

MINIREVIEW

Neurons rely on glucose rather than astrocytic lactate during stimulation

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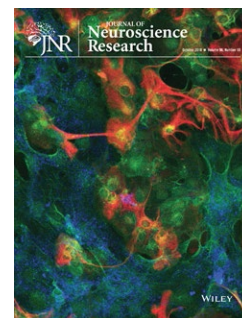
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Abstract

Brain metabolism increases during stimulation, but this increase does not affect all energy metabolism equally. Briefly after stimulation, there is a local increase in cerebral blood flow and in glucose uptake, but a smaller increase in oxygen uptake. This indicates that temporarily the rate of glycolysis is faster than the rate of oxidative metabolism, with a corresponding temporary increase in lactate production. This minireview discusses the long-standing controversy about which cell type, neurons or astrocytes, are involved in this increased aerobic glycolysis. Recent biosensor studies measuring metabolic changes in neurons, in acute brain slices or in vivo, are placed in the context of other data bearing on this question. The most direct measurements indicate that, although both neurons and astrocytes may increase glycolysis after stimulation, neurons do not rely on import of astrocytic-produced lactate, and instead they increase their own glycolytic rate and become net exporters of lactate. This temporary increase in neuronal glycolysis may provide rapid energy to meet the acute energy demands of neurons.

1 | INTRODUCTION

Energy utilization by the brain is both high and highly dynamic. In humans, as much as one-fifth of the total energy is consumed by the brain, a remarkable amount for an organ that accounts for only 2% of the total body mass (Rolfe & Brown, 1997). Additionally, brain metabolism can differ dramatically among circuits, cell-types, and even individual cells according to their moment-to-moment energy demand. For instance, upon stimulation and firing of action potentials, neuronal metabolic burden increases several fold, mainly due to energy demands associated to synaptic transmission (Yu, Herman, Rothman, Agarwal, & Hyder, 2018).¹

It is well established that, in the resting state and on average, the brain consumes glucose and fully oxidizes the resulting glycolytic end products in the mitochondria, as revealed by the nearly stoichiometric (6:1) ratio of O₂ to glucose consumption (Clarke & Sokoloff, 1999; Dalsgaard, 2006; Hyder, Fulbright, Shulman, & Rothman,

2013). Stimulated brain activity induces an increase in cerebral blood flow, which is proportionally matched by glucose utilization in the stimulated area (Fox, Raichle, Mintun, & Dence, 1988; Newberg et al., 2005). Oxygen consumption also increases in stimulated areas of the brain, but surprisingly, not as much as blood flow or glucose consumption. For example, positron emission tomography studies in humans have shown increases of ~50% in blood flow and glucose consumption, but only of ~5% in O₂ consumption (Fox et al., 1988); calibrated functional magnetic resonance imaging estimates a similar value for the increase in both blood flow and glucose metabolism (~45%), but a slightly larger ~16% increase in O₂ consumption (Davis, Kwong, Weisskoff, & Rosen, 1998).²

When glycolysis runs faster than oxidative metabolism, the excess pyruvate and NADH production is managed by the temporary

¹Neurons firing at 1 Hz (a modest rate) in the gray matter of the human brain are calculated to increase their metabolic burden by ~2.7-fold, in order to support processes like restoring the ionic gradients across the cell membrane, neurotransmitter release, vesicle recycling and Ca²⁺ management (Yu, Herman, Rothman, Agarwal, & Hyder, 2018).

²Other studies in the smaller brains of rats (Hyder et al., 1997) found little discrepancy between glucose and oxygen consumption with stimulation. The differences may have to do with the degree of averaging in space and time. If the mismatch between glucose and oxygen metabolism occurs in a small volume of brain but the measurement occurs over a larger volume, focal glucose consumption and lactate production may be balanced by a smaller increase in lactate and oxygen consumption in the neighboring brain areas. Another possible contribution to the difference is that the studies were done in anesthetized animals.

