Intracellular Sodium and Energy Metabolism in Mouse Brain

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Niklas Jonny Gerkau
aus Soltau

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aus dem Institut für Neurobiologie
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Gutachter:

Prof. Dr. rer. nat. Christine R. Rose, Heinrich-Heine-Universität Düsseldorf
Prof. Dr. rer. nat. Christoph Fahlke, Forschungszentrum Jülich
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“Count what is countable, measure what is measurable, and what is not measurable, make measurable.”

Galileo Galilei

Abstract

In the mammalian central nervous system, a dense network of billions of neuronal connections is responsible for information processing. Despite the brain's small mass compared to the rest of the body's weight, it consumes a fifth of the total energy. The major energy consumer of the brain is the sodium potassium ATPase (NKA), which maintains the sodium and potassium gradients across membranes, providing the basis for electrical signaling. As the main extrusion mechanism for sodium ions, the NKA restores the baseline sodium concentration after neural activity and couples the intracellular sodium homeostasis with the cellular energy metabolism. Sodium regulation thus seems to be a critical factor both for signaling as well as for the cellular energy status.

In the past, several studies have addressed intracellular sodium changes in cell culture and tissue slice preparations under physiological and pathophysiological conditions. Still, there is a surprising lack of knowledge on how intracellular sodium changes with activity in different brain areas. Moreover, the influence of the cellular energy level on sodium homeostasis is not understood in detail. The present study addressed these questions, aiming to elucidate sodium influx pathways and the dependence of sodium homeostasis on the cellular metabolism in neurons and astrocytes of the mouse brain. To characterize intracellular ion changes, I employed quantitative, fluorescence-based wide-field and two-photon imaging both in acute tissue slices and in the mouse brain *in vivo*. In addition, whole-cell patch-clamp recordings served to characterize the electrophysiological behavior of the cells under study.

Using these tools, I could elucidate and characterize relevant sodium influx pathways into different neuronal compartments such as axons and spiny dendrites as well as into processes of astrocytes. Besides already proposed mechanisms (among them voltage-gated sodium channels), I was able to identify ENaC channels as hitherto unknown pathways for sodium influx into neural stem cells. Furthermore, I could show that depending on the sodium gradient, the NKCC1 work either in sodium influx or efflux mode. Moreover, my work indicated for the first time that sodium signals in perivascular endfeet are directly related to the breakdown of ATP, emphasizing the close interrelation between sodium homeostasis and cellular metabolism. In addition, I could provide the first quantitative data on intracellular sodium elevations in peri-infarct regions in mouse brain *in vivo*. These propagated in a wavelike manner across the cortex and invaded neurons as well as astrocytes. My data suggested that sodium influx drives reversal of the sodium/calcium exchanger (NCX), triggering a massive secondary calcium elevation while promoting export of sodium. Reported neuroprotective effects of the NCX activity in stroke models might thus be related to its dampening of ischemia-induced sodium loading.

Zusammenfassung

Im ZNS von Säugern bilden Neurone ein dichtes Netzwerk von aber Milliarden Verbindungen, die für die Informationsverarbeitung zuständig sind. Trotz der relativ kleinen Masse des Gehirns zur restlichen Masse des Körpers, verbraucht es dennoch ein Fünftel der Gesamtenergie. Der Hauptkonsument ist die NKA, die mit der Aufrechterhaltung der Natrium- und Kaliumgradienten für die Möglichkeit der Erregungsweiterleitung verantwortlich ist. Nach neuronaler Aktivität stellt die NKA die Ruhekonzentration an Natriumionen in den Zellen wieder her und verbindet somit die Natriumhomöostase mit dem zellulären Energiemetabolismus. Die Natriumregulation ist ein entscheidender Faktor für die zelluläre Signalgebung sowie für den Energiehaushalt der Zelle.

Bisherige Studien konnten intrazelluläre Natriumveränderung in Zellkulturen und Gewebeschnitten unter physiologischen und pathophysiologischen Bedingungen charakterisieren. Trotz dessen, bleiben Fragen über die Natriumänderung bei neuronaler Aktivität in verschiedenen Gehirnregionen ungeklärt. Darüber hinaus, ist der Einfluss des zellulären Energielevels auf die Natriumhomöostase nicht im Detail verstanden. In dieser Studie sollten diese Fragen adressiert werden, mit dem Ziel den Natriumeinstrom und die Abhängigkeit der Natriumhomöostase vom Zellmetabolismus in Neuronen und Astrozyten des Mausgehirns aufzuklären. Zu diesem Zweck, wurde in dieser Studie quantitatives Fluoreszenz-basiertes Weit-feld- und Zwei-Photonen-Imaging an Gewebeschnitten sowie am Mausgehirn *in vivo* durchgeführt. Diese Messungen wurden mit elektrophysiologischen Messungen mit Hilfe des Patch-Clamp Verfahrens komplementiert.

Mit Hilfe dieser Techniken konnten in dieser Studie relevante Natriumeinstromwege in neuronale Axone und Dendriten sowie astrozytäre Ausläufer charakterisiert werden. Neben bereits vorgeschlagenen Mechanismen (unter diesen spannungs-abhängige Natriumkanäle), konnten hier erstmalig ENaC-Kanäle als Natrium-permeable Kanäle in neuronalen Stammzellen charakterisiert werden. Weiterhin konnte gezeigt werden, dass sich die Transportrichtung des NKCC1 in Abhängigkeit vom Natriumgradienten ändert. Weiter konnte erstmalig gezeigt werden, dass Natriumsignale in astrozytären Ausläufern direkt mit ATP-Verbrauch verknüpft sind, was die enge Verbindung zwischen der Natriumhomöostase und dem zellulären Energiemetabolismus verdeutlicht. Außerdem, konnten erstmalig Natriummessungen in der ischämischen Penumbra im Mäusegehirn *in vivo* dargestellt werden. Diese Natriumsignale breiten sich wellenartig über den Kortex aus und betrafen sowohl Neurone als auch Astrozyten. Die erhöhte Natriumkonzentration führte zum Umkehren des NCX und somit zu einem starken Kalziumeinstrom mit einem gleichzeitigen Natriumausstrom. Die schützende Wirkung des NCX während ischämischen Bedingungen ist vor allem auf seine mildernde Wirkung auf den Natriumeinstrom zurück zu führen.

Table of Content

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Introduction and Résumé

"…the cortical mass is 475 g, resulting in a gross consumption of 3.4 x 10^{21} molecules of ATP per minute." (Lennie, 2003)

Over the last 60 years of clinical and experimental research on brain energy metabolism, including studies on humans, it has become clear that glucose is nearly the sole energy substrate that is fully oxidized and used almost exclusively by a single mechanism which regulates ion homeostasis in the brain and in doing so, consumes an inconceivable amount of ATP (Magistretti, 2008). This essential mechanism is the sodium/potassium ATPase (NKA), which maintains the sodium and potassium gradients across membranes. However, as we will see, there are much more mechanisms involved making things more complex…

1. Sodium homeostasis and cellular energy

The brain is the organ with the highest energy need relative to its mass (Harris et al., 2012; Mink et al., 1981): constituting just 2% of the body's weight, under resting conditions it already consumes more than 20% of the total energy and oxygen (Rolfe and Brown, 1997; Sokoloff, 1960). During neuronal information processing the relative energy need is even higher. Most of this energy consumption is required for synaptic transmission, the importance of which is reflected by a 3-fold increase in the brain-body-ratio and an increase in the number of synaptic connections during the evolution from primates to the today's human (Abeles, 1991; Mink et al., 1981). The increased relative energy consumption of the brain during evolution was facilitated by a reduction in energy expenditure for digestion and locomotion, along with an increased energy uptake from higher quality food (Navarrete et al., 2011). The significant drawback from this trade-off is that the relatively high energy needs of the brain make it accessible for conditions of energy deprivation like anoxia or ischemia as will be described as a central part of this study in the last chapter (Ames et al., 1995).

In general, electrical signaling in the brain is based on the movement of ions across plasma membranes as well as intracellular membranes like in neurons and astrocytes of one of the best studied grey matter regions: the hippocampus (see Figure 1).

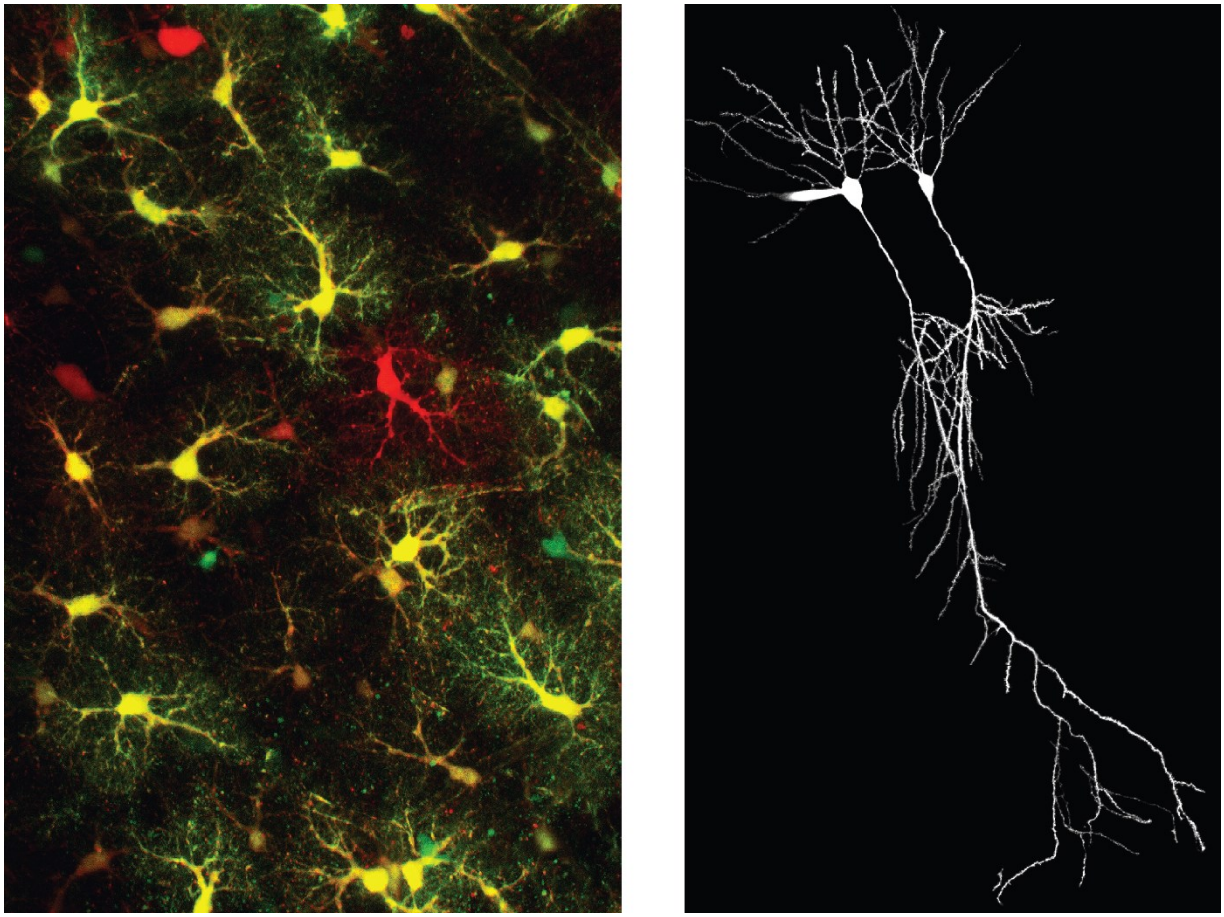

Figure 1: Astrocytes and neurons of the hippocampal CA1 region. Left: astrocytes of the *stratum radiatum* in the CA1 region. Astrocytes are labelled with sulforhodamine 101 (SR101; red) and the green fluorescent protein (GFP; green) under the astrocyte-specific glial fibrillary acidic protein (GFAP) promoter. Double-labelling appears yellow. Notice the astrocytic perivascular endfeet enwrapping some blood vessels (Image was provided from Schreiner and Rose, Institute for Neurobiology, Heinrich-Heine University Düsseldorf). Right: two CA1 pyramidal neurons individually loaded through a patch pipette with the sodium-sensitive fluorescent dye sodium-binding benzofuran isophthalate (SBFI).

