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Sodium Signaling and Astrocyte Energy Metabolism

by

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We would like to dedicate this review to Louis Sokoloff, a pioneer and one of the most influential, knowledgeable, and inspiring scientists in the field of brain energy metabolism.

Main points:

1. Astrocytic sodium is an energy currency and a key mediator of neurometabolic coupling.

2. Fluorescent probes for ions and metabolites are being combined to bridge the mechanistic gap between astrocytic sodium dynamics and the metabolic machinery.

Keywords: membrane transport, Na,K-ATPase, neuron-glia interactions, glycolysis, mitochondria, syncytium, lactate

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Abstract

The Na^+ gradient across the plasma membrane is constantly exploited by astrocytes as a secondary energy source to regulate the intracellular and extracellular milieu, and discard waste products. One of the most prominent roles of astrocytes in the brain is the Na^+-dependent clearance of glutamate released by neurons during synaptic transmission. The intracellular Na^+ load collectively generated by these processes converges at the Na,K-ATPase pump, responsible for Na^+ extrusion from the cell, which is achieved at the expense of cellular ATP. These processes represent pivotal mechanisms enabling astrocytes to increase the local availability of metabolic substrates in response to neuronal activity. This review presents basic principles linking the intracellular handling of Na^+ following activity-related transmembrane fluxes in astrocytes and the energy metabolic pathways involved. We propose a role of Na^+ as an energy currency and as a mediator of metabolic signals in the context of neuron-glia interactions. We further discuss the possible impact of the astrocytic syncytium for the distribution and coordination of the metabolic response, and the compartmentation of these processes in cellular microdomains and subcellular organelles. Finally, we illustrate future avenues of investigation into signaling mechanisms aimed at bridging the gap between Na^+ and the metabolic machinery.

Introduction

The intimate relationship between Na^+ and metabolism goes back to the beginnings of cellular life. As soon as living matter took the form of a thin lipid vesicle enclosing metabolites and macromolecules, it had to deal with the phenomenon proposed by Willard Gibbs and demonstrated experimentally by Frederick Donnan (Donnan 1911; Macknight and Leaf 1977). The presence of non-diffusible negatively charged species, such as metabolites, proteins and nucleic acids, forces an uneven distribution of diffusible ions across the cell membrane, resulting in lower water activity within the cell, net water influx, and cell swelling. Unless an equal and opposite force balances this tendency, cells will burst and die. Bacteria and plants counteract the Donnan effect in thrifty fashion by coating themselves with extracellular walls that withstand hundreds of atmospheres of pressure. Mammalian cells shunned the rigidity of cell walls and instead solved the challenge by bailing out water. This is not done by pumping water itself, but by pumping Na^+ (Skou 1989), which is followed by osmotically obliged water. The Na,K-ATPase pump generates a double-Donnan equilibrium, whereby the asymmetric distribution of non-diffusible anions is exactly compensated by a counter-distribution of Na^+ (Macknight and Leaf 1977). One thing leads to another, and the energy stored in the Na^+ gradient has permitted the emergence of great inventions like action potentials, synaptic currents, memory and cognition. The downside is that bailing out requires constant expenditure of ATP, as evidenced by cytotoxic glial swelling following brain tissue ischemia and traumatic brain injury (Liang et al. 2007). The Na^+ gradient is also exploited as a secondary energy source to clear neurotransmitters such as glutamate and GABA, and recycle waste products such as NH_4^+, functions that are much more developed in astrocytes than in neurons (Weber and Barros 2015).

The maintenance of the transmembrane Na^+ gradient in neural cells represents a large energy cost, estimated to correspond to more than half of the overall ATP hydrolyzed in the brain (Erecinska and Silver 1994; Hevner et al. 1992). Brain Na^+ homeostasis therefore has crucial implications for brain energy metabolism both in health and disease, and has consequences for functional brain imaging techniques, as the signals that they detect are based on energy expenditure coupled to neuronal activity (Magistretti and Allaman 2015). The aims of this review are to examine the relationship between astrocyte energy metabolism and Na^+ fluxes,

to propose a role of Na^+ as an energy currency and to emphasize how little we know about the molecular mechanisms that control energy metabolism in glial cells.

Transmembrane Na^+ fluxes and energy metabolism

Recent years have brought about several discoveries on the role of intracellular Na^+ in neural cells. These advancements have been to a good extent made possible with the emergence of imaging technologies for monitoring Na^+ dynamics *in situ* , and are shedding a new light on cerebral Na^+, revealing it as a component of rapid intracellular as well as intercellular signaling between astrocytes and neurons. Parallel development of genetically-encoded sensors for glucose, ATP, NADH, lactate and pyruvate, have opened the possibility of imaging metabolite levels, and the determination of concentrations and fluxes, also with cellular resolution and in real time (for a review, see San Martin et al. 2014). The brain is conspicuous for fast local fluctuations of ion levels and energy demand, processes which may now be detected and quantified.

Due to their large transmembrane electrochemical gradient, Na^+ ions constitute a major driving force used for both fast electrical signaling in excitable cells and ubiquitously for transmembrane solute transport. Neural cells deploy energy-costly processes to ensure that the intracellular Na^+ concentration remains low, in such a way that the ~10-fold concentration difference with external Na^+ concentration, along with a strongly negative electrical potential, can be used to energize multiple ion channels and transporters. On top of their own housekeeping duties, glial cells have evolved specific transport functions related to their intimate relationship with neurons, and Na^+ is key to these roles (**Figure 1**). Among the most abundant proteins of the brain are GLT-1 and GLAST, the astrocytic Na^+/glutamate co-transporters responsible for nearly instant termination of excitatory neurotransmission. GLT-1 can lower interstitial glutamate to nanomolar levels by coupling the internalization of one neurotransmitter molecule to that of three Na^+ ions (Levy et al. 1998). There is little doubt that the increases in intracellular Na^+ accompany glutamatergic transmission (Chatton et al. 2000; Karus et al. 2015; Langer and Rose 2009) and that this Na^+ entry is coupled to increased energy consumption by astrocytes (Magistretti and Chatton 2005).

