CROSSTALK

CrossTalk proposal: an important astrocyte-to-neuron lactate shuttle couples neuronal activity to glucose utilisation in the brain

L. F. Barros[1] and **B. Weber**[2]
[1] *Centro de Estudios Científicos, Valdivia 5110466, Chile*
[2] *Institute of Pharmacology and Toxicology, University of Zurich, and Neuroscience Center Zurich, Zurich CH-8057, Switzerland*

Email: fbarros@cecs.cl

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We will argue here that there is net lactate transfer from astrocytes to neurons and that this transfer is important for brain function. Following CrossTalk guidelines, we will focus on data published over the last decade.

Experiments in cultured cells

The astrocyte-to-neuron lactate shuttle (ANLS) hypothesis was proposed based on glutamate experiments with cultured cells (Pellerin & Magistretti, 1994). Later on, comparative NMR spectroscopy confirmed that cultured astrocytes are more glycolytic than neurons (Bouzier-Sore *et al.* 2006), a metabolic divergence that has later been explained by constitutive inhibition of phosphofructokinase in neurons but not in astrocytes, which diverts the neuronal glucose flux towards the pentose phosphate pathway (Herrero-Mendez *et al.* 2009). Since then, other neuronal signals have been found to be capable, like glutamate, of commanding the production and/or release of lactate by astrocytes, specifically potassium and ammonium (Bittner *et al.* 2011; Choi *et al.* 2012; Lerchundi *et al.* 2015; Sotelo-Hitschfeld *et al.* 2015). In all cases, the findings in culture were confirmed in slices or *in vivo* (see below). In contrast, we could not find any reports of lactate release by neurons in the presence of physiological lactate, either at rest or during electrical stimulation. But to what extent are cells in culture representative of cells *in vivo*? According to transcriptomic analysis of adult brain cells, the metabolic difference between astrocytes and neurons described in culture is also found *in vivo* (Zhang *et al.* 2014), a difference that becomes accentuated if astrocytes and neurons are cultured together and even more so upon induction of neuronal activity (Mamczur *et al.* 2015; Hasel *et al.* 2017).

Glucose flux experiments in brain tissue

Because lactate is made from glucose, the uptake of glucose informs on the question in hand. Measured in tissue slices, Bergmann glia and astrocytes were found to transport and metabolise fluorescent glucose analogues, NBDGs, faster than neighbouring neurons (Barros *et al.* 2009; Jakoby *et al.* 2014). In a separate study *in vivo*, whisker stimulation caused a stronger increase in NBDG accumulation in astrocytes than in neurons of the somatosensory cortex (Chuquet *et al.* 2010). These results are in line with the much higher cytosolic NADH/NAD^+ of hippocampal astrocytes relative to neurons (Mongeon *et al.* 2016), indicative of stronger astrocytic glycolysis, and with the decrease in neuronal cytosolic NADH/NAD^+ after blocking the neuronal monocarboxylate transporter MCT2 (Diaz-Garcia *et al.* 2017). Also in hippocampal slices, astrocytic glucose consumption could be induced by neuronal stimulation, a phenomenon mediated by the sodium/bicarbonate cotransporter NBCe1 (Ruminot *et al.* 2017). Astrocytic uptake of glucose *in vivo* is further supported by an increased fluorodeoxyglucose (FDG) uptake in response to pharmacological stimulation of the astrocytic glutamate transporter GLT-1 (Zimmer *et al.* 2017). Glial support for neuronal energy metabolism was demonstrated in compact white matter (Saab *et al.* 2016). Activity-dependent glutamate release from axons leads to increased oligodendroglial glucose uptake and energetic support of spiking axons in the form of lactate. As it is widely accepted that most glucose metabolised by brain tissue ends up as CO_2 in neurons and not in glial cells, the preferential uptake of glucose by glial cells implies net carbon transfer from astrocytes to neurons in the form of lactate. Furthermore, direct evidence of lactate consumption by orexinergic neurons in the hypothalamus was shown very recently (Clasadonte *et al.* 2017).