Significance

The brain consumes a large amount of energy daily to support its function, and this energy is derived mainly from controlled digestion of glucose. Using a rapid process called “glycolysis” that emphasizes speed over fuel efficiency, active brain cells can temporarily process glucose to lactate, a high-energy intermediate, and defer the more efficient but slower production of energy from lactate (which requires oxygen). This minireview discusses how specific types of brain cells, neurons, and the surrounding astrocytes, derive energy-on-demand from glucose. Astrocytes are thought to provide important metabolic support for neurons, but neurons may do their own glycolysis when stimulated.

accumulation of lactate (made from the two glycolytic products) (Hertz, Gibbs, & Dienel, 2014)³. Much of the glycolytically derived lactate does not leave the brain, but instead is ultimately consumed by oxidative metabolism in brain cells, probably sometimes in the cells that produced it or their neighbors, and sometimes by somewhat distant cells (Gandhi, Cruz, Ball, & Dienel, 2009; but see Dienel, 2012 on lactate efflux from brain with strong global activation). There is good reason to believe that this oxidative metabolism is often prompt, as oxygen consumption by stimulated brain tissue rises with a similar time course to that of lactate (Hall, Klein-Flügge, Howarth, & Attwell, 2012). Nevertheless, the elevated lactate levels observed upon neuronal stimulation (Cruz, Ball, & Dienel, 2007; Hu & Wilson, 1997; Li & Freeman, 2015) are a clear indication that production of lactate (from glycolysis) is temporarily outstripping its consumption (by oxidative metabolism), a phenomenon known as aerobic glycolysis. This is consistent with the *in vivo* demonstration of the mismatch between glucose and oxygen consumption in the response to stimulation⁴. The temporary uncoupling of glycolysis and oxidative phosphorylation in the active brain may be a fast mechanism to cope with acute increases in energy demand, similar to what occurs when fast-twitch muscle is used intensively (Spriet, 1989). In other words, even though the ATP yield per glucose molecule is lower for glycolysis compared to oxidative metabolism,

glycolysis has a much faster rate of ATP production (Sahlin, Tonkonogi, & Söderlund, 1998).

2 | CELLULAR COMPARTMENTATION OF RAPID AEROBIC GLYCOLYSIS

In which brain cells does the stimulation-induced aerobic glycolysis occur? The brain has two major cell types, neurons and astrocytes, with a division of labor between them. Neurons are almost certainly the site of greatest energy demand (Attwell & Laughlin, 2001; Yu et al., 2018) because their function involves metabolically demanding processes including electrical signaling, managing substantial ion fluxes, and release (and repackaging) of neurotransmitters using synaptic vesicle fusion. But astrocytes also contribute by taking up neurotransmitters and buffering ion fluxes, and they also have substantial direct access to nutrients through their endfeet positioned near blood vessels.

The identity of the cell type that is responsible for rapid aerobic glycolysis during stimulation has been controversial. Although neurons can certainly utilize their own glucose (with some conversion to lactate) when cultured alone (Bak et al., 2009), cultured astrocytes can also metabolize glucose and secrete lactate, particularly upon stimulation, and cultured neurons are capable of oxidizing lactate that is supplied to them exogenously (Magistretti, Sorg, Yu, Martin, & Pellerin, 1993; Pellerin & Magistretti, 1994). The astrocytes are proposed to be stimulated metabolically by the uptake of synaptically released glutamate (Pellerin & Magistretti, 1994) or, in later work, by K^+ ions released by excited neurons (Fernández-Moncada et al., 2018; Ruminot, Schmälzle, Leyton, Barros, & Deitmer, 2017). These observations led to the notion of the “astrocyte-neuron lactate shuttle” (ANLS), whereby stimulated astrocytes supply lactate to neurons as their principal fuel (at least during stimulation).

