CROSSTALK

The Journal of Physiology

Comments on CrossTalk 35: an important astrocyte-to-neuron lactate shuttle couples neuronal activity to glucose utilisation in the brain/lack of evidence supporting an astrocyte-to-neuron lactate shuttle coupling neuronal activity to glucose utilisation in the brain

Comments on 'An important astrocyte-to-neuron lactate shuttle couples neuronal activity to glucose utilisation in the brain'

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There is no doubt that glucose is the key energy substrate to fuel the brain's energy demand, and that both neurons and glial cells are capable of taking up and metabolising glucose as well as glycolysis products (lactate). One major problem in the past has been that experimental findings *in vitro* were obtained under rather unphysiological conditions (e.g. using newly differentiated, nearly embryonic cells maintained at exceedingly high glucose concentrations), and that cultured neurons and glial cells lack their functional morphological specialisation (synapses, myelination), which can only be studied in the intact tissue.

Disrupting mitochondrial respiration of Cox10 mutant mice specifically in cortical neurons (Fukui *et al.* 2007; Fünfschilling *et al.* 2012), oligodendrocytes (Fünfschilling *et al.* 2012) and astrocytes (Supplie *et al.* 2017) revealed that, not surprisingly, neurons require mitochondrial ATP generation to survive *in vivo*. In contrast, oligodendrocytes and astrocytes do not depend on mitochondrial respiration to survive as glycolytic cells. Interestingly, the proposed division of labour with a 'glia-to-neuron lactate shuttle' may be old in evolution. This is at least suggested by genetic studies in *Drosophila*, where neurons cannot maintain their ATP demands without neighbouring glial cells providing glycolysis products, but show full survival in the absence of neuronal glycolysis (Volkenhoff *et al.* 2015). Taken together, we feel there is convincing *in vivo* evidence that neurons depend on glial metabolic support, most likely by supplying glycolytic metabolites such as lactate or pyruvate to fuel neuronal mitochondria. At present we would therefore not feel comfortable with the claim that there is a 'lack of evidence supporting' the glia-to-neuron lactate shuttle hypothesis.

References

Fukui H, Diaz F, Garcia S & Moraes CT (2007). Cytochrome c oxidase deficiency in neurons decreases both oxidative stress and amyloid formation in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* **104**, 14163–14168.

Fünfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Mobius W, Diaz F, Meijer D, Suter U, Hamprecht B, Sereda MW, Moraes CT, Frahm J, Goebbels S & Nave KA (2012). Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature* **485**, 517–521.

Supplie LM, Duking T, Campbell G, Diaz F, Moraes CT, Gotz M, Hamprecht B, Boretius S, Mahad D & Nave KA (2017). Respiration-deficient astrocytes survive as glycolytic cells in vivo. *J Neurosci* **37**, 4231–4242.

Volkenhoff A, Weiler A, Letzel M, Stehling M, Klambt C & Schirmeier S (2015). Glial glycolysis is essential for neuronal survival in drosophila. *Cell Metab* **22**, 437–447.

Additional information

Competing interests

None declared.

Comment on 'An important astrocyte-to-neuron lactate shuttle couples neuronal activity to glucose utilisation in the brain'

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It emerges that the proposed hypotheses can only be verified by a quantitative measurement of labelled metabolite fluxes over time. The most obvious opposition to the astrocyte-to-neuron lactate shuttle (Pellerin & Magistretti, 1994) is the magnitude of glutamate released from the presynaptic terminals (nanomolar) with respect to the produced lactate (in the millimolar range). The measurements recently carried out by Trevisiol *et al.* (2017) showed that blockade of lactate metabolism with D-lactate or AR-C155858 (an inhibitor of the 2-monocarboxylate transporter – MCT1) does not influence ATP consumption by the axon (measured by FRET with a probe by Imamura *et al.* (2009)). Moreover, MCT1 was not found in the astrocyte–axon contacts (Lee *et al.* 2012), which stands against astrocyte-to-neuron lactate transfer. Moreover, from two biochemists' point of view, the delivery of a considerable amount of lactate to the axoplasm would require an equimolar amount of NAD^+ to transform it to pyruvate, and a prompt reoxidation of the produced NADH by the malate/aspartate shuttle, which utilises mitochondrial oxaloacetate. Its shortage would be detrimental, as entry of pyruvate into the mitochondrion would produce great amounts of acetyl-coA, which needs an equimolar amount of oxaloacetate. This would require pyruvate carboxylase activity, competing with pyruvate dehydrogenase. In turn, if acetyl-coA accumulates, it would be transformed to ketone bodies, accumulating as β-hydroxybutyrate, due to the excess of cytosolic NADH. Finally, the very few axonal mitochondria (Perge *et al.* 2012) may not sustain such metabolite and pyridine nucleotide fluxes.

References

Imamura H, Nhat KP, Togawa H, Saito K, Iino R, Kato-Yamada Y, Nagai T & Noji H (2009). Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. *Proc Natl Acad Sci USA* **106**, 15651–15656.

Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang P-W, Pellerin L, Magistretti PJ & Rothstein JD (2012). Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* **487**, 443–448.

Pellerin L & Magistretti PJ (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci USA* **91**, 10625–10629.

 DOI: 10.1113/JP274408

Perge JA, Niven JE, Mugnaini E, Balasubramanian V & Sterling P (2012). Why do axons differ in caliber? *J Neurosci* **32**, 626–638.

Trevisiol A, Saab AS, Winkler U, Marx G, Imamura H, Möbius W, Kusch K, Nave K-A & Hirrlinger J (2017). Monitoring ATP dynamics in electrically active white matter tracts. *Elife* **6**, e24241.

Additional information

Competing interests

None declared.

