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## **Astrocyte-Neuron Metabolic Cooperation Shapes Brain Activity**

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## **SUMMARY**

The brain has almost no energy reserve but its activity coordinates organismal function, a burden that requires precise coupling between neurotransmission and energy metabolism. Deciphering how the brain accomplishes this complex task is crucial to understand central facets of human physiology and disease mechanisms. Each type of neural cell displays a peculiar metabolic signature, forcing the intercellular exchange of metabolites that serve as both energy precursors and paracrine signals. The paradigm of this biological feature is the astrocyte-neuron couple, in which the glycolytic metabolism of astrocytes contrasts with the mitochondrial oxidative activity of neurons. Astrocytes generate abundant mitochondrial reactive oxygen species and shuttle to neurons glycolytically-derived metabolites, such as L-lactate and L-serine, which sustain energy needs, conserve redox status and modulate neurotransmitter-receptor activity. Conversely, early disruption of this metabolic cooperation may contribute to the initiation or progression of several neurological diseases, thus requiring innovative therapies to preserve brain energetics.

## INTRODUCTION

### The brain paradox: high demands, no reserve and an unconsolidated budget

The brain lacks fuel stores and hence requires a continuous supply of energy substrates. Still, it is one of the most active metabolic organs in the body. Oxygen consumption in a normal human brain is approximately 50 ml/min. Since an average human consumes about 250 ml O<sub>2</sub>/min in the basal state, the brain, which represents only about 2% of total body weight, accounts for 20% of the resting total body O<sub>2</sub> consumption (Clarke and Sokoloff, 1994). This number is even larger in children; the developing brain can take up as much as 50% in the first decade of life (Kennedy and Sokoloff, 1957). Similar percentages can be derived from the use of glucose, the main energy substrate of the brain. The global cerebral metabolic rate of glucose (CMRGlc) is roughly 80 mg/min, which corresponds to 0.45 mmol/min, 20% of the resting total body glucose consumption in adults. This rate peaks at age 5-9 yr (Chugani et al., 1987) and corresponds to more than 50% of the body's resting metabolic rate (Figure 1).

What workload requires this enormous energy use? Amazingly, the answer to this simple question is not straightforward. A comprehensive understanding of how energy use is apportioned among the numerous functional processes performed by the different brain cells is not yet available (Harris et al., 2012). The picture is clearer for the heart, muscles and kidneys because we have a better knowledge of the work they perform and of the characteristics of the cells that actually contract or filtrate. Considering the high fixed cost of brain function (10 times that expected vs. its weight alone), tasks directly associated with the ongoing neuronal activity must be identified. Obviously, the function of nervous tissues is to conduct electric signals and these are reflected in the unceasing electrical activity of the brain as was monitored broadly in the electroencephalogram (EEG) recorded from the scalp as early as 1929 in the human brain (Berger, 1929). It is worth mentioning here that Berger's scientific interests followed very closely those of Angelo Mosso on brain circulation, the first to suggest that brain activity is able to elicit changes in cerebral perfusion (Raichle, 1998) and may represent the primary driver of energy consumption. The restoration of the ion movements generated by postsynaptic currents, action potentials, and neurotransmitter uptake are key processes that contribute to the energetic burden (Alle et al., 2009). Experiments performed in the early 80's already identified the high metabolic cost related to the activity of the sodium pump that is responsible for the maintenance of the cell membrane potential, by moving sodium ions out and potassium ions in (Mata et al., 1980). Also at that time, it was established that energy was used in regions enriched in axon terminals, dendrites and synapses, and not in those where neuronal cell bodies are located (Schwartz et al., 1979). It is now assumed that a large majority of the metabolic activity of the brain is devoted to ongoing synaptic processes associated with maintaining a proper balance between excitatory and inhibitory activity (Figure 1).

Since storage levels of both O<sub>2</sub> and glucose in the brain are extremely small compared with their rate of utilization, the brain requires continuous replenishment by the circulation. If cerebral blood flow is interrupted completely, consciousness is lost within less than 10 sec. Indeed, the relationship between metabolism and brain function is so tight that it was already postulated in the 50's that the level of conscious activity parallels the rate of oxygen utilization by the brain (Kety, 1950). All these aforementioned rates represent the average values in the brain as a whole. **For a comprehensive review that compiles numerous rates, readers can refer to (Dienel, 2019).** The brain is not homogeneous, however; it is composed of a variety of cells and discrete structures that either function independently or in close coordination. Rates vary widely throughout the brain, with average values in gray matter approximately four times those in white matter. With the advent of positron emission tomography (PET), it has been possible to map human brain metabolism using the positron emitter [18F]-fluorodeoxyglucose, FDG (Reivich et al., 1979). It is fascinating to observe how images of glucose use at rest map the underlying anatomy so that it is possible to identify gyri, cortical layers, small nuclei and white matter tracts (Figure 1). Perhaps even more important is the fact that changes in energy metabolism and blood flow can be used as a proxy of neuronal activity (Bonvento et al., 2002). Indeed, the widely used functional magnetic resonance imaging technique (fMRI) do not record neuronal activity as one would expect, but detect signals that are related to energy delivery and use (Bandettini, 2012; Logothetis et al., 2001; Ogawa et al., 1990).

### **Functional brain imaging monitors metabolism**

It is not our purpose to review the biological mechanisms underlying fMRI signals (Logothetis, 2008) and to describe the strengths and weaknesses of the methods used to probe the brain at work (Poldrack and Farah, 2015). It is worth mentioning that those functional imaging approaches all rely on the tight relationship between brain activity and energy metabolism/flow, both spatially and temporally. As stated by its name, the fMRI-BOLD (blood-oxygen-level-dependent) effect relies on the local transient increase of the oxyhemoglobin-to-deoxyhemoglobin ratio that occurs during the haemodynamic response associated with neuronal activity. The BOLD-fMRI signal is not a straightforward measure of energy metabolism. The content of deoxyhemoglobin within a voxel is determined by the balance between the supply of oxygenated blood, the rate of oxygen consumption and the fractional blood volume. Hence, the BOLD response is dependent upon hemodynamic (blood flow and volume) and metabolic (oxygen consumption) responses (Barros et al., 2018). It is important to keep in mind that when a BOLD signal is detected in a brain region, blood flow has changed out of proportion to the change in oxygen consumption. Because of this metabolic mismatch, fMRI-BOLD signals change in regions where the actual amount of oxygen remaining in blood vessels is modified during activation (Raichle, 1998). Therefore, during activation, local increases in blood flow (CBF) and glucose utilization (CMRGlc) are not matched by commensurate

increases in oxygen consumption (CMRO<sub>2</sub>). The first description of the BOLD signal was almost contemporary with the provocative publication of the group of Marcus Raichle reporting a “nonoxidative glucose consumption during focal physiologic neural activity” (Fox et al., 1988). Using PET imaging, they found that visual stimulation increased glucose uptake (51%) and blood flow (50%) much more than oxygen consumption (5%) and concluded “transient increases in neural activity cause a tissue uptake of glucose in excess of that consumed by oxidative metabolism”. This result suggests that brain energy metabolism is not a strictly oxidative process operating in all brain cells.

### **Glycolysis and oxidative phosphorylation at rest: the interest of measuring the oxygen–glucose index (OGI)**

The same research group measured CMRO<sub>2</sub> and CMRGlc with PET in 33 normal right-handed adults in the resting awake state. They estimated OGI by dividing relative CMRO<sub>2</sub> by relative CMRGlc in every voxel and calculated a whole-brain molar ratio of 5.32, significantly inferior to 6, a value corresponding to a full oxidation of glucose (Vaishnavi et al., 2010). The other main observation was that the brain exhibits strong regional variations in OGI. However, there is no consensus about the heterogeneity of OGI in the human brain. The analysis of a same datasets (CMRO<sub>2</sub> and CMRGlc measured in the brain of 13 adults) provided opposite conclusion with either uniform OGI (Hyder et al., 2016) or robust regional differences within gray matter (Blazey et al., 2019). Positron emission tomography (PET) is still the method of choice for imaging CMRO<sub>2</sub> in humans (Mintun et al., 1984). It is based on the use of an oxygen gas enriched with the isotope <sup>15</sup>O and it monitors the spatial distribution and accumulation rate of metabolic water (H<sub>2</sub><sup>15</sup>O). Because of the short half-life of <sup>15</sup>O (2 minutes) combined with the limited spatial resolution of the CMRO<sub>2</sub> images, <sup>15</sup>O<sub>2</sub> PET remains challenging and is limited to the study of the primate brain, precluding comprehensive experimental studies in smaller animals such as rats and mice. Another experimental approach based on <sup>17</sup>O magnetic resonance spectroscopic imaging has been developed to probe the CMRO<sub>2</sub> in the rat brain during a two-min inhalation of <sup>17</sup>O<sub>2</sub> (Zhu et al., 2002). It is now possible to determine CMRO<sub>2</sub> in the human brain using dynamic <sup>17</sup>O-MRI with a good reproducibility (Niesporek et al., 2018). This opens the opportunity to combine <sup>17</sup>O-MRI with <sup>18</sup>FDG-PET to obtain OGI maps with good spatial resolution in the rodent and primate brain. This is of great importance because OGI may represent a relevant index of brain metabolism in healthy and pathological conditions. We return to the interest of measuring OGI in aging (Goyal et al., 2017) and in pathological conditions (Vlassenko et al., 2018; Vlassenko et al., 2010) at the end of the review. In the healthy brain, the question as why would brain cells do not fully oxidize glucose when oxygen is abundantly available has still not been fully resolved. Such a metabolic feature is well characterized in the cancer field for decades (Warburg, 1956). One of the major functions of glycolysis is to provide the glycolytic intermediates necessary to biosynthesis in proliferation and growth, and for maintenance of the biomass (Lunt and Vander

Heiden, 2011). However, what could be the purpose of glycolysis in an organ where the vast majority of differentiated neurons do not divide? In order to answer to this question it is necessary to realize that our brain is composed of multiple diverse cells that do not display the same metabolic profile.

