Astrocyte sodium signaling and neuro-metabolic coupling in the brain

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Abstract

At tripartite synapses, astrocytes undergo calcium signaling in response to release of neurotransmitters and this calcium signaling has been proposed to play a critical role in neuron-glia interaction. Recent work has now firmly established that, in addition, neuronal activity also evokes sodium transients in astrocytes, which can be local or global depending on the number of activated synapses and the duration of activity. Furthermore, astrocyte sodium signals can be transmitted to adjacent cells through gap junctions and following release of gliotransmitters. A main pathway for activity-related sodium influx into astrocytes is via high-affinity sodium-dependent glutamate transporters. Astrocyte sodium signals differ in many respects from the well-described glial calcium signals both in terms of their temporal as well as spatial distribution. There are no known buffering systems for sodium ions, nor is there store-mediated release of sodium. Sodium signals thus seem to represent rather direct and unbiased indicators of the site and strength of neuronal inputs. As such they have an immediate influence on the activity of sodium-dependent transporters which may even reverse in response to sodium signaling, as has been shown for GABA transporters for example. Furthermore, recovery from sodium transients through Na^+/K^+ -ATPase requires a measurable amount of ATP, resulting in an activation of glial metabolism. In this review, we present basic principles of sodium regulation and the current state of knowledge concerning the occurrence and properties of activity-related sodium transients in astrocytes. We then discuss different aspects of the relationship between sodium changes in astrocytes and neuro-metabolic coupling, putting forward the idea that indeed sodium might serve as a new type of intracellular ion signal playing an important role in neuron-glia interaction and neuro-metabolic coupling in the healthy and diseased brain.

1. Introduction

Active neurons and glial cells dynamically interact in many ways. One of the most prominent and most widely known examples of such an interaction was described about 30 years ago, through studies demonstrating that transmitters released by active neurons result in the activation of transmitter receptors on astrocytes (Bowman and Kimelberg, 1984, Kettenmann et al., 1984). It took about another 10 years before the advent of imaging techniques enabled the detection of astrocyte calcium signals in response to neuronal transmitters (Nedergaard, 1994). Astrocyte calcium signaling has since taken center stage in research efforts and interests. This is mainly because such signaling can result in the release of gliotransmitters and vasoactive substances by astrocytes, which thereby feedback onto and modulate the neuronal network (see chapters by Panatier/Robitaille and Volterra; this issue).

In addition to calcium signals, neuronal activity is, however, accompanied by a second type of ion signal in astrocytes: these are sodium transients, detected upon neuronal release of glutamate and -to a lesser extent- GABA. The existence of such activity-dependent sodium signals is surprising at first glance (Rose and Karus, 2013). First of all, they occur against a relatively high background sodium concentration (10-15 mM), which is fundamentally different from other ion species involved in signaling (e. g. baseline intracellular calcium or proton concentrations are roughly around 100 nM). Also, as compared to calcium changes, which usually occur in the low μM range, sodium changes are a thousand-fold larger, occurring in the mM range. Furthermore, sodium signals not only differ in their magnitude, but also in their spatial and temporal profiles from classical calcium signaling in astrocytes. Sodium changes are quite long lasting, exhibiting decay times in the range of tens of seconds. Given the high diffusion coefficient for sodium ions measured in mammalian cytosol ($0.6 \mu\text{m}^2/\text{sec}$; (Kushmerick and Podolsky, 1969), sodium transients should, however, dissipate within fractions of a second. Apparently, free diffusion of sodium ions is considerably slowed because of increased tortuosity in the cytosol (Sykova and Nicholson, 2008), and/or binding to plasma membrane transporters such as the Na^+/K^+ -ATPase. Moreover, a recent study has provided evidence for restricted molecular diffusion and the existence of subcellular compartments astrocytes (Nuriya and Yasui, 2013).

There are no known classical buffering mechanisms for sodium ions inside cells and, apart from the Na^+/K^+ -ATPase (see below), there are no explicit sodium-binding proteins present that activate enzymes and enzyme cascades. Because sodium ions are central charge carriers, channel- or transporter-mediated influx of sodium resulting in changes in intracellular sodium concentration in the mM range, directly influences the cellular membrane

potential. In contrast to the situation with calcium ions, there are no intracellular compartments or organelles which serve as storage compartments for sodium and thus there is no comparable release from stores.

When considering sodium signaling, it is also important to bear in mind that many secondary active transport systems depend on the sodium gradient and that sodium transients - that is, a decrease in the inwardly directed sodium gradient- have an immediate impact on the driving force and activity of these transporters. Among those are transporters for regulation of other ions (e. g. sodium/proton exchange (NHE) and sodium/calcium exchange (NCX)) as well as transporters for the re-uptake of transmitters (e. g. high-affinity, sodium-dependent transporters for glutamate or GABA). In fact, it is conceptually astonishing how many highly relevant transporters work close to their equilibrium potential and may reverse upon increases in intracellular sodium. This topic has been comprehensively discussed recently and the reader is kindly referred to these earlier reviews (Kirischuk et al., 2012, Rose and Karus, 2013). One might argue that this is an inherent “weakness” of the system, based on a somewhat faulty design. Instead of this rather unsatisfactory argument, we prefer the interpretation that sodium transients might serve as signals.

A critical aspect in this argumentation is the question of what kind of information content such sodium signals might represent and encode. This point has not been fully been clarified yet and many questions still remain open. An established finding, however, is that extrusion of sodium ions is metabolically relevant because recovery from sodium signals requires a measurable amount of ATP. Thus, sodium increases will cause activation of glial metabolism. Consequently, activity-induced sodium transients are ideally positioned to take an essential signaling role in neuro-metabolic coupling between neurons and astrocytes.

