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PERSPECTIVE

Fueling thought: Management of glycolysis and oxidative phosphorylation in neuronal metabolism

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The brain's energy demands are remarkable both in their intensity and in their moment-to-moment dynamic range. This perspective considers the evidence for Warburg-like aerobic glycolysis during the transient metabolic response of the brain to acute activation, and it particularly addresses the cellular mechanisms that underlie this metabolic response. The temporary uncoupling between glycolysis and oxidative phosphorylation led to the proposal of an astrocyte-to-neuron lactate shuttle whereby during stimulation, lactate produced by increased glycolysis in astrocytes is taken up by neurons as their primary energy source. However, direct evidence for this idea is lacking, and evidence rather supports that neurons have the capacity to increase their own glycolysis in response to stimulation; furthermore, neurons may export rather than import lactate in response to stimulation. The possible cellular mechanisms for invoking metabolic resupply of energy in neurons are also discussed, in particular the roles of feedback signaling via adenosine diphosphate and feedforward signaling by calcium ions.

Our brains perform a wide variety of amazing computational feats, the major research attention of most neurobiologists. By comparison, brain metabolism has been dismissed by the term "housekeeping" and also by the blanket assertion of a principle of homeostasis: the notion that biological systems by their nature tend toward the (effortless) maintenance of constant internal conditions. As biologists, we must question our assumptions: how constant are the internal conditions, and what are the mechanisms that help to maintain them? Understanding these homeostatic mechanisms and their limitations is critical to understanding the myriad ways in which our housekeeping systems can fail and produce the dysfunction associated with disease.

Brain metabolism presents a particular challenge. The human brain is only ∼2% of the body's mass, but it consumes ∼20% of the daily energy budget (Rolfe and Brown, 1997). And there is enormous dynamic variation in the energy consumption of brain cells from moment to moment as the brain performs its signaling. Coordinated changes in brain activity and blood flow account for some of the adaptive response to energy demand (this allows regional brain activity to be monitored using functional magnetic resonance imaging based on blood oxygenation level–dependent [BOLD] signals; Ogawa et al., 1992), but the mechanisms of fuel use and energy generation in individual brain cells are also exquisitely controlled by both feedback and feedforward regulation to produce choreographed changes in the flow through specific biochemical pathways.

Compared with the encyclopedic knowledge of the metabolic enzymes and biochemical pathways amassed by biochemists in the late 20th century, our understanding of metabolic regulation and the actual cellular physiology of these pathways is in its infancy. The Warburg effect—the tendency of some cancer cells to emphasize glycolysis (see text box) of glucose to lactate despite the availability of oxygen—was described in the 1940s (Koppenol et al., 2011), but the underlying key regulatory mechanisms are only now being elucidated (Christofk et al., 2008; Vander Heiden et al., 2009; Hu et al., 2016). In cancer cells, the increased glycolysis appears to be more important for biosynthesis than for producing ATP (Vander Heiden et al., 2009; Liberti and Locasale, 2016; Vander Heiden and DeBerardinis, 2017).

In the brain, acute changes in metabolism occur in response to neuronal stimulation, and the increased energy demand causes a Warburg-like transient dissociation between glycolysis and oxphos. This perspective will focus on the acute changes in metabolism that occur in response to neuronal stimulation. The relationship between neuronal metabolism and that of the neighboring astrocytes (glial cells) will be discussed; however, the focus will be on neurons which comprise most (∼80–90%) of the energy demand of the brain (Attwell and Laughlin, 2001; Yu et al., 2017). For recent reviews and studies on the regulation of astrocytic metabolism, see Bolaños (2016), Dienel and Cruz (2016), and Nortley and Attwell (2017).

This perspective will address two main questions: (1) What is the actual balance in brain cells between glycolysis and oxphos (and are the processes compartmentalized to different cell types)? (2) What cellular mechanisms are responsible for the acute

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Definitions

Glycolysis
The partial metabolism of glucose to pyruvate or lactate. It occurs in the cytosol (and possibly is subcompartmented within the cytosol, perhaps to the intracellular surface of the plasma membrane). Metabolism of glucose to pyruvate yields two net phosphorylations of ADP to ATP and two $2e^-$ (two-electron) reductions of NAD^+ to NADH. Sustained glycolysis requires reoxidation of NADH to NAD^+ either through the coordinated reduction of pyruvate to lactate by lactate dehydrogenase (LDH) or through the action of one of the mitochondrial NADH shuttles.

Oxidative phosphorylation (oxphos)
The metabolism of pyruvate or other oxidative fuels through the mitochondrial TCA cycle, which leads to ATP resynthesis through the action of the electron transport chain (ETC) and ATP synthase.

control of glycolysis and oxphos in neurons? The choice between glycolysis and oxphos may have important consequences for brain function in both health and disease.

What is the balance in brain between glycolysis and oxphos?

Near-stoichiometric utilization of oxygen and glucose in resting brain

For the resting brain, the rates of glycolysis and oxphos appear well matched, with the cerebral metabolic rates (CMRs) of glucose and oxygen corresponding with nearly complete oxidation of glucose to pyruvate to CO_2. The expected stoichiometry for this process is that six O_2 molecules will be consumed for each glucose molecule. The best estimates for the oxygen–glucose index (OGI; OGI = $CMRO_2$/CMRglc) in the resting brain is around ∼5.5 in gray matter (Clarke and Sokoloff, 1994; Hyder et al., 2013), very close to the ideal stoichiometry of 6.

This corresponds well with the textbook picture of total glucose oxidation to CO_2 as it occurs in unicellular organisms, but it may actually be quite unusual in a multicellular organism. Recent work on in vivo metabolite fluxes argues that uncoupling between glycolysis and oxidative metabolism is the norm for all organs except the brain (Hui et al., 2017). Other organs readily export the lactate they derive from glucose metabolism, and they readily use circulating lactate for their oxidative metabolism. In contrast, the brain's tricarboxylic acid (TCA) cycle is fed mainly by metabolites derived from circulating glucose, with relatively little intermediate mixing of lactate from the circulation. This picture of complete glucose oxidation by the brain is nicely compatible with (though not required by) the near-stoichiometric consumption of oxygen and glucose by the brain.

However, the near agreement of these values with total glucose oxidation does not require that each brain cell has an uninterrupted pipeline from glucose to CO_2, just that the organ as a whole consumes its own intermediates. And although it describes the resting brain, it does not take account of the large dynamic changes that occur with activation of specific brain regions.

Transient uncoupling between glycolysis and oxphos during brain activation

The drop in OGI during brain stimulation was the first indication of some transient uncoupling of glycolysis and oxphos in the brain (Fox et al., 1988). With regional stimulation (e.g., of the visual cortex by a flashing checkerboard pattern), positron electron tomography studies reveal that local blood flow increases by ∼50% and that there is a commensurate increase in local glucose consumption. But the increase in oxygen utilization is much less, on the order of 5%, indicating that the additional glucose consumption is not stoichiometrically matched by oxygen but rather that glucose is metabolized nonoxidatively by glycolysis without oxphos. The overall OGI is reduced from the near-stoichiometric values down to ∼5 (Madsen et al., 1999). This is supported by measurements showing local elevation of brain lactate levels with stimulation (Korf and de Boer, 1990; Hu and Wilson, 1997; Mazuel et al., 2017) and even lactate export from the brain (e.g., Duffy et al., 1975, with stronger stimuli such as epileptic seizures).

There are plenty of caveats to bear in mind when assessing the OGI (Madsen et al., 1999; Dienel and Cruz, 2016). First, in addition to glycolysis, the pentose phosphate pathway and glycogen synthesis can both result in net utilization of glucose without molecular oxygen, thereby reducing the OGI. Second, the measurement conditions in some studies could be perturbed by excessive stress in the experimental subjects (meaning that the preconditions for reduced OGI may not be so simple as enhanced activity); furthermore, stimulus strength can have a nonlinear effect on the metabolic response mechanisms. Second, OGI may be exaggerated or suppressed depending on how the stimulated region is defined. For instance, if the unstimulated tissue in the periphery or even within the stimulated region is capable of oxidative metabolism of the excess lactate (Madsen et al., 1999; Gandhi et al., 2009), then including the contribution from this tissue will normalize the OGI. Finally, consistent with stress contributing to the reduced OGI, β-adrenergic blockade by propranolol reduces or eliminates the stimulation-induced change in OGI (Dienel and Cruz, 2016). Nevertheless, multiple studies exhibit net production of lactate from glucose in activated brain, arguing that glycolysis without oxphos constitutes a component of the reduced OGI (Korf and de Boer, 1990; Hu and Wilson, 1997; Mazuel et al., 2017).

Regardless of how universal the result, the ability of brain stimulation to produce dissociation between glucose consumption and oxygen consumption in some experiments has been ample motivation to consider the possible explanations for such dissociation at the level of cellular metabolism.

The source of neuronal energy during activation

One suggestion to explain dissociation in the brain between glycolytic metabolism and oxphos is that the two processes are substantially compartmentalized in different cell types. The astrocyte-to-neuron lactate shuttle (ANLS) hypothesis proposed that neuronal activity leads to increased glycolysis in astrocytes, leading to release of lactate that is taken up by neurons as their primary energy source (Magistretti et al., 1993; Pellerin and Magistretti, 1994, 2012). The ANLS hypothesis has been quite controversial for a long time (Chih et al., 2001; Gjedde and Marrett, 2001; Hertz, 2004; Mangia et al., 2011; Dienel, 2012, 2017; Bak and Walls, 2018; Barros and Weber, 2018; see also Dienel [2017] for strong arguments against ANLS at the tissue level).

Experiments in support of the ANLS often argue for its feasibility rather than providing direct evidence that it occurs.

