Neuroscience 567 (2025) 227–234

Contents lists available at ScienceDirect

Neuroscience

journal homepage: www.elsevier.com/locate/nsc

Review

Mitochondria and astrocyte reactivity: Key mechanism behind neuronal injury☆

Patricia Cassina [a,*], Ernesto Miquel [a], Laura Martínez-Palma [a], Adriana Cassina [b]

[a] Departamento de Histología y Embriología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[b] Departemento de Bioquímica and Centro de Investigaciones Biomédicas (CEINBIO), Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

ARTICLE INFO

Keywords:
Glial metabolism
Astrocytes
Aberrant glial cells
Amyotrophic lateral sclerosis
Chronic pain

ABSTRACT

In this special issue to celebrate the 30th anniversary of the Uruguayan Society for Neuroscience (SNU), we find it pertinent to highlight that research on glial cells in Uruguay began almost alongside the history of SNU and contributed to the understanding of neuron-glia interactions within the international scientific community.

Glial cells, particularly astrocytes, traditionally regarded as supportive components in the central nervous system (CNS), undergo notable morphological and functional alterations in response to neuronal damage, a phenomenon referred to as glial reactivity.

Among the myriad functions of astrocytes, metabolic support holds significant relevance for neuronal function, given the high energy demand of the nervous system. Although astrocytes are typically considered to exhibit low mitochondrial respiratory chain activity, they possess a noteworthy mitochondrial network. Interestingly, both the morphology and activity of these organelles change following glial reactivity.

Despite receiving less attention compared to studies on neuronal mitochondria, recent studies indicate that mitochondria play a crucial role in driving the transition of astrocytes from a quiescent to a reactive state in various neurological disorders. Notably, stimulating mitochondria in astrocytes has been shown to reduce damage associated with the neurodegenerative disease amyotrophic lateral sclerosis.

Here, we focus on studies supporting the emerging paradigm that metabolic reprogramming occurs in astrocytes following damage, which is associated with their phenotypic shift to a new functional state that significantly influences the progression of pathology. Thus, exploring mitochondrial activity and metabolic reprogramming within glial cells may provide valuable insights for developing innovative therapeutic approaches to mitigate neuronal damage. In this review, we focus on studies supporting the emerging paradigm that metabolic reprogramming occurs in astrocytes following damage, which is associated with their phenotypic shift to a new functional state that significantly influences the progression of pathology. Thus, exploring mitochondrial activity and metabolic reprogramming within glial cells may provide valuable insights for developing innovative therapeutic approaches to mitigate neuronal damage.

Introduction

Brain energy metabolism is linked to neuronal activity, a concept that was recognized in the mid-20th century and has been consistently supported since then (McIlwain et al., 1951; Van den Berg et al., 1969). Numerous studies have further supported this hypothesis (Pellerin and Magistretti, 1994; Poitry-Yamate et al., 1995; Theparambil et al., 2024). This process is mediated through the precise metabolic coupling between neurons, glia, and vascular cells (Lauro and Limatola, 2020; Bonvento and Bolaños, 2021; Kugler et al., 2021).

Glial cells, traditionally regarded as supportive elements in the nervous system, are increasingly being recognized as active components of the neural tissue together with neurons. These include ectoderm-originated macroglia (radial glia, astrocytes, oligodendrocytes, NG2-positive progenitors) and microglia, the mesoderm-derived macrophages of the central nervous system (CNS). Through dynamic monitoring of CNS structure and function, glial cells respond to environmental changes and provide homoeostatic support and defense

☆ This article is part of a special issue entitled: 'SNU 30th anniversary' published in Neuroscience.

* Corresponding autor at: Departamento de Histología y Embriología, Facultad de Medicina, Universidad de la República., Avda. Gral. Flores 2125, Montevideo, Uruguay.
E-mail address: pcassina@fmed.edu.uy (P. Cassina).

https://doi.org/10.1016/j.neuroscience.2024.12.058
Received 5 October 2024; Accepted 30 December 2024
Available online 7 January 2025

of the nervous tissue during physiological or damaging conditions (Allen and Lyons, 2018). The number and relative proportions of neurons and different types of glial cells vary by region (e.g., gray matter versus white matter; cerebral versus cerebellar cortex), developmental stage, and species (Von Bartheld et al., 2016). Importantly, growing evidence supports the morphological and functional diversity of glial cells, which is likely influenced by both intrinsic developmental programs and extrinsic interactions with their environment (Trias et al., 2018; Khakh and Deneen, 2019; Reichenbach and Bringmann, 2020; Vidal-Itriago et al., 2022).

In the context of injury and disease, astrocytes and microglia, the two major non-neuronal cell types in the CNS, experience morphological, transcriptional, and functional alterations that modify the final outcome of the damage. This phenomenon is referred to as glial reactivity, first described in the early 20th century with the advent of silver staining techniques for nervous tissue. It continues to be actively studied today due to its significant impact on the progression of neurological pathologies (Liddelow and Barres, 2017; Sofroniew, 2020; Escartin et al., 2021).

During reactivity, astrocytes and microglia release cytokines, chemokines, reactive oxygen species, and secondary messengers, which drive an inflammatory response within the brain or spinal cord, commonly referred to as neuroinflammation. Endothelial and immune cells originating from the periphery also contribute to this process, which has a wide range of immune, physiological, and biochemical effects that can lead to immune cell recruitment, edema, tissue damage, and even cell death (Yang and Zhou, 2019).

The study of reactive astrocytes within the Uruguayan neuroscience community began more than 20 years ago, following one of the earliest observations that astrocyte–motor neuron interactions influence motor neuron survival. This was demonstrated while studying the trophic effects of the glutamatergic antagonist riluzole on primary rat motor neuron cultures (Peluffo et al., 1997). It was observed that the drug's trophic effect was significantly enhanced when motor neurons were co-cultured with astrocytes. This finding contributed significantly to advancing knowledge about riluzole, the first drug approved for the treatment of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by progressive motor neuron loss. Subsequently, a series of publications emerged in the field, which can be reviewed in (Pehar et al., 2005; Olivera-Bravo et al., 2016; Trias et al., 2018). The result of this collaborative work between different generations of scientists led to the inclusion of Uruguayan researchers in the establishment of guidelines for glial research (Escartin et al., 2021).

Glial cells cooperate with neurons in energy metabolism, with astrocytes playing a critical role. Several features of astrocytes contribute to their metabolic differences with neurons, including a higher rate of glycolysis and lactate release, the presence of glutamate uptake transporters, different sensitivities of monocarboxylate transporters, the presence of glycogen, extensive gap junction connections, the expression of distinct isoforms of synthetic enzymes (e.g., isocitrate dehydrogenase, pyruvate carboxylase, pyruvate kinase, lactate dehydrogenase), as well as different glucose uptake mechanisms (Verkhratsky et al., 2021).

In this regard, considering astrocytes as essentially glycolytic cells, little attention has been given to their mitochondrial function. Although astrocytes respond to increased neuronal activity and metabolic demands by enhancing glycolysis and glycogenolysis, they also have a substantial capacity for oxidative (mitochondrial) metabolism. In fact, the mitochondrial network in astrocytes is highly developed and exhibits changes in response to variations in neural function (Jackson and Robinson, 2018). This complex meshwork of mitochondria in astrocytes is conserved across different regions of the CNS despite their significant morphological and functional heterogeneity, suggesting the involvement of tightly regulated mechanisms supporting a relevant cell function (Göbel et al., 2018).

