

A Cellular Perspective on Brain Energy Metabolism and Functional Imaging

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<http://dx.doi.org/10.1016/j.neuron.2015.03.035>

The energy demands of the brain are high: they account for at least 20% of the body's energy consumption. Evolutionary studies indicate that the emergence of higher cognitive functions in humans is associated with an increased glucose utilization and expression of energy metabolism genes. Functional brain imaging techniques such as fMRI and PET, which are widely used in human neuroscience studies, detect signals that monitor energy delivery and use in register with neuronal activity. Recent technological advances in metabolic studies with cellular resolution have afforded decisive insights into the understanding of the cellular and molecular bases of the coupling between neuronal activity and energy metabolism and point at a key role of neuron-astrocyte metabolic interactions. This article reviews some of the most salient features emerging from recent studies and aims at providing an integration of brain energy metabolism across resolution scales.

1. Why Should We Care about Brain Energy Metabolism?

Why should we care about brain energy metabolism or, using a more contemporary term, neuroenergetics? After all, elegant and pioneering studies conducted by Kety and Sokoloff *in vivo* in humans over 60 years ago clearly showed that glucose is the obligatory energy substrate for the brain, where it is almost fully oxidized (Allaman and Magistretti, 2013). Further studies at the whole-organ level have provided some refinements to this view, such as, for example, the fact that under particular conditions, such as fasting, uncontrolled diabetes, or maternal milk diet in newborns, ketone bodies sustain the energetic requirements of the brain (Magistretti, 1999 and references therein).

Technological developments over the last two decades have, however, afforded new insights at the regional, cellular, and molecular levels into neuroenergetics; these developments deserve some renewed attention.

At one end of the resolution spectrum, functional brain imaging techniques have shed new light on the regulation of regional brain energy metabolism in register with neuronal activity. An appreciation of the existence of physiological mechanisms that couple local activity to energy delivery was in fact provided by Charles Sherrington over a century ago in a seminal article (Roy and Sherrington, 1890) where he postulated that “the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variations of functional activity.”

Indeed, the two main functional brain imaging techniques, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), detect signals that are directly related to energy delivery and use (Logothetis et al., 2001; Raichle, 1983, 1998). For PET, the distribution of tracers labeled with positron-

emitting isotopes such as ^{18}F or ^{15}O reveals changes in cerebral blood flow (CBF) using ^{15}O -labeled water, glucose utilization with ^{18}F -labeled 2-deoxyglucose (2-DG), and oxygen consumption with $^{15}\text{O}_2$, which are associated with localized brain activities. For fMRI, the “contrast agent” is endogenous, being provided by changes in the ratios of oxy- and deoxyhemoglobin following activity-dependent processes related to blood flow and volume as well as to metabolic fluxes (Ogawa et al., 1990a). Thus, both techniques provide signals that are surrogate markers of neuronal activity produced by mechanisms that couple neuronal activity to blood flow and metabolism. These mechanisms will be discussed in further detail below with a focus on their cellular and molecular underpinnings.

Furthermore, through the use of high-field magnetic resonance spectroscopy (MRS), often using stable isotopes such as ^{13}C and ^1H , it has become possible to study metabolic fluxes *in vivo* in laboratory animals and humans under physiological and pathological conditions (Duarte et al., 2012; Shulman and Rothman, 2001).

At the other end of the resolution spectrum, studies on homogeneous preparations of the cellular elements of the brain, in particular neurons and astrocytes, such as primary cultures or fluorescence-activated cell sorting (FACS)-purified cells and single-cell analyses by transcriptomics (Cahoy et al., 2008; Lovatt et al., 2007; Zhang et al., 2014) or using nanosensors/fluorescent analogs (Barros et al., 2013; Chuquet et al., 2010; Jakoby et al., 2014) for specific metabolites, have provided means to disentangle the relative contributions of each cell type to the metabolic processes analyzed by PET, fMRI, and MRS. A current challenge for neuroenergetics is to integrate the various levels of resolution scales at which data are gathered to provide a coherent view of brain energy metabolism across resolution scales.

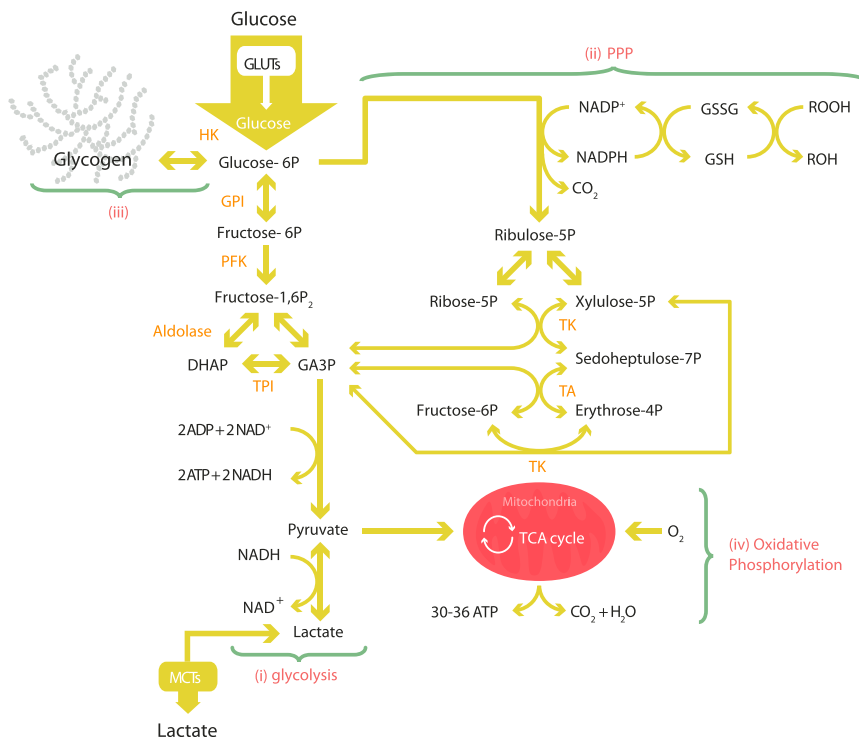


Figure 1. Schematic Representation of Glucose Metabolism

Glucose enters cells through glucose transporters (GLUTs) and is phosphorylated by hexokinase (HK) to produce glucose-6-phosphate (glucose-6P). Glucose-6P can be processed through three main metabolic pathways. First, it can be metabolized through glycolysis (i), giving rise to two molecules of pyruvate and producing ATP and NADH. Pyruvate can then enter mitochondria, where it is metabolized through the tricarboxylic acid cycle and oxidative phosphorylation, producing ATP and CO_2 while consuming oxygen (iv). Under hypoxic conditions or depending on the cellular metabolic profile (see text), pyruvate can also be reduced to lactate by lactate dehydrogenase. This lactate can be released in the extracellular space through monocarboxylate transporters. The complete oxidation of glucose produces larger amounts of energy in the form of ATP in the mitochondria (30–36 ATPs) compared to glycolysis (2 ATPs). Alternatively, glucose-6P can be processed through the pentose phosphate pathway (PPP) (ii), leading to the production of reducing equivalents in the form of NADPH. Note that the PPP and glycolysis are linked at the level of glyceraldehyde-3-phosphate (GA3P) and fructose-6-phosphate (fructose-6P). NADPH produced in the PPP is important for defense against oxidative stress through the metabolism of the tripeptide glutathione (GSH). GSH is an electron donor in several reactions, including the detoxification of ROS (e.g., the reduction of peroxides [ROOH] by glutathione peroxidase). The

oxidized glutathione (GSSG) formed as a result is recycled back to glutathione by the action of glutathione reductase using NADPH as an electron donor. In astrocytes, glucose-6P can also be used to store glucosyl units as glycogen (iii). GPI, glucose-6-phosphate isomerase; PFK, phosphofructokinase; fructose-1,6- P_2 , fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; TPI, triose phosphate isomerase; ribulose-5P, ribulose-5-phosphate; ribose-5P, ribose-5-phosphate; xylulose-5P, xylulose-5-phosphate; TK, transketolase; sedoheptulose-7P, sedoheptulose-7-phosphate; TA, transaldolase; erythrose-4P, erythrose-4-phosphate.

In addition to these technological advances that have re-kindled interest in neuroenergetics, a largely underestimated feature of brain energy metabolism has received sustained attention over the last decade. Indeed, 10% of glucose entering the brain yields lactate in a metabolic process known as aerobic glycolysis, which is the production of lactate in the presence of oxygen (Vaishnavi et al., 2010). In fact, during development, aerobic glycolysis accounts for up to 30% of brain glucose metabolism. In the adult brain, aerobic glycolysis becomes restricted to specific regions, such as the dorsolateral prefrontal cortex, the superior and medial frontal gyrus, or the precuneus and posterior cingulate cortex, where it accounts for up to 25% of glucose utilization, whereas in other areas, such as the cerebellum, it is virtually nonexistent (Bauernfeind et al., 2014; Goyal et al., 2014). At the cellular level, aerobic glycolysis and lactate production are metabolic features of astrocytes but are only marginally expressed in neurons (Bélanger et al., 2011a). We return to this point below, as this metabolic process is currently being revisited and is providing new insights into its possible role in brain function.

2. Cellular Specificity of Energy Metabolism

Given the cellular heterogeneity of the brain, it is not surprising that different cell types have distinctive metabolic profiles. Such differences have been particularly studied in neurons and astrocytes, whereas much remains to be elucidated for oligo-

dendrocytes and microglia. As a first, high-level view, one can say that neurons are predominantly oxidative whereas glycolysis predominates in astrocytes (Bélanger et al., 2011a; Hyder et al., 2006; Zhang et al., 2014). As a reminder, an oxidative profile is characterized by a series of metabolic steps that lead to the full oxidation of glucose or of its metabolites, such as pyruvate and lactate, in the mitochondria, resulting in the production of approximately 30–36 molecules of ATP per glucose molecule (or 14–17 if the substrates are the three-carbon molecules lactate or pyruvate), depending on the degree of coupling of oxidative phosphorylation. This mitochondrial process involves the tricarboxylic acid (TCA) cycle, an electron transfer in the respiratory chain, oxygen consumption, and the production of CO_2 and water (Figure 1). For glycolysis, two molecules of ATP are produced as glucose is processed to pyruvate. Lactate is also formed by glycolysis: under low oxygen tension, pyruvate is converted to lactate in a process that regenerates NAD^+ , an essential cofactor necessary to sustain a glycolytic flux (Figure 1) (Allaman and Magistretti, 2013). However, as noted earlier, through aerobic glycolysis also known as the Warburg effect (Warburg, 1956), lactate can also be formed in the presence of physiological oxygen tension for subsequent extracellular release and transfer to neighboring cells. The existence of a metabolic profile predominant in each cell type had already been identified in pioneering neurochemical studies performed in the 1950s and 1960s by Hyden in individually

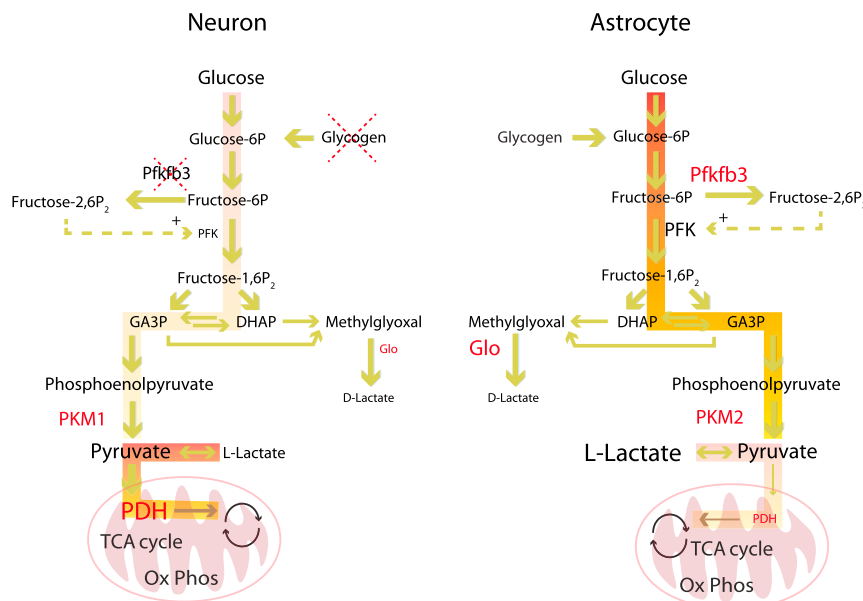


Figure 2. Salient Features that Underlie the Differences in Metabolic Profiles between Neurons and Astrocytes

No glycogen is stored in neurons, whereas astrocytes are the only cells in the brain that store it. In neurons, reduced expression and activity of Pfkfb3, a key positive modulator of glycolysis, contrasts with high levels of expression and activity in astrocytes. Cell-specific differential splicing of pyruvate kinase results in the expression of the PKM1 isoform exclusively in neurons and of the PKM2 isoform in astrocytes. Activity of pyruvate dehydrogenase is high in neurons and low in astrocytes, due to its high degree of phosphorylation in the latter cell type. Taken together, these cell-specific expression and activity profiles result in a limited capacity of glycolysis and in an active TCA cycle and oxidative phosphorylation in neurons, whereas in astrocytes glycolysis is more active and can be upregulated whereas pyruvate processing in the TCA cycle is limited. Expression and activity of the glyoxalase system that can detoxify cells of methylglyoxal are considerably higher in astrocytes. For additional details, see text.

dissected neurons and astrocytes. In these early experiments, Hyden showed that CO_2 is produced in isolated neurons at a much higher rate than in astrocytes and that the enzymatic complement of each cell type was consistent with a predominance of glycolysis in glial cells and oxidation in neurons (Hamberger and Hyden, 1963; Hyden and Lange, 1962). Later studies in primary cultures of each cell type have yielded similar results (Bélanger et al., 2011a). Elegant functional studies using acutely isolated and FACS-purified astrocytes have confirmed the predominantly glycolytic profile of astrocytes (Lovatt et al., 2007; Zhang et al., 2014). Modeling studies based on in vivo magnetic resonance spectroscopy analyses corroborate the distinct and, as we will see later, functionally complementary profile of neurons and astrocytes (Aubert et al., 2007; Hyder et al., 2006; Jolivet et al., 2015).

One point that has created some confusion is that astrocytes are definitely endowed with mitochondria, a cell biological feature that suggests robust oxidative activity (Lovatt et al., 2007; Pysh and Khan, 1972). However, the entry of pyruvate into the TCA cycle is a regulated process, mainly determined by the activity of the multiunit enzyme pyruvate dehydrogenase (PDH) (Figure 2). If the activity of PDH is close to saturation under basal metabolic conditions, then the added pyruvate flux that would follow increased glucose uptake cannot be processed through the TCA cycle, thereby diverting pyruvate to lactate production. The activity of PDH is regulated by phosphorylation at several phosphorylation sites; when phosphorylated, PDH activity is decreased. Work by Halim and collaborators has shown that the degree of phosphorylation of PDH under basal conditions is high in astrocytes (Halim et al., 2010). Recently, Zhang et al. have shown that astrocytes express high levels of the gene encoding pyruvate dehydrogenase kinase 4 (PDK4), an enzyme that maintains PDH in a phosphorylated, meaning less active, state (Zhang et al., 2014) (Figure 2). This situation provides one of the necessary conditions to favor aerobic glycol-

ysis, namely the production of lactate in the presence of normal oxygen tension. Accordingly, the Sokoloff group has shown that dephosphorylation of PDH by dichloroacetate results in a marked increase in oxidative use of glucose by astrocytes and a concomitant decrease in lactate production by these cells (Itoh et al., 2003).

