

## PAST TO FUTURE

## Bioenergetics and redox adaptations of astrocytes to neuronal activity

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Neuronal activity is a high-energy demanding process recruiting all neural cells that adapt their metabolism to sustain the energy and redox balance of neurons. During neurotransmission, synaptic cleft glutamate activates its receptors in neurons and in astrocytes, before being taken up by astrocytes through energy costly transporters. In astrocytes, the energy requirement for glutamate influx is likely to be met by glycolysis. To enable this, astrocytes are constitutively glycolytic, robustly expressing 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), an enzyme that is negligibly present in neurons by continuous degradation because of the ubiquitin-proteasome pathway *via* anaphase-promoting complex/cyclosome (APC)-Cdh1. Additional factors contributing to the glycolytic frame of astrocytes may include 5'-AMP-activated protein kinase (AMPK), hypoxia-inducible factor-1 (HIF-1), pyruvate kinase muscle isoform-2 (PKM2), pyruvate dehydrogenase kinase-4 (PDK4), lactate dehydrogenase-B, or monocarboxylate transporter-4 (MCT4). Neurotransmission-

associated messengers, such as nitric oxide or ammonium, stimulate lactate release from astrocytes. Astrocyte-derived glycolytic lactate thus sustains the energy needs of neurons, which in contrast to astrocytes mainly rely on oxidative phosphorylation. Neuronal activity unavoidably triggers reactive oxygen species, but the antioxidant defense of neurons is weak; hence, they use glucose for oxidation through the pentose-phosphate pathway to preserve the redox status. Furthermore, neural activity is coupled with erythroid-derived erythroid-derived 2-like 2 (Nrf2) mediated transcriptional activation of antioxidant genes in astrocytes, which boost the *de novo* glutathione biosynthesis in neighbor neurons. Thus, the bioenergetics and redox programs of astrocytes are adapted to sustain neuronal activity and survival. Developing therapeutic strategies to interfere with these pathways may be useful to combat neurological diseases.

**Keywords:** AMPK, Cdh1, Glycolysis, GSH, Nrf2, PFKFB3. *J. Neurochem.* (2016) **139** (Suppl. 2), 115–125.

*This article is part of the [60th Anniversary special issue.](#)*

## Neural activity is energy costly

The brain consumes about 20% of inhaled O<sub>2</sub> suggesting a high-energy-demanding tissue. Most of the neural energy expenditure is accounted for by the electrical impulses,

which require continuous re-setting of ion (Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup>) gradients across the plasma membrane of dendrites and axons through primary active transporters, including Na<sup>+</sup>/K<sup>+</sup>- and Ca<sup>2+</sup>-ATPase pumps (Cai and Sheng 2009). Accordingly, it is not unexpected that stimulation of glutamate

Received October 22, 2015; revised manuscript received November 19, 2015; accepted November 23, 2015.

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**Abbreviations used:** 6PG, 6-phosphogluconate; 6PGD, 6-phosphogluconate dehydrogenase; AMPK, AMP-activated protein kinase; ANGS, astrocyte-neuronal glutathione shuttle; ANLS, astrocyte-neuronal lactate shuttle; APC/C, anaphase-promoting complex/cyclosome; ARE, antioxidant response element; Cdk5, cyclin-dependent kinase-5; Cul3, cullin-3; F2,6P<sub>2</sub>, fructose-2,6-bisphosphate; G6PD, glucose-6-phosphate dehy-

drogenase; G6P, glucose-6-phosphate; GCL, glutamate-cysteine ligase; GSH, glutathione; GSSG, oxidized glutathione; Keap1, Kelch-like ECH-associated protein 1; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; MRC, mitochondrial respiratory chain; mTOR, mammalian target of rapamycin; NMDA, *N*-methyl-D-aspartate; NMR, nuclear magnetic resonance; NOS, nitric oxide synthase; Nrf2, nuclear factor (erythroid-derived 2)-like 2; OXPHOS, oxidative phosphorylation; PDK, pyruvate dehydrogenase kinase; PFK1, 6-phosphofructo-1-kinase; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; PKM2, pyruvate kinase muscle-2 isoform; PK, pyruvate kinase; PPP, pentose-phosphate pathway; R5P, ribulose-5-phosphate; ROS, reactive oxygen (and nitrogen) species; TCA, tricarboxylic acid.

receptors in cortical neurons causes rapid ATP consumption (Almeida and Bolaños 2001), which is immediately followed by a rise in mitochondrial O<sub>2</sub> consumption (Thompson *et al.* 2003; Jekabsons and Nicholls 2004; Garcia *et al.* 2005). This suggests that the energy requirements of neurons during activity are met by mitochondrial oxidative phosphorylation (OXPHOS) (Nicholls 2008). Interestingly, prior to the rise in O<sub>2</sub> consumption, there is an increase in intracellular Ca<sup>2+</sup> concentrations (Gleichmann *et al.* 2009), suggesting an early regulatory role for Ca<sup>2+</sup> in the bioenergetics of neurotransmission (Nicholls *et al.* 2007). Furthermore, mitochondrial influx of Ca<sup>2+</sup> alters mitochondrial OXPHOS efficiency; hence, the production of reactive oxygen species (ROS) by this organelle is an inherent phenomenon of neurotransmission (Mattson and Liu 2002).

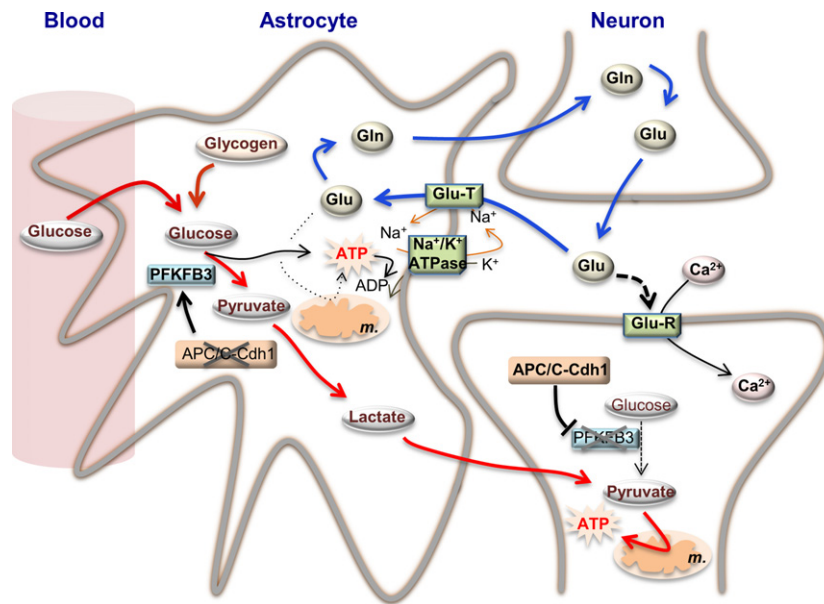
An issue that has remained controversial over decades is the unequivocal identification of the main energetic precursor for neuronal activity. According to *in vivo* studies using functional magnetic resonance imaging and positron emission tomography, glucose is the preferred energy substrate for the brain (Hertz *et al.* 2007). However, the occurrence of a lag between activation of cerebral blood flow and O<sub>2</sub> consumption following neural activity (Fox and Raichle 1986) challenges this notion and lays the foundation for a long-lasting debate (Hertz *et al.* 2007; Pellerin *et al.* 2007; Ivanov *et al.* 2014). The aim of this review was to revisit our current knowledge on the brain's management of the bioenergetics and redox needs that result from neural activity, as well as to identify potential gaps requiring future research.