Evidence *versus* biochemical reasons

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In the first part of this CrossTalk, Barros and Weber have presented around 30 studies performed during the last decade, from *in vitro* to *in vivo* with various techniques, that are in favour of a lactate shuttle between astrocytes and neurons. All data referenced in this short review are summarised in Fig. 1, giving a clear overview of the different steps of this intercellular exchange for which evidence was obtained. In the second part of the CrossTalk, Bak and Walls based their review on two recent publications, already commented on by Barros and Weber, and focused their discussion on two key issues. The important key point concerns the fact that neurons express glucose transporter and do metabolise glucose. Indeed, neurons need glucose. But glycolysis is not the only metabolic route for this sugar since it can also follow the pentose phosphate pathway (PPP). Quantification of the PPP is not easy, and it is always underestimated (Bouzier-Sore & Bolanos, 2015), if not forgotten. This pathway is, however, particularly active and necessary for neuroprotection against reactive oxygen species produced during oxidative metabolism (Bolanos *et al.* 1995), which is high in neurons. On the other hand, neuronal glycolysis is maintained at a low level compared to astrocytes and when this pathway is increased in neurons it leads to reduction in the PPP and to neuronal death (Herrero-Mendez *et al.* 2009). Therefore, the presence of GLUT1 on neurons and glucose consumption is compulsory for neuronal survival and not contradictory to the astrocyte-to-neuron lactate shuttle.

References

Bolanos JP, Heales SJ, Land JM & Clark JB (1995). Effect of peroxynitrite on the mitochondrial respiratory chain: differential susceptibility of neurones and astrocytes in primary culture. *J Neurochem* **64**, 1965–1972.

Bouzier-Sore A-K & Bolanos JP (2015). Uncertainties in pentose-phosphate pathway flux assessment underestimate its contribution to neuronal glucose consumption: relevance for neurodegeneration and aging. *Front Aging Neurosci* **7**, 89.

Herrero-Mendez A, Almeida A, Fernandez E, Maestre C, Moncada S & Bolanos JP (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.

Additional information

Competing interests

I declare that I have no competing interest.

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Titres, isoforms and evolution

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Opponents of the astrocyte-to-neuron lactate shuttle (ANLS) hypothesis claim that ANLS supporters rely too strongly on the kinetic properties of isoforms of lactate dehydrogenase and the monocarboxylate transporter (MCT). According to them, the crucial role in release/uptake of lactate by astrocytes and neurons plays the law of mass action, not a simple distribution of proteins among various cells. Apparently, such a 'thermodynamic' interpretation seems to have solid 'hard-science' foundations.

Obviously, differences in metabolite concentrations (and equilibrium constants) are the driving force for any reaction; however, just the affinity of a protein for its substrate decides whether this driving force would be in use or not.

On the other hand, lactate may be transported even against its chemical gradient ('reverse osmosis') if the membrane were to be semipermeable (e.g. because of MCTs' selectivity) and pressure were applied. Interestingly, it has been shown that astrocytes swell (and thus the pressure increases) during neuronal activity (Florence *et al.* 2012).

Cell-specific expression of various protein isoforms has also an evolutionary aspect. There are numerous examples showing that the variety of the isoforms is an adaptation to a cell/tissue function. However, the opponents of the ANLS suggest that the distribution of different protein isoforms is not important and, in fact, random, although the pattern of such a 'random' distribution recurs in many tissues.

If we want to question that isoform distribution is associated with the function of an organ we ought to deliver very strong evidence that the distribution (and kinetic properties of isoforms) has no association with the function of an organ.

Reference

Florence CM, Baillie LD & Mulligan SJ (2012). Dynamic volume changes in astrocytes are an intrinsic phenomenon mediated by bicarbonate ion flux. *PLoS One* **7**, e51124.

Additional information

Competing interests

The authors declare no conflict of interest.

Homeostasis, mass action and cell numbers

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In their debate both parties bring forward valid arguments. Importantly, the existence of the astrocyte-to-neuron lactate shuttle (Pellerin & Magistretti, 1994) is not disputed. The issue of disagreement is the existence of a *unidirectional* flow of lactate from astrocytes to neurons. Let's step back

and consider how homeostatic biochemical systems work. In general, mass action, i.e. the availability of substrates and products, can drive any transport or biochemical reaction in any direction. Theoretically, because neurons can utilise glucose and produce lactate (Prichard *et al.* 1991), it should be also possible that neurons shuttle lactate to astrocytes if neuronal lactate production was sufficiently high. Although this scenario is unlikely, it illustrates the point that the directionality of shuttles depends on mass action. The key question that remains to be answered is whether astrocytes can outpace neurons in glycolytic activity and lactate production in order to permit a directional flow of lactate from astrocytes to neurons. To assess this equilibrium it is important to also consider cell numbers, which has not been considered in the debate. Because each neuron is contacted by multiple astrocytes, the sheer number of astrocytes might just be able to maintain a sufficient lactate gradient to direct a net flux of lactate into neurons (Belanger *et al.* 2011; Herculano-Houzel, 2014). Based on those considerations and because astrocytes via their endfeet have direct access to blood glucose (Belanger *et al.* 2011) it makes at least biological sense to have a *directional net* transport of lactate from astrocytes to neurons.

References

Belanger M, Allaman I & Magistretti PJ (2011). Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab* **14**, 724–738.

Herculano-Houzel S (2014). The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. *Glia* **62**, 1377–1391.

Pellerin L & Magistretti PJ (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci USA* **91**, 10625–10629.

Prichard J, Rothman D, Novotny E, Petroff O, Kuwabara T, Avison M, Howseman A, Hanstock C & Shulman R (1991). Lactate rise detected by ^{1}H NMR in human visual cortex during physiologic stimulation. *Proc Natl Acad Sci USA* **88**, 5829–5831.

Additional information

Competing interests

The author declares that he has no conflicts of interest.