This set of results highlights the fact that astrocytes have a limited capacity to upregulate the processing of pyruvate, the endproduct of glycolysis, by mitochondria when glucose uptake is stimulated. In a mirror image, experimental evidence has been provided indicating that neurons cannot upregulate glycolysis and that they express high oxidative activity. The groups of Bolaños and Moncada have unraveled the molecular mechanism that underlies the limited capacity of neurons to upregulate glycolysis (Bolaños et al., 2010; Herrero-Mendez et al., 2009). They showed that the enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (Pfkfb3), a key positive modulator of glycolysis in cells, is constantly subject to proteasomal degradation, whereas in astrocytes this enzyme is fully active, thus allowing upregulation of glycolysis (Figure 2). In fact, glucose is mainly processed through the oxidative branch of the pentose phosphate pathway in neurons, rather than through glycolysis. This pathway is the main producer of reducing equivalents in the form of NADPH, which are used to regenerate reduced glutathione, in this way providing the principal molecular effector for scavenging reactive oxygen species (ROS) produced by the marked oxidative activity of neurons. Accordingly, if proteasome degradation of Pfkfb3 is blocked in neurons, resulting in a redirection of glucose processing through glycolysis rather than through the pentose phosphate pathway, neurons undergo oxidative stress and apoptotic death (Bélanger et al., 2011b; Herrero-Mendez et al., 2009). This set of data indicates that glucose is predominantly used in neurons to maintain antioxidant status rather than for bioenergetic purposes.

A recent study performed by the Barres group (Zhang et al., 2014) that produced a transcriptome database of genes expressed in acutely isolated neurons and astrocytes by RNA sequencing and alternative splicing analysis has provided compelling evidence confirming the metabolic complementarity between these two cell types. In particular, this in-depth analysis confirms that the pattern of expression and splicing of selected genes of glucose metabolism confers to astrocytes the ability to dynamically upregulate glycolysis, whereas neurons are locked in a steady state of glycolysis. In addition to confirming the enrichment of Pfkfb3 in astrocytes, Zhang and colleagues found a cell-specific differential splicing of the enzyme pyruvate kinase (PK), with the isoform PKM1 exclusively expressed in neurons and the PKM2 isoform in astrocytes. PK, by promoting the conversion of phosphoenolpyruvate to pyruvate, is a key regulator of glycolysis. The PKM2 isoform, which in addition to astrocytes is also enriched in cells displaying sustained aerobic glycolysis such as cancer cells (Ward and Thompson, 2012), can upregulate the glycolytic flux in response to increased energy demands, whereas PKM1, the form expressed in neurons, does not have this property. As noted earlier, the work of Zhang and colleagues has also confirmed the cell-specific enrichment (greater than 30-fold) in astrocytes of PDK4, the enzyme that phosphorylates pyruvate dehydrogenase, rendering it less active. This fact implies that increases in the glycolytic flux in astrocytes will result in lactate production, because the entry of pyruvate into the TCA cycle cannot be upregulated (Figure 2). Overall, this selective expression of glucose metabolism genes is configured to drive glucose processing in astrocytes toward aerobic glycolysis.

Another distinguishing feature exists between astrocytes and neurons in respect to glycolysis. A byproduct of glycolysis is methylglyoxal (MG) (Figure 2), a highly reactive dicarbonyl compound that promotes the formation of advanced glycation endproducts (AGEs), which are associated with several neurodegenerative disorders (see, e.g., Münch et al., 2012). MG is detoxified by cells through the glyoxalase system comprising glyoxalase 1 (Glo-1) and Glo-2. Both enzymes are expressed at considerably higher levels in astrocytes compared with neurons. Accordingly, enzymatic activity rates are several-fold higher in astrocytes, a feature that renders them, unlike neurons, resistant to MG toxicity and to AGE accumulation (Bélanger et al., 2011b). These features endow astrocytes with the capacity to sustain high levels of glycolysis, as they can detoxify MG, and to protect neurons against MG toxicity (Bélanger et al., 2011b). Interestingly, upregulating glycolysis in neurons by increasing the activity of Pfkfb3 results in increased MG levels and MG-modified proteins (Bélanger et al., 2011b). This set of results suggests that the limited capacity of neurons (unlike astrocytes) to upregulate their glycolytic flux may be related to their weaker ability to detoxify MG.

Altogether, results reviewed in this section converge to indicate that astrocytes and neurons resort to two complementary metabolic pathways to produce energy from glucose. That is, neurons predominantly process glucose through the pentose phosphate pathway to produce the reducing power (NADPH) necessary to scavenge molecules producing oxidative stress that results from their high mitochondrial oxidative activity (Fig-

ures 1 and 2). This pathway implies that neurons utilize substrates, such as pyruvate and lactate, downstream of glycolysis to feed the TCA cycle and the associated oxidative phosphorylation to produce ATP (Figure 2). Consistent with this view, an uncoupling between glycolysis and the TCA cycle has been reported in neurons (Bouzier-Sore et al., 2006; Selak et al., 1985). In contrast, astrocytes, although endowed with a certain degree of oxidative capacity, predominantly process glucose glycolytically to produce lactate and pyruvate. A metabolic complementarity thus appears to exist between astrocytes and neurons whereby glucose, the almost exclusive energy substrate of the brain, is processed differentially by neurons and astrocytes. These cell-specific metabolic pathways also imply a transfer of the three-carbon substrates—lactate and to a lesser extent pyruvate—from astrocytes, where they are produced by glycolysis, to neurons, where they are oxidized to yield ATP.

3. Energy-Consuming Processes in the Brain

Given the main mechanisms described above that yield energy in neurons and astrocytes in the form of ATP or reducing power (NADPH) (Figure 1), it is then necessary to identify the processes that cost energy and to determine how this energy is delivered. Various approaches have been enlisted to establish the “energy budget” of the brain (Attwell and Laughlin, 2001; Harris et al., 2012; Hyder et al., 2013; Jolivet et al., 2009). As a first approximation, it appears that neurons consume 75%–80% of the energy produced whereas the rest is used for glia-based processes (Harris et al., 2012; Hyder et al., 2013). As far as neurons are concerned, energy utilization is related to the activity of ionic pumps that reestablish the electrochemical gradients dissipated by signaling, namely action potentials and synaptic potentials. Which of these two signaling processes dominates energy consumption in neurons has been debated for some time. In theoretical estimates based on the classical Hodgkin-Huxley formalism, Laughlin and Attwell proposed a 50:50 contribution of action potentials and synaptic potentials to neuronal energy consumption in the rat cerebral cortex (Attwell and Laughlin, 2001). These estimates have been revised on the basis of elegant experiments by Geiger and colleagues, who demonstrated that over 80% of the energy in myelinated hippocampal axons is expended by postsynaptic potentials (Alle et al., 2009). These researchers demonstrated the existence of considerable energy efficiency in action potential signaling. Indeed, unlike in unmyelinated giant axons in squid, the temporal overlap of the openings of voltage-sensitive sodium channels (VSNs) and delayed-rectifier potassium channels (DRKCs), the two principal channels underlying the generation of action potentials, is minimal in vertebrate myelinated axons. This means that the positive charges entering through the VSNs are only minimally short-circuited by the opening of the DRKCs, resulting in a limited dissipation of the sodium gradients and hence of the energy-consuming sodium-potassium ATPase (for a commentary, see Magistretti, 2009). Thus, the majority of the energy used by neurons for signaling appears to be consumed at the synapse (Harris et al., 2012). Recent evidence confirms this view by identifying, in addition to postsynaptic potentials, pre-synaptic vesicle recycling as an additional energy-consuming process (Rangaraju et al., 2014). This high energy demand at

the synapse, a site distant from the metabolic machinery of the cell body, implies that local mechanisms must exist to sense synaptic activity and provide the energy substrates necessary to sustain pre- and postsynaptic processes.

4. Neuron-Astrocyte Metabolic Coupling

In the 1970s, the development of the 2-deoxyglucose autoradiographic technique by Sokoloff and colleagues afforded a means to determine local energy utilization in register with activation of specific neuronal circuits in several laboratory animal species (Sokoloff et al., 1977; Sokoloff, 1981). Although the cellular localization of glucose utilization was not possible with this technique, Sokoloff and colleagues could still conclude that most of the glucose utilization visualized with 2-DG was localized in synapse-rich regions of the nervous system. An elegant example of this was provided in experiments in which somatosensory stimulation was shown to elicit accumulation of the label in the synapse-rich dorsal horn rather than in the dorsal root ganglia, where the cell bodies of the sensory neurons are located (Kadekaro et al., 1985). More recent work, particularly that by Logothetis and colleagues (Logothetis et al., 2001), indicates that fMRI signals spatially coincide with electroencephalogram-recorded local field potentials (LFPs), which are electrical markers of synaptic activity. Thus, evidence from in vitro and in vivo biochemical and imaging studies converges to indicate that (1) the major energetic burden of the nervous system is localized at the synapse and (2) molecular mechanisms must exist to couple synaptic activity to local energy delivery. Over the last three decades, evidence has accumulated pointing at astrocytes as key cells for the coupling between synaptic activity and energy metabolism.

Astrocytes belong to the general class of macroglia cells (Kettenmann and Verkhratsky, 2008). They were described initially by Virchow (Virchow, 1856, 1859), and a role in the “distribution of nutrients” to the brain parenchyma was proposed by Golgi and later Andriezen at the turn of the nineteenth century (Andriezen, 1893; Golgi, 1886). Further cytological studies using light and electron microscopes have revealed some particular features that make astrocytes ideally positioned to sense synaptic activity and couple it with the delivery of energy substrates originating from the capillaries. Indeed, astrocytes feature multiple processes that define spherical volumes of cortex approximately 80–100 μm in diameter (Bushong et al., 2002; Nedergaard et al., 2003; Sofroniew and Vinters, 2010). One of these processes, the endfoot, abuts against the capillary wall whereas several others ensheath synaptic contacts (Figure 3). Essentially, the entire outer surface of intraparenchymal capillaries is covered by astrocytic endfeet (Kacem et al., 1998; Simard et al., 2003), leaving only clefts of 20 nm between endfeet (Mathiisen et al., 2010). Recent evidence suggests that aquaporin 4-enriched astrocytic endfeet may provide a low-resistance conduit for solute distribution into the interstitium, through either specific astroglial transporters or through the intercellular cleft between endfeet (Iliff et al., 2012). From the interstitium, solutes are then cleared via paravenous drainage into the cerebrospinal fluid through a sort of intracerebral lymphatic system, the glymphatic system. On the synaptic side, astrocytic lamellar processes ensheath synaptic elements. Such ensheathment of

synapses by the astrocytic process is dynamic and presents activity-dependent characteristics (Genoud et al., 2006). In addition to these morphological features, astrocytes express proteins at strategic sites that confer upon them the capacity to sense synaptic activity through receptors and reuptake sites, notably for glutamate, located on the perisynaptic lamellar processes (Danbolt, 2001; Iadecola and Nedergaard, 2007; Kacem et al., 1998) and to couple this activity with the import of substrates, notably glucose, from the capillary through glucose transporters expressed on the endfoot membranes.

Indeed, in 1994, our group described a mechanism, now known as the astrocyte-neuron lactate shuttle (ANLS), that could account for the coupling between synaptic activity and energy delivery (Pellerin and Magistretti, 1994). We showed that glutamate stimulates glucose uptake and lactate production in astrocytes (Pellerin and Magistretti, 1994). This glutamate-stimulated aerobic glycolysis is triggered by the uptake of glutamate (one of the main functions of astrocytes), which is cotransported with sodium with a stoichiometry of one glutamate to three sodium ions, resulting in the disruption of the sodium gradient. This triggers the activity of the energy-consuming sodium-potassium ATPase (Na^+/K^+ ATPase) at the expense of one ATP per turn of the pump to extrude three sodiums. Glutamate is mainly converted to glutamine by glutamine synthase at the expense of another ATP. This glutamate-glutamine cycle allows a large proportion of glutamate to be recycled to neuronal terminals to replenish the glutamate vesicular pool (Bak et al., 2006; McKenna, 2007; Stobart and Anderson, 2013), whereas the remaining glutamate enters the TCA cycle in astrocytes after conversion to α -ketoglutarate (Bak et al., 2006; McKenna, 2007; Stobart and Anderson, 2013) (Figure 3). Thus, glutamate uptake and recycling put an energetic burden on astrocytes, resulting in a decrease in ATP content (Magistretti and Chatton, 2005). This decrease in the energy charge of the cell promotes glucose uptake and metabolism by disinhibiting key enzymes of glycolysis, such as hexokinase and phosphofructokinase (Figure 1). As noted earlier, the glucose taken up via this mechanism is processed glycolytically to yield lactate. Through specific monocarboxylate transporters (MCTs) (Halestrap, 2013; Pierre and Pellerin, 2005), MCT1 (also present in other glial cells and endothelial cells) and MCT4 (specific for astrocytes), lactate is released, to be taken up by MCT2 (selective for neurons) into neurons and, after conversion to pyruvate, is processed oxidatively in mitochondria to produce 14–17 ATPs per lactate molecule (Figure 3).

The ANLS mechanism thus suggests that the uptake of synaptically released glutamate into astrocytes and the ensuing increase in intracellular sodium represent a key signal for activated neurons to import glucose into astrocytes and produce lactate as an energy substrate. Accordingly, using the whisker-to-barrel somatosensory pathway as a model system combined with two-photon analysis allowing cellular resolution, Chuquet et al. showed that vibrissal stimulation results in an increase in glucose uptake predominantly in astrocytes (Chuquet et al., 2010). In the same physiological system, we showed that when the expression of the astrocytic glutamate transporter GLAST (EAAT1) is downregulated with antisense oligonucleotides in a single barrel, the stimulation of the corresponding vibrissa does not result in a