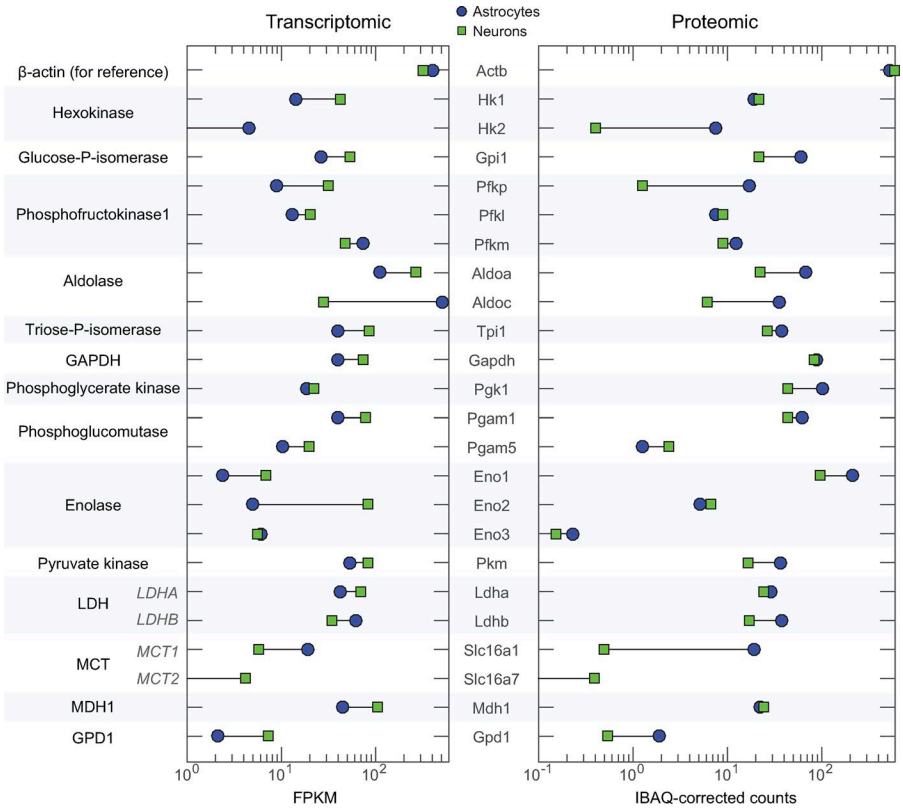

Figure 1. **Expression of ample levels of glycolytic (and related) enzymes in both astrocytes and neurons of the central nervous system.** Left: Transcriptional expression (from quantitative RNA sequencing) of the glycolytic enzymes and the cytosolic dehydrogenases associated with the NADH shuttles. Data from Zhang et al. (2014) use acutely purified neurons and astrocytes from mouse brain; data from Zeisel et al. (2015) tell a qualitatively similar story. Right: Proteomics data (Sharma et al., 2015) from cultured neurons and astrocytes confirm the general pattern from the transcriptomics data. The data plotted are for cultured astrocytes and for cultured neurons at day-in-vitro 15. Common names are given at the left, and gene names are shown between the two graphs. The largest proportional deficit in glycolysis is a ∼3.6-fold lower total aldolase in neurons versus astrocytes. Note that in neurons, the expression level differences for LDH isoforms and for MCT isoforms are actually quite small using either measure of expression. The expression level for the cytosolic malate dehydrogenase (MDH1) is substantially higher than for cytosolic glycerol-P-dehydrogenase (GPD1), consistent with the dominance of the MAS over the glycerol–phosphate shuttle. FPKM, fragments per kilobase million; IBAQ, integrity-based absolute quantitation.

For instance, the initial demonstration of feasibility was from separately cultured astrocytes and neurons: glutamate application to cultured astrocytes triggers [^{3}H]-2-deoxyglucose (2-DG; [^{3}H]–2-DG) uptake and lactate release, and neurons are capable of consuming lactate as a fuel (Magistretti et al., 1993; Pellerin and Magistretti, 1994). The initial proposal was that when glutamate, which is released as a neurotransmitter by neurons at excitatory synapses, is taken up by astrocytes, the cotransported Na^+ ions stimulate the ATP-driven Na^+ pump, which in turn promotes glycolytic production of ATP and lactate.

Other observations adduced in support of the ANLS hypothesis are mainly suggestive. For instance, astrocytes with pericapillary endfeet are ideally positioned to take up glucose from the vasculature; however, recent data with high-pressure cryopreservation show much less astrocytic coverage of both capillaries and synapses than seen with aldehyde fixation, which shrinks the extracellular space (Korogod et al., 2015). Neurons and astrocytes were reported to express different isoforms of LDH with different Michaelis constants (K_ms) for pyruvate (Bittar et al., 1996). This led to the proposal of mainly unidirectional flux from astrocytes to neurons; however, both isoforms are rapidly equilibrative (as argued by Gandhi et al., 2009; Dienel, 2012; Hertz et al., 2014; Bak and Schousboe, 2017), and recent single-cell transcriptomics studies show roughly equal expression of the two isoforms in neurons (Fig. 1). A similar argument was made for monocarboxylate transporter (MCT) isoforms (Pierre et al., 2000); although again, the transporters are both equilibrative, and transcriptomics show comparable levels of MCT1 in both neurons and astrocytes (Fig. 1, left; the proteomics data on the right show an overall deficit of MCTs in neurons, but they are from cells in culture). Moreover, detailed transport modeling shows that MCT2 is capable of ample lactate export from neurons at physiological concentrations (Simpson et al., 2007).

Astrocytes can indeed produce lactate from glucose (Hertz et al., 1988; Magistretti et al., 1993), and this lactate production can be stimulated by glutamate and potassium, which become elevated during neuronal activity (Walz and Mukerji, 1988; Peng et al., 1994; Pellerin and Magistretti, 1996; Bittner et al., 2011). Although they support the feasibility of the ANLS, these results do not demonstrate that astrocyte-to-neuron shuttling actually occurs during brain activation. The alternative possibility, whether or not astrocytes export lactate, is that neurons themselves use glucose as their primary fuel during activation.

Glycolytic capacity of neurons. Neurons express all of the enzymes needed for glucose metabolism (Fig. 1) and can metabolize glucose (Bak et al., 2009). In vitro glycolytic behavior can change with choice of culture media (Sünwoldt et al., 2017) and astrocyte coculture (Mamczur et al., 2015), but gene expression data from acutely dissociated brain cells indicate that neurons are well equipped for glycolysis, with higher levels of mRNA expression than astrocytes for most glycolytic enzymes (Fig. 1, left). At the protein level, cultured neurons and astrocytes have substantial expression of the glycolytic enzymes (Fig. 1, right); here, the pattern is somewhat reversed, with ∼1.5- to threefold lower expression in neurons for some of the enzymes (the outliers are the MCTs, which are required for the ANLS but are 40-fold lower in neurons). Although one specific enzyme for up-regulation of glycolysis, the bifunctional 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (isoform PFKFB3), is posttranslationally down-regulated in neurons and thus cannot

participate in glycolytic stimulation (Herrero-Mendez et al., 2009; Bolaños, 2016), this rules out only one of many mechanisms that neurons can use to stimulate glycolysis. Glycolysis is one of the most ancient metabolic pathways with a wide variety of allosteric and phosphorylation-based controls (Mulukutla et al., 2010; Icard and Lincet, 2012).

If the stimulated brain uses glycolysis as a low-capacity but fast mechanism for ATP production (as argued for fast-twitch skeletal muscle, for instance; Crow and Kushmerick, 1982; Spriet, 1989), it would make sense teleologically for this fast ATP resupply to occur within neurons, where the acute demand for energy is greatest.

Fuel utilization in activated neurons. When presented with a choice of either glucose or lactate as fuel, which do activated neurons use? Using unstimulated cultured neurons, Bouzier-Sore et al. (2006) reported that resting neurons preferentially consume lactate over glucose. However, the use of pure lactate as an alternative fuel, rather than a redox-balanced mixture of lactate and pyruvate, abnormally elevates the low cytosolic NADH redox ratio in neurons (seen in vitro by Hung et al. [2011] and Mongeon et al. [2016] as well as in vivo by Díaz-García et al. [2017]); this decreases the rate of neuronal glycolysis in a way that would not represent the normal effect in the brain of extracellular lactate on neuronal metabolism.

Importantly, this study was done with unstimulated neurons and did not test whether the preferential use of lactate was enhanced by activation as proposed (Bouzier-Sore et al., 2006). Similar experiments using graded neuronal stimulation by NMDA, which produced moderate submicromolar increases in cytosolic calcium, showed that activation increased glucose metabolism over twofold, whereas lactate metabolism declined, consistent with a role for extracellular lactate supporting basal metabolism in neurons but not stimulated activity (Bak et al., 2009).

Neuronal metabolic behavior in intact brain tissue. Regardless of the relative preparedness of the two cell types for metabolism, the most important question is how glucose uptake and lactate movements behave in the two cell types in intact brain tissue, when neurons are stimulated.

Comparison of glucose tracer uptake into neurons and astrocytes. Glucose uptake by neurons and astrocytes can be monitored with tracers based on 2-DG, relying on the assumption that uptake of the tracers and (in some cases) their phosphorylation by hexokinase parallels glucose utilization. High-resolution autoradiographic analysis of [^{14}C]–2-DG uptake in awake but unstimulated rats revealed roughly comparable label uptake in neurons and astrocytes (Nehlig et al., 2004); another in vivo labeling study using the fluorescent glucose analogue 2-NBDG showed stronger signals in neurons (Itoh et al., 2004). Two recent in vivo studies monitored glucose analogue accumulation in somatosensory cortex during whisker stimulation; they used two different glucose analogues and obtained opposite results. The first study (Chuquet et al., 2010) used the fluorescent glucose analogue 6-NBDG; because it cannot be phosphorylated by hexokinase (given that the 6 position was already occupied by the fluorophore), this analogue's accumulation may not correspond with the actual rate of glucose utilization, and it has been shown in some cases to correlate negatively with glucose utilization (DiNuzzo et al., 2013). Baseline accumulation of the analogue into astrocytes and neurons was similar; upon whisker stimulation in anesthetized rats, uptake into both cell types tended to increase, though only significantly for astrocytes (Chuquet et al., 2010). Using a different, phosphorylatable glucose analogue (2DG-IR) in nonanesthetized mice, better labeling was seen in neurons than astrocytes, particularly with whisker stimulation (Lundgaard et al., 2015). A major limitation of all these studies is that the different analogues (which, except for 2-DG itself, are two- to sevenfold larger molecules than glucose) may have preferential transport through the different glucose transporter isoforms that are predominantly expressed in neurons or astrocytes (Vannucci et al., 1997).

Carbon tracing by magnetic resonance spectroscopy in vivo has great chemical specificity but cannot directly measure metabolism separately for neurons and glia, so the conclusions are dependent on the details of multicompartment modeling of metabolism. A deliberate review of the evidence with an optimized model concluded that neurons provide about a third of their own lactate/pyruvate during brain activation, with the other two thirds from astrocytes (Hyder et al., 2006). However, this conclusion depends on the assumption that acetate is metabolized only in glia; a recent study of acetate metabolism in brain slices showed a substantial ability of neurons to metabolize acetate directly, which calls the compartment-specific modeling into question (Rowlands et al., 2017).

Differences in resting metabolic state between neurons and astrocytes. Consistent with most of the cultured cell studies, in resting brain or in quiescent brain slice astrocytes appear to be more glycolytic than neurons, with higher resting cytosolic NADH/NAD$^+$ ($NADH_{CYT}$) ratios (Mongeon et al., 2016) that predispose them to higher resting lactate/pyruvate ratios and higher resting lactate levels (Mächler et al., 2016). The difference in resting $NADH_{CYT}$ makes some baseline redox exchange likely between the two compartments (Cerdán et al., 2006); even with equal total (lactate + pyruvate) levels in the two cell types, astrocytic lactate could exchange for neuronal pyruvate, effectively allowing transfer of the glycolysis-derived NADH reducing equivalents from astrocytes to neurons. In essence, the very active malate–aspartate shuttle (MAS) of neurons could transfer reducing equivalents not only from neuronal glycolysis but also from astrocytic glycolysis. However, neighboring cells are capable of maintaining very different $NADH_{CYT}$ redox, and at rest, the high $NADH_{CYT}$ of astrocytes does not drive neighboring neurons to a correspondingly high level. At present, there are no data to indicate whether there is significant redox exchange between astrocytes and neurons through lactate–pyruvate exchange.

