absence of the normal transmembrane Na^+ gradient, maintained by the ATP-dependent Na^+ pump, the glutamate transporter ceases to remove glutamate and can run in reverse so that it pumps glutamate into the extracellular space (ECS).

(57). Glutamine is released by the glial cells and taken up by the neurons through specific uptake carriers. In the pre-synaptic terminal, glutamine is converted to glutamate through glutaminase, a phosphate-dependent enzyme preferentially localized to synaptosomal mitochondria (58,59). The newly synthesized glutamate is then packed into vesicles and becomes available for release. The glutamate-glutamine cycle is a clear and important example of cooperativity between astrocytes and neurons (Fig. 10.2). It mediates removal of potentially toxic excess glutamate from the extracellular space and provides the neuron with a synaptically inert precursor for resynthesis of glutamate. (Glutamine does not bind to neurotransmitter receptors.)

The cycle is surprisingly rapid. After a 6-min incubation of slices of rabbit hippocampus in [^{14}C] glutamine, half of the radioactivity was in the form of glutamate. Removal of glutamine from the bathing solution of the hippocampal slices decreased glutamate efflux by 60% to 80% after only 6 min (52).

Not all of the glutamate taken up by astrocytes is directly converted to glutamine. Glutamate can also enter the TCA cycle through its conversion to α -ketoglutarate (KG). Three enzymatic reactions can yield KG: one catalyzed by aspartate amino transferase and another by alanine aminotransferase, both reactions involving the transfer of an α -amino group. The third reaction is the direct conversion of glutamate to KG via the action of glutamate dehydrogenase (60) (Fig. 10.2). Theoretically, therefore, neurons *might not* get back in the form of glutamine (from astrocytes) all of the glutamate that they release for two reasons: (a) some of the glutamate diffuses away or is taken up by postsynaptic neurons, or (b) not all of the glutamate that enters astrocytes becomes glutamine. Two possibilities can be considered for stabilizing the pool of vesicular glutamate in neurons. First, contrary to the preceding premise, astrocytes might be able to compensate neurons for their loss of glutamate by appropriate adjustments in glutamine export. This would be possible because the pool of cytosolic glutamate in astrocytes is in equilibrium with TCA cycle intermediates, which in turn can be replenished by the carboxylation of pyruvate derived from glucose. The signal for more glutamine export could be extracellular (glutamine), which would fluctuate in response to the needs of glutamatergic neurons. Second, recent data have demonstrated that neurons can generate glutamate directly from pyruvate obtained from glucose or lactate (61,62). Indeed, lactate produced by astrocytes in response to synaptically released glutamate (see the following) appears to be taken up by neurons and could be a substrate for glutamate formation.

GABA, the most common inhibitory neurotransmitter in the brain, is synthesized from glutamate. Consequently, glutamine and α -ketoglutarate are used for GABA synthesis as well (63). Depolarization-released GABA is preferentially synthesized from glutamine supplied by astrocytes (64). Inhibition of astrocyte glutamine synthetase with methionine

sulfoximine produces a significant decrease in GABA production both *in vivo* and in brain slices (65%); however, because a 90% decrease in glutamine did not fully suppress GABA synthesis, an additional metabolic source is considered to be likely (65).

Astrocytes, it would seem, are essential for normal glutamate- and GABA-mediated synaptic transmission. Indeed, selective inhibition of glial cells in the guinea pig hippocampus using the glial-specific metabolic blocker, fluoroacetate, decreases transmission at glutamate synapses (66). Intracellular recordings verified the integrity of neurons in fluoroacetate-treated slices and the persistence of normal responses to glutamate applied iontophoretically. A modulatory role of astrocytes in excitatory synaptic transmission is supported by this study.

Transmitter Removal

In addition to being the most important excitatory neurotransmitter in the brain, glutamate is also a potent neurotoxin and has been implicated in stroke, amyotrophic lateral sclerosis, and epilepsy. Highly efficient glutamate transporters remove synaptically released glutamate and also keep the extracellular concentration of this amino acid at about 2 μM (67). Glutamate transporters are expressed in oligodendrocytes, neurons, microglia, and astrocytes, but transporters in astrocytes are quantitatively the most important in regulating glutamate at synapses and in the extracellular space (Fig. 10.2). (See ref. 55 for review.)