On neuroimaging signals

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After reading this CrossTalk debate, we decided to contribute by expressing our view about the cellular origin of [^{18}F] fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography (PET) imaging. The radiopharmaceutical [^{18}F]FDG is a glucose analogue that is taken up by brain cells, and it is widely used in research and clinical settings for estimating brain glucose metabolism (Leung, 2004; Krell-Roesch *et al.* 2016). The seminal work by Sokollof (Kuhl *et al.* 1977; Reivich *et al.* 1977) provided the basis for the interpretation of [^{18}F]FDG-PET imaging. Based on Sokollof's compartmental model, it has been widely accepted by researchers and physicians that the [^{18}F]FDG-PET signal would serve as a proxy of neuronal activity (Mosconi *et al.* 2010). However, back in 1996, in the first edition of *Molecular Psychiatry*, Magistretti and Pellerin anticipated that astrocytes could significantly contribute to the [^{18}F]FDG-PET signal (Magistretti & Pellerin, 1996), a phenomenon linked to the now well-recognised astrocyte–neuron lactate shuttle. The CrossTalk proposal by Barros and Weber and the opposing view by Bak and Walls share some concepts, and what seems clear is that astrocytes and neurons take up glucose, which is intuitive since they have all the machinery necessary to deal with glucose. So, why would the [^{18}F]FDG-PET signal reflect only neuronal activity? We are of the opinion that astrocytes should be included as an additional compartment in [^{18}F]FDG-PET modelling. Then, a key question arises. Would the [^{18}F]FDG-PET signal reflect neuronal activity during functional activation? It could, but as an 'indirect index' of neuronal activity and as a 'direct indicator' of glucose metabolism in astrocytes. This view is in line with strong evidence demonstrating that during functional activation glucose metabolism is predominantly enhanced in astrocytes, which supports the lactate transfer from astrocytes to neurons (Voutsinos-Porche *et al.* 2003; Chuquet *et al.* 2010; Zimmer *et al.* 2017). This paradigm shift strongly impacts the interpretation of [^{18}F]FDG-PET in human research. For example, [^{18}F]FDG-PET hypometabolism has been interpreted as biomarker of neuronal dysfunction in brain pathologies. Is this true? Again, it could be. However, it could also be a biomarker of astroglial dysfunction. As stated by Barros and Weber, we also look forward to additional data providing quantitative measurements of these metabolic fluxes.

References

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

Krell-Roesch J, Ruider H, Lowe VJ, Stokin GB, Pink A, Roberts RO, Mielke MM, Knopman DS, Christianson TJ, Machulda MM, Jack CR, Petersen RC & Geda YE (2016). FDG-PET and neuropsychiatric symptoms among cognitively normal elderly persons: the mayo clinic study of aging. *J Alzheimers Dis* **53**, 1609–1616.

Kuhl DE, Phelps ME, Hoffman EJ, Robinson GD Jr & MacDonald NS (1977). Initial clinical experience with ^{18}F-2-fluoro-2-deoxy-D-glucose for determination of local cerebral glucose utilization by emission computed tomography. *Acta Neurol Scand Suppl* **64**, 192–193.

Leung K (2004). [^{18}F]Fluoro-2-deoxy-2-D-glucose. In *Molecular Imaging and Contrast Agent Database (MICAD)*. National Center for Biotechnology Information, Bethesda, MD, USA.

Magistretti PJ & Pellerin L (1996). The contribution of astrocytes to the ^{18}F-2-deoxyglucose signal in PET activation studies. *Mol Psychiatry* **1**, 445–452.

Mosconi L, Berti V, Glodzik L, Pupi A, De Santi S & de Leon MJ (2010). Pre-clinical detection of Alzheimer's disease using FDG-PET, with or without amyloid imaging. *J Alzheimers Dis* **20**, 843–854.

Reivich M, Kuhl D, Wolf A, Greenberg J, Phelps M, Ido T, Casella V, Fowler J, Gallagher B, Hoffman E, Alavi A & Sokoloff L (1977). Measurement of local cerebral glucose metabolism in man with ^{18}F-2-fluoro-2-deoxy-D-glucose. *Acta Neurol Scand Suppl* **64**, 190–191.

Voutsinos-Porche B, Bonvento G, Tanaka K, Steiner P, Welker E, Chatton JY, Magistretti PJ & Pellerin L (2003). Glial glutamate transporters mediate a functional metabolic crosstalk between neurons and astrocytes in the mouse developing cortex. *Neuron* **37**, 275–286.

Zimmer ER, Parent MJ, Souza DG, Leuzy A, Lecrux C, Kim HI, Gauthier S, Pellerin L, Hamel E & Rosa-Neto P (2017). [^{18}F]FDG PET signal is driven by astroglial glutamate transport. *Nat Neurosci* **20**, 393–395.

Additional information

Competing interests

None declared.

Direct evidence against astrocyte-to-neuron lactate shuttling during neuronal activation

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Barros and Weber cite several results in our work (Díaz-García *et al.* 2017) as possibly favouring the astrocyte-to-neuron lactate shuttle (ANLS). But they discount the most prominent and direct evidence, which is quite incompatible with neuronal import of lactate during activation, the central feature of the ANLS. We monitored the transient elevation of cytosolic NADH/NAD$^+$ ($NADH_{CYT}$) in stimulated neurons and found that inhibition of monocarboxylate transport (MCT) or lactate dehydrogenase (LDH) did not impair these transients, but instead *increased* them. This shows that both lactate production (via LDH) and export (via MCT) are important outlets for the $NADH_{CYT}$ elevation produced by neuronal glycolysis, and also rules out that neuronal $NADH_{CYT}$ transients depend on lactate *import*.

Similarly, we observed an activity-dependent transient elevation of intracellular [lactate] in neurons, which was not abolished by MCT inhibition. Clearly this elevation arises from neuronal lactate production and not lactate import. Barros and Weber argue that failure of MCT inhibition to *increase* this transient is evidence against export, but the glycolytic rate in neurons is probably self-limited by accumulation of NADH and lactate, as shown by the more sensitive measurement of $NADH_{CYT}$ and by results elsewhere in our paper.