In the case of microglia, their roles in brain energy homeostasis have been less thoroughly explored. Analysis of a cell-type-specific gene expression database generated from acutely dissociated mouse brains reveals that microglia express the full complement of genes required for both glycolytic and oxidative energy metabolism (Ghosh et al., 2018). As with other mononuclear immune-resident cells, their metabolic state drives their functional phenotypes. Under homeostatic conditions, adenosine triphosphate (ATP) is generated in microglia via glucose metabolism through glycolysis, the tricarboxylic acid cycle (TCA), and mitochondrial oxidative phosphorylation (OXPHOS), but after proinflammatory stimuli, metabolic reprogramming shifts their energy production to glycolysis (Lynch, 2020).

The exact way in which the metabolic contribution of glial cells is affected during glial reactivity remains under investigation. Research on reactive astrocytes has focused on evaluating various molecular and functional parameters across different types of injury to assess their impact on key pathological features in relevant models (Zamanian et al., 2012; Escartin et al., 2021). Among them, the study of energy metabolism and mitochondrial morphology has received increased attention recently and indicates that mitochondria contribute to the transition of glial cells from a quiescent to a reactive state in various neurological disorders. Notably, stimulating mitochondrial respiration in glial cells has been shown to reduce damage associated with ALS and chronic pain (Cassina et al., 2021).

This review will focus on metabolic changes in glial cells, mainly in astrocytes, during reactivity associated with neuronal injury, primarily in ALS and chronic pain, which profoundly modulate their reactive phenotype. This has led to a new paradigm proposing that what is typically characterized as mitochondrial dysfunction may actually represent a metabolic adaptation to damage, which: 1) modifies glial functions under normal conditions, 2) impacts the progression of disease, and 3) may be reversible through mitochondrial-targeted therapies.

This information may contribute to identifying therapies aimed at mitigating the harmful effects of reactive astrocytes, enhancing their neuroprotective and glioprotective actions, and restoring or strengthening their homeostatic, modulatory, and defensive roles.

The metabolic flexibility of glial cells

There is a substantial difference between neurons and glial cells regarding their metabolic flexibility and adaptability in different situations (Cantando et al., 2024). Neurons depend on OXPHOS, and as such, they present a strictly oxidative metabolism (Herrero-Mendez et al., 2009), whereas glial cells such as astrocytes and microglia exhibit a higher metabolic flexibility that is deeply linked to their function and activation state (Amo-Aparicio et al., 2023).

Astrocytes support neuronal function by providing an energy supply and protection against oxidative stress, activities linked to their glycolytic activity, the pentose phosphate pathway, and lipid metabolism (Bonvento and Bolaños, 2021). Astrocytes are thought to primarily meet their energy needs through the glycolysis pathway (Dienel and Cruz, 2006; Bélanger et al., 2011), although recent findings suggest that this process coexists with fatty acid oxidation (Morant-Ferrando et al., 2023). Nonetheless, astrocytes possess a highly developed mitochondrial network that adapts to variations in neural function (Jackson and Robinson, 2018) and demonstrates a substantial capacity for oxidative metabolism (Hertz et al., 2007).

These cells are particularly suited to accomplish energetic metabolism: they exhibit high expression of glucose transporters (Allaman et al., 2011), are connected by gap junction channels (Rouach et al., 2008), and express glucose-metabolizing enzymes in their perivascular regions (Escartin and Rouach, 2013). They possess specific glycolytic enzymes that enable them to derive 80 % of their glucose supply through glycolysis (Bélanger et al., 2011; Dienel, 2019). Astrocytes exhibit higher glycolytic activity due to their elevated expression of the enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (pFKFB3), in contrast to neurons, which express this enzyme at lower levels (Herrero-

Mendez et al., 2009). Pfkfb3 controls both the synthesis and degradation of fructose-2,6-bisphosphate (F2,6BP), a regulatory molecule that controls glycolysis in eukaryotes (Bolaños et al., 2010). F2,6BP, in turn, activates glycolysis through allosteric modulation of phosphofructokinase. In addition, astrocytes predominantly express lactate dehydrogenase 5 (LDH5), facilitating the conversion of pyruvate to lactate, while neurons express LDH1, which favors the reverse reaction (Bittar et al., 1996). The higher nicotinamide adenine dinucleotide reduced (NADH) to oxidized (NAD +) ratio in astrocytes further promotes the reduction of pyruvate to lactate (Mongeon et al., 2016). In response to neuronal activity, astrocytes uptake glutamate, which is recycled through the glutamate-glutamine cycle (Bak et al., 2006; McKenna, 2007). The uptake of glutamate triggers aerobic glycolysis in astrocytes, leading to the release of lactate. According to the astrocyte-neuron lactate shuttle model (Pellerin and Magistretti, 1994), neurons then utilize these extracellular monocarboxylates, directing them into the mitochondrial respiratory chain and OXPHOS to generate energy.

Astrocyte mitochondrial function, while important, does not appear to be essential for cell survival, even in situations of injury. Specifically, when astrocytes and neurons are exposed to concentrations of nitric oxide that inhibit mitochondrial respiration, neurons undergo apoptosis due to insufficient ATP production. In contrast, astrocytes maintain ATP production through glycolysis and survive (Almeida et al., 2001). The ability of the cell to switch to glycolytic metabolism is sufficient to provide ATP for maintaining survival, offering further evidence of the metabolic flexibility of astrocytes. For a complete review of mitochondrial metabolism in astrocytes, see (Rose et al., 2020).

Microglia, the resident immune cells of the CNS, have often been explored from an immunological perspective. However, recent evidence suggests that these cells may also influence brain metabolism. In the adult brain, at the resting, homeostatic state, they continuously extend and retract their highly branched, motile processes to actively monitor the surrounding environment (Nimmerjahn et al., 2005). Upon identifying cellular damage and/or pathogen molecular patterns, microglial activation involves reprogramming of cell metabolism, switching from oxidative phosphorylation to the aerobic glycolytic pathway (Lauro and Limatola, 2020). These metabolic adaptations also include the triggering of a lipid biosynthetic program (Button et al., 2014) and upregulation in oxidant production accompanied by mitochondrial fragmentation (Nair et al., 2019). In addition, brain-resident microglia might also adapt to use glutamine as an alternative metabolic source in unperturbed or glucose-deprived conditions, allowing the maintenance of their critical immune surveillance functions (Bernier et al., 2020). Moreover, recent evidence indicates that microglial metabolic flexibility protects neuronal network function against alterations in local substrate availability during moderate neuroinflammation (Chausse et al., 2024).

These studies indicate that astrocytes, as well as microglia, exhibit wide metabolic flexibility, enabling them to adapt rapidly to metabolic changes occurring during periods of high neuronal demand and/or pathology.

Reduced mitochondrial respiratory activity in reactive astrocytes

Glial reactivity has been regarded as a stereotyped response to injury in the nervous system. However, subsequent transcriptomic analysis from isolated astrocytes showed that reactive astrocyte phenotype strongly depended on the type of inducing injury (Zamanian et al., 2012). In this study, although a core set of genes was upregulated in reactive astrocytes from two different injury models, ischemia and LPS injection, at least 50 % of the altered gene expression was specific to each injury type. Reactive astrocytes in ischemia exhibited a molecular phenotype that suggests that they may be beneficial or protective, whereas reactive astrocytes induced by LPS exhibited a phenotype that suggests that they may be detrimental. These findings demonstrate that, despite well-established commonalities, astrocyte reactive gliosis is a highly heterogeneous state in which astrocyte activities are altered to respond to the specific injury. Since then, research on reactive astrocytes has involved the assessment of multiple molecular and functional parameters, including metabolic studies, plus the determination of impact on pathological hallmarks in relevant models to analyze more precisely the glial response (Escartin et al., 2021). With regard to metabolism, reduced mitochondrial respiration has been identified in a series of neurological diseases, some of which are detailed below.