Five main types of glutamate transporters have been described: GLAST (EAAT 1), GLT-1 (EAAT 2), EAAC 1 (EAAT 3), EAAT 4, and EAAT 5 (55). The latter two appear to be predominantly localized in cerebellum and retina, respectively. All have been cloned, functionally characterized, and their localization and distribution at the regional, cellular, and subcellular levels in the CNS is known (55,68). A detailed review of this fertile field of research is beyond the scope of this chapter. Thus, EAAC 1 transporters are neuronal, mostly localized on the cell body and dendrites, whereas GLAST and GLT-1 are predominantly glial (68). There are regional differences in the expression of GLAST and GLT-1; GLAST is more heavily expressed in the cerebellum and GLT-1 is more prevalent in the forebrain.

Glutamate uptake into astrocytes is driven by the electrochemical gradients of Na^+ and K^+ , with a stoichiometry of 3 Na^+ and 1 H^+ in and 1 K^+ out with the uptake of each glutamate anion (55,69). The resulting increase in $[\text{Na}^+]$; must be corrected by a cycle of the Na^+ pump and ATP consumption (70). One advantage of a system where astrocytes take up most of the glutamate is that the metabolic burden of this work is offloaded from neurons (55).

Several lines of evidence support that astrocytes play an essential role in glutamate uptake in the brain (55): (a) Astrocytes preferentially accumulate glutamate transporter

substrate (71). (b) In the absence of astrocytes, neurons are 100-fold more vulnerable to glutamate toxicity (72). (c) Genetic down-regulation of GLAST or GLT-1, but not the neuronal subtype EAAC1, causes elevated extracellular levels of glutamate and neurotoxicity (73).

As emphasized, astrocytes form a ubiquitous part of all glutamatergic synapses. Astrocyte membrane facing a glutamate synapse expresses higher levels of GLAST than membrane facing other structures such as pia mater or capillaries (74). Most of the glutamate released at synapses appears to be taken up by the adjacent astrocytes (75), although there may be some regions in the brain where up to 20% of glutamate is transported into the postsynaptic neuron (55). The impact of astrocytic glutamate uptake at synapses is most emphatically detected when uptake is blocked. This increases both the amplitude and the duration of the excitatory postsynaptic current (76).

Metabolic Coupling with Neurons

Coupling of Synaptic Activity to Glucose Utilization

The cytoarchitecture of astrocytes is of particular relevance in a discussion about the coupling of synaptic activity to glucose utilization. As mentioned, astrocyte end-feet surround virtually all brain capillaries, whereas other astrocytic processes ensheath synaptic contacts (5,7) or end among axons (4). In addition, astrocytes possess receptors and reuptake sites for a variety of neurotransmitters, including the excitatory neurotransmitter glutamate (77), whereas astrocytic end-feet are enriched in the specific glucose transporter GLUT-1 (6). Thus, astrocytes possess the necessary features to sense synaptic activity, through receptors and reuptake sites for neurotransmitters, and to couple it with the entry of glucose into the brain parenchyma (78). Experimental evidence supporting this function is reviewed here.

Glutamate, the main excitatory neurotransmitter released by activated circuits, is a potent stimulator of glycolysis; that is, of glucose uptake and lactate production, in primary astrocyte cultures. (See refs. 60 and 79 for review.) The metabolic effect of glutamate is not mediated by receptors, but rather by glutamate transporters selectively expressed in astrocytes, in particular GLAST (80). These observations suggest a mechanism whereby astrocytes contribute to the uptake of glucose from the circulation into brain parenchyma in register with synaptic activity: The release of glutamate from synaptically active neurons stimulates glucose uptake in nearby astrocytes. The extent of glucose uptake would be proportional to the extent of activity, thereby “coupling” neuronal activity to glucose utilization (80). The Na-K-ATPase is critical for this coupling. Thus, ouabain, a specific inhibitor of the Na-K-ATPase, completely inhibits the glutamate-evoked glycolysis in astrocytes

