

Serveur Académique Lausannois SERVAL serval.unil.ch

Author Manuscript

Faculty of Biology and Medicine Publication

This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Published in final edited form as:

Title: Astrocyte sodium signaling and neuro-metabolic coupling in the brain.

Authors: Rose CR, Chatton JY

Journal: Neuroscience

Year: 2016 May 26

Issue: 323

Pages: 121-34

DOI: 10.1016/j.neuroscience.2015.03.002

In the absence of a copyright statement, users should assume that standard copyright protection applies, unless the article contains an explicit statement to the contrary. In case of doubt, contact the journal publisher to verify the copyright status of an article.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21

Astrocyte sodium signaling and neuro-metabolic coupling in the brain

Christine R. Rose* & Jean-Yves Chatton#

*Institute of Neurobiology, Faculty of Mathematics and Natural Sciences, Heinrich-Heine-University Düsseldorf, Universitätsstrasse 1, D-40225 Düsseldorf, Germany

#Department of Fundamental Neurosciences, University of Lausanne, rue du Bugnon 9, CH-1005 Lausanne, Switzerland

***Corresponding author:** Prof. Dr. Christine Rosemarie Rose, Institute of Neurobiology, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf, Universitätsstrasse 1, D-40225 Düsseldorf, Germany. Phone: ++49 (211) 81 13416; Fax: ++49 (211) 81 13415; e-mail: rose@uni-duesseldorf.de

Keywords: astrocytes, Na^+/K^+ -ATPase, glutamate transport, neuron-glia interaction, metabolism

1 **Abstract**

2

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

25

26

27

1 **1. Introduction**

2 Active neurons and glial cells dynamically interact in many ways. One of the most prominent
3 and most widely known examples of such an interaction was described about 30 years ago,
4 through studies demonstrating that transmitters released by active neurons result in the
5 activation of transmitter receptors on astrocytes (Bowman and Kimelberg, 1984, Kettenmann
6 et al., 1984). It took about another 10 years before the advent of imaging techniques enabled
7 the detection of astrocyte calcium signals in response to neuronal transmitters (Nedergaard,
8 1994). Astrocyte calcium signaling has since taken center stage in research efforts and interests.
9 This is mainly because such signaling can result in the release of gliotransmitters and vasoactive
10 substances by astrocytes, which thereby feedback onto and modulate the neuronal network (see
11 chapters by Panatier/Robitaille and Volterra; this issue).

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

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

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

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

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

24

25 **2. Sodium homeostasis and regulation**

26 Cellular sodium homeostasis is of the utmost functional importance for the brain and most of
27 brain energy is in fact consumed by the Na^+/K^+ -ATPases (Erecinska and Silver, 1994, Ames,
28 2000, Howarth et al., 2012). By transporting sodium ions out of the cell in exchange for
29 potassium, the activity of the Na^+/K^+ -ATPase establishes a low intracellular sodium
30 concentration against a high sodium concentration in the extracellular space (~ 145 mM; cf. Fig.
31 2; (Skou and Esmann, 1992, Kaplan, 2002, Somjen, 2004)). In hippocampal neurons, baseline
32 sodium concentrations of about 12 mM were reported, whereas data obtained from hippocampal
33 astrocytes indicate a sodium concentration of about 11 mM (e. g. (Rose and Ransom, 1996a,
34 1997b, Chatton et al., 2001, Sheldon et al., 2004, Langer and Rose, 2009). This indicates that,

1 at least in this preparation, there is no significant difference in intracellular sodium
2 concentrations between neurons and astrocytes. The cellular uptake of potassium by the
3 Na^+/K^+ -ATPase results in a high intracellular potassium concentration (>100 mM) as compared
4 to that of the extracellular space (~ 2 mM; (Erecinska and Silver, 1994, Kofuji and Newman,
5 2004)). In light of the essential role of sodium homeostasis for cellular function, it is remarkable
6 that the sodium pump is the *only* transport mechanism for efficient extrusion of sodium across
7 the plasma membrane. Regulation of most other ions, in contrast, involves at least two
8 mechanisms (e. g. plasma membrane Ca^{2+} -ATPase works together with sodium/calcium-
9 exchangers (NCX) to extrude calcium ions and several other transporters in addition to the
10 Na^+/K^+ -ATPase mediate uptake of potassium).

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

23 The transport cycle of the Na^+/K^+ -ATPase has been characterized in great detail in cell
24 culture models and heterologous expression systems, and new crystal structures of defined
25 binding states are continuously being published (Morth et al., 2011, Kanai et al., 2013, Nyblom
26 et al., 2013). Despite its central importance, the pump's functional properties in astrocytes and
27 neurons in the intact brain, including basic attributes such as ion binding affinities or
28 intracellular interaction partners, are poorly understood. One problem that arises in studies
29 addressing these issues is that manipulation of sodium and the Na^+/K^+ -ATPase in the intact
30 tissue directly alters basic physiological cellular parameters and influences extracellular ion
31 homeostasis. Moreover, "the sodium pump" is in fact a protein complex comprised of different
32 subunits (α , β) of which different isoforms and binding partners (γ /FXYP subunits) exist, and
33 each of the possible combinations may result in different functional properties (Blanco and
34 Mercer, 1998, Kaplan, 2002, Crambert and Geering, 2003). Furthermore, the Na^+/K^+ -ATPase

1 is subject to additional regulation and modulation by endogenous ouabain-like compounds
2 binding to α 1-subunits (Kala et al., 2000).

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

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

23

24

25

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

27

28 **3. 1. Sodium signals in the extracellular space and in neurons**

29 Experiments with ion-selective microelectrodes performed in the vertebrate brain *in vivo* and
30 in acute tissue slices, have demonstrated that activity-related opening of sodium-permeable
31 voltage- or ligand-gated ion channels, and the flux of sodium through these channels, can alter
32 the sodium concentration in the extracellular space. At the existing high extracellular baseline
33 sodium concentration, the response characteristics of ion-selective microelectrodes are not
34 favourable for the study of sodium signals evoked by minor or moderate levels of activity. With

1 prolonged afferent stimulation or with induction of epileptiform discharges or spreading
2 depression, however, this technique enabled detection of decreases in the extracellular sodium
3 by up to about 15 mM (Dietzel et al., 1982, Zanotto and Heinemann, 1983, Hablitz and
4 Heinemann, 1989, Kohr and Heinemann, 1989); Fig. 1A).

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

16 Activity-induced sodium transients can also arise in dendrites in response to the opening
17 of voltage-gated sodium channels during back-propagating action potentials, as is the case in
18 hippocampal and cortical neurons ((Jaffe et al., 1992, Rose et al., 1999, Lamy and Chatton, 2011);
19 Fig. 1B; cf. Fig. 2). Action potential-induced sodium transients reached values of 4 mM in apical
20 dendrites of hippocampal neurons following a train of 20 action potentials and monotonically
21 decayed with a time constant of several seconds (Rose et al., 1999). Particularly prominent
22 postsynaptic sodium transients are seen with glutamatergic synaptic activity and opening of
23 ionotropic glutamate receptors generating long-lasting, substantial currents across the membrane
24 ((Callaway and Ross, 1997, Rose and Konnerth, 2001, Bennay et al., 2008, Langer and Rose,
25 2009); Fig. 1C; cf. Fig. 2). In apical dendrites of hippocampal CA1 pyramidal neurons, sodium
26 increases rose by about 10 mM were detected in response to short-burst stimulation (5 pulses at
27 50 Hz) (Rose and Konnerth, 2001). In active spines, stimulus-induced sodium increases even
28 reached to up 35-40 mM following this stimulation paradigm (Rose and Konnerth, 2001). Such
29 activity-induced influx of sodium into neurons through ionotropic glutamate receptors and
30 voltage-gated channels in fact represents the main energy-consuming process in the brain and
31 requires most of the ATP produced (Erecinska and Silver, 1994, Lennie, 2003, Harris et al., 2012).

32

33 **3. 2. Sodium signals in astrocytes**

1 The main mechanism for the inactivation of synaptically released glutamate is its fast binding
2 to and cellular reuptake by high-affinity transporters (Danbolt, 2001, Tzingounis and Wadiche,
3 2007). In the rodent hippocampus, this task is mainly accomplished by astrocytic glutamate
4 transporters (EAATs; excitatory amino acid transporters), namely GLAST
5 (glutamate/aspartate-transporter) and GLT-1 (glutamate-transporter-1; rodent analogues of
6 EAAT1 and EAAT2, respectively; (Gegelashvili and Schousboe, 1998, Bergles et al., 1999,
7 Anderson and Swanson, 2000, Maragakis and Rothstein, 2004, Marcaggi and Attwell, 2004);
8 Fig. 2). High-affinity glutamate uptake is energized by the concomitant inward transport of
9 three sodium ions and a proton, while one potassium ion is transported outward. Consequently,
10 its activation is accompanied by an inward current as well as intracellular acidification and an
11 increase in the intracellular sodium concentration of astrocytes (Rose and Ransom, 1996b,
12 Deitmer and Rose, 2010, Kirischuk et al., 2012).