2. Sodium homeostasis and regulation

Cellular sodium homeostasis is of the utmost functional importance for the brain and most of brain energy is in fact consumed by the Na^+/K^+ -ATPases (Erecinska and Silver, 1994, Ames, 2000, Howarth et al., 2012). By transporting sodium ions out of the cell in exchange for potassium, the activity of the Na^+/K^+ -ATPase establishes a low intracellular sodium concentration against a high sodium concentration in the extracellular space (~145 mM; cf. Fig. 2; (Skou and Esmann, 1992, Kaplan, 2002, Somjen, 2004)). In hippocampal neurons, baseline sodium concentrations of about 12 mM were reported, whereas data obtained from hippocampal astrocytes indicate a sodium concentration of about 11 mM (e. g. (Rose and Ransom, 1996a, 1997b, Chatton et al., 2001, Sheldon et al., 2004, Langer and Rose, 2009).

This indicates that, at least in this preparation, there is no significant difference in intracellular sodium concentrations between neurons and astrocytes. The cellular uptake of potassium by the Na^+/K^+ -ATPase results in a high intracellular potassium concentration (>100 mM) as compared to that of the extracellular space (~ 2 mM; (Erecinska and Silver, 1994, Kofuji and Newman, 2004)). In light of the essential role of sodium homeostasis for cellular function, it is remarkable that the sodium pump is the *only* transport mechanism for efficient extrusion of sodium across the plasma membrane. Regulation of most other ions, in contrast, involves at least two mechanisms (e. g. plasma membrane Ca^{2+} -ATPase works together with sodium/calcium-exchangers (NCX) to extrude calcium ions and several other transporters in addition to the Na^+/K^+ -ATPase mediate uptake of potassium).

Low intracellular sodium concentrations together with the about 10-fold higher extracellular sodium concentration and negative cellular membrane potentials result in inwardly directed electro-chemical gradients for sodium ions across the plasma membrane of both neurons and glial cells. Thus, most of the basic currency of cellular metabolism, ATP, is converted into -and stored as- a strong inward driving force for sodium ions. This enables sodium-dependent electrical signaling and serves to energize many secondary transport processes across the plasma membrane (Rose and Karus, 2013). Changes in intracellular sodium will ultimately feed back on the activity of such sodium-dependent transport processes. Among these are transporters for the re-uptake of glutamate as well as of GABA and glycine, and the latter two may even reverse in response to sodium elevations (Kirischuk et al., 2012, Rose and Karus, 2013). There is also increasing evidence that sodium transients directly modulate intracellular calcium signaling through reversal of NCX (Kirischuk et al., 2012).

The transport cycle of the Na^+/K^+ -ATPase has been characterized in great detail in cell culture models and heterologous expression systems, and new crystal structures of defined binding states are continuously being published (Morth et al., 2011, Kanai et al., 2013, Nyblom et al., 2013). Despite its central importance, the pump's functional properties in astrocytes and neurons in the intact brain, including basic attributes such as ion binding affinities or intracellular interaction partners, are poorly understood. One problem that arises in studies addressing these issues is that manipulation of sodium and the Na^+/K^+ -ATPase in the intact tissue directly alters basic physiological cellular parameters and influences extracellular ion homeostasis. Moreover, “the sodium pump” is in fact a protein complex comprised of different subunits (α , β) of which different isoforms and binding partners (γ/FXVD subunits) exist, and each of the possible combinations may result in different

functional properties (Blanco and Mercer, 1998, Kaplan, 2002, Crambert and Geering, 2003). Furthermore, the Na^+/K^+ -ATPase is subject to additional regulation and modulation by endogenous ouabain-like compounds binding to $\alpha 1$ -subunits (Kala et al., 2000).

It is widely held that both neurons and astrocytes ubiquitously express a Na^+/K^+ -ATPase complex containing the $\alpha 1$ -subunit and that the activity of this complex mediates “housekeeping” functions by setting the baseline sodium concentration and counteracting constitutive sodium influx. In addition to $\alpha 1$, neurons seem to preferentially express the $\alpha 3$ and astrocytes the $\alpha 2$ subunit, both of which have been suggested to handle sodium loads imposed during periods of high activity (Pellerin and Magistretti, 1997, Zahler et al., 1997, Azarias et al., 2013). Earlier studies also suggest that astrocytes are able to efficiently take up potassium from the extracellular space following activity, which is attributed to a higher K_d for extracellular potassium in astrocytes compared to neurons (Kofuji and Newman, 2004, Hertz et al., 2013, Rose and Karus, 2013). While the functional relevance of this property was long acknowledged in the light of the necessity for extracellular potassium clearance only, recent work has established that activation of glial Na^+/K^+ -ATPase by extracellular potassium is also involved in neuro-metabolic coupling and the stimulation of glycolysis by potassium (Bittner et al., 2011).

A third major difference in the mechanisms of sodium homeostasis and regulation between astrocytes and neurons arises from a fundamental difference in the intercellular coupling between these two cell types. In astrocytes, an additional pathway for the recovery from local sodium loads exists. This additional pathway is represented by gap junctions, which can mediate the rapid diffusion and distribution of sodium to neighboring cells (Rose and Ransom, 1997a, Langer et al., 2012).

3. Dynamic changes in extra- and intracellular sodium during neural activity

3. 1. Sodium signals in the extracellular space and in neurons

Experiments with ion-selective microelectrodes performed in the vertebrate brain *in vivo* and in acute tissue slices, have demonstrated that activity-related opening of sodium-permeable voltage- or ligand-gated ion channels, and the flux of sodium through these channels, can alter the sodium concentration in the extracellular space. At the existing high extracellular baseline sodium concentration, the response characteristics of ion-selective microelectrodes are not

favourable for the study of sodium signals evoked by minor or moderate levels of activity. With prolonged afferent stimulation or with induction of epileptiform discharges or spreading depression, however, this technique enabled detection of decreases in the extracellular sodium by up to about 15 mM (Dietzel et al., 1982, Zanotto and Heinemann, 1983, Hablitz and Heinemann, 1989, Kohr and Heinemann, 1989); Fig. 1A).