Much of the work adduced in support of the ANLS has been performed on separately cultured neurons and astrocytes. Some studies on unstimulated cultured neurons (Bouzier-Sore et al., 2006) show preferential labeling of metabolic intermediates from lactate over glucose, when the two metabolites are presented at equal concentrations; however, other studies show preferential labeling from glucose (and a decline in labeling from lactate) when cultured neurons are stimulated (Bak et al., 2009). Even neglecting the likely distortion of metabolism that occurs in prolonged cell culture, as well as the critical dependence on small details of the culture conditions (Sünwoldt, Bosche, Meisel, & Mergenthaler, 2017), these studies on separate neurons and astrocytes support the feasibility of an ANLS; however, they cannot demonstrate the actual direction of transport of lactate between neurons and astrocytes when they are in normal contact in the brain. Incidentally, an opposite proposal of a neuron-to-astrocyte lactate shuttle has been made (Hurley, Lindsay, & Du, 2015; Mangia, Simpson, Vannucci, & Carruthers, 2009); this situation is well-documented in retina, where the radial glia (Müller cells) cannot perform glycolysis normally and rely on photoreceptor-produced lactate (Kanow et al., 2017; Lindsay et al., 2014).

³Few researchers would claim that the energy-rich lactate produced by aerobic glycolysis is a “waste product” (i.e. discarded by the brain), except in extreme circumstances (Duffy, Howse, & Plum, 1975). It is a metabolic intermediate, and indisputably it can be utilized as fuel by reversion to pyruvate and NADH. Most researchers would expect this to occur via the abundant cytosolic lactate dehydrogenase (LDH); others (Schurr, 2018) via a mitochondrial-associated LDH, but the ultimate ability of cells to utilize lactate as oxidative fuel is not in question.

⁴Rabinowitz and colleagues (Hui et al., 2017) have shown that lactate is widely shared between different organs, indicating that in general, glycolysis is not strictly coupled to oxidative metabolism and that lactate is an intermediate reservoir that permits this uncoupling. Interestingly, they found that the brain does not typically share lactate with the circulation, and that instead it uses its own intermediates. But the local accumulation of lactate in the brain with stimulation will nevertheless allow brain cells to exchange lactate with one another, and it also indicates that the rates of glycolysis and oxidative metabolism are temporarily uncoupled, though the magnitude of this uncoupling is uncertain.

Neurons have been shown to lack a key glycolytic regulator, the 6-phosphofructo-2-kinase enzyme PFKFB3 (Herrero-Mendez et al., 2009); the gene is transcribed, but rapid protein degradation results in very low levels in neurons. This has been taken to imply that neuronal glycolysis does not occur, but neurons express ample levels of all of the main glycolytic enzymes (Yellen, 2018) and there are many alternative mechanisms for the regulation of glycolysis (Berg, Tymoczko, & Stryer, 2002).

In vivo tests of lactate and glucose uptake by brain (van Hall et al., 2009; Wyss, Jolivet, Buck, Magistretti, & Weber, 2011) show that the brain as a whole is capable of lactate utilization under conditions of hyperlactemia (likely to occur during intense exercise when muscle exports lactate). Such lactate uptake is also capable of ameliorating neuronal deficits due to insulin-induced hypoglycemia (Wyss et al., 2011), and brain glucose uptake is reduced in hyperlactemia (van Hall et al., 2009; Wyss et al., 2011). Under these conditions, elevated cytosolic NADH:NAD^+ redox will directly inhibit glucose consumption by all cells due to its direct effect on glycolysis. In addition, the ability of the organ to consume either glucose or lactate is not direct evidence of the cellular source (or sink) for lactate, or of the normal course of local lactate production by either astrocytes or neurons under more ordinary metabolic conditions.

We and our colleagues recently addressed the questions of which cell type is performing aerobic glycolysis, and of possible lactate utilization by stimulated neurons, using a fluorescent biosensor of the cytosolic NADH:NAD^+ ratio (NADH_{CYT}) (Díaz-García et al., 2017; Hung, Albeck, Tantama, & Yellen, 2011). Our experiments in both acutely prepared mouse hippocampal slices, and in the cortex of awake mice, demonstrate that NADH_{CYT} exhibits a reliable, transient increase upon neuronal stimulation.

