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MINI-REVIEW

Neuron–astrocyte interactions in the regulation of brain energy metabolism: a focus on NMR spectroscopy

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Abstract

An adequate and timely production of ATP by brain cells is of cardinal importance to support the major energetic cost of the rapid processing of information via synaptic and action potentials. Recently, evidence has been accumulated to support the view that the regulation of brain energy metabolism is under the control of an intimate dialogue between astrocytes and neurons. *In vitro* studies on cultured astrocytes and *in vivo* studies on rodents have provided evidence that glutamate and Na^+ uptake in astrocytes is a key triggering signal regulating glucose use in the brain. With the advent of NMR spectroscopy, it has been possible to provide experimental evidence to show that energy consumption is mainly devoted to glutamatergic neurotransmission and that glutamate-glutamine cycling is coupled in a $\sim 1:1$ molar stoichiometry to glucose oxidation, at least in the cerebral cortex. This improved understanding of neuron–astrocyte metabolic interactions offers the potential for developing novel therapeutic strategies for many neurological disorders that include a metabolic deficit.

Keywords: brain imaging, glia, glucose, glutamate transport, neurodegenerative diseases, position emission tomography.

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Activated neurons oxidize more glucose to face their increased energy need. Until recently, this assertion was the starting point of any textbook devoted to brain energy metabolism (Siesjö 1978). During the last decade, the entrance of astrocytes in the scene of neuroscience has revolutionized many constrained principles (for review see Volterra and Meldolesi 2005). As for the other fields of neuroscience, astrocytes are now important and active partners of neurons in the regulation of brain energy metabolism. Since many comprehensive and authoritative reviews have already been published on this topic (Ames 2000; Magistretti 2003), we will only underline the milestones that have led to the notion that brain energy metabolism is regulated by an intimate dialogue between astrocytes and neurons and specifically review the major contribution of Nuclear Magnetic Resonance (NMR) spectroscopy to this notion. We will finally discuss the importance of such a crosstalk during pathological situations.

From blood to ATP: the long journey of glucose

Although the brain represents only 2% of the body weight, it receives 15% of the cardiac output and accounts for 20% of total body oxygen consumption, and 25% of total body glucose utilization. The respiratory quotient (RQ) of the brain is close to 1, indicating that carbohydrates are the substrates for oxidative metabolism (for references see Magistretti 2003). However, the brain is also able to use additional energy substrates if they are present in sufficient amounts in the blood. For example, the brain can rely on ketone bodies when their blood levels rise in conditions of prolonged starvation or high-fat diet (Nehlig 2004). Blood-borne

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Abbreviations used: α-KG, alpha-ketoglutarate; ALS, amyotrophic lateral sclerosis; EAAT, excitatory amino acid transporter; fMRI, functional magnetic resonance imaging; GLUT, glucose transporter; GS, glutamine synthetase; NMR, nuclear magnetic resonance; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PET, positron emission tomography; RQ, respiratory quotient; TCA, tricarboxylic acid; V_{TCA}, TCA cycle flux; V_{PC}, pyruvate carboxylase flux; V_{cycle}, glutamate/glutamine cycle flux.

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glucose crosses the vascular endothelial cells and enters brain cells through specific hexose transporters of the glucose transporter (GLUT) family (Fig. 1), along its diffusion gradient (Vannucci *et al*. 1997; McEwen and Reagan 2004). The glucose concentration in the blood is around 5 mM (at normoglycemia) and approximately 1 mM in the extracellular space (for references see Leybaert 2005). Besides the 55 kDa GLUT1 which is highly expressed at luminal and abluminal plasma membranes of blood–brain barrier endothelial cells, each cell type can express a different form of GLUTs, suggesting that glucose transport is differentially regulated among brain cells. Following transport, glucose is phosphorylated by the hexokinase. This process merits a great attention because it is the rate-limiting step in glycolysis and as such, it may exert control over glucose metabolism. However, a recent analysis has suggested that glucose transport may also be a rate-limiting step for glucose use (Barros *et al*. 2005). At rest, the baseline metabolic rate of the brain is very high and heterogeneous (Gusnard and Raichle 2001) and most of the energy consumed by the brain is thought to support glutamatergic neurotransmission at least in the cortical grey matter (see paragraph on NMR spectroscopy) (Shulman *et al*. 2004). Physiological changes in the cellular activity of the brain are invariably accompanied by changes in local blood flow and glucose utilization. Such powerful relationships formed the bases for the imaging methods such as Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI) (Raichle 1998).