A contrasting conclusion was reached from two other studies. In one of them, forebrains of animals injected with FDG were used to prepare nerve terminal vesicles. The radioactivity present in the vesicles was compared with the concentration of the neuronal marker *N*-acetylaspartate (NAA) and found to be similar to that of the starting tissue homogenate (Patel *et al.* 2014). Taken at face value, this similarity would mean that glial cells do not consume any glucose, a rather extreme proposition. Without information about isotope and NAA leakage and degradation

L. Felipe Barros qualified as a Medical Doctor in 1988 and obtained his PhD in Sciences in 1993 at the Universidad de Chile, advised by David Yudilevich. From 1993 to 1996 he was a Wellcome Trust Fellow in Steve Baldwin's lab in Leeds, UK. In 1996 he became Assistant Professor and then Associate Professor at the University of Chile. In 2000, he joined the Centro de Estudios Científicos as Principal Investigator. How can organisms manage to balance the flux of matter and energy? To address this question, his team are developing new molecular tools, capable of measuring metabolic parameters with high spatiotemporal resolution. **Bruno Weber** studied and obtained his PhD in Neuroscience in Zurich, Switzerland. He was a postdoctoral fellow at the Max Planck Institute in Tübingen, Germany and is now a professor at the Institute of Pharmacology and Toxicology at the University of Zurich. His group uses a wide range of imaging tools to study the cell-to-cell communication pathways involved in energy metabolism and information processing in cerebral cortex. He is working on dissecting the interaction of neurons and astrocytes with the vascular system, which is responsible for maintaining adequate delivery of oxygen and energy substrates to the brain. As well as studying these systems, the development of imaging systems for *in vivo* research is an additional research focus of the group.

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during membrane disruption/resealing and prolonged density gradient centrifugation, the meaning of the FDG/NAA ratio does not seem straightforward to us. In the second study, the glucose analogue IR2DG800 was found to preferentially stain neurons over astrocytes (Lundgaard *et al.* 2015) when administered into the cerebrospinal fluid, thus bypassing the physiological and more efficient pathway of glucose entry from the circulation via astrocytic endfeet. Critically, IR2DG800 may not be a transported substrate because of its size (molecular mass 1300 Da), which is larger than the GLUT blocker cytochalasin B (molecular mass 480 Da) and much larger than NBDGs (342 Da). Based on its inhomogeneous subcellular distribution, it was concluded that IR2DG800 probably enters cells by endocytosis (Kovar *et al.* 2009). Because of these technical issues, we are doubtful that these two articles provide compelling evidence against ANLS.

Lactate studies *in vivo*

Given the choice, neurons *in vivo* prefer lactate over glucose, as shown by equicaloric substitution of glucose- by lactate-consumption during intravenous infusion of lactate (Van Hall *et al.* 2009; Wyss *et al.* 2011). A similar conclusion was reached based on the rapid use of tissue lactate upon withdrawal of anaesthesia, which was quantified by NMR spectroscopy to be approx. 5 μM/s (Funfschilling *et al.* 2012). As the metabolism of lactate to CO_2 is strictly coupled to the use of oxygen and most oxygen consumption in brain tissue is neuronal, it follows that in these three studies lactate was oxidised by neurons. But what is the source of lactate for neurons under normal conditions, when blood lactate and tissue lactate are low? Neural activation triggers