Based on earlier studies in invertebrate preparations, it was long assumed that physiological activity is not accompanied by measurable changes in intracellular sodium concentrations (e. g. (Hodgkin and Huxley, 1952); many current text books). In intracellular compartments which have a high surface-to-volume ratio such as in vertebrate neurons and glial cells, the situation is quite different. Indeed, dynamic measurements using sodium-sensitive fluorescent dyes have now established that action potentials cause an increase in the sodium concentration in axons due to sodium influx through TTX-sensitive, voltage-gated channels (Lasser-Ross and Ross, 1992, Kole et al., 2008, Fleidervish et al., 2010, Baranauskas et al., 2013). In cortical pyramidal neurons, maximal amplitudes for sodium increases were obtained in the axon initial segment, and computer simulations indicated an increase by about 10 mM with trains of 10-12 action potentials (Kole et al., 2008).

Activity-induced sodium transients can also arise in dendrites in response to the opening of voltage-gated sodium channels during back-propagating action potentials, as is the case in hippocampal and cortical neurons ((Jaffe et al., 1992, Rose et al., 1999, Lamy and Chatton, 2011); Fig. 1B; cf. Fig. 2). Action potential-induced sodium transients reached values of 4 mM in apical dendrites of hippocampal neurons following a train of 20 action potentials and monotonically decayed with a time constant of several seconds (Rose et al., 1999). Particularly prominent postsynaptic sodium transients are seen with glutamatergic synaptic activity and opening of ionotropic glutamate receptors generating long-lasting, substantial currents across the membrane ((Callaway and Ross, 1997, Rose and Konnerth, 2001, Bennay et al., 2008, Langer and Rose, 2009); Fig. 1C; cf. Fig. 2). In apical dendrites of hippocampal CA1 pyramidal neurons, sodium increases rose by about 10 mM were detected in response to short-burst stimulation (5 pulses at 50 Hz) (Rose and Konnerth, 2001). In active spines, stimulus-induced sodium increases even reached to up 35-40 mM following this stimulation paradigm (Rose and Konnerth, 2001). Such activity-induced influx of sodium into neurons through ionotropic glutamate receptors and voltage-gated channels in fact represents the main energy-consuming process in the brain and requires most of the ATP produced (Erecinska and Silver, 1994, Lennie, 2003, Harris et al., 2012).

3. 2. Sodium signals in astrocytes

The main mechanism for the inactivation of synaptically released glutamate is its fast binding to and cellular reuptake by high-affinity transporters (Danbolt, 2001, Tzingounis and Wadiche, 2007). In the rodent hippocampus, this task is mainly accomplished by astrocytic glutamate transporters (EAATs; excitatory amino acid transporters), namely GLAST (glutamate/aspartate-transporter) and GLT-1 (glutamate-transporter-1; rodent analogues of EAAT1 and EAAT2, respectively; (Gegelashvili and Schousboe, 1998, Bergles et al., 1999, Anderson and Swanson, 2000, Maragakis and Rothstein, 2004, Marcaggi and Attwell, 2004); Fig. 2). High-affinity glutamate uptake is energized by the concomitant inward transport of three sodium ions and a proton, while one potassium ion is transported outward. Consequently, its activation is accompanied by an inward current as well as intracellular acidification and an increase in the intracellular sodium concentration of astrocytes (Rose and Ransom, 1996b, Deitmer and Rose, 2010, Kirischuk et al., 2012).

This uptake mechanism was first demonstrated in cultured astrocytes, in which application of glutamate or its transportable agonist D-aspartate readily evoke substantial increases (> 10 mM) in intracellular sodium concentration (Kimelberg et al., 1989, Rose and Ransom, 1996b, Chatton et al., 2000, Chatton et al., 2001). These are suppressed by the glutamate uptake blocker TBOA (Chatton et al., 2001, Bernardinelli and Chatton, 2008) or its higher-affinity version TFB-TBOA (Tsukada et al., 2005, Bozzo and Chatton, 2010). Cytosolic sodium elevations induced by glutamate are accompanied by sodium signals in mitochondria as well, indicating a link between glutamate-evoked sodium signalling and mitochondrial function as discussed below (see chapter 4. 3., this article; (Bernardinelli et al., 2006)). Sodium-dependent glutamate uptake also represents a powerful pathway for the induction of astrocyte sodium signals *in situ*, including astrocytes in cerebellum (Kirischuk et al., 2007, Bennay et al., 2008), hippocampus (Langer and Rose, 2009), neocortex ((Lamy and Chatton, 2011, Unichenko et al., 2013); Fig. 1D), and at the Calyx of Held (Uwechue et al., 2012). Sodium typically increased by several mM in the cell bodies with exogenous application of glutamate or D-aspartate. GABA transporters provide another pathway for sodium influx into astrocytes. GABA uptake is, however, coupled to co-transport of two sodium ions only (Gadea and Lopez-Colome, 2001), and evokes significantly smaller sodium increases as compared to glutamate (Chatton et al., 2003, Unichenko et al., 2012). In cultured mouse cortical astrocytes for example, the maximum amplitude of sodium increases in response to 500 μ M GABA was 4-6 mM, compared with an increase by 25-30 mM in response to an application of glutamate at comparable concentrations (Chatton et al., 2003).