### Energy management of astrocytes during neural activity

Astrocytes are crucial to the metabolic and structural support of the brain (Parpura *et al.* 1994; Allen and Barres 2009), as they are essential partners in neurotransmission and behavior (Perea *et al.* 2014; Oliveira *et al.* 2015). During glutamatergic neurotransmission, astrocytes efficiently take up neuronal-derived glutamate from the synaptic space through secondary, Na<sup>+</sup>-dependent active transporters. Thus, glutamate uptake is energy-costly for astrocytes, as re-setting the Na<sup>+</sup> gradient across the plasma membrane to the resting levels requires Na<sup>+</sup> pumping by the Na<sup>+</sup>/K<sup>+</sup>-ATPase. The mechanism used by astrocytes to couple glutamate uptake with its energy requirement is currently controversial. Intracellular glutamate may follow at least two metabolic fates, namely conversion into  $\alpha$ -ketoglutarate for oxidation within mitochondria *via* the tricarboxylic acid (TCA) cycle, or conversion into glutamine to be released and taken up by neighbor neurons, which convert it back into glutamate (Fig. 1). Estimations, made on the bases of the energetic efficiencies of substrates, initially concluded that the energy required for glutamate uptake exceeds that provided by the mitochondrial oxidation of glutamate alone (Hertz *et al.*

2007). Considering this, the increase in the rate of glucose uptake (Loaiza *et al.* 2003; Porras *et al.* 2008) coupled to glutamate influx by astrocytes, suggests that invoked glycolysis likely supplements the energy needs of the process, which is part of the so-called astrocyte-neuronal lactate shuttle (Pellerin and Magistretti 1994; Magistretti and Allaman 2015) that will be discussed below. Nonetheless, this issue has been the matter of debate. Comparing the use of glucose or lactate in cortical neurons and astrocytes under resting conditions by <sup>13</sup>C-nuclear magnetic resonance (<sup>13</sup>C-NMR), it was concluded that lactate was a preferential TCA cycle substrate over glucose in neurons, whereas astrocytes oxidized less lactate indicating a less active oxidative metabolism than neurons (Bouzier-Sore *et al.* 2006). This notion has been confirmed in resting cerebellar neurons (Bak *et al.* 2006), albeit glucose – not lactate – utilization *via* TCA cycle oxidation increases upon glutamatergic activity in these cells (Bak *et al.* 2006, 2009, 2012). Therefore, it seems likely that cerebellar and cortical neurons have different intrinsic responses against depolarization. Interestingly, extracellular lactate inhibits glucose usage by astrocytes, an effect that is stronger in resting than in K<sup>+</sup>-stimulated conditions (Sotelo-Hitschfeld *et al.* 2012). This suggests the occurrence of a negative feedback regulatory mechanism of glucose consumption in astrocytes by lactate that diverts glucose utilization from resting to active zones. Moreover, astrocytes store glycogen particularly in areas of high synaptic activity (Pellerin and Magistretti 2012), and glycogen-derived lactate (Pellerin *et al.* 2007) can sustain neuronal activity specially during hypoglycemia (Brown and Ransom 2007; Suh *et al.* 2007) (Fig. 1). Neurons also synthesize glycogen that is continuously degraded (Vilchez *et al.* 2007), the physiological function of which is a matter of investigation (Duran and Guinovart 2015).

Therefore, it seems likely that cerebellar and cortical neurons have different intrinsic responses against depolarization. (Bittner *et al.* 2011). However, the nature of the functional coupling between glycolysis and Na<sup>+</sup>/K<sup>+</sup>-ATPase may not be energetic (Fernandez-Moncada and Barros 2014). The effect of glutamate on glycolysis is delayed and persistent, in contrast to the effect that is observed using K<sup>+</sup> at concentrations compatible with those during glutamatergic neurotransmission, which stimulates glycolysis rapidly and reversibly (Bittner *et al.* 2011). Ammonium (NH<sub>4</sub><sup>+</sup>), which is produced stoichiometrically with glutamate, can rapidly activate lactate release from astrocytes, as can glutamate (Lerchundi *et al.* 2015); however, this effect is not coupled with glycolysis but with inhibition of mitochondrial uptake of pyruvate. Thus, the long-lasting effect of glutamate (Bittner *et al.* 2011) suggests the action of adaptive metabolic mechanisms of astrocytes to neurotransmission, which will be discussed below. The mechanisms responsible for the short-term effects on glycolysis and lactate release triggered by K<sup>+</sup> and NH<sub>4</sub><sup>+</sup> deserve further investigation.



**Fig. 1** Bioenergetics adaptations of astrocytes to neurotransmission. Synaptic cleft glutamate (Glu) released by the pre-synaptic neuron acts on glutamate receptors (Glu-R) placed in the post-synaptic neurons, triggering the influx of  $\text{Ca}^{2+}$  (and  $\text{Na}^+$ , not indicated) and causing plasma membrane depolarization, which is needed to propagate the nervous impulse (neuronal activity). To reset basal levels of glutamate in the synaptic cleft, it is taken up by astrocytes through glutamate transporters (Glu-T), which require  $\text{Na}^+$  uptake. Astrocytes convert glutamate into glutamine (Gln), which is released and then taken up by neurons, which convert it back into glutamate (blue arrowed lines). The  $\text{Na}^+/\text{K}^+$ -ATPase hydrolyses ATP to conserve its energy to reset  $\text{Na}^+$  (and  $\text{K}^+$ ) homeostasis. Such ATP can be obtained by glutamate oxidation in the tricarboxylic acid cycle in the mitochondria (*m*). However, strong evidence indicates that glutamate uptake is coupled, via a yet unknown mechanism, with glucose conversion into lactate through the glycolytic pathway (red arrowed lines). Neuronal activity is also coupled with glucose uptake from the blood, and with glycogen conversion into lactate. As neuronal activity-associated glycolytic

activation in astrocytes is coupled with stoichiometric lactate release, it is likely that glycolytic-derived ATP would be in charge of sustaining  $\text{Na}^+/\text{K}^+$  homeostasis during glutamate uptake. Astrocytes are genetically adapted to support a constitutive glycolytic phenotype in view of their low activity of APC/C-Cdh1 (anaphase-promoting complex/cyclosome/Cdh1), the E3 ubiquitin ligase responsible for the degradation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), a pro-glycolytic enzyme. In contrast to astrocytes, neurons express high APC/C-Cdh1 activity hence continuously degrading PFKFB3 causing their low glycolytic phenotype. Thus, neurons need an energy source different from glucose. Evidence obtained from cultured cells and *in vivo* strongly suggest that astrocyte-released lactate support neuronal functions, both serving as a fuel and, possibly, as a signaling molecule. Thus, astrocytes are metabolically adapted to sustain the bioenergetics status of neurons during neural activity. The stoichiometry of the reactions has been omitted for clarity. Likewise, additional factors involved in these adaptations could not be depicted herein and can be found in the main text.

### Is OXPHOS dispensable for energy generation in astrocytes?

Nitric oxide ( $\text{NO}$ ) is a neural messenger (Garthwaite *et al.* 1988) that is formed by neurons following glutamatergic neurotransmission. Experiments performed in astrocytes revealed the inhibition of the mitochondrial respiratory chain (MRC) by a  $\text{NO}$  synthase-mediated mechanism (Bolaños *et al.* 1994). The most susceptible component of the MRC was found to be cytochrome *c* oxidase (complex IV) (Bolaños *et al.* 1994). Subsequent studies confirmed this effect and revealed such inhibition to be reversible (Brown and Cooper 1994; Cleeter *et al.* 1994; Schweizer and Richter 1994), occurring by competition with  $\text{O}_2$  (Brown and Cooper 1994). During endogenous  $\text{NO}$  production inhibiting the

MRC at cytochrome *c* oxidase in astrocytes,  $\sim 90\text{--}100\%$  of the glucose they consumed was released as lactate, and cells remained alive (Bolaños *et al.* 1994). These results suggest that OXPHOS inhibition invokes glycolysis as a survival pathway in astrocytes. In fact, endothelial cells-derived  $\text{NO}$  activates glycolytic lactate from neighbor astrocytes through hypoxia-inducible factor-1 (HIF-1)-mediated induction of glycolytic enzymes and monocarboxylate transporter-4 expression (Brix *et al.* 2012). Glycolytic ATP drives the reverse ATP synthase reaction, which pumps  $\text{H}^+$  into the intermembrane space, thus sustaining the mitochondrial membrane potential ( $\Delta\Psi_m$ ) (Beltran *et al.* 2000; Almeida *et al.* 2001). As long as glycolytic ATP sustains  $\Delta\Psi_m$  astrocytes avoid apoptosis (Almeida *et al.* 2001), confirming that glycolysis is a survival pathway (Bolaños *et al.* 2010).

Neurotransmission-associated  $\text{NH}_4^+$  formation acidifies the mitochondrial matrix in astrocytes leading to inhibition of pyruvate uptake (Lerchundi *et al.* 2015), which likely leads to TCA cycle impairment and mitochondrial energy stress, thus, contributing to glycolytic activation. Therefore, it is tempting to speculate that glycolysis is essential in astrocytes whereas OXPHOS is dispensable for the generation of energy associated with neurotransmission.

In contrast to astrocytes, neurons are highly vulnerable to the inhibition of the MRC. Incubation of neurons and astrocytes with  $\text{NO}$  or its derivative peroxynitrite anion causing identical degree permanent inhibition of cytochrome *c* oxidase and mitochondrial respiration in both cell types dramatically triggered neuronal death, whereas astrocytes remained intact (Bolaños *et al.* 1995; Almeida *et al.* 2001). Interestingly, under these conditions, neurons were unable to up-regulate glycolysis, and neuronal apoptosis was preceded by  $\Delta\Psi_m$  collapse and ATP depletion (Almeida *et al.* 2001). Furthermore, these features were mimicked by stimulation of glutamate receptors, which immediately caused ATP decline (Almeida and Bolaños 2001; Gleichmann *et al.* 2009) that did not result in a concomitant up-regulation of glucose uptake (Porras *et al.* 2004) or glycolytic activation (Delgado-Esteban *et al.* 2000). Thus, during neuronal activity, neurons – in contrast to astrocytes – are tightly dependent on OXPHOS for energy generation and survival.