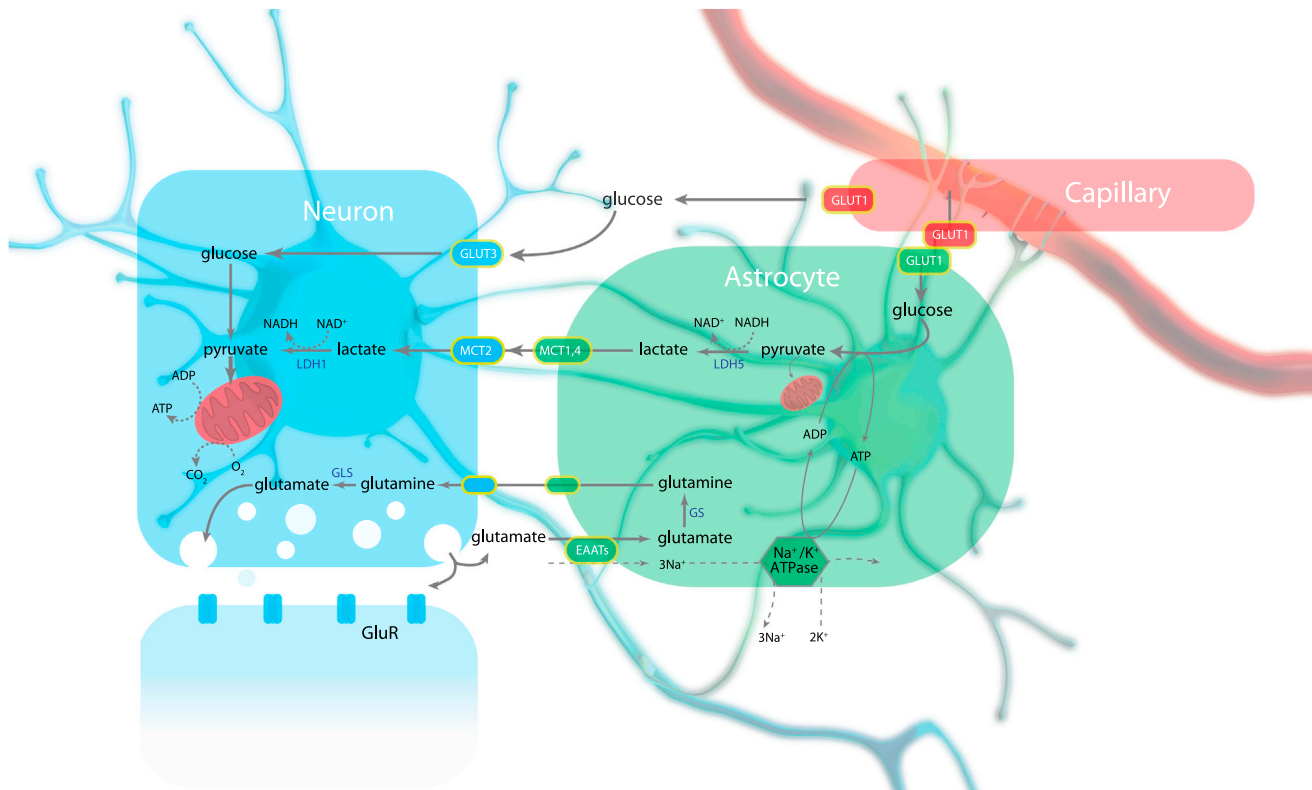


Figure 3. Schematic Representation of the Astrocyte-Neuron Lactate Shuttle

Glutamate released at the synapse activates glutamatergic receptors (GluRs), a process associated with energy expenditure in neuronal compartments. A large proportion of the glutamate released at the synapse is taken up by astrocytes via excitatory amino acid transporters (EAATs; more specifically, GLT-1 and GLAST) using the electrochemical gradient of Na^+ as a driving force, with a stoichiometry of one glutamate to three sodium ions. The disrupted Na^+ homeostasis is reestablished by the action of the Na^+/K^+ ATPase (in particular the $\alpha 2$ subunit; Pellerin and Magistretti, 1997), an ATP-consuming process. Following its uptake by astrocytes, glutamate is converted to glutamine by the action of glutamine synthetase (GS), also an ATP-consuming process, and shuttled to neurons, where it is converted back to glutamate by glutaminases (GLSs). The metabolic burden created by glutamate uptake triggers nonoxidative glucose utilization in astrocytes and glucose uptake from the circulation through the glucose transporter GLUT1 expressed by both capillary endothelial cells and astrocytes. Glycolytically derived pyruvate is converted to lactate by lactate dehydrogenase 5 (mainly expressed in astrocytes) and shuttled to neurons through monocarboxylate transporters (mainly MCT1 and MCT4 in astrocytes and MCT2 in neurons). In neurons, lactate can be used as an energy substrate following its conversion to pyruvate by LDH1 (mainly expressed in neurons). Under basal conditions, neurons can also take up glucose via the neuronal GLUT3. Concomitantly, astrocytes participate in the recycling of synaptic glutamate via the glutamate-glutamine cycle. For additional details, see text and Pellerin and Magistretti (2012).

concomitant increase in the 2-DG signal in the barrel (Cholet et al., 2001). Similar results were obtained in knockout transgenic mice in which the expression of the astrocyte-specific glutamate transporters was impaired (Voutsinos-Porche et al., 2003a, 2003b). These data indicate that astrocytes play a central role in coupling synaptic activity to glucose uptake through a mechanism that is mediated by glutamate transporters; lactate produced through glutamate-stimulated aerobic glycolysis is delivered to active synapses through MCTs (Nagase et al., 2014). Very recently it was shown that pharmacological inhibition of LDH, one of the components of the ANLS (Figure 3), which results in a reduction of lactate release by astrocytes, markedly decreases the excitability of neighboring pyramidal cells (Sada et al., 2015). For a review and discussion of the ANLS mechanism and aerobic glycolysis, readers are referred to Pellerin and Magistretti (2012) and Schurr (2014).

An important point to consider in this context is that ANLS is operative during glutamate-mediated activation. Under baseline conditions, there is no doubt that glucose transport and glucose

utilization operate in neurons mostly to produce reducing equivalents (Herrero-Mendez et al., 2009). Indeed, neurons express glucose transporters of the GLUT3 type as well as hexokinase activity (Mueckler and Thorens, 2013; Zhang et al., 2014). Furthermore, mitochondrial oxidative activity occurs also in astrocytes, as indicated, for example, by the expression of oxidative phosphorylation genes in purified populations of astrocytes (Cahoy et al., 2008; Lovatt et al., 2007). However, during glutamate-mediated activation, two mechanisms contribute to the promotion of glucose uptake into astrocytes and the reduction of its oxidative fate in these cells. First, glutamate uptake into astrocytes results in acidification of the mitochondrial matrix. This glutamate-induced mitochondrial matrix acidification dissipates the cytosol-to-mitochondrial matrix pH gradient, resulting in the decreased capacity of mitochondrial oxidative phosphorylation in astrocytes (Azarias et al., 2011).

Second, Barros and colleagues (Porras et al., 2004) have shown that glutamatergic activity results in decreased glucose utilization in neurons, whereas uptake of saccharide into

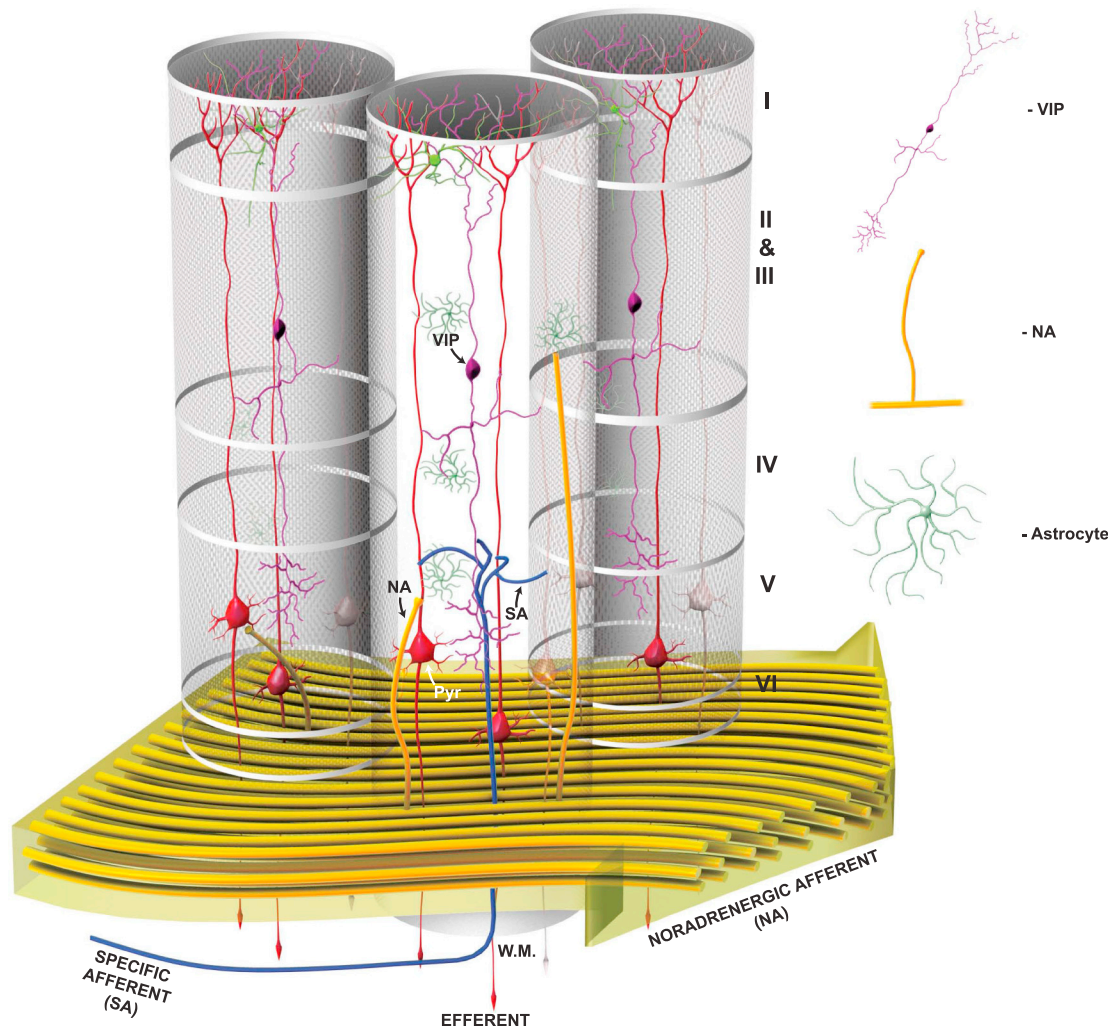


Figure 4. Contrasting Spatial Domains in the Neocortex within which VIP Neurons and Noradrenergic Fibers Exert Their Glycogenolytic Action

Vasoactive intestinal peptide neurons promote glycogenolysis in astrocytes. Given the bipolar nature of the VIP neurons, this effect is restricted to cortical columns. VIP neurons are activated by corticocortical and thalamocortical specific afferents (SAs). The noradrenergic afferent (NA) fibers span horizontally across cortical domains and hence can metabolically prime a vast expanse of neocortex by promoting glycogenolysis in astrocytes. For additional details, see text and [Magistretti and Morrison \(1988\)](#). I–VI refer to cortical layers. W.M., white matter.

astrocytes is increased. This observation indicates that, following glutamatergic activity, there is a shift of glucose utilization away from neurons and toward astrocytes, suggesting that an energy substrate other than glucose (i.e., lactate) must be used by neurons to meet the increased energy requirements associated with activation. This observation is consistent with the redox switch model between astrocytes and neurons proposed by [Cerdán et al. \(2006\)](#). This model suggests that the lactate transfer from astrocytes to neurons serves the purpose not only of fueling the neuronal TCA cycle but also of providing reducing equivalents to neurons. Through this exchange, the intracellular compartments of astrocytes and neurons are coupled and the directionality of the lactate transfer is determined by the redox state of each cell, mostly oxidized in neurons due to the predominance of oxidative metabolism there, whereas rather reduced in astrocytes owing to their high glycolytic activity. Hence, according to

the redox switch model ([Cerdán et al., 2006](#)), the inhibition of glucose transport (and consequently of glycolysis) induced by glutamate in neurons creates thermodynamically favorable conditions for the use of lactate as an energy substrate in this cell type, because these two processes compete for intracellular NAD^+ ([Figures 1 and 2](#)).

Although there is considerable support, both theoretical and experimental, for the existence of an activity-dependent transfer of lactate between astrocytes and neurons (see preceding paragraphs), some aspects of the ANLS mechanism have been challenged on theoretical or modeling grounds ([Dienel, 2012](#); [DiNuzzo et al., 2010](#); [Mangia et al., 2009a](#); [Patel et al., 2014](#)). In particular, although the ANLS does not exclude glucose neuronal use under basal conditions (see above and [Figure 5](#) in an original article by [Pellerin and Magistretti, 1994](#)), some members of the neurochemistry community defend the view that glucose, and



Figure 5. Multiscale Integration of Cellular Events, Metabolic Indices, and BOLD fMRI Signal during Activation

Synaptic activity results in EPSP generation in postsynaptic neurons (1, N) and glutamate-transporter current in astrocytes (1, A) (green circles). The early energy requirements of neurons are met by lactate oxidation in neuronal mitochondria, resulting in decreased extracellular lactate levels (2) and oxygen concentration (2) (red circles). As illustrated by combined PET measurements of glucose utilization, blood flow, and oxygen consumption, an uncoupling between CMRGlu, CBF, and CMRO₂ occurs, resulting in a decreased oxygen extraction fraction (OEF) and increased lactate production (3); the ANLS model provides a metabolic context for these observations with an increased glucose uptake into astrocytes (3) that is processed through aerobic glycolysis, resulting in the replenishment of the extracellular lactate pool (3) (blue circles). A direct glucose uptake by neurons under basal conditions and a potential lactate overflow into the circulation during activation may occur (dotted arrows). The delayed transient aerobic glycolysis occurring in astrocytes, which does not require an increase in oxygen consumption commensurate with glucose utilization, results in excess oxyhemoglobin in the activated area, producing the positive BOLD signal (4) (gold circle). The initial dip, or negative BOLD signal, corresponds to oxygen utilization, resulting in increased deoxyhemoglobin, which occurs during the early phase of synaptic activity (4). CBF: cerebral blood flow; CMRGlu: cerebral metabolic rate of glucose; CMRO₂: cerebral metabolic rate of oxygen; EPSP: excitatory postsynaptic potential.

not lactate, is the main energy substrate for oxidative metabolism in neurons (Dienel, 2012; Hertz et al., 2007). Such an opinion is mainly based on the grounds of modeling studies suggesting that the glucose transport capacity is larger in neurons than in astrocytes (see DiNuzzo et al., 2010; Mangia et al., 2009b; Simpson et al., 2007), a prediction considered to be inconsistent with the ANLS. Such a view is notably based on the determination, using cytochalasin B binding, of glucose-transporter density in cultured neurons (Simpson et al., 2007). However, glucose utilization is mainly determined by rate-limiting steps such as, for example, phosphorylation by hexokinase, implying that glucose metabolism cannot be reduced to the sole determination of the amount of transporters (Barros et al., 2007). Essentially it is not

justifiable to derive quantitative in vivo properties of glucose utilization by determining transport capacity, particularly when considered as a rate-limiting step, from transporter density (for a discussion of these points, see Jolivet et al., 2010). Further stressing the importance of glucose uptake into astrocytes compared with neurons, experiments in transgenic mice presenting a haploinsufficiency of glucose transporters have shown that decreased expression of the neuron-specific glucose transporter GLUT3 does not result in a pathological phenotype and does not affect cerebral glucose utilization (Schmidt et al., 2008; Stuart et al., 2011). In contrast, haploinsufficiency of GLUT1, which is absent in neurons and expressed in astrocytes, results in a severe neurological phenotype (Wang et al., 2006).

There has also been a suggestion that lactate is a poor energy substrate for neurons (Dienel, 2012; Hertz et al., 2007, and references therein) and that neurons are able to increase their glucose utilization rate in different conditions (see, e.g., Dienel, 2012; Patel et al., 2014). The latter interpretation has been discounted rather convincingly by experimental evidence provided by several groups reviewed in the preceding paragraphs (Cerdán et al., 2006; Herrero-Mendez et al., 2009; Porras et al., 2004). The value of lactate as an energy substrate is obviously linked to proper oxygen tension in the tissue as, after its conversion to pyruvate, the metabolic conditions must allow its processing through the TCA cycle and the associated oxygen phosphorylation. Not surprisingly, a study by Ivanov et al. has shown that in vitro, a condition used in the studies challenging the value of lactate as an energy substrate, the energetic role of lactate critically depends on oxygen tension in brain slices and that under appropriate oxygenation conditions lactate sustains oxidative activity even more than glucose (Ivanov et al., 2011). In fact, the capacity of lactate to sustain neuronal activity was already reported over 60 years ago by McIlwain (McIlwain, 1953) and later confirmed by various authors (e.g., Izumi et al., 1997; Schurr et al., 1988). A more detailed discussion of the arguments that challenge some aspects of the ANLS on the basis of in vitro and modeling studies has been provided (Pellerin and Magistretti, 2012).