The main consumption of energy in almost all brain cells is the restoration and maintenance of those ion gradients, even under resting conditions (accounting for ~15-20% of the energy used in the whole rat cerebral cortex, see Figure 2 (Attwell and Laughlin, 2001; Howarth et al., 2012)). However, an even higher energy consumption is required for the gradient restoration after the generation of action potentials, excitatory postsynaptic potentials, and neurotransmitter release, uptake, and recycling (accounting for ~60% of the energy used in the whole rat cerebral cortex (Ames et al., 1995; Attwell and Laughlin, 2001; Erecinska and Silver, 1994; Howarth et al., 2012)).

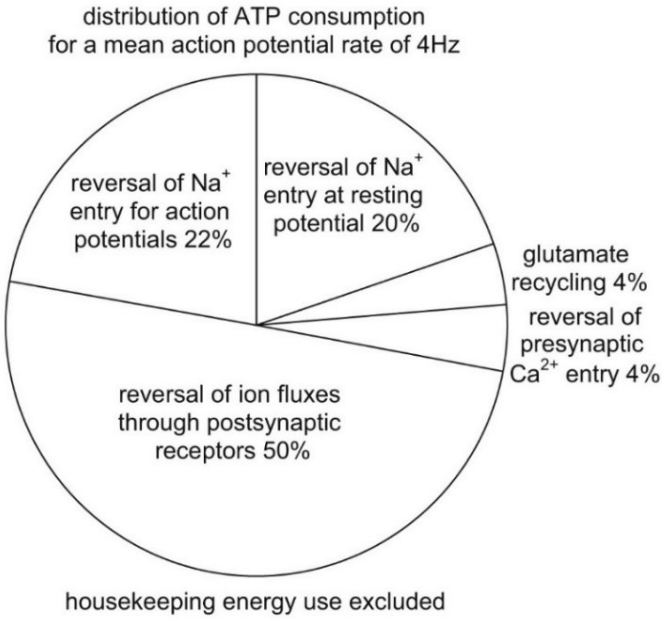

Figure 2: Energy budget for the whole rat cerebral cortex during an action potential rate of 4 Hz. Most of the energy is used to restore ion concentrations to their resting values. Housekeeping tasks are excluded. Reprinted with permission from Elsevier (open access): (Harris et al., 2012).

ATP-consuming ion pumps maintain ion gradients, especially that of sodium, which forms the basis for electrical signaling in both neurons and astrocytes. In addition, there is a significant portion of energy consumed for non-signaling processes, also called “housekeeping” processes, which can take around 25 % (depending on the cell type) of the total energy use in grey matter and up to 40 % for the whole brain. This includes protein and lipid synthesis, proton leak across mitochondrial membranes, and cytoskeleton rearrangements (Engl and Attwell, 2015).

Nevertheless, by far the largest part of the brains’ energy supply is utilized for the maintenance of ion homeostasis, which is not a static state but rather a highly dynamic process (Rose and Karus, 2013). The mechanisms that make the upkeep so energy expensive, and the reasons behind why these ion gradients (in particular ***sodium***) are so necessary to maintain across plasma membranes – even under resting conditions – will be discussed and emphasized in this study.

The Sodium/Potassium ATPase

Under resting conditions, ions are distributed unequally and thus possess electrical and chemical gradients across membranes that are the basis of almost every process in the cells. The maintenance of ion gradients is a highly dynamic process rather than just existing in a static state inside and outside cells. While sodium, chloride and calcium have high concentrations in the extracellular space surrounding the cells, potassium and organic anions have a low concentration. The extracellular ion concentrations are mirrored inside the cells: with low concentrations of sodium, chloride and calcium but high concentrations of potassium and organic anions. The extracellular sodium concentration of about 140-150 mM is 10-fold higher than inside neurons and astrocytes, being about 12-15 mM (see Figure 3 (Kelly and Rose, 2010; Langer and Rose, 2009; Mondragao et al., 2016)).

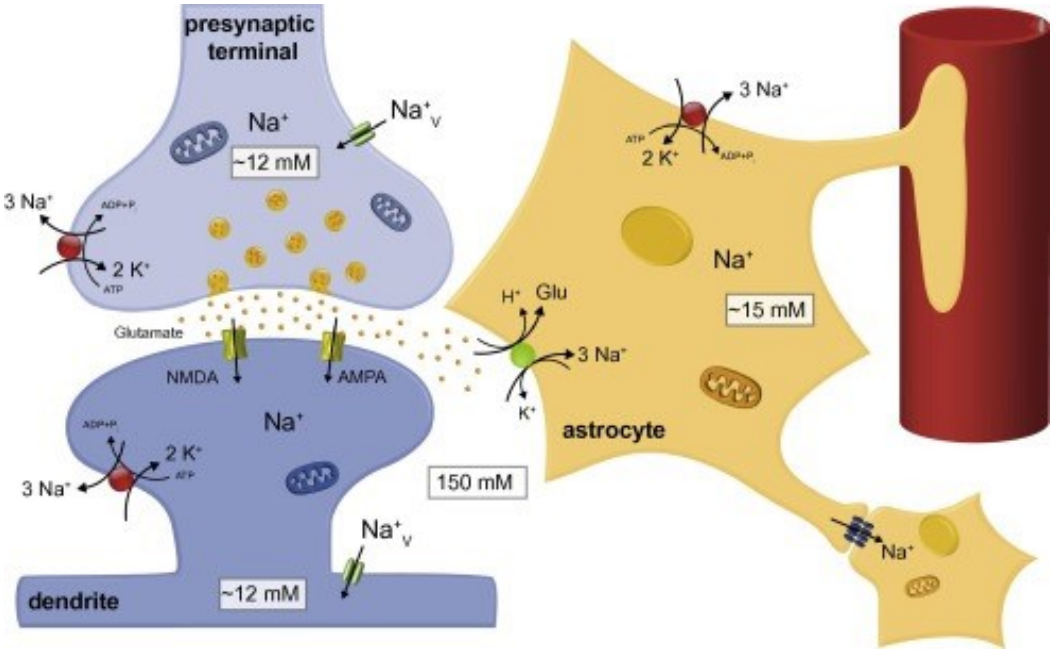

Figure 3: Sodium pathways at glutamatergic synapses. At the pre- and postsynaptic terminals sodium can enter through voltage-gated sodium channels upon action-potential firing. In addition, ionotropic glutamate receptors mediate sodium influx into spines and dendrites. Sodium influx in astrocytes is mainly realized by glutamate transporters. The resting sodium baseline is restored through the activity of the NKA. Sodium ions spread in the astrocytic syncytium via gap junction coupling. Reprinted with permission from Elsevier: (Rose and Chatton, 2016).

The resulting steep inwardly directed sodium gradient is maintained by the action of the NKA pumping three sodium ions out of the cell while importing two potassium

ions. During one pumping cycle, one ATP molecule is hydrolyzed to ADP and free phosphate. This process is electrogenic, providing a net positive charge to the extracellular space and thus the NKA contributes to the negative membrane potential of the cells. The NKA is the only effective mechanism for sodium extrusion under physiological conditions (Mondragao et al., 2016; Rose and Ransom, 1996). The equilibrium potential can be calculated with the Nernst equation, taking into account the aforementioned concentration differences. Consequently, as a result, the equilibrium potential for potassium is around -90 mV and for sodium around +58 mV. Together with a selective permeable membrane leading to potassium leak currents among other ions, this results in a negative membrane potential of about -70 mV in neurons and -80 to -90 mV in astrocytes of the grey matter, which is close to the potassium equilibrium potential. Together with the given intracellular sodium concentrations mentioned above, the inwardly directed driving force for sodium ions is around 120 mV in neurons and around 140 mV in astrocytes. Under physiological conditions, net diffusion of sodium is therefore always inwardly directed, whereas outward movement of sodium ions requires additional energy (Rose and Karus, 2013). Importantly, the ubiquitously expressed NKA is constantly working under resting conditions in both neurons and astrocytes: by blocking its action with specific antagonists (mostly alkaloids like ouabain) the intracellular sodium concentration rapidly increases (Chatton et al., 2000; Kelly and Rose, 2010; Rose and Ransom, 1996). In addition, removal of extracellular potassium, which is also necessary for the proper function of the ion pump, has the same effect on astrocytes (Rose and Ransom, 1996; Walz and Hertz, 1984).

The NKA consists of two main regulatory subunits, α and β subunit (see Figure 4 (Sweadner, 1989; 1992)). The α subunit is the binding site for sodium, potassium, and ATP as well as for the selective inhibitor ouabain (Sweadner, 1992). The β subunit is responsible for full enzymatic activity of the α subunit and is regulated through extracellular phosphorylation sides. Astrocytes predominantly express the α2 and β2 subunit, whereas neurons mainly express the α3 subunit (Blanco and Mercer, 1998). Both cell types express the α1 and β1 subunit (Cameron et al., 1994; Cholet et al., 2002). The difference in the expression profiles between neurons and astrocytes completely change the affinities of the NKA in each particular location. While the α1 and neuronal α3 subunits are already saturated during resting potassium concentrations, the lower affinity astrocytic α2 subunit remains sensitive to raises in extracellular

potassium when in combination with the β1 and to an even greater extent with β2 (as this has a still lower affinity (Rose and Ransom, 1996; 1997)).

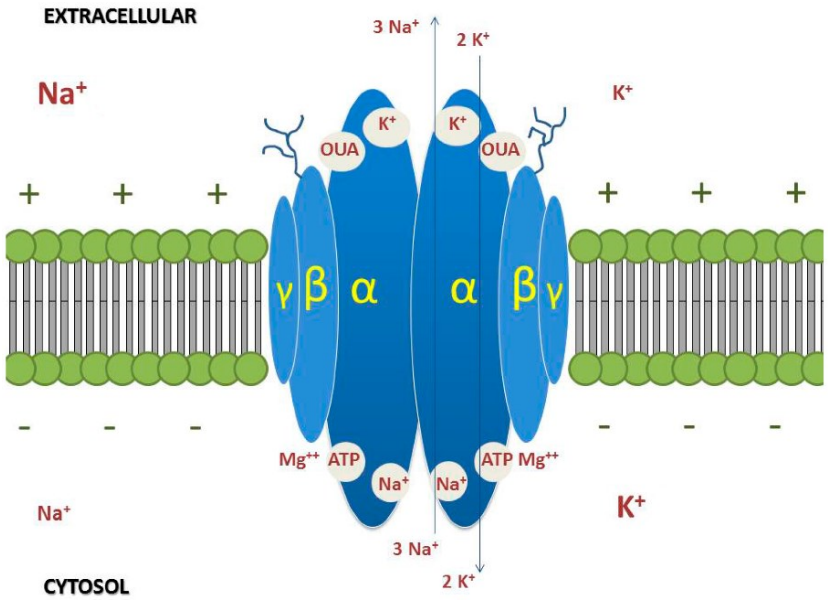

Figure 4: Scheme of the NKA inserted into the plasma membrane. Transport of sodium and potassium is accomplished by ATP hydrolysis and is dependent on the ion distribution on each side of the membrane. Reprinted with permission from MDPI (open access): (Goncalves-de-Albuquerque et al., 2017).

The most prominent NKA subunit combination of α2β2 is mostly expressed on astrocyte processes surrounding synaptic spines, allowing them to respond efficiently to stimulus-evoked potassium increases in the extracellular space (Cholet et al., 2002). This suggests that astrocytes sense neuronal activity and take up potassium after/during synaptic transmission, a concept which is discussed below (Kofuji and Newman, 2009). In addition, subunit constellations like α2β1 in astrocytes and α1β1 in neurons can sense changes in intracellular sodium as they are not fully saturated under the resting sodium concentration. They are therefore activated and can respond to intracellular physiological sodium changes (Larsen et al., 2016). As mentioned above, the maintenance of the resting membrane potential even under resting conditions consumes around 15% of the total energy in the whole rat cortex (see Figure 2 (Attwell and Laughlin, 2001)) and even up to 20% in cultured hippocampal astrocytes (Silver and Erecinska, 1997).