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

1 Astrocytes undergo sodium signaling with stimulation of excitatory synaptic activity
2 and synaptic glutamate release. In acutely isolated tissue slices of the cerebellum, brief
3 stimulation of parallel fibers caused sodium transients of up to 9 mM in Bergmann glial cells
4 ((Kirischuk et al., 2007, Bennay et al., 2008); Fig. 1E). Stimulation of climbing fibers, in contrast,
5 induced global sodium signals in Bergmann glial cells, the amplitude and time course of which
6 did not significantly differ between different branches and the soma (Bennay et al., 2008).
7 Synaptically-induced sodium transients in the mM range were also detected in astrocytes of the
8 CA1 *stratum radiatum* of the juvenile mouse hippocampus (Langer and Rose, 2009). With low
9 stimulation intensities, hippocampal astrocytes displayed differences in amplitude and time
10 course of activity-induced sodium signals between different cellular regions. Under these
11 conditions, sodium signals amounted to 1-2 mM, were confined to one or two primary branches
12 and adjacent fine processes and only weakly invaded the soma. Increasing the number of activated
13 synapses by increasing the stimulation intensity increased the amplitude of sodium transients to
14 up to 6.0 mM and resulted in global sodium transients that included the soma (Langer and Rose,
15 2009). Sodium signals in astrocytes thus seem to represent indicators of the location and strength
16 of synaptic activity.

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

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

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

7 8 **3. 3. Propagation of sodium signals in the astrocyte network**

9 Sodium signals are not restricted to the site of sodium influx. In hippocampal slices, locally-
10 induced sodium signals spread along processes of individual astrocytes at an initial velocity of
11 $>60 \mu\text{m/s}$ (Langer et al., 2012), a value several times higher than that of classical calcium waves
12 (Scemes and Giaume, 2006). Furthermore, sodium signals evoked in individual cells spread in a
13 radial manner to virtually all neighboring astrocytes within a distance of $100 \mu\text{m}$. Intercellular
14 spread of sodium in hippocampal slice preparations has been demonstrated to be primarily based
15 on sodium diffusion through gap junctions composed of Cx30 and Cx43 ((Langer et al., 2012);
16 Fig. 2). In addition, pharmacological inhibition of mGluR1/5 slightly dampened the spread of
17 sodium, whereas inhibition of glutamate uptake or purinergic receptors had no effect, indicating
18 the involvement of gliotransmission and glia-derived glutamate in the spread of sodium signals
19 in the astrocyte network. A prominent role for gliotransmitter release has been demonstrated for
20 the propagation of regenerative sodium waves between astrocytes in primary cell culture
21 (Bernardinelli et al., 2004).

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

33

34

1 **4. Sodium signals and neuro-metabolic coupling**

2 **4. 1. Glutamate transport and energy metabolism**

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

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

25 It should be pointed out that other elements in addition to glutamate transporters could
26 potentially be involved, in particular ionotropic glutamate receptors, which function as ligand-
27 gated channels for cations such as sodium, and are expressed by some astrocytes and Bergmann
28 glia (David et al., 1996, Lalo et al., 2006, Brennan et al., 2009). Indeed, sodium signals in
29 Bergmann glial cells in response to parallel fiber stimulation are reduced by 40% by blockers
30 of AMPA receptors as described above (Bennay et al., 2008). Consequently, one would expect
31 that their activation might significantly draw on the cell's energy. While this question has not
32 yet been addressed for Bergmann glial cells, in astrocytes expressing relatively low levels of
33 AMPA receptors, the rapid transition of this receptor-channel to a non-conducting inactive state
34 following glutamate binding (Wyllie and Cull-Candy, 1994) limits the rise in intracellular

1 sodium concentration. It follows that Na^+/K^+ ATPase activity and its associated ATP hydrolysis
2 remain close to basal levels as reported in cortical astrocytes (Chatton et al., 2000).

3 Enhancement of glucose capture occurs within seconds following glutamate application
4 (Loaiza et al., 2003, Porras et al., 2008). The stimulation involves glutamate transporters and is
5 not mimicked by AMPA receptor or metabotropic glutamate receptor activation (Porras et al.,
6 2008). Interestingly, a co-signaling of sodium and Ca^{2+} was found to be required for glucose
7 transporter stimulation (Fig. 3A), which may indicate the involvement of a signaling event such
8 as Ca^{2+} -dependent phosphorylation occurring on one of the kinase-recognition sites of the
9 glucose transporter 1 (Porras et al., 2008).

10 Several studies have reported the existence of a physical as well as a functional
11 association between glutamate transporters and the Na^+/K^+ -ATPase $\alpha 2$ subunit (Cholet et al.,
12 2002, Porras et al., 2008, Rose et al., 2009, Genda et al., 2011, Bauer et al., 2012, Matos et al.,
13 2013). The physical proximity of the two elements implies that after entering the cell through
14 the glutamate transporter, sodium can be readily handled and extruded by the Na^+/K^+ -ATPase.
15 It should be kept in mind, however, that in spite of the proposed close functional interaction
16 between glutamate transporters and the Na^+/K^+ -ATPase, substantial cytosolic sodium transients
17 are detected (see above). This shows that a substantial number of sodium ions escape the
18 binding to, and export by, the Na^+/K^+ -ATPase and diffuse from their point of entry at the
19 membrane into the cytosol. Nonetheless, because sodium ions entering through glutamate
20 transporters have to be expelled again by the ATP-consuming sodium pump, there should not
21 only be a functional interaction between the two transport systems themselves, but also between
22 glutamate transport and astrocyte energy metabolism in general.

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

32 Apart from activity-dependent stimulation of Na^+/K^+ -ATPase and glial metabolism by
33 intracellular sodium, alternative pathways were described in neuro-metabolic coupling,
34 involving electrogenic sodium bicarbonate cotransport (NBC; (Ruminot et al., 2011, Choi et

1 al., 2012). It has long been known that inward NBC is activated by increases in extracellular
2 potassium and the subsequent depolarization of astrocytes, respectively, resulting in an
3 alkalinization of astrocytes (Deitmer and Rose, 1996, Chesler, 2003). This so-called
4 depolarization-induced alkalinization has been shown to stimulate the phosphofructokinase, and
5 thereby activate glycolysis, bypassing the need for increases in sodium and activation of
6 Na^+/K^+ -ATPase for metabolic stimulation of astrocytes (Ruminot et al., 2011). These pathways
7 might be especially important at for neural activity not involving glutamate.

8 While the neurometabolic coupling model, referred to as the "astrocyte-neuron lactate
9 shuttle" hypothesis (Fig. 4) was initially deduced from studies using primary cultured cells,
10 evidence that the coupling occurs *in vivo* and including the key role of glutamate transporters
11 has been demonstrated as well,. In particular, quantitative autoradiographic studies using ^{14}C -
12 2-deoxyglucose as a tracer were performed by stimulating the whisker-to-barrel pathway in
13 anesthetized rodents while measuring local cerebral glucose utilization (Cholet et al., 2001). A
14 significant increase in glucose utilization was seen in the activated cortical area, specifically in
15 the barrels corresponding to the mechanically stimulated whisker. This somatotopic
16 relationship between enhanced local neuronal activity and glucose capture and utilization
17 disappeared when antisense GLAST oligonucleotide sequences were injected in the rat cortex
18 (Cholet et al., 2001) and was absent in transgenic mice lacking GLAST or GLT-1 isoforms of
19 the glutamate transporter ((Voutsinos-Porche et al., 2003); Fig. 3C). Such neurometabolic
20 interactions were also shown to occur *in vivo* in brain regions other than cortex. The
21 enhancement of intrinsic optical signal measured in the olfactory glomeruli caused by odor
22 application was abolished by a glutamate transporter inhibition (Gurden et al., 2006). On the
23 contrary, the intrinsic optical signals were found to be independent of postsynaptic transmission
24 through ionotropic or metabotropic glutamate receptors. These data indicate that neuronal
25 glutamate release and subsequent sodium-dependent uptake by astrocytes form a critical
26 pathway through which neural activity is linked to metabolic processing.