In the mid-1980s, brain imaging studies using PET indicated that in normal, awake adult humans, visual or somatosensory stimulation results in dramatic increases in blood flow and glucose utilization but minimal increases in oxygen consumption. These results supported the dissident notion that a part of the glucose consumption process during an increased neuronal activity might be non-oxidative (Fox and Raichle 1986; Fox *et al*. 1988). As the supply of oxygen is therefore not matched precisely with the demand, an unexpected consequence is that the actual amount of oxygen remaining in blood vessels at the site of brain activation

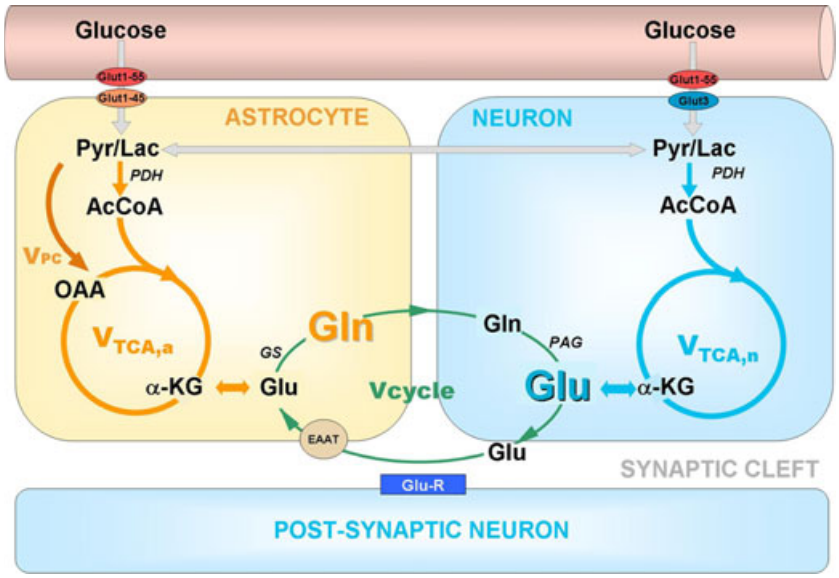

Fig. 1 Neurotransmitter cycling and metabolic fluxes between neurons and astrocytes. Glutamatergic neurotransmission is governed both by the action of glutamate (Glu) upon postsynaptic glutamate receptors (Glu-R) following its release into the synaptic cleft and by the uptake of glutamate by astrocytes via excitatory amino acid transporters (EAAT). In astrocytes, glutamate is converted into glutamine (Gln) by glutamine synthetase (GS). Glutamine is then transported back to neurons and transformed into glutamate by the phosphate-activated glutaminase (PAG). This specific cellular localization of these transporters and enzymes allows to define a neuronal pool of glutamate and an astrocytic pool of glutamine. The quantification of energy metabolism by NMR essentially relies on the detection of ^{13}C accumulation within these two large cerebral pools. Indeed, following injection of ^{13}C-enriched glucose, ^{13}C labeling is incorporated through complete metabolic pathways, including transport via glucose transporters (Glut1–55: glucose transporter 1, endothelial, 55Kd isoform; Glut1–45: glucose transporter 1, astrocytic, 45Kd isoform; Glut3: glucose transporter 3, neuronal), glycolysis, neuronal TCA cycle (through pyruvate dehydrogenase, PDH via acetylCoA, AcCoa) and astrocytic TCA cycle (through PDH and Pyruvate Carboxylase, PC). Since the pool of alpha-ketoglutarate (α-KG) is dynamically equilibrated with the pool of glutamate, different fluxes (filled arrows) can be determined: the neuronal and astrocytic TCA cycle flux (V_{TCA}), the pyruvate carboxylase flux (V_{PC}) and the glutamate/glutamine cycle flux (V_{cycle}). Due to the low sensitivity of NMR detection and since no compartmentation exists for lactate, it is currently not possible to quantify neither the glycolytic flux nor the astrocyte-neuron lactate shuttle (grey arrows) with *in vivo* NMR spectroscopy.