3 | CHANGES OF NADH_{CYT} UPON NEURONAL STIMULATION RESULT FROM GLYCOLYSIS, NOT LACTATE IMPORT

Such an elevation of NADH_{CYT} in stimulated neurons is consistent *either* with direct neuronal glycolysis or with neuronal import of astrocytically produced lactate. To distinguish these possibilities, we used high affinity, specific inhibitors of specific metabolic processes. For instance, we inhibited monocarboxylate transport (MCT) using the compound AR-C155858, which specifically inhibits the plasma membrane transporters MCT1 and MCT2 without inhibiting mitochondrial pyruvate uptake (Compan et al., 2015), unlike the commonly used inhibitor α -cyano-4-hydroxycinnamic acid, which inhibits both processes (Halestrap & Denton, 1975). The equilibrative, bidirectional MCT transporter is required for import or export of lactate by either astrocytes or neurons, and thus plays an essential role in the hypothesized ANLS. The ANLS makes a strong prediction that the neuronal NADH_{CYT} transients revealed by the biosensors should be produced by neuronal lactate import, and that they should disappear (or be strongly

diminished) by MCT inhibition. However, despite control experiments showing that the MCT inhibitor substantially diminished the effect of exogenous lactate, the stimulation-induced NADH transients in neurons were *not* impaired by the inhibitor (Figure 1). Similarly, biosensor monitoring of the intracellular [lactate] in neurons revealed a stimulation-induced lactate transient that was also not diminished by the inhibitor⁵.

With blockade of monocarboxylate transport or of the lactate dehydrogenase enzyme, neuronal NADH_{CYT} transients actually *increased* in size. This is completely inconsistent with a dependence on lactate import, and instead suggests that the source of neuronal NADH during stimulation is neuronal glycolysis, and that neurons are normally *exporters* of lactate when they are stimulated. Thus, inhibition of lactate export via MCTs causes lactate to accumulate in neurons and to exert back-pressure on the LDH reaction, preventing the recycling of NADH to NAD^+ and leading to higher NADH_{CYT} transients. Inhibition of the LDH reaction itself prevents all recycling of glycolytically produced NADH , and the transients are even larger.

One might hypothesize that this evidence of direct neuronal glycolysis, which leads to neuronal NADH and lactate production, is reporting a phenomenon that occurs only under the abnormal conditions of MCT or LDH blockade. In other words, suppose that in unperturbed brain, the ANLS is indeed responsible for the neuronal NADH_{CYT} transients, but that blockade of MCT forces the neurons to find an alternative energy source, and they therefore reluctantly perform glycolysis themselves. However, this idea is inconsistent with the behavior of the glucose concentration in neurons (reported by a different biosensor). Intraneuronal glucose exhibits small dips in concentration whenever neurons are stimulated, consistent with an increased rate of glucose utilization. If neuronal glycolysis is mainly a compensatory phenomenon that occurs when MCTs are inhibited, then these dips should become even more prominent. Instead, though, the dips are *smaller* when MCTs are inhibited, consistent with the idea of increased back-pressure on glycolysis when neuronal NADH_{CYT} levels become too high.

These results (and others in Díaz-García et al., 2017) argue strongly that neuronal NADH_{CYT} signals are produced not by import of astrocytic lactate, but rather by stimulation of neuronal aerobic glycolysis, both in stimulated dentate granule neurons of the hippocampus and in L2/3 neurons of somatosensory barrel cortex (activated by whisker stimulation). Further, these results are consistent with the experimental results of Mazuel et al. (2017) measuring lactate increases in stimulated barrel cortex, with or without knock-down of neuronal MCT2. These experiments showed that prolonged

⁵Barros and Weber (2018) have remarked on the fact that the lactate transient was not increased with MCT inhibition, and taken this as evidence that neurons do not contribute to the accumulation of lactate. However, the reduced dips in glucose with MCT inhibition indicate that the increased NADH_{CYT} exerted back-pressure on glycolysis (as discussed below; this is also the implication of the Mazuel et al. (2017) study discussed below). In addition, the signal-to-noise ratio for the lactate determination is smaller than for NADH_{CYT} , and lactate is a less sensitive measure than NADH_{CYT} . In any case, the failure of the lactate transient to decrease with MCT inhibition is strong evidence against the idea that neuronal lactate is supplied by the ANLS.