Evidence against lactate consumption by activated neurons in intact brain tissue. Fluorescent biosensor measurement of $NADH_{CYT}$ in neurons during excitation has helped to resolve the role of glycolysis and intercellular lactate transport in the acute metabolic response (Díaz-García et al., 2017). With brief neuronal excitation in an acute hippocampal slice, $NADH_{CYT}$ rises rapidly and remains elevated for ∼2–3 min, reflecting a temporary imbalance favoring production of NADH over its recycling back to NAD$^+$. NADH production could be transiently increased either through

increased glycolysis or increased lactate import and oxidation by LDH; NADH recycling could be transiently decreased because of reduced activity of the MAS. Pharmacologic blockade of MCT or of LDH abolishes the elevation of $NADH_{CYT}$ in response to exogenous lactate but not in response to stimulation, which rules out a role of lactate import in producing the transient increases in neuronal $NADH_{CYT}$. These blockers instead lead to enhancement of the $NADH_{CYT}$ transients, suggesting that neurons normally export lactate when stimulated. The $NADH_{CYT}$ transients decrease with inhibition of glycolysis but not of the MAS, indicating that increased neuronal glycolysis upon stimulation is the main cause of the $NADH_{CYT}$ transients. Inhibition of MCT in vivo in the cortical neurons of awake mice also blocks the $NADH_{CYT}$ response to exogenous lactate but not to sensory stimulation. The inferred recruitment of direct neuronal glycolysis in response to stimulation in a relatively intact system is consistent with earlier carbon tracing studies on cultured neurons (Bak et al., 2009, 2012). Moderate chemical stimulation produced a strong increase in the labeling of glutamate from labeled glucose and a decrease in the incorporation of label from labeled lactate, inconsistent with significant lactate utilization by neurons during stimulation.

In summary, experiments on both cultured cells and intact tissue demonstrate that stimulated neurons augment their glucose, not lactate, utilization. Although ANLS thus does not seem to provide the primary source of neuronal energy during brain activation, a role for lactate shuttling from astrocytes to neurons at rest is possible. As discussed, resting $NADH_{CYT}$ and resting lactate is higher in astrocytes than in neurons. Furthermore, although in cultured neurons, unlabeled lactate does not diminish the increased utilization of labeled glucose with stimulation, it does reduce labeling from glucose at rest (Bak et al., 2009). If an ANLS operates at rest, it could also explain why chronic disruption of MCT, LDH, or glycogenolysis in astrocytes can affect neuronal function (Suzuki et al., 2011; Zhang et al., 2016; Mazuel et al., 2017); these and similar results showing that MCT and lactate transport contribute to neuronal health and function provide evidence for some critical biological role for lactate uptake, but do not provide specific evidence for astrocyte-to-neuron lactate movement during activation (Mangia et al., 2011; Hall et al., 2012; Dienel, 2017).

In *Drosophila melanogaster*, glycolysis appears to be more essential in glial cells than it is in neurons; knockdown of glycolytic enzymes in neurons did not affect the viability or the general behavior of flies, whereas knockdown in glial cells produced noticeable phenotypic changes (Volkenhoff et al., 2015). These results could indicate an important role of glial lactate or alanine in fueling neurons; alternatively, knockdown of glial glycolysis could alter gliotransmitter release or could compromise glutamate or K^+ clearance, leading to compromised neuronal health. The acute metabolic response to neuronal activity has not been studied.

A neuron-to-glia lactate shuttle in retina. Although it was initially suggested that in analogy to the ANLS model for the central nervous system, the radial glial cells of the retina (Müller cells) might supply lactate to photoreceptors (Poitry-Yamate et al., 1995; Hurley et al., 2015), the opposite appears to be true: photoreceptors perform aerobic glycolysis and export lactate, which is then oxidized by Müller cells (Hurley et al., 2015; Kanow et al., 2017). Müller cells are deficient in pyruvate kinase, the final enzyme in the glycolytic pathway, and have limited ability to convert glucose to lactate (Lindsay et al., 2014). The high level of glycolysis in photoreceptors is important as they perform substantial biosynthesis during their diurnal degradation and nocturnal regeneration of outer segments (Chinchore et al., 2017). For in-depth reviews of retinal metabolism, see Hurley et al. (2015) and Kanow et al. (2017).

Possible differences in metabolic management of different neuronal compartments. In presynaptic terminals, both glycolysis and oxphos appear important for managing intense activity (Rangaraju et al., 2014; Sobieski et al., 2017). Glycolysis alone can support presynaptic energy needs during very gentle stimulation (Lujan et al., 2016). Many synaptic sites in brain have been shown to lack mitochondria (Chavan et al., 2015) and thus must depend on glycolysis, but ordinarily, brain mitochondria are quite motile, and movement of mitochondria into the vicinity of synaptic sites has been demonstrated to affect both ATP levels and synaptic vesicle fusion in response to trains of stimulation (Sun et al., 2013). Either glucose or lactate can support transmission at high-frequency auditory presynaptic terminals (Lucas et al., 2018); this study argued for lactate shuttling under limited conditions, but this conclusion is weak because MCT blockers that would also block mitochondrial pyruvate uptake were used (Halestrap and Denton, 1975; McKenna et al., 2001). In vivo uptake of 2-fluoro–2-DG into presynaptic terminals of rat brain assessed by postmortem isolation of synaptosomes (Patel et al., 2014) indicated substantial direct metabolism of glucose, consistent with an important role for glucose transporters and glycolysis in presynaptic terminals (Jang et al., 2016; Ashrafi et al., 2017; Ashrafi and Ryan, 2017).

In some postsynaptic structures, most notably the dendritic spines that receive most excitatory synaptic inputs in the mammalian brain, mitochondria were found to be absent in an exhaustive EM survey of a volume of neocortex (Kasthuri et al., 2015), despite prior evidence in cultured neurons that mitochondria can localize to dendritic protrusions, particularly with stimulation (Li et al., 2004). Dendrites are sites of intense energy utilization (Hall et al., 2012), and mitochondria in dendritic shafts likely perform oxphos to supply needed energy. But the absence of mitochondria from dendritic spines does make it questionable whether the role of MCTs, which may be inserted into spine membranes together with AMPA receptors (Bergersen et al., 2001), is to support astrocyte-to-neuron movement of lactate rather than efflux of lactate produced by aerobic glycolysis in the spines. Again, more direct experiments are needed; in this case, the compartmentation of glycolysis and oxphos could be between the dendritic spines and the dendritic shaft.

In myelinated axons, the myelin-forming cells (oligodendrocytes or Schwann cells) have been assumed to bear substantial responsibility for metabolic support (Nave, 2010), particularly as they cover ∼97–99% of the axon surface. Either glucose or lactate can be used as an energy source for white matter (Brown et al., 2001; Saab et al., 2016), and glial glycogen can supply energy to axons during aglycemia (Brown et al., 2003, 2012); however,

in corpus callosum white matter, exogenous lactate could not support axon function in the absence of glucose (Meyer et al., 2018). Once again, the question of whether intercellular glucose movements or lactate movements are involved either in ischemic or normal conditions is more difficult. In aglycemic conditions, glucose supplied to the gap junction–coupled network of oligodendrocytes was more effective than lactate in restoring axon function; however, pharmacologic manipulation of MCTs and glucose transporters gave uncertain answers about how axonal support was provided (Meyer et al., 2018).

Summary. Under ischemic conditions, lactate supplied exogenously or from astrocyte glycogenolysis is capable of providing metabolic support to neurons. However, there is no affirmative evidence that lactate movement from astrocytes to neurons occurs during the ordinary function of the brain, particularly during brain stimulation. In neuronal cell bodies, stimulation leads to enhanced neuronal glucose metabolism, whereas in some myelinated axons, metabolic support from glia may be essential during stimulation. In presynaptic terminals and postsynaptic dendritic compartments, more evidence is needed to understand the relative importance of neuronal and astrocytic glycolysis.

We now turn to the question of what cellular mechanisms may allow the transient excess of glycolysis over oxphos when neurons are stimulated.

What are the contributors to controlling the balance between glycolysis and oxphos?

Neuronal activity requires the expenditure and resupply of metabolic energy. Rapid electrical signaling involves ion movement on the order of thousands of ions per millisecond per open channel, and the steady-state ion concentrations must be restored and maintained by ATP-dependent active transport. Recycling and repackaging of neurotransmitters like glutamate and GABA into synaptic vesicles requires active transport, and the synaptic vesicles themselves must be retrieved, requiring additional energy.

What signals coordinate the metabolic response to this energy demand? The two most likely proximal signals are elevated [ADP] (the product of ATP hydrolysis) and elevated [Ca^{2+}] (which accompanies most neuronal electrical signals by permeation through voltage- and neurotransmitter-activated channels). ADP can be considered a feedback metabolic signal—energetic costs have been incurred and must be paid for—whereas Ca^{2+} can function as a feedforward signal to anticipate energetic costs before they are incurred. A complication in dissecting the roles of these two avenues for metabolic regulation is that Ca^{2+} handling is itself metabolically expensive, so elevated [Ca^{2+}] is usually accompanied by an elevation in ADP.

Both of these agents can regulate glycolysis and oxphos rates via multiple mechanisms. Also, for glycolysis and oxphos to run at different rates, there must be slippage or uncoupling at the interface between them: lactate-pyruvate partitioning controlled by the $NADH_{CYT}$ ratio.

ADP and Ca^{2+} can both depolarize mitochondria

ADP and Ca^{2+} both have well-known effects on mitochondria and oxphos (Nicholls and Ferguson, 2002; Salway, 2004). Proximally, both produce depolarization (diminution) of the mitochondrial membrane potential ($\Delta\psi$) and the related mitochondrial proton driving force ($\Delta\mu_H$; Fig. 2 A). ADP^{3-} enters the mitochondrial matrix through the adenine nucleotide translocase in exchange for ATP^{4-}, equivalent to one positive charge entering the mitochondrion; once inside, it interacts with the ATP synthase (also known as complex V) and is converted to ATP coordinately with the downhill transport of three protons from cytosol to matrix; an additional proton is transported to bring inorganic phosphate into the matrix. The net change with ADP entrance and ATP synthesis is four protons moved downhill plus an additional charge. For Ca^{2+}, entry into the matrix through the mitochondrial calcium uniporter or other Ca^{2+}-permeable channels produces an immediate depolarization of two charges per Ca^{2+} ion; a round trip by Ca^{2+} drains an additional three protons from the electrochemical proton gradient (via 3:1 Na^+/Ca^{2+} exchange and 1:1 Na^+/H^+ exchange).