Astrocytes undergo sodium signaling with stimulation of excitatory synaptic activity and synaptic glutamate release. In acutely isolated tissue slices of the cerebellum, brief stimulation of parallel fibers caused sodium transients of up to 9 mM in Bergmann glial cells ((Kirischuk et al., 2007, Bennay et al., 2008); Fig. 1E). Stimulation of climbing fibers, in contrast, induced global sodium signals in Bergmann glial cells, the amplitude and time course of which did not significantly differ between different branches and the soma (Bennay et al., 2008). Synaptically-induced sodium transients in the mM range were also detected in astrocytes of the CA1 *stratum radiatum* of the juvenile mouse hippocampus (Langer and Rose, 2009). With low stimulation intensities, hippocampal astrocytes displayed differences in amplitude and time course of activity-induced sodium signals between different cellular regions. Under these conditions, sodium signals amounted to 1-2 mM, were confined to one or two primary branches and adjacent fine processes and only weakly invaded the soma. Increasing the number of activated synapses by increasing the stimulation intensity increased the amplitude of sodium transients to up to 6.0 mM and resulted in global sodium transients that included the soma (Langer and Rose, 2009). Sodium signals in astrocytes thus seem to represent indicators of the location and strength of synaptic activity.

Pharmacological approaches have indicated that the sodium influx pathways activated by synaptic stimulation differ between Bergmann glial cells and hippocampal astrocytes, although both express sodium-dependent glutamate transporters (Danbolt, 2001, Schousboe et al., 2004, Schreiner et al., 2014). The glutamate uptake blocker TBOA virtually eliminated sodium transients in hippocampal astrocytes, indicating that they were predominately mediated by this pathway (Langer and Rose, 2009). Further, application of D-aspartate, an agonist of glutamate transport, reliably induced sodium signals in processes of hippocampal astrocytes (Langer and Rose, 2009). In Bergmann glia, in contrast, synaptically-induced sodium signals were reduced by only ~60% with TBOA. The remaining signal was blocked by blockade of AMPA receptors (Bennay et al., 2008), which are highly sodium-permeable, glutamate-gated ion channels, and expressed by these cells (Lalo et al., 2011).

While glutamate uptake and opening of ionotropic glutamate receptors seem to represent the predominant mechanisms for generating sodium signals in astrocytes, additional pathways for sodium influx are clearly present (Rose and Karus, 2013), but their contribution to activity-induced sodium signaling in astrocytes has not been investigated yet. Two interesting candidates are the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ -cotransport (NKCC; (Jayakumar and Norenberg, 2010) and the electrogenic sodium-bicarbonate cotransport (NBC, (Parker and Boron, 2013). Both are stimulated by increases in extracellular potassium, albeit at different concentration levels. Direct

activation of NKCC apparently requires relatively large increases in extracellular potassium (to more than 10 mM, (Hertz et al., 2013), indicating that sodium influx through this pathway will only be relevant during periods of strong synchronized activity. Inward NBC, in contrast, is activated by increases in the extracellular potassium in the low mM range (Pappas and Ransom, 1994), and might therefore generate measurable sodium influx into astrocytes during activity-induced increases in extracellular K^+ , also independent from glutamatergic signaling.

3. 3. Propagation of sodium signals in the astrocyte network

Sodium signals are not restricted to the site of sodium influx. In hippocampal slices, locally-induced sodium signals spread along processes of individual astrocytes at an initial velocity of $>60 \mu\text{m/s}$ (Langer et al., 2012), a value several times higher than that of classical calcium waves (Scemes and Giaume, 2006). Furthermore, sodium signals evoked in individual cells spread in a radial manner to virtually all neighboring astrocytes within a distance of 100 μm . Intercellular spread of sodium in hippocampal slice preparations has been demonstrated to be primarily based on sodium diffusion through gap junctions composed of Cx30 and Cx43 ((Langer et al., 2012); Fig. 2). In addition, pharmacological inhibition of mGluR1/5 slightly dampened the spread of sodium, whereas inhibition of glutamate uptake or purinergic receptors had no effect, indicating the involvement of gliotransmission and glia-derived glutamate in the spread of sodium signals in the astrocyte network. A prominent role for gliotransmitter release has been demonstrated for the propagation of regenerative sodium waves between astrocytes in primary cell culture (Bernardinelli et al., 2004).

Thus, astrocytes possess efficient pathways for the fast redistribution of sodium in the gap-junction coupled network. This sodium diffusion through gap junctions following local synaptic activity and local sodium transients could serve to lower the metabolic load of single cells. With more extensive activity and global sodium increases in neighboring astrocytes, however, this diffusion-based pathway will no longer function effectively. Under these conditions, the generation of global calcium signals and regenerative sodium waves based on gliotransmission might together promote glucose uptake from the blood by enhancing the activity of GLUT1 at astrocyte endfeet (Barros and Deitmer, 2010). Gap junctions will then still enable diffusion of glucose from endfeet to active sites providing the fuel for increased glycolysis ((Rouach et al., 2008); Fig. 4). Accordingly, such sodium signals might not only serve to activate astrocyte metabolism, but also promote neurometabolic coupling as discussed below.

4. Sodium signals and neuro-metabolic coupling

4. 1. Glutamate transport and energy metabolism

As introduced above, maintenance of the transmembrane sodium gradient in brain cells comes with a large energy cost, estimated to account for approximately 40-60% of brain ATP hydrolysis (Hevner et al., 1992). It is therefore expected that any cellular process involving substantial sodium movements into the cell will increase the energy load of cells, which is the case for glutamate transport. Extracellular glutamate clearance constitutes one of the most crucial roles of astrocytes in the brain, notably by continuously preventing a buildup of extracellular glutamate concentration during neuronal activity thereby avoiding excitotoxicity and enabling high frequency glutamatergic signaling. However, glutamate transport activity is also associated with substantial sodium influx into astrocytic cells (Fig. 1D, E; Fig. 2). This increased sodium influx has been proposed to constitute a pivotal element of a mechanism enabling astrocytes to increase the local availability of metabolic substrates in response to neuronal activity, through so-called “neurometabolic coupling”.