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changes. Because the MRI signal intensity is sensitive to the amount of oxygen carried by hemoglobin (Ogawa *et al*. 1990), this change in blood oxygen content at the site of brain activation formed the basis for the blood-oxygen-level-dependent signal that is now routinely employed in many imaging institutes (Raichle 1998). To summarize, in physiological conditions, glucose is the sole substrate for energy production and it is broken down with the help of oxygen to supply the brain with energy. During neuronal activation, glucose may be broken down anaerobically, by the rapid process of glycolysis, to supply energy faster (Gusnard and Raichle 2001). In the next section (vide infra), we will shed light upon the notion that glutamate plays a key role in the regulation of brain energy metabolism through neuron–astrocyte interactions.

Glutamate: from glucose to synaptic vesicles, a process controlled by astrocytes

Glutamate, the major excitatory transmitter in many brain regions including the cortex, possesses several characteristics that make it a unique neurotransmitter in the field of neuroenergetics (see Magistretti 2003). On one hand, it is tightly linked to the metabolism of glucose and on the other hand, glutamate represents a coupling signal to supply active neurons with glucose-derived energy.

Glutamate is an alpha-ketoglutarate [α-KG, an intermediate of the tricarboxylic acid (TCA) cycle] derivative and its pool is dynamically equilibrated with the pool of α–KG via a bidirectional transamination (Fig. 1) so that glutamate can be labeled following *in vivo* administration of any labeled substrate that will enter the TCA cycle (see paragraph on NMR spectroscopy). Glutamate is also a metabolic fuel since it can be oxidatively degraded, after its conversion to α-KG (Waagepetersen *et al*. 2002), to provide up to three-fourths of the ATP that glucose oxidation can produce (for references see Hertz and Zielke 2004; Marcaggi and Attwell 2004). Such an oxidation process is supposed to occur mainly in astrocytes.

Following release in the synaptic cleft, glutamate is avidly taken up by astrocytes to start an 'ecological' process of recycling. Indeed, glutamate re-uptake is efficiently performed by two glial glutamate transporters (EAAT1 or GLAST and EAAT2 or GLT-1) (Danbolt 2001) while the conversion of glutamate into glutamine is under the control of a specific astrocytic enzyme, glutamine synthetase (GS). Glutamine is then transported back to neurons via amino acid transporters (Mackenzie and Erickson 2004), then transformed into glutamate by the phosphate-activated glutaminase and finally packed into vesicles by the vesicular glutamate transporters (Fremeau *et al*. 2004) (Fig. 1). Therefore, the levels of glutamate available to neuronal vesicles are dependent upon astrocyte delivery (in the form of glutamine). The cost of the glutamate recycling is two ATP for each recycled molecule (one ATP for the NA/K ATPase and one ATP for the GS).

Because glutamate can be oxidized and is not fully regenerated through the glutamate-glutamine cycle, its *de novo* synthesis is required to maintain the neuronal glutamate pool. This synthesis requires the major metabolic fuel glucose to provide the carbon backbone as α-KG through the TCA cycle. Since α-KG is used for this synthesis and in order to maintain the TCA flux, a net synthesis of TCA intermediates must occur. This is performed by the enzyme pyruvate carboxylase (PC) which generate oxaloacetate and is located almost exclusively in astrocytes (Hertz and Zielke 2004) (Fig. 1).