## **BRAIN ENERGY METABOLISM: A CELLULAR VIEW**

### **High cellular diversity**

Our nervous system is composed of multiple cell types, including neurons, oligodendrocytes, astrocytes, ependymal cells, peripheral glia (Schwann cells, satellite, and enteric glia), immune cells, and vascular cells. The total number and proportion of cells is still a matter of controversy but it is safe to say that the glia to neuron ratio is close to one at least in the Human brain (von Bartheld et al., 2016). It is probably more important to realize that this number vary across the species and between brain regions. For example, the cerebellum constitutes only 10% of the whole brain volume but it contains more than 50% of the total number of neurons present in the brain. Not only is the brain a very heterogeneous organ in terms of cellular composition but also in terms of wiring, connection and intrinsic neuronal properties. Indeed, cortical networks are mainly composed of both glutamatergic excitatory projection neurons and local GABAergic inhibitory interneurons that display highly heterogeneous features (Tremblay et al., 2016). Electrophysiologically, a large number of firing patterns have been observed in these interneurons, ranging from fast-spiking to a more regular or burst-spiking activities. Obviously, all these different neurons may impose a different workload and associated energy needs (Figure 1). In the human brain, more that 50% is composed of white mater, made up of myelinated axons and glial cells of which there are the myelin-producing oligodendrocytes, astrocytes, microglia, and oligodendrocyte progenitor cells. Regulation of energy metabolism in the white matter may noticeably be different as compared to the gray matter where synaptic activities are occurring. Recent evidence in RNA profiling of acutely isolated and FACS-purified brain cells suggest that glial cells, in particular astrocytes are heterogeneous cells, as neurons (Ben Haim and Rowitch, 2017; Khakh, 2019; Khakh and Sofroniew, 2015). Recent single-cell RNA sequencing performed in the mouse identified at least seven distinct astrocyte types with regionally restricted distribution (Zeisel et al., 2018) and five transcriptomically distinct astrocyte subtypes in cortex and hippocampus (Batiuk et al., 2020). Transcriptional profiling of the adult mouse nervous system allowed the identification of 265 clusters of distinct cell types (Zeisel et al., 2018). It is therefore probably useless to draw any significant conclusion on brain metabolism when considering this organ as a whole. Even if some general functionalities can be put forward, regarding especially the differences between neurons and glial cells, it is likely that the precise mechanisms underlying the neuroenergetic processes must be studied on a cell-by-cell basis.

### **Single-cell approaches to measure energy metabolism**

Glucose is the main energy substrate in the brain. Three rate constants are critical to determine glucose homeostasis - its uptake, its efflux and its phosphorylation. In order to monitor glucose homeostasis, one needs methods for directly measuring glucose levels in living cells with temporal and cellular or even subcellular resolution. In 2003, the group of Wolf Frommer (Fehr et al., 2003) developed one of the first metabolic biosensors that can be used *in vitro* but also *in vivo*. This glucose sensor called FLIPglu-600 sensor is the first of a long list of metabolic biosensors (Arce-Molina et al., 2020; Bittner et al., 2010; San Martin et al., 2014a; San Martin et al., 2013; Zhang et al., 2020). Since these sensors are genetically-encoded, they can be targeted to organelles and subcellular domains by fusion to proteins or targeted sequences (Pendin et al., 2017). The use of FRET sensors to probe energy metabolism in the brain is an emerging technology (San Martin et al., 2014b). In 2014, sensors for six energy metabolites had been validated in mammalian cells: glucose, glutamate, ATP, NADH, lactate, and pyruvate. Since then, new probes have been developed (Luddecke et al., 2017; Mita et al., 2019; Zhang et al., 2018) and others are surely on their way (Zhang et al., 2020). It is now possible to assess central carbon metabolism, including the glycolytic pathway, the pentose phosphate pathway and the tricarboxylic acid (TCA) cycle using more than 15 different types of sensors (Zhang et al., 2020). In addition, other single-wavelength sensors engineered from circularly permuted green fluorescent protein (cpGFP) and periplasmic binding proteins have been made available by the group of Loren Looger (Diaz-Garcia et al., 2019; Keller et al., 2019; Lobas et al., 2019; Marvin et al., 2018; Marvin et al., 2019). Obviously, these tools will help determine how glucose levels and dynamics behave in different brain cells, provided accurate calibrations are performed. These genetically encoded fluorescent sensors can be applied for estimating the relative abundances of metabolites at the single-cell level, subcellular level, and for some of them even *in vivo*. By expressing the lactate sensor Laconic in both cortical astrocytes and neurons with AAVs, it has been possible for the first time to provide *in vivo* evidence for a lactate gradient from astrocytes to neurons (Machler et al., 2016), although the actual magnitude of such a gradient remains to be quantified. *In vivo* two-photon imaging of metabolic biosensors in non-anesthetized animals is a real asset to increase our knowledge about the study of metabolism at cellular resolution in behaving animals (Diaz-Garcia et al., 2017; Machler et al., 2016; Zuend et al., 2020).

### **OXPHOS is more dispensable for astrocytes than for neurons**

A key metabolic difference between neurons and glial cells relies on their dependence on the mitochondrial oxidative phosphorylation (OXPHOS) for survival. Thus, astrocytes survive relatively well upon OXPHOS inhibition by nitric oxide (NO) (Almeida et al., 2001; Bolaños et al., 1994; San Martin et al., 2017), a potent reversible inhibitor of mitochondrial complex IV (CIV) (Bolaños et al., 1994; Brown et al., 1995). The consequent bioenergetic stress activates 5'-AMP-activated protein kinase (AMPK) to up-regulate glycolysis at the level of 6-phosphofructo-1-kinase (PFK1) (Almeida et



al., 2004), a key regulatory step of the pathway (Uyeda, 1979). This metabolic switch partially compensates the loss of mitochondrial ATP, which is hydrolyzed by ATPase (complex V) to maintain the mitochondrial membrane potential ( $\Delta\psi_m$ ) and evade apoptosis (Almeida et al., 2004). In contrast to astrocytes, inhibiting OXPHOS does not seem to activate glycolysis in neurons to the same extent (Bolaños et al., 1995) and therefore cannot maintain ATP, causing  $\Delta\psi_m$  collapse and apoptotic death (Almeida et al., 2001; Almeida et al., 2004). These observations, which have been confirmed *in vivo* (Fiebig et al., 2019; Supplie et al., 2017), suggest that glycolysis is a key survival pathway and indicate that OXPHOS is more dispensable for astrocytes than for neurons (Bolaños et al., 2010). Understanding the molecular mechanism that regulates this cell-specific metabolic profile is, therefore, of critical importance. Thus, the reduced ability of neurons to invoke glycolysis upon OXPHOS inhibition is due, at least partially, to their virtual absence of a key glycolytic-promoting enzyme, namely 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) (Almeida et al., 2004). PFKFB3 catalyzes the formation of fructose-2,6-bisphosphate (F2,6P<sub>2</sub>) (Hue and Rider, 1987), the most potent allosteric positive effector of PFK1 (Van Schaftingen et al., 1982). PFKFB3 is highly expressed in astrocytes (Almeida et al., 2004; Herrero-Mendez et al., 2009; Zhang et al., 2014), but it is unstable in neurons due to continuous ubiquitylation and proteasomal degradation by the anaphase-promoting complex/cyclosome-Cdh1 (APC/C-Cdh1) (Herrero-Mendez et al., 2009), an E3 ubiquitin ligase that coordinates the cell division. Altogether, these studies support the notion that astrocytes bioenergetically rely mainly on glycolysis whereas neurons mainly rely on OXPHOS.

### **PFKFB3 stability regulates glycolytic flux and antioxidant neuronal protection**

What is the advantage of neurons to show a relatively lower glycolytic flux than astrocytes? To address this question, it is important to consider that glycolysis flux is interconnected with that of the pentose-phosphate pathway (PPP) at the level of glucose-6-phosphate (G6P) (Figure 2). By contributing to a relatively low neuronal glycolysis during PFKFB3 protein degradation, G6P utilization through PPP may be favored (Herrero-Mendez et al., 2009). In fact, by over-expressing PFKFB3 in neurons, the glycolytic flux is increased while that of PPP is decreased. This latter effect is counteracted by co-expressing glucose-6-phosphate dehydrogenase (G6PD), a rate-limiting step of PPP (Herrero-Mendez et al., 2009). The benefits of favoring PPP is related with its function at regenerating NADPH(H<sup>+</sup>), an essential glutathione reductase cofactor in glutathione (GSH) reduction from its oxidized form (GSSG) (Ben-Yoseph et al., 1996). Sustaining this delicate balance between glycolysis and PPP fluxes is therefore a critical factor in antioxidant protection, particularly for neurons. Thus, compared with astrocytes, neurons are weakly equipped with the antioxidant machinery governed by the nuclear factor erythroid 2-related factor-2 (Nrf2) transcription factor, which is responsible for the expression of glutamate-cysteine ligase (GCL) (Jimenez-Blasco et al., 2015), the rate-limiting step of the *de novo* GSH biosynthesis. Accordingly, the total GSH pool of

neurons is lower than in astrocytes (Raps et al., 1989), forcing neurons to invest part of the redox potential of glucose in regenerating GSH *via* PPP, rather than synthesizing GSH *de novo*, which astrocytes can do (Jimenez-Blasco et al., 2015). In fact, disrupting the glycolysis/PPP balance is sufficient to trigger redox stress and, eventually, neuronal death. For instance, inactivation of APC/C-Cdh1 by knocking down Cdh1 cofactor stabilizes PFKFB3, promoting a PPP to glycolysis shift causing redox stress (Herrero-Mendez et al., 2009). Moreover, Cdh1 hyperphosphorylation causes its disassembly from APC/C, resulting in the inhibition of its activity (Jaquenoud et al., 2002). N-Methyl-D-aspartate (NMDA) glutamate-subtype of neuronal receptors (NMDAR) over-activation, a well-known trigger of excitotoxicity, initiates a cascade of events leading to aberrant activation of cyclin-dependent kinase-5 (Cdk5), which hyperphosphorylates Cdh1 causing APC/C inhibition (Maestre et al., 2008). In concordance, during excitotoxic insults, APC/C-Cdh1 inhibition stabilizes PFKFB3 causing a PPP-glycolysis switch and neuronal oxidative damage (Burmistrova et al., 2019; Rodriguez-Rodriguez et al., 2012). In summary, the glycolytic flux must be kept relatively low to contribute maintaining the antioxidant status and survival of neurons; in contrast, glycolytic flux is important for the survival of astrocytes. This cell-specific metabolic expertise suggest that astrocytes and neurons must cooperate to sustain neurotransmission and brain function.