In vitro studies have indicated that glutamate uptake into astrocytes cause a significant increase in their glucose uptake (Pellerin and Magistretti, 1994), which depends on the activity of the Na^+/K^+ -ATPase. This observation prompted subsequent studies, which suggested that the glutamate transporter-mediated sodium influx constituted was causal to enhanced ATP consumption. It was for instance estimated from both experimental and modeling data (Chatton et al., 2000) that glutamate, applied at physiologically relevant concentrations, increases Na^+/K^+ -ATPase activity by a factor of two to three. If one considers that the pump, at rest, is responsible for about half of the consumption of cellular ATP (Hevner et al., 1992) this large increase in ATP hydrolysis is bound to have important consequences on the energy budget of the cells, and the subsequent adjustment of their metabolic pathways.

It should be pointed out that other elements in addition to glutamate transporters could potentially be involved, in particular ionotropic glutamate receptors, which function as ligand-gated channels for cations such as sodium, and are expressed by some astrocytes and Bergmann glia (David et al., 1996, Lalo et al., 2006, Brennan et al., 2009). Indeed, sodium signals in Bergmann glial cells in response to parallel fiber stimulation are reduced by 40% by blockers of AMPA receptors as described above (Bennay et al., 2008). Consequently, one would expect that their activation might significantly draw on the cell's energy. While this question has not yet been addressed for Bergmann glial cells, in astrocytes expressing

relatively low levels of AMPA receptors, the rapid transition of this receptor-channel to a non-conducting inactive state following glutamate binding (Wyllie and Cull-Candy, 1994) limits the rise in intracellular sodium concentration. It follows that Na^+/K^+ ATPase activity and its associated ATP hydrolysis remain close to basal levels as reported in cortical astrocytes (Chatton et al., 2000).

Enhancement of glucose capture occurs within seconds following glutamate application (Loaiza et al., 2003, Porras et al., 2008). The stimulation involves glutamate transporters and is not mimicked by AMPA receptor or metabotropic glutamate receptor activation (Porras et al., 2008). Interestingly, a co-signaling of sodium and Ca^{2+} was found to be required for glucose transporter stimulation (Fig. 3A), which may indicate the involvement of a signaling event such as Ca^{2+} -dependent phosphorylation occurring on one of the kinase-recognition sites of the glucose transporter 1 (Porras et al., 2008).

Several studies have reported the existence of a physical as well as a functional association between glutamate transporters and the Na^+/K^+ -ATPase $\alpha 2$ subunit (Cholet et al., 2002, Porras et al., 2008, Rose et al., 2009, Genda et al., 2011, Bauer et al., 2012, Matos et al., 2013). The physical proximity of the two elements implies that after entering the cell through the glutamate transporter, sodium can be readily handled and extruded by the Na^+/K^+ -ATPase. It should be kept in mind, however, that in spite of the proposed close functional interaction between glutamate transporters and the Na^+/K^+ -ATPase, substantial cytosolic sodium transients are detected (see above). This shows that a substantial number of sodium ions escape the binding to, and export by, the Na^+/K^+ -ATPase and diffuse from their point of entry at the membrane into the cytosol. Nonetheless, because sodium ions entering through glutamate transporters have to be expelled again by the ATP-consuming sodium pump, there should not only be a functional interaction between the two transport systems themselves, but also between glutamate transport and astrocyte energy metabolism in general.

This tight dynamic connection was further demonstrated by experiments in which intracellular sodium and ATP hydrolysis were simultaneously measured in primary astrocyte cultures (Chatton and Magistretti, 2005). Glutamate application caused a rapid intracellular sodium rise which was accompanied, without significant delay, by a sharp increase in ATP hydrolysis, the two processes occurring with the same kinetics (Fig. 3B). In the presence of glutamate, inhibition of the Na^+/K^+ -ATPase using the cardiac glycoside ouabain caused the expected rapid intracellular sodium rise due to the absence of sodium efflux mechanisms, and interestingly, it also caused a concomitant large *decrease* in ATP hydrolysis, which reversed once the pump activity was restored (Fig. 3B).

Apart from activity-dependent stimulation of Na^+/K^+ -ATPase and glial metabolism by intracellular sodium, alternative pathways were described in neuro-metabolic coupling, involving electrogenic sodium bicarbonate cotransport (NBC; (Ruminot et al., 2011, Choi et al., 2012). It has long been known that inward NBC is activated by increases in extracellular potassium and the subsequent depolarization of astrocytes, respectively, resulting in an alkalinization of astrocytes (Deitmer and Rose, 1996, Chesler, 2003). This so-called depolarisation-induced alkalinization has been shown to stimulate the phosphofructokinase, and thereby activate glycolysis, bypassing the need for increases in sodium and activation of Na^+/K^+ -ATPase for metabolic stimulation of astrocytes (Ruminot et al., 2011). These pathways might be especially important at for neural activity not involving glutamate.

While the neurometabolic coupling model, referred to as the "astrocyte-neuron lactate shuttle" hypothesis (Fig. 4) was initially deduced from studies using primary cultured cells, evidence that the coupling occurs *in vivo* and including the key role of glutamate transporters has been demonstrated as well,. In particular, quantitative autoradiographic studies using ^{14}C -2-deoxyglucose as a tracer were performed by stimulating the whisker-to-barrel pathway in anesthetized rodents while measuring local cerebral glucose utilization (Cholet et al., 2001). A significant increase in glucose utilization was seen in the activated cortical area, specifically in the barrels corresponding to the mechanically stimulated whisker. This somatotopic relationship between enhanced local neuronal activity and glucose capture and utilization disappeared when antisense GLAST oligonucleotide sequences were injected in the rat cortex (Cholet et al., 2001) and was absent in transgenic mice lacking GLAST or GLT-1 isoforms of the glutamate transporter ((Voutsinos-Porche et al., 2003); Fig. 3C). Such neurometabolic interactions were also shown to occur *in vivo* in brain regions other than cortex. The enhancement of intrinsic optical signal measured in the olfactory glomeruli caused by odor application was abolished by a glutamate transporter inhibition (Gurden et al., 2006). On the contrary, the intrinsic optical signals were found to be independent of postsynaptic transmission through ionotropic or metabotropic glutamate receptors. These data indicate that neuronal glutamate release and subsequent sodium-dependent uptake by astrocytes form a critical pathway through which neural activity is linked to metabolic processing.