They also argue incorrectly that higher $NADH_{CYT}$ redox in astrocytes than in neurons (Mongeon *et al.* 2016) would make lactate export from neurons impossible. While higher redox should indeed make the [lactate]/[pyruvate] ratio higher in astrocytes, elevated production of pyruvate in neurons could easily result in a higher absolute [lactate] in neurons. Moreover, dispersion of lactate away from the activated region (Cruz *et al.* 2007) should further reduce extracellular [lactate] and facilitate export.

References

Cruz NF, Ball KK & Dienel GA (2007). Functional imaging of focal brain activation in conscious rats: impact of [^{14}C]glucose metabolite spreading and release. *J Neurosci Res* **85**, 3254–3266.

Díaz-García CM, Mongeon R, Lahmann C, Koveal D, Zucker H & Yellen G (2017). Neuronal stimulation triggers neuronal glycolysis and not lactate uptake. *Cell Metab* **26**, 361–374.e4.

Mongeon R, Venkatachalam V & Yellen G (2016). Cytosolic NADH-NAD$^+$ redox visualized in brain slices by two-photon fluorescence lifetime biosensor imaging. *Antioxid Redox Signal* **25**, 553–563.

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Competing interests

None declared.

Astrocyte–neuron lactate shuttling is a minor flux in brain *in vivo*

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Strong evidence *against* astrocyte–neuron lactate (ANL) shuttling (Dienel, 2012, 2017*a*, *b*; Dienel & Cruz, 2016) was not adequately addressed in these CrossTalk discussions. First, quantitative analyses of fates of lactate generated during activation exclude substantial oxidation. Sensory stimulation increased glucose and oxygen utilisation by 50% and 5%, respectively (Fox *et al.* 1988); respiration cannot support oxidation of the lactate from this excess glucose. When oxidised, lactate label is incorporated into amino acids, but this is not the case in activated brain; rapid lactate release predominates. Astrocytes are poised to disperse and discharge lactate because lactate uptake and shuttling among gap junction-coupled astrocytes is 2- to 5-fold *greater* than neuronal uptake and ANL shuttling. High capillary coverage by coupled astrocytic endfeet facilitates lactate release to blood. Lactate contributes <10% to total oxidation in resting human brain, and increased influx from blood is irrelevant to ANL shuttling.

Second, neurons are glucose consumers and lactate producers. Bak *et al.* showed activated cultured neurons choose to oxidise glucose, not lactate. Activated synaptosomes substantially upregulate glycolysis and respiration, and release lactate. Diaz-Garcia *et al.* proved activated neurons are not fuelled by extracellular lactate; they generate and release lactate.

Third, the evidence supporting ANL shuttling has flaws. Mächler *et al.* emphasised their ANL concentration gradient but ignored the equivalent astrocyte–blood gradient. Zimmer *et al.* didn't have cellular resolution, precluding quantification of astrocytic–neuronal energetics (Dienel *et al.* 2017). ANL shuttling was never measured in memory studies, where rescue with supra-pathological lactate doses causes neuronal shutdown. Thus, the importance of ANL shuttling to brain function lacks unequivocal proof.

References

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

Dienel GA (2017*a*). Lack of appropriate stoichiometry: strong evidence against an energetically-important astrocyte-neuron lactate shuttle in brain. *J Neurosci Res* **95**, 2103–2125.

Dienel GA (2017*b*). The metabolic trinity, glucose–glycogen–lactate, links astrocytes and neurons in brain energetics, signaling, memory, and gene expression. *Neurosci Lett* **637**, 18–25.

Dienel GA, Behar KL & Rothman DL (2017). Cellular origin of [^{18}F]FDG-PET imaging signals during ceftriaxone-stimulated glutamate uptake: astrocytes and neurons. *Neuroscientist* (in press; https://doi.org/10.1177/1073858417749375).

Dienel GA & Cruz NF (2016). Aerobic glycolysis during brain activation: adrenergic regulation and influence of norepinephrine on astrocytic metabolism. *J Neurochem* **138**, 14–52.

Fox PT, Raichle ME, Mintun MA & Dence C (1988). Nonoxidative glucose consumption during focal physiologic neural activity. *Science* **241**, 462–464.

Additional information

Competing interests

None.

Is the threshold for astrocyte activation central to the astrocyte neuron lactate shuttle?

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Both sides of the debate are arguing on related but disparate points: Bak and Walls for the idea that neurons readily metabolise glucose and do not exclusively rely on lactate, and Barros and Weber for the idea that there is a 'net' transfer of lactate from astrocytes to neurons. Both ideas can co-exist and the data to date suggest they likely do under certain situations. Focusing on two recent papers in the field using new metabolic Förster resonance energy transfer (FRET) sensors, the existence of a lactate gradient from astrocytes to neurons *in vivo* has been demonstrated under anaesthetised, resting conditions (Mächler, 2016). Other evidence provides a convincing case that an astrocyte-to-neuron lactate shuttle (ANLS) is likely not utilised in slices or *in vivo* during brief, low intensity neural activity (3–10 s) (Díaz-García, 2017). Importantly, each employs specific methodology and essential to the advancement of this field is to determine the exact physiological conditions in which ANLS is utilised or not. The latter paper did not examine astrocyte signals and several lines of evidence suggest astrocytes exhibit an activation threshold to synaptic activity (Paukert, 2014; Institoris et al. 2015; Otsu, 2015; Srinivasan, 2015), which could explain the lack of observable ANLS in some experiments. In our opinion, a 'killer experiment' would entail an astrocyte selective knockdown of LDH combined with metabolic FRET sensors in awake animals, looking at resting state, sensory/motor activity, learning paradigms and startle, only some of which excite astrocytes (Paukert, 2014; Srinivasan, 2015). Such data will provide insight as to which cell-type is the main producer and consumer of lactate in the brain under each specific physiological circumstance.