In conclusion, glutamate is a signaling molecule for excitatory transmission when acting upon neuronal/glial receptors but since its synthesis is closely linked to glucose metabolism, glutamate may also be involved in the regulation of brain energy metabolism. Experiments performed 12 years ago on cell cultures have indeed demonstrated that glutamate may be a coupling signal between neuronal activity and glucose uptake (Pellerin and Magistretti 1994).

Glutamate/Na^+ uptake into astrocytes: a key triggering signal for glucose use

The precise molecular mechanisms responsible for the increase in glucose use by the active brain cells have remained unclear until recently. Results obtained from *in vivo* studies have indicated that the increase in glucose use varies in proportion to the magnitude of neuronal activity (Sokoloff 1999). Neuronal excitation generates action potentials produced by a depolarization-induced rapid inward Na^+ current and K^+ efflux. Numerous experiments have been conducted in cell cultures to determine the effects of elevated extracellular K^+ and/or intracellular Na^+ on glucose metabolism. In cultured neurons, membrane depolarization by elevated extracellular K^+ concentration causes rapid Na^+ influx through voltage-sensitive Na^+ channels. The consequent increases in intracellular Na^+ concentration and/or extracellular K^+ stimulate Na^+, K^+-ATPase activity, which in turn stimulates energy metabolism and the rate of glucose utilization in neurons (Takahashi *et al*. 1995). The direct increase of intracellular Na^+ by opening voltage-sensitive Na^+ channels with veratridine also stimulates neuronal glucose uptake (Takahashi *et al*. 1995; Honegger and Pardo 1999). In contrast, astrocytic glucose metabolism is not stimulated by high extracellular K^+, since these cells do not produce action potential and, indeed, have a large capability to buffer K^+ (Seifert *et al*. 2006). However, glucose metabolism is stimulated by increased intracellular Na^+ in astrocytes as in neurons. Na^+-mediated stimulation of glucose use can be triggered by the carboxylic Na^+ ionophore monensin (Yarowsky *et al*. 1986), by veratridine (Takahashi *et al*. 1995), also by cyclothiazide which prevents desensi-

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tization of AMPA receptors thereby increasing Na^+ influx (Chatton *et al*. 2000; Voutsinos-Porche *et al*. 2003b) and finally by the cognitive-enhancing drug CX546 that increases Na^+ influx through AMPA receptors (Pellerin and Magistretti 2005).

Apart from depolarization and ionic fluxes, neurotransmission also involves release of specific neurotransmitters such as glutamate. Indeed, the presence of glutamate itself can raise glucose uptake in astrocytes in a dose-dependent manner (Pellerin and Magistretti 1994; Takahashi *et al*. 1995). The effect of glutamate is mediated by the Na^+-dependent glutamate uptake system in astrocytes (GLAST and GLT-1) independently of glutamate receptors, which may provide a new mechanism of neurometabolic coupling. Glutamate uptake is powered by the cotransport of three Na^+ ions and one H^+ while one K^+ is counter-transported (Attwell and Gibb 2005). Additional experiments have confirmed that it is indeed the intracellular concentration of Na^+ and not glutamate itself that activates the Na^+, K^+-ATPase pump, decreases ATP levels and activates glycolysis in astrocytes (Chatton *et al*. 2000; Voutsinos-Porche *et al*. 2003b) upon glutamate release. Results from experiments performed *in vivo* have supported such hypothesis by showing that the glucose use evoked by either somatosensory (Voutsinos-Porche *et al*. 2003a; Voutsinos-Porche *et al*. 2003b) or visual stimulation (Herard *et al*. 2005) is strongly decreased in mice deficient for GLT-1. Owing to the fact that astrocytes are highly connected cells through gap junctions (Giaume and McCarthy 1996), it has been recently proposed that astrocytes function as a network for concerted neurometabolic coupling through the generation of intercellular Na^+ metabolic waves (Bernardinelli *et al*. 2004). However, it is important to consider that the uptake of glutamate *per se* does not consume energy and that only the associated glutamine synthesis and reestablishment of the Na^+ gradient will use ATP (no more than 3% of the total ATP consumed, see Attwell and Laughlin 2001).