Amyotrophic lateral sclerosis

ALS is a human neurodegenerative disease characterized by motor neuron death and glial reactivity. Since the earliest identification of mitochondrial abnormalities in tissues of individuals with ALS, mitochondrial dysfunction has been recognized as a key factor in the pathogenesis of the disease (Cozzolino et al., 2013; Islam, 2017; Smith et al., 2019; Obrador et al., 2021). A range of structural and functional mitochondrial abnormalities has been documented in ALS patients, including aggregates of dysfunctional mitochondria found in skeletal muscle, lumbar motor neurons, and motor nerve terminals (Manfredi and Xu, 2005). Similar findings have been reproduced in animal models. In the classical mutated SOD1 models (SOD1-G93A), as well as in models with mutated TDP-43 or FUS proteins, mitochondrial fragmentation and vacuolization were observed (Smith et al., 2019). In these models, mitochondrial defects arise even before motor symptoms appear, with significant reductions in respiratory activity and ATP synthesis in mitochondria isolated from the spinal cord (Cassina et al., 2008; Cassina et al., 2021).

A reduction in mitochondrial respiration observed in ALS animal models has been described in whole spinal cord samples, irrespective of cell type (Jung et al., 2002; Mattiazzi et al., 2002). However, it is clear that mitochondrial defects occur in both neurons (Menzies et al., 2002) and glial cells (Cassina et al., 2008; Miquel et al., 2012; Martínez-Palma et al., 2019). Astrocytes expressing mutant SOD1-G93A show impaired mitochondrial respiratory function, reduced ATP synthesis, and increased production of reactive oxygen and nitrogen species, such as nitric oxide (NO), superoxide, and peroxynitrite (Cassina et al., 2008; Miquel et al., 2012; Miquel et al., 2014). These changes result in nitro-oxidative damage to mitochondrial proteins, further exacerbating the dysfunction and energy metabolism impairments (Smith et al., 2019). Importantly, these mitochondrial deficits in astrocytes are associated with decreased motor neuron survival in a co-culture setting, highlighting the relevance of astrocytic mitochondrial activity on their ability to support motor neurons, with evidence for a link between mitochondrial respiratory activity and a neurotrophic-neurotoxic phenotype balance in astrocytes (Cassina et al., 2008; Martínez-Palma et al., 2019; Miquel et al., 2024). This link is also present in aberrant glial cells isolated from the spinal cord of symptomatic SOD1-G93A-expressing rats, which exhibit an even greater neurotoxic activity toward motor neurons in culture, which is also associated with their mitochondrial activity (Díaz-Amarilla et al., 2011; Martínez-Palma et al., 2019). Furthermore, the selective inhibition of mitochondrial activity in non-transgenic astrocytes results in reduced trophic support for motor neurons in co-culture, further underscoring the importance of mitochondrial function in glial cells (Cassina et al., 2008).

How reduced mitochondrial respiration in astrocytes could account for their neurotrophic-neurotoxic state in ALS needs to be further resolved. Mitochondrial activity profoundly affects ATP production, cellular redox balance, and lipid metabolism. Altered mitochondrial activity causes an increase in reactive oxygen and nitrogen species, leading to nitro-oxidative damage in SOD1-G93A astrocytes. This triggers downstream signaling events that promote a neurotoxic phenotype. In fact, targeting oxidative stress with mitochondria-targeted antioxidants can prevent astrocyte-mediated motor neuron death in culture and extend survival *in vivo* in SOD1-G93A mice (Cassina et al., 2008; Miquel et al., 2014). The reduced mitochondrial respiration characteristic of

proinflammatory states is often associated with elevated lactate levels in various neuropathological conditions (Chen et al., 2022). Excessive lactate transmission into neurons can lower pH levels, resulting in a failure of mitochondrial function and apoptosis, which ultimately impacts brain function (Schwartz et al., 2020). Notably, astrocytes derived from SOD1-G93A mice produce more extracellular lactate in culture compared to non-transgenic astrocytes (Valbuena et al., 2017). Then, while lactate is essential for maintaining neuronal energy supply under physiological conditions, astrocyte-mediated toxicity in ALS models does not depend on reduced lactate production. In fact, enhancing glial mitochondrial respiratory activity (and lowering lactate production) using dichloroacetate (DCA), an orally available drug with therapeutic applications, including inherited mitochondrial disorders (James et al., 2017), reduced astrocyte-mediated motor neuron death in culture and extended survival in SOD1-G93A mice (Miquel et al., 2012; Martínez-Palma et al., 2019). DCA specifically inhibits Pyruvate Dehydrogenase kinases (PDK)(Knoechel et al., 2006). In turn, PDK inhibits, by phosphorylation, the activity of the pyruvate dehydrogenase complex (PDH), which is responsible for the conversion of pyruvate to acetyl-CoA. As such, PDK inhibition keeps PDH in its active, unphosphorylated state, feeding the TCA cycle and mitochondrial respiratory activity. SOD1-G93A astrocytes treated with DCA improved their mitochondrial respiratory activity and restored their ability to support motor neuron survival (Miquel et al., 2012). Additionally, activation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and subsequent transcription of antioxidant response element (ARE)-containing genes via electrophilic nitro-fatty acids reduced SOD1-G93A astrocyte-mediated toxicity to motor neurons in culture (Diaz-Amarilla et al., 2016), highlighting the importance of redox balance in astrocyte-mediated motor neuron death.

Mitochondrial activity and cytosolic oxidant levels can modulate the formation and release of exosomes, a subtype of extracellular vesicles, by mechanisms involving activation of neutral sphingomyelinase activity, a critical step in exosome formation (Cutler et al., 2002; Alessenko et al., 2005; Khodorova et al., 2013). This could establish another link between mitochondrial respiration and astrocyte-mediated neurotoxic mechanisms. There is evidence that the exosomal signaling pathway may contribute to modulating astrocyte-mediated toxicity in ALS (Basso et al., 2013; Ferrara et al., 2018; Marton et al., 2023). For example, astrocytes in the SOD1-G93A ALS model show an increase in exosome release and transport the human SOD1-G93A protein to motor neurons (Basso et al., 2013). Furthermore, SOD1-G93A astrocyte-derived exosomes are sufficient to reduce neurite outgrowth and survival of motor neurons in the presence of glial cell line-derived neurotrophic factor (GDNF) to the level exhibited upon trophic factor deprivation, with a role for the microRNA miRNA 155-5p in exosome-mediated motor neuron death (Marton et al., 2023).

Another possibility is that mitochondrial respiration contributes to astrocyte-mediated toxicity by affecting lipid metabolism in astrocytes. Lipid metabolism has been linked to astrocyte-mediated motor neuron death (Agrawal et al., 2022). Astrocytes are the main site for lipid oxidation and storage in CNS (Liu et al., 2017), and dysregulation of astrocytic lipid metabolism has been described in ALS (Chaves-Filho et al., 2019; Velebit et al., 2020). Elevated oxidative stress and mitochondrial dysfunction in neurons induce the transfer of lipids to neighboring astrocytes and the accumulation of lipid droplets in glial cells (Liu et al., 2017). Lipid droplets are dynamic organelles that can serve as storage sites for lipids and are regulated in response to different cellular and physiological conditions, including mitochondrial dysfunction (Renne and Hariri, 2021). Lipid droplet content increases in astrocytes under hypoxia conditions (Smolič et al., 2021) or upon oxidative stress (Islam et al., 2019). A recent study demonstrated that astrocytes become reactive in response to oxidative stress, secreting long-chain saturated fatty acids which are neurotoxic (Guttenplan et al., 2021). In addition, lipid droplets were reduced when mitochondrial OXPHOS activity was stimulated in SOD1-G93A astrocytes, suggesting a link between mitochondria, lipid storage, and astrocyte-mediated toxicity (Miquel et al., 2024).

