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PAST TO FUTURE

Bioenergetics and redox adaptations of astrocytes to neuronal activity

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Abstract
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.
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Neural activity is energy costly

The brain consumes about 20% of inhaled O_2 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^+, K^+, and Ca^{2+}) gradients across the plasma membrane of dendrites and axons through primary active transporters, including Na^+/K^+- and Ca^{2+}-ATPase pumps (Cai and Sheng 2009). Accordingly, it is not unexpected that stimulation of glutamate

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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; $F2,6P_2$, fructose-2,6-bisphosphate; G6PD, glucose-6-phosphate dehydrogenase; 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.

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receptors in cortical neurons causes rapid ATP consumption (Almeida and Bolaños 2001), which is immediately followed by a rise in mitochondrial O_2 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_2 consumption, there is an increase in intracellular Ca^{2+} concentrations (Gleichmann *et al.* 2009), suggesting an early regulatory role for Ca^{2+} in the bioenergetics of neurotransmission (Nicholls *et al.* 2007). Furthermore, mitochondrial influx of Ca^{2+} 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_2 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^+-dependent active transporters. Thus, glutamate uptake is energy-costly for astrocytes, as re-setting the Na^+ gradient across the plasma membrane to the resting levels requires Na^+ pumping by the Na^+/K^+-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 α-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 ^{13}C-nuclear magnetic resonance (^{13}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^+-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^+/K^+-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^+ at concentrations compatible with those during glutamatergic neurotransmission, which stimulates glycolysis rapidly and reversibly (Bittner *et al.* 2011). Ammonium (NH_4^+), 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^+ and NH_4^+ deserve further investigation.

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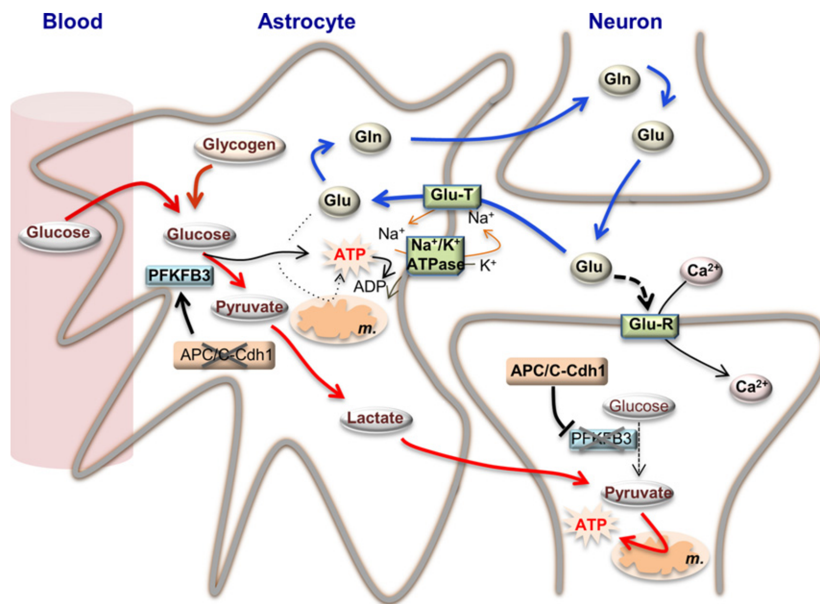