Besides stimulation of glycolysis in astrocytes, can glutamate also regulate the transport of glucose in both neurons and astrocytes? To address this question, glucose transport was studied simultaneously in cultured hippocampal neurons and neighboring astrocytes using a real-time assay based on confocal epifluorescence microscopy and fluorescent glucose analogs (Porras *et al*. 2004). These authors found that glutamate, although stimulating glucose transport in astrocytes, strongly inhibited glucose transport in neurons, by a mechanism that is dependant on glutamate receptors. These results suggest that glutamate is not only able to activate astrocytic glycolysis but could also differentially regulate glucose transport in astrocytes and neurons.

If there is a consensus about the notion that the entry of Na^+, which is related, via a close stoichiometry, to the uptake of glutamate in astrocytes, is a key signaling metabolic event, a strong controversy still exists about the existence of a metabolic compartmentalization between glycolytic astrocytes and oxidative neurons (see for references Bonvento *et al*. 2005). The hypothesis initially put forward by Pellerin and Magistretti was that, upon glutamate release, astrocytes will export lactate to the neurons where it is converted back to pyruvate in order to fuel mitochondria (Pellerin and Magistretti 1994). The controversy over this so called astrocyte neuron lactate shuttle is not the topic of the current review and many recent reviews have been already published (Hertz 2004; Bonvento *et al*. 2005; Schurr 2006).

We will now discuss how results obtained by NMR spectroscopy have provided convincing arguments in favor of a central role of astrocytes and glutamate in the regulation of brain metabolism.

Astrocyte contribution to the regulation of energy metabolism as revealed *in vivo* by ^{13}C NMR spectroscopy

Intimacy of astrocytes/neurons dialogue partially undisclosed by NMR

Since the mid-1980s, NMR spectroscopy has proven a unique tool to measure brain metabolic fluxes *in vivo*. The most common NMR approach relies on the infusion of ^{13}C-enriched substrates of brain metabolism, mainly glucose but occasionally acetate or ketone bodies. The natural abundance of ^{13}C being 1.1%, isotopic incorporation into brain metabolites can be measured during i.v. infusion of highly ^{13}C-enriched substrates (Rothman *et al*. 1985). The ^{13}C measurement of metabolic rates has strong similarities with radio-isotopic imaging techniques such as ^{14}C-2-DG autoradiography or ^{18}F-FDG-PET which measure glucose consumption (Boumezbeur *et al*. 2005). However the NMR approach differs from radiotracer detection in several ways and may advocate NMR as the instrument of choice for *in vivo* monitoring of the dialogue between astrocytes and neurons.

NMR spectroscopy has the unique property to specifically identify the molecule and the atomic position in this molecule at which the ^{13}C label accumulates. This contrasts with nuclear techniques for which radioactivity is measured, independently of the metabolite the radioactive tracer is attached to. Thanks to this property, fully metabolized substrates, i.e. [1–^{13}C]glucose, that are metabolized the same way as endogenous non-labeled substrates, can be used. In contrast, radio-isotopic techniques like PET or autoradiography must use non-metabolizable analogs like ^{18}F-FDG or ^{14}C-2-DG to measure glucose use following acquisition of a single time-activity curve originating from all radio-labeled atomic positions.