Mitochondrial depolarization is important because in a resting mitochondrion, the ETC that pumps protons out of the matrix while consuming O_2, and matrix NADH is nearly stalled by the buildup of the electrochemical proton gradient ($\Delta\mu_H$), which is itself a combination of the electrical ($\Delta\psi$) and chemical (ΔpH) gradients. The depolarization and proton movements accompanying ADP or Ca^{2+} entry will diminish $\Delta\mu_H$ and allow the stalled ETC to function again, consuming O_2 and matrix NADH (Fig. 2).

The consumption of O_2 and matrix NADH correspond with two of the readily measured metabolic responses to neuronal stimulation. Oxygen consumption is monitored with a Clark-style electrochemical sensor of O_2 concentration; in brain slices, [O_2] shows a pronounced dip with stimulation, corresponding with increased O_2 consumption (Hall et al., 2012; Liotta et al., 2012; Ivanov et al., 2014).

NADH changes can be monitored using the autofluorescence evoked by shortwave light: NADH, in addition to the chemically related but very differently behaving NADPH are weakly fluorescent upon UV illumination and yield the signal referred to as NAD(P)H autofluorescence. Because mitochondrial matrix has a higher concentration of NADH than cytosol, and because of the higher fluorescence of protein-bound NADH, mitochondrial NADH is considered to be the major source of the NAD(P)H signal (though this is contested; see Kasischke et al., 2004; Brennan et al., 2006; Blacker et al., 2014; and the discussion below). Upon stimulation of neurons in a brain slice (Kann et al., 2003; Shuttleworth et al., 2003; Kasischke et al., 2004) or individual dissociated neurons (Duchen, 1992), the NAD(P)H signal has a stereotyped response: a rapid dip in fluorescence occurring within the first few seconds that yields in ∼10 s to a prolonged increase above baseline (overshoot) lasting for 2–3 min (Fig. 2 B).

Both the increased O_2 consumption and the initial NAD(P)H dip are generally agreed to reflect increased ETC activity upon mitochondrial depolarization, but the relative importance of ADP and Ca^{2+} in initiating these signals is not agreed upon. Duchen (1992), working on dissociated sensory neurons, argued that the main effect on oxygen consumption and the NAD(P)H dip is through Ca^{2+}. Upon neuronal stimulation with KCl, he observed mitochondrial depolarizations lasting 10–60 s and NAD(P)H dips lasting a few seconds followed by overshoots lasting ∼2 min. Oligomycin, which blocks the ATP synthase that links ADP influx

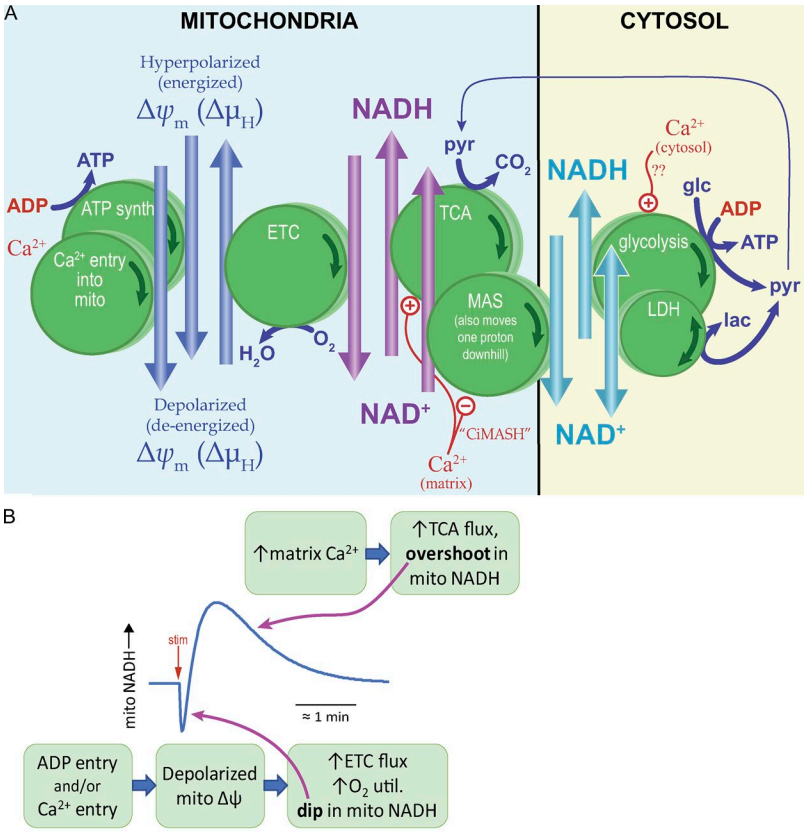

Figure 2. **Relationships of some major bioenergetic pools in the neuronal cytosol and mitochondria and their regulation. (A)** This schematic shows key bioenergetic processes as waterwheels or gears as well as their interaction with three major bioenergetic reservoirs: the membrane potential across the mitochondrial inner membrane ($\Delta\psi_m$) and the related electrochemical gradient for protons across the mitochondrial inner membrane ($\Delta\mu_H$); the mitochondrial NADH/NAD+ redox; and the cytosolic NADH/NAD+ redox. Both in the mitochondrion and cytosol, ADP and Ca^{2+} are the proximal signals for metabolic demand. As explained in the text, both ADP and Ca^{2+} entry into mitochondria contribute to mitochondrial depolarization; this allows the ETC to consume mitochondrial NADH and O_2 and to restore the mitochondrial $\Delta\mu_H$. **(B)** The impact of neuronal stimulation on these pathways as measured experimentally by monitoring NAD(P)H autofluorescence is diagrammed. The consumption of mitochondrial NADH leads to the dip in mitochondrial NADH. The increase in mitochondrial [Ca^{2+}] stimulates the TCA cycle and produces an overshoot in NADH production. In the cytosol, NADH/NAD+ redox is controlled by glycolysis (which increases NADH), the MAS, which decreases NADH, and the LDH reaction, which can run in either direction.

to mitochondrial depolarization, had no major effects, whereas removal of extracellular Ca^{2+} or blockade of the mitochondrial uniporter with ruthenium red abolished all of the transients, leading to the conclusion that ADP is unimportant for engaging the mitochondrial machinery but rather that intramitochondrial Ca^{2+} is the main control.

Kann et al. (2003) did analogous measurements in organotypic hippocampal slice cultures with electrical stimulation. Although intracellular and mitochondrial Ca^{2+} signals were abolished by removal of extracellular Ca^{2+}, the NAD(P)H transients were reduced in amplitude by about half but not abolished. They concluded that in addition to a modulatory feedforward effect of Ca^{2+}, there remains an intrinsic regulation of NADH production including the effect of ADP, though they did not test the importance of ADP directly. Similar experiments on acute hippocampal slices found little or no reduction in the NAD(P)H signals with removal of Ca^{2+} (Shuttleworth et al., 2003).

Other studies that considered mainly the compartmental origin of the NAD(P)H signal rather than the mechanism of production have made a strong case that both phases of the NAD(P)H signal are mostly from mitochondrial rather than cytosolic NADH. An early study using two-photon microscopy of the NAD(P)H signal had claimed that although the dip occurred in neuronal mitochondria, the overshoot was evidence of glycolytic metabolism in astrocytic cytosol (Kasischke et al., 2004). Despite the ability, in principle, to segregate signals based on the actual spatial locations of astrocytes, they performed a pixel-by-pixel analysis with a low signal-to-noise ratio to argue that pixels showing a dip were likely to have a smaller overshoot. However, in a later study with covisualization of neurons, astrocytes, and NAD(P)H, the transient changes in NAD(P)H appeared spatially homogenous (Kasischke et al., 2011). A study that instead used pharmacological inhibition of glycolysis (but in the presence of 1 mM pyruvate as a mitochondrial fuel, which will incidentally suppress cytosolic NADH transients) showed that much of the overshoot signal could not arise from glycolysis and instead must be mitochondrial in origin (Brennan et al., 2006; Shuttleworth, 2010). Extended intense stimulation may increase demand to the point that some glycolytic NADH also contributes to the autofluorescence signal; in addition, the quantitative contributions of glycolysis and oxphos can vary with oxygenation (Ivanov et al., 2014).

If both components of the NAD(P)H signal are substantially mitochondrial in origin, then what accounts for the overshoot? One candidate is the known effect of matrix Ca^{2+} in stimulating the matrix dehydrogenases including pyruvate dehydrogenase and the TCA dehydrogenases (McCormack et al., 1990). This feedforward effect of Ca^{2+} would increase the rate of matrix NADH production (and thus the NAD(P)H signal) and thereby increase the supply of electrons for the ETC, leading to mitochondrial hyperpolarization (Ivannikov and Macleod, 2013). This agrees with the results of Duchen (1992) and with the experimental and modeling studies in slice by Kann et al. (2003) (see also Berndt et al., 2015). However, it is hard to reconcile this with the clear finding by Shuttleworth et al. (2003) that the NAD(P)H overshoot in slices did not disappear with the prolonged elimination of extracellular Ca^{2+}, although the mitochondrial Ca^{2+} transient did disappear. Lactate produced by astrocytes has also been suggested

as the source of the NAD(P)H overshoot (Galeffi et al., 2007), but this study used a poorly selective blocker of MCT that also affects mitochondrial pyruvate uptake; this hypothesis is also incompatible with the finding that glycolytic blockade (which is not selective between neurons and astrocytes) failed to block the overshoot (Brennan et al., 2006).

Other possible sources of the NAD(P)H overshoot in the mitochondrial compartment would be an increased supply of pyruvate to mitochondria or increased NADH shuttling from the cytosol. An increased supply of pyruvate could occur with up-regulation of glycolysis (though the increased production of pyruvate would be somewhat offset by conversion to lactate because of elevated $NADH_{CYT}$). Increased NADH shuttling, particularly for small elevations of cytosolic Ca^{2+}, could occur by stimulation of aralar, a principal component of the MAS (Pardo et al., 2006), although elevated matrix Ca^{2+} can produce the opposite effect (Ca^{2+} inhibition of MAS or "CiMASH;" Bak et al., 2012); however, these effects on MAS would also not explain the production of the NAD(P)H overshoot when Ca^{2+} is removed (Shuttleworth et al., 2003).

Clearly, we do not have a complete understanding of the oxphos-related mitochondrial signals that result from neuronal excitation. The mechanisms that are engaged by neuronal excitation can vary with the degree of stimulation and with the environmental conditions of [glucose] and [O_2] during experiments, which usually vary from the in vivo condition, and the mechanisms may also vary with cell type. It would also be valuable to have a more compartmental- and cell-specific study of mitochondrial NADH than the NAD(P)H autofluorescence.