The temporal sequence by which the ANLS becomes operative deserves some attention. Indeed, as will be reviewed later in the discussion on the cellular determinants of the signals detected by functional brain imaging techniques, in vivo optical imaging methods have demonstrated the existence of an oxidative metabolism in the early phases of synaptic activity (Malonek and Grinvald, 1996); these observations are consistent with the presence of an “initial dip” in the fMRI BOLD (blood-oxygen-level-dependent) signal, corresponding to decreased deoxyhemoglobin concentrations (Hu and Yacoub, 2012, and references therein), meaning a transient increase in oxygen consumption. In vitro studies in slices have also indicated a rapid and transient increase in oxygen consumption concomitant with synaptic activity (Hall et al., 2012). It is therefore likely that the immediate energy requirements of synaptic activity are met by the oxidation of lactate present in the extracellular space, and that the ANLS mechanism provides an efficient and spatiotemporally precise way to replenish the extracellular lactate pool in register with synaptic activity.

The respective phenotypic features of astrocytes and neurons that underlie the metabolic interactions that have been reviewed thus far are subject to developmental regulation and plasticity. For example, the different molecular determinants that confer to astrocytes the ability to upregulate aerobic glycolysis in response to glutamate—namely the glutamate transporter GLAST, the $\alpha 2$ subunit of the Na^+/K^+ ATPase, and MCT1—are upregulated as astrocytes differentiate and acquire the capacity to increase aerobic glycolysis in response to glutamate (Brunet et al., 2004). Similarly, in the presence of neurons, astrocytes increase the expression levels of mRNA and proteins for enzymes critical for glycolysis (phosphofructokinase, aldolase, and pyruvate kinase), glycogen metabolism (glycogen synthase and glycogen phosphorylase), and glutamine synthetase (Mamczur et al., 2015). This upregulation in astrocytes is paralleled by a

simultaneous decrease in the capacity of neurons to metabolize glucose and glutamine.

In summary, these observations indicate that, in the brain, glycolysis is concentrated in astrocytes to sustain, via lactate export, neuronal energy needs in register with activation. As nicely formulated by Barros, glycolysis is “outsourced” by neurons to astrocytes (Barros, 2013).

Other intercellular signals in addition to glutamate promote aerobic glycolysis in astrocytes, such as arachidonic acid, noradrenaline, and potassium (Bittner et al., 2011; Hutchinson et al., 2007; Obel et al., 2012; Ruminot et al., 2011; Takahashi et al., 1995; Yu et al., 1993). In particular, through elegant studies using high-temporal-resolution Förster resonance energy transfer (FRET) glucose nanosensors, Barros and colleagues showed that concentrations of K^+ reached in the extracellular space during neuronal activity stimulate a rapid (within seconds) increase in the glycolytic rate of astrocytes, both in cultures and slices (Bittner et al., 2011). There appears to be a temporal complementarity between the K^+ - and glutamate-evoked aerobic glycolysis in astrocytes, the former affording a rapid and transient processing of glucose whereas the latter is delayed and sustained (Bittner et al., 2011). The glycolytic action of K^+ in astrocytes involves the mobilization of the $\text{Na}^+/\text{HCO}_3^-$ cotransporter NBCe1 (Ruminot et al., 2011; as later confirmed by Choi et al., 2012).

Another mechanism of neuron-glia metabolic coupling involves the mobilization of glycogen, the storage form of glucose exclusively localized in astrocytes. A restricted set of neurotransmitters is able to promote glycogenolysis in astrocytes, the main ones being vasoactive intestinal peptide (VIP) and noradrenaline (NA) (Magistretti et al., 1981). The intracellular signaling mechanism triggered by VIP and NA to promote glycogenolysis is the adenylate-cyclase cascade. Glycogenolysis is also promoted by certain neuromodulators such as the purinergic agonists adenosine and ATP (Sorg et al., 1995; Sorg and Magistretti, 1991) as well as by activity-dependent increases in extracellular K^+ (Hof et al., 1988). The morphology of the neuronal circuits that contain VIP and NA in the neocortex is of particular interest in relation to energy metabolism regulation. Indeed, VIP neurons represent a homogeneous population of bipolar cells that are present throughout the neocortex. Like most cortical interneurons, they receive thalamocortical and corticocortical afferents (Magistretti and Morrison, 1988); what makes them particular is that their radially oriented dendritic arbor spans across virtually all cortical layers, implying that the VIP neurons are in a position to translate the afferent volley to the neocortex into a metabolic message to astrocytes by mobilizing energy reserves from glycogen within radially restricted volumes corresponding to functional columns. In contrast, the noradrenergic fibers, which all originate in the locus coeruleus in the brainstem, span the entire cortical volume horizontally from the frontal to the occipital poles, meaning that when activated, they mobilize glycogen globally throughout the cortical mantle and across functionally distinct areas, resulting in an energetic priming state of the neocortex (Figure 4).

In summary, at least two main mechanisms exist for coupling neuronal activity to energy delivery by astrocytes: aerobic glycolysis triggered by glutamate and a few other signals including K^+ , and VIP/NA-stimulated glycogenolysis. Both processes lead to

the release of lactate from astrocytes for use by neurons (Barros, 2013; Magistretti, 2006).

An additional spatiotemporal dimension of neuron-glia metabolic coupling in brain energy metabolism to be considered is the role of the gap-junctional communication that astrocytes entertain with other astrocytes and with oligodendrocytes (Fünfschilling et al., 2012; Giaume et al., 2010, 2013). Through gap junctions, metabolites can be distributed at distances throughout the glial syncytium. For example, in the hippocampus, glucose delivery by a patch pipette to a single astrocyte can sustain neuronal activity at distances (Rouach et al., 2008). This effect is mimicked by lactate and is abolished in the presence of the MCT inhibitor α -cyano-4-hydroxycinnamic acid, suggesting that glucose present in the astrocytic network is metabolized to lactate, transported through the astrocytic syncytium, and used by neurons to sustain their synaptic activity (Rouach et al., 2008). Astrocytic metabolic waves, analogous to the well-characterized calcium waves, have been described using a fluorescent analog of glucose (Bernardinelli et al., 2004). A similar intercellular trafficking of lactate across gap junctions necessary for axonal function has been shown to occur between astrocytes and oligodendrocytes (Fünfschilling et al., 2012; Saab et al., 2013).

As part of a discussion on the role of neuron-glia interactions in brain energy metabolism, one should mention the existence of mechanisms that regulate the local delivery of energy substrates to the active neuropil through the local regulation of blood flow. Since the formulation of the hypothesis by Roy and Sherrington that a coupling existed between the local vascular supply and functional activity, the search for the identification of “chemical products,” as stated by the authors (Roy and Sherrington, 1890), that would mediate such coupling has been ongoing. For extensive treatment of the subject, readers are referred to recent reviews (see, e.g., Attwell et al., 2010; Cauli and Hamel, 2010; Filosa and Iddings, 2013; Hillman, 2014; Kleinfeld et al., 2011; Petzold and Murthy, 2011).

5. The Emerging Role of Lactate in Neuroenergetics

Glucose is the main energy substrate for the adult brain under physiological conditions. However, as noted earlier, evidence now shows that the three-carbon monocarboxylate lactate plays an important role in neuroenergetics. Following the original observation by Henry McIlwain in the 1950s in brain slices (McIlwain, 1953), evidence accrued from both in vitro and in vivo studies over the past two decades indicates that lactate is also an efficient energy substrate capable of sustaining neuronal activity (Boumezbeur et al., 2010; Bouzier et al., 2000; Bouzier-Sore et al., 2006; Itoh et al., 2003; Qu et al., 2000; Schurr et al., 1997; Schurr and Gozal, 2011; Serres et al., 2005; also reviewed in Bélanger et al., 2011a; Pellerin et al., 2007; Pellerin and Magistretti, 2012).

With a plasma level of approximately 1.0 mM, lactate crossing the blood-brain barrier via MCT1 (Pierre and Pellerin, 2005) is fully oxidized and can provide up to 8%–10% of the brain's energy requirements (Boumezbeur et al., 2010; Gallagher et al., 2009; Smith et al., 2003; van Hall et al., 2009). This contribution may be even greater at supraphysiological lactate concentrations (i.e., following intravenous lactate infusion), with a near-

linear relationship (Boumezbeur et al., 2010; van Hall et al., 2009). During moderate-to-vigorous exercise, resulting in blood lactate concentrations of up to 10 mM, the human brain takes up and oxidizes lactate to a considerably larger extent than under normal conditions, supplying up to 20%–25% of total brain energy demands; this increase in lactate oxidation occurs at the expense of blood glucose utilization (van Hall et al., 2009). Overall, these data demonstrate that plasma lactate can be an efficient energy substrate for the adult human brain. In addition, if formed within the brain parenchyma via the ANLS from glucose that has crossed the blood-brain barrier or from astrocytic glycogen, lactate may in fact become the preferred energy substrate for activated neurons.

Quantitative studies in primary cultures have shown that neuronal oxidative metabolism is largely supported by lactate even in the presence of equicaloric amounts of glucose (Bouzier-Sore et al., 2003, 2006; Itoh et al., 2003). In rat cerebral cortex using [1 - ^{11}C]L-lactate and [18F]fluorodeoxyglucose as substrates, it was shown that in vivo, lactate is readily oxidized in an activity-dependent manner; furthermore, increasing plasma lactate results in the reduction of cerebral glucose utilization (Smith et al., 2003; Wyss et al., 2011). In line with the latter observations, NMR studies using [3 - ^{13}C]lactate in vivo in humans (reviewed in Bouzier-Sore and Pellerin, 2013) indicate that lactate is metabolized in a predominantly neuronal compartment (Boumezbeur et al., 2010). This in vivo lactate use by the brain is in fact correlated with the level of neuronal activity (Serres et al., 2004). For example, during whisker stimulation in rats, an increase in lactate produced from plasma ^{13}C -labeled glucose occurs in the corresponding somatosensory cortex (Sampol et al., 2013).

Such glucose-derived lactate use by neurons had also been demonstrated by Tsacopoulos and colleagues in the well-compartmentalized guinea pig retina containing photoreceptors attached to Mueller cells (glia); they showed that lactate, formed glycolytically from glucose, is released by Mueller cells to fuel activated photoreceptors (reviewed in Tsacopoulos and Magistretti, 1996).

Consistent with its role as a neuronal energy substrate, lactate exerts a neuroprotective role when the energy supply is defective. In slices, lactate can preserve neuronal integrity and function during glucose depletion (Berthet et al., 2009; Izumi et al., 1994, 1997; Schurr et al., 1988) or under posthypoxic conditions (Schurr et al., 1997). In vivo, blockade of lactate transport exacerbates delayed neuronal damage in a rat model of cerebral ischemia (Schurr et al., 2001) whereas, as a mirror effect, direct intracerebroventricular or intravenous administration of lactate protects the mouse brain against ischemic damage (Berthet et al., 2009, 2012). In line with this, exogenous supplementation of lactate shows promising perspectives for intervention in humans with traumatic brain injuries (Bouzat et al., 2014; Bouzat and Oddo, 2014). Brown and collaborators have shown that axonal function is preserved under aglycemic conditions through a mechanism involving mobilization of astrocytic glycogen and the associated lactate transfer to axons (Brown et al., 2005; Evans et al., 2013; Tekkök et al., 2005). Consistent with these observations, strategies aiming at increasing glycogen levels in astrocytes have been successfully used to mitigate neuronal loss

under glucose-deprived conditions both in vitro and in vivo (Suh et al., 2007; Swanson and Choi, 1993). Enhancing astrocyte-neuron lactate shuttling through the overexpression of the glucose transporter GLUT1 in astrocytes and MCT2 in neurons in a coculture model was shown to be neuroprotective against excitotoxic insults (Bliss et al., 2004). Thus, boosting lactate transfer from astrocytes to neurons may represent an effective neuroprotective therapeutic strategy not only under glucose-deprivation conditions but more generally against neuronal insults associated with intracellular energy deficiency such as excitotoxicity. In contrast, conditions under which lactate delivery to neurons is impaired lead to neuronal damage. For example, disruption of MCT1 expression in oligodendroglia (which are coupled to astrocytes through gap junctions), resulting in an impairment in the delivery of lactate, produces axonal damage and neuronal loss in animal and cell-culture models (Fünfschilling et al., 2012; Lee et al., 2012), an observation that may be relevant to the pathophysiology of amyotrophic lateral sclerosis (Lee et al., 2012).

In addition to its role as an energy substrate, lactate has also recently been shown to exert intercellular signaling functions (for a review, see Barros, 2013; Bergersen and Gjedde, 2012). Lactate activates glucose-sensing and orexin neurons in the hypothalamus as well as GABAergic neurons in the subfornical organ (see Bélanger et al., 2011a, and references therein). Although the mechanism(s) of action of lactate in this instance is not entirely characterized, it appears to critically involve ATP-sensitive K^+ channels (K_{ATP}) (see Bélanger et al., 2011a, and references therein). Increased ATP concentration, produced by lactate oxidation, triggers the closure of K_{ATP} channels, leading to membrane depolarization and neuronal activation, providing a mechanism that links lactate release by astrocytes, lactate utilization by neurons, and modulation of neuronal excitability. These observations add another level of interaction between astrocytes and neurons with regard to energy metabolism. Other regulatory processes of neuronal excitability by lactate shuttling between astrocytes and neurons may also operate (Bozzo et al., 2013; Erlichman et al., 2008; Nagase et al., 2014; Tang et al., 2014; Yang et al., 2014). For example, a pH-dependent effect of lactate has been reported to modulate the activity of the chemosensory (pH-dependent) neurons of the retrotrapezoid nucleus in the ventral medulla, which controls breathing rate (Erlichman et al., 2008). Binding of lactate to the GPR81 receptor (Bozzo et al., 2013) or potentiation of the NMDA receptor through lactate-induced red-ox changes (NAD/NADH ratio) (Yang et al., 2014) has been associated with modulation of neuronal activity.

Of importance regarding higher brain functions, astrocyte-derived lactate is required for learning and memory processes. Suzuki et al. (2011) have demonstrated that the transfer of glycogen-derived lactate from astrocytes to neurons is required for long-term memory formation and for the in vivo maintenance of long-term potentiation. Intrahippocampal injection of DAB (a potent inhibitor of glycogen phosphorylase and hence of lactate production from glycogen) specifically impaired long-term memory formation (while leaving short-term memory unaffected) in an inhibitory avoidance test in rats. A similar result was obtained by disrupting the expression of the astrocyte-

specific lactate transporter MCT4. These memory-inhibiting effects were fully rescued by intrahippocampal administration of lactate but not by equicaloric glucose. Disruption of the expression of the neuron-specific lactate transporter MCT2 also leads to amnesia, which, however, is not rescued by exogenous lactate, indicating that the import of lactate into neurons is necessary for long-term memory formation. In keeping with the behavioral effects observed, the in vivo induction of long-term potentiation and of genes known to be required for memory formation, such as phospho-CREB, Arc, and phospho-cofilin, was prevented by inhibition of glycogenolysis and MCT4 expression in a lactate-reversible manner (Suzuki et al., 2011). These data provide in vivo evidence of the existence of an astrocyte-neuron lactate transfer for physiological functions such as plasticity and memory (Suzuki et al., 2011). Similar results have been obtained in a different (working memory) learning paradigm (Newman et al., 2011). Following up on these observations on the role of lactate in memory, Yang et al. showed that lactate, but not glucose, stimulates the expression of plasticity-related genes such as Arc, Zif268, or brain-derived neurotrophic factor through a mechanism involving potentiation of NMDA receptor activity (Yang et al., 2014), providing a likely molecular mechanism accounting for the effect of lactate in long-term memory.