There are many computational and mathematical models which attempt to estimate the exact number of consumed ATP molecules both at rest and in action. Many

of them are based on the original work of Attwell and Laughlin (2001) which was recently expanded to and updated by Howarth et al. (2012). Compared to the 15% energy consumption under rest, 16 of the ~60% required for synaptic processes is used for action potentials, emphasizing the importance and the need for a high ATP turnover rate even under resting conditions (Harris et al., 2012; Howarth et al., 2012). From the known membrane potentials and input resistances for each cell type, it can be calculated that the energy for maintaining the resting potential is higher in neurons than in astrocytes (3.42 x 10^8 vs 1.02 x 10^8 ATP/cell/s; see Figure 5 (Attwell and Laughlin, 2001)). For this and many other models, about 75% of the total energy is consumed by the NKA to retain the sodium and potassium gradients and to maintain resting membrane potential.

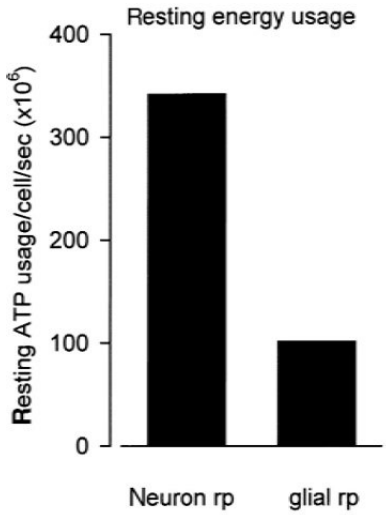

Figure 5: Energy consumption under resting conditions in neurons and astrocytes of the grey matter. The resting energy use in neurons is higher compared to astrocytes. Reprinted with permission from Elsevier (open access): (Harris et al., 2012).

Sodium influx during synaptic transmission

In addition to its activity under resting conditions, the NKA counteracts the sodium and potassium changes during electrical signaling. During action potential firing, excitatory potentials and astrocytic glutamate uptake, sodium is highly increased in both neurons and astrocytes (Fleidervish et al., 2010; Langer et al., 2017; Langer and Rose, 2009; Langer et al., 2012; Ona-Jodar et al., 2017; Rose and Ransom, 1996). As a consequence of the activity-induced increases in intracellular sodium concentrations,

the NKA has to work harder in order to preserve baseline conditions, thereby expending even more energy than during resting states. Physiological glutamate changes can already increase the NKA activity by two to three fold (Deitmer and Rose, 2010). Therefore, this chapter will guide through the main sodium influx pathways and therefore energy consuming processes during synaptic activity starting from the axon, across spines and dendrites and finally to astrocytes.

The action potential generation and propagation is accomplished by the opening of TTX-sensitive voltage-gated sodium channels that are expressed in several different types of neuronal axons (Jaffe et al., 1992; Ona-Jodar et al., 2017; Rose and Konnerth, 2001). The concentration differences and the resulting steep inwardly directed electrochemical gradient for sodium ions results in a strong movement of sodium ions into the cells upon opening of voltage-gated sodium channels (see Figure 6 (Fleidervish et al., 2010; Kole et al., 2008; Lasser-Ross and Ross, 1992)). This was shown, for example, in cultured rat hippocampal neurons (Rose and Ransom, 1997), in cortical pyramidal neurons in acute brain slices (Fleidervish et al., 2010), in hippocampal CA1 neurons also in brain slices (Lamy and Chatton, 2011; Rose, 2002; Rose et al., 1999), and in this study for the first time also in mitral and granule cells of the olfactory bulb of rats (see Figure 6 (Ona-Jodar et al., 2017)). Activity-induced sodium transients can also arise in dendrites, in response to the opening of voltage-gated sodium channels through back-propagating action potentials, as has been shown in hippocampal, cortical, and olfactory bulb neurons as in this study (see Figure 6 (Jaffe et al., 1992; Ona-Jodar et al., 2017; Rose et al., 1999)). The increased intracellular sodium concentration is restored by the NKA, a processes which constitutes about 16% of the total energy expenditure within the whole cortex (Attwell and Laughlin, 2001; Howarth et al., 2012).

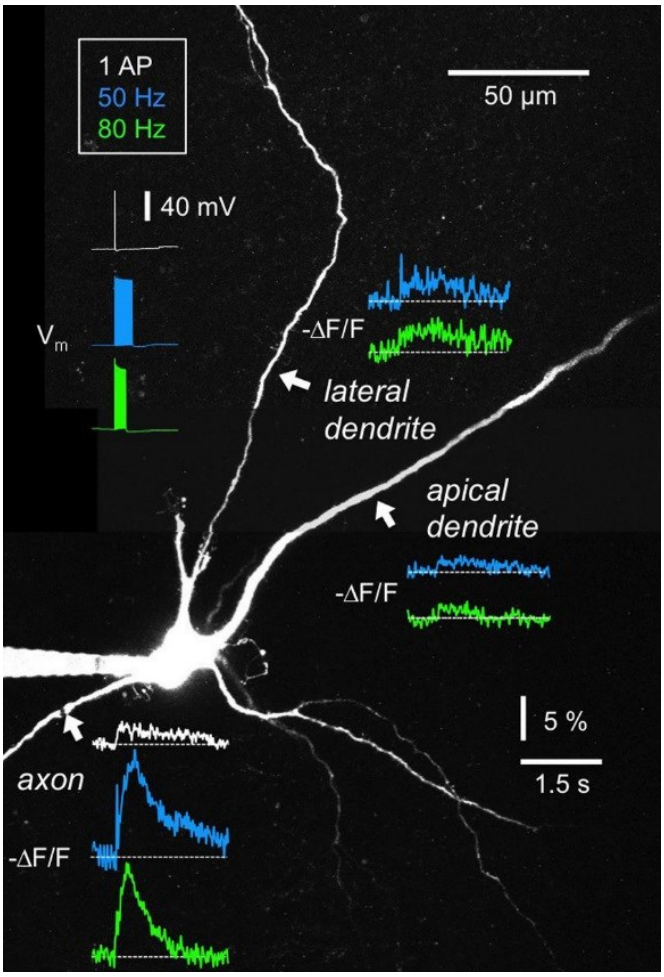

Figure 6: Sodium signals in mitral cell axons and dendrites. Two-photon image of a mitral cell filled with SBFI. Upper left: Voltage traces for three different somatic stimulation protocols. Sodium responses in the axon and dendrites to the three stimulation protocols. Reprinted with permission from Front. Cell. Neurosci. (open access): (Ona-Jodar et al., 2017).

Around 85% of all neurons in the brains grey matter are excitatory (Abeles, 1991), therefore, mainly glutamatergic signaling will be discussed in the following sections (see Figure 3). Most of the energy required for action potentials is used for the presynaptic events including generation and propagation/self-regeneration of the action potentials themselves (Attwell and Laughlin, 2001; Harris et al., 2012). In addition, presynaptic voltage-gated calcium channels open, resulting in calcium influx into the presynaptic terminal and triggering the induction of neurotransmitter release. To restore the intracellular calcium concentration, the plasma membrane bound calcium ATPase (PMCA) is activated and by hydrolyzing ATP, pumps calcium back out across the plasma membranes. In addition, the sodium/calcium exchanger (NCX) exchanges one calcium ion for three sodium ions (Blaustein and Golovina, 2001). The energy used by the PMCA accounts for a small part of the total energy (about 5% (Attwell and Laughlin,

2001)). Even less energy is consumed for the exocytosis of vesicles (<1%), where at least 400 different molecules and proteins are involved (Marsh and McMahon, 1999). The vesicles are filled with neurotransmitters (e.g. glutamate), which are released into the synaptic cleft during synaptic activity.

Once glutamate is released into the synaptic cleft, it binds postsynaptically to ionotropic glutamate receptors: non-NMDA receptors (AMPA and Kainate) and to NMDA receptors. Although AMPA and Kainate receptors have a low channel conductance (~10 pS) but also short channel opening times(~1 ms) (Spruston et al., 1995a)) they are the first to open during signaling, and therefore they constitute the fast component of synaptic transmission. As mentioned above, the steep inwardly directed driving force for sodium ions (120-140 mV) results in a strong influx into the postsynaptic terminal and therefore a depolarization of the plasma membrane (Callaway and Ross, 1997; Linden et al., 1993). In consequence of this membrane depolarization, the magnesium block of NMDA receptors is removed and the slower but more powerful component of synaptic transmission is activated (higher channel conductance (~50 pS) and much longer opening times (~50 ms) (Malenka, 1994; Spruston et al., 1995a; Spruston et al., 1995b)). The resulting excitatory postsynaptic potential and the increased intracellular sodium concentration is afterwards restored by the NKA (Rose and Konnerth, 2001). In addition, because of the calcium permeability of NMDA receptors and partially of AMPA receptors (depending on subunit composition) for calcium ions, the increased intracellular calcium concentration has to be restored by the PMCA and NCX (Koester and Sakmann, 1998; Yuste and Denk, 1995). Calcium influx through NMDA receptors and somatic calcium channels is responsible for the induction of synaptic plasticity in terms of long-term potentiation and depression (LTP and LTD (Nicholls et al., 2012)). Furthermore, glutamate also binds to metabotropic glutamate receptors expressed on the post synapse and on astrocytes, which can, for example, activate phospholipase C to generate inositol-3-phosphate (IP_3), resulting in further release of calcium from internal stores, termed calcium induced calcium release (CICR) (Clapham, 2007; Rakers and Petzold, 2017). The restoration of ions involved in metabotropic glutamate signaling is not that expensive process compare to the total energy consumption and is assumed to require less than 10% of the total energy spent for postsynaptic signaling (Attwell and Laughlin, 2001).

Calcium, as one of the best described second messengers in the brain, is involved in several processes such as the regulation of enzymes and transcription

factors but also gliotransmission and blood flow as it will be described in the next chapter. Therefore, it is critical that calcium is tightly regulated through energy consuming transporters as well as through the NCX and therefore it plays a central part in this study. The intracellular calcium concentration is roughly 150 nM, whereas the extracellular space contains around 2 mM and the endoplasmatic reticulum, as the major calcium store around 0.6-0.8 mM (see Figure 7; for review see: (Clapham, 2007)).

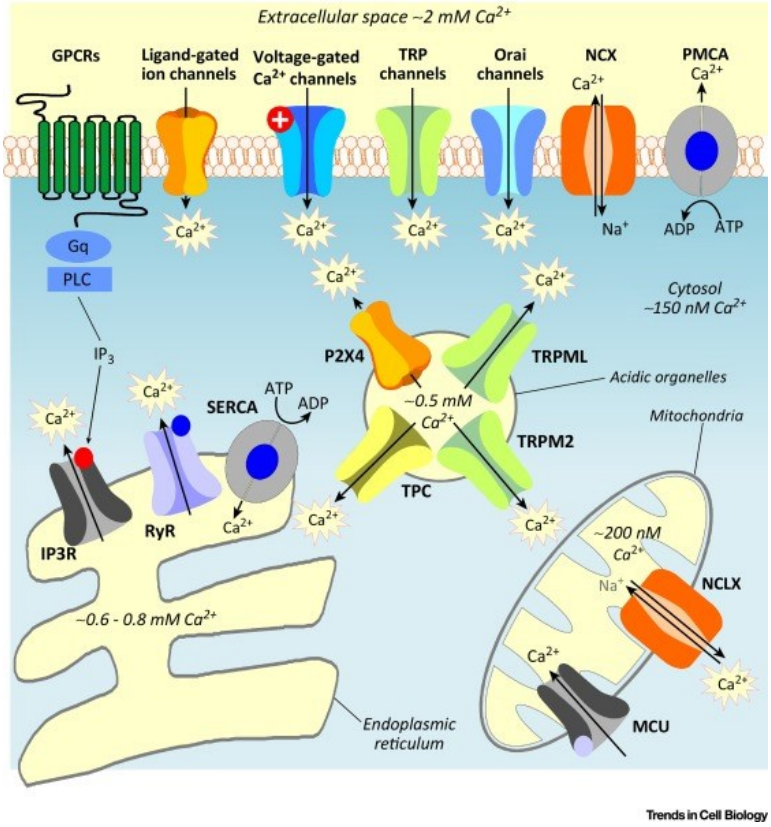

Figure 7: Calcium signaling pathways in astrocytes. Calcium influx and efflux is mediated by a plethora of channels, receptors and transporters that are bound either to the plasma membrane, membranes of ER, acidic organelles or mitochondria. See text for further abbreviations and explanation (GPCRs – G-protein coupled calcium receptors; TRP channels – transient receptor potential channels; Orai channels – calcium release-activated calcium channel; IP3R – inositol triphosphate receptor; RyR – ryanodine receptor; P2X4 – purinergic receptor; TPC – two pore channel; MCU – mitochondrial calcium channel). Reprinted with permission from Elsevier: (Shigetomi et al., 2016).