Active neurons release K^+, which depolarizes astrocytes, leading to activation of the NBCe1, the abundant Na^+/HCO_3^- co-transporter (**Figure 1**) that, along with the plasma membrane

Na^+/H^+ exchanger (NHE), deals with the protons captured by GLT-1/GLAST and the plasma membrane Ca^{2+} pump (PMCA). NBCe1 along with NHE transporters represent the major acid-base regulation system in neural cells (Ruffin et al. 2014). Na^+ also enters cells via the Na^+/Ca^{2+} exchanger (NCX), a major mechanism of Ca^{2+} regulation during glutamate and ATP mediated signaling, expressed both by neurons and glial cells, including astrocytes, microglia and oligodendrocytes (Annunziato et al. 2013). Another crucial Na^+-dependent transport system expressed at the astrocyte membrane is the $Na^+/K^+/2Cl^+$ cotransporter (NKCC) which contributes to the electroneutral uptake of Na^+, K^+ and Cl^-, and the control of the extracellular K^+ concentration as well as the cell volume (Kofuji and Newman 2004). Of note, the main inhibitory neurotransmitter GABA is also in part cleared from the brain interstitium by a Na^+-coupled cotransporter located in astrocytes although with kinetics that are different from those operative for glutamate, which result in only marginal and transient changes in Na^+ homeostasis (Chatton et al. 2003). These Na^+ transport mechanisms are discussed in detail elsewhere in this issue of Glia. The continuous influx of Na^+ through these and other entry pathways can be evidenced for instance by experimentally inhibiting the Na,K-ATPase, which leads in the resting state to a rapid rise of cytosolic Na^+ (**Figure 2A**).

Other Na^+ conductances potentially contribute to background Na^+ entry in astrocytes and therefore to a sustained energy expenditure. Among them, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, permeable to both Na^+ and K^+, have their expression strongly upregulated in reactive astrocytes following ischemic insult (Honsa et al. 2014). The passive conductance of astrocytes was also recently shown to be to a large extent due to a heterodimer of two pore forming channels (K2P) composed of TWIK-1 and TREK-1 proteins (Hwang et al. 2014). This complex K^+ channel has a non-conventional pore domain that was shown in cardiomyocytes to become Na^+ permeable when extracellular K^+ levels fall below ~3mM (Ma et al. 2011), a situation that could occur during pathological hypokalemia. In brain tissue following high frequency burst of activity (Heinemann and Lux 1975), K^+ was shown to undershoot to values as low as ~2mM. However, it remains to be determined whether the size and duration (0.5-4 min) of the K^+ undershoot are sufficient to alter the ion selectivity of the channel.

Finally, transient receptor potential vanilloid type 1 (TRPV1) was shown to be functionally expressed by astrocytes and to preferentially conduct Na^+ (Huang et al. 2010). The

conductances cited above were identified and mainly studied for their roles in defining the membrane properties of cells. Determining their flux relative to those of housekeeping and activity-dependent Na^+ influx pathways and thus their impact on bioenergetics, will help to figure out the extent to which Na^+ plays a tonic and a phasic role in energy control.

Na^+ distribution and energy metabolism

Cytosolic and sub-cellular level

Na^+ concentration in the astrocyte cytosol is in the range 10-17mM (see e.g. Chatton et al. 2000; Rose and Ransom 1996; Rose and Ransom 1997b; Unichenko et al. 2012), whereas the interstitial Na^+ concentration was measured to be around 150mM (Dietzel et al. 1982), thereby creating an approximately ten-fold concentration gradient across the plasma membrane. Protoplasmic astrocytes have slender processes extending for tens of micrometers towards synaptic regions, prompting the question of whether inside these structures Na^+ may behave as a local signal, like Ca^{2+} and H^+, possibly controlling local metabolism. According to Brownian diffusion, the answer is negative, because GLT-1-mediated Na^+ entry is too slow compared with diffusion (Barros and Martinez 2007). However, a Na^+-sensitive dye did detect local Na^+ transients in astrocytes in hippocampal slices (Langer and Rose 2009). This conflict between theory and experiment may be reconciled by the unexpected delay observed for the diffusion of non-charged fluorescent probes between astrocytic soma and end-feet, the processes that ensheathe capillaries (Nuriya and Yasui 2013). Conceivably, the diffusion of Na^+ may be even more restricted if the putative cytosolic sieve between end feet and somata selects by charge. Similar diffusional restrictions appear to be present in neurons, as Na^+ gradients were imaged even between adjacent dendritic spines, in conflict with the known diffusion coefficients measured for neuronal cytosol (Rose and Konnerth 2001). Thus, if restricted Na^+ diffusion in astrocytes is further substantiated, processes would emerge as semi-autonomous in terms of Na^+ handling and possibly of energy metabolism.

In contrast to Ca^{2+} and H^+ ions, which are heavily buffered, most Na^+ ions are free. The question of whether Na^+ could distribute differentially or be accumulated into intracellular organelles has yet to be clarified. However, limited experimental approaches can currently help the evaluation of the fate of intracellular Na^+ and its impact on the energy metabolism.