6. Contribution of Astrocytes to Producing the Signals Detected by Functional Brain Imaging Techniques

PET detects metabolic or vascular signals, glucose utilization, blood flow, and oxygen consumption, and fMRI detects oxy/deoxyhemoglobin ratios (BOLD) and hemodynamic changes. These signals are produced by the coupling between neuronal activity and the vascular and metabolic responses reviewed above. In other words, these imaging signals are surrogate markers of neuronal activity. It is therefore critical to understand the relationship between neuronal activity and these markers. Given the critical role that astrocytes play in the coupling of neuronal activity with metabolic and vascular responses, it follows that the role of astrocytes in producing functional brain imaging signals deserves close attention (Figley and Stroman, 2011; Magistretti, 2000; Magistretti and Pellerin, 1999).

Functional brain imaging techniques are used to monitor brain activity under both basal and task-evoked conditions. In the latter, the increased region-specific signal is taken to reflect local increases in neuronal activity. Electrophysiological measurements made in parallel to hemodynamic signals detected by fMRI have led to the conclusion that the imaging signals are closely associated with afferent inputs and local processing as detected by LFPs rather than with single- or multiunit recordings produced by output spiking activity (Logothetis et al., 2001; Raichle and Mintun, 2006). Activity at synapses resulting from integration of afferent inputs thus appears to be the cellular site where the signals detected by fMRI are generated. As noted earlier, a similar localization has been shown for imaging of glucose utilization with the 2-DG technique (Kadekaro et al., 1985). This conclusion is substantiated by the analysis of energy costs associated with neuronal activity, which has clearly identified synaptic signaling as the main site for energy utilization,

accounting for 80% of the total cost (Alle et al., 2009). A recent observation adding to this view shows that postsynaptic potentials contribute to the energy expenditure associated with synaptic signaling, as do presynaptic processes, particularly the filling of synaptic vesicles with neurotransmitters (Rangaraju et al., 2014).

Synapses are largely ensheathed by astrocyte processes that can detect synaptically released neurotransmitters, in particular glutamate, through receptors and reuptake sites. Electrochemical and magnetic resonance spectroscopy studies have unequivocally demonstrated increases in glutamate levels associated with modality-specific increases in inputs to a given region (Hu et al., 1994; Just et al., 2013; Miele et al., 1996; Schaller et al., 2013). This is not surprising, as about 80% of synaptic inputs to a given cortical region are glutamatergic. As reviewed in the preceding sections, several of the molecular steps have been described indicating how glutamate implements neurometabolic coupling by acting on astrocytes. This set of mechanisms provides a parsimonious and highly efficient mechanism to ensure energy delivery when and where needed, because the predominant signal of increased synaptic activity, and hence increased local energy demands, is glutamate (Magistretti and Pellerin, 1999). As a corollary of this phenomenology, it follows that functional brain imaging signals not only depend on neuronal function but also on a proper sequence of signaling cascades that takes place in astrocytes. Thus, alterations of such imaging signals observed in pathological states should be understood as reflecting abnormal processes occurring in neurons as well as in astrocytes.

The physiological mechanisms underlying one of the components of the fMRI signal, namely BOLD signal, deserve some particular consideration, as they are highly relevant to this discourse. Observations made in the late 1980s by Fox and Raichle (Fox et al., 1988; Fox and Raichle, 1986) and more recently further validated with higher-resolution technologies by Lin et al. (2010) have established that during activation a certain degree of metabolic “uncoupling” occurs, whereby the activity-dependent local increases in CBF and glucose utilization (cerebral metabolic rate of glucose; CMRGlu) are not matched by commensurate increases in oxygen consumption (cerebral metabolic rate of oxygen; CMRO₂) (Buxton, 2010; Lin et al., 2010). This occurrence predicts that in the activated region, the capillaries on the venous side should be enriched in oxyhemoglobin, as the excess oxygen carried by the increased blood flow is not being consumed (Figure 5). This in turn results in decreased relative levels of deoxyhemoglobin. Consideration of these physiological phenomena and of the biophysical properties of the two main forms of hemoglobin led Seiji Ogawa to develop the now popular BOLD fMRI technique (Ogawa et al., 1990a, 1990b). Indeed, because of the differential magnetic properties between oxy- (diamagnetic) and deoxy- (paramagnetic) hemoglobin, the magnetic susceptibility detected by MRI is modified, producing a signal specific for the activated region (Figure 5).

As previously discussed (Magistretti, 2000; Magistretti and Pellerin, 1999), the glutamate-stimulated aerobic glycolysis as formulated by the ANLS mechanism provides a metabolic context with cellular resolution for the origin of the BOLD signal.

Transport into astrocytes of synaptically released glutamate promotes glucose uptake with spatiotemporal precision into the brain parenchyma; impairment of such glutamate transport into astrocytes abolishes the activity-evoked increase in the 2-DG signal (Cholet et al., 2001; Voutsinos-Porche et al., 2003a, 2003b). Glucose is processed through aerobic glycolysis in astrocytes, implying that pyruvate formed through the glycolytic pathway is converted to lactate rather than entering the TCA cycle and the subsequent oxygen-consuming process of oxidative phosphorylation. The occurrence of this metabolic process is substantiated by the elevated lactate levels detected by micro-sensors and microdialysis in rodents and by MRS in humans in the activated area (Hu and Wilson, 1997; Just et al., 2013; Prichard et al., 1991; Sappey-Marinié et al., 1992; Schaller et al., 2013; Suzuki et al., 2011). The occurrence of this glycolytic step in astrocytes also implies that during the activation, oxygen consumption will not increase, in accordance with observed increases in oxyhemoglobin in the activated region. Given the oxidative nature of neurons, the released lactate will be oxidized, resulting in a delayed increase in oxygen consumption.

BOLD fMRI studies have also reported the occurrence of an initial dip, or negative BOLD signal, in the very early stages of activation. This is consistent with the occurrence of an early oxidative use of lactate followed by delayed production of lactate that transiently exceeds consumption.

Consistent with this observation, analysis of lactate transients indicates an initial lactate dip followed by a delayed decay (Hu and Wilson, 1997; Mangia et al., 2003) (Figure 5). Such a sequence of events has been supported by two-photon imaging analyses of NADH in hippocampal slices. Activation of the intra-hippocampal Schaffer collateral pathway induces a biphasic NADH fluorescence response consisting of a rapid and transient decrease of NADH levels, reaching a minimum (dip) 10 s after stimulation, followed by a sustained overshoot of NADH, peaking at 60 s (Kasischke et al., 2004). Decreases in NADH fluorescence reflect a rapid and transient activation of oxidative metabolism, whereas a delayed sustained increased NADH fluorescence reflects the activation of their glycolytic rate (Kasischke et al., 2004). Interestingly, the temporal sequence of these cell-specific NADH transients closely parallels the extracellular temporal sequence of the lactate transients observed in vivo in human and rodent brains, characterized by an early decrease (Hu and Wilson, 1997; Mangia et al., 2003) followed by a sustained increase (Hu and Wilson, 1997; Lin et al., 2010; Prichard et al., 1991) (Figure 5).

In summary, glutamate released by synaptic activity produces excitatory postsynaptic potentials (EPSPs) in target neurons (Figure 5, inset 1, N) and is avidly taken up by astrocytes (Figure 5, inset 1, A). The immediate energy needs of activated neurons are met by oxidation of lactate present in the extracellular space, resulting in a transient decrease in lactate levels and oxygen concentration (Figure 5, inset 2). The decreased deoxyhemoglobin levels produce the initial dip of the BOLD signal (Figure 5, inset 4). The occurrence of a delayed transient aerobic glycolysis in astrocytes resulting in increased glucose uptake and lactate production as described by the ANLS provides a temporal window during which oxygen is not consumed commensurately with glucose metabolism (Figure 5, inset 3), providing a transient

relative decrease in deoxyhemoglobin (due to the increased oxyhemoglobin concentration) that results in positive BOLD signal (Figure 5, inset 4). An additional intriguing link between increases in lactate during activation and the BOLD signal have been provided by von Pfösti et al. (2012), by showing that increases in plasma lactate concentrations dose-dependently produce a BOLD signal detected by fMRI in nonhuman primates (von Pfösti et al., 2012).

7. Evolutionary Considerations

Energy utilization by the brain markedly varies across species (Herculano-Houzel, 2011). Thus, glucose utilization increases from 0.3–1.2 $\mu\text{mol}/\text{min}$ in rodents (rats and mice), to 30–60 $\mu\text{mol}/\text{min}$ in nonhuman primates, to 450 $\mu\text{mol}/\text{min}$ in humans. Interestingly, the percentage of energy resources allocated to the brain also similarly varies, with 2% of whole-body energy consumption being used to meet the energy demands of the rodent brain, 9%–12% for nonhuman primates, and 20% for humans (Mink et al., 1981). This increase in relative energy utilization by the brain is actually greater than that predicted by the increase in body size (Karbowski, 2007; Martin, 1981). In addition to brain size, a massive, nonlinear development in neocortex size has occurred in humans, which accounts for over 40% of whole-brain energy utilization (Lennie, 2003).

There is ample evidence indicating that the distinctive appearance of higher cognitive functions in humans when compared to other nonhuman primate species (Tomasello and Call, 1997) is largely related to the massive expansion of the neocortex (Jerison, 1973; Stephan et al., 1988), suggesting that the emergence of higher cognitive functions in humans is closely associated with a considerable increase of energy consumption devoted to neural processing (Aiello and Wheeler, 1995; Goodman and Sterner, 2010). Another element associated with the expansion of the human brain and in particular of the neocortex is the large increase in the number of neurons in this brain area over the course of evolution. Comparatively, rodents have 10–31 million neurons, nonhuman primates 1.7–5.5 billion, and humans \sim 16 billion (Herculano-Houzel, 2011). Not only does the number of neurons in the neocortex increase but the complexity of their dendritic arborization—the number of spines and synapses—increases more than linearly in humans. These facts bear at least two implications: first, because of the massive neuronal arborization increase, the density of neurons per unit volume is likely to be lower in humans. Consistent with this fact, the energy utilization (i.e., glucose use) per unit volume is considerably lower in humans compared with non/human primates and rodents with, for example, 0.89 and 0.68 $\mu\text{mol}/\text{g}\cdot\text{min}$ in mouse and rat, respectively, 0.44 $\mu\text{mol}/\text{g}\cdot\text{min}$ in baboons, and 0.31 $\mu\text{mol}/\text{g}\cdot\text{min}$ in humans (Herculano-Houzel, 2011). A question that is still debated is the energy consumption per neuron: is it constant across species, or do neurons with larger arborizations consume more energy? According to the former view, brain size is essentially determined by the number of neurons and the increase in energy utilization with increasing brain size in a simple linear relationship with neuron number (Herculano-Houzel, 2011). Another view attributes the increase in energy utilization by larger brains not only to the sheer number of neurons but also to their larger dendritic arborization in more evolved brains (Sherwood et al.,

2006). Notwithstanding this debate, the evidence gathered so far does point to a relative constancy of energy requirements per neuron across species, compounded, however, by the demands that are imposed for maintaining resting potentials across a large dendritic spatial expanse. In particular, the high density of synaptic contacts on dendritic spines in humans resulting, upon activation, in electrochemical ion-gradient disruption imposes even higher energy demands (Elston et al., 2006; Friede and van Houten, 1962; Harrison et al., 2002).

Given the existence of a tight metabolic coupling between neurons and glia, astrocytes in particular, consideration of the contribution of this cell type to brain energy metabolism evolution is warranted. The glia/neuron ratio increases across evolution, with a 1.6 ratio in the human neocortex (Sherwood et al., 2006). The increase is only slightly above what should be expected from an allometric increase; the fact remains, however, that there are more astrocytes, the predominant sites of glucose uptake, in the human cortex than in lower species. Furthermore, there is evidence that each human neocortical neuron consumes close to three times more ATP per neuron both to maintain resting membrane potential and to fire (Lennie, 2003). Given the critical role that astrocytes play in sustaining the energetic requirements of neurons, it is not surprising that the overall astrocyte/neuron ratio has increased in humans.

Gene profiling techniques have provided a powerful tool to identify enrichments in the expression of certain genes that are correlated with higher cognitive functions when comparing human with nonhuman primates. Interestingly, the picture that emerges is that there is a massive increase in the expression of genes involved in energy production in the human neocortex (Cáceres et al., 2003; Uddin et al., 2004), particularly those involved in the electron chain transfer of ATP-producing oxidative phosphorylation (Grossman et al., 2004; Khaitovich et al., 2006). These observations indicate that throughout evolution, the human neocortex, which is so intimately related to the higher functional abilities that characterize humans, has evolved toward an energy-producing profile, which favors the oxidative production of ATP. This is a highly energy-efficient metabolic pathway that, however, bears the intrinsic risk of producing more potentially damageable reactive oxygen species. Metabolomic analysis likewise indicates an over 4-fold increase in metabolic changes that are specific to the human prefrontal cortex compared with nonhuman primate species (Fu et al., 2011). Furthermore, studies across organs and species show an acceleration of metabolome evolution in the human prefrontal cortex and skeletal muscle compared to nonprimate species in a way that indicates, in humans, a preferential allocation of energy production to the brain compared to muscle (Bozek et al., 2014). It also appears that brain energy metabolism is inversely correlated during development, suggesting that the high metabolic cost of brain function is compensated by a slowing of the body growth rate (Kuzawa et al., 2014).

Cross-species comparisons of the level of expression of the isoforms of lactate dehydrogenase (LDH) indicate that, in the human neocortex, the metabolic substrate for feeding the tricarboxylic acid cycle and eventually the electron transport chain is lactate (Duka et al., 2014). LDH is a tetrameric enzyme formed by various combinations of two units, A and B, which are encoded

by two distinct genes. The one with predominantly A subunits (LDHA) provides high throughput for lactate production, and is accordingly the predominant form found in lactate-producing cells such as skeletal myocytes. In contrast, the LDHB form is expressed in cells that take up lactate and metabolize it to pyruvate to produce energy, such as cardiac myocytes. In the rodent brain, neurons selectively express the LDHB form, whereas the LDHA form is almost exclusively found in astrocytes (Bittar et al., 1996). Western blot analyses of the expression of the LDH isoforms indicate that the synaptosomal fraction of the human neocortex contains predominantly the LDHB form, consistent with a predominant use of lactate as a substrate for energy production by neurons (Duka et al., 2014).

8. Conclusions

Considerable progress has been made over the last two decades in understanding the cellular, and in some cases molecular, mechanisms of brain energy metabolism. The main realization is that, not surprisingly, different cell types have distinct metabolic profiles, with a particular distinction between neurons and astrocytes. This relative cell specificity of energy metabolism implies the existence of rich and coordinated cell-cell signaling mechanisms in the regulation of metabolic processes. Novel techniques, with even higher spatial and temporal resolution, will provide new tools to refine our understanding of intercellular metabolic exchanges and possibly reveal a higher level of regulation in subcellular compartments. From a more global perspective, functional brain imaging techniques, which have also blossomed over the last two decades, along with magnetic resonance spectroscopy investigations have provided new insights into brain energy metabolism. Indeed, although such techniques by monitoring metabolic processes provide a marker of neuronal activity, they also have revealed some unexpected characteristics of brain metabolism such as the uncoupling between glucose utilization and oxygen consumption along with the enrichment in oxyhemoglobin in activated areas. It is therefore of great importance to bridge the gap in resolution scales between the cellular and molecular techniques with those, such as imaging techniques, that monitor metabolism at the local, multicellular, and circuit level. This is certainly a challenge that is worth pursuing, to better understand both the fundamental physiological phenomena of brain function as well as the significance of alterations in functional brain imaging signals that are detected in several neuropsychiatric disorders. Furthermore, as reviewed in the preceding section, an association exists between the emergence of higher cognitive functions in humans and a considerable expansion of the neocortex accompanied by an increase in glucose utilization and the expression of energy metabolism genes. If nothing else, such associations warrant a sustained effort to understand better the cellular and molecular determinants of neuroenergetics, with particular attention to the human brain.