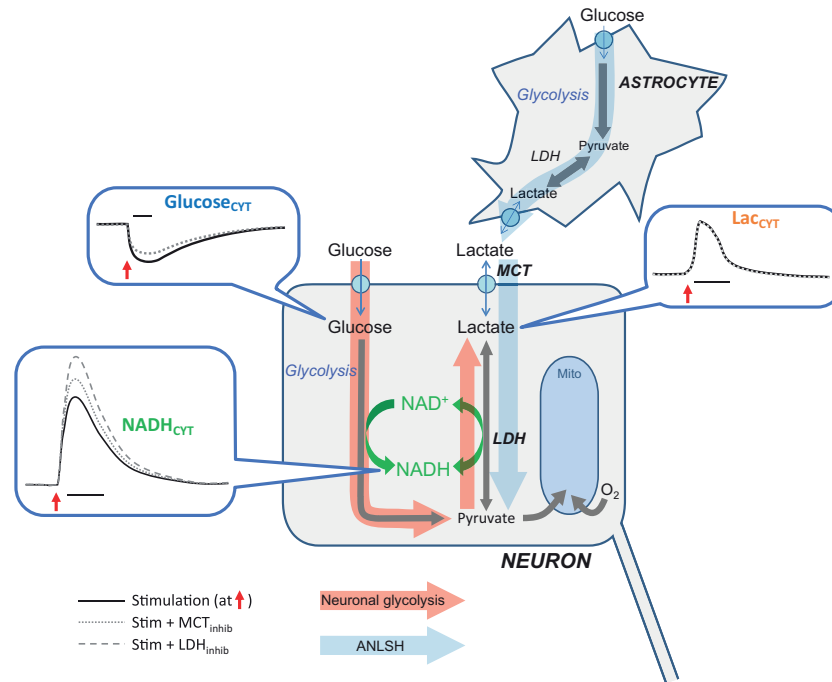


FIGURE 1 Two hypotheses for how neurons respond metabolically to stimulation. One is that stimulated astrocytes produce lactate, which is then utilized by neurons (ANLS hypothesis, blue arrows). The other is that stimulated neurons themselves use glucose to perform glycolysis (red arrows). The insets depict the behavior of the biosensor data from Díaz-García et al. (2017). They show that stimulation leads to an elevation in neuronal NADH_{CYT} and Lac_{CYT} (solid lines); these changes are consistent with both hypotheses. When the monocarboxylate transporter (MCT) is inhibited, however, NADH_{CYT} transients become larger, and Lac_{CYT} transients are preserved. This is inconsistent with the ANLSH, which predicts that they should diminish or disappear. The accumulation of NADH_{CYT} and Lac_{CYT} is instead due to direct neuronal glycolysis. The NADH_{CYT} transient is enhanced because MCT blockade prevents export of lactate that would normally relieve accumulation on the NADH_{CYT} ; the enhancement is even greater with blockade of LDH. The dips in neuronal cytosolic glucose, produced by an increased glycolytic rate, indicate that rather than being upregulated by MCT blockade (and putative deprivation of astrocytic lactate), neuronal glycolysis is diminished by buildup of NADH_{CYT} . Time scale bar = 1 min

stimulation increased the lactate content of barrel cortex, but that this effect was absent when neuronal MCT2 was knocked down. As discussed by the authors, this would be consistent with a neuronal source of lactate (as we hypothesize), but inconsistent with the ANLS-related notion that neurons are instead a sink for lactate (in which case the lactate increases would have become even larger). They also note that inhibition of lactate export from neurons would diminish lactate accumulation by inhibiting glycolysis, as seen in the biosensor studies⁶.

The recent biosensor experiments are the most direct investigation of the cellular site of aerobic glycolysis in stimulated brain, and they argue strongly that stimulated neurons rely not on astrocytically derived lactate but rather on their own ability to perform glycolysis. Further investigation is needed to learn whether similar

results are observed in dendritic or presynaptic compartments of neurons, or in axons, particularly those in white matter that are surrounded by myelin and seem quite likely to rely on surrounding glial cells (oligodendrocytes) for metabolic support (Meyer et al., 2018; Saab et al., 2016; Trevisiol et al., 2017). Moreover, there is evidence that stimulated astrocytes also increase glucose utilization (Chuquet, Quilichini, Nimchinsky, & Buzsáki, 2010; Loaiza, Porras, & Barros, 2003) and NADH_{CYT} (Köhler, Winkler, Sicker, & Hirrlinger, 2018), in support of the possibility of an ANLS. But further experiments are needed to learn whether increased astrocytic glycolysis produces transport of lactate from astrocytes to neurons, or, if instead, both cell types when stimulated export lactate that may then be oxidized by their unstimulated neighbors and/or by themselves at a future time.