The precise nature of the local regulation of energy metabolism is not only important for the understanding of brain function under normal physiological and pathological conditions, but also for a correct interpretation of functional brain imaging approaches, that use one or the other form of local metabolic responses to neuronal activity to generate activation maps of the brain (Figley and Stroman, 2011). In particular, the technique of ^{18}F -

2DG positron emission tomography (2DG-PET) yields images of brain regions with increased glucose utilization that are correlates of increased neuronal electrical activity. Therefore, the cellular localization of glucose uptake, as well as its link with electrical activity, are of prime importance. One of the important issues to clarify is whether the measured metabolic signals assumed to reflect excitatory activity, *i.e.* glutamate release and reuptake, and the ensuing metabolic response, also encompasses inhibitory activity. Indeed, after release from inhibitory neurons, GABA is removed from the extracellular space by sodium-dependent transporters expressed to a large extent by astrocytes (Larsson et al., 1980, Gadea and Lopez-Colome, 2001). The sodium influx associated with GABA uptake could in principle lead to the same metabolic response as glutamate. However, it has been demonstrated that the neurometabolic coupling mechanism described for glutamate cannot be directly transposed to GABA (Chatton et al., 2003), the main reason being a strikingly different kinetics of transport, rendering GABA transporters unable to sufficiently activate Na^+/K^+ -ATPase.

4. 2. Sodium as an intracellular second messenger

At the cellular level, sodium is most commonly viewed as essential for providing a driving force for transmembrane transport systems, for the generation and maintenance of membrane electrical potential, and for being a key component in the generation of fast inward currents in excitable cells. However, to some extent, it can be argued that sodium can be considered as a second messenger, a role more commonly attributed to Ca^{2+} . This less conventional view of sodium is prompted by the analysis of the initial hypothesis of intracellular signal transduction presented by Earl Sutherland in his Nobel lecture (Sutherland, 1972). As discussed by Orlov and Hamet (2006), any intracellular molecule can be considered a potential second messenger as long as it fulfills three main criteria. (a) The modulation of the intracellular concentration of this molecule following the onset of an external stimulus precedes the cellular responses, and normalizes upon cessation of the stimulus. (b) In the absence of the investigated external stimulus, the transient modulation of intracellular second messenger is *per se* sufficient to evoke cellular responses. (c) The interaction of second messengers with their intracellular targets is necessary for the manifestation of cellular responses.

Thus, intracellular sodium, increasing in response to glutamate stimulation, causing an increased energy demand and leading to enhanced glucose uptake and utilization, could arguably be considered an intracellular second messenger for energy metabolism. In support of this notion, the signaling role of intracellular sodium has been demonstrated in an entirely different context, namely that of the sensing of peripheral circulating sodium levels by

neurons of the subfornical organ (SFO), one of the circumventricular organs (Shimizu et al., 2007). This specialized structure lacks a blood-brain barrier and the neurons that comprise it are exposed to the chemical environment of the peripheral circulation. Astrocytes in the SFO express the Na_x channel, an atypical type of sodium channel that enables these specialized cells to act as sensors of sodium in the extracellular medium. A physiological increase of sodium level in body fluids activates a sodium signaling pathway in astrocytes, similar to that of the ANLS, triggering an enhanced glucose uptake and glycolytic response, leading to release of lactate. Lactate then increases the firing rate of local GABAergic neurons, which in turn regulate the activity of SFO efferent neurons involved in the central control of peripheral natriuremia.

Recently, the concept of sodium-glutamate transporter-mediated signaling via its associated lactate release has been extended beyond a purely energy metabolic mechanism. It was shown that lactate can spread far beyond the area of neural activity and the associated domain of glucose consumption (Cruz et al., 2007). Furthermore, released lactate has been shown to activate a class of G_i -protein coupled receptor, termed the hydrocarboxylic acid receptor 1 (HCAR1, formerly GPR81) first described in adipose tissue (Ahmed et al., 2009, Liu et al., 2009). HCA1 is expressed by brain cells (Bozzo et al., 2013, Lauritzen et al., 2014) and has been demonstrated to underlie a lactate-mediated negative modulation of neuronal activity (Bozzo et al., 2013). Evidence for a lactate-mediated signaling mechanism, possibly engaging another kind of receptor, was found in the *locus coeruleus* (Tang et al., 2014). Furthermore, lactate released as a consequence of enhanced glycogenolysis and glycolysis is also critical for long-term memory formation by inducing molecular changes, including the induction of phospho-CREB, Arc, and phospho-cofilin (Suzuki et al., 2011, Yang et al., 2014). Such lactate-mediated signaling between glial cells and neurons may constitute a signal for a multicellular metabolic recruitment (Barros, 2013) or for providing a metabolic feedback on neuronal activity (Bozzo et al., 2013, Tang et al., 2014).