Partitioning between lactate production and pyruvate oxidation depends on $NADH_{CYT}$

Uncoupling between glycolysis and oxphos involves the partitioning between pyruvate (the primary substrate for glucose-driven oxphos) and lactate. Lactate is generally thought to be a terminal metabolite for glycolysis that must be converted back to pyruvate in the cytosol to serve as a substrate for oxphos (Nelson and Cox, 2017), though this is debated by some (Schurr, 2006). Because interconversion between pyruvate and lactate by LDH requires coordinate interconversion between NADH and NAD^+, and because the rate of glycolysis depends on some form of recycling of NADH to NAD^+, the $NADH_{CYT}$ ratio is a key parameter for the uncoupling.

High $NADH_{CYT}$ limits pyruvate availability for oxidation by forcing pyruvate conversion to lactate. For instance, it has been argued that pyruvate is limiting (i.e., that it falls below the Km for the mitochondrial pyruvate carrier) in Ins-1E and HEK cells because of the high $NADH_{CYT}$ in these cells (Compan et al., 2015). High $NADH_{CYT}$ can also limit the rate of glycolysis; this is commonly described as a deficiency in cytosolic NAD^+ that is needed for glycolysis, but because $NADH_{CYT}$ rarely reaches as high as 0.1 (or even 0.01), this effect is probably better described as inhibition of the forward rate of the GAPDH reaction by the product NADH. In dentate granule neurons, MCT inhibition led to higher peaks of $NADH_{CYT}$ as well as smaller dips in glucose, suggesting that the elevated $NADH_{CYT}$ also exerted back pressure on glycolysis, presumably on the GAPDH equilibrium (Díaz-García et al., 2017). Both of these effects suggest that elevated $NADH_{CYT}$ by itself can both affect glucose flux through glycolysis and shunt pyruvate toward lactate and away from utilization for oxphos.

What factors can elevate $NADH_{CYT}$? As discussed, there are three main influences corresponding with the dominant cytosolic NADH dehydrogenases in neurons (Fig. 2 A). First, glycolysis produces NADH at the GAPDH step. This NADH will accumulate in the cell (and ultimately inhibit continued glycolysis) unless it is recycled by one of two recycling routes. The first is LDH: conversion of NADH to NAD^+ coordinately with production of lactate from pyruvate; this is the primary mechanism by which cells can perform glycolysis without oxphos, as it deprives mitochondria of their primary substrate pyruvate. The second recycling route is through malate dehydrogenase, usually as the first step in the MAS. The shuttle is the primary route for moving reducing equivalents across the mitochondrial inner membrane (because NADH itself does not cross).

Some key puzzles to solve

One key unsolved question is how neuronal glycolysis is triggered by neuronal activity. Is it through feedback regulation of the glycolytic enzymes by changes in cytosolic ATP, ADP, and AMP or through feedforward regulation by intracellular Ca^{2+} (and if so, through which of many possible signaling pathways)? Neuronal glycolytic regulation probably does not involve a key regulatory enzyme, PFKFB3 (Herrero-Mendez et al., 2009), but there are many other points of regulation in this ancient metabolic pathway. An additional avenue for glycolytic regulation could involve indirect stimulation through activation of mitochondrial metabolism (itself involving a still unclear combination of feedback through ADP and feedforward through Ca^{2+}).

Another question is a more teleological "why" question: Why do astrocytes have higher cytosolic NADH redox than neurons, and what are the implications? Bolaños (2016) has thoroughly reviewed the causes of elevated astrocyte redox including PFK FB3 regulation of glycolysis and pyruvate dehydrogenase kinase regulation of mitochondrial oxidation. One additional possibility is that like cancer cells, astrocytes perform glycolysis not primarily for energy but rather for anabolic biosynthesis: For instance, astrocytes and not neurons express the phosphoglycerate dehydrogenase involved in serine biosynthesis (Ehmsen et al., 2013), which branches off of the main glycolytic pathway. This conversion of glucose to serine produces twice as much NADH as conversion of glucose to pyruvate (although flux through the first pathway may be smaller). Another likely contributor to elevated cytosolic NADH redox in astrocytes is diminished shuttling of redox equivalents to mitochondria via the MAS. The function of the MAS is antagonized by glutamate oxidation (LaNoue and Williamson, 1971; Du et al., 2013), and there is ample evidence of glutamate oxidation by astrocytes (Hertz et al., 2007; Dienel, 2017).

What are the implications of higher cytosolic NADH redox in astrocytes? The clearest case for glial supply of energy to neurons is in ischemic situations, as shown best for white matter axons that can be supported by glial glycogen in the absence of an external supply of glucose (Brown et al., 2003).

Shuttling of lactate from astrocytes to neurons may be physiologically important as a chronic resting process, but the most

direct evidence argues that upon neuronal stimulation, the classical ANLS hypothesis does not apply and that instead, there is an increase in neuronal glycolysis accompanied by a smaller increase in neuronal oxphos. The best direct evidence on this point is for neuronal cell bodies in both hippocampus and cerebral cortex, and it remains possible that an activity-dependent ANLS could function in specific subcellular compartments during activation; however, at this time, there is more evidence for direct glucose utilization in presynaptic terminals than evidence for lactate uptake. This question about the geography of fuel utilization by neurons is a valuable target for future research.

Finally, why might neurons temporarily allow glycolysis to exceed oxphos in the face of acute energy demand? One speculation is that glycolysis, although it produces a lower yield of ATP than oxphos, provides a faster resupply of energy. Some evolutionary support for this speculation comes from muscle: fast-twitch muscle, requiring the greatest acute supply of energy, tends to be far more glycolytic and to have fewer mitochondria than the slow-twitch muscle used for postural control (Crow and Kushmerick, 1982; Spriet, 1989). Neurons possess many mitochondria and rely chronically on them as a major source of ATP (Hall et al., 2012), but perhaps, like fast-twitch muscle fibers, they use glycolysis together with the formidable and fast phosphocreatine/creatine kinase system to ensure prompt resupply of ATP. There is also evidence that the glycolytic enzymes may be localized right where the neuron most requires acute resupply of ATP: at the plasma membrane and on the synaptic vesicles where major ion pumping is performed by transport ATPases (Ikemoto et al., 2003; Dhar-Chowdhury et al., 2007; Hinckelmann et al., 2016). Certainly glycolysis alone is insufficient to power neuronal ion pumping (Hall et al., 2012), but it may nevertheless play an important role in fast refueling. Further work is needed to test the idea that glycolysis is critical for fast energy resupply.

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References

Ashrafi, G., and T.A. Ryan. 2017. Glucose metabolism in nerve terminals. *Curr. Opin. Neurobiol.* 45:156–161. https://doi.org/10.1016/j.conb.2017.03.007

Ashrafi, G., Z. Wu, R.J. Farrell, and T.A. Ryan. 2017. GLUT4 mobilization supports energetic demands of active synapses. *Neuron.* 93:606–615. https://doi.org/10.1016/j.neuron.2016.12.020

Attwell, D., and S.B. Laughlin. 2001. An energy budget for signaling in the grey matter of the brain. *J. Cereb. Blood Flow Metab.* 21:1133–1145. https://doi.org/10.1097/00004647-200110000-00001

Bak, L.K., and A. Schousboe. 2017. Misconceptions regarding basic thermodynamics and enzyme kinetics have led to erroneous conclusions regarding the metabolic importance of lactate dehydrogenase isoenzyme expression. *J. Neurosci. Res.* 95:2098–2102. https://doi.org/10.1002/jnr.23994

Bak, L.K., and A.B. Walls. 2018. CrossTalk opposing view: lack of evidence supporting an astrocyte-to-neuron lactate shuttle coupling neuronal activity to glucose utilisation in the brain. *J. Physiol.* 596:351–353. https://doi.org/10.1113/JP274945

Bak, L.K., A.B. Walls, A. Schousboe, A. Ring, U. Sonnewald, and H.S. Waagepetersen. 2009. Neuronal glucose but not lactate utilization is positively correlated with NMDA-induced neurotransmission and fluctuations in cytosolic Ca^{2+} levels. *J. Neurochem.* 109(Suppl 1):87–93. https://doi.org/10.1111/j.1471-4159.2009.05943.x

Bak, L.K., L.F. Obel, A.B. Walls, A. Schousboe, S.A.A. Faek, F.S. Jajo, and H.S. Waagepetersen. 2012. Novel model of neuronal bioenergetics: postsynaptic utilization of glucose but not lactate correlates positively with Ca^{2+} signalling in cultured mouse glutamatergic neurons. *ASN Neuro.* 4:e00083. https://doi.org/10.1042/AN20120004

Barros, L.F., and B. Weber. 2018. CrossTalk proposal: an important astrocyte-to-neuron lactate shuttle couples neuronal activity to glucose utilisation in the brain. *J. Physiol.* 596:347–350. https://doi.org/10.1113/JP274944

Bergersen, L., O. Waerhaug, J. Helm, M. Thomas, P. Laake, A.J. Davies, M.C. Wilson, A.P. Halestrap, and O.P. Ottersen. 2001. A novel postsynaptic density protein: the monocarboxylate transporter MCT2 is co-localized with delta-glutamate receptors in postsynaptic densities of parallel fiber-Purkinje cell synapses. *Exp. Brain Res.* 136:523–534. https://doi.org/10.1007/s002210000600

Berndt, N., O. Kann, and H.-G. Holzhütter. 2015. Physiology-based kinetic modeling of neuronal energy metabolism unravels the molecular basis of NAD(P)H fluorescence transients. *J. Cereb. Blood Flow Metab.* 35:1494–1506. https://doi.org/10.1038/jcbfm.2015.70

Bittar, P.G., Y. Charnay, L. Pellerin, C. Bouras, and P.J. Magistretti. 1996. Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. *J. Cereb. Blood Flow Metab.* 16:1079–1089. https://doi.org/10.1097/00004647-199611000-00001

Bittner, C.X., R. Valdebenito, I. Ruminot, A. Loaiza, V. Larenas, T. Sotelo-Hitschfeld, H. Moldenhauer, A. San Martín, R. Gutiérrez, M. Zambrano, and L.F. Barros. 2011. Fast and reversible stimulation of astrocytic glycolysis by K^+ and a delayed and persistent effect of glutamate. *J. Neurosci.* 31:4709–4713. https://doi.org/10.1523/JNEUROSCI.5311-10.2011

Blacker, T.S., Z.F. Mann, J.E. Gale, M. Ziegler, A.J. Bain, G. Szabadkai, and M.R. Duchen. 2014. Separating NADH and NADPH fluorescence in live cells and tissues using FLIM. *Nat. Commun.* 5:3936. https://doi.org/10.1038/ncomms4936