Chronic pain

Chronic pain is another condition linked to central nervous system damage and glial activation. Typically defined as pain persisting for three months or longer (Treede et al., 2019), it is the leading cause of disability worldwide when considering all pain-related conditions, including low back pain, headaches, and neck pain (James et al., 2018). In particular, neuropathic pain is an especially severe type of chronic pain resulting from damage or disease affecting the somatosensory nervous system, caused by conditions like diabetes, human immunodeficiency virus (HIV) infection, and chemotherapy, among others (Cavalli et al., 2019).

The role of mitochondrial dysfunction in sensory processing and the establishment of chronic pain is relatively unexplored. Some reviews have approached a comprehensive understanding of how mitochondrial dysfunction connects inflammation and damage-associated pathways to neuronal sensitization and persistent pain (Silva Santos Ribeiro et al., 2022; Willemen et al., 2023). Moreover, morphological and functional alterations in mitochondria have been reported in patients and animal models of pathological pain (Flatters, 2015). However, very few reports analyzed the mitochondrial respiratory function at the central nervous system level. For example, reduced oxygen consumption by mitochondria has been described in paclitaxel-induced painful neuropathy, measured at isolated dorsal ganglia cells (Duggett et al., 2017) and at the sciatic nerve (Zheng et al., 2011), but an increase in oxygen consumption was measured in peripheral nerves after partial nerve ligation (Lim et al., 2015).

When analyzing high-resolution respirometry in spinal cord tissue, a decrease in mitochondrial respiratory activity was detected at the lesion site in mice with chronic constriction of the sciatic nerve. Additionally, in rats injected with Freund's adjuvant in the posterior paw, a decrease in mitochondrial respiration in the spinal cord was observed on the ipsilateral side of the lesion but not on the contralateral side (Lagos-Rodríguez et al., 2020). These findings clearly associate a decrease in mitochondrial oxygen consumption with the site of the first synapse in the pain pathways in animals with chronic pain.

As in the ALS model, stimulation of mitochondrial respiration by DCA administration reduced the levels of phosphorylated PDH and increased the mitochondrial oxygen consumption at the spinal cord level (Lagos-Rodríguez et al., 2020). Importantly, this treatment attenuated the hyperalgesia and mechanical allodynia, indicating that PDK inhibition affects the synaptic plasticity associated with central sensitization in chronic pain. Therefore, PDK modulation can be proposed as a new target to reduce pain. In agreement with these results, the genetic ablation of PDK2/4 (the astrocyte isoforms of PDK) reduced the pain-associated behavior in mice with formalin paw injection. Thermal hypersensitivities and glial reactivity in the spinal cord were significantly reduced in Pdk2/4-deficient mice (Jha et al., 2016). DCA treatment led to decreased astrocyte and microglia reactivity, so a potential effect of DCA on microglial cells cannot be ruled out. Additionally, DCA has been shown to shift glucose metabolism toward the TCA cycle and oxidative phosphorylation in astrocytes (Miquel et al., 2012) and in T cells in mice affected by experimental autoimmune encephalomyelitis (Gerriets et al., 2015). Recently, DCA treatment has been shown to improve cognitive dysfunction and neuronal loss in a sepsis mouse model by reducing proinflammatory phenotype in microglia and blood macrophages, and also prevented the decrease in mitochondrial membrane potential triggered by LPS and ATP in BV2 cells (Huang et al., 2024). Astrocytes and also microglia are involved in synapsis structure and physiology (Ball et al., 2022; Oliveira and Araque, 2022). In particular, perisynaptic astrocytic processes are key elements in regulating synaptic transmission as components of tripartite synapses (Ko et al., 2023). In fact, recent data show that astrocytes modified GLT1 expression during

chronic pain conditions (Benson et al., 2023). To date, several astrocyte-secreted mediators that induce synaptic plasticity have been identified (Sancho et al., 2021). However, further studies are needed to fully understand the impact of modulating metabolism on the synapsis plasticity linked to chronic pain.

Metabolic reprogramming of astrocytes: A target to modify disease

As described above, astrocytes have the ability to undergo significant metabolic changes in pathological states. This metabolic reprogramming lets them meet increased energy demands and support their reactive functions associated with those states (Calì et al., 2024). We have proposed that this metabolic reprogramming, characterized by reduced mitochondrial respiratory activity with a decreased capacity to respond to energetic demands, is particularly relevant in ALS. We have demonstrated that it occurs in SOD1-G93A-expressing astrocytes and that it is critically associated with their phenotype shift towards a motor neuron-degeneration-inducing astrocyte (Cassina et al., 2021). This was previously described as mitochondrial dysfunction in SOD1-G93A-expressing astrocytes but can now be considered an adaptive metabolic reprogramming associated with a new functional state, a concept not exclusive to ALS. In fact, metabolic reprogramming was shown in animal models of Huntington and Duchenne muscular dystrophy (Polyzos et al., 2019; Bellissimo et al., 2022).

It is important to highlight that the reduction in mitochondrial respiration is not irreversible; in fact, it can be reversed with mitochondria-targeted therapies (Cassina et al., 2008; Miquel et al., 2012; Miquel et al., 2014; Miquel et al., 2024). As such, the signaling and phenotypical consequences of that metabolic reprogramming can also be modulated, with evidence for a reduction in SOD1-G93A astrocyte-mediated motor neuron death in culture after stimulation of mitochondrial respiratory activity or mitochondria-targeted antioxidants.

A key point in building our hypothesis was the finding that specific silencing of isoform 2 of pyruvate dehydrogenase kinase (PDK2), which is highly expressed in astrocytes but shows low expression in neurons and microglia, caused a switch in mitochondrial metabolism in SOD1-G93A astrocytes. This change was associated with a recovery in the ability of astrocytes to support neuronal survival (Miquel et al., 2024). Silencing PDK2, as well as previous work involving DCA treatment in SOD1-G93A-expressing astrocytes, induced a significant transformation in the mitochondrial network of the astrocytes. The network shifted from clustered, fragmented organelles with decreased OXPHOS to a branched, connected, respiratory-active elongated network resembling that of non-transgenic astrocytes (Miquel et al., 2024). These results underscore the flexibility and adaptability of astrocyte metabolism and mitochondrial activity.

Although astrocyte OXPHOS is not required for survival, its activity has been reported as a source of reactive oxygen species due to inefficient complex activity and electron transfer (Lopez-Fabuel et al., 2016). Following glycolysis, pyruvate is typically diverted to anaplerotic reactions to synthesize TCA cycle intermediates instead of being converted into acetyl-CoA. This process is constrained by reduced PDH activity due to phosphorylation via PDK (Halim et al., 2010; Jakoby et al., 2014). This scenario suggests that in vivo therapies targeting the prevention of PDH phosphorylation could have a greater impact on astrocytes, as their pool of PDH remains phosphorylated, compared to microglia or neurons, where PDH is generally dephosphorylated.

Recent studies further support this hypothesis. For example, analyzing the proteomic profile and secreted metabolome of primary spinal cord astrocytes derived from SOD1-G93A mice, compared to mice overexpressing wild-type human SOD1 (hSOD1-WT), revealed that certain metabolic pathways were predominantly altered. These include fatty acid biosynthesis, regulated by the availability of acetyl-CoA, and amino acid and glutathione (GSH) metabolism, many of which are mitochondrial processes (Stella et al., 2021).

Additionally, a study investigating the tissue metabolome in the lumbar spinal cord of two ALS mouse models with differing disease progression rates—SOD1-G93A C57BL/6JOlaHsd (C57-G93A, slow progression) and SOD1-G93A-129SvHsd (129S-G93A, fast progression)—revealed differences in metabolites involved in energy and lipid metabolism (Valbuena et al., 2019). Notably, more significant metabolic trajectory differences were observed after symptom onset than those attributed to mouse genetic background, indicating that shifts in energy metabolism correlate with increased glial reactivity.