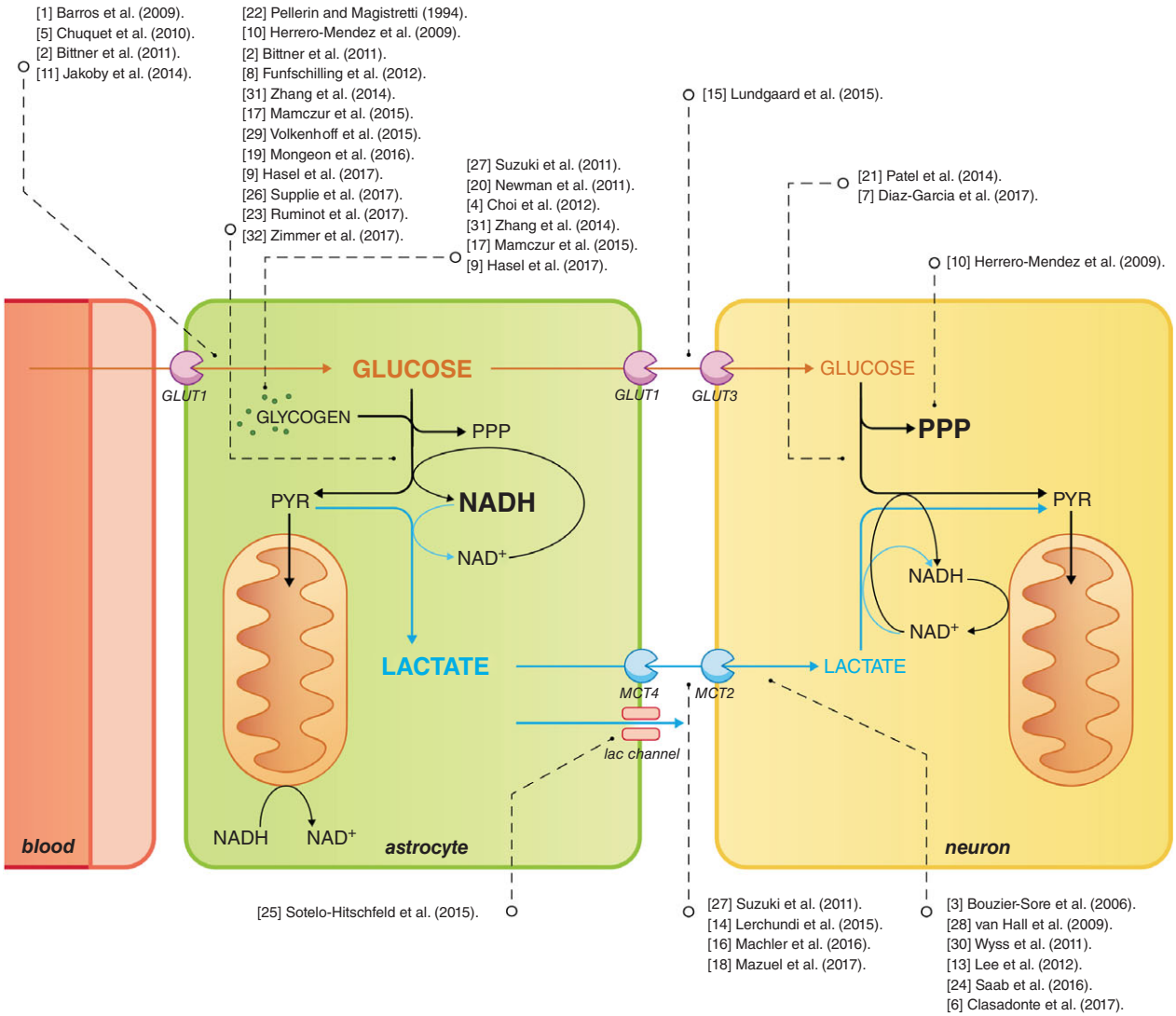

Figure 1. Working model of glucose and lactate exchange between astrocytes and neurons
Dashed arrows connect the relevant pathways with the respective published work. For the sake of simplicity, only the main findings of the papers are considered. Please refer to the main text for details. We apologise to authors of relevant work that had to be omitted because of the maximum 30 references allowed by CrossTalk guidelines. GLUT1, GLUT3: glucose transporters GLUT1 and GLUT3; lac channel: lactate channel; MCT2, MCT4: monocarboxylate transporters MCT2 and MCT4; PPP: pentose phosphate pathway; PYR: pyruvate.