Electron probe microanalysis of cryosections from slice cultures (Pivovarova et al. 2002; Pozzo-Miller et al. 1997) estimated a mitochondrial Na^+ resting level of approximately 11-12mM (*i.e.* 25mmol/kg dry weight), similar to the 19mM value found in mitochondria of living cultured astrocytes (Bernardinelli et al. 2006) using a mitochondria-specific Na^+-sensitive fluorescent indicator. During neuronal activity (Pivovarova et al. 2002) or in response to glutamate application to astrocytes (Bernardinelli et al. 2006), mitochondrial Na^+ concentration can reach over 30mM. The Na^+ entry into mitochondria involves its exchange with Ca^{2+} by the mitochondrial Na^+/Ca^{2+} exchanger, and may also be partly mediated by mitochondrial cation conductances such as mitochondrial K_{ATP} channels (Bernardinelli et al. 2006). In physiological conditions, Na^+ changes resulting from plasma membrane fluxes, in particular due to Na^+/glutamate transport activity, appear to be faithfully transmitted to the mitochondrial matrix (**Figure 3A**). Whereas the Na,K-ATPase controls cytosolic Na^+, deriving energy directly from the hydrolysis of ATP (*see below*), mitochondrial Na^+ is mainly regulated by mitochondrial Na^+/H^+ exchangers (Bernardinelli et al. 2006), powered by the proton gradient generated by the respiratory chain.

It is therefore likely that mitochondria–and possibly the endoplasmic reticulum— dynamically take up and release Na^+ during activity. The participation of organelles could have functional role for intracellular buffering of this ion. In the case of mitochondria, the powerhouses of cells, it has been proposed that these Na^+ concentration increases influence their oxidative energy production in a dynamic way, either cell-wide or at the level of single mitochondrion. Indeed, it was described single mitochondria in several cells types, ranging from cardiac myocytes to plant cells, exhibit spontaneous transients often named 'flashes' (for a critical review, see Schwarzlander et al. 2012), corresponding to rapid fluctuations of either matrix pH or superoxide production. Astrocytes were shown early on (**Figure 3B**) to exhibit spontaneous mitochondrial Na^+ transients (Azarias et al. 2008). Those were proposed to be one component of a chain of events occurring at the level of single mitochondria, which involves—in addition to Na^+—the rapid fluctuation of mitochondrial matrix pH, electrical potential, and superoxide production, and which was proposed to be related to the availability of ATP in the microdomain surrounding the mitochondria (Azarias and Chatton 2011).

Tissue and network level

Astrocytes are extensively connected by gap junctions, which enable them to function as a coordinated cellular ensemble, by means of apposed connexins proteins, mainly of the isoforms 43 and 30. In astrocytes, gap junctions between adjacent astrocyte processes enable forming a network named the syncytium. Gap junctions allow ions like Na^+, K^+ or Ca^{2+}, as well as small molecules such as second messengers, or metabolites, to diffuse from one cell to another (for a review, see Giaume et al. 2010). The permeability of the gap junctions may be dynamically regulated by several factors, among them endothelin (Blomstrand and Giaume 2006) or plasma membrane depolarization (De Pina-Benabou et al. 2001), a fact that suggests a constant reshaping of the astrocyte network properties, which could be used to constrain and adapt it to the relevant areas.

Because of their permeability to small cations, gap junctions allow for equalizing Na^+ concentrations across cells to approximately the same intracellular concentration (Rose and Ransom 1997a); conversely, blocking gap junctions was shown to lead to rapidly diverging resting levels of intracellular Na^+ of individual cells, and therefore to differential responses to external stimuli. It can be deduced that the extensive mutual connectivity of astrocytes allow them to coordinate physiological responses as well distribute the energy burden of astrocyte Na^+-homeostasis.

With their ability to spread Na^+ across the astrocyte network (**Figure 2C**), the possible role of gap junctions in coordinating the intracellular Na^+ responses to neuronal activity within the network has been investigated in primary cultures (Bernardinelli et al. 2004) as well as in brain slices (Langer et al. 2012). It was shown that stimulation of a single astrocyte in culture generated a Ca^{2+} wave that was accompanied by an intercellular Na^+ wave (Bernardinelli et al. 2004). The mechanism was found to involve the Ca^{2+}-dependent release of glutamate and ATP from cells with elevated Ca^{2+}. The accompanying Na^+ wave was attributable to the Na^+-dependent reuptake of glutamate by surrounding astrocytes, as well as the direct diffusion of Na^+ from cell to cell through gap junctions. In parallel, the main drive of the regenerative Ca^{2+} wave was found to be the Ca^{2+}-dependent ATP release and paracrine stimulation of purinoceptors as previously described (Anderson et al. 2004; Guthrie et al. 1999). The cellular Na^+ increases occurring across the glial network propagating the waves was shown to be energy costly, inasmuch as it caused an increased glucose uptake in cells reached by the waves, as evidenced using the fluorescent glucose analogue 2-NBD-glucose (Bernardinelli et

al. 2004). Furthermore, in situations where glutamate transporters were inhibited, the Na^+ wave—but not the Ca^{2+} wave—was inhibited, and so was the spatially-correlated enhanced glucose uptake, underlying the tight link between glutamate uptake, intracellular Na^+, and energy demands in astrocytes. As highlighted in the perspective article by Charles (2005), these spatially coupled mechanisms could serve to recruit surrounding astrocytes to deliver the energy metabolite, notably lactate, to distant neurons. The astrocyte metabolic wave triggered by glutamatergic transmission could mediate the delivery of lactate to GABAergic neurons, inasmuch as Na^+-dependent GABA uptake into astrocytes was shown not to trigger enhanced glucose uptake in those cells as does glutamate (see above and in Chatton et al. 2003).