References

Díaz-García CM, Mongeon R, Lahmann C, Koveal D, Zucker H & Yellen G (2017). Neuronal stimulation triggers neuronal glycolysis and not lactate uptake. *Cell Metab* **26**, 361–374.e4.

Institoris Á, Rosenegger DG & Gordon GR (2015). Arteriole dilation to synaptic activation that is sub-threshold to astrocyte endfoot Ca^{2+} transients. *J Cereb Blood Flow Metab* **35**, 1411–1415.

Mächler P, Wyss MT, Elsayed M, Stobart J, Gutierrez R, von Faber-Castell A, Kaelin V, Zuend M, San Martín A, Romero-Gómez I, Baeza-Lehnert F, Lengacher S, Schneider BL, Aebischer P, Magistretti PJ, Barros LF & Weber B (2016). In vivo evidence for a lactate gradient from astrocytes to neurons. *Cell Metab* **23**, 94–102.

Otsu Y, Couchman K, Lyons DG, Collot M, Agarwal A, Mallet JM, Pfrieger FW, Bergles DE & Charpak S (2015). Calcium dynamics in astrocyte processes during neurovascular coupling. *Nat Neurosci* **18**, 210–218.

Paukert M, Agarwal A, Cha J, Doze VA, Kang JU & Bergles DE (2014). Norepinephrine controls astroglial responsiveness to local circuit activity. *Neuron* **82**, 1263–1270.

Srinivasan R, Huang BS, Venugopal S, Johnston AD, Chai H, Zeng H, Golshani P & Khakh BS (2015). Ca^{2+} signaling in astrocytes from *Ip3r2*$^{-/-}$ mice in brain slices and during startle responses in vivo. *Nat Neurosci* **18**, 708–717.

Additional information

Competing interests

None declared.

Brief comment

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Although the astrocyte-to-neuron lactate shuttle (ANLS) hypothesis was based on the anatomical argument that glucose utilisation in the brain must pass through astrocytes, these cells do not fully cover the vasculature, and neurons can obtain glucose from the interstitial space. Even if astrocytes take up glucose, it can be directly transferred to nearby neurons (Gandhi *et al.* 2009).

Neurons take up more near-infrared 2-deoxyglucose (2DG-IR) than astrocytes (Lundgaard *et al.* 2015). Granted, 2DG-IR uptake might occur via endocytosis after binding to GLUT1, there is still suggested to be a larger density of glucose carriers in neurons than astrocytes, in line with glucose being the primary energy source for neurons. The difference in 2DG-IR uptake between neurons and astrocytes in the wakeful state is almost abolished by anaesthesia, which hampers interpreting of data from anaesthetised animals (Lundgaard *et al.* 2015; Machler *et al.* 2016).

Pyruvate oxidation in the tricarboxylic acid (TCA) cycle increases in astrocytes during stimulation. Furthermore, the astrocytic TCA cycle rate correlates with the glutamate–glutamine cycle rate (Sonnay *et al.* 2018). In awake rats, visual stimulation increases the uptake of [2-^{14}C]acetate, a putative astrocyte-specific substrate, into activated structures (Dienel *et al.* 2007). Thus, the coupling of oxidative metabolism in astrocytes with neuronal activity is in line with astrocytic *consumption* of lactate. Alternatively, the astrocytic TCA cycle rate stimulation might be necessary for the oxidation of glutamate taken up from the synapse, eventually leading to pyruvate/lactate production (Sonnewald, 2014).

In summary, the ANLS is not the only way of fuelling neurons.

References

Gandhi GK, Cruz NF, Ball KK & Dienel GA (2009). Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. *J Neurochem* **111**, 522–536.

Lundgaard I, Li B, Xie L, Kang H, Sanggaard S, Haswell JD, *et al* (2015). Direct neuronal glucose uptake heralds activity-dependent increases in cerebral metabolism. *Nat Commun* **6**, 6807.

Machler P, Wyss MT, Elsayed M, Stobart J, Gutierrez R, von Faber-Castell A, *et al* (2016). In vivo evidence for a lactate gradient from astrocytes to neurons. *Cell Metab* **23**, 94–102.

Sonnay S, Poirot J, Just N, Clerc AC, Gruetter R, Rainer G & Duarte JMN (2018). Astrocytic and neuronal oxidative metabolism are coupled to the rate of glutamate-glutamine cycle in the tree shrew visual cortex. *Glia* **66**, 477–491.

Dienel GA, Schmidt KC & Cruz NF (2007). Astrocyte activation in vivo during graded photic stimulation. *J Neurochem* **103**, 1506–1522.

Sonnewald U (2014). Glutamate synthesis has to be matched by its degradation – where do all the carbons go? *J Neurochem* **131**, 399–406.

Additional information

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The astrocyte-to-neuron lactate shuttle means different things to different neurons

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We have here two opposite views on the astrocyte-to-neuron lactate shuttle (ANLS) hypothesis. Rather than arguing on who is right, it would be more interesting to understand why some populations of neurons use lactate as energy substrate. While different neuronal populations in distinct brain regions utilise lactate to sustain their activity (Bélanger *et al.* 2011; Magistretti & Allaman, 2015; Clasadonte *et al.* 2017), a recent study has demonstrated that two populations of hippocampal neurons identified as consumers and non-consumers of lactate can coexist in the same region (Sada *et al.* 2015), suggesting that the ANLS cannot be generalised to all neurons. Additionally, we have recently shown that astrocyte-derived lactate is used as energy substrate by orexin neurons located in the lateral hypothalamus (Clasadonte *et al.* 2017), and that this process is critical for driving the normal daily cycle of wakefulness. This points to a physiological role for astrocytic lactate, which is more essential than merely as an 'opportunistic' energy source. More effort is needed to investigate the relevance of the ANLS to different brain areas, to better understand the role of these neuroglial metabolic cooperations in the regulation of brain functions under normal and pathological conditions, and to explore why the ANLS pathway is more relevant to a subset of neuronal populations. In my opinion, there is some truth to both sides of the debate with the ANLS being a genuine source of energy substrate for some neurons and an opportunistic pathway in others.