27 The precise nature of the local regulation of energy metabolism is not only important
28 for the understanding of brain function under normal physiological and pathological conditions,
29 but also for a correct interpretation of functional brain imaging approaches, that use one or the
30 other form of local metabolic responses to neuronal activity to generate activation maps of the
31 brain (Figley and Stroman, 2011). In particular, the technique of ^{18}F -2DG positron emission
32 tomography (2DG-PET) yields images of brain regions with increased glucose utilization that
33 are correlates of increased neuronal electrical activity. Therefore, the cellular localization of
34 glucose uptake, as well as its link with electrical activity, are of prime importance. One of the

1 important issues to clarify is whether the measured metabolic signals assumed to reflect
2 excitatory activity, *i.e.* glutamate release and reuptake, and the ensuing metabolic response, also
3 encompasses inhibitory activity. Indeed, after release from inhibitory neurons, GABA is
4 removed from the extracellular space by sodium-dependent transporters expressed to a large
5 extent by astrocytes (Larsson et al., 1980, Gadea and Lopez-Colome, 2001). The sodium influx
6 associated with GABA uptake could in principle lead to the same metabolic response as
7 glutamate. However, it has been demonstrated that the neurometabolic coupling mechanism
8 described for glutamate cannot be directly transposed to GABA (Chatton et al., 2003), the main
9 reason being a strikingly different kinetics of transport, rendering GABA transporters unable to
10 sufficiently activate Na^+/K^+ -ATPase.

11

12 **4. 2. Sodium as an intracellular second messenger**

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

27 Thus, intracellular sodium, increasing in response to glutamate stimulation, causing an
28 increased energy demand and leading to enhanced glucose uptake and utilization, could
29 arguably be considered an intracellular second messenger for energy metabolism. In support of
30 this notion, the signaling role of intracellular sodium has been demonstrated in an entirely
31 different context, namely that of the sensing of peripheral circulating sodium levels by neurons
32 of the subfornical organ (SFO), one of the circumventricular organs (Shimizu et al., 2007). This
33 specialized structure lacks a blood-brain barrier and the neurons that comprise it are exposed to
34 the chemical environment of the peripheral circulation. Astrocytes in the SFO express the Na_x

1 channel, an atypical type of sodium channel that enables these specialized cells to act as sensors
2 of sodium in the extracellular medium. A physiological increase of sodium level in body fluids
3 activates a sodium signaling pathway in astrocytes, similar to that of the ANLS, triggering an
4 enhanced glucose uptake and glycolytic response, leading to release of lactate. Lactate then
5 increases the firing rate of local GABAergic neurons, which in turn regulate the activity of SFO
6 efferent neurons involved in the central control of peripheral natriuremia.

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

23

24 **4. 3. Mitochondrial sodium and energy metabolism**

25 When considering the role of intracellular sodium in cellular energy metabolism, it should be
26 kept in mind that the main powerhouses of cells are the mitochondria that host the tricarboxylic
27 acid cycle and are the site of the oxidative phosphorylation. Whereas the glycolysis discussed
28 above yields only two ATP molecules per glucose consumed, oxidative phosphorylation
29 produces seventeen-fold more ATP per glucose molecule. In astrocytes, glutamate transporters
30 co-compartmentalize with the Na⁺/K⁺-ATPase, as well as with glycolytic enzymes and
31 mitochondria (Genda et al., 2011, Bauer et al., 2012). In addition, it has been demonstrated that
32 movements of mitochondria stabilize at sites of glutamate uptake (Jackson et al., 2014). This
33 raises the question of the functional purpose of this proximity of mitochondria with the major
34 membrane sodium influx pathway.

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

16 The roles of mitochondrial sodium influx may extend beyond the glutamate-evoked
17 responses discussed above. Indeed, it was observed, that single mitochondria in astrocytes
18 display spontaneous sodium spiking activity (Azarias et al., 2008) that coincide with rapid
19 mitochondrial transients of pH and reactive oxygen species, possibly related to the availability
20 of ATP in the mitochondrial microdomain (Azarias et al., 2011). Similar single mitochondrial
21 spiking events were found in a variety of cell types and organisms, including plants
22 (Schwarzlander et al., 2012) and *C. elegans* (Shen et al., 2014). However, the mechanistic
23 nature and function of these events is still debated (Schwarzlander et al., 2012, Schwarzlander
24 et al., 2014). Mitochondrial spiking activity is increasingly thought to represent a novel
25 frequency-coded readout of metabolism at the single mitochondria level (Schwarzlander et al.,
26 2014).

27 While mitochondria do not appear to accumulate sodium, their ability to take up and
28 release sodium ions may enable them to influence the kinetics of local sodium fluctuations as
29 proposed for Ca^{2+} in neuronal presynaptic boutons (Scotti et al., 1998). Other subcellular
30 compartments may be relevant for cellular sodium regulation, such as the endoplasmic
31 reticulum, a major Ca^{2+} store critically involved in the fast release and reuptake of Ca^{2+} ions
32 during agonist-evoked responses or calcium-induced calcium release. While experimentally
33 difficult to address, quantitative X-ray microanalysis studies of cryosections of hippocampal
34 slices estimated that ER sodium levels in dendrites are somewhat higher than in the cytoplasm

1 (~19 mM) and can substantially increase during trains of action potentials (Pozzo-Miller et al.,
2 1997).

5 **5. Consequences of sodium homeostasis disturbances in the diseased brain**

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

16 The $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) that contributes to a large extent to the establishment
17 of the large inwardly directed Ca^{2+} gradient, is expressed by both neurons and glia (Annunziato
18 et al., 2013). When intracellular sodium rises to abnormal concentrations, the transporter
19 reverses its mode of operation and drives the entry of Ca^{2+} from the extracellular milieu. This
20 so-called reversal mode of NCX (Philipson and Nicoll, 2000) may have several deleterious
21 consequences for brain cells. In particular, excessive intracellular Ca^{2+} rise leads to
22 mitochondrial dysfunction (Putney et al., 2002) or further glutamate release by glia (Paluzzi et
23 al., 2007), which in turn contributes to the worsening of pathological conditions such as those
24 found in ischemic insults. The $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporters (NKCC) are expressed in neurons,
25 where they regulate the intracellular Cl^- concentration and therefore neuronal excitability. In
26 astrocytes, this transporter plays an important role in the control of extracellular K^+
27 concentration as well as cell volume regulation (Kofuji and Newman, 2004, Jayakumar and
28 Norenberg, 2010). As introduced above, stimulation of NKCC1 activity such as that occurring
29 during ischemia, may lead to the loss of sodium homeostasis with the consequence of increasing
30 cytoplasmic Ca^{2+} following stimulation of NCX reverse mode activation (Lenart et al., 2004).
31 Aberrant activation of NKCC1 is also believed to cause deleterious astrocyte swelling during
32 epileptic activity (Hochman, 2012).

33 Finally, the most important regulator of sodium homeostasis, the Na^+/K^+ -ATPase, is
34 critically susceptible to ATP depletion, such as that which occurs during ischemic stroke or

1 severe hypoglycemia, which ultimately leads to the loss of the sodium and K^+ transmembrane
2 gradients in these conditions. Na^+/K^+ -ATPase gene mutations have also been linked to familial
3 hemiplegic migraine (Capendeguy and Horisberger, 2004). Impaired Na^+/K^+ ATPase activity
4 is inevitably accompanied by the failure of transporters discussed above (NCX, NKCC, etc.)
5 that are critical for the proper brain function at the cellular and network level.

6

7

8

9

1 Acknowledgements

2 Work in the authors' laboratories is supported by grants from the Deutsche
3 Forschungsgemeinschaft to CRR (DFG: Ro2327/8-1) and the Swiss National Science
4 Foundation (SNF) to JYC. We thank Jennifer I. Luebke for her critical reading of the
5 manuscript.