The occurrence of intercellular glial Ca^{2+} waves *in vivo* under physiological conditions has been questioned. Recent studies indicated that Ca^{2+} waves do exist *in vivo*. For instance, in the Bergmann glia intercellular Ca^{2+} waves are triggered by specific motor behaviors of the animal (Nimmerjahn et al. 2009); in hippocampal CA1 *stratum oriens*, astrocytes were shown to generate Ca^{2+} waves across the astrocyte network by mechanisms that involve purinergic receptors and were suppressed by tetrodotoxin (Kuga et al. 2011). Whether such waves could drive the propagation of Na^+ and metabolic waves *in vivo* is uncertain. Langer et al. (2012) found that electrical stimulation of single astrocytes in CA1 *stratum radiatum* slices led to a Na^+ wave that depended on gap junctions, but not on glutamate release and reuptake or ATP, as found in primary astrocytes (**Figure 2C**).

The spread of Na^+ through the astrocyte syncytium is also a potentially important means of distributing the metabolic cost of Na^+/glutamate reuptake. Along these lines, it has been demonstrated that the astrocyte network allows distributing lactate and glucose molecules in activated areas (Rouach et al. 2008) in order to sustain activity (**Figure 4**). It is conceivable that intercellular Na^+ fluxes paralleled these processes, which would be important to test.

It is noteworthy that gap junctional proteins are observed at the interface between astrocytes and oligodendrocytes (Massa and Mugnaini 1982) and enable functional coupling between the two cell types (Rash et al. 2001). It has been more recently demonstrated that this so-called panglial coupling is essential for the maintenance of myelin (Tress et al. 2012). Such heterologous coupling was not found between astrocytes and NG2 cell pairs, or astrocyte-

interneuron pairs (Xu et al. 2010), which is an indication that astrocytes and oligodendrocytes have complementary functions. This heterologous coupling indicates that cation and metabolic substrates could travel between the two cell types, allowing periaxonal membranes to have access to a larger functional glial network than the one formed solely by oligodendrocytes (**Figure 4**). This is particularly relevant considering the fact that the brain tissue is a large energy consumer and contains few energy reserves. Such a metabolic coupling between astrocytes and oligodendrocytes has been shown to occur for the distribution of lactate across this cellularly heterogeneous syncytium, and to play a role in the maintenance of axonal function (Saab et al. 2013).

The Na,K-ATPase as an ion and energy hub

Eventually, the intracellular Na^+ load generated must converge at the Na,K-ATPase pump, the mechanism chiefly responsible for Na^+ extrusion from the cell (**Figure 1**). It is at the Na^+ pump where the energy debt acquired by intracellular Na^+ accumulation must be paid. The Na^+ pump of astrocytes has an unusually low affinity for K^+ (Larsen et al. 2014), which compounded with relatively low resting levels of K^+ in brain interstitium (2.5 to 3mM, compared to 4 to 5mM in plasma), makes it responsive to physiological variations of extracellular K^+, in contrast with the neuronal Na^+ pump, which is saturated at resting extracellular K^+ concentration and is therefore solely activated by intracellular Na^+ (Kofuji and Newman 2004).

The fueling of the Na^+ pump has been a subject of debate. There are only three significant standing sources of ATP in mammalian cells: mitochondria, and the glycolytic enzymes 3-phosphoglycerate kinase and pyruvate kinase. In the case of neurons, which spend 70% of their ATP on pumping Na^+ but produce only 5% of their ATP through glycolysis, it follows that their Na^+ pump must be chiefly fueled by mitochondrial ATP (Erecinska and Silver 1994), a prediction that has been supported experimentally (Hall et al. 2012). However, astrocytes are more glycolytic and handle less Na^+ than neurons, opening the possibility of preferential fueling of the Na^+ pump by glycolytic ATP, a concept deeply ingrained in cell physiology since the influential erythrocyte study of Mercer and Durham (1981). On the other hand, a study designed directly to test this possibility with the aid of high resolution FRET sensors failed to support preferential glycolytic fueling of the Na^+ pump in astrocytes (Fernandez-Moncada and Barros 2014), a result consistent with previous observations in these cells (Rose et al. 1998;

Silver and Erecinska 1997), with the constraints of Brownian diffusion (Barros and Martinez 2007; Martinez et al. 2010) and with the notion that some of the energy used for the extrusion of glutamate-linked Na^+ entry is generated by the mitochondrial oxidation of glutamate itself (McKenna et al. 1996). It should nevertheless be noted that activation of aerobic glycolysis associated with the uptake of glutamate, is independent from its metabolism in the TCA cycle, as it is mimicked by D-aspartate which is transported into astrocytes by the same transporters but is not metabolized (Pellerin and Magistretti 1994).

If the close structural and functional relationship between the glycolytic machinery, the glutamate transporter, and the Na^+ pump (Genda et al. 2011; Rose et al. 2009) does not relate to preferential fueling: what does it mean? The answer to this question may well be bidirectional flux of information. For example the stimulations of astrocytic glycolysis mediated by primary engagement of GLT-1/GLAST and the NBCe1 are both abrogated by pharmacological blockage of the Na^+ pump (Bittner et al. 2011; Pellerin and Magistretti 1994) indicating that information flows from surface transporters to the Na^+ pump, and then to the glycolytic machinery. According to this view, intracellular Na^+ and extracellular K^+ behave as the respective readouts of presynaptic and postsynaptic activity, information that is integrated at the Na^+ pump, which controls glycolysis and mitochondria via energy status (Magistretti and Chatton 2005). The surface transporters also affect astrocytic pH and HCO_3^- leading to parallel modulation of glycolysis (Ruminot et al. 2011), mitochondrial respiration (Azarias et al. 2011), and glycogen degradation (Choi et al. 2012). Information also flows in the opposite direction, as shown by inhibition of GLT-1 transport activity by a peptide that disrupts the association of the glycolytic enzyme hexokinase with mitochondria (Jackson et al. 2015).