References

Bélanger M, Allaman I & Magistretti PJ (2011). Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab* **14**, 724–738.

Clasadonte J, Scemes E, Wang Z, Boison D & Haydon PG (2017). Connexin 43-mediated astroglial metabolic networks contribute to the regulation of the sleep-wake cycle. *Neuron* **95**, 1365–1380.

Magistretti PJ & Allaman I (2015). A cellular perspective on brain energy metabolism and functional imaging. *Neuron* **86**, 883–901.

Sada N, Lee S, Katsu T, Otsuki T & Inoue T (2015). Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy. *Science* **347**, 1362–1367.

Additional information

Competing interests

None declared.

Neurons utilise glucose for antioxidant rather than energetic purposes

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Glucose conversion to lactate rate in neurons is one-fourth of that in astrocytes (Almeida *et al.* 2004) because the glycolysis-promoting enzyme PFKFB3 is unstable in neurons (Herrero-Mendez *et al.* 2009). This is due to the ubiquitin ligase APC/C-Cdh1 (Herrero-Mendez *et al.* 2009), the activity of which is much higher in neurons than in astrocytes – where PFKFB3 is more stable. Admittedly, neurons are equipped with the full glycolytic machinery; thus, PFKFB3 overexpression is sufficient to convert glucose to lactate, although at half the rate observed in astrocytes (Herrero-Mendez *et al.* 2009). Similarly, glutamate receptor overactivation, which inactivates APC/C-Cdh1, stabilises endogenous PFKFB3 in neurons (Rodriguez-Rodriguez *et al.* 2012). Glycolysis is therefore amenable to regulation in neurons, although in a pathological-like context. Forced expression of PFKFB3 shifts glucose-6-phosphate (G6P) consumption from the pentose-phosphate pathway (PPP) to glycolysis, leaving neurons with a weaker ability to regenerate $NADPH(H^+)$ from $NADP^+$, resulting in impaired glutathione (GSH) regeneration, oxidative damage and neuronal death (Herrero-Mendez *et al.* 2009; Rodriguez-Rodriguez *et al.* 2012). Thus, keeping the glycolytic rate low prevents competition with the PPP for G6P, and promotes neuronal survival. It seems that intracellular glucose metabolism in neurons is therefore adapted to optimise the scarce glucose available likely dictated by the low capacity GLUT3 transporter (Maher *et al.* 1996). Given that, when compared with astrocytes, neurons have a very weak ability to *de novo* synthesise GSH (Jimenez-Blasco *et al.* 2015), regenerating GSH at the expense of metabolising glucose through PPP is critical (Rodriguez-Rodriguez *et al.* 2013). Thus, the oxidation of alternative substrates –such as lactate – by neurons to obtain energy does not seem to be accessorial, but compulsory.

References

Almeida A, Moncada S & Bolaños JP (2004). Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. *Nat Cell Biol* **6**, 45–51.

Herrero-Mendez A, Almeida A, Fernandez E, Maestre C, Moncada S & Bolaños JP (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.

Jimenez-Blasco D, Santofimia-Castaño P, Gonzalez A, Almeida A & Bolaños JP (2015). Astrocyte NMDA receptors activity sustains neuronal survival through a Cdk5-Nrf2 pathway. *Cell Death Differ* **22**, 1877–1889.

Maher F, Davies-Hill TM & Simpson IA (1996). Substrate specificity and kinetic parameters of GLUT3 in rat cerebellar granule neurons. *Biochem J* **315**, 827–831.

Rodriguez-Rodriguez P, Fernandez E, Almeida A & Bolaños JP (2012). Excitotoxic stimulus stabilizes PFKFB3 causing pentose-phosphate pathway to glycolysis shift and neurodegeneration. *Cell Death Differ* **19**, 1582–1589.

Rodriguez-Rodriguez P, Fernandez E & Bolaños JP (2013). Underestimation of the pentose-phosphate pathway in intact primary neurons as revealed by metabolic flux analysis. *J Cerebr Blood Flow Metabol* **33**, 1843–1845.

Additional information

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Nerve terminal glucose phosphorylation remains a significant challenge to astrocyte–neuron lactate shuttling

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Barros and Weber dismiss the evidence of Patel *et al.* (2014) for direct consumption of glucose in neurons, which challenges astrocyte–neuron lactate shuttling. In the Patel study, rats were infused with 2-fluorodeoxyglucose (2FDG) under control and seizure conditions. Glucose phosphorylation in neurons was calculated by measuring in synaptosomes prepared from the rat brains the ratio of 2FDG6P to *N*-acetylaspartate (NAA is found only in neurons). Glucose phosphorylation in synaptosomes was found to increase proportionately with the increase in total brain glucose phosphorylation indicating activity-dependent direct neuronal glucose consumption. Furthermore, the 2FDG6P/NAA ratio was similar in synaptosomes and whole brain suggesting that neuronal glucose phosphorylation could account for the large majority of the functional energetic needs of the neuron. In contrast, if functional glucose phosphorylation went through astrocytes, the 2FDG6P/NAA ratio in synaptosomes would be substantially lower than in whole brain (where 2FDG6P would be trapped in the astrocytes). Barros and Weber argued that the 2FDG6P/NAA ratio might be artificially high, perhaps due to selective NAA loss during synaptosome isolation. However, their explanation is inconsistent with the increase in 2FDG6P/NAA ratio during seizure. Furthermore, expressing 2FDG6P as a ratio with the sum of glutamate and GABA – also enriched in neurons – led to the same finding, arguing against selective loss of NAA. In summary, glucose consumption by neurons can support neuronal oxidation in a 1:1 relationship with glutamate–glutamine cycling (Sibson *et al.* 1998; Patel *et al.* 2004; Rothman *et al.* 2011), and thus, is not compatible with a major role of the astrocyte-to-neuron lactate shuttle.