The low intracellular calcium concentration is maintained by ATP-consuming ion pumps such as the PMCA and, as already mentioned, by exchangers like the NCX and its potassium-dependent variant NCKX. Both exchangers rely on the sodium gradient as the driving force. Furthermore, calcium is stored in the ER and the mitochondria, where its concentration is maintained by the sarcoendoplasmic calcium ATPase (SERCA) and the NCLX respectively (see Figure 7).

However, it is sodium influx into neurons during spike activity and excitatory postsynaptic potentials as the most energy-expensive process in the brain and responsible for AP generation and propagation (Erecinska and Silver, 1994; Harris et al., 2012). In contrast, astrocytes do not experience fast sodium-based electrical signaling and maintenance of glial membrane potential requires only a minor part of the total costs of cortical computation in human cortex (Lennie, 2003).

Sodium-dependent glutamate uptake

In addition to neurons and their pre- and postsynaptic compartments, astrocytes - as already mentioned - play a crucial role in network circuitry summarized in the concept of the tripartite synapse first introduced by Araque and collaborators 1999 (Araque et al., 1999). Most of the synapses in grey matter are partially or fully enwrapped by astrocytic processes depending on the brain region (60-99% (Witcher et al., 2007)). Hence, astrocytes are well-positioned to detect and integrate neuronal activity with their perisynaptic processes and to respond by various mechanisms ranging from homeostatic control over morphological rearrangement to active feedback (Haber et al., 2006; Henneberger et al., 2010; Perez-Alvarez et al., 2014). This range of responses includes removal of glutamate as well as the uptake and redistribution (via gap junctions) of potassium that is released by neurons during neuronal activity (Kimelberg, 2010). These mechanisms are critical to prevent hyperexcitability and to protect against excitotoxic damage to the synapse. One of the most important players in this neuron-glia interaction are glutamate transporters that remove glutamate from the synaptic cleft by co-transporting sodium and protons while counter-transporting potassium (see Figure 8; for review see: (Danbolt, 2001; Rose et al., 2018)).

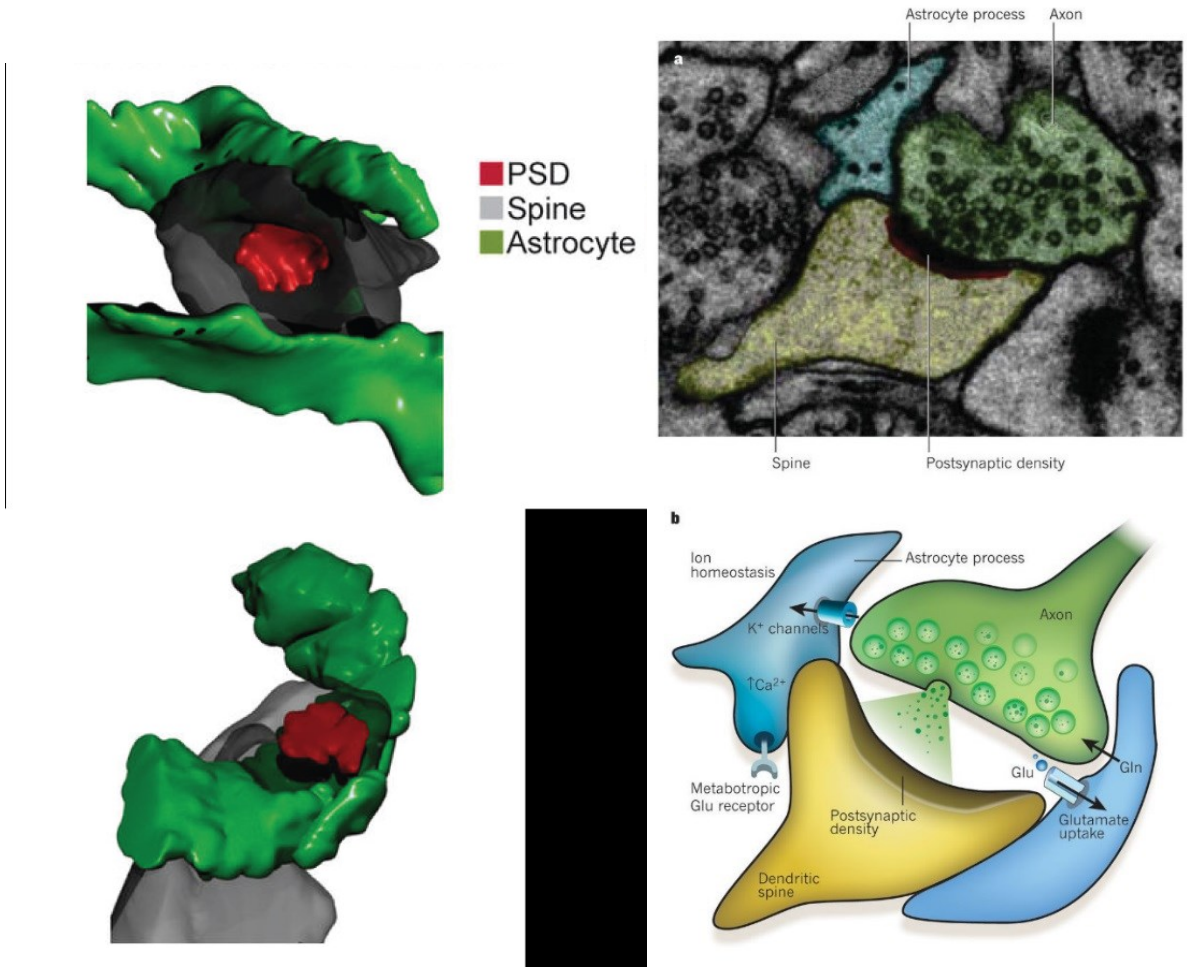

Figure 8: Tripartite Synapse. Astrocytic endfeet enwrap the pre and post synapses of neurons. Potassium clearance from the synaptic cleft can occur through direct uptake and then through spatial buffering across the astrocytic syncytium via gap junctions. In addition, astrocytes take up glutamate and sodium to prevent excitotoxicity. The glutamate is converted to glutamine and transferred back to neurons. Reprinted with permission from Springer Nature: (Eroglu and Barres, 2010; Pannasch et al., 2014).

Astrocytes express two types of glutamate transporters: GLAST (glutamate-aspartate transporter; EEAT1 human homolog) and GLT-1 (glutamate transporter 1; EEAT2) (see Figure 10 (Danbolt, 2001; Karus et al., 2017; Rose et al., 2018; Schreiner et al., 2014)). The relative expression levels of these transporters changes during development: GLAST is mainly expressed in neonatal brain, while GLT-1 is upregulated in older brains (Furuta et al., 1997; Karus et al., 2017; Schreiner et al., 2014). Therefore, during development, sodium increases mediated by glutamate uptake is due to GLAST in neonatal brains and due to GLT-1 in older brains as it was shown in this study (Karus et al., 2017). The uptake of glutamate is not only relevant to protect against excitotoxicity but is also important in coupling neuronal activity to astrocytic metabolism as described below (neuro-metabolic coupling; reviewed in: e.g. (Rose and Chatton, 2016)). The co-

transport of sodium into the astrocytes results in strong and detectable sodium transients in astrocytic somata (Langer and Rose, 2009; Unichenko et al., 2013), and into their perisynaptic and perivascular endfeet, which was shown the first time in this study (see Figure 9 (Langer et al., 2017; Langer et al., 2012)).

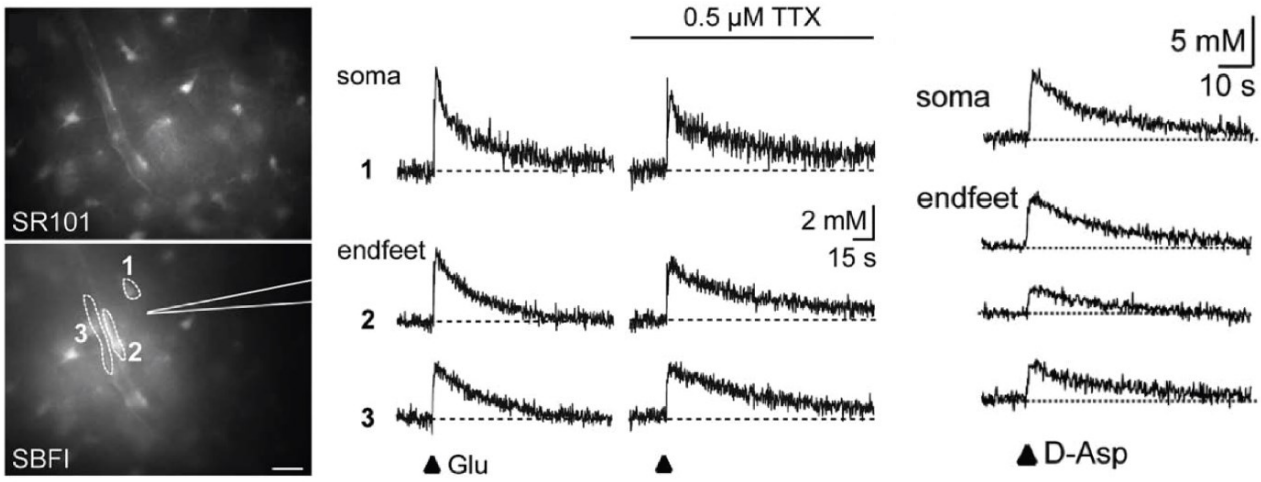

Figure 9: Astrocytic sodium signals. Left: image of SR101 and SBFI fluorescence of astrocytes and their perivascular endfeet enwrapping a blood vessel. Middle and right: induction of sodium signals through application of glutamate (Glu) in the absence and presence of tetrodotoxin (TTX), a specific antagonist for voltage-gated sodium channels, or D-aspartate (D-Asp) a glutamate transporter agonist. Reprinted with permission from John Wiley and Sons: (Langer et al., 2017).

The increased sodium concentration is again restored by the NKA under the consumption of ATP. There is evidence for a close spatial association and interaction between the NKA and GLAST/GLT-1 indicating that the NKA can handle fast sodium loads caused by the glutamate uptake (Cholet et al., 2002). Another possibility for astrocytes to manage high sodium increases is the distribution within the astrocytic syncytium via gap junctions (Langer et al., 2012; Moshrefi-Ravasdjani et al., 2017), as it was also described for potassium in the concept of “spatial buffering” (Walz, 2000). As already mentioned, the extracellular potassium increase during neuronal activity and the affinity of the α2 subunit of the NKA expressed by astrocytes also stimulates the NKA and additionally contributes to neuro-metabolic coupling. In addition, glutamate transporters not only take up glutamate and sodium but also protons which results in an acidification of the intracellular milieu during synaptic activity (Azarias et al., 2011; Rose and Ransom, 1996). Furthermore, neurons also express glutamate transporters

(EAAT3 and 4 (Kanai and Hediger, 1992; Rose et al., 2018)), however the majority of glutamate uptake is mediated by astrocytes.