A tight coupling between Na^+ entry and Na,K-ATPase activity was revealed by the massive intracellular Na^+ increase evoked by glutamate when pump activity was inhibited by ouabain (**Figure 2A&B**). A mathematical model of the dynamics of intracellular Na^+ homeostasis in response to glutamate indicated that already at 10µM glutamate markedly increases the energetic burden put on astrocytes (Chatton et al. 2000). Accordingly, marked increases in intracellular ATP consumption temporally match the increases in intracellular Na^+ (Magistretti and Chatton 2005). Further stressing the key role of glutamate uptake and its coupling to the energy consuming activation of the Na,K-ATPase, decreased expression of the glial glutamate

transporters, GLAST or GLT-1, either by using oligonucleotide antisense (Cholet et al. 2001) or in knockout mice (Voutsinos-Porche et al. 2003) decreases glucose utilization *in vivo* in the somatosensory barrel cortex following activation of whiskers.

Na^+ and the control of astrocytic metabolism

As discussed above, there is abundant evidence that neurons control astrocytic energy metabolism via surface Na^+-coupled cotransporters and the Na,K-ATPase pump; it is also well established that mammalian astrocytes are primarily fueled by glucose, which is partly oxidized to CO_2 and partly fermented to lactate, and that they store energy in the form of glycogen (Magistretti and Allaman 2015; Weber and Barros 2015). According to the original astrocyte neuron lactate shuttle (ANLS) model (Pellerin and Magistretti 1994), astrocytes generate and release lactate in register with neuronal activity. Lactate can then serve as a metabolic substrate for activated neurons. Considerable experimental evidence supports the ANLS model (e.g. Jolivet et al. 2015; Magistretti and Allaman 2015; Pellerin and Magistretti 2012), the most recent one being the observation of a substantial standing gradient of lactate between astrocytes and neurons in the somatosensory cortex of living mice (Mächler et al. 2015). The extent, timing, brain localization and relative weight of the multiple intercellular and intracellular signaling mechanisms involved in ANLS are under active experimental investigation. There have been several attempts to model ANLS based on theoretical considerations, but the results have shown to be too sensitive to initial assumptions (Dienel 2012; DiNuzzo et al. 2010; Jolivet et al. 2015; Magistretti and Allaman 2015; Patel et al. 2014; e.g. Pellerin and Magistretti 2012). Overall, several lines of experimental evidence show that Na^+ is a signal that triggers glucose uptake and lactate production by astrocytes in a process known as aerobic glycolysis, a biochemical pathway also known as Warburg effect. Interestingly, aerobic glycolysis is a hallmark of rapidly proliferating cells such as cancer cells (Warburg 1956). Goyal et al. (2014) recently demonstrated that periods and regions of the brain where aerobic glycolysis is most active correspond to a high expression of plasticity genes in particular those associated with synaptic remodeling. Consistent with this, during early brain development, aerobic glycolysis is particularly high peaking at 5 years of age, when it represents 30% of glucose utilization. At adulthood, aerobic glycolysis is restricted to the specific brain areas such as the dorsolateral prefrontal cortex, superior and medial frontal gyrus, precuneus and posterior cingulate cortex, while it is absent in the cerebellum

(Vaishnavi et al. 2010). Interestingly, these regions with high aerobic glycolysis in the adult correspond to a brain network known as the Default Mode Network (DMN), which is more active in the absence of a task-specific activation (Raichle et al. 2001). Another intriguing aspect of the DMN is that it is the region most subject to β-amyloid deposition (Vlassenko et al. 2010).

The signaling mechanisms that bridge the gap between Na^+ and the metabolic machinery are not fully understood (**Figure 1**). The first metabolic control point beyond the blood-brain barrier is the astrocytic glucose transporter GLUT-1, which is activated by glutamate via the Na^+/glutamate transporter (Loaiza et al. 2003). The stimulation of GLUT-1 by glutamate may be mimicked by coincidental rises of intracellular Na^+ and Ca^{2+} (Porras et al. 2008), but the link between these ion changes and the activity of the glucose transporter is not known. Downstream of GLUT-1 is glycolysis, which is also activated by glutamate via the Na^+/glutamate transporter (Pellerin and Magistretti 1994; Voutsinos-Porche et al. 2003). Whereas the observation (**Figure 2B**) that glutamate can induce ATP hydrolysis in ouabain-sensitive fashion (Magistretti and Chatton 2005) suggests that the stimulation of astrocytic glycolysis may be explained by direct effects of adenine nucleotides on regulatory glycolytic enzymes, the actual control points remain to be identified. Other pending issues are the spatiotemporal segregation of the effects of glutamate and K^+ on glycolysis, the respective roles of Na^+, Ca^{2+}, H^+ and adenine nucleotides on the control of mitochondrial metabolism (Brand and Nicholls 2011), and the relative weight of non-signaling metabolism in astrocytes, including housekeeping and *de novo* synthesis of building blocks for synaptic formation and growth (Goyal et al. 2014).