References

Patel AB, de Graaf RA, Mason GF, Kanamatsu T, Rothman DL, Shulman RG & Behar KL (2004). Glutamatergic neurotransmission and neuronal glucose oxidation are coupled during intense neuronal activation. *J Cereb Blood Flow Metab* **24**, 972–985.

Patel AB, Lai JC, Chowdhury GM, Hyder F, Rothman DL, Shulman RG & Behar KL (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.

Sibson NR, Dhankhar A, Mason GF, Rothman DL, Behar KL & Shulman RG (1998). Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *Proc Natl Acad Sci USA* **95**, 316–321.

Rothman DL, De Feyter HM, de Graaf RA, Mason GF & Behar KL (2011). ^{13}C MRS studies of neuroenergetics and neurotransmitter cycling in humans. *NMR Biomed* **24**, 943–957.

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None.

Eyes wide shut: lack of vision, not evidence

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Barros and Weber collected from the recent literature compelling *in vitro*, *ex vivo* and *in vivo* evidence (from *videre* = see) supporting the concept of a net lactate transfer from astrocytes to neurons. This represents a scholarly overview of the most recent data, with a critical assessment of the few experimental studies that were not consistent with the astrocyte-to-neuron lactate shuttle (ANLS). Although none of the evidence taken separately is sufficient to prove the existence of the ANLS, collectively they form a solid and convincing case in favour of its validity as a model. Despite several attempts in the last 25 years to suggest major flaws and weaknesses in the ANLS model or the experimental evidence that supports it, no serious contender has emerged as a competing model. Bak and Walls pursue this tradition by arguing, based on disputable biochemical arguments and *in vitro* data, that the ANLS model has no substantiated basis. However, they do not provide explanations for several observations that are consistent with the ANLS model. They do not propose any alternative model either. But more surprisingly, they argue that the ANLS biases the interpretation of data. As with any useful scientific model, the ANLS allows us to make testable predictions and serves to explore new hypotheses. The extent of its validity remains to be evaluated in the face of new data (and not fallacious arguments). With the current state of the art, it is preferable to move forward, guided by the light of a solid model, rather than to stay still in the dark.

Additional information

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The author declares no conflict of interest.

Astrocyte-derived lactate is required to support orexinergic neuron activity and to prevent narcolepsy

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I had been listening to the astrocyte–neuron lactate shuttle (ANLS) debate from a distance until the results of a recent experiment made me focus my attention on its importance in the lateral hypothalamus (Clasadonte *et al.* 2017).

Deletion of connexin 43 (Cx43) from astrocytes in the lateral hypothalamus leads to a narcoleptic-like phenotype of excessive daytime sleepiness that is rescued by *in vivo* delivery of lactate. *In situ* brain slice studies, in the presence of physiological glucose, show that in Cx43 KO

mice orexinergic neurons are silent rather than tonically active. Delivery of lactate fully rescues orexinergic neuron activity. Pharmacological studies show that this rescue requires monocarboxylate transporters (MCTs) and lactate dehydrogenase. Thus, this *in vivo* and *in situ* study shows that even in the presence of physiological glucose, lactate is *required* to support neuronal activity and that it is not simply acting as an 'opportunistic' substrate (Dienel, 2012). To ask whether lactate can be delivered from the astrocyte to the neuron we used wild-type slices and performed double patch clamp experiments while depleting glucose from the aCSF. Glucose depletion leads to a loss of neuronal activity which is rescued by extracellular lactate. In glucose-deprived conditions whole cell dialysis of lactate into a single astrocyte caused an MCT-dependent rescue of neuronal activity. This study adds strong evidence in support of the idea that astrocytic lactate is delivered to neurons, and that neuronal lactate utilisation is required, even in the presence of extracellular glucose, and that lactate utilisation is not merely opportunistic.

References

Clasadonte J, Scemes E, Wang Z, Boison D & Haydon PG (2017). Connexin 43-mediated astroglial metabolic networks contribute to the regulation of the sleep-wake cycle. *Neuron* **95**, 1365–1380.

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

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The astrocyte–neuron lactate shuttle: a paradigm shift in brain physiology

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The astrocyte-to-neuron lactate shuttle (ANLS) has revealed that (a) astrocytes play a central role in glucose uptake into the brain and (b) that lactate is a fuel for neuronal activity (Magistretti & Allaman, 2018). Further evidence has shown that lactate is also a signal for neuronal plasticity (Suzuki *et al.* 2011; Yang *et al.* 2014). The discovery of the ANLS and its numerous extensions has provided a novel view of brain energy metabolism validated by dozens of articles published over 20 years by diverse groups confirming the role of astrocytes in glucose uptake into the brain parenchyma, their processing of glucose through aerobic glycolysis and the transfer of the produced lactate from astrocytes to neurons (for references see Baros & Weber, 2018). The challenges to the ANLS mostly came from over-interpretations by some suggesting that all glucose enters astrocytes and all synapses are the site of the ANLS. The ANLS model includes glucose utilisation by neurons under basal conditions and the likely absence of the ANLS at inhibitory synapses (Magistretti & Allaman, 2018). Furthermore the challenges to the ANLS have been mostly theoretical (see as an example Dienel, 2017) and the little experimental evidence against it presents serious methodological issues discussed in Baros & Weber (2018). It is possible that some lactate produced by astrocytes acts as a gliotransmitter on cognate receptors on neurons or may be released into the circulation. It remains that the discovery of the ANLS has brought a paradigm shift in brain physiology placing astrocytes centre stage in brain energy metabolism, a fact that has been validated across species and conditions (see for example Volkenhoff *et al.* 2015).

References

Barros LF & Weber B (2018). CrossTalk proposal: an important astrocyte-to-neuron lactate shuttle couples neuronal activity to glucose utilisation in the brain. *J Physiol* **596**, 347–350.