(80). Glutamate stimulates astrocytic Na-K-ATPase (81) by increasing intracellular Na^+ concentration ($[\text{Na}^+]_i$) via Na^+ -dependent glutamate uptake by glutamate transporters (55,69) (Figs. 10.2 and 10.3). The overall stoichiometry of the molecular steps involved in the coupling between glutamate uptake and glucose utilization are as follows: one glutamate is taken up with 3 Na^+ , whereas one glucose consumed through glycolysis produces two ATPs. One ATP is used by the Na^+/K^+ ATPase for the extrusion of 3 Na^+ ; the other ATP could be used for the synthesis of glutamine from glutamate by glutamine synthase (Fig. 10.3) (82). The glutamate-stimulated glycolytic processing of glucose results in approximately two lactate molecules produced per one glucose molecule; that is, an expected stoichiometric relationship between glucose and lactate. This scheme is, of course, primarily relevant for synaptic regions of the brain (i.e., gray matter). The mechanism that “couples” axonal activity to glucose utilization in white matter is not established. Because axonal activity produces proportional increases in $[\text{K}^+]_o$ (28) and increases in $[\text{K}^+]_o$ increase astrocyte glucose utilization (35,83), it is tempting to speculate that activity-dependent changes in $[\text{K}^+]_o$ in white matter play an analogous role to glutamate release in gray matter.

Is the lactate released by astrocytes in this model used as fuel by neurons? A vast array of experimental data indicate that *in vitro*, lactate in the absence of glucose can adequately maintain synaptic (84–86) or axonal activity (87,88). If astrocytes transfer lactate to neurons as a fuel source (Fig. 10.3), several conditions must be met. There must be appropriate enzymes for the creation of lactate in astrocytes and its use in neurons, and appropriate transport mechanisms for the movement of lactate. Indeed one isoform of lactate dehydrogenase (LDH5), which is enriched in lactate-producing glycolytic tissues such as skeletal muscle, is predominantly localized in astrocytes in the human brain, whereas the other isoform, LDH1, expressed in highly oxidative tissues such the heart, which utilize lactate, is mostly found in neurons (89). Monocarboxylate transporters (MCTs) mediate the exchange of lactate between astrocytes and neurons. These transporters show a cell-specific distribution, with MCT1 predominantly present in astrocytes, whereas MCT2 is enriched in neurons (90,91).

Glutamate-mediated neuron–glia metabolic interactions provide an initial basis to better understand the cellular and molecular steps involved in neuro metabolic coupling (Fig. 10.3). In particular, the model proposed according to which the activity-dependent synaptic release of glutamate triggers glucose uptake into the brain parenchyma coupled with a transient production of lactate, provides a possible basis for functional brain imaging techniques such as positron emission tomography (PET) (78,82). Indeed, a host of PET human studies have indicated an activity-dependent partial uncoupling between glucose utilization (^{18}F -deoxyglucose PET) and oxygen consumption (^{15}O PET), whereas magnetic resonance spectroscopy (MRS) analyses show a tran-