Recent studies show that the detrimental effects of reactive astrogliosis are not only due to pro-inflammatory and neurotoxic gain of function but also a loss of their homeostatic functions. This pathological remodeling could be linked to epigenetic remodeling of chromatin. LPS-induced and traumatic brain injury-induced reactive astrocytes exhibit not only increased expression of pro-inflammatory factors, but also persistent DNA hypermethylation, leading to a decreased expression of homeostatic genes, including those involved in the lactate shuttle to neurons (Cuautle et al., 2024). ALS astrocytes are known to exist in a pro-inflammatory state, suggesting a possible link between inflammation and epigenetic reprogramming in ALS, a valuable mechanistic insight that would need to be explored.

Moreover, recent findings identified tissue-specific changes in the relative expression and daily expression patterns of metabolic enzymes, including Pfkfb3, in SOD1-G93A mice (Killoy et al., 2021). As mentioned earlier, Pfkfb3 controls the synthesis and degradation of F2,6BP, the allosteric activator of phosphofructokinase that regulates glycolysis. Thus, altered Pfkfb3 expression may contribute to disrupted glycolysis and gluconeogenesis seen in the spinal cord of hSOD1-G93A mice. This data supports the idea that in the neurodegenerative inflammatory environment, there is a metabolic remodeling of energy metabolism pathways, including not only OXPHOS but also glycolysis, glycogenolysis, and others.

All these metabolic alterations across different pathways, observed in ALS models or other conditions involving glial reactivity, demonstrate that metabolic remodeling occurs in response to injury. Consequently, the activation of a specific pathway—in this case, the Krebs cycle and OXPHOS—suggests a metabolic modulation that restores astrocytic function to its pre-injury or resting state. However, how this modulation influences other homeostatic activities of astrocytes requires further research.

Final remarks

Metabolic studies in astrocytes allow us to conclude that, unlike neurons, these cells exhibit metabolic flexibility that enables them to survive damage and contribute to repair. However, this flexibility entails phenotypic modifications that may influence the course of the pathology. Mitochondria are, therefore, key organelles in determining the glial phenotype in ALS, chronic pain, and potentially other CNS diseases. Treatments that modify mitochondrial metabolism produce phenotypic changes in glial cells that alter the course of the pathology, as represented in the graphical abstract of this review. Gaining a better understanding of the metabolic reprogramming that glial cells undergo during pathology could help identify targets for designing alternative therapies for these diseases, which currently lack effective pharmacological treatments.

CRediT authorship contribution statement

Patricia Cassina: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Ernesto Miquel:** Writing – review & editing, Writing – original draft. **Laura Martínez-Palma:** Writing – review & editing, Writing – original draft. **Adriana Cassina:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Reference

Agrawal, I., Lim, Y.S., Ng, S.-Y., Ling, S.-C., 2022. Deciphering lipid dysregulation in ALS: from mechanisms to translational medicine. Transl. Neurodegener. 11, 48.

Alessenko, A.V., Shupik, M.A., Gutner, U.A., Bugrova, A.E., Dudnik, L.B., Shingarova, L. N., Mikoyan, A., Vanin, A.F., 2005. The relation between sphingomyelinase activity, lipid peroxide oxidation and NO-releasing in mice liver and brain. FEBS Lett. 579, 5571–5576.

Allaman, I., Bélanger, M., Magistretti, P.J., 2011. Astrocyte–neuron metabolic relationships: for better and for worse. Trends Neurosci. 34, 76–87.

Allen, N.J., Lyons, D.A., 2018. Glia as architects of central nervous system formation and function. Science 362, 181–185.

Almeida, A., Almeida, J., Bolaños, J.P., 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. 98, 15294–15299.

Amo-Aparicio, J., Dinarello, C.A., Lopez-Vales, R., 2023. Metabolic reprogramming of the inflammatory response in the nervous system: the crossover between inflammation and metabolism. Neural Regen. Res. 19, 2189–2201.

Bak, L.K., Schousboe, A., Waagepetersen, H.S., 2006. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis, and ammonia transfer. J. Neurochem. 98, 641–653.

Ball, J.B., Green-Fulgham, S.M., Watkins, L.R., 2022. Mechanisms of microglia-mediated synapse turnover and synaptogenesis. Prog. Neurobiol. 218, 102336.

Basso, M., Pozzi, S., Tortarolo, M., Fiordaliso, F., Bisighini, C., Pasetto, L., Spaltro, G., Lidonnici, D., Gensano, F., Battaglia, E., Bendotti, C., Bonetto, V., 2013. Mutant copper-zinc superoxide dismutase (SOD1) induces protein secretion pathway alterations and exosome release in astrocytes: implications for disease spreading and motor neuron pathology in amyotrophic lateral sclerosis. J. Biol. Chem. 288, 15699–15711.

Bélanger, M., Allaman, I., Magistretti, P.J., 2011. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell Metab. 14, 724–738.

Bellissimo, C.A., Garibotti, M.C., Perry, C.G.R., 2022. Mitochondrial stress responses in Duchenne muscular dystrophy: metabolic dysfunction or adaptive reprogramming? Am. J. Physiol. Cell Physiol. 323, C718–C730.

Benson, C.A., King, J.F., Kauer, S.D., Waxman, S.G., Tan, A.M., 2023. Increased astrocytic GLT-1 expression in tripartite synapses is associated with SCI-induced hyperreflexia. J. Neurophysiol. 130, 1358–1366.

Bernier, L.-P., York, E.M., Kamyabi, A., Choi, H.B., Weilinger, N.L., MacVicar, B.A., 2020. Microglial metabolic flexibility supports immune surveillance of the brain parenchyma. Nat. Commun. 11, 1559.

Bittar, P.G., Charnay, Y., Pellerin, L., Bouras, C., Magistretti, P.J., 1996. Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. J. Cereb. Blood Flow Metab. 16, 1079–1089.

Bolaños, J.P., Almeida, A., Moncada, S., 2010. Glycolysis: a bioenergetic or a survival pathway? Trends Biochem. Sci. 35, 145–149.

Bonvento, G., Bolaños, J.P., 2021. Astrocyte-neuron metabolic cooperation shapes brain activity. Cell Metab. 33, 1546–1564.

Button, E.B., Mitchell, A.S., Domingos, M.M., Chung, J.-H.-J., Bradley, R.M., Hashemi, A., Marvyn, P.M., Patterson, A.C., Stark, K.D., Quadrilatero, J., Duncan, R. E., 2014. Microglial cell activation increases saturated and decreases monounsaturated fatty acid content, but both lipid species are proinflammatory. Lipids 49, 305–316.

Calì, C., Cantando, I., Veloz Castillo, M.F., Gonzalez, L., Bezzi, P., 2024. Metabolic reprogramming of astrocytes in pathological conditions: implications for neurodegenerative diseases. Int. J. Mol. Sci. 25, 8922.

Cantando, I., Centofanti, C., D'Alessandro, G., Limatola, C., Bezzi, P., 2024. Metabolic dynamics in astrocytes and microglia during post-natal development and their implications for autism spectrum disorders. Front. Cell. Neurosci. 18, 1354259.

Cassina, P., Cassina, A., Pehar, M., Castellanos, R., Gandelman, M., De León, A., Robinson, K.M., Mason, R.P., Beckman, J.S., Barbeito, L., Radi, R., 2008. Mitochondrial dysfunction in SOD1 ^{G93A} -bearing astrocytes promotes motor neuron degeneration: prevention by mitochondrial-targeted antioxidants. J. Neurosci. 28, 4115–4122.

Cassina, P., Miquel, E., Martínez-Palma, L., Cassina, A., 2021. Glial Metabolic reprogramming in amyotrophic lateral sclerosis. Neuroimmunomodulation 28, 204–212.