## **ASTROCYTE-NEURON METABOLISM REGULATES SYNAPTIC ACTIVITY, PLASTICITY AND BEHAVIOR**

### **Glucose breakdown: beyond ATP production**

The degradation of glucose occurs through glycolysis, the Embden–Meyerhof pathway, defined as a series of ten reactions leading to two molecules of pyruvate per glucose and then via the Krebs cycle (Figure 2). This does not mean that every single molecule of glucose when transported from the blood via the endothelial glucose transporter GLUT1 will follow these two metabolic pathways in series. After phosphorylation, G6P may form glycogen or enter the PPP (Figure 2). In addition, glycolysis is branching off and on at multiple points, likely suggesting that pyruvate is not made at the same rate that glucose is consumed, as elegantly reminded by Felipe Barros (Barros et al., 2020). As we already mentioned above, glycolytic intermediates are providing carbons to generate biomass (Lunt and Vander Heiden, 2011). Generating new cells requires the replication of all cellular contents, including nucleic acids, proteins, and lipids. Glucose is the main precursor for the chemical constituents that are used to build macromolecules essential for cell division. For example, G6P is a significant carbon source for purine and pyrimidine nucleotides, as it provides five out of ten or nine carbons required for their biosynthesis, respectively. The glycolytic intermediates dihydroxyacetone phosphate (DHAP) and 3-phosphoglycerate (3PG) are also the principal sources of carbon for lipid precursors such as glycerol-3-phosphate (G3P) (crucial for the biosynthesis of the phospholipids) and of sphingolipids, respectively (Figure 2). What fraction of glucose is then shunted to the production

of macromolecules? Current knowledge comes from the study of cancer cells and it has been estimated that the rate of utilization of glycolytic intermediates for biosynthesis of macromolecules accounts for 10% of the glycolytic rate. This is a mean value and it can vary a lot depending on the status of the cell. For example, in HEK cells, the flux that produces L-serine from 3PG is operating at the same speed that the one leading to 2-phosphoenolpyruvate (PEP), suggesting that the relative fluxes are comparable (Locasale et al., 2011). Besides cancer cells mostly studied *in vitro*, data concerning the proportion of glucose that escapes from glycolysis *in vivo* in the brain are scarce. Using PET imaging it has been calculated that a significant fraction (10–15%) of brain glucose is shunted away from the ATP-producing pathway (OXPHOS) and may be used to support other functions than providing ATP (Shannon et al., 2016). This may be particularly true during brain development that is lasting for years after birth in humans, to support the processes required for synapse formation and growth (Goyal et al., 2014). Accordingly, this fraction of glucose that is not fully oxidized decreases in normal human aging (Goyal et al., 2017). The extent to which adult humans, produce and recruit new neurons in adulthood is very limited (Bhardwaj et al., 2006; Sorrells et al., 2018); so what is the fate of this significant fraction of glucose that escape from glycolysis in the brain? A fraction of glucose is derived into the PPP (Bouzier-Sore and Bolaños, 2015; Rodriguez-Rodriguez et al., 2012). Since glial cells, in particular astrocytes, show a large capacity to up-modulate the glycolytic flux, it follows that most of this glucose is processed into metabolic pathways that are operating in astrocytes. Recent experiments suggest that products of astrocyte glycolysis operate as signaling molecules in the brain.

### **Cell-specific regulation of glucose metabolism sustains lactate release from astrocytes: the astrocyte-neuron lactate shuttle (ANLS)**

Formerly considered a waste product of anaerobic metabolism in muscle, lactate is now known to form continuously under aerobic conditions in many organs. In the brain, it took a long time for lactate to be recognized as (i) an alternative energy substrate for neural cells, (ii) a preferred substrate over glucose to sustain neuronal activity and (iii) a signaling molecule critical for synaptic plasticity and memory (Magistretti and Allaman, 2018). Excellent historical and comprehensive reviews have been published that relate how lactate switched from a bad reputation molecule to a sweet energy substrate despite numerous disagreements among investigators in the field (Brooks, 2018; Schurr, 2014). One central metabolic shuttle has attracted a lot of attention these last 25 years and is related to the transfer of lactate from astrocytes to neurons (Pellerin and Magistretti, 1994). This so-called astrocyte-to-neuron lactate shuttle (ANLS) has been quite controversial for a long time and is still debated (Barros and Weber, 2018; Yellen, 2018) but is now widely confirmed experimentally. In neurons, OXPHOS is very important for energy homeostasis since these cells weakly invoke glycolysis as an energy-generating pathway (Bolaños et al., 2010). Accordingly,

neurons are imposed to replace glycolytically-derived pyruvate to fuel mitochondria. Given their relatively high glycolytic activity, astrocytes release lactate both under resting conditions (Almeida et al., 2004) and upon glutamatergic activation (Pellerin and Magistretti, 1994; Zuend et al., 2020). The precise mechanism that links glutamatergic neurotransmission with astrocytic release of lactate is unclear. Glutamate increases glucose uptake and glucose to lactate conversion in astrocytes via a mechanism involving sodium/potassium ATPase (Pellerin and Magistretti, 1994), suggesting an energy-dependent mechanism. However, other mediators have been proposed to contribute to lactate release. For instance, NO, which is formed and released by neurons during glutamatergic neurotransmission (Garthwaite et al., 1988) inhibits CIV in astrocytes (Bolaños et al., 1994) at nanomolar concentrations (San Martin et al., 2017) by reversibly competing with oxygen (Brown et al., 1995). This effect causes the activation of glycolysis (Almeida et al., 2001) via the AMPK-PFKFB3 pathway (Almeida et al., 2004), releasing lactate, which could be taken up by neurons (Figure 3). *In vivo*, AMPK in astrocytes has recently been shown to phosphorylate and inactivate thioredoxin-interacting protein (TXNIP), a mechanism that facilitates glucose transporter-1 (GLUT1) translocation to the plasma membrane to sustain glycolytic-released lactate and neuronal survival (Muraleedharan et al., 2020). Interestingly, it has also been shown that glutamatergic neurotransmission might account for astrocytic lactate release in the absence of glycolytic stimulation. Thus, ammonium ( $\text{NH}_4^+$ ) is produced stoichiometrically with glutamate during neurotransmission (Richter and Dawson, 1948; Schousboe et al., 2013) and is released from neurons along with the neurotransmitter (Rothman et al., 2012). In astrocytes,  $\text{NH}_4^+$  inhibits mitochondrial pyruvate uptake, causing cytosolic pyruvate to lactate formation that, after release can be taken up by neurons (Lerchundi et al., 2015). **Synaptic activity can also stimulate neuronal glucose consumption (Diaz-García et al. Cell Metab. 26, 361-374, 2017; Baeza-Lehnert et al. Cell Metab. 29:668-680, 2019). Whereas Diaz-García et al. (2017) inferred qualitative glycolytic activation from a rise in cytosolic NADH, Baeza-Lehnert et al. (2019) provided quantitative measurement of glucose consumption. As the temporal resolution in Baeza-Lehnert et al. was higher, they were able to detect a 15 s delay between activation of mitochondrial pyruvate consumption and activation of glucose consumption by neuronal activity (Baeza-Lehnert et al., 2019). Both studies agreed in showing that neurons do not export lactate upon stimulation but increase their lactate levels when exposed to extracellular lactate. Noticeably, neither study addressed what fraction of glucose is used via the PPP; this prevents from accurately ascertaining the respective contribution of the glycolytic and PPP fluxes to the observed increase in glucose consumption by synaptic activity.** How these cascades of events take place during an increase in neuronal activity in specific circuits remains to be fully elucidated *in vivo*. Still, neurons and astrocytes are metabolically adapted cells to cooperate in fuel handling to meet the brain energy requirements of neurotransmission (Figure 3).