### Astrocytes are constitutively glycolytic

Glycolysis is mainly regulated by the enzymatic activities of hexokinase, 6-phosphofructo-1-kinase (PFK1), and pyruvate kinase (PK). In resting astrocytes, PFK1 specific activity is ~fourfold that found in resting neurons, and the levels of PFK1 positive effector, fructose-2,6-bisphosphate ( $\text{F2,6P}_2$ ) is twofold (Almeida *et al.* 2004). There are four isoforms of the enzyme responsible for the synthesis of  $\text{F2,6P}_2$ , 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB). However, isoform 3 (PFKFB3) is, by far, the most abundant one found in astrocytes (Almeida *et al.* 2004). Interestingly, PFKFB3 has a high, ~700-fold kinase versus bisphosphatase ratio (Pilkis *et al.* 1995). Hence, PFKFB3 levels are directly proportional to  $\text{F2,6P}_2$  synthesizing activity. PFKFB3 knockdown abolishes the ability of astrocytes to up-regulate glycolysis upon mitochondrial inhibition, suggesting that PFKFB3 is necessary and sufficient to maintain the glycolytic phenotype of astrocytes (Almeida *et al.* 2004) (Fig. 1).

The transcriptome of the brain has been analyzed at single-cell level by RNA-sequencing, and it was found that PFKFB3, PK muscle-2 isoform, lactate dehydrogenase B-isoform (LDHB), and pyruvate dehydrogenase kinase 4-isoform (PDK4) were highly expressed in astrocytes when compared with neurons (Zhang *et al.* 2014). This confirms previous findings on PFKFB3 levels and activity (Almeida

*et al.* 2004), strengthening the notion that astrocytes are constitutively glycolytic (at the light of PFKFB3, PK muscle-2 isoform, and LDHB mRNA levels) and independent on the OXPHOS (as judged by the high mRNA levels of pyruvate dehydrogenase kinase 4-isoform, which phosphorylates and inhibits pyruvate dehydrogenase). In another study, the isoforms of LDH were shown to be differentially expressed in neurons and astrocytes, with neurons preferentially expressing LDH1 (mainly pyruvate-producing tissues) and astrocytes expressing LDH5 (associated with high lactate-producing tissues) (Pellerin *et al.* 1998). Furthermore, astrocytes were shown to express the monocarboxylate transporters-1 and -4 (MCT1 and MCT4), which are responsible for lactate efflux, whereas neurons express MCT2, which is specialized for lactate influx (Pierre and Pellerin 2005). These data, taken together, support the notion that astrocytes continuously produce lactate through glycolysis and explain the presence of an intracellular lactate reservoir in these cells (Sotelo-Hitschfeld *et al.* 2015), which is released through a channel upon extracellular rise in  $\text{K}^+$  or depolarization (Sotelo-Hitschfeld *et al.* 2015).

Upon cellular energy stress, 5'-AMP concentrations increase and facilitate activation 5'-AMP-activated protein kinase (AMPK) by phosphorylation of a Thr<sup>172</sup> residue on its catalytic  $\alpha 1$  subunit by the liver kinase-1 (Sanders *et al.* 2007), which is a protein kinase with tumor suppression activity (Partanen *et al.* 2009). Active AMPK catalyzes the phosphorylation of several key metabolic regulatory enzymes, including PFKFB (Lage *et al.* 2008). In astrocytes, AMPK becomes phosphorylated,  $\text{F2,6P}_2$  levels elevated, and glycolysis activated within minutes of the inhibition of mitochondrial respiration by  $\text{NO}$  or cyanide (Almeida *et al.* 2004). Knockdown of the  $\alpha 1$  subunit of AMPK, or PFKFB3, abolishes  $\text{F2,6P}_2$  elevation and glycolysis activation (Almeida *et al.* 2004). Thus, energy stress up-regulates glycolysis through AMPK-mediated activation of PFKFB3 in astrocytes. There is a likely possibility that the glycolytic activation caused by glutamate uptake (Pellerin and Magistretti 1994) occurs through this AMPK-PFKFB3 pathway, as suggested before (Ronnelt *et al.* 2009), but this needs to be directly elucidated. Interestingly, the ATP/AMP ratio, as well as  $\text{O}_2$  levels and nutrients, such as glucose and amino acids, regulate the mammalian target of rapamycin (mTOR) (Wullschleger *et al.* 2006). Beside controlling key cellular functions, including energy metabolism or autophagy, mTOR regulates synaptic plasticity, memory storage, and cognition, this being the subject of a recent review (Bockaert and Marin 2015). Besides its critical importance in the tissue energy homeostasis, no evidence for the cellular localization and specific functions of mTOR on the bioenergetics and redox adaptations of brain cells to neural activity has been reported so far. The possible involvement of mTOR pathways in the control of brain energy metabolism during neural activity is therefore interesting and worthy of exploration.

## Neurons are less glycolytic than astrocytes

While the widely spread notion is that glucose utilization through glycolysis by neurons is low and they depend on OXPHOS for energy generation (Knott *et al.* 2008), results obtained from different biologic preparations and brain regions are apparently controversial. Studies performed in rat cerebellar neurons in culture (Budd and Nicholls 1996), cortex synaptosomes (Kauppinen and Nicholls 1986; Choi *et al.* 2009), and retinal neurons (Xu *et al.* 2007; Bringmann *et al.* 2009) proposed glucose as the main energetic substrate. Noticeably, fast axonal transport of vesicles has a need for locally supplied ATP, which is met by glycolysis not mitochondria (Zala *et al.* 2013), and thus is a process that specifically requires glyceraldehyde-3-phosphate dehydrogenase. In addition, neurons have recently been reported to require glucose for synaptic activity (Lundgaard *et al.* 2015), although it remains unclear whether the metabolic fate of neuronally used glucose is glycolysis or pentose-phosphate pathway (PPP) – known to be essential for antioxidant protection (Herrero-Mendez *et al.* 2009) (see below). Moreover, there is question as to the reliability of the fluorescent probe, used in Lundgaard *et al.* (2015), to specifically assess glucose transport—in contrast to carrier endocytosis (Tadi *et al.* 2015). Furthermore, recently, using the genetically encoded lactate probe Laconic, it has been provided the first *in vivo* evidence for a lactate gradient from astrocytes to neurons (Maechler *et al.* 2016), a prerequisite for the occurrence of the ANLS. Accordingly, glucose does not seem to be used for the energy generation needed during neural activity, at least by cortical and hippocampal neurons, except for in fast vesicle transport through the axons (Zala *et al.* 2013).

Studies aimed at understanding why neurons do not rely on glycolysis for energy generation found PFKFB3 protein to be virtually absent in neurons (Almeida *et al.* 2004), both in culture and *in vivo*, because of the continuous destabilization by the ubiquitin-proteasome pathway (Herrero-Mendez *et al.* 2009). Thus, PFKFB3 – not PFKFB1, -2 or -4 – contains a <sup>142</sup>Lys-Glu-Asn (*KEN*) box that targets it for ubiquitination by the anaphase-promoting complex/cyclosome (APC/C)-Cdh1 (Herrero-Mendez *et al.* 2009), which is an E3 ubiquitin ligase known for its roles in the regulation of mitosis, meiosis (Pesin and Orr-Weaver 2008), tumor suppression, and genome stability (Garcia-Higuera *et al.* 2008). Besides these roles, APC/C-Cdh1 regulates important functions in neurons, such as axonal growth (Konishi *et al.* 2004; Harmey *et al.* 2009; Huynh *et al.* 2009), cortical neurogenesis and size (Delgado-Esteban *et al.* 2013), and survival (Almeida *et al.* 2005; Stegmuller and Bonni 2005; Maestre *et al.* 2008). In cortical neurons, Cdh1 knockdown leads to PFKFB3 accumulation, which is sufficient to increase glycolysis (Herrero-Mendez *et al.* 2009). This was the first observation to describe a role for a cell cycle-related

protein (APC/C-Cdh1) in metabolism, which was mimicked by PFKFB3 full-length cDNA over-expression (Herrero-Mendez *et al.* 2009). In contrast, Cdh1 protein levels and APC/C-Cdh1 ubiquitylating activity are very low in astrocytes, which explains their high levels of PFKFB3 and glycolytic activity (Herrero-Mendez *et al.* 2009) (Fig. 1).