6

7

8

1 **References**

- 2
- 3 Ahmed K, Tunaru S, Offermanns S (2009) GPR109A, GPR109B and GPR81, a family of
4 hydroxy-carboxylic acid receptors. *Trends Pharmacol Sci* 30:557-562.
- 5 Ames A, 3rd (2000) CNS energy metabolism as related to function. *Brain Res Brain Res Rev*
6 34:42-68.
- 7 Anderson CM, Swanson RA (2000) Astrocyte glutamate transport: review of properties,
8 regulation, and physiological functions. *Glia* 32:1-14.
- 9 Annunziato L, Boscia F, Pignataro G (2013) Ionic transporter activity in astrocytes, microglia,
10 and oligodendrocytes during brain ischemia. *J Cereb Blood Flow Metab* 33:969-982.
- 11 Azarias G, Kruusmagi M, Connor S, Akkuratov EE, Liu XL, Lyons D, Brismar H, Broberger
12 C, Aperia A (2013) A specific and essential role for Na,K-ATPase alpha3 in neurons
13 co-expressing alpha1 and alpha3. *J Biol Chem* 288:2734-2743.
- 14 Azarias G, Perreten H, Lengacher S, Poburko D, Demarex N, Magistretti PJ, Chatton J-Y
15 (2011) Glutamate transport decreases mitochondrial pH and modulates oxidative
16 metabolism in astrocytes. *Journal of Neuroscience* 31:3550-3559.
- 17 Azarias G, Van De Ville D, Unser M, Chatton J-Y (2008) Spontaneous Na⁺ transients in
18 individual mitochondria of intact astrocytes. *Glia* 56:342-353.
- 19 Baranauskas G, David Y, Fleidervish IA (2013) Spatial mismatch between the Na⁺ flux and
20 spike initiation in axon initial segment. *Proc Natl Acad Sci U S A* 110:4051-4056.
- 21 Barros LF (2013) Metabolic signaling by lactate in the brain. *Trends Neurosci*.
- 22 Barros LF, Deitmer JW (2010) Glucose and lactate supply to the synapse. *Brain Res Rev*
23 63:149-159.
- 24 Bauer DE, Jackson JG, Genda EN, Montoya MM, Yudkoff M, Robinson MB (2012) The
25 glutamate transporter, GLAST, participates in a macromolecular complex that supports
26 glutamate metabolism. *Neurochem Int* 61:566-574.
- 27 Bennay M, Langer J, Meier SD, Kafitz KW, Rose CR (2008) Sodium signals in cerebellar
28 Purkinje neurons and Bergmann glial cells evoked by glutamatergic synaptic
29 transmission. *Glia* 56:1138-1149.
- 30 Bergles DE, Diamond JS, Jahr CE (1999) Clearance of glutamate inside the synapse and
31 beyond. *Curr Opin Neurobiol* 9:293-298.
- 32 Bernardi P (1999) Mitochondrial transport of cations: channels, exchangers, and permeability
33 transition. *Physiol Rev* 79:1127-1155.

- 1 Bernardinelli Y, Azarias G, Chatton JY (2006) In situ fluorescence imaging of glutamate-
2 evoked mitochondrial Na⁺ responses in astrocytes. *Glia* 54:460-470.
- 3 Bernardinelli Y, Chatton JY (2008) Differential effects of glutamate transporter inhibitors on
4 the global electrophysiological response of astrocytes to neuronal stimulation. *Brain Res*
5 1240:47-53.
- 6 Bernardinelli Y, Magistretti PJ, Chatton JY (2004) Astrocytes generate Na⁺-mediated
7 metabolic waves. *Proc Natl Acad Sci U S A* 101:14937-14942.
- 8 Bittner CX, Valdebenito R, Ruminot I, Loaiza A, Larenas V, Sotelo-Hitschfeld T, Moldenhauer
9 H, San Martin A, Gutierrez R, Zambrano M, Barros LF (2011) Fast and reversible
10 stimulation of astrocytic glycolysis by K⁺ and a delayed and persistent effect of
11 glutamate. *J Neurosci* 31:4709-4713.
- 12 Blanco G, Mercer RW (1998) Isozymes of the Na-K-ATPase: heterogeneity in structure,
13 diversity in function. *Am J Physiol* 275:F633-650.
- 14 Bowman CL, Kimelberg HK (1984) Excitatory amino acids directly depolarize rat brain
15 astrocytes in primary culture. *Nature* 311:656-659.
- 16 Bozzo L, Chatton JY (2010) Inhibitory effects of (2S, 3S)-3-[3-[4-
17 (trifluoromethyl)benzoylamino]benzyloxy]aspartate (TFB-TBOA) on the astrocytic
18 sodium responses to glutamate. *Brain Res* 1316:27-34.
- 19 Bozzo L, Puyal J, Chatton JY (2013) Lactate modulates the activity of primary cortical neurons
20 through a receptor-mediated pathway. *PLoS One* 8:e71721.
- 21 Brennan AM, Suh SW, Won SJ, Narasimhan P, Kauppinen TM, Lee H, Edling Y, Chan PH,
22 Swanson RA (2009) NADPH oxidase is the primary source of superoxide induced by
23 NMDA receptor activation. *Nat Neurosci* 12:857-863.
- 24 Callaway JC, Ross WN (1997) Spatial distribution of synaptically activated sodium
25 concentration changes in cerebellar Purkinje neurons. *J Neurophysiol* 77:145-152.
- 26 Capendeguy O, Horisberger J-D (2004) Functional effects of Na⁺,K⁺-ATPase gene mutations
27 linked to familial hemiplegic migraine. *Neuromolecular medicine* 6:105-116.
- 28 Chatton J-Y, Magistretti PJ (2005) Relationship between L-glutamate-regulated intracellular
29 Na⁺ dynamics and ATP hydrolysis in astrocytes. *Journal of Neural Transmission*
30 112:77-85.
- 31 Chatton JY, Marquet P, Magistretti PJ (2000) A quantitative analysis of L-glutamate-regulated
32 Na⁺ dynamics in mouse cortical astrocytes: implications for cellular bioenergetics. *Eur*
33 *J Neurosci* 12:3843-3853.

- 1 Chatton JY, Pellerin L, Magistretti PJ (2003) GABA uptake into astrocytes is not associated
2 with significant metabolic cost: implications for brain imaging of inhibitory
3 transmission. *Proc Natl Acad Sci U S A* 100:12456-12461.
- 4 Chatton JY, Shimamoto K, Magistretti PJ (2001) Effects of glial glutamate transporter
5 inhibitors on intracellular Na⁺ in mouse astrocytes. *Brain Res* 893:46-52.
- 6 Chesler M (2003) Regulation and modulation of pH in the brain. *Physiol Rev* 83:1183-1221.
- 7 Choi HB, Gordon GR, Zhou N, Tai C, Rungta RL, Martinez J, Milner TA, Ryu JK, McLarnon
8 JG, Tresguerres M, Levin LR, Buck J, Macvicar BA (2012) Metabolic Communication
9 between Astrocytes and Neurons via Bicarbonate-Responsive Soluble Adenylyl
10 Cyclase. *Neuron* 75:1094-1104.
- 11 Cholet N, Pellerin L, Magistretti PJ, Hamel E (2002) Similar perisynaptic glial localization for
12 the Na⁺,K⁺-ATPase alpha 2 subunit and the glutamate transporters GLAST and GLT-
13 1 in the rat somatosensory cortex. *Cereb Cortex* 12:515-525.
- 14 Cholet N, Pellerin L, Welker E, Lacombe P, Seylaz J, Magistretti P, Bonvento G (2001) Local
15 injection of antisense oligonucleotides targeted to the glial glutamate transporter
16 GLAST decreases the metabolic response to somatosensory activation. *J Cereb Blood
17 Flow Metab* 21:404-412.
- 18 Crambert G, Geering K (2003) FXYD proteins: new tissue-specific regulators of the ubiquitous
19 Na,K-ATPase. *Sci STKE* 2003:RE1.
- 20 Cruz NF, Ball KK, Diemel GA (2007) Functional imaging of focal brain activation in conscious
21 rats: impact of [(14)C]glucose metabolite spreading and release. *J Neurosci Res*
22 85:3254-3266.
- 23 Danbolt NC (2001) Glutamate uptake. *Prog Neurobiol* 65:1-105.
- 24 David JC, Yamada KA, Bagwe MR, Goldberg MP (1996) AMPA receptor activation is rapidly
25 toxic to cortical astrocytes when desensitization is blocked. *Journal of Neuroscience*
26 16:200-209.
- 27 Deitmer JW, Rose CR (1996) pH regulation and proton signalling by glial cells. *Prog Neurobiol*
28 48:73-103.
- 29 Deitmer JW, Rose CR (2010) Ion changes and signalling in perisynaptic glia. *Brain Res Rev*
30 63:113-129.
- 31 Dietzel I, Heinemann U, Hofmeier G, Lux HD (1982) Stimulus-induced changes in
32 extracellular Na⁺ and Cl⁻ concentration in relation to changes in the size of the
33 extracellular space. *Exp Brain Res* 46:73-84.
- 34 Erecinska M, Silver IA (1994) Ions and energy in mammalian brain. *Prog Neurobiol* 43:37-71.