We will concentrate here on the notion that lactate can exert energy-independent functions in brain signaling (Bergersen and Gjedde, 2012). Given the evidence identifying astrocytes as the main brain cells (i) producing lactate, (ii) releasing lactate via specific monocarboxylate transporters (MCTs) or lactate channel and (iii) storing glucose as glycogen, two experiments performed in 2011 have used pharmacological tools to assess the potential role of glycogen-derived lactate in memory consolidation (Newman et al., 2011; Suzuki et al., 2011). In the rat hippocampus, pharmacological inhibition of glycogen phosphorylase using DAB (1,4-dideoxy-1,4-imino-d-arabinitol) or downregulation of the astrocytic MCTs (MCT1 and MCT4) impaired memory in two different learning tasks, inhibitory avoidance and spatial memory. Such blockade of lactate production from astrocytic glycogen also inhibited *in vivo* hippocampal long-term potentiation (LTP). Perhaps more importantly is the fact that lactate but not glucose was able to significantly rescue memory and synaptic plasticity, strongly suggesting that the release of lactate by astrocytes modulates neuronal functions via an energy-independent action. In fact, a number of studies have provided details about potential mechanisms underlying the role of lactate in neuronal excitability, plasticity and memory consolidation. First,  $\beta$ 2-adrenergic receptors expressed in hippocampal astrocytes are necessary for the learning-evoked release of lactate from astrocytes (Gao et al., 2016). Second, lactate is probably released by astrocytes via a high-capacity anion channel to ensure point-to-point local signaling (Sotelo-Hitschfeld et al., 2015). Third, lactate activates signaling cascades in neurons through a G protein-coupled receptor called hydrocarboxylic acid receptor 1 (HCAR1) that is negatively coupled to adenylyl cyclase (AC) (Bozzo et al., 2013; Lauritzen et al., 2014; Morland et al., 2015) and probably via yet another unknown receptor that activates AC (Tang et al., 2014). Fourth, lactate stimulates expression of synaptic-plasticity-related genes in neurons through a mechanism involving redox changes and potentiation of NMDAR activity (Yang et al., 2014). Obviously, it is difficult teasing apart the roles that lactate plays in neuronal plasticity and excitability, independently of its metabolic effects. Additional studies are needed to draw a comprehensive view of whether and how lactate can modulate synaptic activity in specific neural circuits at different time scales. Study of the non-metabolic functions of lactate in physiology is a very active field (Barros, 2013; Mosienko et al., 2015). Lactate-derived lactylation of histone lysine residues has been recently shown to represent an additional epigenetic modification that can stimulate gene transcription from chromatin (Zhang et al., 2019). This clearly expands the range of functions of lactate as well as its role in various pathophysiological conditions. It has been very recently observed that lactate can act upon neuronal metabotropic GABA-B receptors at least in the spinal cord. Such activation increases cAMP signaling and promotes neuronal regrowth after axotomy (Li et al., 2020). These results show that lactate can act as an inverse activator of GABA-B receptors, reducing the tonic effect of GABA and therefore determining the CNS inhibitory environment.

### **Delivery of energy metabolites via astrocyte networks**

A growing number of studies now indicate that astrocyte bioenergetics represents a powerful primary signaling contributor to brain activity, plasticity and behavior (Belanger et al., 2011). Because astrocytes occupy very restricted and independent spatial territories (Bushong et al., 2002), it is mandatory that they work in coordination as functional networks to sustain complex behaviors. The identification of astrocyte networks, closely associated with neuronal circuits (Giaume et al., 2010), has prompted us to reconsider how neuron-to-astrocyte metabolic interactions could operate in the brain. In a seminal paper published in 2008, Nathalie Rouach and Christian Giaume discovered that supply of energetic metabolites involves groups of connected astrocytes to reach more efficiently and distally the sites of high neuronal demand (Rouach et al., 2008). They observed that two gap-junction subunit proteins (connexin 43, Cx43 and connexins 30, Cx30) provide an activity-dependent intercellular pathway for the delivery of energetic metabolites from blood vessels to distal neurons. Interestingly, this trafficking of metabolites is regulated by glutamatergic synaptic activity **and direct delivery of either glucose or lactate to one astrocyte is capable of sustaining glutamatergic synaptic transmission even in the absence of extracellular glucose**. Since, a number a studies have consistently corroborated these initial findings and confirmed that astroglial metabolic networks play an important role in sustaining complex behaviors and are dysregulated in many brain diseases (see last paragraph). Among those complex behaviors, sleep is universal as it is present in vertebrate and invertebrate animals. Adenosine is a transmitter involved in the homeostatic drive for sleep following prolonged wakefulness (Porkka-Heiskanen et al., 1997). In 2009, experiments suggested that astrocytes are the source of adenosine that will act upon neuronal A1 receptors to promote sleep (Halassa et al., 2009). This was one of the first experimental demonstration of active glial involvement in mammalian behavior (Halassa and Haydon, 2010). Eight years after, the same group provided evidence that an astrocyte network operating in the lateral hypothalamus and mediated by Cx43, was critically involved in the trafficking of lactate required for a proper functioning of orexin neurons, a population of neurons involved in the circadian sleep/wake cycle and arousal (Clasadonte et al., 2017). It appears that the delivery of energy substrates through gap junction-mediated astrocytic networks is a prime candidate for efficiently sustaining the energy needs of neurons during activity.

### **Cell-specific regulation of glucose metabolism sustains L-Serine biosynthesis in astrocytes**

Another brain metabolic shuttle has been described in 2003 by Furuya and Watanabe (Furuya and Watanabe, 2003) but until recently has received much less attention than the ANLS. Probably because L-serine was known for a long time to sustain cell growth more efficiently than any other amino acid (Lockart and Eagle, 1959), L-serine has attracted more interest in oncology than in Neuroscience. However, L-serine plays an important role in neurotransmission. It is the precursor of

two co-agonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. These two molecules, D-serine and glycine gate synaptic and extra-synaptic NMDARs, respectively (Mothet et al., 2000; Papouin et al., 2012). While synaptic NMDARs facilitates synaptic plasticity such as long-term potentiation (LTP, a persistent strengthening of synapses underlying synaptic plasticity)(Panatier et al., 2006), extra-synaptic NMDARs are mostly involved in neurodegenerative disorders and cell death (Hardingham and Bading, 2010). An intense controversy as whether D-serine is released by astrocytes, neurons or by both cells (Papouin et al., 2017; Wolosker et al., 2017) led to the rediscovery of the metabolic pathway that produces L-serine in the brain (Ehmsen et al., 2013). This pathway, called the phosphorylated pathway (PP) was described in 1965 (Bridgers, 1965) in the mammalian brain. The PP starts from the glycolytic intermediate 3PG, and through the action of three enzymes, gives rise to L-serine (Figure 2). In the brain, the initial precursor 3PG probably comes only from glucose (through glycolysis). L-serine synthesis is predominantly performed in astrocytes in the mature brain. Initial immunohistochemical staining of PHGDH in the mouse brain revealed its exclusive expression in astrocytes (Yamasaki et al., 2001). The astrocytic expression of PHGDH has been recently confirmed in human and non-human primates (Le Douce et al., 2020) and was reported for PSAT1 as well (Maugard et al., 2020). The first study demonstrating that L-serine availability determines the rate of D-serine synthesis in brain was published by the group of Furuya who generated mice with a conditional deletion of PHGDH into astrocytes (Yang et al., 2010). In these animals, the levels of L- and D-serine were approximately 10% of those measured in control animals in the cortex and hippocampus. These data provide strong evidence that L-serine synthesized endogenously via the PP in astrocytes is a key rate-limiting factor for maintaining steady state levels of D-serine in the adult mouse brain. Remarkably, they provided evidence that intraperitoneal L-serine administration induced an increase of both L-serine and D-serine in the brain of PHGDH KO mice, indicating that D-serine can be synthesized from L-serine that originates outside the brain when its endogenous biosynthesis is limiting. Inhibition of astrocytic PHGDH either pharmacologically (Neame et al., 2019) or genetically (Le Douce et al., 2020) suppress the biosynthesis of L-and D-serine and alter NMDAR synaptic potentials and long-term potentiation (LTP) in the hippocampus. Spatial memory assessed with the hippocampal-dependent Morris Water Maze is also affected in mice conditionally lacking PHGDH in astrocytes (Le Douce et al., 2020). The role of D-serine is not limited to synaptic plasticity because NMDARs play very important roles in the physiological and pathological processes in the brain. Recent observations suggest that D-serine binding on NMDA-Rs affects neuronal proliferation in the dentate gyrus (Zhao et al., 2019), synaptogenesis (Diniz et al., 2012), dendrite maturation, spine stability as well as spine density (Sultan et al., 2015).

Serine is also essential for the synthesis of glycine, a co-agonist of extra-synaptic NMDARs. In addition, glycine acts as an inhibitory neurotransmitter via ionotropic glycine receptors located mostly in brain stem and spinal cord (Rajendra et al., 1997). The conversion between L-serine and glycine is catalyzed by serine hydroxymethyltransferase (SHMT) and is coupled to the folate cycle (Ducker and Rabinowitz, 2017). L-serine conversion to glycine generates *N*-5,*N*-10-methylenetetrahydrofolate (5,10-meTHF) from tetrahydrofolate (THF), making L-serine a one-carbon unit donor.

Therefore, the PP is of significant importance in the brain not only because it contributes to the vast majority of L-serine production but also because it uses and produces co-factors (NADH/NAD<sup>+</sup>, glutamate and  $\alpha$ -KG) that play key role in modulating cellular signaling, metabolic and neurotransmission processes. As compared to other organs, the brain interstitial fluid (ISF) and cerebrospinal fluid (CSF) expose brain cells to a microenvironment that is depleted in amino acids relative to the plasma. Indeed the concentrations of serine in mouse CSF and ISF are approximately 4- and 10-fold lower than in the plasma. It is critical to determine how these conditions are affecting the dependency of brain cells to the PP.

L-serine, being the precursor of glycine and cysteine, provides carbons for the synthesis of antioxidant GSH ( $\gamma$ -L-glutamyl-L-cysteinyl-glycine). The conversion of serine to glycine have the combined advantage of providing GSH synthesis and the NADPH-reducing power to maintain GSH in its reduced form. Indeed, the one-carbon folate cycle generates NADPH during the oxidation of 10-formyl-THF to CO<sub>2</sub> via the activity of 10-formyltetrahydrofolate dehydrogenase ALDH1L1 (cytosolic) and ALDH1L2 (mitochondrial form) (Ducker and Rabinowitz, 2017). It is worth mentioning here that ALDH1L1 has been identified as a specific astrocyte marker (Cahoy et al., 2008) and that the ALDH1L1 promoter was used to create transgenic mice for the study of astrocyte function (bacterial artificial chromosome (BAC) ALDH1L1 eGFP mice, BAC - translating ribosome affinity purification (bacTRAP) mice and Aldh1l1-Cre/ERT2 BAC transgenic mice)(Yu et al., 2020).