^{13}C-NMR provides several ^{13}C time-courses for identified carbon positions, supplying sufficient information to use complex metabolic models to derive metabolic fluxes

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including all major pathways labeled by ^{13}C-glucose. Indeed, upon i.v. administration of [1–^{13}C]glucose, ^{13}C is incorporated into glycolysis, neuronal TCA cycle (through pyruvate dehydrogenase, PDH), astrocytic TCA cycle (through PDH and PC), and substrate exchange between neurons and astrocytes (glutamate/glutamine cycle, see Fig. 1). *In vivo* NMR performed during [1–^{13}C]glucose administration allows to distinguish ^{13}C accumulation within ~10 different atomic positions in different molecules: glutamate C2, C3 and C4, glutamine C2, C3 and C4, glucose C1, lactate C3, aspartate C2 and C3 (Behar *et al*. 1986), glutamine (Gruetter *et al*. 1994; Gruetter *et al*. 2001) and to a lesser extent aspartate or glycogen (Choi *et al*. 1999). In particular, the possibility to resolve glutamate labeling from glutamine labeling is of great interest for the study of the dialogue between astrocytes and neurons. Indeed, glutamate and glutamine are strongly compartmentalized (in neurons for glutamate and in astrocytes for glutamine) and they are directly connected to energy metabolism and neurotransmission (see former paragraph).

These two features of ^{13}C NMR have allowed quantitative determination of fluxes through energy metabolism and neurotransmission in rat and human cerebral cortex. Using neuron/astrocyte bi-compartment models (Sibson *et al*. 1997; Shen *et al*. 1999; Gruetter *et al*. 2001) fluxes within each cell type and fluxes connecting both cell populations were determined: the neuronal and astrocytic TCA cycle flux (V_{TCA}), the pyruvate carboxylase flux (V_{PC}) and the glutamate/glutamine cycle flux (V_{cycle}) (Fig. 1). Those early studies – based on [1–^{13}C]glucose – were shortly followed by alternate labeling strategies using [2–^{13}C]glucose or [2–^{13}C]acetate which provided independent measures with increased sensitivity to particular pathways (anaplerosis for [2–^{13}C]glucose, astrocytic TCA cycle for [2–^{13}C]acetate) (Sibson *et al*. 2001; Lebon *et al*. 2002). All studies yielded consistent values, demonstrating that the glutamate/glutamine cycle is a major metabolic flux in the mammalian brain, and that glutamate neurotransmitter replenishment is essentially accomplished by the neuron/astrocyte glutamate/glutamine cycle. The glutamate/α-KG cycle was shown to account for less than 10% of substrate cycling between astrocytes and neurons (Lebon *et al*. 2002). By unambiguously demonstrating the crucial role of astrocytes in maintaining the synaptic homeostasis of glutamate, these ^{13}C NMR studies brought a significant contribution to the understanding of the neuron/astrocyte dialogue.

The second major contribution results from the unique ability of NMR to derive both the rate of glucose oxidation (V_{TCA}) and the rate of neurotransmission (V_{cycle}) from the ^{13}C time-courses collected during the same experiment. About 20 years before NMR made it possible to measure those two metabolic rates *in vivo*, ^{14}C-2-DG studies demonstrated that glucose metabolism and electrical activity were tightly coupled in regions rich in synapses but not in cell bodies (see for references Sokoloff 1999). These observations supported the concept of coupling between energy metabolism and neurotransmission. From the mid-1990s, NMR allowed *in vivo* exploration of the relationship between glucose consumption and neurotransmission in rodents at different levels of electrical activity (Sibson *et al*. 1998; Choi *et al*. 2002; Oz *et al*. 2004; Patel *et al*. 2004) and in various brain regions (de Graaf *et al*. 2004). Altogether these studies demonstrated a roughly linear relationship between glucose oxidation and neurotransmission in the rat brain. In spite of controversies on the value of glucose consumption at isoelectricity (intercept of the 'neurotransmission – glucose oxidation' curve) and on the exact slope of the relationship (Choi *et al*. 2002; Gruetter 2002), ^{13}C NMR studies provided strong evidence to suggest that energy consumption is mostly devoted to neurotransmission in the cerebral cortex, and that changes in neurotransmission result in proportional changes in glucose metabolism (Hyder *et al*. 2006). At the same time, independent *in vitro* studies on cultured astrocytes and *ex vivo* studies on rodents suggested that astrocytic glutamate uptake may regulate energy metabolism. In this context, the NMR-observed relationship between glucose oxidation and glutamate/glutamine neurotransmission cycling brings strong arguments in favor of a tight coupling, *in vivo*, between neuronal activity and energy metabolism mediated by glutamate transport from the synaptic cleft into astrocytes.