## Metabolic fate of neuronal glucose

The rate of [6-<sup>14</sup>C]glucose incorporated into <sup>14</sup>CO<sub>2</sub> (i.e., an index of glucose that is oxidized in the TCA cycle after having been converted into pyruvate) is negligible in cortical neurons when compared with astrocytes (Garcia-Nogales *et al.* 2003; Herrero-Mendez *et al.* 2009). Glucose is not actively transformed into lactate either, as judged by the low rate of [U-<sup>14</sup>C]glucose incorporation into <sup>14</sup>C-lactate in neurons (Herrero-Mendez *et al.* 2009). Lastly, glycolytic rate, assessed as the rate of <sup>3</sup>H<sub>2</sub>O formation from [3-<sup>3</sup>H] glucose and thus accurately reflecting the flux of glucose through glycolysis (Bouzier-Sore and Bolanos 2015), is ~ 4-5-fold lower in neurons than in astrocytes (Herrero-Mendez *et al.* 2009). It seems, therefore, likely that the neuronal ability to perform glycolysis is limited. Interestingly, over-expression of PFKFB3 leading to up-regulation of glycolysis causes oxidative stress and neuronal death (Herrero-Mendez *et al.* 2009), whereas over-activation of glutamate receptors, which inhibits APC/C-Cdh1 (Maestre *et al.* 2008), stabilizes endogenous PFKFB3 protein causing neuronal death that can be rescued by knocking down PFKFB3 (Rodriguez-Rodriguez *et al.* 2012). Together, these results strongly suggest that high glycolysis is not safe for neurons.

PPP converts glucose-6-phosphate (G6P) into ribulose-5-phosphate (R5P) in three consecutive steps, the first one catalyzed by G6P dehydrogenase (G6PD), which forms 6-phosphogluconolactone; 6-phosphogluconolactone is then converted into 6-phosphogluconate (6PG) by a lactonase, followed by its decarboxylation into R5P by 6PG dehydrogenase (Wamelink *et al.* 2008; Bouzier-Sore and Bolanos 2015). Two of these reactions (G6PD and 6PG dehydrogenase) occur, each, at the expense of one mole of NADPH(H<sup>+</sup>) regenerated per mole of substrate, and constitute the oxidative branch of the PPP. Thus, this branch of the PPP can regenerate 2 moles of NADPH(H<sup>+</sup>) per mole of G6P entering the pathway with the loss of one carbon atom as CO<sub>2</sub>. This branch (the non-oxidative branch) is followed by a series of reactions that produce, from three moles of R5P, one mole of the glycolytic intermediates fructose-6-phosphate (F6P) and glyceraldehyde-3-phosphate (Wamelink *et al.* 2008; Bouzier-Sore and Bolanos 2015). The oxidative PPP branch is therefore a process that conserves the glucose redox energy in reducing NADP<sup>+</sup> to NADPH(H<sup>+</sup>), a necessary cofactor for antioxidant (GSH) regeneration (Wamelink *et al.* 2008).

G6PD catalyzes the PPP rate-limiting reaction (Eggelston and Krebs 1974). A large body of evidence now supports the

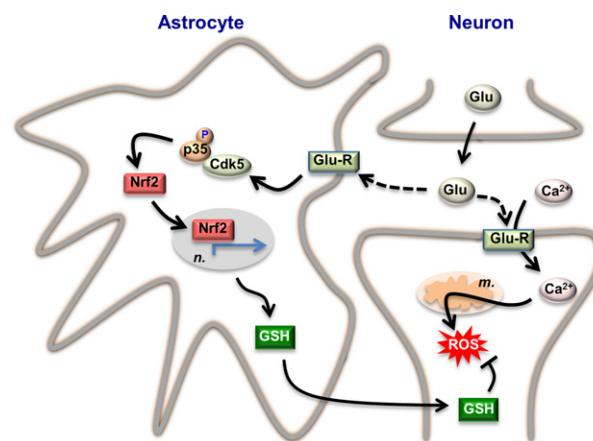
notion that the PPP has antioxidant and cytoprotective roles. In cortical neurons, exogenous  $H_2O_2$  stimulates PPP activity and regenerates  $NADPH(H^+)$  and GSH, thus contributing to neuroprotection (Ben-Yoseph *et al.* 1996). By stimulating glutamate receptors in neurons, GSH becomes oxidized to oxidized glutathione, which triggers an increase in the rate of glucose oxidation through the PPP to regenerate neuroprotective  $NADPH(H^+)$  and GSH (Delgado-Esteban *et al.* 2000). At low doses, peroxyxynitrite activates G6PD, causing an enhancement of neuroprotective PPP activity in neurons (Garcia-Nogales *et al.* 2003). Moreover, inhibition of APC/C-Cdh1 activity leading to PFKFB3 stabilization or PFKFB3 over-expression shifts glucose-6-phosphate utilization from PPP to glycolysis causing neuronal death (Herrero-Mendez *et al.* 2009). Taken together, these results strongly suggest that neurons use glucose through the PPP as an essential survival pathway (Vaughn and Deshmukh 2008; Herrero-Mendez *et al.* 2009). Thus, by using glucose preferentially through the PPP for antioxidant purposes (Herrero-Mendez *et al.*, 2009), neurons can mainly rely on ANLS-derived lactate as metabolic fuel (Kasparov 2016; Maechler *et al.* 2016). Furthermore, APC/C-Cdh1 is likely to coordinate the metabolic adaptation of neurons and astrocytes to the astrocyte-neuronal lactate shuttle (Pellerin and Magistretti 1994, 2012; Bouzier-Sore *et al.* 2003; Magistretti 2006; Allaman *et al.* 2011) (Fig. 1).

### An astrocyte-neuronal glutathione shuttle couples neural activity with redox balance

Neurotransmission unavoidably increases mitochondrial ROS in neurons, possibly because of mitochondrial  $Ca^{2+}$  influx (Mattson and Liu 2002). However, neuronal antioxidant machinery is generally weak (Makar *et al.* 1994; Bolaños *et al.* 1995, 1996), albeit provided with some intrinsic defense (Papadia *et al.* 2008; Deighton *et al.* 2014; Baxter *et al.* 2015). The antioxidant defense of neurons is repressed because of continuous protein destabilization of the master antioxidant transcriptional activator, nuclear factor-erythroid 2-related factor-2 (Nrf2), by Cullin 3/Kelch-like ECH-associated protein 1 (Bell *et al.* 2015; Jimenez-Blasco *et al.* 2015). In contrast, Nrf2 is highly stable in neighbor astrocytes, which explains their robust antioxidant defense and resistance against oxidative stress (Habas *et al.* 2013; Jimenez-Blasco *et al.* 2015), although this notion has been disputed (Haskew-Layton *et al.* 2010). Moreover, astrocytes release GSH precursors, which neurons can use for the *de novo* GSH biosynthesis (Dringen *et al.* 1999), a system that contributes to neuroprotective ischemic preconditioning (Bell *et al.* 2011). However, a definitive answer as to whether astrocytes sense neurotransmission to activate the release of GSH precursors upon neuronal activity has long remained elusive. Like post-synaptic neurons, astrocytes express glutamate receptors (Conti *et al.* 1996; Schipke *et al.*

2001; Seifert and Steinhäuser 2001; Verkhatsky and Kirchhoff 2007; Lee *et al.* 2010; Palygin *et al.* 2011), though their function in these glial cells has been enigmatic.

Recently, it was shown that subtle and persistent stimulation of glutamate receptors in astrocytes, through a mechanism not requiring extracellular  $Ca^{2+}$  influx, up-regulates a signal transduction pathway involving phospholipase C-mediated endoplasmic reticulum release of  $Ca^{2+}$  and protein kinase C $\delta$  activation. Through phosphorylation, active protein kinase C $\delta$  promotes the stabilization of p35, a cyclin-dependent kinase-5 (Cdk5) cofactor. Active p35/Cdk5 complex in the cytosol phosphorylates Nrf2 at Thr<sup>395</sup>, Ser<sup>433</sup>, and Thr<sup>439</sup> that is sufficient to promote Nrf2 translocation to the nucleus and induce the expression of antioxidant genes. Furthermore, this Cdk5-Nrf2 transduction pathway boosts GSH metabolism in astrocytes efficiently protecting closely spaced neurons against oxidative damage. These results demonstrate that neural activity is coupled with astrocyte release of GSH for neuronal *de novo* GSH biosynthesis (astrocyte-neuronal glutathione shuttle or



**Fig. 2** Redox adaptation of astrocytes to neurotransmission. Synaptic cleft glutamate (Glu) released by the pre-synaptic neuron acts on glutamate receptors (Glu-R) placed both in the post-synaptic neuron and astrocytes. Part of intracellular  $Ca^{2+}$  at the post-synaptic neurons is removed from the cytosol by entering mitochondria, and this causes mitochondrial production of reactive oxygen species (ROS). Synaptic cleft glutamate interacts with its receptors placed in astrocytes, triggering a cascade of events *via* cyclin-dependent kinase-5 (Cdk5)-mediated phosphorylation of Nrf2 (nuclear factor-erythroid 2-related factor-2), which enters the nucleus (n.) and binds to the antioxidant responsive elements (ARE) to promote the expression of antioxidant genes (green arrowed lines). This pathway leads to the biosynthesis and release of glutathione (GSH), whose precursors are taken up by neurons for the *de novo* GSH biosynthesis necessary to detoxify neuronal activity-mediated mitochondrial ROS. Thus, through this astrocyte-neuronal glutathione shuttle, astrocytes sustain the redox status of neurons during neural activity. The stoichiometry of the reactions has been omitted for clarity. Likewise, additional factors involved in these adaptations could not be depicted herein and can be found in the main text.