a surge in brain tissue lactate, which is detected in humans and rodents by multiple techniques, including NMR spectroscopy, microdialysis, enzyme-based microprobes and genetically encoded sensors. For example, a memory task caused a rapid increase in interstitial lactate (Newman *et al.* 2011) that, assuming a resting lactate level of 1 mM, may be estimated to be about 10 μM/s. This means that some cells released lactate at a speed commensurate with the rate of glucose consumption of the tissue. Our interpretation is that the lactate was released by astrocytes, which have preferential access to blood-borne glucose and also contain glycogen that may be metabolised to lactate. Moreover, astrocytes maintain high resting levels of intracellular lactate, a dynamic reservoir that can be quickly mobilised upon neuronal demand via a lactate-permeable ion channel gated by extracellular potassium (Sotelo-Hitschfeld *et al.* 2015; Ruminot *et al.* 2017). In contrast, neurons, which are separated from blood glucose by astrocytes and do not possess glycogen stores, are poised to import lactate, as they maintain lower resting lactate levels (Machler *et al.* 2016) and lower NADH/NAD^+ than astrocytes, thus favouring lactate to pyruvate conversion (Mongeon *et al.* 2016). Stimulation of neuronal glycolysis by electrical activity was inferred from a rise in neuronal NADH/NAD^+ that was insensitive to MCT2 blockage, which was interpreted as evidence against ANLS (Diaz-Garcia *et al.* 2017). However, the same study reported a parallel rise in neuronal lactate that was also unaffected by MCT2 blockage, which implies lack of lactate release. Thus, if neurons do not contribute to the activity-dependent interstitial lactate surge, the surge may only come from glial cells. Worthy of note is that neurons remained much more oxidised than resting astrocytes even at the peak of their activity-dependent NADH/NAD^+ rise (Mongeon *et al.* 2016; Diaz-Garcia *et al.* 2017), which also conspires against reversal of pre-stimulation ANLS. Furthermore, astrocytes are likely to become even more reduced during activity, as judged by their NADH/NAD^+ response to high extracellular potassium (Sotelo-Hitschfeld *et al.* 2015).

Genetic and pharmacological evidence

In between the lactate pools of glial cells and neurons are the monocarboxylate transporters (MCTs), which would be redundant were there no intercellular lactate transfer. However several studies have reported perturbation of neuronal function and viability in response to pharmacological or genetic disruption of MCTs in astrocytes, oligodendrocytes or neurons (Newman *et al.* 2011; Suzuki *et al.* 2011; Funfschilling *et al.* 2012; Lee *et al.* 2012; Mazuel *et al.* 2017). Significantly, the deletion of MCTs in glial cells but not in neurons could be rescued by lactate, meaning that maintaining function requires neurons to have access to extracellular lactate. In the same vein, inhibition of glycogen degradation resulted in memory deficits that were also rescued by exogenous lactate (Newman *et al.* 2011; Suzuki *et al.* 2011). Even more dramatic are the effects of genetic inhibition of mitochondrial respiration in mice, which is lethal for neurons and innocuous for astrocytes (Funfschilling *et al.* 2012; Supplie *et al.* 2017), and also the genetic deletion of glycolytic enzymes in fruit fly, which is deleterious in glia but innocuous in neurons (Volkenhoff *et al.* 2015).

In summary, while some of the jurors on ANLS may still be out, we are of the opinion that fresh evidence from numerous laboratories using diverse techniques and experimental models, *in vitro* and *in vivo*, supports an important transfer of lactate from astrocytes and other glial cells to neurons (Fig. 1). We look forward to quantitative measurement of these fluxes and their dependence on brain states in the near future.

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Additional information

Competing interests

None declared.

Author contributions

Both authors have contributed to the conception or design of the work, acquisition or analysis or interpretation of data for the work, and drafting the work or revising it critically for important intellectual content. Both authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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