Large parts of the conversation remain undisclosed

^{13}C NMR presents several striking advantages over radiotracer techniques for the exploration of brain energy metabolism, mostly due to the NMR signal reflecting the molecular environment of each detected nucleus. But the price for this fine biochemical probing is the intrinsic low sensitivity of NMR detection. A practical consequence is the inability to detect trace amounts of the label by NMR. Quantification of energy metabolism by NMR essentially relies on the detection of ^{13}C accumulation within the large cerebral pools of glutamate (~10 mM) and glutamine (~3 mM). The fact that ^{13}C label undergoes glycolysis does not imply that the glycolytic flux can be determined by NMR, simply because the concentration of glycolytic intermediates is far too low to be detected by ^{13}C NMR, even when it becomes highly enriched. Notwithstanding, if glycolytic intermediates could be detected by ^{13}C NMR, the absence of clear compartmentation of lactate would not allow NMR spectroscopy to assign a glycolytic flux to any cell type. Therefore *in vivo* NMR is currently unable to resolve the controversy on the existence of a lactate shuttle. Although *in vitro* and *ex vivo* NMR studies do not allow quantitative measurements of metabolic fluxes, they can offer experimental arguments in favor of a preferential lactate consumption by neurons. Performing NMR spectroscopy on cell cultures has several advantages over *in vivo* NMR:

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specific cell types can be studied (astrocytes or neurons), ^{13}C precursors that barely cross the BBB can be used, and increased sensitivity can be achieved by high resolution NMR analysis of cell suspensions. Based on this approach, evidence for lactate being preferentially metabolized by neurons has accumulated (Waagepetersen *et al*. 2000a; Bouzier-Sore *et al*. 2003) and the concept of a lactate/alanine shuttle between astrocytes and neurons was proposed (Waagepetersen *et al*. 2000b; Zwingmann *et al*. 2000; Zwingmann and Leibfritz 2003). Through this shuttle, alanine would be a nitrogen carrier from glutamatergic neurons to astrocytes, providing astrocytes with nitrogen for glutamine synthesis. Lactate would provide neurons with carbon chains for oxidation. *Ex vivo* ^{13}C NMR – which consists in high resolution analysis of brain extracts labeled *in vivo* – also supported the existence of a significant lactate flux from astrocytes to neurons. Using combinations of ^{13}C-labeled and unlabeled glucose and lactate administered *in vivo*, the implication of the astrocyte-neuron lactate shuttle in the coupling between cerebral activity and energy metabolism has been demonstrated (Serres *et al*. 2003; 2005). *In vivo* observation of the lactate/alanine shuttle and quantification of the lactate flux from astrocytes to neurons are beyond the current sensitivity of *in vivo* NMR. The question as to whether high field *in vivo* NMR system will help resolving these issues remains open.

Another consequence of NMR intrinsic low sensitivity is the need to perform ^{13}C NMR measurements in large brain voxels (typically ~25 mL in humans) which are two to three orders of magnitude larger than the spatial resolution of human PET systems (~0.1 mL). For rodent studies, high-field NMR systems significantly reduce the gap. Indeed the NMR signal increases with the magnetic field (due to increased spin polarization), whereas the radiotracer activity does not increase when reducing the size of the PET system. However, the typical spatial resolution of ^{13}C detection in rats (~50 μL) remains larger than micro-PET (~5 μL) by one order of magnitude. This is the main reason why ^{13}C NMR has been having a hard time exploring focal neural activation, whereas PET studies provided convincing data on the energy basis of cerebral activation (Fox *et al*. 1988). Several groups have attempted to quantify changes in oxidative metabolism associated with focal activation (Hyder *et al*. 1996; Chen *et al*. 2001; Chhina *et al*. 2001). However the sensitivity of *in vivo* NMR is limited for the accurate quantification of energy metabolism and neurotransmission upon focal activation. It must be noted that the exploration of the coupling between neurotransmission and energy metabolism was not performed by varying the degree of focal neural activation, but by modulating whole brain activity using various anesthetics. This observation does not minimize the interest of this landmark work, but studying the relationship between neurotransmission and energy metabolism upon focal neural activation remains a challenge for ^{13}C NMR spectroscopy.