Bolaños, J.P. 2016. Bioenergetics and redox adaptations of astrocytes to neuronal activity. *J. Neurochem.* 139(Suppl 2):115–125. https://doi.org/10.1111/jnc.13486

Bouzier-Sore, A.-K., P. Voisin, V. Bouchaud, E. Bezancon, J.-M. Franconi, and L. Pellerin. 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. https://doi.org/10.1111/j.1460-9568.2006.05056.x

Brennan, A.M., J.A. Connor, and C.W. Shuttleworth. 2006. NAD(P)H fluorescence transients after synaptic activity in brain slices: predominant role of mitochondrial function. *J. Cereb. Blood Flow Metab.* 26:1389–1406. https://doi.org/10.1038/sj.jcbfm.9600292

Brown, A.M., R. Wender, and B.R. Ransom. 2001. Metabolic substrates other than glucose support axon function in central white matter. *J. Neurosci. Res.* 66:839–843. https://doi.org/10.1002/jnr.10081

Brown, A.M., S.B. Tekkök, and B.R. Ransom. 2003. Glycogen regulation and functional role in mouse white matter. *J. Physiol.* 549:501–512. https://doi.org/10.1113/jphysiol.2003.042416

Brown, A.M., R.D. Evans, J. Black, and B.R. Ransom. 2012. Schwann cell glycogen selectively supports myelinated axon function. *Ann. Neurol.* 72:406–418. https://doi.org/10.1002/ana.23607

Cerdán, S., T.B. Rodrigues, A. Sierra, M. Benito, L.L. Fonseca, C.P. Fonseca, and M.L. García-Martín. 2006. The redox switch/redox coupling hypothesis. *Neurochem. Int.* 48:523–530. https://doi.org/10.1016/j.neuint.2005.12.036

Chavan, V., J. Willis, S.K. Walker, H.R. Clark, X. Liu, M.A. Fox, S. Srivastava, and K. Mukherjee. 2015. Central presynaptic terminals are enriched in ATP but the majority lack mitochondria. *PLoS One.* 10:e0125185. https://doi.org/10.1371/journal.pone.0125185

Chih, C.P., P. Lipton, and E.L. Roberts Jr. 2001. Do active cerebral neurons really use lactate rather than glucose? *Trends Neurosci.* 24:573–578. https://doi.org/10.1016/S0166-2236(00)01920-2

Chinchore, Y., T. Begaj, D. Wu, E. Drokhlyansky, and C.L. Cepko. 2017. Glycolytic reliance promotes anabolism in photoreceptors. *eLife.* 6:e25946. https://doi.org/10.7554/eLife.25946

Christofk, H.R., M.G. Vander Heiden, N. Wu, J.M. Asara, and L.C. Cantley. 2008. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature.* 452:181–186. https://doi.org/10.1038/nature06667

Chuquet, J., P. Quilichini, E.A. Nimchinsky, and G. Buzsáki. 2010. Predominant enhancement of glucose uptake in astrocytes versus neurons during activation of the somatosensory cortex. *J. Neurosci.* 30:15298–15303. https://doi.org/10.1523/JNEUROSCI.0762-10.2010

Clarke, D.D., and L. Sokoloff. 1994. Circulation and energy metabolism of the brain. *In* Basic neurochemistry: molecular, cellular and medical aspects. G.J. Siegel, B.W. Agranoff, R.W. Albers, S.K. Fisher, and M.D. Uhler, editors. Lippincott, Williams, and Wilkins, Philadelphia. 637–669.

Compan, V., S. Pierredon, B. Vanderperre, P. Krznar, I. Marchiq, N. Zamboni, J. Pouyssegur, and J.-C. Martinou. 2015. Monitoring Mitochondrial Pyruvate Carrier Activity in Real Time Using a BRET-Based Biosensor: Investigation of the Warburg Effect. *Mol. Cell.* 59:491–501. https://doi.org/10.1016/j.molcel.2015.06.035

Crow, M.T., and M.J. Kushmerick. 1982. Chemical energetics of slow- and fast-twitch muscles of the mouse. *J. Gen. Physiol.* 79:147–166. https://doi.org/10.1085/jgp.79.1.147

Dhar-Chowdhury, P., B. Malester, P. Rajacic, and W.A. Coetzee. 2007. The regulation of ion channels and transporters by glycolytically derived ATP. *Cell. Mol. Life Sci.* 64:3069–3083. https://doi.org/10.1007/s00018-007-7332-3

Díaz-García, C.M., R. Mongeon, C. Lahmann, D. Koveal, H. Zucker, and G. Yellen. 2017. Neuronal Stimulation Triggers Neuronal Glycolysis and Not Lactate Uptake. *Cell Metab.* 26:361–374. https://doi.org/10.1016/j.cmet.2017.06.021

Dienel, G.A. 2012. Brain lactate metabolism: the discoveries and the controversies. *J. Cereb. Blood Flow Metab.* 32:1107–1138. https://doi.org/10.1038/jcbfm.2011.175

Dienel, G.A. 2017. Lack of appropriate stoichiometry: Strong evidence against an energetically important astrocyte-neuron lactate shuttle in brain. *J. Neurosci. Res.* 95:2103–2125. https://doi.org/10.1002/jnr.24015

Dienel, G.A., and N.F. Cruz. 2016. Aerobic glycolysis during brain activation: adrenergic regulation and influence of norepinephrine on astrocytic metabolism. *J. Neurochem.* 138:14–52. https://doi.org/10.1111/jnc.13630

DiNuzzo, M., F. Giove, B. Maraviglia, and S. Mangia. 2013. Glucose metabolism down-regulates the uptake of 6-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose (6-NBDG) mediated by glucose transporter 1 isoform (GLUT1): theory and simulations using the symmetric four-state carrier model. *J. Neurochem.* 125:236–246. https://doi.org/10.1111/jnc.12164

Du, J., W. Cleghorn, L. Contreras, J.D. Linton, G.C.-K. Chan, A.O. Chertov, T. Saheki, V. Govindaraju, M. Sadilek, J. Satrústegui, and J.B. Hurley. 2013. Cytosolic reducing power preserves glutamate in retina. *Proc. Natl. Acad. Sci. USA.* 110:18501–18506. https://doi.org/10.1073/pnas.1311193110

Duchen, M.R. 1992. $Ca^{(2+)}$-dependent changes in the mitochondrial energetics in single dissociated mouse sensory neurons. *Biochem. J.* 283:41–50. https://doi.org/10.1042/bj2830041

Duffy, T.E., D.C. Howse, and F. Plum. 1975. Cerebral energy metabolism during experimental status epilepticus. *J. Neurochem.* 24:925–934. https://doi.org/10.1111/j.1471-4159.1975.tb03657.x

Ehmsen, J.T., T.M. Ma, H. Sason, D. Rosenberg, T. Ogo, S. Furuya, S.H. Snyder, and H. Wolosker. 2013. D-serine in glia and neurons derives from 3-phosphoglycerate dehydrogenase. *J. Neurosci.* 33:12464–12469. https://doi.org/10.1523/JNEUROSCI.4914-12.2013

Fox, P.T., M.E. Raichle, M.A. Mintun, and C. Dence. 1988. Nonoxidative glucose consumption during focal physiologic neural activity. *Science.* 241:462–464. https://doi.org/10.1126/science.3260686

Galeffi, F., K.A. Foster, M.P. Sadgrove, C.J. Beaver, and D.A. Turner. 2007. Lactate uptake contributes to the NAD(P)H biphasic response and tissue oxygen response during synaptic stimulation in area CA1 of rat hippocampal slices. *J. Neurochem.* 103:2449–2461. https://doi.org/10.1111/j.1471-4159.2007.04939.x

Gandhi, G.K., N.F. Cruz, K.K. Ball, and G.A. Dienel. 2009. Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. *J. Neurochem.* 111:522–536. https://doi.org/10.1111/j.1471-4159.2009.06333.x

Gjedde, A., and S. Marrett. 2001. Glycolysis in neurons, not astrocytes, delays oxidative metabolism of human visual cortex during sustained checkerboard stimulation in vivo. *J. Cereb. Blood Flow Metab.* 21:1384–1392. https://doi.org/10.1097/00004647-200112000-00002

Halestrap, A.P., and R.M. Denton. 1975. The specificity and metabolic implications of the inhibition of pyruvate transport in isolated mitochondria and intact tissue preparations by alpha-Cyano-4-hydroxycinnamate and related compounds. *Biochem. J.* 148:97–106. https://doi.org/10.1042/bj1480097

Hall, C.N., M.C. Klein-Flügge, C. Howarth, and D. Attwell. 2012. Oxidative phosphorylation, not glycolysis, powers presynaptic and postsynaptic mechanisms underlying brain information processing. *J. Neurosci.* 32:8940–8951. https://doi.org/10.1523/JNEUROSCI.0026-12.2012

Herrero-Mendez, A., A. Almeida, E. Fernández, C. Maestre, S. Moncada, and J.P. Bolaños. 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. https://doi.org/10.1038/ncb1881

Hertz, L. 2004. The astrocyte-neuron lactate shuttle: a challenge of a challenge. *J. Cereb. Blood Flow Metab.* 24:1241–1248. https://doi.org/10.1097/00004647-200411000-00008

Hertz, L., J. Drejer, and A. Schousboe. 1988. Energy metabolism in glutamatergic neurons, GABAergic neurons and astrocytes in primary cultures. *Neurochem. Res.* 13:605–610. https://doi.org/10.1007/BF00973275

Hertz, L., L. Peng, and G.A. Dienel. 2007. Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J. Cereb. Blood Flow Metab.* 27:219–249. https://doi.org/10.1038/sj.jcbfm.9600343

Hertz, L., M.E. Gibbs, and G.A. Dienel. 2014. Fluxes of lactate into, from, and among gap junction-coupled astrocytes and their interaction with noradrenaline. *Front. Neurosci.* 8:261. https://doi.org/10.3389/fnins.2014.00261

Hinckelmann, M.-V., A. Virlogeux, C. Niehage, C. Poujol, D. Choquet, B. Hoflack, D. Zala, and F. Saudou. 2016. Self-propelling vesicles define glycolysis as the minimal energy machinery for neuronal transport. *Nat. Commun.* 7:13233. https://doi.org/10.1038/ncomms13233

Hu, Y., and G.S. Wilson. 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. https://doi.org/10.1046/j.1471-4159.1997.69041484.x

Hu, H., A. Juvekar, C.A. Lyssiotis, E.C. Lien, J.G. Albeck, D. Oh, G. Varma, Y.P. Hung, S. Ullas, J. Lauring, et al. 2016. Phosphoinositide 3-kinase regulates glycolysis through mobilization of aldolase from the actin cytoskeleton. *Cell.* 164:433–446. https://doi.org/10.1016/j.cell.2015.12.042