Cavalli, E., Mammana, S., Nicoletti, F., Bramanti, P., Mazzon, E., 2019. The neuropathic pain: an overview of the current treatment and future therapeutic approaches. Int. J. Immunopathol. Pharmacol. 33 (205873841983838).

Chausse, B., Malorny, N., Lewen, A., Poschet, G., Berndt, N., Kann, O., 2024. Metabolic flexibility ensures proper neuronal network function in moderate neuroinflammation. Sci. Rep. 14, 14405.

Chaves-Filho, A.B., Pinto, I.F.D., Dantas, L.S., Xavier, A.M., Inague, A., Faria, R.L., Medeiros, M.H.G., Glezer, I., Yoshinaga, M.Y., Miyamoto, S., 2019. Alterations in lipid metabolism of spinal cord linked to amyotrophic lateral sclerosis. Sci. Rep. 9, 11642.

Chen, X., Zhang, Y., Wang, H., Liu, L., Li, W., Xie, P., 2022. The regulatory effects of lactic acid on neuropsychiatric disorders. Discov. Ment. Health 2, 8.

Cozzolino, M., Ferri, A., Valle, C., Carrì, M.T., 2013. Mitochondria and ALS: implications from novel genes and pathways. Mol. Cell. Neurosci. 55, 44–49.

Cuautle, D.G., Donna, S., Cieri, M.B., Villarreal, A., Ramos, A.J., 2024. Pathological remodeling of reactive astrocytes: involvement of DNA methylation and downregulation of homeostatic genes. J. Neurochem.:jnc.16164.

Cutler, R.G., Pedersen, W.A., Camandola, S., Rothstein, J.D., Mattson, M.P., 2002. Evidence that accumulation of ceramides and cholesterol esters mediates oxidative stress-induced death of motor neurons in amyotrophic lateral sclerosis. Ann. Neurol. 52, 448–457.

Diaz-Amarilla, P., Miquel, E., Trostchansky, A., Trias, E., Ferreira, A.M., Freeman, B.A., Cassina, P., Barbeito, L., Vargas, M.R., Rubbo, H., 2016. Electrophilic nitro-fatty acids prevent astrocyte-mediated toxicity to motor neurons in a cell model of familial amyotrophic lateral sclerosis via nuclear factor erythroid 2-related factor activation. Free Radic. Biol. Med. 95, 112–120.

Díaz-Amarilla, P., Olivera-Bravo, S., Trias, E., Cragnolini, A., Martínez-Palma, L., Cassina, P., Beckman, J., Barbeito, L., 2011. Phenotypically aberrant astrocytes that promote motoneuron damage in a model of inherited amyotrophic lateral sclerosis. PNAS 108, 18126–18131.

Dienel, G.A., 2019. Brain glucose metabolism: integration of energetics with function. Physiol. Rev. 99, 949–1045.

Dienel, G.A., Cruz, N.F., 2006. Astrocyte activation in working brain: energy supplied by minor substrates. Neurochem. Int. 48, 586–595.

Duggett, N.A., Griffiths, L.A., Flatters, S.J.L., 2017. Paclitaxel-induced painful neuropathy is associated with changes in mitochondrial bioenergetics, glycolysis, and an energy deficit in dorsal root ganglia neurons. Pain 158, 1499–1508.

Escartin, C., et al., 2021. Reactive astrocyte nomenclature, definitions, and future directions. Nat. Neurosci. 24, 312–325.

Escartin, C., Rouach, N., 2013. Astroglial networking contributes to neurometabolic coupling. Front Neuroenergetics 5.

Ferrara, D., Pasetto, L., Bonetto, V., Basso, M., 2018. Role of extracellular vesicles in amyotrophic lateral sclerosis. Front. Neurosci. 12, 574.

Flatters, S.J.L., 2015. The contribution of mitochondria to sensory processing and pain. In: Progress in Molecular Biology and Translational Science. Elsevier, pp. 119–146. https://doi.org/10.1016/bs.pmbts.2014.12.004.

Gerriets, V.A., et al., 2015. Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. J. Clin. Invest. 125, 194–207.

Ghosh, S., Castillo, E., Frias, E.S., Swanson, R.A., 2018. Bioenergetic regulation of microglia. Glia 66, 1200–1212.

Göbel, J., Motori, E., Bergami, M., 2018. Spatiotemporal control of mitochondrial network dynamics in astroglial cells. Biochem. Biophys. Res. Commun. 500, 17–25.

Guttenplan, K.A., Weigel, M.K., Prakash, P., Wijewardhane, P.R., Hasel, P., Rufen-Blanchette, U., Münch, A.E., Blum, J.A., Fine, J., Neal, M.C., Bruce, K.D., Gitler, A.D., Chopra, G., Liddelow, S.A., Barres, B.A., 2021. Neurotoxic reactive astrocytes induce cell death via saturated lipids. Nature 599, 102–107.

Halim, N.D., Mcfate, T., Mohyeldin, A., Okagaki, P., Korotchkina, L.G., Patel, M.S., Jeoung, N.H., Harris, R.A., Schell, M.J., Verma, A., 2010. Phosphorylation status of pyruvate dehydrogenase distinguishes metabolic phenotypes of cultured rat brain astrocytes and neurons: inhibited glucose oxidation in astrocytes. Glia 58, 1168–1176.

Herrero-Mendez, A., Almeida, A., Fernández, E., Maestre, C., Moncada, S., 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., 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.

Huang, X., Zheng, Y., Wang, N., Zhao, M., Liu, J., Lin, W., Zhu, Y., Xie, X., Lv, Y., Wang, J., Mo, Y., 2024. Dichloroacetate prevents sepsis associated encephalopathy by inhibiting microglia pyroptosis through PDK4/NLRP3. Inflammation. https://doi.org/10.1007/s10753-024-02105-3.

Islam, M.T., 2017. Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. Neurol. Res. 39, 73–82.

Islam, A., Kagawa, Y., Miyazaki, H., Shil, S.K., Umaru, B.A., Yasumoto, Y., Yamamoto, Y., Owada, Y., 2019. FABP7 protects astrocytes against ROS toxicity via lipid droplet formation. Mol. Neurobiol. 56, 5763–5779.

Jackson, J.G., Robinson, M.B., 2018. Regulation of mitochondrial dynamics in astrocytes: mechanisms, consequences, and unknowns. Glia 66, 1213–1234.

Jakoby, P., Schmidt, E., Ruminot, I., Gutierrez, R., Barros, L.F., Deitmer, J.W., 2014. Higher transport and metabolism of glucose in astrocytes compared with neurons: a multiphoton study of hippocampal and cerebellar tissue slices. Cereb. Cortex 24, 222–231.

James, S.L., et al., 2018. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1789–1858.

James, M.O., Jahn, S.C., Zhong, G., Smeltz, M.G., Hu, Z., Stacpoole, P.W., 2017. Therapeutic applications of dichloroacetate and the role of glutathione transferase zeta-1. Pharmacol. Ther. 170, 166–180.

Jha, M.K., Rahman, M.H., Park, D.H., Kook, H., Lee, I., Lee, W., Suk, K., 2016. Pyruvate dehydrogenase kinase 2 and 4 gene deficiency attenuates nociceptive behaviors in a mouse model of acute inflammatory pain. J. Neurosci. Res. 94, 837–849.

Jung, C., Higgins, C.M.J., Xu, Z., 2002. Mitochondrial electron transport chain complex dysfunction in a transgenic mouse model for amyotrophic lateral sclerosis. J. Neurochem. 83, 535–545.

Khakh, B.S., Deneen, B., 2019. The emerging nature of astrocyte diversity. Annu. Rev. Neurosci. 42, 187–207.