4. 3. Mitochondrial sodium and energy metabolism

When considering the role of intracellular sodium in cellular energy metabolism, it should be kept in mind that the main powerhouses of cells are the mitochondria that host the tricarboxylic acid cycle and are the site of the oxidative phosphorylation. Whereas the glycolysis discussed above yields only two ATP molecules per glucose consumed, oxidative phosphorylation produces seventeen-fold more ATP per glucose molecule. In astrocytes, glutamate transporters co-compartmentalize with the Na^+/K^+ -ATPase, as well as with

glycolytic enzymes and mitochondria (Genda et al., 2011, Bauer et al., 2012). In addition, it has been demonstrated that movements of mitochondria stabilize at sites of glutamate uptake (Jackson et al., 2014). This raises the question of the functional purpose of this proximity of mitochondria with the major membrane sodium influx pathway.

Energized mitochondria generate and maintain a large electrical gradient—of typically of -180mV— across their inner membrane. As a consequence, cations such as sodium should have a strong tendency to be taken up into the mitochondrial matrix. However, it has been long thought that mitochondria possess a very low permeability for monovalent cations, a property enabling them to generate the large proton motive force necessary for oxidative phosphorylation (Bernardi, 1999). Nevertheless, measurements of intramitochondrial sodium performed inside living astrocytes have revealed that cytosolic sodium changes are faithfully transmitted to the mitochondrial matrix (Fig. 3D) (Bernardinelli et al., 2006). Despite the large electronegativity of mitochondria, the resting intramitochondrial sodium levels are maintained only slightly higher (~19 mM) than in the cytosol mainly by the activity of the mitochondrial Na^+/H^+ exchanger (Pozzo-Miller et al., 1997, Bernardi, 1999, Bernardinelli et al., 2006). The consequence of the mitochondrial sodium influx following cellular glutamate uptake is not obvious. A likely action is through the ensuing acidification of mitochondria, which reduces mitochondrial respiration, therefore contributing to the glycolytic metabolic response (Azarias et al., 2011, Perreten Lambert et al., 2014).

The roles of mitochondrial sodium influx may extend beyond the glutamate-evoked responses discussed above. Indeed, it was observed, that single mitochondria in astrocytes display spontaneous sodium spiking activity (Azarias et al., 2008) that coincide with rapid mitochondrial transients of pH and reactive oxygen species, possibly related to the availability of ATP in the mitochondrial microdomain (Azarias et al., 2011). Similar single mitochondrial spiking events were found in a variety of cell types and organisms, including plants (Schwarzlander et al., 2012) and *C. elegans* (Shen et al., 2014). However, the mechanistic nature and function of these events is still debated (Schwarzlander et al., 2012, Schwarzlander et al., 2014). Mitochondrial spiking activity is increasingly thought to represent a novel frequency-coded readout of metabolism at the single mitochondria level (Schwarzlander et al., 2014).

While mitochondria do not appear to accumulate sodium, their ability to take up and release sodium ions may enable them to influence the kinetics of local sodium fluctuations as proposed for Ca^{2+} in neuronal presynaptic boutons (Scotti et al., 1998). Other subcellular compartments may be relevant for cellular sodium regulation, such as the endoplasmic

reticulum, a major Ca^{2+} store critically involved in the fast release and reuptake of Ca^{2+} ions during agonist-evoked responses or calcium-induced calcium release. While experimentally difficult to address, quantitative X-ray microanalysis studies of cryosections of hippocampal slices estimated that ER sodium levels in dendrites are somewhat higher than in the cytoplasm (~19 mM) and can substantially increase during trains of action potentials (Pozzo-Miller et al., 1997).

5. Consequences of sodium homeostasis disturbances in the diseased brain

Disturbance of the cellular sodium handling is highly likely to impact the functional integrity of neurons and other brain cells and hence may plausibly play a causal role in the generation of pathophysiological neurological or psychiatric conditions. Conversely, altered sodium homeostasis is known to result from pathological brain conditions, in particular during cerebral ischemia. These situations may be connected to specific sodium-dependent membrane transporters or linked with intracellular homeostasis. Several plasma membrane transport systems, such as the sodium-glutamate transporters or those involved in brain pH regulation (Na^+/H^+ exchangers and $\text{Na}^+/\text{HCO}_3^-$ cotransporters), critically depend on the integrity of brain sodium homeostasis. Other sodium-dependent transporters stand out for their involvement in certain brain pathologies.

The $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) that contributes to a large extent to the establishment of the large inwardly directed Ca^{2+} gradient, is expressed by both neurons and glia (Annunziato et al., 2013). When intracellular sodium rises to abnormal concentrations, the transporter reverses its mode of operation and drives the entry of Ca^{2+} from the extracellular milieu. This so-called reversal mode of NCX (Philipson and Nicoll, 2000) may have several deleterious consequences for brain cells. In particular, excessive intracellular Ca^{2+} rise leads to mitochondrial dysfunction (Putney et al., 2002) or further glutamate release by glia (Paluzzi et al., 2007), which in turn contributes to the worsening of pathological conditions such as those found in ischemic insults. The $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporters (NKCC) are expressed in neurons, where they regulate the intracellular Cl^- concentration and therefore neuronal excitability. In astrocytes, this transporter plays an important role in the control of extracellular K^+ concentration as well as cell volume regulation (Kofuji and Newman, 2004, Jayakumar and Norenberg, 2010). As introduced above, stimulation of NKCC1 activity such as that occurring during ischemia, may lead to the loss of sodium homeostasis with the consequence of increasing cytoplasmic Ca^{2+} following stimulation of NCX reverse mode

activation (Lenart et al., 2004). Aberrant activation of NKCC1 is also believed to cause deleterious astrocyte swelling during epileptic activity (Hochman, 2012).

Finally, the most important regulator of sodium homeostasis, the Na^+/K^+ -ATPase, is critically susceptible to ATP depletion, such as that which occurs during ischemic stroke or severe hypoglycemia, which ultimately leads to the loss of the sodium and K^+ transmembrane gradients in these conditions. Na^+/K^+ -ATPase gene mutations have also been linked to familial hemiplegic migraine (Capendeguy and Horisberger, 2004). Impaired Na^+/K^+ ATPase activity is inevitably accompanied by the failure of transporters discussed above (NCX, NKCC, etc.) that are critical for the proper brain function at the cellular and network level.