- 1 Figley CR, Stroman PW (2011) The role(s) of astrocytes and astrocyte activity in
2 neurometabolism, neurovascular coupling, and the production of functional
3 neuroimaging signals. *Eur J Neurosci* 33:577-588.
- 4 Fleidervish IA, Lasser-Ross N, Gutnick MJ, Ross WN (2010) Na⁺ imaging reveals little
5 difference in action potential-evoked Na⁺ influx between axon and soma. *Nat Neurosci*
6 13:852-860.
- 7 Gadea A, Lopez-Colome AM (2001) Glial transporters for glutamate, glycine, and GABA: II.
8 GABA transporters. *J Neurosci Res* 63:461-468.
- 9 Gegelashvili G, Schousboe A (1998) Cellular distribution and kinetic properties of high-affinity
10 glutamate transporters. *Brain Res Bull* 45:233-238.
- 11 Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R,
12 Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB (2011) Co-
13 compartmentalization of the astroglial glutamate transporter, GLT-1, with glycolytic
14 enzymes and mitochondria. *J Neurosci* 31:18275-18288.
- 15 Gurden H, Uchida N, Mainen ZF (2006) Sensory-evoked intrinsic optical signals in the
16 olfactory bulb are coupled to glutamate release and uptake. *Neuron* 52:335-345.
- 17 Hablitz JJ, Heinemann U (1989) Alterations in the microenvironment during spreading
18 depression associated with epileptiform activity in the immature neocortex. *Brain Res*
19 *Dev Brain Res* 46:243-252.
- 20 Harris JJ, Jolivet R, Attwell D (2012) Synaptic energy use and supply. *Neuron* 75:762-777.
- 21 Hertz L, Xu J, Song D, Yan E, Gu L, Peng L (2013) Astrocytic and neuronal accumulation of
22 elevated extracellular K⁽⁺⁾ with a 2/3 K⁽⁺⁾/Na⁽⁺⁾ flux ratio-consequences for energy
23 metabolism, osmolarity and higher brain function. *Front Comput Neurosci* 7:114.
- 24 Hevner RF, Duff RS, Wong-Riley MT (1992) Coordination of ATP production and
25 consumption in brain: parallel regulation of cytochrome oxidase and Na⁺, K⁽⁺⁾-
26 ATPase. *Neurosci Lett* 138:188-192.
- 27 Hochman D (2012) The extracellular space and epileptic activity in the adult brain: explaining
28 the antiepileptic effects of furosemide and bumetanide. *Epilepsia* 53 Suppl 1:18-25.
- 29 Hodgkin A, Huxley A (1952) A quantitative description of membrane current and its
30 application to conduction and excitation in nerve. *J Physiol* 117:500-544.
- 31 Howarth C, Gleeson P, Attwell D (2012) Updated energy budgets for neural computation in the
32 neocortex and cerebellum. *J Cereb Blood Flow Metab* 32:1222-1232.

- 1 Jackson JG, O'Donnell JC, Takano H, Coulter DA, Robinson MB (2014) Neuronal activity and
2 glutamate uptake decrease mitochondrial mobility in astrocytes and position
3 mitochondria near glutamate transporters. *J Neurosci* 34:1613-1624.
- 4 Jaffe DB, Johnston D, Lasser-Ross N, Lisman JE, Miyakawa H, Ross WN (1992) The spread
5 of Na⁺ spikes determines the pattern of dendritic Ca²⁺ entry into hippocampal neurons.
6 *Nature* 357:244-246.
- 7 Jayakumar AR, Norenberg MD (2010) The Na-K-Cl Co-transporter in astrocyte swelling.
8 *Metab Brain Dis* 25:31-38.
- 9 Kala G, Kumarathasan R, Peng L, Leenen FH, Hertz L (2000) Stimulation of Na⁺,K⁺-ATPase
10 activity, increase in potassium uptake, and enhanced production of ouabain-like
11 compounds in ammonia-treated mouse astrocytes. *Neurochem Int* 36:203-211.
- 12 Kanai R, Ogawa H, Vilsen B, Cornelius F, Toyoshima C (2013) Crystal structure of a Na⁺-
13 bound Na⁺,K⁺-ATPase preceding the E1P state. *Nature* 502:201-206.
- 14 Kaplan JH (2002) Biochemistry of Na,K-ATPase. *Annu Rev Biochem* 71:511-535.
- 15 Kettenmann H, Backus KH, Schachner M (1984) Aspartate, glutamate and gamma-
16 aminobutyric acid depolarize cultured astrocytes. *Neurosci Lett* 52:25-29.
- 17 Kimelberg HK, Pang S, Treble DH (1989) Excitatory amino acid-stimulated uptake of ²²Na⁺
18 in primary astrocyte cultures. *J Neurosci* 9:1141-1149.
- 19 Kirischuk S, Kettenmann H, Verkhratsky A (2007) Membrane currents and cytoplasmic sodium
20 transients generated by glutamate transport in Bergmann glial cells. *Pflugers Arch*
21 454:Kirischuk et al., *Pflügers Arch*; 245-252.
- 22 Kirischuk S, Parpura V, Verkhratsky A (2012) Sodium dynamics: another key to astroglial
23 excitability? *Trends Neurosci* 35:497-506.
- 24 Kofuji P, Newman EA (2004) Potassium buffering in the central nervous system. *Neuroscience*
25 129:1045-1056.
- 26 Kohr G, Heinemann U (1989) Effects of NMDA antagonists on picrotoxin-, low Mg²⁺- and
27 low Ca²⁺-induced epileptogenesis and on evoked changes in extracellular Na⁺ and
28 Ca²⁺ concentrations in rat hippocampal slices. *Epilepsy Res* 4:187-200.
- 29 Kole MH, Ilschner SU, Kampa BM, Williams SR, Ruben PC, Stuart GJ (2008) Action potential
30 generation requires a high sodium channel density in the axon initial segment. *Nat*
31 *Neurosci* 11:178-186.
- 32 Kushmerick MJ, Podolsky RJ (1969) Ionic mobility in muscle cells. *Science* 166:1297-1298.
- 33 Lalo U, Pankratov Y, Kirchhoff F, North RA, Verkhratsky A (2006) NMDA receptors mediate
34 neuron-to-glia signaling in mouse cortical astrocytes. *J Neurosci* 26:2673-2683.

- 1 Lalo U, Pankratov Y, Parpura V, Verkhratsky A (2011) Ionotropic receptors in neuronal-
2 astroglial signalling: what is the role of "excitable" molecules in non-excitable cells.
3 *Biochim Biophys Acta* 1813:992-1002.
- 4 Lamy CM, Chatton JY (2011) Optical probing of sodium dynamics in neurons and astrocytes.
5 *Neuroimage* 58:572-578.
- 6 Langer J, Rose CR (2009) Synaptically induced sodium signals in hippocampal astrocytes in
7 situ. *J Physiol* 587:5859-5877.
- 8 Langer J, Stephan J, Theis M, Rose CR (2012) Gap junctions mediate intercellular spread of
9 sodium between hippocampal astrocytes in situ. *Glia* 60:239-252.
- 10 Larsson OM, Hertz L, Schousboe A (1980) GABA uptake in astrocytes in primary cultures:
11 coupling with two sodium ions. *Journal of Neuroscience Research* 5:469-477.
- 12 Lasser-Ross N, Ross WN (1992) Imaging voltage and synaptically activated sodium transients
13 in cerebellar Purkinje cells. *Proc Biol Sci* 247:35-39.
- 14 Lauritzen KH, Morland C, Puchades M, Holm-Hansen S, Hagelin EM, Lauritzen F, Attramadal
15 H, Storm-Mathisen J, Gjedde A, Bergersen LH (2014) Lactate receptor sites link
16 neurotransmission, neurovascular coupling, and brain energy metabolism. *Cereb Cortex*
17 24:2784-2795.
- 18 Lenart B, Kintner DB, Shull GE, Sun D (2004) Na-K-Cl cotransporter-mediated intracellular
19 Na^+ accumulation affects Ca^{2+} signaling in astrocytes in an in vitro ischemic model. *J*
20 *Neurosci* 24:9585-9597.
- 21 Lennie P (2003) The cost of cortical computation. *Curr Biol* 13:493-497.
- 22 Liu C, Wu J, Zhu J, Kuei C, Yu J, Shelton J, Sutton SW, Li X, Yun SJ, Mirzadegan T, Mazur
23 C, Kamme F, Lovenberg TW (2009) Lactate inhibits lipolysis in fat cells through
24 activation of an orphan G-protein-coupled receptor, GPR81. *J Biol Chem* 284:2811-
25 2822.
- 26 Loaiza A, Porras OH, Barros LF (2003) Glutamate triggers rapid glucose transport stimulation
27 in astrocytes as evidenced by real-time confocal microscopy. *J Neurosci* 23:7337-7342.
- 28 Maragakis NJ, Rothstein JD (2004) Glutamate transporters: animal models to neurologic
29 disease. *Neurobiol Dis* 15:461-473.
- 30 Marcaggi P, Attwell D (2004) Role of glial amino acid transporters in synaptic transmission
31 and brain energetics. *Glia* 47:217-225.
- 32 Matos M, Augusto E, Agostinho P, Cunha RA, Chen JF (2013) Antagonistic Interaction
33 between Adenosine A2A Receptors and Na^+/K^+ -ATPase- $\alpha 2$ Controlling
34 Glutamate Uptake in Astrocytes. *J Neurosci* 33:18492-18502.