Khodorova, A., Nicol, G.D., Strichartz, G., 2013. The p75NTR signaling cascade mediates mechanical hyperalgesia induced by nerve growth factor injected into the rat hind paw. Neuroscience 254, 312–323.
Killoy, K.M., Pehar, M., Harlan, B.A., Vargas, M.R., 2021. Altered expression of clock and clock-controlled genes in a hSOD1-linked amyotrophic lateral sclerosis mouse model. FASEB J. 35, e22262.
Knoechel, T.R., Tucker, A.D., Robinson, C.M., Phillips, C., Taylor, W., Bungay, P.J., Kasten, S.A., Roche, T.E., Brown, D.G., 2006. Regulatory roles of the N-terminal domain based on crystal structures of human pyruvate dehydrogenase kinase 2 containing physiological and synthetic ligands. Biochemistry 45, 402–415.
Ko, H.-G., Chun, H., Han, S., Kaang, B.-K., 2023. Role of spinal astrocytes through the perisynaptic astrocytic process in pathological pain. Mol. Brain 16, 81.
Kugler, E.C., Greenwood, J., MacDonald, R.B., 2021. The "Neuro-Glial-Vascular" unit: the role of glia in neurovascular unit formation and dysfunction. Front. Cell Dev. Biol. 9, 732820.
Lagos-Rodríguez, V., Martínez-Palma, L., Marton, S., Miquel, E., Escobar-Pintos, R., Cassina, A., Lago, N., Cassina, P., 2020. Mitochondrial bioenergetics, glial reactivity, and pain-related behavior can be restored by dichloroacetate treatment in rodent pain models. Pain 161, 2786–2797.
Lauro, C., Limatola, C., 2020. Metabolic reprograming of microglia in the regulation of the innate inflammatory response. Front. Immunol. 11, 493.
Liddelow, S.A., Barres, B.A., 2017. Reactive astrocytes: production, function, and therapeutic potential. Immunity 46, 957–967.
Lim, T.K.Y., Rone, M.B., Lee, S., Antel, J.P., Zhang, J., 2015. Mitochondrial and bioenergetic dysfunction in trauma-induced painful peripheral neuropathy. Mol. Pain 11.
Liu, L., MacKenzie, K.R., Putluri, N., Maletić-Savatić, M., Bellen, H.J., 2017. The glia-neuron lactate shuttle and elevated ROS promote lipid synthesis in neurons and lipid droplet accumulation in glia via APOE/D. Cell Metab. 26, 719–737.e6.
Lopez-Fabuel, I., Le Douce, J., Logan, A., James, A.M., Bonvento, G., Murphy, M.P., Almeida, A., Bolaños, J.P., 2016. Complex I assembly into supercomplexes determines differential mitochondrial ROS production in neurons and astrocytes. Proc. Natl. Acad. Sci. 113, 13063–13068.
Lynch, M.A., 2020. Can the emerging field of immunometabolism provide insights into neuroinflammation? Prog. Neurobiol. 184, 101719.
Manfredi, G., Xu, Z., 2005. Mitochondrial dysfunction and its role in motor neuron degeneration in ALS. Mitochondrion 5, 77–87.
Martínez-Palma, L., Miquel, E., Lagos-Rodríguez, V., Barbeito, L., Cassina, A., Cassina, P., 2019. Mitochondrial modulation by dichloroacetate reduces toxicity of aberrant glial cells and gliosis in the SOD1G93A rat model of amyotrophic lateral sclerosis. Neurotherapeutics 16, 203–215.
Marton, S., Miquel, E., Acosta-Rodríguez, J., Fontenla, S., Libisch, G., Cassina, P., 2023. SOD1 ^{G93A} astrocyte-derived extracellular vesicles induce motor neuron death by a miRNA-155-5p-mediated mechanism. ASN Neuro 15 (17590914231197527).
Mattiazzi, M., D'Aurelio, M., Gajewski, C.D., Martushova, K., Kiaei, M., Beal, M.F., Manfredi, G., 2002. Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. J. Biol. Chem. 277, 29626–29633.
McIlwain, H., Anguiano, G., Cheshire, J.D., 1951. Electrical stimulation *in vitro* of the metabolism of glucose by mammalian cerebral cortex. Biochem. J. 50, 12–18.
McKenna, M.C., 2007. The glutamate-glutamine cycle is not stoichiometric: fates of glutamate in brain. J. Neurosci. Res. 85, 3347–3358.
Menzies, F.M., Cookson, M.R., Taylor, R.W., Turnbull, D.M., Chrzanowska-Lightowlers, Z.M.A., Dong, L., Figlewicz, D.A., Shaw, P.J., 2002. Mitochondrial dysfunction in a cell culture model of familial amyotrophic lateral sclerosis. Brain J. Neurol. 125, 1522–1533.
Miquel, E., Cassina, A., Martínez-Palma, L., Bolatto, C., Trías, E., Gandelman, M., Radi, R., Barbeito, L., Cassina, P., 2012. Modulation of astrocytic mitochondrial function by dichloroacetate improves survival and motor performance in inherited amyotrophic lateral sclerosis ferreira ST, ed. PLoS One 7, e34776.
Miquel, E., Cassina, A., Martínez-Palma, L., Souza, J.M., Bolatto, C., Rodríguez-Bottero, S., Logan, A., Smith, R.A.J., Murphy, M.P., Barbeito, L., Radi, R., Cassina, P., 2014. Neuroprotective effects of the mitochondria-targeted antioxidant MitoQ in a model of inherited amyotrophic lateral sclerosis. Free Radic. Biol. Med. 70, 204–213.
Miquel, E., Villarino, R., Martínez-Palma, L., Cassina, A., Cassina, P., 2024. Pyruvate dehydrogenase kinase 2 knockdown restores the ability of amyotrophic lateral sclerosis-linked SOD1G93A rat astrocytes to support motor neuron survival by increasing mitochondrial respiration. Glia 72, 999–1011.
Mongeon, R., Venkatachalam, V., Yellen, G., 2016. Cytosolic NADH-NAD $^{+}$ redox visualized in brain slices by two-photon fluorescence lifetime biosensor imaging. Antioxid. Redox Signal. 25, 553–563.
Morant-Ferrando, B., Jimenez-Blasco, D., Alonso-Batan, P., Agulla, J., Lapresa, R., Garcia-Rodriguez, D., Yunta-Sanchez, S., Lopez-Fabuel, I., Fernandez, E., Carmeliet, P., Almeida, A., Garcia-Macia, M., Bolaños, J.P., 2023. Fatty acid oxidation organizes mitochondrial supercomplexes to sustain astrocytic ROS and cognition. Nat. Metab. 5, 1290–1302.
Nair, S., Sobotka, K.S., Joshi, P., Gressens, P., Fleiss, B., Thornton, C., Mallard, C., Hagberg, H., 2019. Lipopolysaccharide-induced alteration of mitochondrial morphology induces a metabolic shift in microglia modulating the inflammatory response in vitro and in vivo. Glia 67, 1047–1061.
Nimmerjahn, A., Kirchhoff, F., Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in Vivo. Science 308, 1314–1318.
Obrador, E., Salvador-Palmer, R., López-Blanch, R., Jihad-Jebbar, A., Vallés, S.L., Estrela, J.M., 2021. The link between oxidative stress, redox status, bioenergetics and mitochondria in the pathophysiology of ALS. Int. J. Mol. Sci. 22, 6352.
Oliveira, J.F., Araque, A., 2022. Astrocyte regulation of neural circuit activity and network states. Glia 70, 1455–1466.