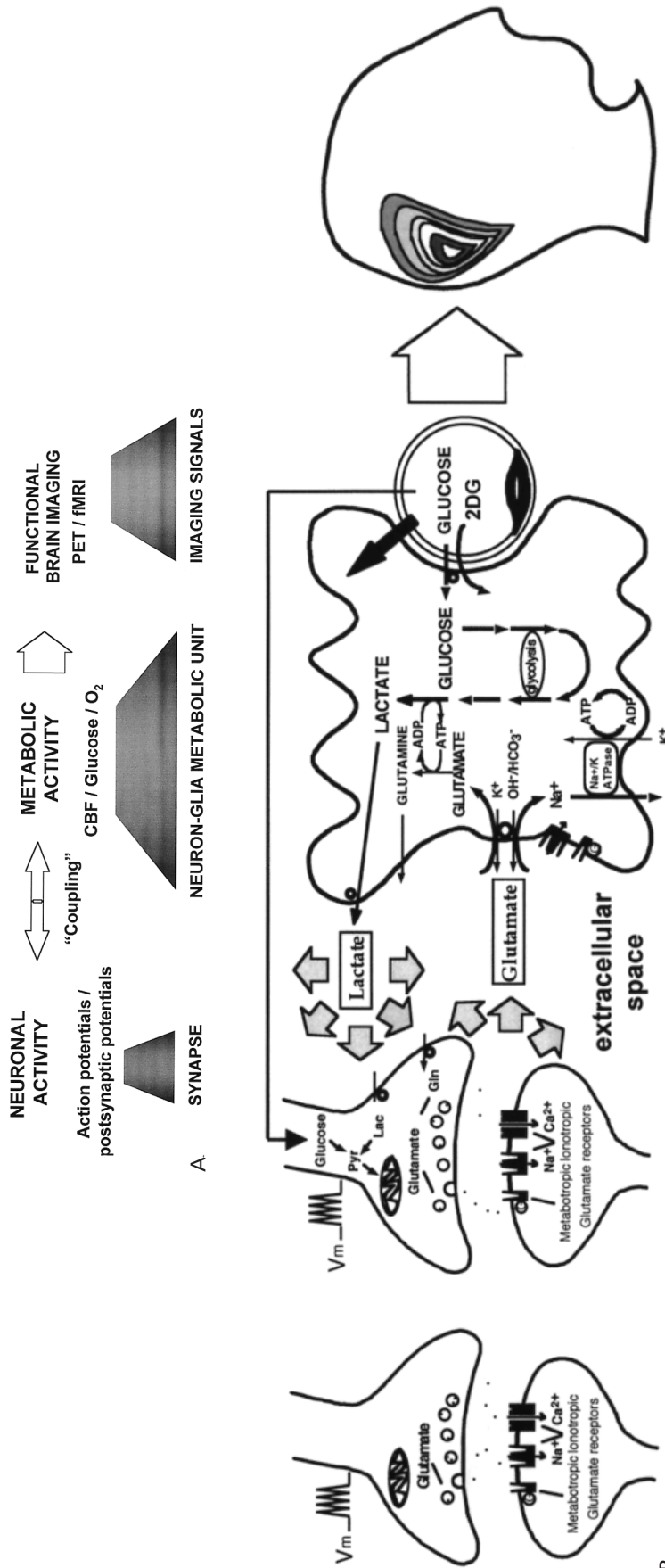


FIGURE 10.3. Schematic representation of the mechanism for glutamate-induced glycolysis in astrocytes during physiologic activation and its relevance for functional brain imaging. At glutamatergic synapses, presynaptically released glutamate depolarizes postsynaptic neurons by acting at specific receptor subtypes. The action of glutamate is terminated by an efficient glutamate uptake system located primarily in astrocytes. Glutamate is cotransported with Na⁺, resulting in an increase in the intra-astrocytic concentration of Na⁺, leading to an activation of the astrocyte Na⁺/K⁺ ATPase. Activation of the Na⁺/K⁺ ATPase stimulates glycolysis (i.e., glucose utilization and lactate production). Lactate, once released by astrocytes, can be taken up by neurons and serve them as an adequate energy supply. The proposed model of glutamate-induced glycolysis in astrocytes implies that the activity-linked uptake of 18FDG monitored with PET, reflects primarily an astrocyte-based signal. Because neuronally released glutamate triggers the cascade of events that leads to glucose uptake, the 18FDG-PET signal faithfully reflects activation of neuronal circuits. The model is also consistent with the transient lactate peak detected during activation by MRS in humans and by microdialysis and electrochemical detection in animals (see text for details).

sient lactate production. (See ref. 79 for review.) In addition, recent MRS studies 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 glucose oxidation and the cycling of glutamate to glutamine (a process that occurs exclusively in astrocytes) using ^{15}C MRS has revealed a striking stoichiometric relationship of 1:1 between glutamate cycling (a reflection of synaptic activity) and glucose utilization (92). According to these data, for each glutamate released from active terminals and taken up by astrocytes one glucose would be oxidized.

Implications for Imaging

The model proposed on the basis of studies at the cellular level 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 (Fig. 10.3).