Once the glutamate is taken up by the astrocytes through glutamate transporters, it is converted to the non-reactive form glutamine by the glial-specific enzyme glutamine synthetase (GS; (Martinez-Hernandez et al., 1977)). The glutamine is then transported out of the astrocytes through the sodium-dependent transporter SN1 and taken up by neurons through system A transporters (illustrated in Figure 15 (Broer and Brookes, 2001; Chaudhry et al., 1999)). In neurons, glutamine is the basis for the generation of glutamate to replenish stores, or in GABAergic synapses to GABA (Bak et al., 2006; Hertz et al., 1999; Schousboe et al., 2004). This route, also called glutamate-glutamine-cycle, is another key element of neuron-glia interaction.

Sodium-dependent secondary active transport mechanisms

In addition to glutamate uptake, and NKA activity, there are several other ways for sodium ions to pass the plasma membrane of astrocytes (as well as neurons). Like neurons, astrocytes also express several voltage- and ligand-gated sodium channels that contribute to the sodium loading. Depending on the brain region, astrocytes express TTX-sensitive and TTX-resistant voltage-gated sodium channels (Sontheimer, 1992; Sontheimer and Waxman, 1992), along with ligand-gated sodium channels like AMPA- and NMDA-receptors, P2X purinoceptors and several acetylcholine-sensitive channels (nAChRs) (see Figure 10 (Verkhratsky, 2010)). Furthermore, sodium influx into astrocytes is mediated by several sodium-permeable channels such as transient receptor potential channels (TRP (Nilius and Owsianik, 2011)), acid-sensitive ion channels (ASICs (Krishtal, 2015)) or epithelial sodium channel/degenerines, which was characterized for the first time as a sodium-permeable channel in this study (ENaCs (Fyfe et al., 1998)). All of these channels seem to be expressed in astrocytes, although their contribution to astroglial function remains largely unexplored.

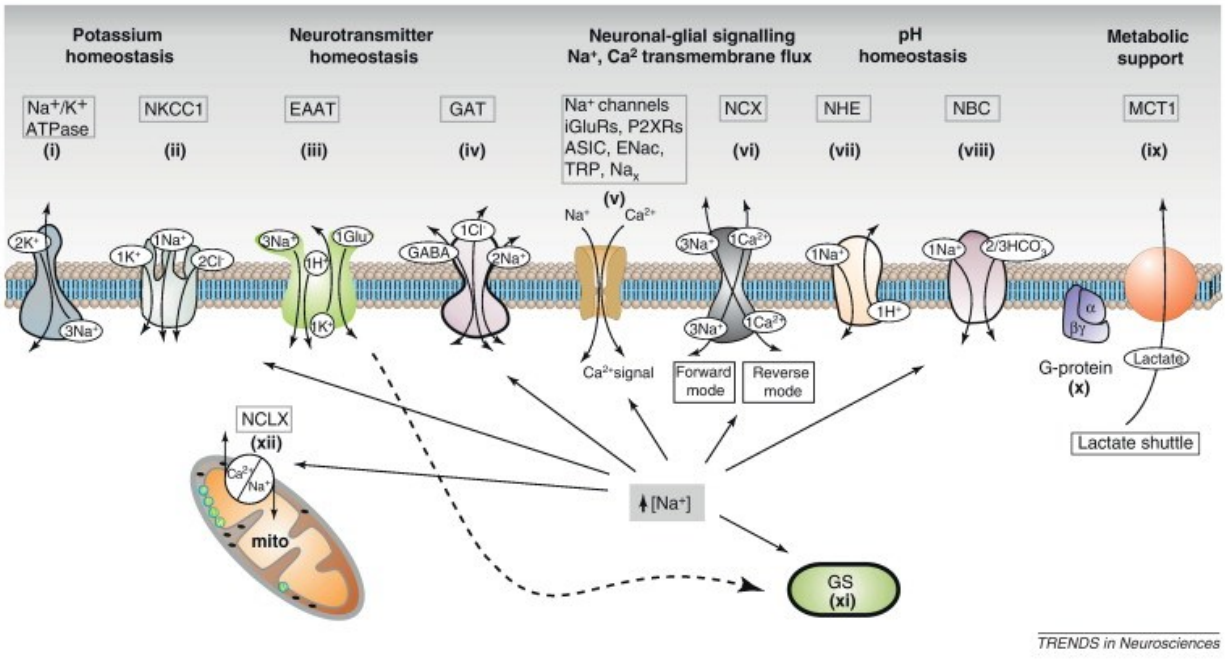

Figure 10: Sodium-related ion homeostasis. Sodium can enter the cell through several channels, transporters and exchangers. There is only one effective extrusion mechanism: the NKA. See text for further abbreviations and explanation (GAT – GABA transporter). Reprinted with permission from Elsevier: (Kirischuk et al., 2012).

However, the steep inwardly directed electrochemical sodium gradient that is maintained – at rest as well as in action – by the NKA, provides the energy for sodium-dependent secondary active transport mechanisms that have been described in much greater detail than the above mentioned channels and receptors concerning sodium fluxes into astrocytes and neurons (Rose and Chatton, 2016; Rose and Karus, 2013). Research has shown that sodium homeostasis and fluctuations are tightly connected to various processes such as calcium signaling, pH transients and the course of synaptic transmission as described above.

One possibility for sodium to enter astrocytes is via the sodium-potassium-dichloride-cotransporter (NKCC1 (Kelly et al., 2009; Untiet et al., 2017; Walz, 2000)) that is also responsible for setting the relatively high chloride concentration in astrocytes of neonatal brains and additionally participates in volume regulation (Walz, 2002). Under resting conditions this transporter appears to work in the importing mode (forward), since its pharmacological inhibition with bumetanide results in a drop of the intracellular sodium concentration in cerebellar Bergmann glia cells as it was shown in this study, and in astrocytes of the hippocampus, indicating a constant sodium influx (see Figure 11 (Kelly et al., 2009; Untiet et al., 2017)).

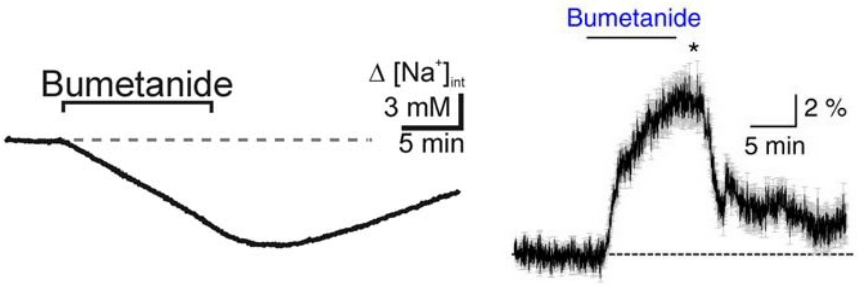

Figure 11: Outward- and inward-directed sodium transport through NKCC1. Inhibition of the NKCC1 with the antagonist bumetanide either results in a drop in the intracellular sodium concentration as for Bergmann glia cells (Untiet et al., 2017) or for an increase in the intracellular sodium concentration in choroid plexus (Steffensen et al., 2018). Reprinted with permission from John Wiley and Sons and Springer Nature (open access): (Untiet et al., 2017); (Steffensen et al., 2018)

The NKCC1 is involved in the uptake of potassium from the extracellular space and plays an important role in astrocytic swelling that mediates an increase in intracellular sodium (Kahle et al., 2009; Kelly et al., 2009; Larsen et al., 2014). In other tissue, like the choroid plexus, where the ion distribution is completely different compared to cerebellum or hippocampus, the NKCC1 is mediating a sodium efflux under resting conditions as it was shown in this study (see Figure 11; Steffensen et al., 2018).

Sodium influx is also tightly coupled to intracellular pH regulation. One of the known transporters is the sodium-bicarbonate-cotransporter (NBC) that imports two sodium and three bicarbonate ions into most cells (Deitmer and Rose, 1996). The NBC functions and reverses close to the resting membrane potential, since its reversal potential is around -70 to -80 mV. Thus, the NBC can work either as a sodium importer or exporter. The NBC is the major mechanism for recovery from an alkalosis in astrocytes (Theparambil et al., 2015). Another transporter for pH regulation (and of course sodium regulation) is the sodium-proton exchanger (NHE) which imports sodium and exports protons (Chesler, 2003). The activation of the NHE is the major mechanism for recovery from acidosis in astrocytes after metabolic inhibition (Bondarenko et al., 2005).

Besides potassium, chloride, pH and volume regulation, there is a very important ion that is also regulated together with and by the sodium gradient: calcium. Sodium and calcium are directly connected through the NCX which is, as already described above (Blaustein and Golovina, 2001; Blaustein and Lederer, 1999; Khananshvili, 2014), expressed in astrocytes as well as in neurons (Blaustein and Lederer, 1999).

This transporter is regarded as a “low-affinity, high-capacity” calcium exporter under physiological conditions (Carafoli and Longoni, 1987), but can reverse upon an increased intracellular sodium concentration and function as a calcium importer, a function which is addressed in this study and will be discussed in much more detail below (Annunziato et al., 2013; Boscia et al., 2016; Gerkau et al., 2017a; Kirischuk et al., 1997; Rojas et al., 2007).

To summarise this first section, maintenance of ion gradients, especially of sodium ions, are necessary for processes related to electrical signaling in neurons and astrocytes. Besides action potential generation and propagation, glutamate uptake and secondary active transport mechanisms are also realized by the flow of sodium ions and the energy provided by this movement. The most important regulatory unit involved is the NKA, consuming most of the energy in order to realize all of the above mentioned processes. The export of sodium and the energy needed for this process couples neurons to astrocytes as well as sodium to metabolism. The last point will be discussed in more detail in the next chapter.

2. Sodium influx and cellular energy production

The enormous energy needed by the ATP-hydrolysing ions pumps such as the NKA and related ATP-consuming processes must be met by cellular ATP breakdown. The main energy substrate of the brain is glucose which is delivered through the blood vessels and is first taken up by endothelial cells (see Figure 12). Afterwards, the facilitative glucose transporter 1 (GLUT1) which is expressed at perivascular endfeet, transport the glucose out of the endothelial cells into astrocytes (Kacem et al., 1998; Mergenthaler et al., 2013). Perivascular endfeet of astrocytes constitute almost a complete coverage of blood vessels (Mathiisen et al., 2010; McCaslin et al., 2011), and therefore they contribute to the generation (during brain development) and maintenance (during adulthood) of the blood-brain-barrier (BBB (Abbott et al., 2006; Alvarez et al., 2013)).

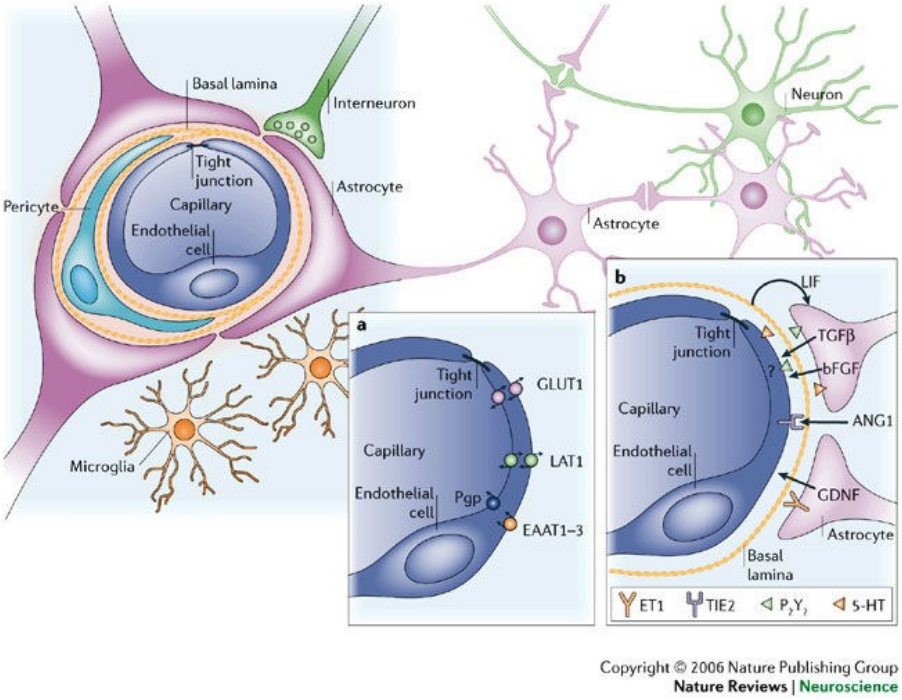

Figure 12: Schematic illustration of perivascular endfeet. Astrocyte endfeet almost completely cover blood vessels and take up substrates from the blood. See text for abbreviations and further explanation (LAT1 – large neutral amino acid transporter; Pgp – permeability glycoprotein; LIF – leukemia inhibitory factor; 5-HT – 5-hydroxytryptamine; ANG1 – angiopoetin 1; bFGF – basic fibroblast growth factor; Et1 – endothelin 1; GDNF – glial cell line-derived neurotrophic factor; TGFβ – transforming growth factor β; TIE2 – endothelium-specific receptor tyrosine kinase 2). Modified and reprinted with permission from Springer Nature: (Abbott et al., 2006).