L-serine is also the head group of the phospholipid phosphatidylserine and a mandatory substrate for ceramide synthesis, the common backbone of sphingolipids. Together with cholesterol, these lipids are particularly enriched in synaptic membranes and are required for the complex branching morphology of astrocytes, allowing them to contact the synaptic cleft, contributing to synaptic physiology. The importance of epigenetic regulation in memory is well established (Day and Sweatt, 2010). Metabolism influences epigenetics by regulating the availability of co-factors required for key epigenetic enzymes (Li et al., 2018). This is true for L-serine metabolism since it is linked to one-carbon metabolism, providing methionine as a precursor for S-adenosylmethionine (SAM), the major methyl donor regulating methylation status of nucleic acids and histone proteins (Kim and Park, 2018). The role of L-serine in supporting methylation through maintenance of nucleotide levels has



been recently highlighted in cancer cells (Maddocks et al., 2016). Interestingly, lactate-derived lactylation of histone lysine residues was recently discovered (Zhang et al., 2019). Such histone acylation may represent a novel epigenetic modification that directly stimulates gene transcription from chromatin. No information is yet available on the interplay between L-serine, lactate and epigenetics in the brain, despite increasing evidence that, epigenetic mechanisms are critical for the maintenance of a healthy brain and are dysregulated in the course of many neurodegenerative diseases.

### **Signaling by mitochondria: OXPHOS assembly can modulate bioenergetic efficiency and reactive oxygen species (ROS) production in astrocytes and neurons that affect ANLS**

Besides their essential role at conserving energy as ATP, mitochondria are the site of signaling processes. An important mitochondrial-derived signal are reactive oxygen species (ROS) (Murphy, 2009) that, by altering the redox status of cysteine residues, can modulate protein synthesis and function (Topf et al., 2018). Therefore, understanding the mechanism(s) that control mitochondrial-derived ROS (mROS) is thus very relevant in the context of brain metabolism and function. As indicated previously, each brain cell type shows a specific gene expression pattern that links cellular function with energy conservation. In particular, the different relative dependence of astrocytes and neurons on glycolysis or OXPHOS suggests that the bioenergetic efficiency of OXPHOS is cell specific, not identical across the brain, and this can affect mROS production. Thus, the bioenergetic efficiency of OXPHOS is in part controlled by the structural organization of the mitochondrial respiratory chain (MRC) complexes in supercomplexes (SC) (Lapunte-Brun et al., 2013). The efficiency of NADH(H<sup>+</sup>)-derived electrons transfer through complex I (CI) to complex III (CIII) is facilitated by super-assembling these two complexes (Lapunte-Brun et al., 2013; Maranzana et al., 2013). Blue-native gel electrophoresis followed by proteomic analysis of mitochondrial proteins isolated from astrocytes and neurons revealed that CI-CIII super-assembly differs between these two cell types (Figure 4). In neurons, CI is bound to CIII more tightly than is in astrocytes, hence improving the bioenergetic mitochondrial efficiency of neurons (Lopez-Fabuel et al., 2016). Conversely, the proportion of free CI -i.e., the CI that is not bound to CIII- is higher in astrocytes than in neurons, implying lower bioenergetic efficiency and higher production of ROS by mitochondria in astrocytes when compared with neurons (Lopez-Fabuel et al., 2016). Mechanistically, such a difference in mROS production between astrocytes and neurons, which is around one order of magnitude (Lopez-Fabuel et al., 2016), may be the consequence of the weak transfer of electrons from free CI to ubiquinone in the astrocytic MRC (Lopez-Fabuel et al., 2016; Maranzana et al., 2013). Given the signaling properties of mROS and the relatively high production of these species by astrocytes, it was conjectured to play a physiological function (Lopez-Fabuel et al., 2016). To investigate this possibility, mROS abundance was modulated by genetically engineering a mouse model that expresses a mitochondrial-tagged

form of the antioxidant enzyme catalase (mCat) in astrocytes (Vicente-Gutierrez et al., 2019). Metabolomics and flux analyses of these mice revealed a shift in astrocytic glucose metabolism consisting of increased PPP and decreased glycolysis (Vicente-Gutierrez et al., 2019). Moreover, the mechanism responsible for this metabolic switch, caused by mROS modulation, involves increased nuclear abundance of histone deacetylase-4 (HDAC4), a transcription factor that represses miR-206 (Winbanks et al., 2011), a negative regulator of G6PD (Coda et al., 2015). In consequence, endogenous mROS production by astrocytes seems to physiologically down-regulate G6PD to sustain glycolysis. Furthermore, sustaining high mROS in astrocytes is important not only for the control of metabolism, but also for neuronal integrity, function and organismal behavior (Vicente-Gutierrez et al., 2019). The impact of astrocytic mROS on behavior was recently confirmed in a different biological setting. Thus, astrocytic mROS production can be altered *in vivo* by activating the cannabinoid receptors-1 that are present in mitochondria (mtCB<sub>1</sub>) (Jimenez-Blasco et al., 2020). Mechanistically, activation of mtCB<sub>1</sub> triggers the dephosphorylation of NDUFS4 (Jimenez-Blasco et al., 2020), a CI subunit (Mimaki et al., 2012). Notably, NDUFS4 dephosphorylation destabilizes CI N-module (Jimenez-Blasco et al., 2020), a structural domain of the complex that is necessary for NADH(H<sup>+</sup>) oxidation (Guaras and Enriquez, 2017) and, therefore, for ROS formation at CI (Murphy, 2009). Consequently, the loss of CI N-module by mtCB<sub>1</sub> activation decreases mROS which, by inactivating the pro-glycolytic transcription factor hypoxia-inducible factor-1 (HIF1), modulates glycolysis and leads to reduced lactate release, causing neuronal bioenergetic deficit and social impairment (Jimenez-Blasco et al., 2020). These observations indicate an essential function for astrocytes, *via* the control of mROS (Jimenez-Blasco et al., 2020) and lactate (Jimenez-Blasco et al., 2020; Suzuki et al., 2011) production, on organismal behavior (Jimenez-Blasco et al., 2020; Suzuki et al., 2011), see Figure 4.

## **DYSREGULATION OF ASTROCYTE-NEURON METABOLIC COOPERATION CONTRIBUTES TO BRAIN DISEASES**

Considering the strong metabolic cooperation that exists between neurons and astrocytes and its implication for neuronal activity, one would predict that disruption of this metabolic dialogue might cause and/or contribute to brain diseases (Allaman et al., 2011). We will summarize our current knowledge on how a loss of metabolic cooperation between astrocytes and neurons is involved in neurodegeneration, aging, inflammation, stress and psychiatric disorders and we will discuss whether enhancing brain metabolism might be a valuable strategy to promote neuroprotection in various CNS disorders.

### **Impaired energy metabolism is a hallmark of neurodegenerative diseases**

Neurological disorders represent major causes of death and disability worldwide. They are the leading cause of DALYs (disability-adjusted life-years) and second leading cause of deaths (Collaborators, 2019). Among these disorders, neurodegenerative diseases (NDs) are incurable and debilitating conditions that result in the progressive dysfunction and death of nerve cells. The loss of specific neurons, glial cells and synapses in distinct brain regions induces diverse clinical manifestations. Movement disorders are observed in amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD) and spinocerebellar ataxias (SCAs) while cognitive deficits occur in Alzheimer's disease (AD), frontotemporal dementia (FTD) and dementia with Lewy bodies (LBD). However, most of these diseases share several subcellular dysfunctions including the aggregation of proteins, a loss of proteostasis and impaired axonal transport. Increasing evidence accumulated during the last 40 years suggests that metabolic alterations strongly influence the initiation and progression of neurodegenerative disorders. PET-FDG imaging studies performed in the 80's have documented reduced glucose utilization in brain regions affected in patients with most types of NDs. More recent studies have determined that these metabolic changes occur early in the course of the disease, so it is reasonable to say that they are not merely secondary to neuronal loss and rather play a role in the disease process (see (Bonvento et al., 2017) for references). For example, PET-FDG imaging studies performed in individuals recruited in the Dominantly Inherited Alzheimer Network (DIAN) provided evidence that glucose hypometabolism emerges 15 years before the expected onset of dementia (Gordon et al., 2018). A recent multicenter consortium study analyzed more than 2,000 human brain tissues and 400 CSF samples by quantitative mass spectrometry (MS)-based proteomics and provided a comprehensive understanding of the biological changes associated with the different stages (asymptomatic and symptomatic) of the disease (Johnson et al., 2020). They found that the "protein module" most strongly associated with AD pathology and cognitive impairment was related to sugar metabolism. Even more interestingly, this sugar module was enriched in both astrocytes and microglia highlighting the central role of glial biology in the metabolic defects occurring in AD. Multiple proteins from this "astrocyte/microglial metabolism" module were also found to be elevated in cerebrospinal fluid in early stages of the disease and may serve as useful biomarkers. Most of them are involved in glycolysis, including Lactate dehydrogenase B (LDHB), Pyruvate kinase isozymes M1/M2 (PKM) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). It is intriguing to mention that among 60 metabolites that were detected by LC-MS in the cerebrospinal fluid (CSF) of 22 patients suffering from AD and 33 controls, statistically significant differences between the 2 groups were only detected for metabolites of the glycolysis, including phosphoenolpyruvate, 2- and 3-phosphoglycerate, pyruvate and dihydroxyacetone phosphate (Bergau et al., 2019). While this latter study should be replicated in a larger cohort, all the aforementioned results led us to suggest that AD