Results obtained in a variety of *in vivo* paradigms both in laboratory animals and 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 and hippocampus during physiologic sensory stimulation (93). 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 (93). In humans, MRS reveals a transient lactate peak in primary visual cortex during physiologic activation of the visual system (94). Thus, microdialysis and MRS data *in vivo* support the notion of a transient glycolytic processing of glucose during activation. In addition, some PET studies have shown that oxygen consumption does not increase commensurately with blood flow and glucose utilization in activated brain areas (95), suggesting the occurrence of an activity-dependent glycolytic processing of glucose. In contrast, using ^{13}C -glucose MRS, recent data are consistent with a significant increase in oxygen utilization during activation (96). These contrasting views relevant to the degree of oxygen utilization during activation can be reconciled by the model proposed (80), which suggests that glucose imported into the brain parenchyma during activation undergoes a *transient* glycolysis in astrocytes, resulting in the production of lactate that is then oxidized by neurons. This latter process would imply a metabolic recoupling with increased oxygen consumption. The spatial and temporal “window” during which a transient glycolysis occurs and a lactate peak can be detected, may depend on the rapidity and degree of recoupling existing between astrocytic glycolysis and neuronal oxidative phosphorylation.

This operational model for coupling is consistent with

the notion that the signals detected during physiologic activation in humans with FDG PET may result from signaling and metabolic exchanges between neurons (glutamate release) and astrocytes (glycolysis) (82). This conclusion does not question the validity of these techniques for monitoring neuronal function because the triggering event is neuronal glutamate release; rather, this conclusion provides a cellular and molecular basis for these functional brain-imaging techniques (Fig. 10.3).

Glycogen

Another facet of neuron–glia metabolic interactions concerns glycogen. Glycogen, the storage form of glucose, is the largest energy reserve of the brain and it is almost exclusively localized in astrocytes. Although the levels of glycogen are low compared to muscle or liver, they appear to vary in register with synaptic activity and are tightly regulated by a variety of neurotransmitters (97). For example, somatosensory stimulation readily mobilizes glycogen in the corresponding somatosensory cortex as well as in subcortical relays (98). In contrast, during anesthesia glycogen levels increase dramatically. Plastic adaptations in glycogen regulation appear to occur in astrocytes as a consequence of acute or slow neuronal loss; indeed, glycogen deposits are often observed in reactive astrocytes present in acutely lesioned areas, as well as at sites of slow neurodegeneration, such as those observed in Alzheimer’s disease (97). These latter observations suggest that impaired synaptic activity associated with neuronal loss results in a glycogen-sparing situation in astrocytes; active mechanisms driving glycogen metabolism toward increased resynthesis may also be operative under such conditions. The mechanisms that underlie the regulation of glycogen metabolism at the cellular and molecular levels have been partially characterized. (See ref. 97 for review.) Whereas the role of brain glycogen remains to be fully elucidated, recent results indicate that astrocytic glycogen in white matter is readily available to axons, mainly as lactate, and sustains their function during glucose withdrawal (99). It also seems likely that glycogen mobilization by certain neurotransmitters is an adaptation designed to provide additional energy substrate to synaptically active neurons (see the following).

It is appealing to think that astrocytic glycogen serves to provide fuel to the brain when glucose is in short supply. Indeed, astrocytic glycogen *in vitro* is rapidly degraded when glucose is withdrawn (100), and glycogen falls rapidly *in vivo* during ischemia, with a time course that is closely related to (87) ATP depletion and accumulation of lactate (101). Some experimental evidence supports this concept. Neurons grown in astrocyte-rich cultures are less severely injured by glucose withdrawal than are neurons in astrocyte-poor cultures (102). This benefit appeared to derive from the presence of greater amounts of glycogen in the astrocyte-rich cultures because depleting glycogen negated the benefit

(102). Two possible mechanisms for this outcome were suggested but not tested: (a) Astrocytes themselves utilize the energy from glycogen breakdown to prevent the accumulation of toxic levels of glutamate (removing it by a sodium-gradient-dependent transporter); or (b) Glycogen provides fuel to neurons to sustain their energy metabolism.