Olivera-Bravo S, Isasi E, Fernández A, Casanova G, Rosillo JC, Barbeito L (2016) Astrocyte Dysfunction in Developmental Neurometabolic Diseases. In: Glial Cells in Health and Disease of the CNS (Von Bernhardi R, ed), pp 227–243 Advances in Experimental Medicine and Biology, vol 949. Springer, Cham. https://doi.org/10.1007/978-3-319-40764-7_11.
Pehar, M., Vargas, M.R., Cassina, P., Barbeito, A.G., Beckman, J.S., Barbeito, L., 2005. Complexity of astrocyte-motor neuron interactions in amyotrophic lateral sclerosis. Neurodegener Dis 2, 139–146.
Pellerin, L., Magistretti, P.J., 1994. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc. Natl. Acad. Sci. 91, 10625–10629.
Peluffo, H., Estevez, A., Barbeito, L., Stutzmann, J.M., 1997. Riluzole promotes survival of rat motoneurons in vitro by stimulating trophic activity produced by spinal astrocyte monolayers. Neurosci. Lett. 228, 207–211.
Poitry-Yamate CL, Poitry S, Tsacopoulos M (1995) Lactate Released by Miiller Glial Cells Is Metabolized by Photoreceptors from Mammalian Retina.
Polyzos, A.A., Lee, D.Y., Datta, R., Hauser, M., Budworth, H., Holt, A., Mihalik, S., Goldschmidt, P., Frankel, K., Trego, K., Bennett, M.J., Vockley, J., Xu, K., Gratton, E., McMurray, C.T., 2019. Metabolic reprogramming in astrocytes distinguishes region-specific neuronal susceptibility in huntington mice. Cell Metab. 29, 1258–1273.e11.
Reichenbach, A., Bringmann, A., 2020. Comparative anatomy of glial cells in mammals. In: Evolutionary Neuroscience. Elsevier, pp. 397–439. https://doi.org/10.1016/B978-0-12-820584-6.00016-7.
Renne, M.F., Hariri, H., 2021. Lipid droplet-organelle contact sites as hubs for fatty acid metabolism, trafficking, and metabolic channeling. Front. Cell Dev. Biol. 9, 726261.
Rose, J., Brian, C., Pappa, A., Panayiotidis, M.I., Franco, R., 2020. Mitochondrial metabolism in astrocytes regulates brain bioenergetics, neurotransmission and redox balance. Front Neurosci. 14, 536682.
Rouach, N., Koulakoff, A., Abudara, V., Willecke, K., Giaume, C., 2008. Astroglial metabolic networks sustain hippocampal synaptic transmission. Science 322, 1551–1555.
Sancho, L., Contreras, M., Allen, N.J., 2021. Glia as sculptors of synaptic plasticity. Neurosci. Res. 167, 17–29.
Schwartz, L., Peres, S., Jolicoeur, M., Da Veiga, M.J., 2020. Cancer and Alzheimer's disease: intracellular pH scales the metabolic disorders. Biogerontology 21, 683–694.
Silva Santos Ribeiro, P., Willemen, H.L.D.M., Eijkelkamp, N., 2022. Mitochondria and sensory processing in inflammatory and neuropathic pain. Front. Pain Res. 3, 1013577.
Smith, E.F., Shaw, P.J., De Vos, K.J., 2019. The role of mitochondria in amyotrophic lateral sclerosis. Neurosci. Lett. 710, 132933.
Smolič, T., Zorec, R., Vardjan, N., 2021. Pathophysiology of lipid droplets in neuroglia. Antioxidants 11, 22.
Sofroniew, M.V., 2020. Astrocyte reactivity: subtypes, states, and functions in CNS innate immunity. Trends Immunol. 41, 758–770.
Stella, R., Bonadio, R.S., Cagnin, S., Massimino, M.L., Bertoli, A., Peggion, C., 2021. Perturbations of the proteome and of secreted metabolites in primary astrocytes from the hSOD1(G93A) ALS mouse model. Int. J. Mol. Sci. 22, 7028.
Theparambil, S.M., Kopach, O., Braga, A., Nizari, S., Hosford, P.S., Sagi-Kiss, V., Hadjihambi, A., Konstantinou, C., Esteras, N., Gutierrez Del Arroyo, A., Ackland, G. L., Teschemacher, A.G., Dale, N., Eckle, T., Andrikopoulos, P., Rusakov, D.A., Kasparov, S., Gourine, A.V., 2024. Adenosine signalling to astrocytes coordinates brain metabolism and function. Nature 632, 139–146.
Treede, R.-D., et al., 2019. Chronic pain as a symptom or a disease: the IASP classification of chronic pain for the international classification of diseases (ICD-11). Pain 160, 19–27.
Trias, E., Barbeito, L., Yamanaka, K., 2018. Phenotypic heterogeneity of astrocytes in motor neuron disease. Clin. Exp. Neuroimmunol. 9, 225–234.
Valbuena, G.N., Tortarolo, M., Bendotti, C., Cantoni, L., Keun, H.C., 2017. Altered metabolic profiles associate with toxicity in SOD1G93A astrocyte-neuron Co-cultures. Sci. Rep. 7, 50.
Valbuena, G.N., Cantoni, L., Tortarolo, M., Bendotti, C., Keun, H.C., 2019. Spinal cord metabolic signatures in models of fast- and slow-progressing SOD1G93A amyotrophic lateral sclerosis. Front. Neurosci. 13, 1276.
Van den Berg, C.J., Krzalić, L., Mela, P., Waelsch, H., 1969. Compartmentation of glutamate metabolism in brain. evidence for the existence of two different tricarboxylic acid cycles in brain. Biochem. J. 113, 281–290.
Velebit, J., Horvat, A., Smolič, T., Prpar Mihevc, S., Rogelj, B., Zorec, R., Vardjan, N., 2020. Astrocytes with TDP-43 inclusions exhibit reduced noradrenergic cAMP and Ca2+ signaling and dysregulated cell metabolism. Sci. Rep. 10, 6003.
Verkhratsky A, Parpura V, Li B, Scuderi C (2021) Astrocytes: The Housekeepers and Guardians of the CNS. In: Astrocytes in Psychiatric Disorders (Li B, Parpura V, Verkhratsky A, Scuderi C, eds), pp 21–53 Advances in Neurobiology, vol 26. Springer, Cham. https://doi.org/10.1007/978-3-030-77375-5_2.
Vidal-Itriago, A., Radford, R.A.W., Aramideh, J.A., Maurel, C., Scherer, N.M., Don, E.K., Lee, A., Chung, R.S., Graeber, M.B., Morsch, M., 2022. Microglia morphophysiological diversity and its implications for the CNS. Front. Immunol. 13, 997786.
Von Bartheld, C.S., Bahney, J., Herculano-Houzel, S., 2016. The search for true numbers of neurons and glial cells in the human brain: a review of 150 years of cell counting. J. Comp. Neurol 524, 3865–3895.
Willemen, H.L.D.M., Santos Ribeiro, P.S., Broeks, M., Meijer, N., Versteeg, S., Tiggeler, A., De Boer, T.P., Małecki, J.M., Falnes, P.Ø., Jans, J., Eijkelkamp, N., 2023.

Inflammation-induced mitochondrial and metabolic disturbances in sensory neurons control the switch from acute to chronic pain. Cell Rep. Med. 4, 101265.
Yang, Q., Zhou, J., 2019. Neuroinflammation in the central nervous system: symphony of glial cells. Glia 67, 1017–1035.
Zamanian, J.L., Xu, L., Foo, L.C., Nouri, N., Zhou, L., Giffard, R.G., Barres, B.A., 2012. Genomic analysis of reactive astrogliosis. J. Neurosci. 32, 6391–6410.
Zheng, H., Xiao, W.H., Bennett, G.J., 2011. Functional deficits in peripheral nerve mitochondria in rats with paclitaxel- and oxaliplatin-evoked painful peripheral neuropathy. Exp. Neurol. 232, 154–161.