- 1 Morth JP, Pedersen BP, Buch-Pedersen MJ, Andersen JP, Vilsen B, Palmgren MG, Nissen P
2 (2011) A structural overview of the plasma membrane Na⁺,K⁺-ATPase and H⁺-
3 ATPase ion pumps. *Nat Rev Mol Cell Biol* 12:60-70.
- 4 Nedergaard M (1994) Direct signaling from astrocytes to neurons in cultures of mammalian
5 brain cells. *Science* 263:1768-1771.
- 6 Nuriya M, Yasui M (2013) Endfeet serve as diffusion-limited subcellular compartments in
7 astrocytes. *J Neurosci* 33:3692-3698.
- 8 Nyblom M, Poulsen H, Gourdon P, Reinhard L, Andersson M, Lindahl E, Fedosova N, Nissen
9 P (2013) Crystal structure of Na⁺, K⁽⁺⁾-ATPase in the Na⁽⁺⁾-bound state. *Science*
10 342:123-127.
- 11 Orlov SN, Hamet P (2006) Intracellular monovalent ions as second messengers. *J Membr Biol*
12 210:161-172.
- 13 Paluzzi S, Alloisio S, Zappettini S, Milanese M, Raiteri L, Nobile M, Bonanno G (2007) Adult
14 astroglia is competent for Na⁺/Ca²⁺ exchanger-operated exocytotic glutamate release
15 triggered by mild depolarization. *J Neurochem* 103:1196-1207.
- 16 Pappas CA, Ransom BR (1994) Depolarization-induced alkalinization (DIA) in rat
17 hippocampal astrocytes. *J Neurophysiol* 72:2816-2826.
- 18 Parker MD, Boron WF (2013) The divergence, actions, roles, and relatives of sodium-coupled
19 bicarbonate transporters. *Physiol Rev* 93:803-959.
- 20 Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis:
21 a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci U S*
22 *A* 91:10625-10629.
- 23 Pellerin L, Magistretti PJ (1997) Glutamate uptake stimulates Na⁺,K⁺-ATPase activity in
24 astrocytes via activation of a distinct subunit highly sensitive to ouabain. *J Neurochem*
25 69:2132-2137.
- 26 Perreten Lambert H, Zenger M, Azarias G, Chatton JY, Magistretti PJ, Lengacher S (2014)
27 Control of Mitochondrial pH by Uncoupling Protein 4 in Astrocytes Promotes Neuronal
28 Survival. *J Biol Chem*.
- 29 Philipson KD, Nicoll DA (2000) Sodium-calcium exchange: a molecular perspective. *Annu*
30 *Rev Physiol* 62:111-133.
- 31 Porras OH, Ruminot I, Loaiza A, Barros LF (2008) Na⁺-Ca²⁺ cosignaling in the stimulation of
32 the glucose transporter GLUT1 in cultured astrocytes. *Glia* 56:59-68.

- 1 Pozzo-Miller LD, Pivovarov NB, Leapman RD, Buchanan RA, Reese TS, Andrews SB (1997)
2 Activity-dependent calcium sequestration in dendrites of hippocampal neurons in brain
3 slices. *J Neurosci* 17:8729-8738.
- 4 Putney LK, Denker SP, Barber DL (2002) The changing face of the Na⁺/H⁺ exchanger, NHE1:
5 structure, regulation, and cellular actions. *Annu Rev Pharmacol Toxicol* 42:527-552.
- 6 Rose CR, Karus C (2013) Two sides of the same coin: sodium homeostasis and signaling in
7 astrocytes under physiological and pathophysiological conditions. *Glia* 61:1191-1205.
- 8 Rose CR, Konnerth A (2001) NMDA receptor-mediated Na⁺ signals in spines and dendrites. *J*
9 *Neurosci* 21:4207-4214.
- 10 Rose CR, Kovalchuk Y, Eilers J, Konnerth A (1999) Two-photon Na⁺ imaging in spines and
11 fine dendrites of central neurons. *Pflugers Arch* 439:201-207.
- 12 Rose CR, Ransom BR (1996a) Intracellular sodium homeostasis in rat hippocampal astrocytes.
13 *J Physiol* 491 (Pt 2):291-305.
- 14 Rose CR, Ransom BR (1996b) Mechanisms of H⁺ and Na⁺ changes induced by glutamate,
15 kainate, and D-Aspartate in rat hippocampal astrocytes. *J Neurosci* 16:5393-5404.
- 16 Rose CR, Ransom BR (1997a) Gap junctions equalize intracellular Na⁺ concentration in
17 astrocytes. *Glia* 20:299-307.
- 18 Rose CR, Ransom BR (1997b) Regulation of intracellular sodium in cultured rat hippocampal
19 neurones. *J Physiol* 499 (Pt 3):573-587.
- 20 Rose EM, Koo JC, Antflick JE, Ahmed SM, Angers S, Hampson DR (2009) Glutamate
21 transporter coupling to Na,K-ATPase. *J Neurosci* 29:8143-8155.
- 22 Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C (2008) Astroglial metabolic
23 networks sustain hippocampal synaptic transmission. *Science* 322:1551-1555.
- 24 Ruminot I, Gutierrez R, Pena-Munzenmayer G, Anazco C, Sotelo-Hitschfeld T, Lerchundi R,
25 Niemeyer MI, Shull GE, Barros LF (2011) NBCe1 mediates the acute stimulation of
26 astrocytic glycolysis by extracellular K⁺. *J Neurosci* 31:14264-14271.
- 27 Scemes E, Giaume C (2006) Astrocyte calcium waves: what they are and what they do. *Glia*
28 54:716-725.
- 29 Schousboe A, Sarup A, Bak LK, Waagepetersen HS, Larsson OM (2004) Role of astrocytic
30 transport processes in glutamatergic and GABAergic neurotransmission. *Neurochem*
31 *Int* 45:521-527.
- 32 Schreiner AE, Durry S, Aida T, Stock MC, Ruther U, Tanaka K, Rose CR, Kafitz KW (2014)
33 Laminar and subcellular heterogeneity of GLAST and GLT-1 immunoreactivity in the
34 developing postnatal mouse hippocampus. *J Comp Neurol* 522:204-224.

- 1 Schwarzlander M, Logan DC, Johnston IG, Jones NS, Meyer AJ, Fricker MD, Sweetlove LJ
2 (2012) Pulsing of membrane potential in individual mitochondria: a stress-induced
3 mechanism to regulate respiratory bioenergetics in Arabidopsis. *Plant Cell* 24:1188-
4 1201.
- 5 Schwarzlander M, Wagner S, Ermakova YG, Belousov VV, Radi R, Beckman JS, Buettner GR,
6 Demaurex N, Duchen MR, Forman HJ, Fricker MD, Gems D, Halestrap AP, Halliwell
7 B, Jakob U, Johnston IG, Jones NS, Logan DC, Morgan B, Muller FL, Nicholls DG,
8 Remington SJ, Schumacker PT, Winterbourn CC, Sweetlove LJ, Meyer AJ, Dick TP,
9 Murphy MP (2014) The 'mitoflash' probe cpYFP does not respond to superoxide. *Nature*
10 514:E12-14.
- 11 Scotti AL, Chatton J-Y, Reuter H (1998) Roles of $\text{Na}^+/\text{Ca}^{2+}$ exchange and of mitochondria in
12 the regulation of presynaptic Ca^{2+} and spontaneous glutamate release. *Phil Trans R Soc*
13 *Lond B* 354:357-364.
- 14 Sheldon C, Cheng YM, Church J (2004) Concurrent measurements of the free cytosolic
15 concentrations of H^+ and Na^+ ions with fluorescent indicators. *Pflugers Arch*
16 449:307-318.
- 17 Shen EZ, Song CQ, Lin Y, Zhang WH, Su PF, Liu WY, Zhang P, Xu J, Lin N, Zhan C, Wang
18 X, Shyr Y, Cheng H, Dong MQ (2014) Mitoflash frequency in early adulthood predicts
19 lifespan in *Caenorhabditis elegans*. *Nature* 508:128-132.
- 20 Shimizu H, Watanabe E, Hiyama TY, Nagakura A, Fujikawa A, Okado H, Yanagawa Y, Obata
21 K, Noda M (2007) Glial Na^+ channels control lactate signaling to neurons for brain
22 $[\text{Na}^+]$ sensing. *Neuron* 54:59-72.
- 23 Skou JC, Esmann M (1992) The Na^+/K^+ -ATPase. *J Bioenerg Biomembr* 24:249-261.
- 24 Somjen GG (2004) *Ions in the brain: Normal function, seizures, and stroke*: Oxford University
25 Press, New York.
- 26 Sutherland EW (1972) Studies on the mechanism of hormone action. *Science* 177:401-408.
- 27 Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, Alberini CM (2011)
28 Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell*
29 144:810-823.
- 30 Sykova E, Nicholson C (2008) Diffusion in brain extracellular space. *Physiol Rev* 88:1277-
31 1340.
- 32 Tang F, Lane S, Korsak A, Paton JF, Gourine AV, Kasparov S, Teschemacher AG (2014)
33 Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nat Commun*
34 5:3284.