ACKNOWLEDGMENTS

Research on brain energy metabolism in P.J.M.'s laboratory has been supported over the years by the Swiss National Science Foundation and by the University of Lausanne, EPFL, CHUV, the NCCR Synapsy, the Biaggi and Panacée foundations, and KAUST. The authors wish to thank Ivan

Gromiko and Heno Hwang for their support in drawing figures and Virginia Unkefer for editorial review, all of the academic writing services at KAUST. For the section on evolution, the authors are grateful to Dr. Alain Prochiantz for pointing out some key references and to Dr. Patrick Hof for critical reading.

REFERENCES

- Aiello, L.C., and Wheeler, P. (1995). The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. *Curr. Anthropol.* 36, 199–221.
- Allaman, I., and Magistretti, P.J. (2013). Brain energy metabolism. In *Fundamental Neuroscience*, L.R. Squire, D. Berg, F.E. Bloom, S. du Lac, A. Ghosh, and N.C. Spitzer, eds. (San Diego: Academic Press), pp. 261–284.
- Alle, H., Roth, A., and Geiger, J.R. (2009). Energy-efficient action potentials in hippocampal mossy fibers. *Science* 325, 1405–1408.
- Andriezen, W.L. (1893). On a system of fibre-like cells surrounding the blood vessels of the brain of man and mammals, and its physiological significance. *Int. Monatsschr. Anat. Physiol.* 10, 532–540.
- Attwell, D., and Laughlin, S.B. (2001). An energy budget for signaling in the grey matter of the brain. *J. Cereb. Blood Flow Metab.* 27, 1133–1145.
- Attwell, D., Buchan, A.M., Chappak, S., Lauritzen, M., Macvicar, B.A., and Newman, E.A. (2010). Glial and neuronal control of brain blood flow. *Nature* 468, 232–243.
- Aubert, A., Pellerin, L., Magistretti, P.J., and Costalat, R. (2007). A coherent neurobiological framework for functional neuroimaging provided by a model integrating compartmentalized energy metabolism. *Proc. Natl. Acad. Sci. USA* 104, 4188–4193.
- Azarias, G., Perreten, H., Lengacher, S., Poburko, D., Demareux, N., Magistretti, P.J., and Chatton, J.Y. (2011). Glutamate transport decreases mitochondrial pH and modulates oxidative metabolism in astrocytes. *J. Neurosci.* 31, 3550–3559.
- Bak, L.K., Schousboe, A., and Waagepetersen, H.S. (2006). The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J. Neurochem.* 98, 641–653.
- Barros, L.F. (2013). Metabolic signaling by lactate in the brain. *Trends Neurosci.* 36, 396–404.
- Barros, L.F., Bittner, C.X., Loaiza, A., and Porras, O.H. (2007). A quantitative overview of glucose dynamics in the gliovascular unit. *Glia* 55, 1222–1237.
- Barros, L.F., San Martín, A., Sotelo-Hitschfeld, T., Lerchundi, R., Fernández-Moncada, I., Ruminot, I., Gutiérrez, R., Valdebenito, R., Ceballos, S., Alegría, K., et al. (2013). Small is fast: astrocytic glucose and lactate metabolism at cellular resolution. *Front. Cell. Neurosci.* 7, 27.
- Bauernfeind, A.L., Barks, S.K., Duka, T., Grossman, L.I., Hof, P.R., and Sherwood, C.C. (2014). Aerobic glycolysis in the primate brain: reconsidering the implications for growth and maintenance. *Brain Struct. Funct.* 219, 1149–1167.
- Bélanger, M., Allaman, I., and Magistretti, P.J. (2011a). Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab.* 14, 724–738.
- Bélanger, M., Yang, J., Petit, J.M., Laroche, T., Magistretti, P.J., and Allaman, I. (2011b). Role of the glyoxalase system in astrocyte-mediated neuroprotection. *J. Neurosci.* 31, 18338–18352.
- Bergersen, L.H., and Gjedde, A. (2012). Is lactate a volume transmitter of metabolic states of the brain? *Front. Neuroenergetics* 4, 5.
- Bernardinelli, Y., Magistretti, P.J., and Chatton, J.Y. (2004). Astrocytes generate Na⁺-mediated metabolic waves. *Proc. Natl. Acad. Sci. USA* 101, 14937–14942.
- Berthet, C., Lei, H., Thevenet, J., Gruetter, R., Magistretti, P.J., and Hirt, L. (2009). Neuroprotective role of lactate after cerebral ischemia. *J. Cereb. Blood Flow Metab.* 29, 1780–1789.
- Berthet, C., Castillo, X., Magistretti, P.J., and Hirt, L. (2012). New evidence of neuroprotection by lactate after transient focal cerebral ischaemia: extended

benefit after intracerebroventricular injection and efficacy of intravenous administration. *Cerebrovasc. Dis.* 34, 329–335.

Bittar, P.G., Charnay, Y., Pellerin, L., Bouras, C., and Magistretti, P.J. (1996). Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. *J. Cereb. Blood Flow Metab.* 16, 1079–1089.

Bittner, C.X., Valdebenito, R., Ruminot, I., Loaiza, A., Larenas, V., Sotelo-Hitschfeld, T., Moldenhauer, H., San Martín, A., Gutiérrez, R., Zambrano, M., and Barros, L.F. (2011). Fast and reversible stimulation of astrocytic glycolysis by K⁺ and a delayed and persistent effect of glutamate. *J. Neurosci.* 31, 4709–4713.

Bliss, T.M., Ip, M., Cheng, E., Minami, M., Pellerin, L., Magistretti, P., and Sapolsky, R.M. (2004). Dual-gene, dual-cell type therapy against an excitotoxic insult by bolstering neuroenergetics. *J. Neurosci.* 24, 6202–6208.

Bolaños, J.P., Almeida, A., and Moncada, S. (2010). Glycolysis: a bioenergetic or a survival pathway? *Trends Biochem. Sci.* 35, 145–149.

Boumezeur, F., Petersen, K.F., Cline, G.W., Mason, G.F., Behar, K.L., Shulman, G.I., and Rothman, D.L. (2010). The contribution of blood lactate to brain energy metabolism in humans measured by dynamic ¹³C nuclear magnetic resonance spectroscopy. *J. Neurosci.* 30, 13983–13991.

Bouzat, P., and Oddo, M. (2014). Lactate and the injured brain: friend or foe? *Curr. Opin. Crit. Care* 20, 133–140.

Bouzat, P., Sala, N., Suys, T., Zerlauth, J.B., Marques-Vidal, P., Feihl, F., Bloch, J., Messerer, M., Levivier, M., Meuli, R., et al. (2014). Cerebral metabolic effects of exogenous lactate supplementation on the injured human brain. *Intensive Care Med.* 40, 412–421.

Bouzier, A.K., Thiaudiere, E., Biran, M., Rouland, R., Canioni, P., and Merle, M. (2000). The metabolism of [3-(13)C]lactate in the rat brain is specific of a pyruvate carboxylase-deprived compartment. *J. Neurochem.* 75, 480–486.

Bouzier-Sore, A.K., and Pellerin, L. (2013). Unraveling the complex metabolic nature of astrocytes. *Front. Cell. Neurosci.* 7, 179.

Bouzier-Sore, A.K., Voisin, P., Canioni, P., Magistretti, P.J., and Pellerin, L. (2003). Lactate is a preferential oxidative energy substrate over glucose for neurons in culture. *J. Cereb. Blood Flow Metab.* 23, 1298–1306.

Bouzier-Sore, A.K., Voisin, P., Bouchaud, V., Bezancon, E., Franconi, J.M., and Pellerin, L. (2006). Competition between glucose and lactate as oxidative energy substrates in both neurons and astrocytes: a comparative NMR study. *Eur. J. Neurosci.* 24, 1687–1694.

Bozek, K., Wei, Y., Yan, Z., Liu, X., Xiong, J., Sugimoto, M., Tomita, M., Pääbo, S., Pleszek, R., Sherwood, C.C., et al. (2014). Exceptional evolutionary divergence of human muscle and brain metabolomes parallels human cognitive and physical uniqueness. *PLoS Biol.* 12, e1001871.

Bozzo, L., Puyal, J., and Chatton, J.Y. (2013). Lactate modulates the activity of primary cortical neurons through a receptor-mediated pathway. *PLoS ONE* 8, e71721.

Brown, A.M., Sickmann, H.M., Fosgerau, K., Lund, T.M., Schousboe, A., Waagepetersen, H.S., and Ransom, B.R. (2005). Astrocyte glycogen metabolism is required for neural activity during aglycemia or intense stimulation in mouse white matter. *J. Neurosci. Res.* 79, 74–80.

Brunet, J.F., Grollmund, L., Chatton, J.Y., Lengacher, S., Magistretti, P.J., Villemure, J.G., and Pellerin, L. (2004). Early acquisition of typical metabolic features upon differentiation of mouse neural stem cells into astrocytes. *Glia* 46, 8–17.

Bushong, E.A., Martone, M.E., Jones, Y.Z., and Ellisman, M.H. (2002). Proto-plasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J. Neurosci.* 22, 183–192.

Buxton, R.B. (2010). Interpreting oxygenation-based neuroimaging signals: the importance and the challenge of understanding brain oxygen metabolism. *Front. Neuroenergetics* 2, 8.

Cáceres, M., Lachuer, J., Zapala, M.A., Redmond, J.C., Kudo, L., Geschwind, D.H., Lockhart, D.J., Preuss, T.M., and Barlow, C. (2003). Elevated gene expression levels distinguish human from non-human primate brains. *Proc. Natl. Acad. Sci. USA* 100, 13030–13035.

Cahoy, J.D., Emery, B., Kaushal, A., Foo, L.C., Zamanian, J.L., Christopherson, K.S., Xing, Y., Lubischer, J.L., Krieg, P.A., Krupenko, S.A., et al. (2008). A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci.* 28, 264–278.

Cauli, B., and Hamel, E. (2010). Revisiting the role of neurons in neurovascular coupling. *Front. Neuroenergetics* 2, 9.

Cerdán, S., Rodrigues, T.B., Sierra, A., Benito, M., Fonseca, L.L., Fonseca, C.P., and García-Martín, M.L. (2006). The redox switch/redox coupling hypothesis. *Neurochem. Int.* 48, 523–530.

Choi, H.B., Gordon, G.R., Zhou, N., Tai, C., Rungta, R.L., Martinez, J., Milner, T.A., Ryu, J.K., McLarnon, J.G., Tresguerres, M., et al. (2012). Metabolic communication between astrocytes and neurons via bicarbonate-responsive soluble adenylyl cyclase. *Neuron* 75, 1094–1104.

Cholet, N., Pellerin, L., Welker, E., Lacombe, P., Seylaz, J., Magistretti, P., and Bonvento, G. (2001). Local injection of antisense oligonucleotides targeted to the glial glutamate transporter GLAST decreases the metabolic response to somatosensory activation. *J. Cereb. Blood Flow Metab.* 21, 404–412.

Chuquet, J., Quilichini, P., Nimchinsky, E.A., and Buzsáki, G. (2010). Predominant enhancement of glucose uptake in astrocytes versus neurons during activation of the somatosensory cortex. *J. Neurosci.* 30, 15298–15303.

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

Dienel, G.A. (2012). Brain lactate metabolism: the discoveries and the controversies. *J. Cereb. Blood Flow Metab.* 32, 1107–1138.

DiNuzzo, M., Mangia, S., Maraviglia, B., and Giove, F. (2010). Changes in glucose uptake rather than lactate shuttle take center stage in subserving neuroenergetics: evidence from mathematical modeling. *J. Cereb. Blood Flow Metab.* 30, 586–602.

Duarte, J.M., Lei, H., Mlynárik, V., and Gruetter, R. (2012). The neurochemical profile quantified by in vivo ¹H NMR spectroscopy. *Neuroimage* 61, 342–362.

Duka, T., Anderson, S.M., Collins, Z., Raghanti, M.A., Ely, J.J., Hof, P.R., Wildman, D.E., Goodman, M., Grossman, L.I., and Sherwood, C.C. (2014). Synaptosomal lactate dehydrogenase isoenzyme composition is shifted toward aerobic forms in primate brain evolution. *Brain Behav. Evol.* 83, 216–230.

Elston, G.N., Benavides-Piccione, R., Elston, A., Zietsch, B., Defelipe, J., Manger, P., Casagrande, V., and Kaas, J.H. (2006). Specializations of the granular prefrontal cortex of primates: implications for cognitive processing. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* 288, 26–35.

Erllichman, J.S., Hewitt, A., Damon, T.L., Hart, M., Kuraszcz, J., Li, A., and Leiter, J.C. (2008). Inhibition of monocarboxylate transporter 2 in the reticulospinal nucleus in rats: a test of the astrocyte-neuron lactate-shuttle hypothesis. *J. Neurosci.* 28, 4888–4896.

Evans, R.D., Brown, A.M., and Ransom, B.R. (2013). Glycogen function in adult central and peripheral nerves. *J. Neurosci. Res.* 91, 1044–1049.

Figley, C.R., and Stroman, P.W. (2011). The role(s) of astrocytes and astrocyte activity in neurometabolism, neurovascular coupling, and the production of functional neuroimaging signals. *Eur. J. Neurosci.* 33, 577–588.

Filosa, J.A., and Iddings, J.A. (2013). Astrocyte regulation of cerebral vascular tone. *Am. J. Physiol. Heart Circ. Physiol.* 305, H609–H619.

Fox, P.T., and Raichle, M.E. (1986). Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc. Natl. Acad. Sci. USA* 83, 1140–1144.

Fox, P.T., Raichle, M.E., Mintun, M.A., and Dence, C. (1988). Nonoxidative glucose consumption during focal physiologic neural activity. *Science* 241, 462–464.

Friede, R.L., and van Houten, W.H. (1962). Neuronal extension and glial supply: functional significance of glia. *Proc. Natl. Acad. Sci. USA* 48, 817–821.

Fu, X., Giavalisco, P., Liu, X., Catchpole, G., Fu, N., Ning, Z.B., Guo, S., Yan, Z., Somel, M., Pääbo, S., et al. (2011). Rapid metabolic evolution in human prefrontal cortex. *Proc. Natl. Acad. Sci. USA* 108, 6181–6186.

Fünfschilling, U., Supplie, L.M., Mahad, D., Boretius, S., Saab, A.S., Edgar, J., Brinkmann, B.G., Kassmann, C.M., Tzvetanova, I.D., Möbius, W., et al. (2012).

- Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature* 485, 517–521.
- Gallagher, C.N., Carpenter, K.L., Grice, P., Howe, D.J., Mason, A., Timofeev, I., Menon, D.K., Kirkpatrick, P.J., Pickard, J.D., Sutherland, G.R., and Hutchinson, P.J. (2009). The human brain utilizes lactate via the tricarboxylic acid cycle: a ^{13}C -labelled microdialysis and high-resolution nuclear magnetic resonance study. *Brain* 132, 2839–2849.
- Genoud, C., Quairiaux, C., Steiner, P., Hirling, H., Welker, E., and Knott, G.W. (2006). Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. *PLoS Biol.* 4, e343.
- Giaume, C., Koulakoff, A., Roux, L., Holcman, D., and Rouach, N. (2010). Astroglial networks: a step further in neuroglial and gliovascular interactions. *Nat. Rev. Neurosci.* 11, 87–99.
- Giaume, C., Leybaert, L., Naus, C.C., and Sáez, J.C. (2013). Connexin and pannexin hemichannels in brain glial cells: properties, pharmacology, and roles. *Front. Pharmacol.* 4, 88.
- Golgi, C. (1886). *Sulla fine anatomia degli organi centrali del sistema nervoso* (Milano: Hoepli).
- Goodman, M., and Sterner, K.N. (2010). Phylogenomic evidence of adaptive evolution in the ancestry of humans. *Proc. Natl. Acad. Sci. USA* 107 (Suppl 2), 8918–8923.
- Goyal, M.S., Hawrylycz, M., Miller, J.A., Snyder, A.Z., and Raichle, M.E. (2014). Aerobic glycolysis in the human brain is associated with development and neonatal gene expression. *Cell Metab.* 19, 49–57.
- Grossman, L.I., Wildman, D.E., Schmidt, T.R., and Goodman, M. (2004). Accelerated evolution of the electron transport chain in anthropoid primates. *Trends Genet.* 20, 578–585.
- Halestrap, A.P. (2013). Monocarboxylic acid transport. *Compr. Physiol.* 3, 1611–1643.
- Halim, N.D., Mcfate, T., Mohyeldin, A., Okagaki, P., Korotchikina, L.G., Patel, M.S., Jeoung, N.H., Harris, R.A., Schell, M.J., and Verma, A. (2010). Phosphorylation status of pyruvate dehydrogenase distinguishes metabolic phenotypes of cultured rat brain astrocytes and neurons. *Glia* 58, 1168–1176.
- Hall, C.N., Klein-Flügge, M.C., Howarth, C., and Attwell, D. (2012). Oxidative phosphorylation, not glycolysis, powers presynaptic and postsynaptic mechanisms underlying brain information processing. *J. Neurosci.* 32, 8940–8951.
- Hamberger, A., and Hyden, H. (1963). Inverse enzymatic changes in neurons and glia during increased function and hypoxia. *J. Cell Biol.* 16, 521–525.
- Harris, J.J., Jolivet, R., and Attwell, D. (2012). Synaptic energy use and supply. *Neuron* 75, 762–777.
- Harrison, K.H., Hof, P.R., and Wang, S.S. (2002). Scaling laws in the mammalian neocortex: does form provide clues to function? *J. Neurocytol.* 31, 289–298.
- Herculano-Houzel, S. (2011). Scaling of brain metabolism with a fixed energy budget per neuron: implications for neuronal activity, plasticity and evolution. *PLoS ONE* 6, e17514.
- Herrero-Mendez, A., Almeida, A., Fernández, E., Maestre, C., Moncada, S., and Bolaños, J.P. (2009). The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat. Cell Biol.* 11, 747–752.
- Hertz, L., Peng, L., and Dienel, G.A. (2007). Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J. Cereb. Blood Flow Metab.* 27, 219–249.
- Hillman, E.M. (2014). Coupling mechanism and significance of the BOLD signal: a status report. *Annu. Rev. Neurosci.* 37, 161–181.
- Hof, P.R., Pascale, E., and Magistretti, P.J. (1988). K^+ at concentrations reached in the extracellular space during neuronal activity promotes a Ca^{2+} -dependent glycogen hydrolysis in mouse cerebral cortex. *J. Neurosci.* 8, 1922–1928.
- Hu, Y., and Wilson, G.S. (1997). A temporary local energy pool coupled to neuronal activity: fluctuations of extracellular lactate levels in rat brain monitored with rapid-response enzyme-based sensor. *J. Neurochem.* 69, 1484–1490.
- Hu, X., and Yacoub, E. (2012). The story of the initial dip in fMRI. *Neuroimage* 62, 1103–1108.
- Hu, Y., Mitchell, K.M., Albahadily, F.N., Michaelis, E.K., and Wilson, G.S. (1994). Direct measurement of glutamate release in the brain using a dual enzyme-based electrochemical sensor. *Brain Res.* 659, 117–125.
- Hutchinson, D.S., Summers, R.J., and Gibbs, M.E. (2007). Beta2- and beta3-adrenoceptors activate glucose uptake in chick astrocytes by distinct mechanisms: a mechanism for memory enhancement? *J. Neurochem.* 103, 997–1008.
- Hyden, H., and Lange, P.W. (1962). A kinetic study of the neuroglia relationship. *J. Cell Biol.* 13, 233–237.
- Hyder, F., Patel, A.B., Gjedde, A., Rothman, D.L., Behar, K.L., and Shulman, R.G. (2006). Neuronal-glial glucose oxidation and glutamatergic-GABAergic function. *J. Cereb. Blood Flow Metab.* 26, 865–877.
- Hyder, F., Rothman, D.L., and Bennett, M.R. (2013). Cortical energy demands of signaling and non-signaling components in brain are conserved across mammalian species and activity levels. *Proc. Natl. Acad. Sci. USA* 110, 3549–3554.
- Iadecola, C., and Nedergaard, M. (2007). Glial regulation of the cerebral microvasculature. *Nat. Neurosci.* 10, 1369–1376.
- Iliff, J.J., Wang, M., Liao, Y., Plogg, B.A., Peng, W., Gundersen, G.A., Benveniste, H., Vates, G.E., Deane, R., Goldman, S.A., et al. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β . *Sci. Transl. Med.* 4, 147ra111.
- Itoh, Y., Esaki, T., Shimoji, K., Cook, M., Law, M.J., Kaufman, E., and Sokoloff, L. (2003). Dichloroacetate effects on glucose and lactate oxidation by neurons and astroglia in vitro and on glucose utilization by brain in vivo. *Proc. Natl. Acad. Sci. USA* 100, 4879–4884.
- Ivanov, A., Mukhtarov, M., Bregestovski, P., and Zilberter, Y. (2011). Lactate effectively covers energy demands during neuronal network activity in neonatal hippocampal slices. *Front. Neuroenergetics* 3, 2.
- Izumi, Y., Benz, A.M., Zorumski, C.F., and Olney, J.W. (1994). Effects of lactate and pyruvate on glucose deprivation in rat hippocampal slices. *Neuroreport* 5, 617–620.
- Izumi, Y., Benz, A.M., Katsuki, H., and Zorumski, C.F. (1997). Endogenous monocarboxylates sustain hippocampal synaptic function and morphological integrity during energy deprivation. *J. Neurosci.* 17, 9448–9457.
- Jakoby, P., Schmidt, E., Ruminot, I., Gutiérrez, R., Barros, L.F., and Deitmer, J.W. (2014). Higher transport and metabolism of glucose in astrocytes compared with neurons: a multiphoton study of hippocampal and cerebellar tissue slices. *Cereb. Cortex* 24, 222–231.
- Jerison, H.J. (1973). *Evolution of the Brain and Intelligence* (New York: Academic Press).
- Jolivet, R., Magistretti, P.J., and Weber, B. (2009). Deciphering neuron-glia compartmentalization in cortical energy metabolism. *Front. Neuroenergetics* 1, 4.
- Jolivet, R., Allaman, I., Pellerin, L., Magistretti, P.J., and Weber, B. (2010). Comment on recent modeling studies of astrocyte-neuron metabolic interactions. *J. Cereb. Blood Flow Metab.* 30, 1982–1986.
- Jolivet, R., Coggan, J.S., Allaman, I., and Magistretti, P.J. (2015). Multi-time-scale modeling of activity-dependent metabolic coupling in the neuron-glia-vasculature ensemble. *PLoS Comput. Biol.* 11, e1004036.
- Just, N., Xin, L., Frenkel, H., and Gruetter, R. (2013). Characterization of sustained BOLD activation in the rat barrel cortex and neurochemical consequences. *Neuroimage* 74, 343–351.
- Kacem, K., Lacombe, P., Seylaz, J., and Bonvento, G. (1998). Structural organization of the perivascular astrocyte endfeet and their relationship with the endothelial glucose transporter: a confocal microscopy study. *Glia* 23, 1–10.

- Kadekaro, M., Crane, A.M., and Sokoloff, L. (1985). Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. *Proc. Natl. Acad. Sci. USA* 82, 6010–6013.
- Karbowsky, J. (2007). Global and regional brain metabolic scaling and its functional consequences. *BMC Biol.* 5, 18.
- Kasischke, K.A., Vishwasrao, H.D., Fisher, P.J., Zipfel, W.R., and Webb, W.W. (2004). Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. *Science* 305, 99–103.
- Kettenmann, H., and Verkhratsky, A. (2008). Neuroglia: the 150 years after. *Trends Neurosci.* 31, 653–659.
- Khaitovich, P., Tang, K., Franz, H., Kelso, J., Hellmann, I., Enard, W., Lachmann, M., and Pääbo, S. (2006). Positive selection on gene expression in the human brain. *Curr. Biol.* 16, R356–R358.
- Kleinfeld, D., Blinder, P., Drew, P.J., Driscoll, J.D., Muller, A., Tsai, P.S., and Shih, A.Y. (2011). A guide to delineate the logic of neurovascular signaling in the brain. *Front. Neuroenergetics* 3, 1.
- Kuzawa, C.W., Chugani, H.T., Grossman, L.I., Lipovich, L., Muzik, O., Hof, P.R., Wildman, D.E., Sherwood, C.C., Leonard, W.R., and Lange, N. (2014). Metabolic costs and evolutionary implications of human brain development. *Proc. Natl. Acad. Sci. USA* 111, 13010–13015.
- Lee, Y., Morrison, B.M., Li, Y., Lengacher, S., Farah, M.H., Hoffman, P.N., Liu, Y., Tsingalia, A., Jin, L., Zhang, P.W., et al. (2012). Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* 487, 443–448.
- Lennie, P. (2003). The cost of cortical computation. *Curr. Biol.* 13, 493–497.
- Lin, A.L., Fox, P.T., Hardies, J., Duong, T.Q., and Gao, J.H. (2010). Nonlinear coupling between cerebral blood flow, oxygen consumption, and ATP production in human visual cortex. *Proc. Natl. Acad. Sci. USA* 107, 8446–8451.
- Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., and Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412, 150–157.
- Lovatt, D., Sonnewald, U., Waagepetersen, H.S., Schousboe, A., He, W., Lin, J.H., Han, X., Takano, T., Wang, S., Sim, F.J., et al. (2007). The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. *J. Neurosci.* 27, 12255–12266.
- Magistretti, P.J. (1999). Brain energy metabolism. In *Fundamental Neuroscience*, M.J. Zigmond, F.E. Bloom, S.C. Landis, J.L. Roberts, and L.R. Squire, eds. (San Diego: Academic Press), pp. 389–413.
- Magistretti, P.J. (2000). Cellular bases of functional brain imaging: insights from neuron-glia metabolic coupling. *Brain Res.* 886, 108–112.
- Magistretti, P.J. (2006). Neuron-glia metabolic coupling and plasticity. *J. Exp. Biol.* 209, 2304–2311.
- Magistretti, P.J. (2009). Neuroscience. Low-cost travel in neurons. *Science* 325, 1349–1351.
- Magistretti, P.J., and Morrison, J.H. (1988). Noradrenaline- and vasoactive intestinal peptide-containing neuronal systems in neocortex: functional convergence with contrasting morphology. *Neuroscience* 24, 367–378.
- Magistretti, P.J., and Pellerin, L. (1999). Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 354, 1155–1163.
- Magistretti, P.J., and Chatton, J.Y. (2005). Relationship between L-glutamate-regulated intracellular Na^+ dynamics and ATP hydrolysis in astrocytes. *J. Neural Transm.* 112, 77–85.
- Magistretti, P.J., Morrison, J.H., Shoemaker, W.J., Sapin, V., and Bloom, F.E. (1981). Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: a possible regulatory mechanism for the local control of energy metabolism. *Proc. Natl. Acad. Sci. USA* 78, 6535–6539.
- Malonek, D., and Grinvald, A. (1996). Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: implications for functional brain mapping. *Science* 272, 551–554.
- Mamczur, P., Borsuk, B., Paszko, J., Sas, Z., Mozrzymas, J., Wiśniewski, J.R., Gizak, A., and Rakus, D. (2015). Astrocyte-neuron crosstalk regulates the expression and subcellular localization of carbohydrate metabolism enzymes. *Glia* 63, 328–340.
- Mangia, S., Garreffa, G., Bianciardi, M., Giove, F., Di Salle, F., and Maraviglia, B. (2003). The aerobic brain: lactate decrease at the onset of neural activity. *Neuroscience* 118, 7–10.
- Mangia, S., Giove, F., Tkáč, I., Logothetis, N.K., Henry, P.G., Olman, C.A., Maraviglia, B., Di Salle, F., and Ugurbil, K. (2009a). Metabolic and hemodynamic events after changes in neuronal activity: current hypotheses, theoretical predictions and in vivo NMR experimental findings. *J. Cereb. Blood Flow Metab.* 29, 441–463.
- Mangia, S., Simpson, I.A., Vannucci, S.J., and Carruthers, A. (2009b). The in vivo neuron-to-astrocyte lactate shuttle in human brain: evidence from modeling of measured lactate levels during visual stimulation. *J. Neurochem.* 109 (Suppl 1), 55–62.
- Martin, R.D. (1981). Relative brain size and basal metabolic rate in terrestrial vertebrates. *Nature* 293, 57–60.
- Mathiisen, T.M., Lehre, K.P., Danbolt, N.C., and Ottersen, O.P. (2010). The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. *Glia* 58, 1094–1103.
- McIlwain, H. (1953). Substances which support respiration and metabolic response to electrical impulses in human cerebral tissues. *J. Neurol. Neurosurg. Psychiatry* 16, 257–266.
- McKenna, M.C. (2007). The glutamate-glutamine cycle is not stoichiometric: fates of glutamate in brain. *J. Neurosci. Res.* 85, 3347–3358.
- Miele, M., Boutelle, M.G., and Fillenz, M. (1996). The source of physiologically stimulated glutamate efflux from the striatum of conscious rats. *J. Physiol.* 497, 745–751.
- Mink, J.W., Blumenschine, R.J., and Adams, D.B. (1981). Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. *Am. J. Physiol.* 241, R203–R212.
- Mueckler, M., and Thorens, B. (2013). The SLC2 (GLUT) family of membrane transporters. *Mol. Aspects Med.* 34, 121–138.
- Münch, G., Westcott, B., Menini, T., and Gugliucci, A. (2012). Advanced glycation endproducts and their pathogenic roles in neurological disorders. *Amino Acids* 42, 1221–1236.
- Nagase, M., Takahashi, Y., Watabe, A.M., Kubo, Y., and Kato, F. (2014). On-site energy supply at synapses through monocarboxylate transporters maintains excitatory synaptic transmission. *J. Neurosci.* 34, 2605–2617.
- Nedergaard, M., Ransom, B., and Goldman, S.A. (2003). New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci.* 26, 523–530.
- Newman, L.A., Korol, D.L., and Gold, P.E. (2011). Lactate produced by glycolysis in astrocytes regulates memory processing. *PLoS ONE* 6, e28427.
- Obel, L.F., Müller, M.S., Walls, A.B., Sickmann, H.M., Bak, L.K., Waagepetersen, H.S., and Schousboe, A. (2012). Brain glycogen—new perspectives on its metabolic function and regulation at the subcellular level. *Front. Neuroenergetics* 4, 3.
- Ogawa, S., Lee, T.M., Kay, A.R., and Tank, D.W. (1990a). Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc. Natl. Acad. Sci. USA* 87, 9868–9872.
- Ogawa, S., Lee, T.M., Nayak, A.S., and Glynn, P. (1990b). Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magn. Reson. Med.* 14, 68–78.
- Patel, A.B., Lai, J.C., Chowdhury, G.M., Hyder, F., Rothman, D.L., Shulman, R.G., and Behar, K.L. (2014). Direct evidence for activity-dependent glucose phosphorylation in neurons with implications for the astrocyte-to-neuron lactate shuttle. *Proc. Natl. Acad. Sci. USA* 111, 5385–5390.
- Pellerin, L., and Magistretti, P.J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc. Natl. Acad. Sci. USA* 91, 10625–10629.