ANGS) as a strategy for balancing the neuronal redox status (Jimenez-Blasco *et al.* 2015) (Fig. 2).

### Pathophysiological implications of disruption in energy and redox adaptations of astrocytes

Disruption of the metabolic and redox adaptations of astrocytes and neurons to neural activity cause neuronal death and is likely to play important roles in neurological diseases. A condition underlying several of these diseases is the excitotoxic phenomenon, in which there is a  $\text{Ca}^{2+}$ -dependent component following glutamate receptor over-activation-mediated neuronal death (Choi 1987). Besides the rapid increase in cytosolic  $\text{Ca}^{2+}$  by *N*-methyl-D-aspartate (NMDA) receptor over-stimulation, there is a delayed  $\text{Ca}^{2+}$  deregulation process, which persists even after glutamate removal from the synaptic cleft, which is responsible for the activation of secondary cascades, notably those involving calpain (Brustovetsky *et al.* 2010). Calpain triggers the proteolytic cleavage of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger, a major plasma membrane system for  $\text{Ca}^{2+}$  extruding, thus impairing  $\text{Ca}^{2+}$  homeostasis and leading to neuronal death (Bano *et al.* 2005; Brustovetsky *et al.* 2010). Furthermore, the increase in intracellular  $\text{Ca}^{2+}$  causes a mitochondrial  $\text{Ca}^{2+}$  overload responsible for enhanced ROS formation and cytochrome *c* release, both playing a crucial role in glutamate-induced excitotoxicity (Luetjens *et al.* 2000). Oxidative stress associated with excitotoxicity also leads to mitochondrial fragmentation, an observation that concurs in several neurodegenerative diseases (Knott *et al.* 2008; Nguyen *et al.* 2011). Moreover, mitochondrial dynamics imbalance can trigger NMDA receptor up-regulation, further contributing to propagate the excitotoxic process (Nguyen *et al.* 2011).

A bioenergetics-redox component in this excitotoxicity cascade has also been shown. Upon a glutamatergic excitotoxic stimulus in cortical neurons, APC/C-Cdh1 is inhibited by Cdk5-mediated phosphorylation of Cdh1 (Maestre *et al.* 2008), a process that is triggered by  $\text{Ca}^{2+}$ -dependent calpain activation (Maestre *et al.* 2008). This leads to PFKFB3 stabilization triggering neuronal death as a consequence of the PPP inhibition that leads to oxidative stress (Rodriguez-Rodriguez *et al.* 2012). Interestingly, PFKFB3 knockdown in cultured human astrocytes leads to extracellular  $\beta$ -amyloid accumulation (Fu *et al.* 2015). Moreover, PFKFB3 is increased in the astrocytes of an Alzheimer's disease mouse model that over-expresses  $\beta$ -amyloid (Fu *et al.* 2015). Specific inhibition of GSH released from astrocyte triggers neuronal death in co-culture systems (Jimenez-Blasco *et al.* 2015), and disruption of astrocyte function in adult mice causes oxygen and nitrogen redox species imbalance of neurons *in vivo* (Schreiner *et al.* 2015). Glucose metabolism is altered in the brains of Alzheimer's disease patients (Silverman *et al.* 2001), and  $\beta$ -amyloid causes an enhancement in the flux of glucose

utilization *via* the PPP (Soucek *et al.* 2003). Interestingly, increased G6PD levels are found in the surviving pyramidal neurons of hippocampal slices from the post-mortem brains of Alzheimer's disease patients (Palmer 1999; Russell *et al.* 1999). In a cell model of Huntington's disease there is evidence for inhibition of the PPP and decreased mitochondrial  $\text{NADH}(\text{H}^+)/\text{NAD}^+$  ratio (Ferreira *et al.* 2011). Together, these findings strongly suggest that during the oxidative stress associated with human brain pathologies, the consumption of glucose through the PPP is critical for maintaining the neuronal antioxidant redox status and survival. Thus, besides their critical role in controlling energy metabolism, astrocyte redox metabolism is also adapted to protect neurons against oxidative stress and neurodegeneration, likely playing an important role in neurological disorders.

### Concluding remarks and perspectives

Astrocytes and neurons are metabolically programmed to spatiotemporally deal with the energy and redox requirements of neural activity. Astrocytes show a prominent constitutive glycolytic metabolism whereas neurons do not, and this is coordinated by the E3 ubiquitin ligase APC/C-Cdh1 (Herrero-Mendez *et al.* 2009). High Cdh1 levels keep APC/C active, thus destabilizing the pro-glycolytic enzyme PFKFB3; whereas low Cdh1 levels in astrocytes allows PFKFB3 stabilization responsible for the high glycolytic phenotype (Herrero-Mendez *et al.* 2009; Bolaños *et al.* 2010) (Fig. 1). In addition, messengers such as  $\text{NO}$  or  $\text{NH}_4^+$  also contribute to the glycolytic phenotype of astrocytes (Almeida *et al.* 2004; Brix *et al.* 2012; Lerchundi *et al.* 2015), and an appropriate redox balance is critically important to sustain antioxidant protection during neural activity. Neurons spare glucose for its oxidation through the PPP, which serves to regenerate GSH and exert protection against oxidative stress (Vaughn and Deshmukh 2008; Herrero-Mendez *et al.* 2009). In addition, neurons constitutively destabilize Nrf2, whereas astrocytes accumulate it (Jimenez-Blasco *et al.* 2015). Thus, neurons rely on astrocyte-derived precursors for *de novo* GSH biosynthesis (Dringen *et al.* 1999), and in astrocytes neural activity triggers the signaling pathway needed to activate this redox regulatory system (Jimenez-Blasco *et al.* 2015) (Fig. 2). Such mechanisms do not exclude the occurrence of other, still unknown systems helping neural cells adapt their metabolism to the necessary rapid changes occurring during neural activity. These may include mTOR (Bockaert and Marin 2015) and/or hypoxia-inducible factor-1 (Brix *et al.* 2012) signaling pathways, the importance of which is still elusive. In any case, the metabolic status of neural cells would favor the necessary spatiotemporal changes in energy homeostasis following neural activity to satisfy neuronal needs. Recent studies in *Drosophila* (Volkenhoff *et al.* 2015)

and in mice (Sada *et al.* 2015; Tadi *et al.* 2015) confirm the occurrence of the metabolic adaptations of astrocytes *in vivo*. The use of novel fluorescent probes has confirmed the key role of glycolytic-derived lactate from astrocytes during neural activity at a real-time resolution (Sotelo-Hitschfeld *et al.* 2015), suggesting dual roles for lactate as metabolic fuel (Pellerin and Magistretti 2012) and intercellular messenger (Barros 2013). Further advances in similar tools would be desirable to investigate roles for neuronal use of glucose through the PPP as an antioxidant strategy during neural activity (Bouzier-Sore and Bolanos 2015). Thus prominent neurochemical advances have been made over the past few decades in our understanding of the physiological mechanisms that coordinate the metabolic adaptations of neural cells to neurotransmission. Key conserved pathways have been identified that are regulated by specific molecules, the disruption of which causes neural problems. Therefore, it seems reasonable to move forward and develop therapeutic interventions aimed to interfere with these targets for the treatment of neurological disorders.

### Acknowledgments and conflict of interest disclosure

J.P.B. is funded by the MINECO (SAF2013-41177-R; RTC-2015-3237-1), the Instituto de Salud Carlos III (RD12/0043/0021), the E.U. SP3-People-MC-ITN programme (608381), the E.U. BATCure grant (666918), the European Regional Development Fund, and the NIH/NIDA (1R21DA037678-01). The author has no conflict of interest to declare.

All experiments were conducted in compliance with the ARRIVE guidelines.