has a strong metabolic component (Demetrius and Driver, 2013). It is however important to consider that AD is a complex disease and cannot be defined as a clinical-pathologic entity (Jack et al., 2018). The genetics of AD provide strong initial evidence for a major pathway centered on A $\beta$  generation, aggregation, and clearance (Sierksma et al., 2020). To date, over 50 risk loci with genome-wide significance are associated with AD, strongly suggesting that AD is a disease of multiple components (Sims et al., 2020). Among these loci, Apolipoprotein  $\epsilon$ 4 allele (*APOE4*) is the only common high-risk genetic variant. Indeed, homozygous  $\epsilon$ 4 individuals have a 12-fold higher risk for developing late-onset AD relative to individuals who have 2 copies of the  $\epsilon$ 3 allele (see review by Chen et al., Neuron 2020). Astrocytes produce and secrete most of the ApoE in the form of lipidated ApoE in the normal CNS. While the ability of the ApoE4 protein to increase the aggregation and decrease the clearance of A $\beta$  has been well documented, numerous data suggest that ApoE4 negatively affects additional processes in the brain, including blood-brain barrier and bioenergetics (Montagne et al., 2020; Williams et al., 2020). Clinical studies have shown that glucose metabolism is altered in ApoE4 carriers' years before amyloid plaques, tau pathology and memory deficits appear (Reiman et al., 2005). A recent study suggest that ApoE exerts isoform-specific effects on glucose utilization, lactate synthesis and de novo biosynthesis pathways in astrocytes (Williams et al., 2020). It is now recognized that AD is a multicomponent disease and that multiple deficits probably combine to trigger the genesis of this disease. The convergence of most of these deficits towards glia energy metabolism (Figure 5) is opening the way for the development of therapeutic strategies preserving or rescuing neuron-glia energetics (see last paragraph). A similar conclusion can be drawn for patients suffering from Huntington Disease (HD) in which PET provides evidence for reduced uptake of [18F]FDG in premanifest HD brain (Lopez-Mora et al., 2016), probably involving a reduction of glycolysis (Powers et al., 2007). Recent observations obtained by us and others points to a metabolic shift in HD astrocytes as an important contributor to the disease (Boussicault et al., 2014; Polyzos et al., 2019).

### **Effect of aging and inflammation on neuron-glia energetics**

There is an important distinction between the changes associated with healthy ageing and those occurring in NDs. As compared to AD, during healthy ageing, some cognitive abilities decline (episodic and working memory) whereas others (short-term memory, autobiographical memory, semantic knowledge and emotional processing) remain relatively stable (Hedden and Gabrieli, 2004). With respect to brain glucose metabolism, changes observed during healthy ageing are less marked and do not involve the same brain regions as those seen in AD. In healthy ageing, the most consistent finding is decreased frontal metabolism, whereas in AD, the parietal lobe and precuneus are the most markedly affected (Mosconi, 2013). Interestingly, age-related decreases in brain glucose uptake exceed that of oxygen use (Goyal et al., 2017). At the level of the whole brain, the fraction of

non-oxidative glucose gradually decreases with age, approximating zero near the age of 60. This change occurs in the absence of pathology, therefore suggesting that it represents a biomarker of normal aging. The cellular and molecular correlates of such decrease are yet to be identified. It is striking to observe that whereas the topography of the other metabolic and hemodynamic variables (CMRGlc, CMRO<sub>2</sub>, and CBF) remains stable during normal aging, the topography of the OGI varies considerably with age. Aging does not affect the whole brain evenly. The hippocampus, a brain structure with a key role in memory processing is particularly vulnerable to the aging process. Hippocampal LTP is impaired in aged individuals, consistent with age-related cognitive deficits (Barnes, 2003). Alterations of functional plasticity associated with cognitive impairments observed across the aging process naturally raised the question for a role of D-serine (Mothet et al., 2006).

### **Effect of stress on neuron-glia energetics**

Stress engages multiple neural and endocrine systems to promote adaptation and survival of the organism (Bains et al., 2015). The circuits that underlie these multiple processes as well as the key molecular players have been well characterized (McEwen and Akil, 2020). The impact of stress on neuroplasticity is also well established. For example, stress, via the direct action of the circulating stress hormone corticosterone on glucocorticoid receptors (GR) located in the hippocampus inhibits LTP and induces amnesia. However, release of glucocorticoids at the time of learning may promote learning and memory (Schwabe et al., 2012). Glucocorticoid receptor (GR) is a ligand-regulated vertebrate transcriptional regulatory factor (TRF) that modulate rates of transcription of target genes (Weikum et al., 2017). Expression of the glucocorticoid receptor is ubiquitous in the brain with a high level found in mouse astrocytes (Zhang et al., 2014). Despite the large amount of data obtained on the role of stress on learning and memory, our understanding of the cellular and molecular targets of glucocorticoids following acute stress, remains fragmented. A recent study provided convincing evidence that the impairment synaptic plasticity induced by stress is mediated by a deficit in synaptic energy directly linked to the ANLS (Murphy-Royal et al., 2020). The authors report that an acute 20-min swim stress induces hypertrophy of astrocytes, modifies calcium signaling, decreases the expression of astrocytic gap junction channels and impairs functional coupling in a glucocorticoid-dependent manner. The authors also show that L-lactate delivery through astrocytes rescues stress-induced impairment of LTP in the hippocampus. These results highlight the critical role of astroglial metabolic networks in regulating synaptic activity not only in health (Rouach et al., 2008) but also in disease. Astrocyte networks may therefore represent an endogenously protective mechanism that serves as a metabolic buffer against bioenergetic stress to distribute the impact of degenerative processes and preserve neuronal function (Cooper et al., 2020).

### **Metabolic aspects of psychiatric disorders**

Psychiatric illnesses are common and chronic mental disorders that affect the lives of millions of individuals worldwide. Common mental disorders refer to two main diagnostic categories: depressive disorders (Malhi and Mann, 2018) and anxiety disorders (Craske and Stein, 2016). The proportion of the global population with depression in 2015 was estimated to be 4.4% and 3.6% for anxiety (World Health Organization). In North America and Europe alone, mental illness accounts for up to 40% of all years lost to disability. Mental disorders feature biological and psychological factors that are numerous, complex and unknown. Still, most of these illnesses are associated with impairments of synaptic plasticity and energy metabolism (Manji et al., 2012; Sullivan et al., 2018; Zuccoli et al., 2017). Most of the previous studies converge towards the hypothesis that a hypoactive astrocyte phenotype exists in some psychiatric disorders resulting in a reduction in the number and density of glial cells in some brain regions (Czeh and Nagy, 2018; Zhou et al., 2019). Low levels of the well-known astrocyte marker, glial fibrillary acidic protein (GFAP) have been reported in several limbic regions (Zhou et al., 2019). Expression of the astrocyte gene and proteins related to glutamate and GABA (glutamine synthetase, SLC1A2 and SLC1A3, the two glial glutamate transporters (Choudary et al., 2005) is reduced in the cerebral cortex from individuals who had suffered from major depressive disorder. Similarly, Cx43 and Cx30, the two gap junction proteins and aquaporin-4 (AQP4), a protein located predominantly in astrocytic endfeet, are down regulated in depressed patients (Kim et al., 2018). It is therefore likely that astrocytes participate in the pathology of depression and anxiety disorders. The central hypothesis postulates that depressive symptoms are due to a dysfunction of monoaminergic systems, which includes serotonin, dopamine and noradrenaline (Krishnan and Nestler, 2008). Second generation medications, such as serotonin selective reuptake inhibitors (SSRIs) and norepinephrine selective reuptake inhibitors are still widely used. Astrocytes express various 5-HT and adrenergic receptors as well as serotonin and noradrenaline transporters, meaning that they may contribute to depression and anxiety by modulating the monoaminergic systems. Few studies have supported the notion that astrocyte energy metabolism is directly involved. Indirect evidence comes from the observation that peripheral administration of L-lactate produces antidepressant-like effects in different animal models of depression (Carrard et al., 2018). The biological effects underlying the antidepressant-like effects of L-lactate are yet to be discovered (Magistretti and Allaman, 2018).

### **Towards metabolic therapeutic strategies?**

Given the central role of energy metabolism in brain functioning, a broad range of strategies aiming at rescuing brain energy have been explored (Camandola and Mattson, 2017). Successful treatments that improve brain energetics in preclinical models of many neurodegenerative diseases as well as clinical trials reporting improved brain function by targeting energy metabolism have been recently reviewed (Cunnane et al., 2020). While previous studies indicated that the metabolic alterations

were consecutive or at best contemporary with neuronal dysfunctions, it is now recognized that impaired brain energy metabolism may precede the onset of clinical symptoms, at least in AD, so that they can contribute to the disease process (Figure 5). This paradigm shift has been corroborated by the discovery that molecules produced from glycolysis and mitochondria in astrocytes operate as signaling molecules in the brain and regulate neurotransmission, plasticity and behavior. This opens the door of early therapeutic strategies improving energetics and signaling. A clear advantage of such strategies is that they are easily transferable to the clinic. The neuroprotective role of lactate has been already reviewed (Magistretti and Allaman, 2018). As an example, our recent observation points to the therapeutic potential of L-serine for AD. Indeed, a 2-month diet enriched with 10% L-serine completely rescues synaptic plasticity as well as spatial memory in a mouse model of AD (Le Douce et al., 2020). Previous clinical studies have already demonstrated the safety and efficacy of a chronic treatment with serine in children suffering from the infantile serine deficiency phenotype (de Koning et al., 2004). In those infants, L-serine therapy has proven to be beneficial in ameliorating symptoms if started before neurological damages occur (El-Hattab, 2016).