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Figure Captions

Fig. 1. Activity-induced sodium signals. (A) extracellular sodium signals in the sensorimotor cortex (depth 600 μm) of cats induced by electrical stimulation of the cortical surface with 20 Hz, 0.2 ms for 10 s. (B), (C) sodium signals in neurons. (B) Left: 3D reconstruction of a layer 2/3 pyramidal neuron patched with ANG-containing electrolyte. Scale bar, 30 μm . Right: Dendritic fluorescence transients recorded during backpropagation of action potentials (top). Space over time display of the matching linescan recording (bottom). (C) Top left: Image of a spiny dendrite of a CA1 pyramidal cell. Closed arrowheads indicate "active" and open arrowheads "passive" spines from which measurements were taken; scale bar: 5 μm . Top right: Suprathreshold stimulation (5 pulses at 50 Hz) induced 5 APs as measured at the soma. Upper traces: Average activity-induced sodium transient in spines (average of 19) and in the dendrite. Middle traces: sodium transients in 3 single "passive" spines. Lower traces: sodium transients in 3 single "active" spines. (D), (E) sodium signals in astrocytes. (D) Top: Fluorescence image of astrocytes in a cortical slice double-stained with sulforhodamine 101 (left panel) and ANG (center panel). Scale bar, 30 μm . Magnification of an ANG stained astrocyte (right panel). Scale bar, 5 μm . Bottom: Sample trace of astrocytic fluorescence response to glutamate puffs of increasing durations. (E) inward current and sodium transients in different processes of a Bergmann glial cell during parallel fibers stimulation (50 Hz, 100 ms). Signals were largest in processes that were located close to the stimulation pipette. Taken from: (A) Dietzel et. al. (1982), (B) Lamy & Chatton 2011, (C) Rose et al. 2001, (D) Lamy & Chatton 2011, (E) Bennay et al. 2008.

Fig. 2. Pathways for sodium influx at glutamatergic synapses. At glutamatergic synapses, action-potentials induce sodium influx into neurons through TTX-sensitive voltage-gated sodium channels. If dendrites carry back-propagating action-potentials, these mediate sodium influx through voltage-gated channels, too. At the postsynaptic site, ionotropic glutamate receptor channels (NMDA and AMPA) represent major sodium influx pathways into dendrites and spines. At the same time, activation of high-affinity, sodium-dependent glutamate transporters results in sodium uptake into astrocytes, resulting in sodium signals in these cells, which can spread to neighboring astrocytes through gap junctions.

Fig. 3: Sodium signals and astrocyte metabolism. (A) Upper traces: calcium (filled symbols) and sodium (open symbols) signals in astrocytes in culture, induced by application of

endothelin-1 and/or gramicidin during the periods indicated. The lower traces show that only coincidence of both signals causes stimulation of glucose transport (as monitored by uptake of 6-NBDG). (B) Interrelationship between intracellular magnesium concentration (determined by fluorescence imaging with MagnesiumGreen) and sodium (determined by imaging with SBFI). Whereas application of glutamate causes an increase in both sodium and magnesium (the latter indicating a reduction in ATP content), inhibition of the sodium pump by ouabain causes a further rise in sodium, but a decrease in free magnesium, indicative of a recovery of intracellular ATP levels. (C) Enhancement of glucose utilization and lactate formation induced by glutamate application depend on the expression of glutamate transporters. Glucose utilization, evaluated by intracellular accumulation of 2-Deoxyglucose (top), and lactate release (bottom) under control conditions and after application of glutamate (Glu) in cortical astrocytes in culture derived from wildtype (+/+), heterozygous (+/-), and GLAST mutant mice (-/-). (D) Sodium signals in mitochondria. Glutamate induces an increase in cytosolic sodium (determined by SBFI) as well as intramitochondrial sodium (determined by CoroNaRed) in cultured astrocytes. Diazoxide, a K_{ATP} channel opener caused an increase in mitochondrial sodium only. Taken from: (A), Porras et al (2008); (B), Magistretti & Chatton (2005); (C), Voutsinos-Porche et al. (2003); (D), Bernardinelli et al (2006).

Fig. 4: Major pathways involved in neuro-metabolic coupling between astrocytes and neurons. At glutamatergic synapses, activation of glutamate transport generates sodium signals in astrocytes which are also transmitted to mitochondria. Cytosolic sodium increases cause activation of Na^+/K^+ -ATPase, which results in increased consumption of ATP. In addition, protons entering cells during glutamate transport activity are transmitted to mitochondria where they weaken the respiratory chain. The enhanced ATP consumption by astrocytes is followed by increased uptake of glucose from the blood. Lactate dehydrogenase (LDH) converts the resulting pyruvate to lactate which is then shuttled from astrocytes to active neurons, where it serves as metabolite substrate for ATP production after its conversion to pyruvate by the LDH. In addition, release of K^+ by active neurons into the extracellular space, causes astrocyte membrane depolarization and influx of bicarbonate through Na^+ -bicarbonate cotransporters. The resulting glial alkalization further stimulates glycolytic enzymes toward the production and release of lactate.

A stimulus-induced sodium changes in the ECS **B** AP-induced sodium changes **C** sodium changes in spines and dendrites **D** cortical astrocytes **E** Bergmann glial cell **Fig. 1:** Rose & Chatton

Fig. 2: Rose & Chatton

A GLUT1 stimulation by coincidental sodium and calcium signals

C glucose utilization and lactate formation in GLAST mutant mice

B correlation between changes in intracellular free magnesium and sodium

D sodium entry into mitochondria

Fig. 3: Rose & Chatton

Fig. 4: Rose & Chatton