The role of astrocytic glycogen in CNS white matter has been analyzed using the rat optic nerve, a representative white matter tract. Optic nerve function, measured as the compound action potential (CAP), persists for about 40 min in the absence of glucose (87,103), suggesting the presence of an intrinsic energy reserve such as astrocytic glycogen. The theory was tested that during glucose withdrawal, astrocytic glycogen is converted to lactate and transported into axons to act as an energy source. Glycogen content of the optic nerve falls during glucose withdrawal, compatible with rapid use in the absence of glucose. Up-regulation of glycogen content increases latency to CAP failure and improves CAP recovery after glucose withdrawal, whereas down-regulation of glycogen content has the opposite effects (99). Lactate can replace glucose as an energy source and appears to be transported by MCT out of astrocytes and into axons in the absence of glucose. These results indicate that astrocytic glycogen in white matter is readily available to axons, mainly as lactate, during glucose withdrawal (Fig. 10.4). Conceptually, they provide “proof of principle”

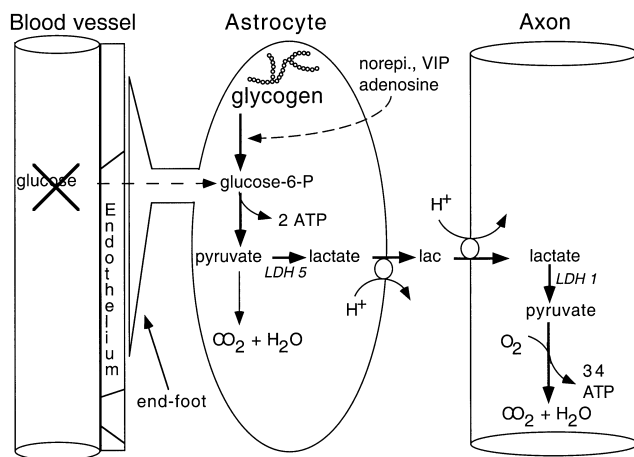


FIGURE 10.4. Schematic illustration of how astrocytic glycogen appears to fuel axons in the absence of glucose. Blood glucose first encounters astrocytic end-feet as it is transported into the brain. In the absence of glucose, astrocytic glycogen is broken down to lactate, which is transported to the extracellular space via a monocarboxylate transporter (MCT). It is then taken up by an MCT in axons and is oxidatively metabolized to produce energy needed to sustain excitability. LDH5 preferentially reduces pyruvate to lactate, whereas LDH1 preferentially oxidizes lactate to pyruvate. Neurotransmitters such as norepinephrine, vasoactive intestinal peptide, and adenosine, promote glycogenolysis. This scheme recognizes that astrocytes can subsist, at least transiently, on glycolytic energy metabolism, whereas axons require oxidative metabolism.

that astrocytic glycogen is an energy reserve that can be shared with, and thus can prolong the function of, neural elements in times of need.

Glycogen levels in astrocytes are tightly regulated by various neurotransmitters. Several monoamine neurotransmitters, namely noradrenaline, serotonin, and histamine, are glycogenolytic in the brain, in addition to certain peptides such as vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP), as well as adenosine and ATP (104,105). The effects of all these neurotransmitters are mediated by specific receptors coupled to second messenger pathways such as adenylate cyclase, for the β -adrenergic, VIP/PACAP and adenosine A2 receptors, or phospholipase C for α -1 adrenergic receptors (106). The initial rate of glycogenolysis activated by VIP and noradrenaline is between 5 and 10 nmol/mg prot/min (106), a value that is remarkably close to glucose utilization of the gray matter as determined by the 2-DG autoradiographic method (107). This correlation raises the possibility that the glycosyl units mobilized in response to the glycogenolytic neurotransmitters are in register with the energy demands of the neuropil.

In addition to the rapid (within seconds) glycogenolysis, VIP, noradrenaline, and adenosine induce a long-lasting plastic response resulting in massive glycogen resynthesis (108). This effect is expressed after several hours and is transcriptionally regulated, involving the expression of new genes: two immediate-early and two late genes, all being induced in a cAMP-dependent manner in astrocytes. The immediate-early genes, C/EBP β and δ are members of a family of transcription factors called CCAAT/enhancer binding protein. This family of transcription factors is predominantly involved in two types physiologic responses: inflammation, through the control of expression of several acute phase response genes, and energy metabolism, in particular through the regulation of cAMP-sensitive genes controlling glucose metabolism in peripheral tissues (109). The late genes regulated by VIP and NA are glycogen synthase (110) and protein targeting to glycogen (PTG) (111). The physiologic function of PTG appears to be that of a chaperone protein coordinating the activity and compartmentalization of glycogen-synthesizing enzymes (111).