Fig. 1 Bioenergetics adaptations of astrocytes to neurotransmission. Synaptic cleft glutamate (Glu) released by the pre-synaptic neuron acts on glutamate receptors (Glu-R) placed in the post-synaptic neurons, triggering the influx of Ca^{2+} (and Na^+, not indicated) and causing plasma membrane depolarization, 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 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 Na^+/K^+-ATPase hydrolyses ATP to conserve its energy to reset Na^+ (and 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 Na^+/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 (•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 •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 O_2 (Brown and Cooper 1994). During endogenous •NO production inhibiting the MRC at cytochrome *c* oxidase in astrocytes, ~ 90–100% 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 •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 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 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 •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 ($F2,6P_2$) is twofold (Almeida *et al.* 2004). There are four isoforms of the enzyme responsible for the synthesis of $F2,6P_2$, 6-phospho-fructo-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 $F2,6\text{-}P_2$ 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 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^{172} residue on its catalytic α1 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, $F2,6P_2$ levels elevated, and glycolysis activated within minutes of the inhibition of mitochondrial respiration by •NO or cyanide (Almeida *et al.* 2004). Knockdown of the α1 subunit of AMPK, or PFKFB3, abolishes $F2,6P_2$ 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 (Ronnett *et al.* 2009), but this needs to be directly elucidated. Interestingly, the ATP/AMP ratio, as well as 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 ^{142}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 ubiquitine 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-^{14}C]glucose incorporated into $^{14}CO_2$ (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-^{14}C]glucose incorporation into ^{14}C-lactate in neurons (Herrero-Mendez *et al.* 2009). Lastly, glycolytic rate, assessed as the rate of ^3H_2O formation from [3-^{3}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^+) 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^+) per mole of G6P entering the pathway with the loss of one carbon atom as CO_2. 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 $NADP^+$ to NADPH(H^+), a necessary cofactor for antioxidant (GSH) regeneration (Wamelink *et al.* 2008).

G6PD catalyzes the PPP rate-limiting reaction (Eggleston 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, peroxynitrite 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; Verkhratsky 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 Cδ activation. Through phosphorylation, active protein kinase Cδ promotes the stabilization of p35, a cyclin-dependent kinase-5 (Cdk5) cofactor. Active p35/Cdk5 complex in the cytosol phosphorylates Nrf2 at Thr^{395}, Ser^{433}, and Thr^{439} 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

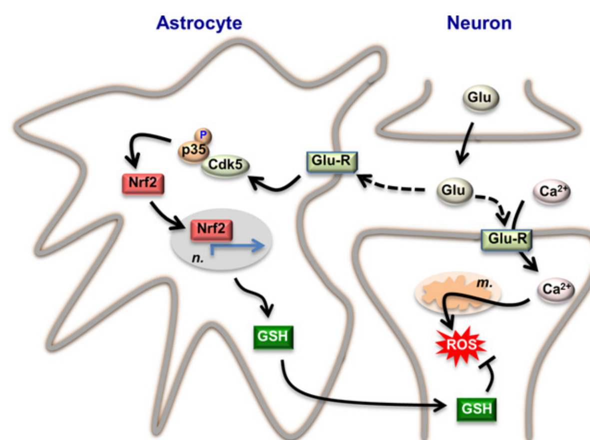

Fig. 2 Redox adaptation of astrocytes to neurotransmission. Synaptic cleft glutamate (Glu) released by the pre-synaptic neuron acts on glutamate receptors (Glu-R) placed both in the post-synaptic neuron and astrocytes. Part of intracellular Ca^{2+} at the post-synaptic neurons is removed from the cytosol 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 Ca^{2+}-dependent component following glutamate receptor over-activation-mediated neuronal death (Choi 1987). Besides the rapid increase in cytosolic Ca^{2+} by *N*-methyl-D-aspartate (NMDA) receptor over-stimulation, there is a delayed 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 Na^{+}/Ca^{2+} exchanger, a major plasma membrane system for Ca^{2+} extruding, thus impairing Ca^{2+} homeostasis and leading to neuronal death (Bano *et al.* 2005; Brustovetsky *et al.* 2010). Furthermore, the increase in intracellular Ca^{2+} causes a mitochondrial 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 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 ß-amyloid accumulation (Fu *et al.* 2015). Moreover, PFKFB3 is increased in the astrocytes of an Alzheimer's disease mouse model that over-expresses ß-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 ß-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 $NADH(H^{+})/NAD^{+}$ 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 $^{\bullet}NO$ or 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

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