### References

- Allaman I., Belanger M. and Magistretti P. J. (2011) Astrocyte-neuron metabolic relationships: for better and for worse. *Trends Neurosci.* **34**, 76–87.
- Allen N. J. and Barres B. A. (2009) Neuroscience: Glia - more than just brain glue. *Nature* **457**, 675–677.
- Almeida A. and Bolaños J. P. (2001) A transient inhibition of mitochondrial ATP synthesis by nitric oxide synthase activation triggered apoptosis in primary cortical neurons. *J. Neurochem.* **77**, 676–690.
- Almeida A., Almeida J., Bolaños J. P. and Moncada S. (2001) Different responses of astrocytes and neurons to nitric oxide: the role of glycolytically-generated ATP in astrocyte protection. *Proc. Natl Acad. Sci. USA* **98**, 15294–15299.
- Almeida A., Moncada S. and Bolaños J. P. (2004) Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. *Nat. Cell Biol.* **6**, 45–51.
- Almeida A., Bolaños J. P. and Moreno S. (2005) Cdh1/Hct1-APC is essential for the survival of postmitotic neurons. *J. Neurosci.* **25**, 8115–8121.
- Bak L. K., Schousboe A., Sonnewald U. and Waagepetersen H. S. (2006) Glucose is necessary to maintain neurotransmitter homeostasis during synaptic activity in cultured glutamatergic neurons. *J. Cereb. Blood Flow Metab.* **26**, 1285–1297.
- Bak L. K., Walls A. B., Schousboe A., Ring A., Sonnewald U. and Waagepetersen H. S. (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.
- Bak L. K., Obel L. F., Walls A. B., Schousboe A., Faek S. A., Jajo F. S. and Waagepetersen H. S. (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.
- Bano D., Young K. W., Guerin C. J., Lefevre R., Rothwell N. J., Naldini L., Rizzuto R., Carafoli E. and Nicotera P. (2005) Cleavage of the plasma membrane  $Na^+/Ca^{2+}$  exchanger in excitotoxicity. *Cell* **120**, 275–285.
- Barros L. F. (2013) Metabolic signaling by lactate in the brain. *Trends Neurosci.* **36**, 396–404.
- Baxter P. S., Bell K. F., Hasel P., Kaindl A. M., Fricker M., Thomson D., Cregan S. P., Gillingwater T. H. and Hardingham G. E. (2015) Synaptic NMDA receptor activity is coupled to the transcriptional control of the glutathione system. *Nat. Commun.* **6**, 6761.
- Bell K. F., Al-Mubarak B., Fowler J. H. *et al.* (2011) Mild oxidative stress activates Nrf2 in astrocytes, which contributes to neuroprotective ischemic preconditioning. *Proc. Natl Acad. Sci. USA* **108**, E1–E2.
- Bell K. F., Al-Mubarak B., Martel M. A. *et al.* (2015) Neuronal development is promoted by weakened intrinsic antioxidant defences due to epigenetic repression of Nrf2. *Nat. Commun.* **6**, 7066.
- Beltran B., Mathur A., Duchon M. R., Erusalimsky J. D. and Moncada S. (2000) The effect of nitric oxide on cell respiration: a key to understanding its role in cell survival or death. *Proc. Natl Acad. Sci. USA* **97**, 14602–14607.
- Ben-Yoseph O., Boxer P. A. and Ross B. D. (1996) Assessment of the role of the glutathione and pentose phosphate pathways in the protection of primary cerebrocortical cultures from oxidative stress. *J. Neurochem.* **66**, 2329–2337.
- Bittner C. X., Valdebenito R., Ruminot I. *et al.* (2011) Fast and reversible stimulation of astrocytic glycolysis by  $K^+$  and a delayed and persistent effect of glutamate. *J. Neurosci.* **31**, 4709–4713.
- Bockaert J. and Marin P. (2015) mTOR in Brain Physiology and Pathologies. *Physiol. Rev.* **95**, 1157–1187.
- Bolaños J. P., Peuchen S., Heales S. J. R., Land J. M. and Clark J. B. (1994) Nitric oxide-mediated inhibition of the mitochondrial respiratory chain in cultured astrocytes. *J. Neurochem.* **63**, 910–916.
- Bolaños J. P., Heales S. J. R., Land J. M. and Clark J. B. (1995) Effect of peroxynitrite on the mitochondrial respiratory chain: differential susceptibility of neurones and astrocytes in primary cultures. *J. Neurochem.* **64**, 1965–1972.
- Bolaños J. P., Heales S. J. R., Peuchen S., Barker J. E., Land J. M. and Clark J. B. (1996) Nitric oxide-mediated mitochondrial damage: a potential neuroprotective role for glutathione. *Free Radic. Biol. Med.* **21**, 995–1001.
- Bolaños J. P., Almeida A. and Moncada S. (2010) Glycolysis: a bioenergetic or a survival pathway?. *Trends Biochem. Sci.* **35**, 145–149.
- Bouzier-Sore A. K. and Bolanos J. P. (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.
- Bouzier-Sore A. K., Voisin P., Canioni P., Magistretti P. J. and Pellerin L. (2003) Lactate is a preferential oxidative energy substrate over glucose for neurons in culture. *J. Cereb. Blood Flow Metab.* **23**, 1298–1306.