Although GLUT1 is not fully active under resting conditions it still takes up sufficient amounts of glucose to cover the energy demands of the cells. During neuronal activity, local glucose uptake from the blood is increased (Chuquet et al., 2010; Langer et al., 2017; Magistretti, 2006). This is achieved by incorporating additional GLUT1 transporters into the membrane but also by stimulation of transporter activity with glutamate or angiotensin II (Leybaert et al., 2007). The glutamatergic stimulation is mainly achieved by calcium signals in astrocytes upon neuronal activity, which lead to the release of vasoactive agents produced from arachidonic acid (AA; see Figure 13). This in turn leads to either vasodilation of blood vessels by prostaglandin E2 (PGE_2) or vasoconstriction by 20-hydroxyeico-sateraenoic acid (20-HETE (Belanger et al., 2011; Koehler et al., 2009; Zonta et al., 2003)). The balance between vasoconstriction and vasodilation is dictated by brain metabolism (Gordon et al., 2008). Neuronal activity activates dilation under low-oxygen conditions and activates constriction under high oxygen-conditions. This suggests that increased cerebral blood flow is driven by glycolytic, and not oxidative metabolism under physiological conditions (Lin et al., 2010). In addition, it was also found that co-signaling of sodium and calcium is required in order to stimulate GLUT1 (Porras et al., 2004). Although neurons express glucose transporter 3 (GLUT3 (Vannucci et al., 1997)), astrocytes are thought to take up most of the glucose delivered through the BBB.

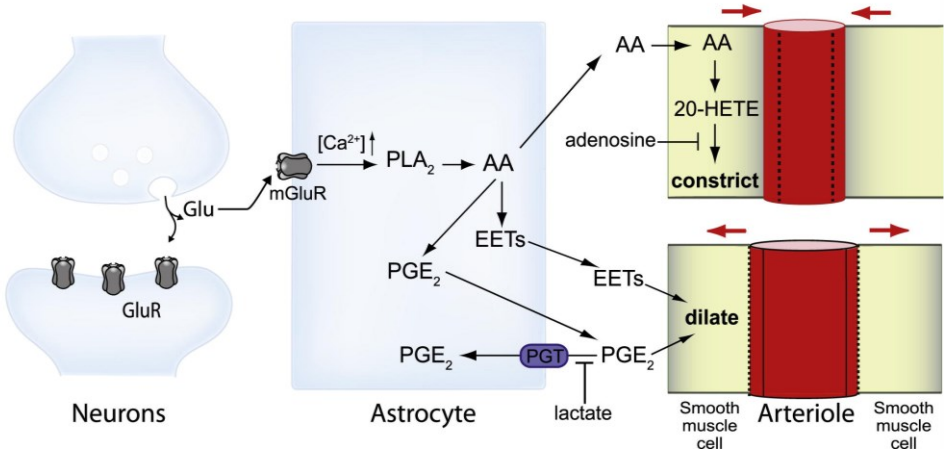

Figure 13: Astrocytes regulate the diameter of blood vessels through glutamate-mediated and metabotropic calcium signaling via the release of vasoactive agents. See text for abbreviations and further explanation (PLA2 – phospholipase A2; PGT – prostaglandin transporter). Reprinted with permission from Elsevier: (Belanger et al., 2011).

Although, glucose is the main energy substrate of the brain, certain circumstances can illicit the utilization of alternative substrates, such as ketones during

development or starvation (Magistretti, 2008; Nehlig, 2004) or lactate during periods of high physical activity (van Hall et al., 2009). In addition, glutamate or acetate can also be used as energy substrates (Zielke et al., 2009). A certain amount of the glutamate taken up by astrocytes is not converted into glutamine, but rather converted to α-ketoglutarate and used as a metabolite of the TCA cycle (Schousboe et al., 2014; Sonnewald, 2014; Sonnewald et al., 1993).

Glycolysis vs. oxidative phosphorylation

Once the glucose is inside the astrocyte (or neuron) it is phosphorylated by hexokinase to form glucose-6-phosphate (G6P), which then can be further metabolized in three different ways (see Figure 14). Firstly, G6P is utilized in the glycolytic pathway, giving rise to two molecules of ATP and two molecules of pyruvate. Secondly, G6P can be converted into ketones through the pentose phosphate pathway (PPP), which serves as an alternative route for neurons to glycolysis and acts to maintain their antioxidant status (Herrero-Mendez et al., 2009; Rodriguez-Rodriguez et al., 2012). Lastly, G6P is utilized through glycogenesis and then can be stored as glycogen in astrocytes to serve as an alternative energy source in times of high energy demands, for example, during neuronal activity (Brown and Ransom, 2007; Gibbs et al., 2006; Hertz et al., 2015). This study shows that elevated extracellular potassium during neuronal activity stimulates glycogenolysis and thereby stimulates the astrocytic NKA (Hertz et al., 2015). Following the first possibility, pyruvate is transported into mitochondria to feed the tricarboxylic acid cycle (TCA) and thereby produces the majority of ATP through oxidative phosphorylation. While glycolysis produces only 2 ATP molecules per glucose molecule, the oxidative phosphorylation produces 30-36 ATP molecules.

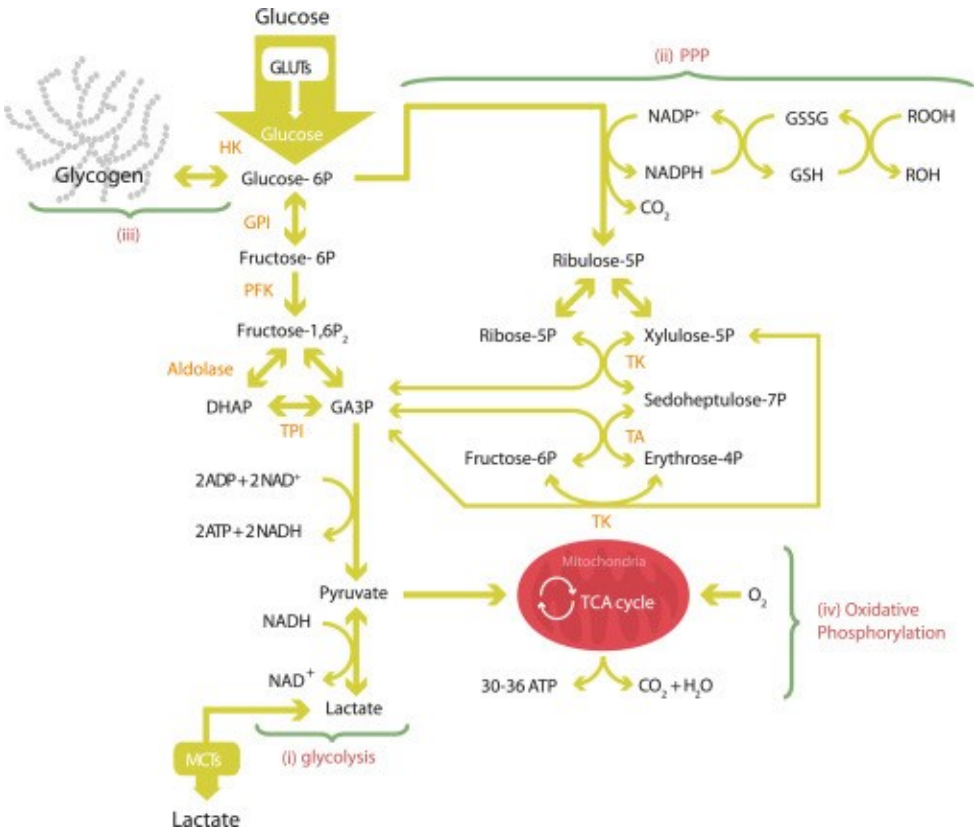

Figure 14: Glucose - as the main energy substrate - is fully metabolized through glycolysis and oxidative phosphorylation. In addition, glucose can be stored as glycogen or fill up ketone bodies through PPP. Lactate is one product of the glucose turnover that serves as a metabolite for neurons and is provided by astrocytes. See text for abbreviations and further explanation (HK – hexokinase; GPI – glucose-6-phosphate isomerase; PFK – phosphofructokinase; TPI – triose phosphate isomerase; TK – transketolase; TA – transaldolase; DHAP – dihyroxyacetone phosphate; GA3P – glyceraldehyde-3-phosphate). Reprinted with permission from Elsevier: (Magistretti and Allaman, 2015).

Glucose vs. Lactate

In addition to the TCA cycle, pyruvate can be converted to lactate by the enzyme lactate dehydrogenase (LDH) which then leaves the astrocytes through monocarboxylate transporters (MCT4) and is in turn taken up by neurons via the MCT2. Once lactate is in the neurons, it can be converted back into pyruvate and fed into the TCA cycle in order to produce ATP (Belanger et al., 2011). While astrocytes express LHD5, which preferentially produces lactate, neurons express LHD1 which rather converts lactate into pyruvate (Bittar et al., 1996). It was shown that lactate production is increased by higher glycolytic activity (Pellerin and Magistretti, 1994), after depolarization of astrocytes by influx of sodium and following the activation of the inward mode of the sodium-dependent NBC, thereby leading to alkalinization (Ruminot et al.,

2011). This concept of the “astrocyte-neuron-lactate shuttle” (ANLS, see Figure 15), first introduced by Pellerin and Magistretti 1994, has received considerable experimental support, but still is under great debate concerning the necessity of lactate as an energy substrate (Dienel and Cruz, 2016; Dienel and McKenna, 2014; Machler et al., 2016; Pellerin and Magistretti, 2012). Nevertheless, it has been shown that glucose uptake into astrocytes is much higher than transport into neurons, but that neurons require much more energy than astrocytes, since they undergo electrical signaling and have to export much more sodium to resting values as described in the previous chapter (Chuquet et al., 2010). Importantly, astrocytes and neurons are able to fully oxidize both glucose and lactate, which perfectly fits to the observation that both cell types have more or less the same number of mitochondria (Lovatt et al., 2007; Zielke et al., 2009).

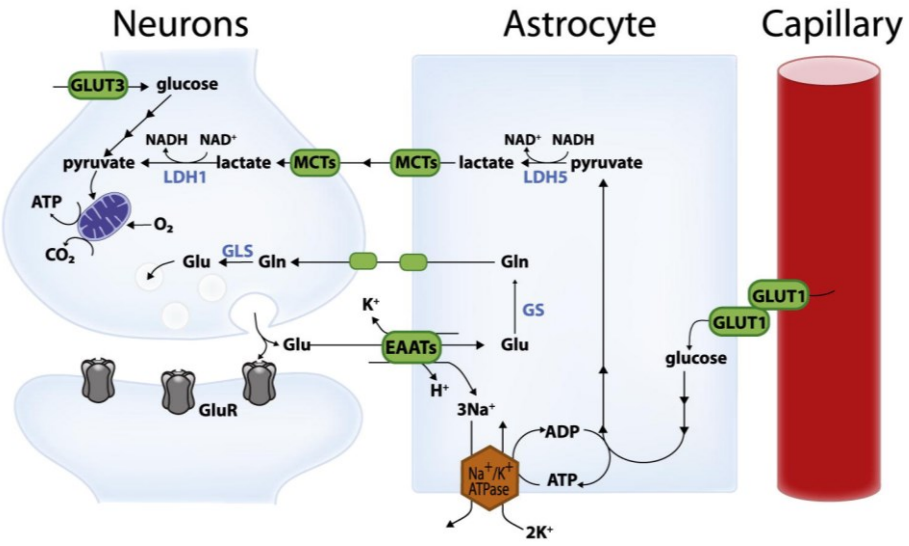

Figure 15: Glucose that is taken up by astrocytes is partially converted to lactate to provide energy back to neurons. The glucose uptake and turnover is stimulated by neuronal activity. (see text for abbreviations and further explanation). Reprinted with permission from Elsevier: (Belanger et al., 2011).