## **CONCLUDING REMARKS**

Brain activity controls organismal function, which compels the brain to meet the energy needs of energetically costly neurotransmission through the continuous and spatiotemporal support of metabolic fuel. The transformation of the chemical energy of fuel molecules into useful energy is strictly regulated and this regulation differs between cells, in particular between non-proliferative and proliferative cells. In the brain, the excitable cells that generate action potentials and activate synaptic connections are non-proliferative and secure their metabolic needs by preferentially using OXPHOS. This metabolic feature renders neurons susceptible and a cellular consortium composed of neurons and astrocytes is at work to overcome this vulnerability. These two cell types are metabolically coupled to coordinately meet the energy needs of neurotransmission, preserve the redox balance and modulate the neurotransmitter-receptors activity. To sustain this cellular cooperation, the enzymes of glycolysis are naturally up-regulated in astrocytes when compared with neurons, whereas the mitochondrial respiratory chain is structurally organized in a more bioenergetically-efficient shape in neurons. Accordingly, astrocytes are well suited to convert glucose from the blood into many glycolytically-derived intermediates, which can be shuttled to neurons where they can be used for oxidizing or biosynthetic purposes. Two notable examples of these metabolites are L-lactate and L-serine. Thus, according to both in vitro and in vivo evidence, astrocytic-released L-lactate can be taken up by neurons, which use this metabolite as a mitochondrial fuel to obtain energy and/or as a redox modulator. L-Serine is an important glycolytically-derived precursor of the NMDARs co-agonists D-serine and glycine, as well as for GSH

biosynthesis. Notably, the apparently inefficient astrocytic mitochondria are, however, important sources of ROS that modulate molecular targets that decisively contribute to the metabolic coupling with neurons. Further characterizing the specific molecular players and mechanisms responsible for the metabolic brain cell consortiums in vivo will identify novel cellular couplings for the control of energy balance and brain function in health and disease.

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## FIGURE LEGENDS

### **Fig. 1. Main features of brain energy metabolism**

Despite its relatively small size (1400 grams), the human brain accounts for about 20% of the calories consumed by the whole body. Most of the energy comes from glucose catabolism. PET-FDG (positron emission tomography [18F]-fluorodeoxyglucose) images of glucose use at rest distinctly map the underlying anatomy (fusion with MRI image) with a large amplitude between regions, an indication for the existence of a tight and local coupling with the underlying activity. Most brain energy is used at synapses (60%) to sustain the effective and rapid transfer of information. The subcellular processes that contribute to the non-signaling energy use are not yet well characterized. Brain cells are very heterogeneous in terms of morphology and function. Neurons display a large number of firing patterns, ranging from fast-spiking to a more regular activities. Glial cells (astrocytes, microglia and oligodendrocytes) do not present electrical activities. All these different cells impose a different workload and associated energy needs. Consequently, each cell type displays a unique metabolic



signature and the regulation of energy metabolism is both ensured by a tight collaboration between cells (between astrocytes via gap junctions) and between astrocytes and neurons, as illustrated here. Astrocytes break down glucose (Glc) to pyruvate (Pyr) and lactate (Lac) and then shuttle Lac to neurons for oxidation. These glial cells take up glutamate (Glu) and  $\text{NH}_4^+$  released from synapses and produce glutamine (Gln), which is sent back to neurons. Created with BioRender.com.

**Fig. 2. Schematic summary of main glucose-metabolizing pathways.**

Glucose entering cells is metabolized through glycolysis and pentose-phosphate pathway (PPP), or may be stored as glycogen. PPP can render glycolytic intermediates, a proportion of which may re-enter the PPP in a cyclin-like manner to conserve the redox potential of glucose as NADPH(H<sup>+</sup>), which is useful to regenerate glutathione (GSH) from its oxidized form (GSSG) or for fatty acid synthesis. PPP intermediates are also nucleotide precursors. Some glycolytic intermediates may also serve as precursors for other biosynthetic metabolic pathways. For instance, dihydroxyacetone phosphate (DHAP) may generate glycerol-3-phosphate (G3P), a phospholipid precursor; and 2-phosphoglycerate (2PG) may be used to generate L-serine, D-serine and GSH. The last glycolytic intermediate, pyruvate (Pyr), can be reduced to L-lactate, which is normally released from cells, or it can enter mitochondria, where it is oxidized to acetyl-Coenzyme A (acetyl-CoA) and further oxidized through the tricarboxylic acid cycle (TCAC) to conserve its redox potential as NADH(H<sup>+</sup>). TCAC is the hub for many biosynthetic pathways, one of which is the fatty acid synthesis, which requires the efflux of the TCAC intermediate to the cytosol. Several TCAC intermediates are amino acid precursors. The NADH(H<sup>+</sup>) regenerated from the TCAC is used by the mitochondrial respiratory chain (MRC) to conserve its energy in the form of ATP by the oxidative phosphorylation (OXPHOS). For simplicity and clarity, the stoichiometry of the pathways has been omitted.

Enzymes:

- 1, hexokinase
- 2, phosphoglucose isomerase
- 3, 6-phosphofructo-1-kinase
- 4, pyruvate kinase
- 5, lactate dehydrogenase
- 6, pyruvate dehydrogenase complex
- 7, glucose-6-phosphate dehydrogenase (G6PD)
- 8, phosphoglycerate dehydrogenase
- 9, phosphoserine aminotransferase-1
- 10, phosphoserine phosphatase

Abbreviations:

- 1-acyl-G3P , 1-acyl-glycerol-3-phosphate
- 1,3P2G , 1,3-bisphosphoglycerate
- 2PG, 2-phosphoglycerate
- 3PG, 3-phosphoglycerate

3PHP, 3-phosphohydroxypruvate  
6PG, 6-phosphogluconate  
Acetyl-CoA , acetyl-Coenzyme A  
DHAP, dihydroxyacetone-phosphate  
E4P, erithrose-4-phosphate  
ePS, 3-phosphoserine  
F1,6P2, fructose-1,6-bisphosphate  
F6P, fructose-6-phosphate  
g-Glu-Cys , g-glutamyl-cysteine  
G1P, glucose-1-phosphate  
G6P, glucose-6-phosphate  
GA3P, glyceraldehyde-3-phosphate  
Gly, glycine  
GSH, reduced glutathione  
GSSG, oxidized form  
L-Lac, L-lactate  
L-Ser, L-serine  
MRC , mitochondrial respiratory chain  
PEP, phosphoenol-pyruvate  
PPP, pentose-phosphate pathway  
Pyr, pyruvate  
R5P, ribose-5-phosphate  
Ru5P , ribulose-5-phosphate  
S7P, sedoheptulose-7-phosphate  
TCAC , tricarboxylic acid cycle  
Xu5P , xylulose-5-phosphate

**Fig. 3. Different regulation of glycolysis explains the astrocyte-neuron metabolic coupling.**

In astrocytes, the conversion of F6P into F1,6P<sub>2</sub> by PFK1 is very active due to the normally high levels of fructose-2,6-bisphosphate (F1,6P<sub>2</sub>). F1,6P<sub>2</sub> is a potent allosteric positive effector of PFK1, and in astrocytes it is synthesized from F6P by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3). PFKFB3 expression in astrocytes is very high under normal conditions and accounts for the glycolytic phenotype of these cells. Glycolysis in astrocytes can be further stimulated by several signals. For instance, the neural messenger nitric oxide (NO), which can be formed by neurons during glutamatergic neurotransmission, can reach astrocytes. NO inhibits mitochondrial complex IV in astrocytes, increasing the AMP/ATP ratio that stimulates 5'-AMP-activated protein kinase (AMPK), which in turn activates PFKFB3. AMPK also phosphorylates, and inhibits, thioredoxin interacting protein (TXNIP), enabling expression and surface translocation of the astrocytic glucose transporter (GLUT1), promoting glucose uptake and L-Lac release from astrocytes. The release of L-Lac by astrocytes can also be stimulated by NH<sub>4</sub><sup>+</sup>, which is also formed by neurons during glutamatergic neurotransmission. NH<sub>4</sub><sup>+</sup> enters mitochondria and reduces pH, inhibiting Pyr transport to mitochondria hence facilitating Pyr conversion into Lac. In neurons, PFK1 activity is very low because PFKFB3 protein is continuously degraded, under normal conditions, due to the high activity present in neurons of the E3 ubiquitin ligase anaphase-promoting complex/cyclosome-Cdh1 (APC/C-Cdh1). Such mechanism favors G6P to enter the PPP and to be recycled in a large proportion, since in neurons the activity of the reversible enzyme, PGI, is very high, which facilitates F6P isomerization to G6P. By facilitating G6P oxidation through the PPP, neurons regenerate GSH from its oxidized form, which is a mechanism that protects these cells from oxidative stress. The weak glycolytic activity of neurons may reduce Pyr formation and therefore obtain limited energy production in mitochondria from glucose metabolism. However, this may be compensated by the uptake of L-Lac from astrocytes, since neurotransmitter glutamate stimulates L-Lac release from astrocytes (astrocyte-neuron lactate shuttle; ANLS). In neurons, L-Lac can be converted into Pyr and be used as a mitochondrial fuel to obtain the energy needed for neurotransmission. Besides providing energy, L-Lac can exert its influence on glutamatergic neurotransmission by acting on NMDA receptors. Similarly, D-serine (D-ser) synthesized from 3-phosphoglycerate 3PG in astrocytes is known to directly regulate the opening of NMDA receptors and to actively contribute to synaptic plasticity and memory.

**Fig. 4. The structural conformation of the mitochondrial respiratory chain dictates cell-specific bioenergetic efficiency and ROS production in astrocytes and neurons.**

In astrocytes, the proportion of mitochondrial complex I (I) that is free -i.e., not bound to complex III (III)- is higher than in neurons. This structural feature determines a relatively low bioenergetic efficiency of astrocytic mitochondria as compared to neurons. Therefore, the proportion of electrons entering complex I from NADH(H<sup>+</sup>) that are transferred to O<sub>2</sub> to form superoxide (O<sub>2</sub><sup>•-</sup>) is higher than in neurons. Thus, the production of mitochondrial ROS by astrocytes is much higher (~1 order to magnitude) than that in neurons. In neurons, complex I assembly to complex III is tighter than in astrocytes, determining a higher bioenergetic efficiency and lower mitochondrial ROS production. The high production of mitochondrial ROS in astrocytes regulates metabolism, sustaining neuronal function and organismal behavior. Created with BioRender.com.