Hui, S., J.M. Ghergurovich, R.J. Morscher, C. Jang, X. Teng, W. Lu, L.A. Esparza, T. Reya, L. Zhan, J.Y. Guo, et al. 2017. Glucose feeds the TCA cycle via circulating lactate. *Nature.* 551:115–118. https://doi.org/10.1038/nature24057

Hung, Y.P., J.G. Albeck, M. Tantama, and G. Yellen. 2011. Imaging cytosolic NADH-$NAD(^{+})$ redox state with a genetically encoded fluorescent biosensor. *Cell Metab.* 14:545–554. https://doi.org/10.1016/j.cmet.2011.08.012

Hurley, J.B., K.J. Lindsay, and J. Du. 2015. Glucose, lactate, and shuttling of metabolites in vertebrate retinas. *J. Neurosci. Res.* 93:1079–1092. https://doi.org/10.1002/jnr.23583

Hyder, F., A.B. Patel, A. Gjedde, D.L. Rothman, K.L. Behar, and R.G. Shulman. 2006. Neuronal-glial glucose oxidation and glutamatergic-GABAergic function. *J. Cereb. Blood Flow Metab.* 26:865–877. https://doi.org/10.1038/sj.jcbfm.9600263

Hyder, F., R.K. Fulbright, R.G. Shulman, and D.L. Rothman. 2013. Glutamatergic function in the resting awake human brain is supported by uniformly high oxidative energy. *J. Cereb. Blood Flow Metab.* 33:339–347. https://doi.org/10.1038/jcbfm.2012.207

Icard, P., and H. Lincet. 2012. A global view of the biochemical pathways involved in the regulation of the metabolism of cancer cells. *Biochim. Biophys. Acta.* 1826:423–433. https://doi.org/10.1016/j.bbcan.2012.07.001

Ikemoto, A., D.G. Bole, and T. Ueda. 2003. Glycolysis and glutamate accumulation into synaptic vesicles. Role of glyceraldehyde phosphate dehydrogenase and 3-phosphoglycerate kinase. *J. Biol. Chem.* 278:5929–5940. https://doi.org/10.1074/jbc.M211617200

Itoh, Y., T. Abe, R. Takaoka, and N. Tanahashi. 2004. Fluorometric determination of glucose utilization in neurons in vitro and in vivo. *J. Cereb. Blood Flow Metab.* 24:993–1003. https://doi.org/10.1097/01.WCB.0000127661.07591.DE

Ivannikov, M.V., and G.T. Macleod. 2013. Mitochondrial free Ca^{2+} levels and their effects on energy metabolism in Drosophila motor nerve terminals. *Biophys. J.* 104:2353–2361. https://doi.org/10.1016/j.bpj.2013.03.064

Ivanov, A.I., A.E. Malkov, T. Waseem, M. Mukhtarov, S. Buldakova, O. Gubkina, M. Zilberter, and Y. Zilberter. 2014. Glycolysis and oxidative phosphorylation in neurons and astrocytes during network activity in hippocampal slices. *J. Cereb. Blood Flow Metab.* 34:397–407. https://doi.org/10.1038/jcbfm.2013.222

Jang, S., J.C. Nelson, E.G. Bend, L. Rodríguez-Laureano, F.G. Tueros, L. Cartagenova, K. Underwood, E.M. Jorgensen, and D.A. Colón-Ramos. 2016. Glycolytic enzymes localize to synapses under energy stress to support synaptic function. *Neuron.* 90:278–291. https://doi.org/10.1016/j.neuron.2016.03.011

Kann, O., S. Schuchmann, K. Buchheim, and U. Heinemann. 2003. Coupling of neuronal activity and mitochondrial metabolism as revealed by NAD(P)H fluorescence signals in organotypic hippocampal slice cultures of the rat. *Neuroscience.* 119:87–100. https://doi.org/10.1016/S0306-4522(03)00026-5

Kanow, M.A., M.M. Giarmarco, C.S. Jankowski, K. Tsantilas, A.L. Engel, J. Du, J.D. Linton, C.C. Farnsworth, S.R. Sloat, A. Rountree, et al. 2017. Biochemical adaptations of the retina and retinal pigment epithelium support a metabolic ecosystem in the vertebrate eye. *eLife.* 6:e28899. https://doi.org/10.7554/eLife.28899

Kasischke, K.A., H.D. Vishwasrao, P.J. Fisher, W.R. Zipfel, and W.W. Webb. 2004. Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. *Science.* 305:99–103. https://doi.org/10.1126/science.1096485

Kasischke, K.A., E.M. Lambert, B. Panepento, A. Sun, H.A. Gelbard, R.W. Burgess, T.H. Foster, and M. Nedergaard. 2011. Two-photon NADH imaging exposes boundaries of oxygen diffusion in cortical vascular supply regions. *J. Cereb. Blood Flow Metab.* 31:68–81. https://doi.org/10.1038/jcbfm.2010.158

Kasthuri, N., K.J. Hayworth, D.R. Berger, R.L. Schalek, J.A. Conchello, S. Knowles-Barley, D. Lee, A. Vázquez-Reina, V. Kaynig, T.R. Jones, et al. 2015. Saturated reconstruction of a volume of neocortex. *Cell.* 162:648–661. https://doi.org/10.1016/j.cell.2015.06.054

Koppenol, W.H., P.L. Bounds, and C.V. Dang. 2011. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat. Rev. Cancer.* 11:325–337. https://doi.org/10.1038/nrc3038

Korf, J., and J. de Boer. 1990. Lactography as an approach to monitor glucose metabolism on-line in brain and muscle. *Int. J. Biochem.* 22:1371–1378. https://doi.org/10.1016/0020-711X(90)90225-R

Korogod, N., C.C.H. Petersen, and G.W. Knott. 2015. Ultrastructural analysis of adult mouse neocortex comparing aldehyde perfusion with cryo fixation. *eLife.* 4. https://doi.org/10.7554/eLife.05793

LaNoue, K.F., and J.R. Williamson. 1971. Interrelationships between malate-aspartate shuttle and citric acid cycle in rat heart mitochondria. *Metabolism.* 20:119–140. https://doi.org/10.1016/0026-0495(71)90087-4

Li, Z., K. Okamoto, Y. Hayashi, and M. Sheng. 2004. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell.* 119:873–887. https://doi.org/10.1016/j.cell.2004.11.003

Liberti, M.V., and J.W. Locasale. 2016. The Warburg Effect: How does it benefit cancer cells? *Trends Biochem. Sci.* 41:211–218. https://doi.org/10.1016/j.tibs.2015.12.001

Lindsay, K.J., J. Du, S.R. Sloat, L. Contreras, J.D. Linton, S.J. Turner, M. Sadilek, J. Satrústegui, and J.B. Hurley. 2014. Pyruvate kinase and aspartate-glutamate carrier distributions reveal key metabolic links between neurons and glia in retina. *Proc. Natl. Acad. Sci. USA.* 111:15579–15584. https://doi.org/10.1073/pnas.1412441111

Liotta, A., J. Rösner, C. Huchzermeyer, A. Wojtowicz, O. Kann, D. Schmitz, U. Heinemann, and R. Kovács. 2012. Energy demand of synaptic transmission at the hippocampal Schaffer-collateral synapse. *J. Cereb. Blood Flow Metab.* 32:2076–2083. https://doi.org/10.1038/jcbfm.2012.116

Lucas, S.J., C.B. Michel, V. Marra, J.L. Smalley, M.H. Hennig, B.P. Graham, and I.D. Forsythe. 2018. Glucose and lactate as metabolic constraints on presynaptic transmission at an excitatory synapse. *J. Physiol.* 596:1699–1721. https://doi.org/10.1113/JP275107

Lujan, B., C. Kushmerick, T.D. Banerjee, R.K. Dagda, and R. Renden. 2016. Glycolysis selectively shapes the presynaptic action potential waveform. *J. Neurophysiol.* 116:2523–2540. https://doi.org/10.1152/jn.00629.2016

Lundgaard, I., B. Li, L. Xie, H. Kang, S. Sanggaard, J.D.R. Haswell, W. Sun, S. Goldman, S. Blekot, M. Nielsen, et al. 2015. Direct neuronal glucose uptake heralds activity-dependent increases in cerebral metabolism. *Nat. Commun.* 6:6807. https://doi.org/10.1038/ncomms7807

Mächler, P., M.T. Wyss, M. Elsayed, J. Stobart, R. Gutierrez, A. von Faber-Castell, V. Kaelin, M. Zuend, A. San Martín, I. Romero-Gómez, et al. 2016. *In vivo* evidence for a lactate gradient from astrocytes to neurons. *Cell Metab.* 23:94–102. https://doi.org/10.1016/j.cmet.2015.10.010

Madsen, P.L., N.F. Cruz, L. Sokoloff, and G.A. Dienel. 1999. Cerebral oxygen/glucose ratio is low during sensory stimulation and rises above normal during recovery: excess glucose consumption during stimulation is not accounted for by lactate efflux from or accumulation in brain tissue. *J. Cereb. Blood Flow Metab.* 19:393–400. https://doi.org/10.1097/00004647-199904000-00005

Magistretti, P.J., O. Sorg, N. Yu, J.L. Martin, and L. Pellerin. 1993. Neurotransmitters regulate energy metabolism in astrocytes: implications for the metabolic trafficking between neural cells. *Dev. Neurosci.* 15:306–312. https://doi.org/10.1159/000111349

Mamczur, P., B. Borsuk, J. Paszko, Z. Sas, J. Mozrzymas, J.R. Wiśniewski, A. Gizak, and D. Rakus. 2015. Astrocyte-neuron crosstalk regulates the expression and subcellular localization of carbohydrate metabolism enzymes. *Glia.* 63:328–340. https://doi.org/10.1002/glia.22753

Mangia, S., M. DiNuzzo, F. Giove, A. Carruthers, I.A. Simpson, and S.J. Vannucci. 2011. Response to 'comment on recent modeling studies of astrocyte-neuron metabolic interactions': much ado about nothing. *J. Cereb. Blood Flow Metab.* 31:1346–1353. https://doi.org/10.1038/jcbfm.2011.29

Mazuel, L., J. Blanc, C. Repond, V. Bouchaud, G. Raffard, N. Déglon, G. Bonvento, L. Pellerin, and A.-K. Bouzier-Sore. 2017. A neuronal MCT2 knockdown in the rat somatosensory cortex reduces both the NMR lactate signal and the BOLD response during whisker stimulation. *PLoS One.* 12:e0174990. https://doi.org/10.1371/journal.pone.0174990

McCormack, J.G., A.P. Halestrap, and R.M. Denton. 1990. Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiol. Rev.* 70:391–425. https://doi.org/10.1152/physrev.1990.70.2.391

McKenna, M.C., I.B. Hopkins, and A. Carey. 2001. Alpha-cyano-4-hydroxycinnamate decreases both glucose and lactate metabolism in neurons and astrocytes: implications for lactate as an energy substrate for neurons. *J. Neurosci. Res.* 66:747–754. https://doi.org/10.1002/jnr.10084