However, neurons and astrocytes prefer different metabolic pathways under physiological conditions (see Figure 16 (Belanger et al., 2011; Lovatt et al., 2007)). Neurons have significantly higher energy needs due to strong sodium influx and its restoration to resting concentrations, and therefore a higher rate of oxidative phosphorylation (Boumezbeur et al., 2010; Bouzier-Sore et al., 2006). Therefore, neurons show a preference for metabolize lactate over glucose when both substrates

are available (Bouzier-Sore et al., 2006; Itoh et al., 2003). In addition, neurons have a lower glycolytic rate than astrocytes because they produce less of the intermediate fructose-1,6-bisphosphate, an activator of phosphofructokinase, and are also unable to be upregulated that intermediate (Almeida et al., 2004; Herrero-Mendez et al., 2009). In contrast, astrocytes have a lower rate of oxidative metabolism but a higher rate for glycolysis, which is also due to their contact to blood vessels and the resultant higher glucose uptake (Herrero-Mendez et al., 2009; Itoh et al., 2003). Astrocytes preferentially produce and release lactate over producing pyruvate and feeding it into the TCA cycle (Bouzier-Sore et al., 2006; Itoh et al., 2003; Lovatt et al., 2007; Pellerin and Magistretti, 1994). In addition to glucose metabolism, the maintenance of the cytosolic redox state of the cells is highly important. The correct ratio between oxidized and non-oxidized NADH (or NADPH and GSH) directly or indirectly influences numerous processes involving energy homeostasis and signal processing (for review see: (Hirrlinger and Dringen, 2010)).

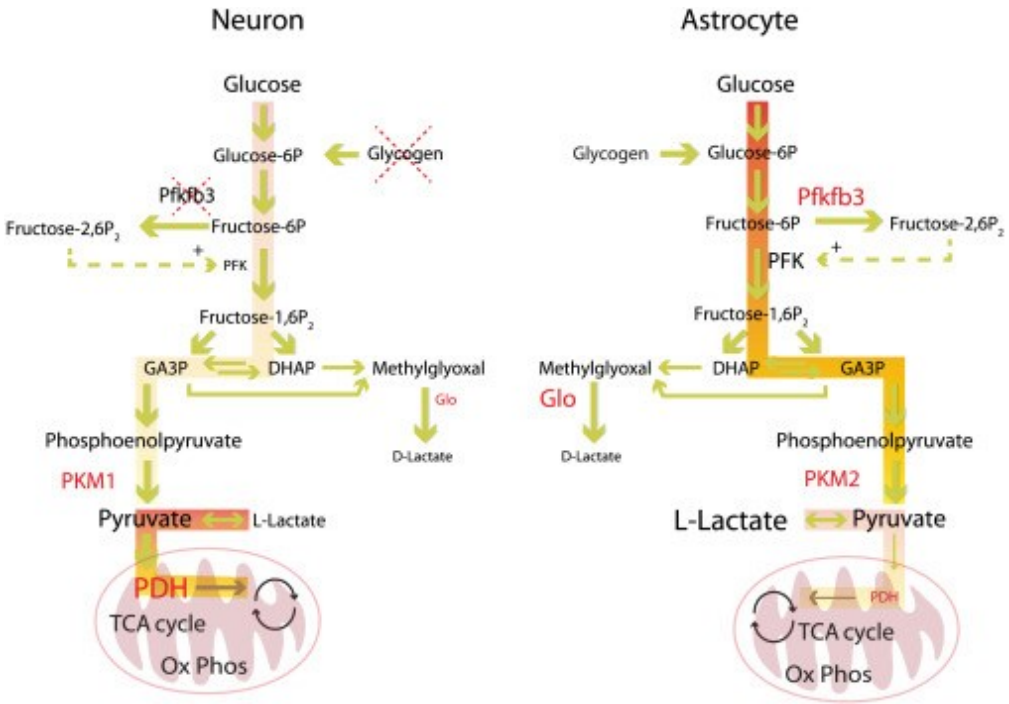

Figure 16: Neurons and astrocytes prefer different metabolic pathways for their glucose turnover. While neurons prefer oxidative phosphorylation, since they need more energy, astrocytes mainly use glycolysis. See text for abbreviations and further explanation (Pfkfb3 – 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; Glo – glyoxalase; PKM1/2 – pyruvate kinase 1/2; PDH – pyruvate dehydrogenase). Reprinted with permission from Elsevier: (Magistretti and Allaman, 2015).

Another important difference in energy metabolism between neurons and astrocytes is the possibility to store energy (Magistretti et al., 1993). Glycogen is the largest energy reserve of the brain and can give rise to ATP under anaerobic conditions, but is stored almost exclusively in astrocytes. Theoretically, neurons also have the potential to produce glycogen through glycogen synthase but it is kept inactive (Vilchez et al., 2007). There is also evidence for glycogen use under physiological conditions, since its amount is increased during sleep and anaesthesia and decreased during neuronal activity (Brown, 2004; Magistretti et al., 1981; Magistretti et al., 1993). Glycogen is strongly involved in energy supply of neurons in the form of lactate during sustained neuronal activity (Brown et al., 2005; Machler et al., 2016).

Coming back to the role of sodium and its impact on the metabolism, it can be summarized as follows: Increased neuronal activity results in the release of glutamate from neurons, which is then taken up by astrocytes from the synaptic cleft through specific glutamate transporters. Together with glutamate, the intracellular sodium concentration is increased by this process (Langer et al., 2017; Langer and Rose, 2009; Langer et al., 2012). This results in the activation of the NKA, thereby also increasing ATP consumption (Langer et al., 2017; Magistretti and Chatton, 2005), glucose uptake from the blood and glycolytic rate. The glycolysis is also activated by astrocytic depolarization and sodium-dependent NBC-induced alkalization (Ruminot et al., 2011). The activated glycolysis results in the production of lactate, which is released into the extracellular space and taken up by neurons in order to meet their high energy demands, based on the ANLS hypothesis (Machler et al., 2016; Magistretti, 2009). Therefore, as sodium has different consecutive tasks within the astrocyte and later within the neurons, it was termed as a “second messenger” for energy metabolism (Rose and Chatton, 2016).

To sum up this second part, besides processes related to electrical signaling, sodium ions and maintenance of the sodium gradient are tightly coupled to cellular energy metabolism. Sodium signaling serves as a sensor for electrical signaling and couples the energy demand to its production and accessibility. In addition, sodium signaling thereby also couples neurons to astrocytes and thus serves as a key component of the neurovascular unit. Therefore, it is obvious that the dysregulation of sodium homeostasis will have a huge impact on cellular signaling as well as on cellular metabolism, which is what will be discussed in detail in the next and last chapter.

3. Sodium dysregulation and energy failure

All the processes and mechanisms described above are highly interrelated and require a steady energy supply. This in turn is dependent on a steady supply of oxygen and glucose, since the majority of ATP is produced through oxidative phosphorylation. Interruption of the glucose and oxygen supply for a time span of only a few seconds to minutes results in a breakdown of the cells ATP content as well as substrate storages leading to cellular damage. Mechanisms involved in this are a central aspect in cerebral ischemic stroke and hypoglycaemia in patients (see Figure 17 (Moskowitz et al., 2010; Nedergaard and Dirnagl, 2005)).

Stroke/Ischemia

Calcium is known to mediate cellular damage by increased influx through glutamate receptors, especially NMDA receptors, and voltage-gated calcium channels. This is due to the decreased removal of glutamate from the synaptic cleft, termed “excitotoxicity” (Choi and Rothman, 1990; Lee et al., 1999). In tissue undergoing stroke, metabolism is completely disturbed - especially in the ischemic core, where the blood flow is reduced to less than 20% of normal values (Hossmann, 1994). In the ischemic core, neurons and astrocytes are highly depolarized, since the energy for maintaining especially the sodium gradients is rapidly depleted. Those cells undergo an anoxic depolarization and cannot repolarize, resulting in loss of cell integrity and finally in cell death (necrosis). In the penumbra (the area surrounding the ischemic core), cell death can be prevented by timely reperfusion, as the blood flow is only reduced to 50% of normal values (Hossmann, 1994; Obrenovitch, 1995). However, still imposes a huge metabolic demand on the cells. In this region, cells are able to repolarize, but at the expense of further energy consumption. However, these cells can be depolarized again, if the extracellular build-up of glutamate and potassium continues. As summarized in figure 18, almost all events following an ischemic stroke start with excessive release of glutamate and the subsequent activation of neuronal glutamate receptors. This results in an increased intracellular calcium and sodium concentrations, further calcium release from internal stores, acidosis, the production of reactive oxygen species (ROS), cell swelling and finally cell death.

The sustained increased extracellular concentration of glutamate and potassium results in depolarization waves propagating across the cortex. These are termed “peri-infarct depolarization” (PID) and were first described by Leao 1944 as strong cortical stimulations that lasted over minutes (Leao, 1944). Several PIDs can occur, which further increases the metabolic demands on the cells within the penumbra (see Figure 17 and 19 (Dirnagl et al., 1999)).

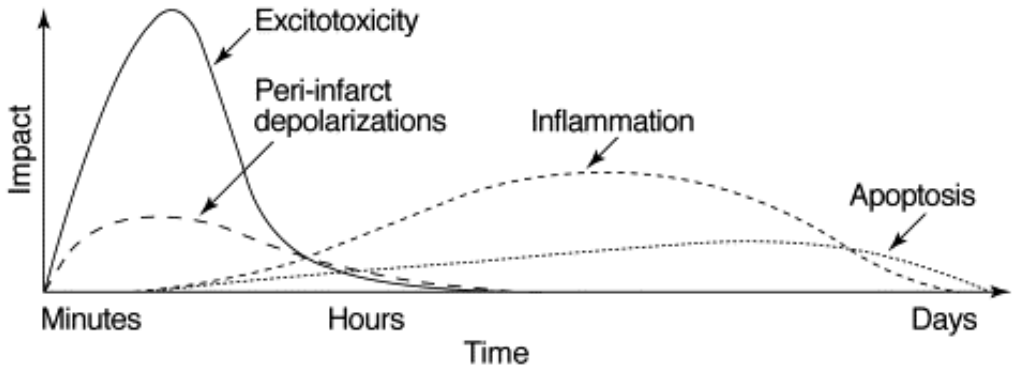

Figure 17: Time-course of damaging events in focal ischemic stroke. The very first consequence after the onset of stroke is excitotoxicity and PIDs invading the penumbra, leading to cellular damage. On a later time-scale things like inflammation and apoptosis also play an important role. Reprinted with permission from Elsevier: (Dirnagl et al., 1999).

It is not only in tissue undergoing stroke, but also in less dramatic pathological conditions like epileptic seizures, that the energy requirement exceeds the energy production and therefore induces metabolic stress in both neurons and astrocytes (Karus et al., 2015; Rouach et al., 2008).

However, it is not only calcium that is involved in the dysfunction and injury of both neurons and astrocytes under energy deprivation. Many studies indicate that changes in intracellular sodium followed by disturbances of intra- and extracellular potassium as well as impaired pH homeostasis are among the very first consequences of energy failure during stroke (Gerkau et al., 2017a; Hansen, 1985; Hertz, 2008; Leng et al., 2014; Somjen, 2002). Subsequently, many other processes that are also related to calcium will be impaired such as glutamate homeostasis, function of pre- and postsynaptic terminals and surrounding astrocytes. The failure of these ultimately results in calcium influx and excitotoxicity (Annunziato et al., 2013; Dirnagl et al., 1999). As described in the previous chapters, the NKA is main mechanism for the extrusion of

sodium and is responsible for the maintenance of the intracellular sodium and potassium concentrations. During energy shortage, almost all available ATP is broken down and is thereafter no longer able to feed the NKA. Consequently, the intracellular sodium concentration is increased, a situation mimicked experimentally by inhibiting the NKA. As a result, the driving force for sodium is decreased, which in turn effects all secondary active transport mechanisms that are dependent on sodium and on the energy stored in its gradient (Rose and Karus, 2013).