Glycogen metabolism in astrocytes appears to be under the dynamic control of at least two neurotransmitters, VIP and NA, with the balance between short-term (glycogenolysis) and transcriptionally regulated long-term effects (glycogen resynthesis) setting the intracellular levels of glycogen.

Calcium Waves and Glial Signaling

The phenomenon of inducible waves of increased $[Ca^{2+}]_i$; moving through adjacent astrocytes was first reported in tissue culture by Cornell-Bell and associates (112). Such waves, stimulated by glutamate application or a mechanical

stimulus, are also known to occur in other cell types (113). It is of special interest that astrocyte Ca^{2+} waves may be elicited by activity in adjacent neurons (114). The mechanism of these waves, which move through cells at a rate of 10 to 20 $\mu\text{m}/\text{sec}$, is not entirely understood (115). It appears to involve the intracellular formation and intercellular transmission of inositol-1,4,5-trisphosphate (IP_3), and the release of an extracellular messenger substance, perhaps ATP (115). This phenomenon has become more interesting with the discovery that it is seen in intact brain tissue such as the retina (116).

The function of intercellular Ca^{2+} waves in astrocytes could be to coordinate the activity of these glial cells. A more intriguing possibility would be that the Ca^{2+} wave could influence neurons in its vicinity. Indeed, Ca^{2+} elevation in astrocytes can cause increases in neuronal $[\text{Ca}^{2+}]_i$ (117) and induce action potentials in hippocampal neurons (118,119). The most compelling demonstration to date of glia-to-neuron signaling mediated by Ca^{2+} waves, however, has come from studies in the rat retina (116). Mechanical stimulation of astrocyte Ca^{2+} waves led to changes in light-induced ganglion cell firing, usually inhibition, when the Ca^{2+} wave reached the neuron. The likely mechanism was the release of glutamate from the astrocytes with stimulation of inhibitory interneurons projecting to the ganglion cells. The importance of this fascinating observation for the normal operation of the nervous system remains unclear, but it suggests a unique form of glial modulation of neuronal activity.

Astrocytes and Synaptic Plasticity

Considerable attention has been given in recent years to the mechanisms of synaptic plasticity. It is now clear that plastic events outlast the early stages of nervous system development and are maintained, to different degrees, throughout adult life, providing the basis for the processes of learning and memory (120). Most experimental paradigms both *in vivo* and *in vitro*, such as the study of long-term potentiation in brain slices, have thoroughly explored the mechanisms of plasticity occurring as a consequence of intense activation of neuronal circuits purportedly associated with the learning process (12). For example, structural rearrangements at synapses characterized by an enhanced number of axodendritic synaptic contacts have been demonstrated (121), and the relative contribution of presynaptic adaptations (increased neurotransmitter release) versus postsynaptic ones (increased responsiveness of the target elements) have been thoroughly investigated (122). The involvement of a variety of molecules, including glutamate and its receptors, nitric oxide, arachidonic acid, certain neurotrophins, and cell adhesion molecules, has been proposed (123).

However, few studies have explored the possible adaptations that may occur in glial elements, in particular at the astrocytic profiles that ensheath synapses, as a consequence