Dienel GA (2017). Lack of appropriate stoichiometry: strong evidence against an energetically important astrocyte-neuron lactate shuttle in brain. *J Neurosci Res* **95**, 2103–2125.

Magistretti PJ & Allaman I (2018). Lactate in the brain: from metabolic end-product to signalling molecule. *Nat Rev Neurosci* **19**, 235–249.

Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ & Alberini CM (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* **144**, 810–823.

Volkenhoff A, Weiler A, Letzel M, Stehling M, Klämbt C & Schirmeier S (2015). Glial glycolysis is essential for neuronal survival in *Drosophila*. *Cell Metab* **22**, 437–447.

Yang J, Ruchti E, Petit JM, Jourdain P, Grenningloh G, Allaman I & Magistretti PJ (2014). Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proc Natl Acad Sci USA* **111**, 12228–12233.

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Cell-to-cell lactate shuttle in the brain: is it worth debating?

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The astrocyte-neuron lactate shuttle hypothesis has taken over in terms of popularity since Pellerin and Magistretti proposed it in 1994 (Pellerin & Magistretti, 1994). At the time, it was an elegant new concept, which has stimulated groundbreaking developments and fueled almost 25 years of debate. Today, scientists do acknowledge that glucose is the obligatory fuel of the brain, yet they dispute whether neurons or astrocytes take up glucose and which cell type metabolizes glucose to lactate for the other cell's benefit, as effectively reviewed in Barros & Weber (2018) and Bak & Walls (2018). As a matter of fact, a transcellular lactate flux in vivo has never been directly

measured but only inferred on the basis of experimental evidence often full of caveats that are prone to ambiguous interpretation. Our group has contributed to this debate by providing highly relevant measurements of changes of metabolites levels in the human brain during increased neuronal activity (Mangia et al, 2007), not mentioned in the present papers. Despite their unprecedented sensitivity and accuracy, such measures could still not attain the spatial resolution needed to solve the debate. Nevertheless, after embarking on extensive theoretical modeling efforts that put these measurements into the context of compartmentalized metabolism (Mangia et al. 2011), it became apparent to us that the cell-to-cell lactate shuttle (CCLS) in whichever direction does not stand out as a quantitatively predominant mechanism of fuel delivery. Therefore, we genuinely keep wondering whether it is worth insisting that the CCLS must be one way or another and how exactly clarifying this point would advance our understanding of brain function.

References

Bak LK & Walls AB (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.

Barros LF & Weber B (2018). CrossTalk proposal: an important astrocyte-to-neuron lactate shuttle couples neuronal activity to glucose utilisation in the brain. *J Physiol* **596**, 347–350.

Mangia S, DiNuzzo M, Giove F, Carruthers A, Simpson IA & Vannucci SJ (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.

Mangia S, Tkac I, Gruetter R, Van de Moortele PF, Maraviglia B & Ugurbil K (2007). Sustained neuronal activation raises oxidative metabolism to a new steady-state level: evidence from 1H NMR spectroscopy in the human visual cortex. *J Cereb Blood Flow Metab* **27**, 1055–1063.

Pellerin L & Magistretti P.J.(1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci U S A* **91**, 10625–10629.

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Evolutionary conservation of a glia–neuron lactate shuttle suggests it is fundamental to nervous system function

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I read the CrossTalk debate on the existence of an astrocyte–neuron lactate shuttle (ANLS) with great interest. There is no doubt that lactate is produced in the brain and that it can be metabolised by neurons. The unidirectional flux from glia to neuron and its role *in vivo*, however, are debated. Important evidence for an ANLS *in vivo* that has been briefly mentioned by Barros and Weber, but neglected by Bak and Walls, comes from *Drosophila*. As in mammals, *Drosophila* neurons and glia readily take up glucose (Volkenhoff *et al.* 2017). However, glycolysis is dispensable in neurons *in vivo* (Volkenhoff *et al.* 2015), meaning that neurons are functional even when limited to alternative energy sources. In contrast, knockdown of glial glycolysis induces severe neurodegeneration caused by neuronal cell death, and lethality, indicating that metabolic support by lactate-producing glial cells is essential *in vivo* (Volkenhoff *et al.* 2015). Furthermore, neuronal activation leads to elevated neuronal pyruvate consumption *in vivo*, which is not coupled to an increase in the glycolytic rate of these neurons, suggesting substrate input from another source, e.g. the glia (Plaçais *et al.* 2017). Finally, mutants lacking a glial MCT are short-lived and display locomotion deficits, phenotypes that could imply neurodegeneration (Delgado *et al.* 2018). These studies strongly suggest that an essential glia–neuron lactate shuttle exists in *Drosophila in vivo*, supporting the data obtained from mammals. Thus, glia–neuron lactate shuttling seems to be a conserved feature of complex nervous systems, further indicating that it is a fundamental mechanism.

References

Delgado MG, Oliva C, López E, Ibacache A, Galaz A, Delgado R, *et al* (2018). Chaski, a novel *Drosophila* lactate/pyruvate transporter required in glia cells for survival under nutritional stress. *Sci Rep* **8**, 1186.

Plaçais P-Y, de Tredern É, Scheunemann L, Trannoy S, Goguel V, Han K-A, *et al* (2017). Upregulated energy metabolism in the *Drosophila* mushroom body is the trigger for long-term memory. *Nat Commun* **8**, 15510.

Volkenhoff A, Hirrlinger J, Kappel JM, Klämbt C & Schirmeier S (2017). Live imaging using a FRET glucose sensor reveals glucose delivery to all cell types in the *Drosophila* brain. *J Insect Physiol* (in press; https://doi.org/10.1016/j.jinsphys.2017.07.010).

Volkenhoff A, Weiler A, Letzel M, Stehling M, Klämbt C & Schirmeier S (2015). Glial glycolysis is essential for neuronal survival in *Drosophila*. *Cell Metab* **22**, 437–447.

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