- 1 Tsukada S, Iino M, Takayasu Y, Shimamoto K, Ozawa S (2005) Effects of a novel glutamate
2 transporter blocker, (2S, 3S)-3-[3-[4-(trifluoromethyl)benzoylamino]benzyloxy]
3 aspartate (TFB-TBOA), on activities of hippocampal neurons. *Neuropharmacology*
4 48:479-491.
- 5 Tzingounis AV, Wadiche JI (2007) Glutamate transporters: confining runaway excitation by
6 shaping synaptic transmission. *Nat Rev Neurosci* 8:935-947.
- 7 Unichenko P, Dvorzhak A, Kirischuk S (2013) Transporter-mediated replacement of
8 extracellular glutamate for GABA in the developing murine neocortex. *Eur J Neurosci*
9 38:3580-3588.
- 10 Unichenko P, Myakhar O, Kirischuk S (2012) Intracellular Na(+) concentration influences
11 short-term plasticity of glutamate transporter-mediated currents in neocortical
12 astrocytes. *Glia* 60:605-614.
- 13 Uwechue NM, Marx MC, Chevy Q, Billups B (2012) Activation of glutamate transport evokes
14 rapid glutamine release from perisynaptic astrocytes. *J Physiol* 590:2317-2331.
- 15 Voutsinos-Porche B, Bonvento G, Tanaka K, Steiner P, Welker E, Chatton JY, Magistretti PJ,
16 Pellerin L (2003) Glial glutamate transporters mediate a functional metabolic crosstalk
17 between neurons and astrocytes in the mouse developing cortex. *Neuron* 37:275-286.
- 18 Wyllie DJ, Cull-Candy SG (1994) A comparison of non-NMDA receptor channels in type-2
19 astrocytes and granule cells from rat cerebellum. *J Physiol* 475:95-114.
- 20 Yang J, Ruchti E, Petit JM, Jourdain P, Grenningloh G, Allaman I, Magistretti PJ (2014) Lactate
21 promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proc*
22 *Natl Acad Sci U S A* 111:12228-12233.
- 23 Zahler R, Zhang ZT, Manor M, Boron WF (1997) Sodium kinetics of Na,K-ATPase alpha
24 isoforms in intact transfected HeLa cells. *J Gen Physiol* 110:201-213.
- 25 Zanutto L, Heinemann U (1983) Aspartate and glutamate induced reductions in extracellular
26 free calcium and sodium concentration in area CA1 of 'in vitro' hippocampal slices of
27 rats. *Neurosci Lett* 35:79-84.

28

29

30

31

1 **Figure Captions**

2

3 **Fig. 1.** Activity-induced sodium signals. (A) extracellular sodium signals in the sensorimotor
 4 cortex (depth 600 μm) of cats induced by electrical stimulation of the cortical surface with 20
 5 Hz, 0.2 ms for 10 s. (B), (C) sodium signals in neurons. (B) Left: 3D reconstruction of a layer
 6 2/3 pyramidal neuron patched with ANG-containing electrolyte. Scale bar, 30 μm . Right:
 7 Dendritic fluorescence transients recorded during backpropagation of action potentials (top).
 8 Space over time display of the matching linescan recording (bottom). (C) Top left: Image of a
 9 spiny dendrite of a CA1 pyramidal cell. Closed arrowheads indicate "active" and open
 10 arrowheads "passive" spines from which measurements were taken; scale bar: 5 μm . Top right:
 11 Suprathreshold stimulation (5 pulses at 50 Hz) induced 5 APs as measured at the soma. Upper
 12 traces: Average activity-induced sodium transient in spines (average of 19) and in the dendrite.
 13 Middle traces: sodium transients in 3 single "passive" spines. Lower traces: sodium transients
 14 in 3 single "active" spines. (D), (E) sodium signals in astrocytes. (D) Top: Fluorescence image
 15 of astrocytes in a cortical slice double-stained with sulforhodamine 101 (left panel) and ANG
 16 (center panel). Scale bar, 30 μm . Magnification of an ANG stained astrocyte (right panel). Scale
 17 bar, 5 μm . Bottom: Sample trace of astrocytic fluorescence response to glutamate puffs of
 18 increasing durations. (E) inward current and sodium transients in different processes of a
 19 Bergmann glial cell during parallel fibers stimulation (50 Hz, 100 ms). Signals were largest in
 20 processes that were located close to the stimulation pipette. Taken from: (A) Dietzel et. al.
 21 (1982), (B) Lamy & Chatton 2011, (C) Rose et al. 2001, (D) Lamy & Chatton 2011, (E) Bennay
 22 et al. 2008.

23

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

32

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

1 endothelin-1 and/or gramicidin during the periods indicated. The lower traces show that only
2 coincidence of both signals causes stimulation of glucose transport (as monitored by uptake of
3 6-NBDG). (B) Interrelationship between intracellular magnesium concentration (determined by
4 fluorescence imaging with MagnesiumGreen) and sodium (determined by imaging with SBFI).
5 Whereas application of glutamate causes an increase in both sodium and magnesium (the latter
6 indicating a reduction in ATP content), inhibition of the sodium pump by ouabain causes a
7 further rise in sodium, but a decrease in free magnesium, indicative of a recovery of intracellular
8 ATP levels. (C) Enhancement of glucose utilization and lactate formation induced by glutamate
9 application depend on the expression of glutamate transporters. Glucose utilization, evaluated
10 by intracellular accumulation of 2-Deoxyglucose (top), and lactate release (bottom) under
11 control conditions and after application of glutamate (Glu) in cortical astrocytes in culture
12 derived from wildtype (+/+), heterozygous (+/-), and GLAST mutant mice (-/-). (D) Sodium
13 signals in mitochondria. Glutamate induces an increase in cytosolic sodium (determined by
14 SBFI) as well as intramitochondrial sodium (determined by CoroNaRed) in cultured astrocytes.
15 Diazoxide, a K_{ATP} channel opener caused an increase in mitochondrial sodium only. Taken
16 from: (A), Porras et al (2008); (B), Magistretti & Chatton (2005); (C), Voutsinos-Porche et al.
17 (2003); (D), Bernardinelli et al (2006).