In the last section, we will briefly point out the potential relevance of this metabolic crosstalk between neurons and astrocytes during various pathological conditions.

Neuron–astrocyte metabolic interactions during pathological conditions

The brain, which has important energy needs and low glycogen levels [located in astrocytes (see Brown 2004)], is highly vulnerable to energetic dysfunction. Many brain diseases have an energetic component (Beal 2000) but the role of the metabolic impairment in the disease process is highly variable. It can be very straightforward as during a stroke when the acute disruption in energy supply will lead to a massive cellular death in the infarct core (Lo *et al*. 2003). In some genetic diseases, the mutation of a key metabolic enzyme or transporter (ex: GLUT1 deficiency syndrome, see Wang *et al*. 2005) triggers neuronal dysfunction. Alternatively, the impact of metabolic impairment in the disease process can be indirect and exacerbate other pathological events such as reactive oxygen species production, calcium deregulation and in particular glutamate excitotoxicity (Beal 2000). Many reviews have addressed the deleterious role of metabolic impairment during pathogenic processes. However, the focus is usually on one partner (neurons) and the impact of a dysfunctional dialogue between astrocytes and neurons has been underestimated until recently (Seifert *et al*. 2006).

Given the key role played by glutamate in the regulation of brain energy metabolism and the fact that glutamatergic homeostasis is maintained through exchanges between astrocytes and neurons, any deregulation of this dialogue is likely to induce combined (and potentially additive) deleterious effects for brain function. In addition, glutamate can by itself be neurotoxic. If present at too high concentration in the synaptic cleft, it is a potential neurotoxin and can trigger neuronal death by a process called excitotoxicity. During stroke, glutamate is massively released from synaptic vesicles and from cytoplasmic pools through transporters working in a reverse mode (Jabaudon *et al*. 2000; Rossi *et al*. 2000), and different types of transmembrane channels (see References in (Volterra and Meldolesi 2005). The reversal of glutamate transporters not only hampers the removal of glutamate from the extracellular space, it also exacerbates the shortage in energetic substrates by suppressing the coupling signal for glucose entry into brain parenchyma. A dysfunction of the homeostasis, recycling and metabolism of glutamate is also involved during the course of many chronic neurodegenerative diseases. Abnormalities in glutamate homeostasis and metabolic function are both hallmarks of neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS) (Maragakis and Rothstein 2004). In ALS patients for example, the regions of selective death of motoneurons (spinal cord and motor

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cortex) have a lower capacity for glutamate uptake (Rothstein *et al*. 1992) and express GLT-1 at a reduced level (Rothstein *et al*. 1995). Again, a reduction in glutamate uptake would lead to excitotoxicity but would also reduce glucose supply and exacerbate energy impairment. Increasing the level of expression of glutamate transporters with β-lactam has proven an efficient therapeutic approach in ALS. This drug delayed neuronal loss and muscle strength and increased mouse survival in an animal model of ALS (Rothstein *et al*. 2005).

Such a strategy could also improve brain energetic supply by bolstering the signal for glucose uptake. More generally, reinforcing the efficiency of the metabolic dialogue between neurons and astrocytes may be beneficial for neuronal survival. Indeed, it has recently been shown that a viral vector-driven overexpression of lactate transporters on neurons and glucose transporters on astrocytes efficiently protects neurons from excitotoxicity *in vitro* (Bliss *et al*. 2004).

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