- Pellerin, L., and Magistretti, P.J. (1997). Glutamate uptake stimulates Na^+ , K^+ -ATPase activity in astrocytes via activation of a distinct subunit highly sensitive to ouabain. *J. Neurochem.* 69, 2132–2137.
- Pellerin, L., and Magistretti, P.J. (2012). Sweet sixteen for ANLS. *J. Cereb. Blood Flow Metab.* 32, 1152–1166.
- Pellerin, L., Bouzier-Sore, A.K., Aubert, A., Serres, S., Merle, M., Costalat, R., and Magistretti, P.J. (2007). Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia* 55, 1251–1262.
- Petzold, G.C., and Murthy, V.N. (2011). Role of astrocytes in neurovascular coupling. *Neuron* 71, 782–797.
- Pierre, K., and Pellerin, L. (2005). Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *J. Neurochem.* 94, 1–14.
- Porras, O.H., Loaiza, A., and Barros, L.F. (2004). Glutamate mediates acute glucose transport inhibition in hippocampal neurons. *J. Neurosci.* 24, 9669–9673.
- Prichard, J., Rothman, D., Novotny, E., Petroff, O., Kuwabara, T., Avison, M., Howseman, A., Hanstock, C., and Shulman, R. (1991). Lactate rise detected by ^1H NMR in human visual cortex during physiologic stimulation. *Proc. Natl. Acad. Sci. USA* 88, 5829–5831.
- Pysh, J.J., and Khan, T. (1972). Variations in mitochondrial structure and content of neurons and neuroglia in rat brain: an electron microscopic study. *Brain Res.* 36, 1–18.
- Qu, H., Håberg, A., Haraldseth, O., Unsørd, G., and Sonnewald, U. (2000). ^{13}C MR spectroscopy study of lactate as substrate for rat brain. *Dev. Neurosci.* 22, 429–436.
- Raichle, M.E. (1983). Positron emission tomography. *Annu. Rev. Neurosci.* 6, 249–267.
- Raichle, M.E. (1998). Behind the scenes of functional brain imaging: a historical and physiological perspective. *Proc. Natl. Acad. Sci. USA* 95, 765–772.
- Raichle, M.E., and Mintun, M.A. (2006). Brain work and brain imaging. *Annu. Rev. Neurosci.* 29, 449–476.
- Rangaraju, V., Calloway, N., and Ryan, T.A. (2014). Activity-driven local ATP synthesis is required for synaptic function. *Cell* 156, 825–835.
- Rouach, N., Koulakoff, A., Abudara, V., Willecke, K., and Giaume, C. (2008). Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* 322, 1551–1555.
- Roy, C.S., and Sherrington, C.S. (1890). On the regulation of the blood-supply of the brain. *J. Physiol.* 11, 85–158.
- Ruminot, I., Gutiérrez, R., Peña-Münzenmayer, G., Añazco, C., Sotelo-Hitschfeld, T., Lerchundi, R., Niemeyer, M.I., Shull, G.E., and Barros, L.F. (2011). NBCe1 mediates the acute stimulation of astrocytic glycolysis by extracellular K^+ . *J. Neurosci.* 31, 14264–14271.
- Saab, A.S., Tzvetanova, I.D., and Nave, K.A. (2013). The role of myelin and oligodendrocytes in axonal energy metabolism. *Curr. Opin. Neurobiol.* 23, 1065–1072.
- Sada, N., Lee, S., Katsu, T., Otsuki, T., and Inoue, T. (2015). Targeting LDH enzymes with a stiripentol analog to treat epilepsy. *Science* 347, 1362–1367.
- Sampol, D., Ostrofet, E., Jobin, M.L., Raffard, G., Sanchez, S., Bouchaud, V., Franconi, J.M., Bonvento, G., and Bouzier-Sore, A.K. (2013). Glucose and lactate metabolism in the awake and stimulated rat: a ^{13}C -NMR study. *Front. Neuroenergetics* 5, 5.
- Sappey-Marini, D., Calabrese, G., Fein, G., Hugg, J.W., Biggins, C., and Weiner, M.W. (1992). Effect of photic stimulation on human visual cortex lactate and phosphates using ^1H and ^{31}P magnetic resonance spectroscopy. *J. Cereb. Blood Flow Metab.* 12, 584–592.
- Schaller, B., Mecke, R., Xin, L., Kunz, N., and Gruetter, R. (2013). Net increase of lactate and glutamate concentration in activated human visual cortex detected with magnetic resonance spectroscopy at 7 tesla. *J. Neurosci. Res.* 91, 1076–1083.
- Schmidt, S., Richter, M., Montag, D., Sartorius, T., Gawlik, V., Hennige, A.M., Scherneck, S., Himmelbauer, H., Lutz, S.Z., Augustin, R., et al. (2008). Neuronal functions, feeding behavior, and energy balance in $\text{Slc2a3}^{+/-}$ mice. *Am. J. Physiol. Endocrinol. Metab.* 295, E1084–E1094.
- Schurr, A. (2014). Cerebral glycolysis: a century of persistent misunderstanding and misconception. *Front. Neurosci.* 8, 360.
- Schurr, A., and Gozal, E. (2011). Aerobic production and utilization of lactate satisfy increased energy demands upon neuronal activation in hippocampal slices and provide neuroprotection against oxidative stress. *Front. Pharmacol.* 2, 96.
- Schurr, A., West, C.A., and Rigor, B.M. (1988). Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* 240, 1326–1328.
- Schurr, A., Payne, R.S., Miller, J.J., and Rigor, B.M. (1997). Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: further in vitro validation. *J. Neurochem.* 69, 423–426.
- Schurr, A., Payne, R.S., Miller, J.J., Tseng, M.T., and Rigor, B.M. (2001). Blockade of lactate transport exacerbates delayed neuronal damage in a rat model of cerebral ischemia. *Brain Res.* 895, 268–272.
- Selak, I., Skaper, S.D., and Varon, S. (1985). Pyruvate participation in the low molecular weight trophic activity for central nervous system neurons in glia-conditioned media. *J. Neurosci.* 5, 23–28.
- Serres, S., Bezancon, E., Franconi, J.M., and Merle, M. (2004). Ex vivo analysis of lactate and glucose metabolism in the rat brain under different states of depressed activity. *J. Biol. Chem.* 279, 47881–47889.
- Serres, S., Bezancon, E., Franconi, J.M., and Merle, M. (2005). Ex vivo NMR study of lactate metabolism in rat brain under various depressed states. *J. Neurosci. Res.* 79, 19–25.
- Sherwood, C.C., Stimpson, C.D., Raghanti, M.A., Wildman, D.E., Uddin, M., Grossman, L.I., Goodman, M., Redmond, J.C., Bonar, C.J., Erwin, J.M., and Hof, P.R. (2006). Evolution of increased glia-neuron ratios in the human frontal cortex. *Proc. Natl. Acad. Sci. USA* 103, 13606–13611.
- Shulman, R.G., and Rothman, D.L. (2001). ^{13}C NMR of intermediary metabolism: implications for systemic physiology. *Annu. Rev. Physiol.* 63, 15–48.
- Simard, M., Arcuino, G., Takano, T., Liu, Q.S., and Nedergaard, M. (2003). Signaling at the gliovascular interface. *J. Neurosci.* 23, 9254–9262.
- Simpson, I.A., Carruthers, A., and Vannucci, S.J. (2007). Supply and demand in cerebral energy metabolism: the role of nutrient transporters. *J. Cereb. Blood Flow Metab.* 27, 1766–1791.
- Smith, D., Pernet, A., Hallett, W.A., Bingham, E., Marsden, P.K., and Amiel, S.A. (2003). Lactate: a preferred fuel for human brain metabolism in vivo. *J. Cereb. Blood Flow Metab.* 23, 658–664.
- Sofroniew, M.V., and Vinters, H.V. (2010). Astrocytes: biology and pathology. *Acta Neuropathol.* 119, 7–35.
- Sokoloff, L. (1981). Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. *J. Cereb. Blood Flow Metab.* 1, 7–36.
- Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M.H., Patlak, C.S., Pettigrew, K.D., Sakurada, O., and Shinohara, M. (1977). The ^{14}C deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* 28, 897–916.
- Sorg, O., and Magistretti, P.J. (1991). Characterization of the glycogenolysis elicited by vasoactive intestinal peptide, noradrenaline and adenosine in primary cultures of mouse cerebral cortical astrocytes. *Brain Res.* 563, 227–233.
- Sorg, O., Pellerin, L., Stolz, M., Beggah, S., and Magistretti, P.J. (1995). Adenosine triphosphate and arachidonic acid stimulate glycogenolysis in primary cultures of mouse cerebral cortical astrocytes. *Neurosci. Lett.* 188, 109–112.
- Stephan, H., Baron, G., and Frahm, H. (1988). Comparative size of brains and brain components. In *Comparative Primate Biology*, H.D. Steklis and J. Erwin, eds. (New York: Alan R. Liss), pp. 1–38.
- Stobart, J.L., and Anderson, C.M. (2013). Multifunctional role of astrocytes as gatekeepers of neuronal energy supply. *Front. Cell. Neurosci.* 7, 38.

- Stuart, C.A., Ross, I.R., Howell, M.E., McCurry, M.P., Wood, T.G., Ceci, J.D., Kennel, S.J., and Wall, J. (2011). Brain glucose transporter (Glut3) haploinsufficiency does not impair mouse brain glucose uptake. *Brain Res.* 1384, 15–22.
- Suh, S.W., Bergher, J.P., Anderson, C.M., Treadway, J.L., Fosgerau, K., and Swanson, R.A. (2007). Astrocyte glycogen sustains neuronal activity during hypoglycemia: studies with the glycogen phosphorylase inhibitor CP-316,819 ([*R**,*S**]-5-chloro-*N*-[2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmethyl)propyl]-1*H*-indole-2-carboxamide). *J. Pharmacol. Exp. Ther.* 321, 45–50.
- Suzuki, A., Stern, S.A., Bozdagi, O., Huntley, G.W., Walker, R.H., Magistretti, P.J., and Alberini, C.M. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810–823.
- Swanson, R.A., and Choi, D.W. (1993). Glial glycogen stores affect neuronal survival during glucose deprivation in vitro. *J. Cereb. Blood Flow Metab.* 13, 162–169.
- Takahashi, S., Driscoll, B.F., Law, M.J., and Sokoloff, L. (1995). Role of sodium and potassium ions in regulation of glucose metabolism in cultured astroglia. *Proc. Natl. Acad. Sci. USA* 92, 4616–4620.
- Tang, F., Lane, S., Korsak, A., Paton, J.F., Gourine, A.V., Kasparov, S., and Teichmayer, A.G. (2014). Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nat. Commun.* 5, 3284.
- Tekkök, S.B., Brown, A.M., Westbroek, R., Pellerin, L., and Ransom, B.R. (2005). Transfer of glycogen-derived lactate from astrocytes to axons via specific monocarboxylate transporters supports mouse optic nerve activity. *J. Neurosci. Res.* 81, 644–652.
- Tomasello, M., and Call, J. (1997). *Primate Cognition* (New York: Oxford University Press).
- Tsacopoulos, M., and Magistretti, P.J. (1996). Metabolic coupling between glia and neurons. *J. Neurosci.* 16, 877–885.
- Uddin, M., Wildman, D.E., Liu, G., Xu, W., Johnson, R.M., Hof, P.R., Kapatos, G., Grossman, L.I., and Goodman, M. (2004). Sister grouping of chimpanzees and humans as revealed by genome-wide phylogenetic analysis of brain gene expression profiles. *Proc. Natl. Acad. Sci. USA* 101, 2957–2962.
- Vaishnavi, S.N., Vlassenko, A.G., Rundle, M.M., Snyder, A.Z., Mintun, M.A., and Raichle, M.E. (2010). Regional aerobic glycolysis in the human brain. *Proc. Natl. Acad. Sci. USA* 107, 17757–17762.
- van Hall, G., Stromstad, M., Rasmussen, P., Jans, O., Zaar, M., Gam, C., Quistorff, B., Secher, N.H., and Nielsen, H.B. (2009). Blood lactate is an important energy source for the human brain. *J. Cereb. Blood Flow Metab.* 29, 1121–1129.
- Virchow, R. (1856). *Gesammelte Abhandlungen zur Wissenschaftlichen Medizin* (Frankfurt: Meidinger Sohn).
- Virchow, R. (1859). *Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre* (Berlin: August Hirschwald).
- von Pfösti, V., Li, J., Zaldivar, D., Goense, J., Zhang, X., Serr, N., Logothetis, N.K., and Rauch, A. (2012). Effects of lactate on the early visual cortex of non-human primates, investigated by pharmacology-MRI and neurochemical analysis. *Neuroimage* 61, 98–105.
- Voutsinos-Porche, B., Bonvento, G., Tanaka, K., Steiner, P., Welker, E., Chatton, J.Y., Magistretti, P.J., and Pellerin, L. (2003a). Glial glutamate transporters mediate a functional metabolic crosstalk between neurons and astrocytes in the mouse developing cortex. *Neuron* 37, 275–286.
- Voutsinos-Porche, B., Knott, G., Tanaka, K., Quairiaux, C., Welker, E., and Bonvento, G. (2003b). Glial glutamate transporters and maturation of the mouse somatosensory cortex. *Cereb. Cortex* 13, 1110–1121.
- Wang, D., Pascual, J.M., Yang, H., Engelstad, K., Mao, X., Cheng, J., Yoo, J., Noebels, J.L., and De Vivo, D.C. (2006). A mouse model for Glut-1 haploinsufficiency. *Hum. Mol. Genet.* 15, 1169–1179.
- Warburg, O. (1956). On the origin of cancer cells. *Science* 123, 309–314.
- Ward, P.S., and Thompson, C.B. (2012). Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. *Cancer Cell* 21, 297–308.
- Wyss, M.T., Jolivet, R., Buck, A., Magistretti, P.J., and Weber, B. (2011). In vivo evidence for lactate as a neuronal energy source. *J. Neurosci.* 31, 7477–7485.
- Yang, J., Ruchti, E., Petit, J.M., Jourdain, P., Grenningloh, G., Allaman, I., and Magistretti, P.J. (2014). Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proc. Natl. Acad. Sci. USA* 111, 12228–12233.
- Yu, N., Martin, J.L., Stella, N., and Magistretti, P.J. (1993). Arachidonic acid stimulates glucose uptake in cerebral cortical astrocytes. *Proc. Natl. Acad. Sci. USA* 90, 4042–4046.
- Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O’Keeffe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N., et al. (2014). An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* 34, 11929–11947.