- Bouzier-Sore A. K., Voisin P., Bouchaud V., Bezancon E., Franconi J. M. and Pellerin L. (2006) Competition between glucose and lactate as oxidative energy substrates in both neurons and astrocytes: a comparative NMR study. *Eur. J. Neurosci.* **24**, 1687–1694.
- Bringmann A., Pannicke T., Biedermann B., Francke M., Iandiev I., Grosche J., Wiedemann P., Albrecht J. and Reichenbach A. (2009) Role of retinal glial cells in neurotransmitter uptake and metabolism. *Neurochem. Int.* **54**, 143–160.
- Brix B., Mesters J. R., Pellerin L. and Jöhren O. (2012) Endothelial cell-derived nitric oxide enhances aerobic glycolysis in astrocytes via HIF-1 $\alpha$ -mediated target gene activation. *J. Neurosci.* **32**, 9727–9735.
- Brown G. C. and Cooper C. E. (1994) Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett.* **356**, 295–298.
- Brown A. M. and Ransom B. R. (2007) Astrocyte glycogen and brain energy metabolism. *Glia* **55**, 1263–1271.
- Brustovetsky T., Bolshakov A. and Brustovetsky N. (2010) Calpain activation and Na<sup>+</sup>/Ca<sup>2+</sup> exchanger degradation occur downstream of calcium deregulation in hippocampal neurons exposed to excitotoxic glutamate. *J. Neurosci. Res.* **88**, 1317–1328.
- Budd S. L. and Nicholls D. G. (1996) A reevaluation of the role of mitochondria in neuronal Ca<sup>2+</sup> homeostasis. *J. Neurochem.* **66**, 403–411.
- Cai Q. and Sheng Z. H. (2009) Molecular motors and synaptic assembly. *Neuroscientist* **15**, 78–89.
- Choi D. W. (1987) Ionic dependence of glutamate neurotoxicity in cortical cell culture. *J. Neurosci.* **7**, 369–379.
- Choi S. W., Gerencser A. A. and Nicholls D. G. (2009) Bioenergetic analysis of isolated cerebrocortical nerve terminals on a microgram scale: spare respiratory capacity and stochastic mitochondrial failure. *J. Neurochem.* **109**, 1179–1191.
- Cleeter M. W. J., Cooper J. M., Darley-Usmar V. M., Moncada S. and Schapira A. H. (1994) Reversible inhibition of cytochrome *c* oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Lett.* **345**, 50–54.
- Conti F., DeBiasi S., Minelli A. and Melone M. (1996) Expression of NR1 and NR2A/B subunits of the NMDA receptor in cortical astrocytes. *Glia* **17**, 254–258.
- Deighton R. F., Markus N. M., Al-Mubarak B., Bell K. F., Papadia S., Meakin P. J., Chowdhry S., Hayes J. D. and Hardingham G. E. (2014) Nrf2 target genes can be controlled by neuronal activity in the absence of Nrf2 and astrocytes. *Proc. Natl Acad. Sci. USA* **111**, E1818–E1820.
- Delgado-Esteban M., Almeida A. and Bolaños J. P. (2000) D-Glucose prevents glutathione oxidation and mitochondrial damage after glutamate receptor stimulation in rat cortical primary neurons. *J. Neurochem.* **75**, 1618–1624.
- Delgado-Esteban M., Garcia-Higuera I., Maestre C., Moreno S. and Almeida A. (2013) APC/C-Cdh1 coordinates neurogenesis and cortical size during development. *Nat. Commun.* **4**, 2879.
- Dringen R., Pfeiffer B. and Hamprecht B. (1999) Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. *J. Neurosci.* **19**, 562–569.
- Duran J. and Guinovart J. J. (2015) Brain glycogen in health and disease. *Mol. Aspects Med.* **46**, 70–77. doi: 10.1016/j.mam.2015.08.007.
- Eggleston L. V. and Krebs H. A. (1974) Regulation of the pentose phosphate cycle. *Biochem. J.* **138**, 425–435.
- Fernandez-Moncada I. and Barros L. F. (2014) Non-preferential fuelling of the Na<sup>(+)</sup>/K<sup>(+)</sup>-ATPase pump. *Biochem. J.* **460**, 353–361.
- Ferreira I. L., Cunha-Oliveira T., Nascimento M. V., Ribeiro M., Proenca M. T., Januario C., Oliveira C. R. and Rego A. C. (2011) Bioenergetic dysfunction in Huntington's disease human cybrids. *Exp. Neurol.* **231**, 127–134.
- Fox P. T. and Raichle M. E. (1986) Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc. Natl Acad. Sci. USA* **83**, 1140–1144.
- Fu W., Shi D., Westaway D. and Jhamandas J. H. (2015) Bioenergetic mechanisms in astrocytes may contribute to amyloid plaque deposition and toxicity. *J. Biol. Chem.* **290**, 12504–12513.
- Garcia O., Almeida A., Massieu L. and Bolaños J. P. (2005) Increased mitochondrial respiration maintains the mitochondrial membrane potential and promotes survival of cerebellar neurons in an endogenous model of glutamate receptor activation. *J. Neurochem.* **92**, 183–190.
- Garcia-Higuera I., Machado E., Dubus P., Canamero M., Mendez J., Moreno S. and Malumbres M. (2008) Genomic stability and tumour suppression by the APC/C cofactor Cdh1. *Nat. Cell Biol.* **10**, 802–811.
- Garcia-Nogales P., Almeida A. and Bolaños J. P. (2003) Peroxynitrite protects neurons against nitric oxide-mediated apoptosis. A key role for glucose-6-phosphate dehydrogenase activity in neuroprotection. *J. Biol. Chem.* **278**, 864–874.
- Garthwaite J., Charles S. L. and Chess-Williams R. (1988) Endothelium-derived relaxing factor release on activation of NMDA receptors suggests a role as intercellular messenger in the brain. *Nature* **336**, 385–387.
- Gleichmann M., Collis L. P., Smith P. J. and Mattson M. P. (2009) Simultaneous single neuron recording of O<sub>2</sub> consumption, [Ca<sup>2+</sup>]<sub>i</sub> and mitochondrial membrane potential in glutamate toxicity. *J. Neurochem.* **109**, 644–655.
- Habas A., Hahn J., Wang X. and Margeta M. (2013) Neuronal activity regulates astrocytic Nrf2 signaling. *Proc. Natl Acad. Sci. USA* **110**, 18291–18296.
- Harmey D., Smith A., Simanski S., Moussa C. Z. and Ayad N. G. (2009) The anaphase promoting complex induces substrate degradation during neuronal differentiation. *J. Biol. Chem.* **284**, 4317–4323.
- Haskew-Layton R. E., Payappilly J. B., Smirnova N. A. et al. (2010) Controlled enzymatic production of astrocytic hydrogen peroxide protects neurons from oxidative stress via an Nrf2-independent pathway. *Proc. Natl Acad. Sci. USA* **107**, 17385–17390.
- Herrero-Mendez A., Almeida A., Fernandez E., Maestre C., Moncada S. and Bolaños J. P. (2009) The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat. Cell Biol.* **11**, 747–752.
- Hertz L., Peng L. and Dienel G. A. (2007) Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J. Cereb. Blood Flow Metab.* **27**, 219–249.
- Huynh M. A., Stegmüller J., Litterman N. and Bonni A. (2009) Regulation of Cdh1-APC function in axon growth by Cdh1 phosphorylation. *J. Neurosci.* **29**, 4322–4327.
- Ivanov A. I., Malkov A. E., Waseem T., Mukhtarov M., Buldakova S., Gubkina O., Zilberter M. and Zilberter Y. (2014) Glycolysis and oxidative phosphorylation in neurons and astrocytes during network activity in hippocampal slices. *J. Cereb. Blood Flow Metab.* **34**, 397–407.
- Jekabsons M. B. and Nicholls D. G. (2004) *In situ* respiration and bioenergetic status of mitochondria in primary cerebellar granule neuronal cultures exposed continuously to glutamate. *J. Biol. Chem.* **279**, 32989–33000.
- Jimenez-Blasco D., Santofimia-Castano P., Gonzalez A., Almeida A. and Bolaños J. P. (2015) Astrocyte NMDA receptors' activity

- sustains neuronal survival through a Cdk5-Nrf2 pathway. *Cell Death Differ.* **22**, 1877–1889.
- Kasparov S. (2016) Are Astrocytes the Pressure-Reservoirs of Lactate in the Brain? (2016) *Cell Metabolism*, <http://dx.doi.org/10.1016/j.cmet.2015.11.001>
- Kauppinen R. A. and Nicholls D. G. (1986) Failure to maintain glycolysis in anoxic nerve terminals. *J. Neurochem.* **47**, 1864–1869.
- Knott A. B., Perkins G., Schwarzenbacher R. and Bossy-Wetzel E. (2008) Mitochondrial fragmentation in neurodegeneration. *Nat. Rev. Neurosci.* **9**, 505–518.
- Konishi Y., Stegmüller J., Matsuda T., Bonni S. and Bonni A. (2004) Cdh1-APC controls axonal growth and patterning in the mammalian brain. *Science* **303**, 1026–1030.
- Lage R., Dieguez C., Vidal-Puig A. and Lopez M. (2008) AMPK: a metabolic gauge regulating whole-body energy homeostasis. *Trends Mol. Med.* **14**, 539–549.
- Lee M., Ting K., Adams S., Brew B., Chung R. and Guillemin G. (2010) Characterisation of the expression of NMDA receptors in human astrocytes. *PLoS ONE* **30**, e14123.
- Lerchundi R., Fernandez-Moncada I., Contreras-Baeza Y. *et al.* (2015) NH<sub>4</sub><sup>+</sup> triggers the release of astrocytic lactate via mitochondrial pyruvate shunting. *Proc. Natl Acad. Sci. USA* **112**, 11090–11095.
- Loaiza A., Porras O. H. and Barros L. F. (2003) Glutamate triggers rapid glucose transport stimulation in astrocytes as evidenced by real-time confocal microscopy. *J. Neurosci.* **23**, 7337–7342.
- Luetjens C. M., Bui N. T., Sengpiel B., Munstermann G., Poppe M., Krohn A. J., Bauerbach E., Krieglstein J. and Prehn J. H. (2000) Delayed mitochondrial dysfunction in excitotoxic neuron death: cytochrome *c* release and a secondary increase in superoxide production. *J. Neurosci.* **20**, 5715–5723.
- Lundgaard I., Li B., Xie L. *et al.* (2015) Direct neuronal glucose uptake heralds activity-dependent increases in cerebral metabolism. *Nat. Commun.* **6**, 6807.
- Maechler P., Wyss M. T., Elsayed M., Stobart J., Gutierrez R., von Faber-Castell A., Kaelin V., Zuend M., San Martin A., Romero-Gomez I., Baeza-Lehnert F., Lengacher S., Schneider B. L., Aebischer P., Magistretti P. J., Barros L. F. and Weber B. (2016) *In Vivo* Evidence for a Lactate Gradient from Astrocytes to Neurons. *Cell Metabolism*, <http://dx.doi.org/10.1016/j.cmet.2015.10.010>
- Maestre C., Delgado-Esteban M., Gomez-Sanchez J. C., Bolaños J. P. and Almeida A. (2008) Cdk5 phosphorylates Cdh1 and modulates cyclin B1 stability in excitotoxicity. *EMBO J.* **27**, 2736–2745.
- Magistretti P. J. (2006) Neuron-glia metabolic coupling and plasticity. *J. Exp. Biol.* **209**, 2304–2311.
- Magistretti P. J. and Allaman I. (2015) A cellular perspective on brain energy metabolism and functional imaging. *Neuron* **86**, 883–901.
- Makar T. K., Nedergaard M., Preuss A., Gelbard A. S., Perumal A. S. and Cooper A. J. L. (1994) Vitamin E, ascorbate, glutathione, glutathione disulfide, and enzymes of glutathione metabolism in cultures of chick astrocytes and neurons: evidence that astrocytes play an important role in antioxidative processes in the brain. *J. Neurochem.* **62**, 45–53.
- Mattson M. P. and Liu D. (2002) Energetics and oxidative stress in synaptic plasticity and neurodegenerative disorders. *Neuromolecular Med.* **2**, 215–231.
- Nguyen D., Alavi M. V., Kim K. Y. *et al.* (2011) A new vicious cycle involving glutamate excitotoxicity, oxidative stress and mitochondrial dynamics. *Cell Death Dis.* **2**, e240.
- Nicholls D. G. (2008) Oxidative stress and energy crises in neuronal dysfunction. *Ann. N. Y. Acad. Sci.* **1147**, 53–60.
- Nicholls D. G., Johnson-Cadwell L., Vesce S., Jekabsons M. and Yadava N. (2007) Bioenergetics of mitochondria in cultured neurons and their role in glutamate excitotoxicity. *J. Neurosci. Res.* **85**, 3206–3212.
- Oliveira J. F., Sardinha V. M., Guerra-Gomes S., Araque A. and Sousa N. (2015) Do stars govern our actions? Astrocyte involvement in rodent behavior. *Trends Neurosci.* **38**, 535–549.
- Palmer A. M. (1999) The activity of the pentose phosphate pathway is increased in response to oxidative stress in Alzheimer's disease. *J. Neural. Transm.* **106**, 317–328.
- Palygin O., Lalo U. and Pankratov Y. (2011) Distinct pharmacological and functional properties of NMDA receptors in mouse cortical astrocytes. *Br. J. Pharmacol.* **163**, 1755–1766.
- Papadia S., Soriano F. X., Leveille F. *et al.* (2008) Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nat. Neurosci.* **11**, 476–487.
- Parpura V., Basarsky T. A., Liu F., Jęftinija K., Jęftinija S. and Haydon P. G. (1994) Glutamate-mediated astrocyte-neuron signalling. *Nature* **369**, 744–747.
- Partanen J. I., Nieminen A. I. and Klefstrom J. (2009) 3D view to tumor suppression: Lkb1, polarity and the arrest of oncogenic *c-Myc*. *Cell Cycle* **8**, 716–724.
- Pellerin L. and Magistretti P. J. (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc. Natl Acad. Sci. USA* **91**, 10625–10629.
- Pellerin L. and Magistretti P. J. (2012) Sweet sixteen for ANLS. *J. Cereb. Blood Flow Metab.* **32**, 1152–1166.
- Pellerin L., Pellegrini G., Bittar P. G., Charnay Y., Bouras C., Martin J. L., Stella N. and Magistretti P. J. (1998) Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. *Dev. Neurosci.* **20**, 291–299.
- Pellerin L., Bouzier-Sore A. K., Aubert A., Serres S., Merle M., Costalat R. and Magistretti P. J. (2007) Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia* **55**, 1251–1262.
- Perea G., Sur M. and Araque A. (2014) Neuron-glia networks: integral gear of brain function. *Front. Cell. Neurosci.* **8**, 378.
- Pesin J. A. and Orr-Weaver T. L. (2008) Regulation of APC/C activators in mitosis and meiosis. *Annu. Rev. Cell Dev. Biol.* **24**, 475–499.
- Pierre K. and Pellerin L. (2005) Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *J. Neurochem.* **94**, 1–14.
- Pilkis S. J., Claus T. H., Kurland I. J. and Lange A. J. (1995) 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase: a metabolic signaling enzyme. *Annu. Rev. Biochem.* **64**, 799–835.
- Porras O. H., Loaiza A. and Barros L. F. (2004) Glutamate mediates acute glucose transport inhibition in hippocampal neurons. *J. Neurosci.* **24**, 9669–9673.
- Porras O. H., Ruminot I., Loaiza A. and Barros L. F. (2008) Na<sup>+</sup>-Ca<sup>2+</sup> cosignaling in the stimulation of the glucose transporter GLUT1 in cultured astrocytes. *Glia* **56**, 59–68.
- Rodriguez-Rodriguez P., Fernandez E., Almeida A. and Bolaños J. P. (2012) Excitotoxic stimulus stabilizes PFKFB3 causing pentose-phosphate pathway to glycolysis switch and neurodegeneration. *Cell Death Differ.* **19**, 1582–1589.
- Ronnett G. V., Ramamurthy S., Kleman A. M., Landree L. E. and Aja S. (2009) AMPK in the brain: its roles in energy balance and neuroprotection. *J. Neurochem.* **109**(Suppl 1), 17–23.
- Russell R. L., Siedlak S. L., Raina A. K., Bautista J. M., Smith M. A. and Perry G. (1999) Increased neuronal glucose-6-phosphate dehydrogenase and sulfhydryl levels indicate reductive compensation to oxidative stress in Alzheimer disease. *Arch. Biochem. Biophys.* **370**, 236–239.
- Sada N., Lee S., Katsu T., Otsuki T. and Inoue T. (2015) Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy. *Science* **347**, 1362–1367.
- Sanders M. J., Grondin P. O., Hegarty B. D., Snowden M. A. and Carling D. (2007) Investigating the mechanism for AMP activation