of learning paradigms known to affect synaptic plasticity. The structural rearrangements occurring at synaptic contacts are likely to affect the morphology of the associated astrocytic profiles. Such an astrocytic structural plasticity has been well documented in the hypothalamus. Here, on physiologic stimulation (lactation, dehydration) the astrocytic profiles surrounding the soma and dendrites of oxytocin-containing neurons retract, allowing a marked increase in membrane surface available for synaptic contacts (124). This structural modification, in which a clear role for certain cell-adhesion molecules (e.g., PSA-NCAM, F3, and tenascin) has been shown (125), is reversible, being associated with the period of stimulation. A similar structural astrocytic plasticity has been shown in the arcuate nucleus during the estrous cycle (124). Evidence for activity-dependent astrocytic plasticity is beginning to be demonstrated also in extrahypothalamic areas of the brain. Striking structural modifications of astrocyte morphology surrounding synaptic contacts occur in parallel to neuronal plastic adaptations in brain areas activated by simple behavioral paradigms of learning. Thus, in animals reared in complex environments, the size and the number of GFAP-immunoreactive astrocytes was found to be increased in the visual cortex as compared to animals raised in normal laboratory cage environment (126–128). In addition, the extent of contact between astrocytic processes and synapses is increased under the same conditions (129). Similar astrocytic modifications are also observed in the cerebellum following synaptic plasticity induced by motor-skill learning (130). Following a spatial learning task (the Morris water maze test), an increase in the density of GFAP-immunoreactive astrocytes is observed in the hippocampus (131). This increase in GFAP is correlated with enhanced expression of basic-fibroblast growth factor. Considering the purported role of LTP in spatial learning (132), it is worth noting that induction of LTP *in vivo* by repeated high-frequency stimulation of the perforant pathway in the hippocampus causes a similar increase in numerical density, higher surface density, and closer apposition of astrocyte processes to the synaptic clefts, or dendritic spines in the potentiated synapses (133). The concept that glial cells, and in particular astrocytes, could contribute to plastic changes occurring after learning and memory or developmental paradigms has already been the subject of reviews (134).

In some of these learning paradigms involving structural plastic responses in astrocytes, long-lasting adaptations in local energy metabolism have been reported. Thus, increases in capillary formation, capillary branching, and surface area were reported in visual cortex following complex experience (127). Angiogenesis was also demonstrated in the cerebellum after motor skill learning (135), thus suggesting that a long-lasting enhancement in the supply of energy substrates (mostly glucose and O_2) is required in the activated area. Several studies have reported long-lasting changes in glucose utilization following various learning and memory tasks

(136–139). Spatial discrimination training was reported to cause persistent increases in glucose utilization, as measured with the 2-deoxyglucose autoradiographic technique in regions such as the hippocampus and various cortical areas (140).

GLIAL CELL FUNCTION: IMPLICATIONS FOR PSYCHIATRY

From the overview presented in the preceding paragraphs, it clearly appears that all facets of intercellular communication in the brain are relevant to glial cell, and specifically astrocyte function. Thus, the presence on astrocytes of receptors for all chemical classes of neurotransmitters (amino acids, monoamines, and peptides) indicates that the receptor-mediated effects of psychoactive drugs can involve astrocytes. To take a simple example, increasing synaptic levels of noradrenaline with certain classes of antidepressants, by activating β -adrenergic receptors on astrocytes, is likely to result in induction of genes that regulate glycogen metabolism. Plasticity is no longer exclusive to neurons, providing the conceptual background for considering the activity- and drug-induced adaptations observed in the function of specific circuits as also involving astrocyte-based mechanisms. As recently reviewed, alterations in excitatory neurotransmission, which appear to be involved in neuropsychiatric disorders such as schizophrenia or Alzheimer's disease, may well include an astroglial component (141). Recent neuroimaging studies have shown that the volume of the subgenual prefrontal cortex is reduced in familial forms of major depressive and in bipolar disorders. This decrease in cortical volume could be ascribed to a marked decrease in the number of glial cells (142). Functional imaging in patients suffering from both clinical conditions show a significant decrease in glucose utilization determined by ^{18}F -deoxyglucose PET (143). In addition to providing indirect evidence for the involvement of glia in certain psychiatric disorders, this set of observations is consistent with the proposed role of astrocytes in the ^{18}F -deoxyglucose PET signal (80). Chronic treatment with neuroleptics has been associated with increased glial density in the primate prefrontal cortex (144). Although these examples still need validation in terms of their relevance in the pathophysiology of certain neuropsychiatric disorders, they provide, along with advances in the understanding of glial cell biology, sufficient grounds for opening the scope of psychiatric neuroscience to the study of what Joseph Coyle and Robert Schwarcz have recently called "mind glue" (the term glia, meaning glue in German) (141). This is a major step indeed from "neuron-centric" dominated psychiatric theory and one that is justified by the available facts.

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