Conclusion and perspectives

The molecular mechanisms involved in the control of energy metabolism in astroglial cells are only scarcely understood. Nevertheless, several pieces of evidence show that the Na,K-ATPase is a major energy consumer in these cells, which argues in favor of proposing Na^+ as energy currency and mediator of metabolic signals in the framework of neuron-glia interactions. Several future avenues of investigation in this field are highlighted in **Box 1**. It could be added that not only astrocyte Na^+ is involved in the modulation of energy metabolism and aerobic glycolysis, but also other intracellular cations, such as H^+ (Azarias et al. 2011; Ruminot et al. 2011) and NH_4^+ (Lerchundi et al. 2015). We anticipate that exploring

the spatiotemporal distribution, physiological and pathophysiological roles of these ancient signals across brain areas will be an important undertaking and also a lot of fun. In the words of Louis Sokoloff "*There is great joy embedded in the process of solving scientific problems, perhaps, as much as in the success of final solving them*".

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BOX 1: Key questions to be addressed

- ***What is the link between neuronal activity and astrocytic energy metabolism?*** *Synaptic activity results in increased extracellular levels of glutamate and K^+, which stimulate Na^+-coupled transporters and the Na,K-ATPase pump in astrocytes. Although it is well established that these events lead to activation of astrocytic glucose transport and glycolysis and that Na^+ ions are key mediators, the molecular mechanisms that control the metabolic machinery are unknown.*
- ***Is the astrocytic heterogeneity observed based on morphology or expression of glial markers correlated with differential energy metabolic properties?*** *The repertoire of astroglial cells is both morphologically and functionally diverse. While differences at the level of transporters, ion channels, and receptors are increasingly investigated, the bioenergetic impact in the various populations is so far elusive, as is the impact on local neuronal network activity.*
- ***How are microdomain intracellular Na^+ changes related to the production of energy equivalents?*** *Whether a localized intracellular Na^+ rise, expected to activate adjacent sodium pumps, engages energy metabolic pathways (glycolytic enzymes, mitochondria, etc.) locally or globally inside cells is so far not known.*
- ***How broadly*** **across the syncytium are energy metabolic responses distributed?** *Astrocytes are extensively coupled by gap junctions that allow them to exchange ions and small molecules such as energy intermediates by passive diffusion. Gap junction proteins are not homogeneously expressed by astrocytes, which often lead to anisotropic coupling between cells. Moreover, a number of factors can regulate their opening. The issue of whether and by which mechanisms the syncytium is able to constrain or distribute the metabolic load within tissue is to be determined.*

Figure legends

Figure 1: Role of Na^+ in the neuronal control of astrocytic energy metabolism. Active neurons release glutamate and K^+, which, via GLT1/GLAST, NBCe1, and other surface transporters and channels, induce the influx of Na^+ into astrocytes. Intracellular Na^+ together with extracellular K^+ activate the Na^+ pump and change the ATP/ADP ratio, which along with H^+, Ca^{2+} and HCO_3^-, modulate glucose transport, glycolysis, glycogen degradation, glucose oxidation and the production of lactate. Classic biochemistry mapped the pathways of glucose metabolism, but the mechanisms that link the neuronal cues and their second messengers to the metabolic machinery remain largely unknown.

Figure 2: Intracellular Na^+ handling in astrocytes and its distribution across the syncytium. (**A**) Na^+ influx into astrocytes depends on glutamate uptake along with various Na^+-dependent transport systems and is regulated by the Na,K-ATPase. Segment ***a*** shows that when the Na^+ efflux through the Na^+ pump is inhibited by ouabain, intracellular concentration rises due to influx through background Na^+-dependent transport systems and conductances. Glutamate application leads to a robust Na^+ rise which persists while glutamate is present. At the onset of glutamate washout, Na^+ is rapidly extruded from the cell by the Na,K-ATPase (segment ***b***) which is associated with substantial ATP hydrolysis. (*Modified from:* Chatton, Marquet, Magistretti. (2000) Eur J Neurosci. 2000 12:3843-53). (**B**) Tight coupling between intracellular Na^+ concentration changes (dotted line), the cellular ATP levels (plain line), indirectly determined by fluorescence imaging of free Mg^{2+}, and the Na,K-ATPase activity. Application of glutamate causes the synchronized increase in both Na^+ and ATP hydrolysis, while inhibition of the Na,K-ATPase by ouabain brings about a further increase in Na^+ and a corresponding drop of ATP hydrolysis, both reversible upon restoration of pump activity. (*From:* Magistretti and Chatton, (2005) J. Neural Transm. 2005 Jan;112:77-85). (**C**) Following single cell intracellular Na^+ rise in astrocytes, Na^+ is able to spread across the astrocyte syncytium (*left panel*) to encompass a large volume (*right panel*). This Na^+ distribution is to a large extent dictated by the gap junction coupling between cells. (*Modified from*: Langer, Stephan,Theis, Rose (2011) Glia. 60:239-252).

Figure 3: Mitochondrial Na^+ exhibits highly dynamic regulations. (**A**) At the level of the mitochondrial population in single cells, Na^+ was found to rise in the mitochondrial matrix (measured using the fluorescent indicator CoroNa Red, plain trace) in synchrony with cytosolic Na^+ (measured using the cytosolic dye SBFI, dotted trace). The application of the mitochondrial Na^+/H^+ exchanger inhibitor (EIPA) responsible for regulating matrix Na^+, led to large mitochondrial Na^+ rise, further accelerated by the extracellular application of glutamate. (*Modified from:* Bernardinelli et al. (2006) Glia. 54:460-70). (**B**) Single mitochondrial Na^+ undergo spontaneous spiking as displayed by false color fluorescence changes of a mitochondria-selective Na^+ indicator (CoroNa Red). Panels 1-4 show higher magnification temporal series and highlight single mitochondria exhibiting reversible Na^+ transients. (*Modified from:* Azarias et al. (2008) Glia. 56:342-53).