4 | POSSIBLE LACTATE SHUTTLING IN RESTING CONDITIONS

An additional question in need of further study is whether there is shuttling of lactate from astrocytes to neurons at rest, when neurons are not stimulated. Biosensor measurements of NADH_{CYT} at

⁶Note, however, that this was not the final conclusion of the Mazuel et al. (2017) paper. The authors dismissed the neuronal source of lactate, because it disagreed with the ANLS. They observed a diminished BOLD signal and concluded that the smaller lactate accumulation was because of diminished neuronal activity. However, the neurons may have been impaired because their glycolysis was inhibited by the inability to export lactate. Alternatively, neuronal activity may not have been impaired, but the BOLD signal used as a proxy for activity might have been diminished because the blockade of lactate movements forced better agreement between glucose utilization and oxygen utilization, and thus a smaller BOLD signal.

rest in neurons and astrocytes (Mongeon, Venkatachalam, & Yellen, 2016) suggest that astrocytes may be more glycolytic than neurons, as judged by elevated NADH_{CYT} levels in astrocytes compared to neurons. However, cytosolic NADH redox is a function not only of the production of NADH by glycolysis but also its recycling by the mitochondrial NADH shuttles (as well as by lactate production). Recent data suggest that these shuttles may be much more active in neurons than in astrocytes: inhibition of malate-aspartate (MAS) and glycerol-phosphate shuttles caused only a small increase in the baseline level of astrocytic NADH_{CYT} (Köhler et al., 2018), in contrast to the much larger effect seen for MAS blockade in neurons (Díaz-García et al., 2017). Thus, even if neuronal glycolysis were very active at rest, the lower resting NADH_{CYT} of neurons may result from increased NADH shuttling.

In vivo biosensor studies in anesthetized mice (Mächler et al., 2016) inferred higher resting lactate levels in astrocytes than in neurons, suggestive of a possible resting flux of lactate from astrocytes to neurons (Barros & Weber, 2018; Mächler et al., 2016; Magistretti & Allaman, 2018). In our studies, we also observed a small decrease in resting neuronal NADH_{CYT} upon the start of MCT inhibition, consistent with a possible contribution of the ANLS to resting neuronal metabolism (Figure 3 of Díaz-García et al., 2017). An alternative explanation is that cells with different redox states may exchange redox equivalents (a “redox switch”) without net carbon transfer, in the form of simultaneous and opposing fluxes of lactate and pyruvate (Cerdán et al., 2006).

5 | CONCLUSION

In the stimulated neurons we have studied, the experimental results rule out a role for lactate import in producing the intracellular neuronal transients of NADH_{CYT} and lactate, and instead point to neuronal glycolysis as the immediate response of neurons to stimulation. Given the observation in exercising muscle that the peak rate of ATP production from glycolysis exceeds that from oxidative phosphorylation (Sahlin et al., 1998)⁷, it may make sense that neuronal glycolysis, rather than the slower oxidative energy production from astrocyte-derived lactate, is the best “first-responder” to the acute energy needs of neurons during activity. In the end, though, it is likely that brain stimulation results in a coordinated increase in all possible sources of metabolic energy, glycolytic and oxidative, and of all cell types, to support the energetically expensive function of this important organ.

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⁷This is true even after adjusting for the lower yield of ATP from glucose than from glycogen.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Both authors contributed to the design and preparation of this review. *Writing – Original Draft*, C.M.D.-G. and G.Y.; *Writing – Review and Editing*, C.M.D.-G. and G.Y.; *Visualization*, G.Y.; *Funding Acquisition*, C.M.D.-G. and G.Y.

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