Meyer, N., N. Richter, Z. Fan, G. Siemonsmeier, T. Pivneva, P. Jordan, C. Steinhäuser, M. Semtner, C. Nolte, and H. Kettenmann. 2018. Oligodendrocytes in the Mouse Corpus Callosum Maintain Axonal Function by Delivery of Glucose. *Cell Reports.* 22:2383–2394. https://doi.org/10.1016/j.celrep.2018.02.022

Mongeon, R., V. Venkatachalam, and G. Yellen. 2016. Cytosolic NADH-NAD^+ Redox Visualized in Brain Slices by Two-Photon Fluorescence Lifetime Biosensor Imaging. *Antioxid. Redox Signal.* 25:553–563. https://doi.org/10.1089/ars.2015.6593

Mulukutla, B.C., S. Khan, A. Lange, and W.-S. Hu. 2010. Glucose metabolism in mammalian cell culture: new insights for tweaking vintage pathways. *Trends Biotechnol.* 28:476–484. https://doi.org/10.1016/j.tibtech.2010.06.005

Nave, K.-A. 2010. Myelination and support of axonal integrity by glia. *Nature.* 468:244–252. https://doi.org/10.1038/nature09614

Nehlig, A., E. Wittendorp-Rechenmann, and C.D. Lam. 2004. Selective uptake of [^{14}C]2-deoxyglucose by neurons and astrocytes: high-resolution microautoradiographic imaging by cellular 14C-trajectography combined with immunohistochemistry. *J. Cereb. Blood Flow Metab.* 24:1004–1014. https://doi.org/10.1097/01.WCB.0000128533.84196.D8

Nelson, D.L., and M.M. Cox. 2017. Lehninger Principles of Biochemistry. Seventh edition. W. H. Freeman, Houndmills, Basingstoke, New York, NY. 1328 pp.

Nicholls, D.G., and S.J. Ferguson. 2002. Bioenergetics 3. Third edition. Elsevier

Nortley, R., and D. Attwell. 2017. Control of brain energy supply by astrocytes. *Curr. Opin. Neurobiol.* 47:80–85. https://doi.org/10.1016/j.conb.2017.09.012

Ogawa, S., D.W. Tank, R. Menon, J.M. Ellermann, S.G. Kim, H. Merkle, and K. Ugurbil. 1992. Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc. Natl. Acad. Sci. USA.* 89:5951–5955. https://doi.org/10.1073/pnas.89.13.5951

Pardo, B., L. Contreras, A. Serrano, M. Ramos, K. Kobayashi, M. Iijima, T. Saheki, and J. Satrústegui. 2006. Essential role of aralar in the transduction of small Ca^{2+} signals to neuronal mitochondria. *J. Biol. Chem.* 281:1039–1047. https://doi.org/10.1074/jbc.M507270200

Patel, A.B., J.C.K. Lai, G.M.I. Chowdhury, F. Hyder, D.L. Rothman, R.G. Shulman, and K.L. Behar. 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. https://doi.org/10.1073/pnas.1403576111

Pellerin, L., and P.J. Magistretti. 1994. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc. Natl. Acad. Sci. USA.* 91:10625–10629. https://doi.org/10.1073/pnas.91.22.10625

Pellerin, L., and P.J. Magistretti. 1996. Excitatory amino acids stimulate aerobic glycolysis in astrocytes via an activation of the Na^+/K^+ ATPase. *Dev. Neurosci.* 18:336–342. https://doi.org/10.1159/000111426

Pellerin, L., and P.J. Magistretti. 2012. Sweet sixteen for ANLS. *J. Cereb. Blood Flow Metab.* 32:1152–1166. https://doi.org/10.1038/jcbfm.2011.149

Peng, L., X. Zhang, and L. Hertz. 1994. High extracellular potassium concentrations stimulate oxidative metabolism in a glutamatergic neuronal culture and glycolysis in cultured astrocytes but have no stimulatory effect in a GABAergic neuronal culture. *Brain Res.* 663:168–172. https://doi.org/10.1016/0006-8993(94)90475-8

Pierre, K., L. Pellerin, R. Debernardi, B.M. Riederer, and P.J. Magistretti. 2000. Cell-specific localization of monocarboxylate transporters, MCT1 and MCT2, in the adult mouse brain revealed by double immunohistochemical labeling and confocal microscopy. *Neuroscience.* 100:617–627. https://doi.org/10.1016/S0306-4522(00)00294-3

Poitry-Yamate, C.L., S. Poitry, and M. Tsacopoulos. 1995. Lactate released by Müller glial cells is metabolized by photoreceptors from mammalian retina. *J. Neurosci.* 15:5179–5191. https://doi.org/10.1523/JNEUROSCI.15-07-05179.1995

Rangaraju, V., N. Calloway, and T.A. Ryan. 2014. Activity-driven local ATP synthesis is required for synaptic function. *Cell.* 156:825–835. https://doi.org/10.1016/j.cell.2013.12.042

Rolfe, D.F., and G.C. Brown. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* 77:731–758. https://doi.org/10.1152/physrev.1997.77.3.731

Rowlands, B.D., M. Klugmann, and C.D. Rae. 2017. Acetate metabolism does not reflect astrocytic activity, contributes directly to GABA synthesis, and is increased by silent information regulator 1 activation. *J. Neurochem.* 140:903–918. https://doi.org/10.1111/jnc.13916

Saab, A.S., I.D. Tzvetavona, A. Trevisiol, S. Baltan, P. Dibaj, K. Kusch, W. Möbius, B. Goetze, H.M. Jahn, W. Huang, et al. 2016. Oligodendroglial NMDA receptors regulate glucose import and axonal energy metabolism. *Neuron.* 91:119–132. https://doi.org/10.1016/j.neuron.2016.05.016

Salway, J.G. 2004. Metabolism at a glance. Third edition. Wiley-Blackwell

Schurr, A. 2006. Lactate: the ultimate cerebral oxidative energy substrate? *J. Cereb. Blood Flow Metab.* 26:142–152. https://doi.org/10.1038/sj.jcbfm.9600174

Sharma, K., S. Schmitt, C.G. Bergner, S. Tyanova, N. Kannaiyan, N. Manrique-Hoyos, K. Kongi, L. Cantuti, U.-K. Hanisch, M.-A. Philips, et al. 2015. Cell type- and brain region-resolved mouse brain proteome. *Nat. Neurosci.* 18:1819–1831. https://doi.org/10.1038/nn.4160

Shuttleworth, C.W. 2010. Use of NAD(P)H and flavoprotein autofluorescence transients to probe neuron and astrocyte responses to synaptic activation. *Neurochem. Int.* 56:379–386. https://doi.org/10.1016/j.neuint.2009.12.015

Shuttleworth, C.W., A.M. Brennan, and J.A. Connor. 2003. NAD(P)H fluorescence imaging of postsynaptic neuronal activation in murine hippocampal slices. *J. Neurosci.* 23:3196–3208. https://doi.org/10.1523/JNEUROSCI.23-08-03196.2003

Simpson, I.A., A. Carruthers, and S.J. Vannucci. 2007. Supply and demand in cerebral energy metabolism: the role of nutrient transporters. *J. Cereb. Blood Flow Metab.* 27:1766–1791. https://doi.org/10.1038/sj.jcbfm.9600521

Sobieski, C., M.J. Fitzpatrick, and S.J. Mennerick. 2017. Differential presynaptic ATP supply for basal and high-demand transmission. *J. Neurosci.* 37:1888–1899. https://doi.org/10.1523/JNEUROSCI.2712-16.2017

Spriet, L.L. 1989. ATP utilization and provision in fast-twitch skeletal muscle during tetanic contractions. *Am. J. Physiol.* 257:E595–E605.

Sun, T., H. Qiao, P.-Y. Pan, Y. Chen, and Z.-H. Sheng. 2013. Motile axonal mitochondria contribute to the variability of presynaptic strength. *Cell Reports.* 4:413–419. https://doi.org/10.1016/j.celrep.2013.06.040

Sünwoldt, J., B. Bosche, A. Meisel, and P. Mergenthaler. 2017. Neuronal culture microenvironments determine preferences in bioenergetic pathway use. *Front. Mol. Neurosci.* 10:305. https://doi.org/10.3389/fnmol.2017.00305

Suzuki, A., S.A. Stern, O. Bozdagi, G.W. Huntley, R.H. Walker, P.J. Magistretti, and C.M. Alberini. 2011. Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell.* 144:810–823. https://doi.org/10.1016/j.cell.2011.02.018

Vander Heiden, M.G., and R.J. DeBerardinis. 2017. Understanding the intersections between metabolism and cancer biology. *Cell.* 168:657–669. https://doi.org/10.1016/j.cell.2016.12.039

Vander Heiden, M.G., L.C. Cantley, and C.B. Thompson. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 324:1029–1033. https://doi.org/10.1126/science.1160809

Vannucci, S.J., F. Maher, and I.A. Simpson. 1997. Glucose transporter proteins in brain: delivery of glucose to neurons and glia. *Glia.* 21:2–21. https://doi.org/10.1002/(SICI)1098-1136(199709)21:1%3C2::AID-GLIA2%3E3.0.CO;2-C

Volkenhoff, A., A. Weiler, M. Letzel, M. Stehling, C. Klämbt, and S. Schirmeier. 2015. Glial glycolysis is essential for neuronal survival in Drosophila. *Cell Metab.* 22:437–447. https://doi.org/10.1016/j.cmet.2015.07.006

Walz, W., and S. Mukerji. 1988. Lactate production and release in cultured astrocytes. *Neurosci. Lett.* 86:296–300. https://doi.org/10.1016/0304-3940(88)90499-5

Yu, Y., P. Herman, D.L. Rothman, D. Agarwal, and F. Hyder. 2017. Evaluating the gray and white matter energy budgets of human brain function. *J. Cereb. Blood Flow Metab.* https://doi.org/10.1177/0271678X17708691

Zeisel, A., A.B. Muñoz-Manchado, S. Codeluppi, P. Lönnerberg, G. La Manno, A. Juréus, S. Marques, H. Munguba, L. He, C. Betsholtz, et al. 2015. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science.* 347:1138–1142. https://doi.org/10.1126/science.aaa1934

Zhang, Y., K. Chen, S.A. Sloan, M.L. Bennett, A.R. Scholze, S. O'Keeffe, H.P. Phatnani, P. Guarnieri, C. Caneda, N. Ruderisch, 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. https://doi.org/10.1523/JNEUROSCI.1860-14.2014

Zhang, Y., Y. Xue, S. Meng, Y. Luo, J. Liang, J. Li, S. Ai, C. Sun, H. Shen, W. Zhu, et al. 2016. Inhibition of lactate transport erases drug memory and prevents drug relapse. *Biol. Psychiatry.* 79:928–939. https://doi.org/10.1016/j.biopsych.2015.07.007