**Fig. 5. Early metabolic deficits contribute to synaptic failure in Alzheimer Disease**

In the healthy brain, the tight metabolic cooperation between astrocytes and neurons sustain synaptic activity both by providing ATP and astrocytes-derived signaling molecules (Lactate and L-serine). In the course of Alzheimer Disease, at least 15 years before the appearance of symptoms, glucose delivery and use are reduced in specific brain regions (see text). Besides a shortage of ATP production, dysfunction of the metabolic dialogue between neurons and astrocytes can directly contribute to synaptic failure and networks miscommunication. Early brain energy rescue strategies are probably well suited to counteract such metabolic dyshomeostasis. Created with BioRender.com.

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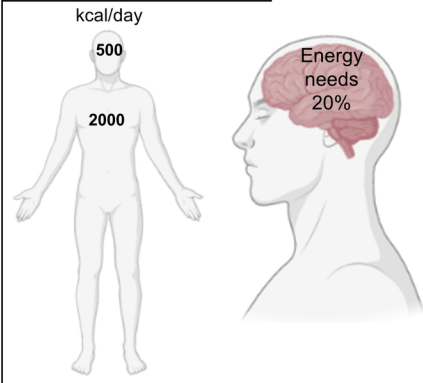
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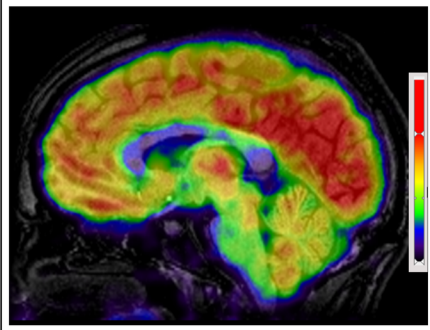
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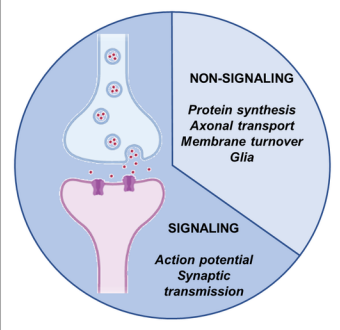
### BODY AND BRAIN USE



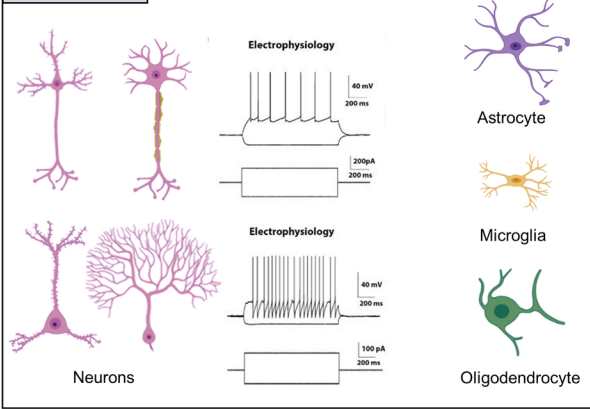
### BRAIN GLUCOSE USE



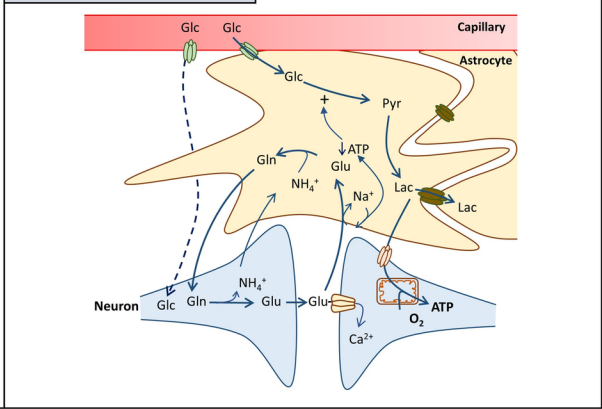
### BRAIN BUDGET

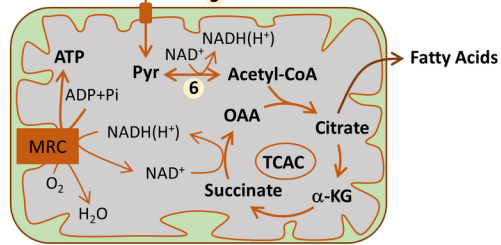
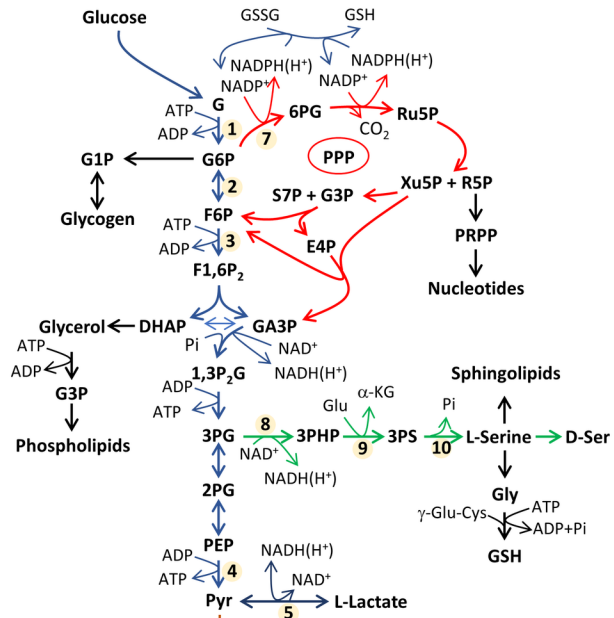


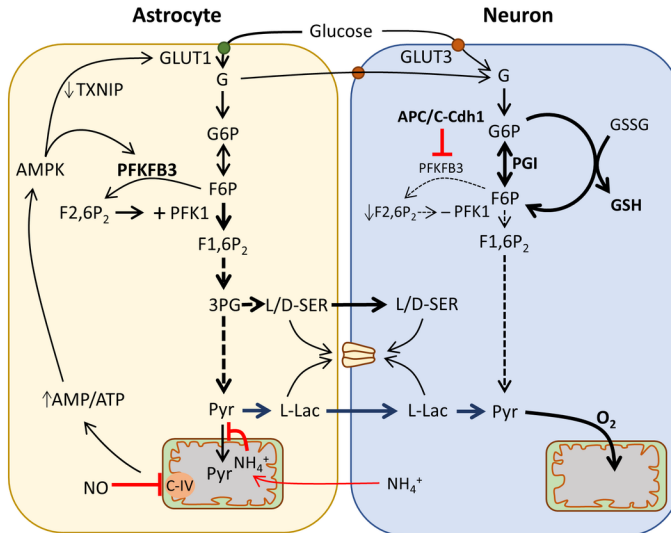
### CELL DIVERSITY

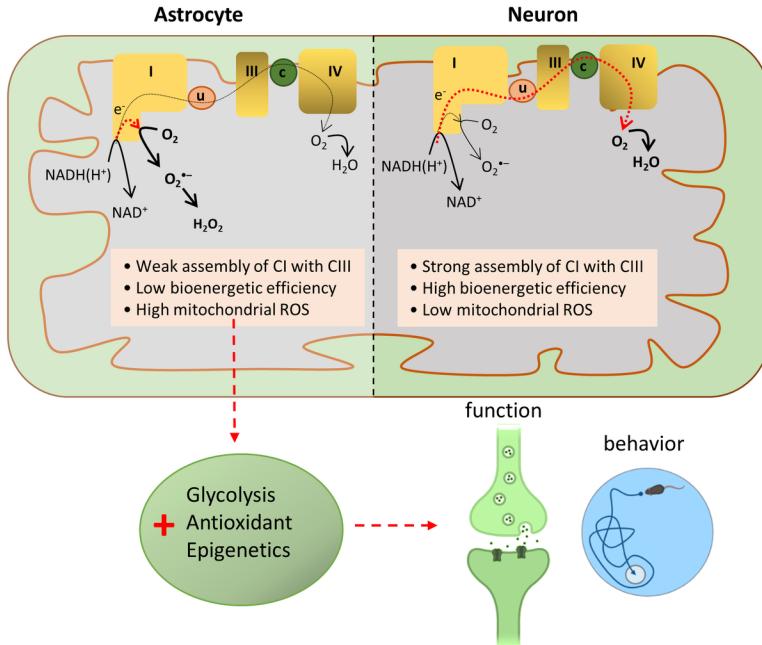


### CELL-CELL INTERACTIONS

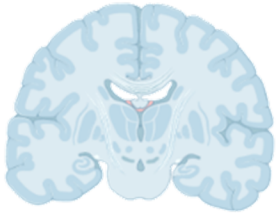








## Healthy brain



### Glucose delivery and use

Oxidation in neurons:

- ATP production
- Antioxidant

Glycolysis in astrocytes:

- Lactate production
- L-serine biosynthesis



### Signaling

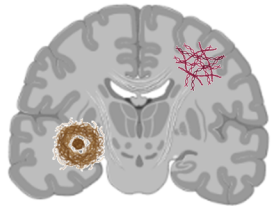
Oxidation in neurons:

- Synaptic activity (ions)
- Antioxidant (ROS)

Glycolysis in astrocytes:

- Glutamate recycling
- NMDA activity
- Lipids/epigenetics

## Alzheimer brain



### Early metabolic defects

Oxidation in neurons:

- ATP production

Alteration of glycolysis in astrocytes:

- Proteins
- Metabolites
- Genetics (ApoE4)



### Signaling impairments

- Excitatory–inhibitory imbalance
- Impaired synaptic plasticity
- Mitochondrial defects
- Oxidative damages