Figure 4: Glial network involvement in the handling of cytosolic Na^+ and in distributing the metabolic response. The scheme depicts how **(1)** the release of the neurotransmitter glutamate by neurons is followed by its Na^+-dependent clearance performed mainly by astrocytes; **(2)** This Na^+ influx impacts on the energy metabolism as it strongly stimulates the Na,K-ATPase and its associated ATP hydrolysis; **(3)** It follows an enhancement of glucose uptake, and its processing by aerobic glycolytic pathways; **(4)** Glial syncytium, which comprises the heterocellular coupling to oligodendrocytes by connexins, allows the spatial distribution of ions and metabolites such as lactate; **(5)** Lactate is transferred to neurons by means of monocarboxylate transporters for its subsequent use as oxidative substrate. **N**: neuron; **A**: astrocyte; **B**: blood capillary; **O**: oligodendrocyte.

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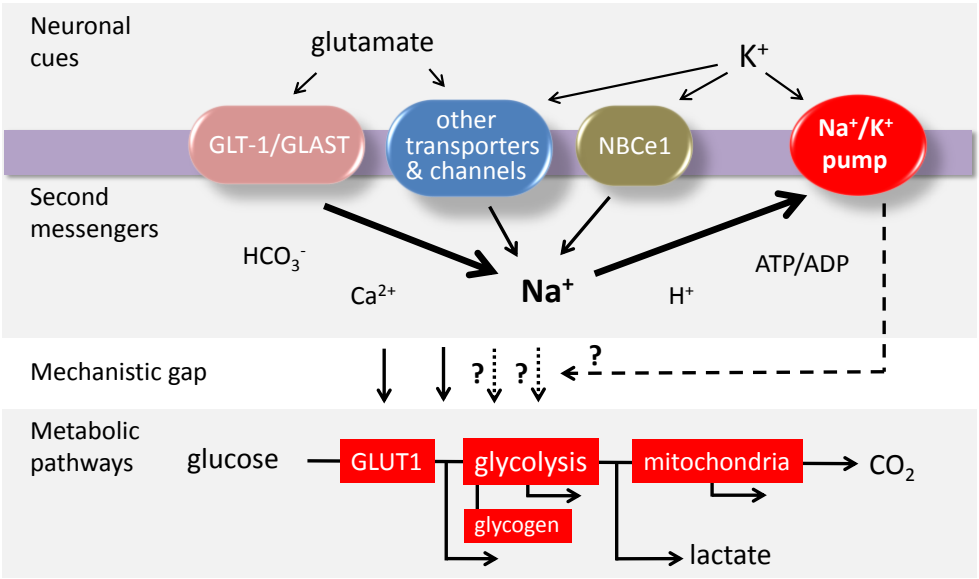
Neuronal cues
glutamate
K+
GLT-1/GLAST
other transporters & channels
NBCe1
Na+/K+ pump
Second messengers
HCO3-
Ca2+
Na+
H+
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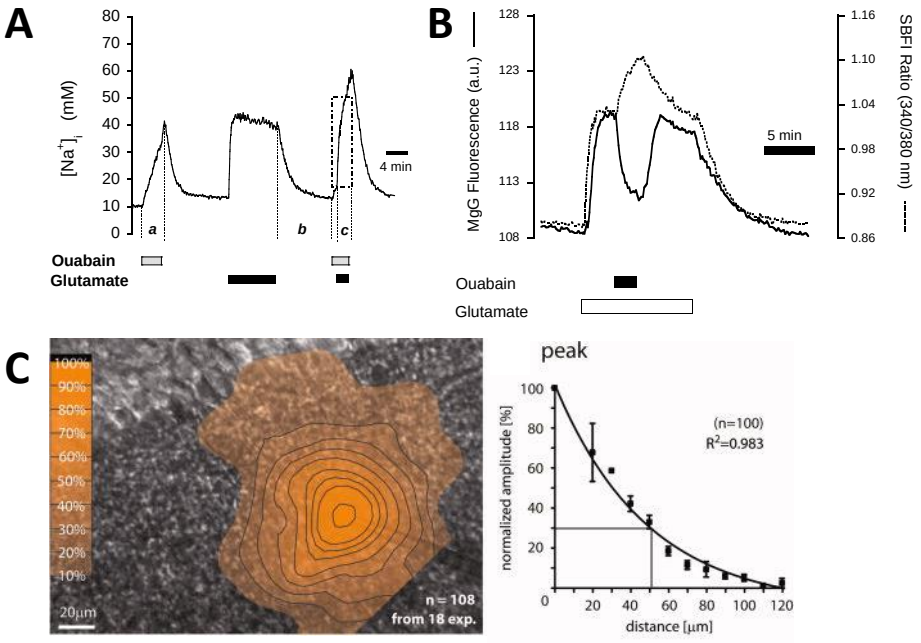
Metabolic pathways
glucose
GLUT1
glycolysis
mitochondria
CO2
glycogen
lactate