- of the AMP-activated protein kinase cascade. *Biochem. J.* **403**, 139–148.
- Schipke C., Ohlemeyer C., Matyash M., Nolte C., Kettenmann H. and Kirchhoff F. (2001) Astrocytes of the mouse neocortex express functional *N*-methyl-D-aspartate receptors. *FASEB J.* **15**, 1270–1272.
- Schreiner B., Romanelli E., Liberski P. *et al.* (2015) Astrocyte depletion impairs redox homeostasis and triggers neuronal loss in the adult CNS. *Cell Rep.* **12**, 1377–1384.
- Schweizer M. and Richter C. (1994) Nitric oxide potently and reversibly deenergizes mitochondria at low oxygen tension. *Biochem. Biophys. Res. Commun.* **204**, 169–175.
- Seifert G. and Steinhäuser C. (2001) Ionotropic glutamate receptors in astrocytes. *Prog. Brain Res.* **132**, 287–299.
- Silverman D. H., Small G. W., Chang C. Y. *et al.* (2001) Positron emission tomography in evaluation of dementia: regional brain metabolism and long-term outcome. *JAMA* **286**, 2120–2127.
- Sotelo-Hitschfeld T., Fernandez-Moncada I. and Barros L. F. (2012) Acute feedback control of astrocytic glycolysis by lactate. *Glia* **60**, 674–680.
- Sotelo-Hitschfeld T., Niemeyer M. I., Machler P. *et al.* (2015) Channel-mediated lactate release by K(+)-stimulated astrocytes. *J. Neurosci.* **35**, 4168–4178.
- Soucek T., Cumming R., Dargusch R., Maher P. and Schubert D. (2003) The regulation of glucose metabolism by HIF-1 mediates a neuroprotective response to amyloid beta peptide. *Neuron* **39**, 43–56.
- Stegmuller J. and Bonni A. (2005) Moving past proliferation: new roles for Cdh1-APC in postmitotic neurons. *Trends Neurosci.* **28**, 596–601.
- Suh S. W., Bergher J. P., Anderson C. M., Treadway J. L., Fosgerau K. and Swanson R. A. (2007) Astrocyte glycogen sustains neuronal activity during hypoglycemia: studies with the glycogen phosphorylase inhibitor CP-316,819 ([R-R\*, S\*]-5-chloro-N-[2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmethyl)propyl]-1H-indole-2-carboxamide). *J. Pharmacol. Exp. Ther.* **321**, 45–50.
- Tadi M., Allaman I., Lengacher S., Grenningloh G. and Magistretti P. J. (2015) Learning-induced gene expression in the hippocampus reveals a role of neuron-astrocyte metabolic coupling in long term memory. *PLoS ONE* **10**, e0141568.
- Thompson J. K., Peterson M. R. and Freeman R. D. (2003) Single-neuron activity and tissue oxygenation in the cerebral cortex. *Science* **299**, 1070–1072.
- Vaughn A. E. and Deshmukh M. (2008) Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome *c*. *Nat. Cell Biol.* **10**, 1477–1483.
- Verkhatsky A. and Kirchhoff F. (2007) NMDA Receptors in glia. *Neuroscientist* **13**, 28–37.
- Vilchez D., Ros S., Cifuentes D. *et al.* (2007) Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. *Nat. Neurosci.* **10**, 1407–1413.
- Volkenhoff A., Weiler A., Letzel M., Stehling M., Klambt C. and Schirmeier S. (2015) Glial glycolysis is essential for neuronal survival in drosophila. *Cell Metab.* **22**, 437–447.
- Wamelink M. M., Struys E. A. and Jakobs C. (2008) The biochemistry, metabolism and inherited defects of the pentose phosphate pathway: a review. *J. Inher. Metab. Dis.* **31**, 703–717.
- Wullschlegel S., Loewith R. and Hall M. N. (2006) TOR signaling in growth and metabolism. *Cell* **124**, 471–484.
- Xu Y., Ola M. S., Berkich D. A., Gardner T. W., Barber A. J., Palmieri F., Hutson S. M. and LaNoue K. F. (2007) Energy sources for glutamate neurotransmission in the retina: absence of the aspartate/glutamate carrier produces reliance on glycolysis in glia. *J. Neurochem.* **101**, 120–131.
- Zala D., Hinckelmann M. V., Yu H., Lyra da Cunha M. M., Liot G., Cordelieres F. P., Marco S. and Saudou F. (2013) Vesicular glycolysis provides on-board energy for fast axonal transport. *Cell* **152**, 479–491.
- Zhang Y., Chen K., Sloan S. A. *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.