18

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

32

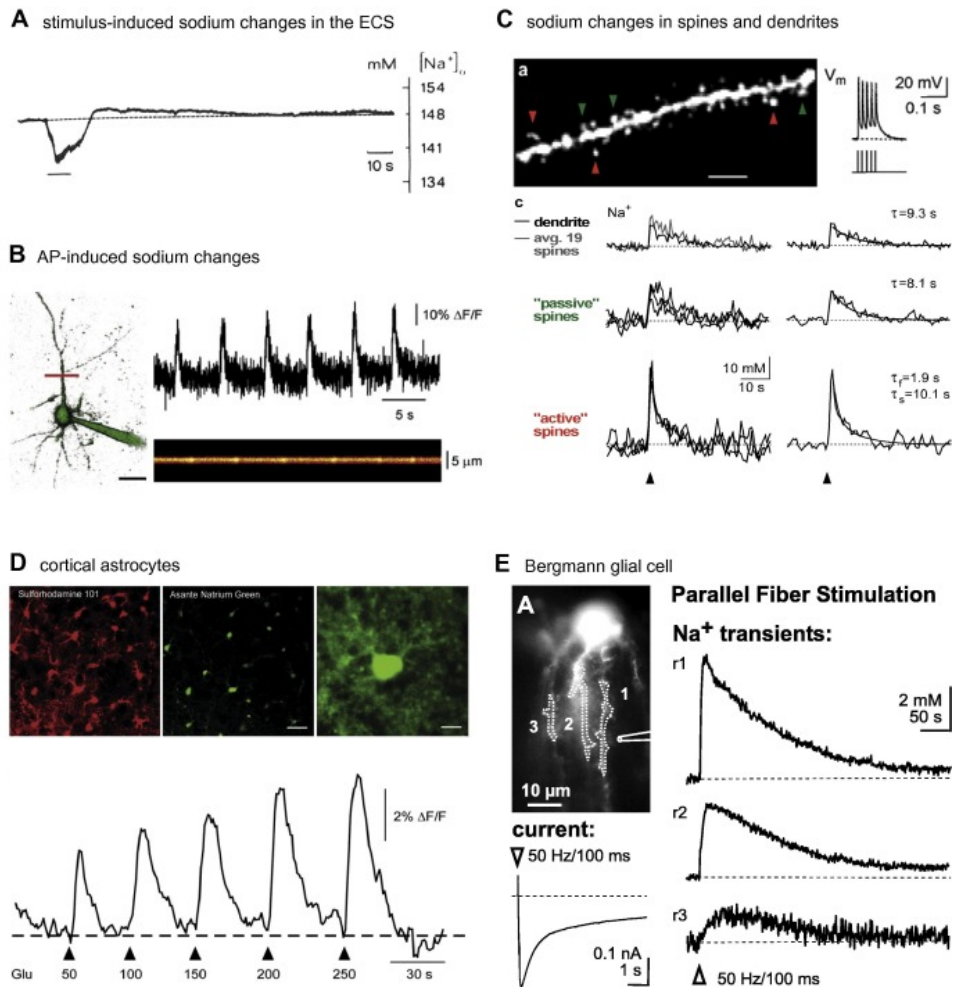


Figure 1

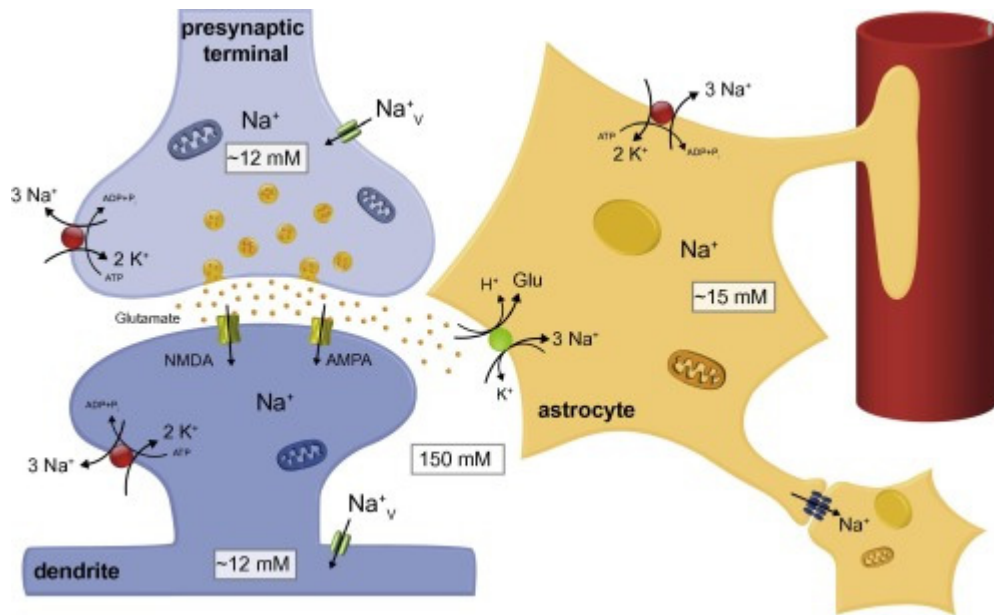
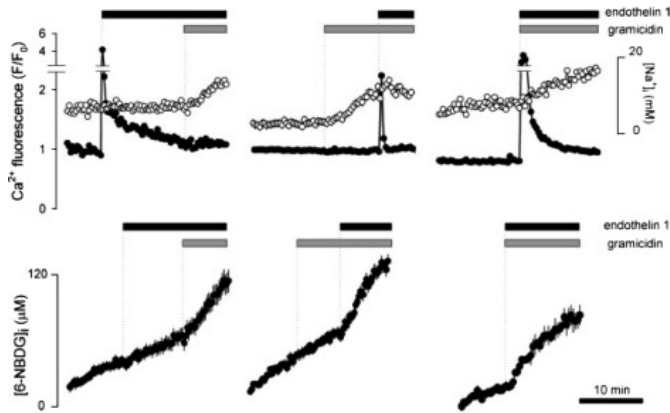
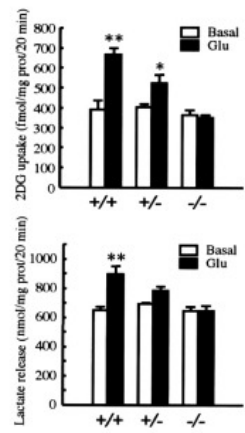


Figure 2

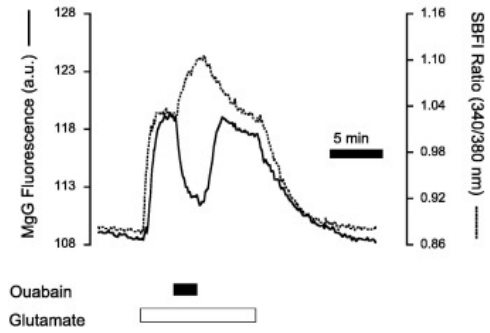
A GLUT1 stimulation by coincidental sodium and calcium signals



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

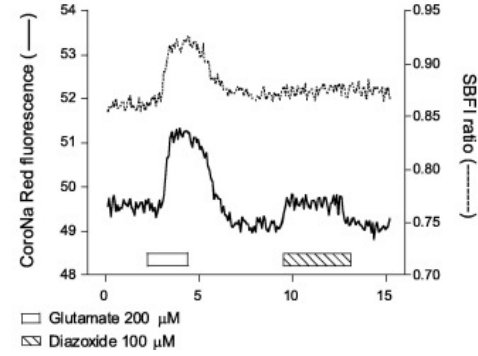


Figure 3

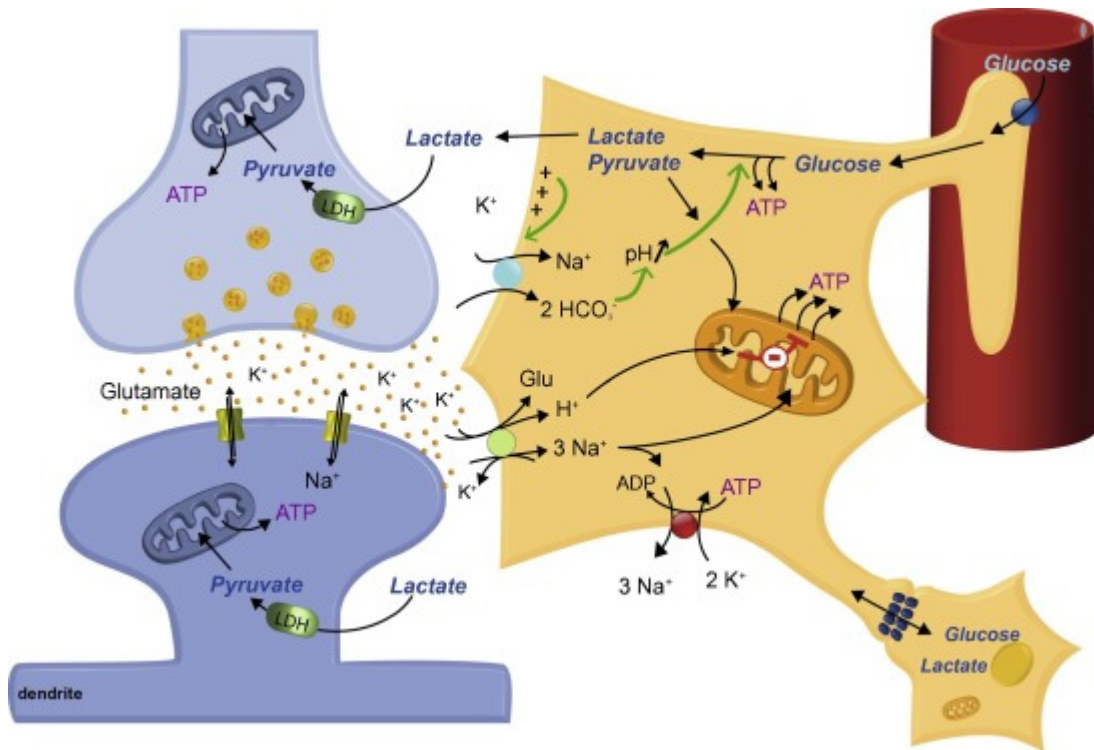


Figure 4