Figure 1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b
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Ouabain
Glutamate
B
128
123
118
113
108
MgG Fluorescence (a.u.)
1.16
1.10
1.04
0.98
0.92
0.86
SBFI Ratio (340/380 nm)
5 min
Ouabain
Glutamate
C
100%
90%
80%
70%
60%
50%
40%
30%
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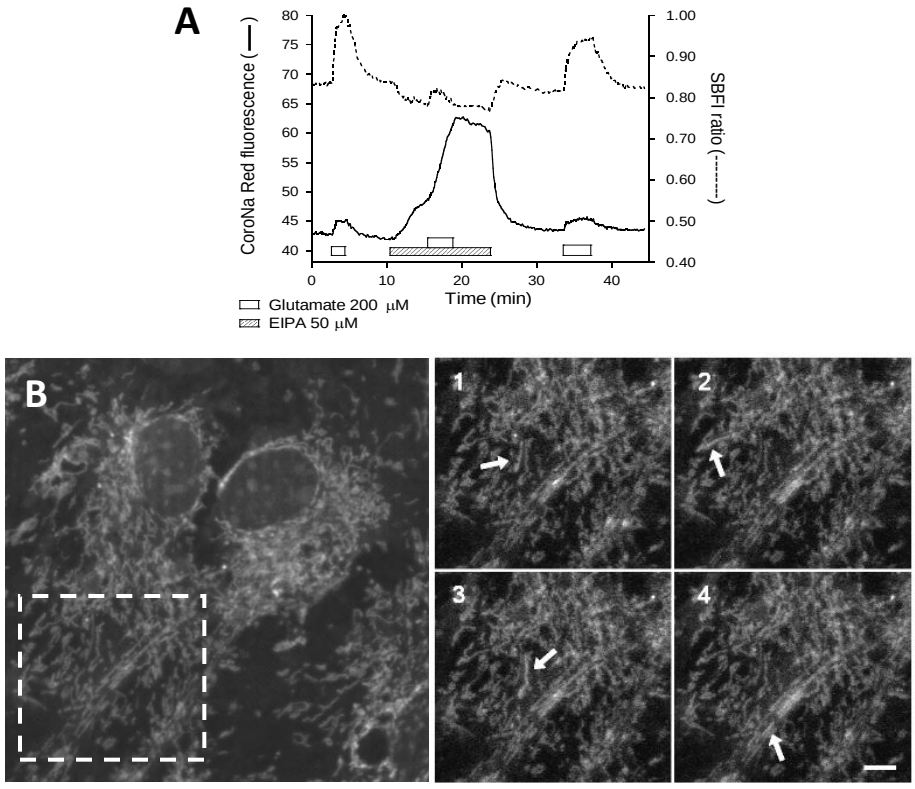
Figure 2


A
CoroNa Red fluorescence (—)
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Time (min)
Glutamate 200 μM
EIPA 50 μM
B
1
2
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Figure 3

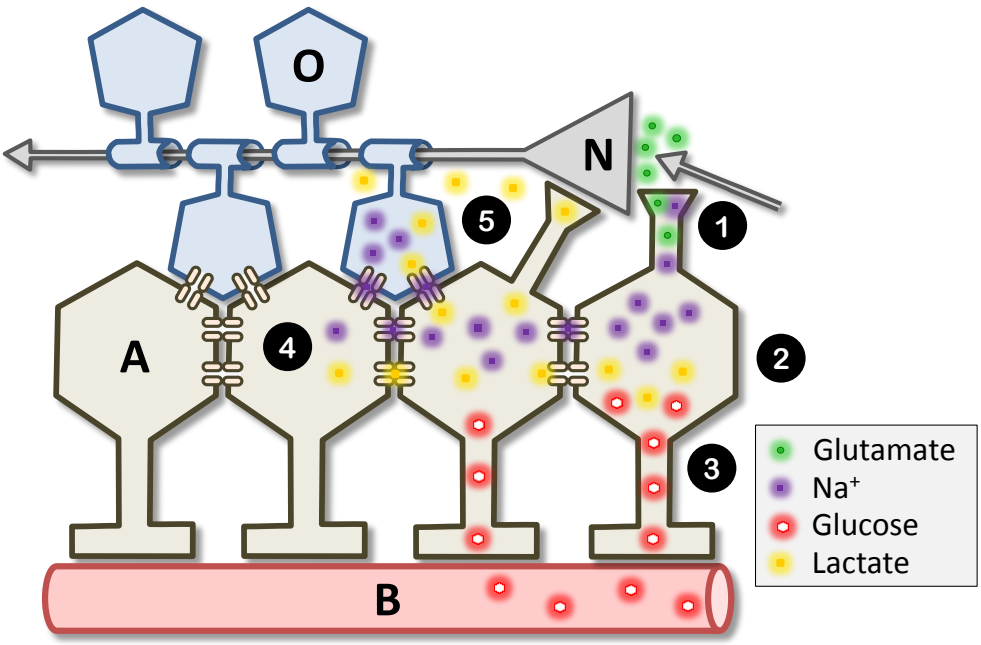
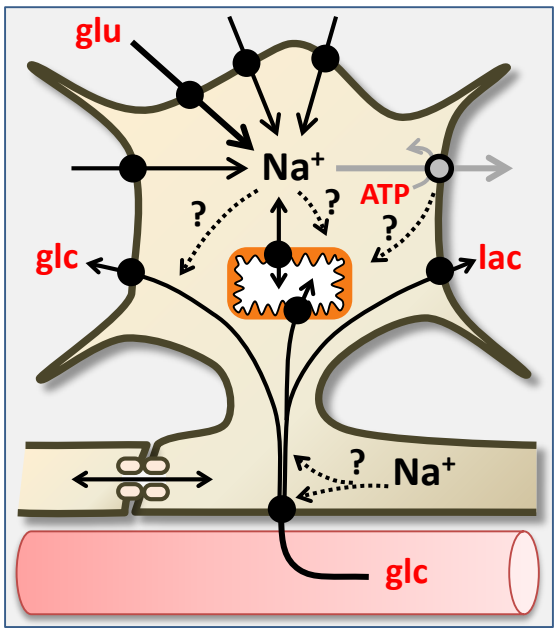


Figure 4


glu
Na+
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