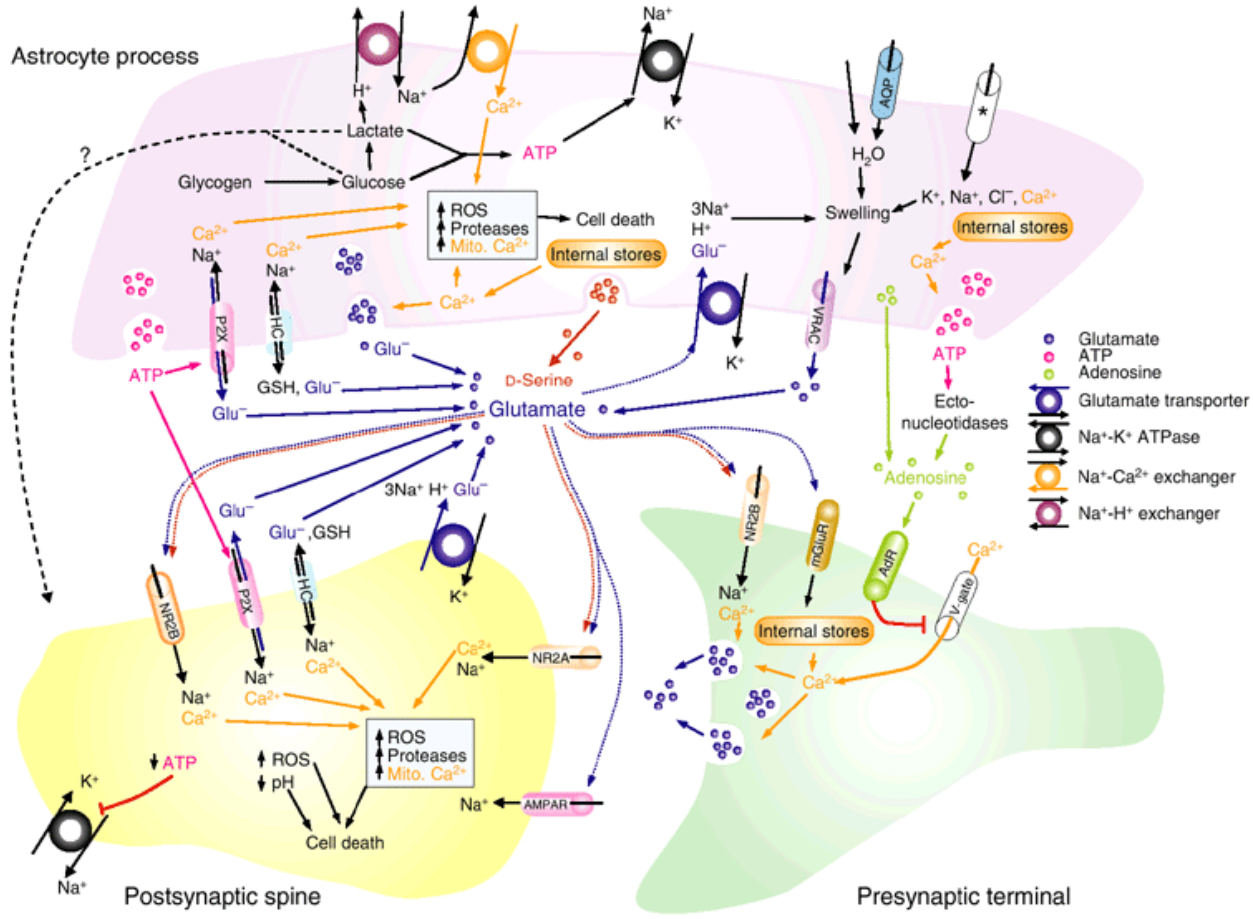

Figure 18: Proposed pathways in neurons and astrocytes after the onset of ischemia leading to increased extracellular glutamate concentration and increased intracellular calcium levels. Important players are the NKA, glutamate transporters and ionotropic glutamate receptors. Increased extracellular glutamate leads to metabotropic signalling, transporter activation or even reverse of transporters as well as second scale signalling cascades, leading to cell death. See text for abbreviations and further explanation (AQP – aquaporin channel; GSH – glutathione; HC – connexion hemichannel; NR2A/B – NMDA receptor). Reprinted with permission from Springer Nature: (Rossi et al., 2007).

Since neurons and astrocytes prefer different metabolic pathways to meet their energy demands, they also react differently to periods of energy deprivation. In addition, they also differ in sodium influx mechanisms and the magnitude of sodium influx. The strongest effect on the intracellular sodium concentration is observed in neurons during anoxia in combination with ischemia, since neurons preferentially use oxidative phosphorylation to cover their energy needs and are therefore strongly dependent on a stable oxygen supply. Cultured mammalian neurons that are treated for only a few minutes with anoxia, have an increased sodium concentration of 20-30 mM and, if at all, baseline concentration recovered only long after the anoxia onset (Calabresi et al., 1999; Friedman and Haddad, 1994a). Thereby different sodium-dependent secondary mechanisms like the NHE and NCX play an important role in sodium regulation, which is a central part of this study (Gerkau et al., 2017a; Sheldon et al., 2004). Under physiological conditions, as during energy deprivation, a significant component of sodium influx is mediated by ionotropic glutamate receptors (Rose and Konnerth, 2001; Mondragao et al., 2016; Karus et al., 2015). Hypoxia also has an impact on astrocytes and they experience strong sodium loading - as shown in culture conditions (Longuemare et al., 1999; Rose et al., 1998). For most of the models, trying to mimic stroke-like conditions in culture or slices, the reperfusion with normal saline after the ischemia cocktail has an even more dramatic effect. This is mainly due to the activation of the NKCC1 and NHE, at least in astrocytes (Bondarenko and Chesler, 2001; Kintner et al., 2004; Rose et al., 1998). Such a strong and long-lasting sodium increase also has a significant influence on many already described secondary active transport mechanisms that are dependent on an intact resting membrane potential. Since some of the sodium-dependent transporters work in close proximity to the equilibrium potential, a reduction in the sodium gradient can easily reverse their direction of function. This applies especially for sodium-dependent uptake of neurotransmitters such as GABA, glycine and glutamate (Richerson and Wu, 2003; Rossi et al., 2000). For the first two, it is quite easy to reverse their transporters operational direction, whereas glutamate transporters in contrast have an equilibrium potential of around +60 mV (Danbolt, 2001; Nicholls and Attwell, 1990). Thus, only very strong shifts in ion concentrations, like those present during severe ischemic core conditions, might reverse glutamate transporters (Somjen, 2004). In these conditions, the supply of oxygen and glucose is at its lowest with neurons dying within minutes and in doing so release further glutamate. In addition to the already depolarized tissue surrounding the

ischemic core, PIDs invade the penumbra and there also inflate energy consumption (see Figure 19 (Mergenthaler et al., 2004)). Therefore, the penumbra is of great interest in preclinical and clinical studies, which aim to reduce depolarization and further metabolic stress, especially to neurons, since they are more vulnerable to cell death than other cell types (Pulsinelli, 1985; Rossi et al., 2007). An important point of focus for these studies are astrocytes, since they take up most of the glutamate from the synaptic cleft and therefore may shape excitotoxicity and PID development (Nedergaard and Dirnagl, 2005). Therefore, the strong sodium increase into neurons and astrocytes are the central part of this study.

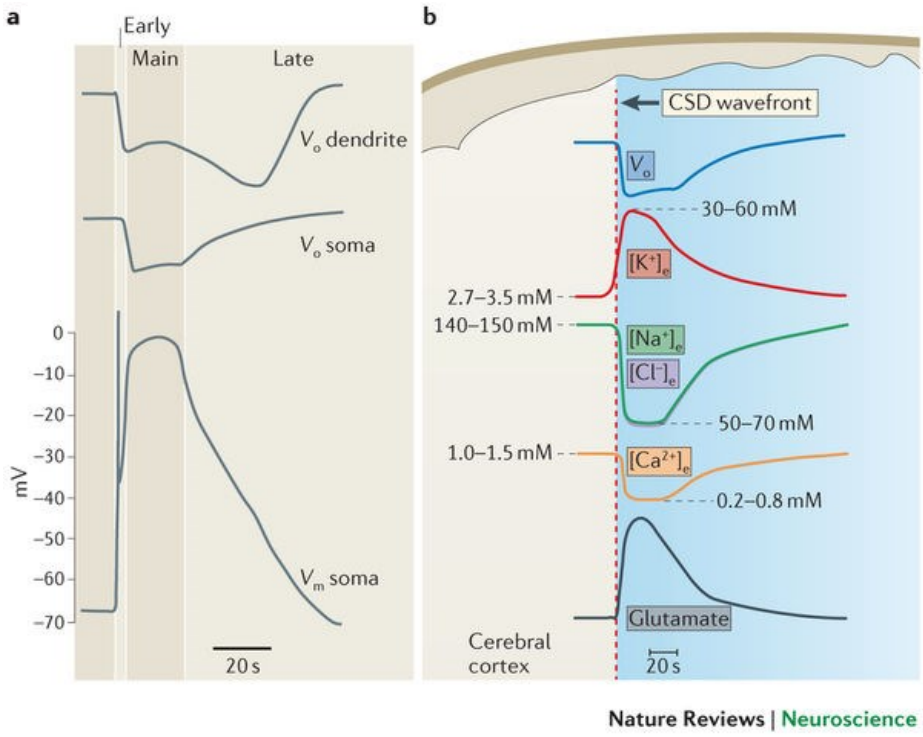

Figure 19: Electrophysiological and ionic changes during cortical spreading depression (CSD) or peri-infarct depolarization (PID). Left: extracellular potential in close proximity to the dendrites and close to the soma of a pyramidal cell during CSD, as well as the membrane potential of a specific pyramidal neurons. Right: Changes of the ion concentrations in the extracellular space during a CSD/PID. (see text for abbreviations and further explanation). Reprinted with permission from Springer Nature: (Pietrobon and Moskowitz, 2014).

Compared to the strong sodium increases present during almost complete blockage of energy metabolism, inhibiting just one pathway results in comparably mild changes. Blocking glycolysis only, results in small baseline increases in the sodium concentration in astrocytes, whereas neurons remain more or less unaffected under

those conditions (Karus et al., 2015; Rose et al., 1998). Additional activity elicited by external application of glutamate results in prolonged and slowed sodium transients, which is due to lower energy availability for the NKA and therefore slower extrusion rates compared to normal conditions (Karus et al., 2015; Mondragao et al., 2016). Neurons and astrocytes obviously require intact glycolytic activity for the proper export of sodium, mediated by the NKA during neuronal activity. However, under conditions of low energy availability, it can be compensated for by other metabolites or the breakdown of glycogen (Karus et al., 2015; Mondragao et al., 2016). Additional externally applied lactate fully compensated the energy demands of neurons but not of astrocytes, supporting the ANLS idea that astrocytes release lactate and that neurons take it up in order to continue functioning (Barros, 2013; Pellerin and Magistretti, 2012; Rose and Chatton, 2016). Thus, the sodium concentration is a perfect indicator for the energy status of a certain cell type and the preferred metabolic pathway they used. The described mechanisms indicate that sodium loading is one of the very first consequences of low energy conditions. Over and above that, it is not completely clear what the long-term consequences of a disturbed sodium homeostasis are on their own, apart from very strong increases leading to over-depolarization. In contrast it is quite clear that a disturbed sodium homeostasis impacts a lot of other mechanisms downstream, such as like calcium and pH homeostasis.

To sum up this third and final part, the maintenance of ionic gradients and especially that of sodium ions is vital for proper cell function, including synaptic transmission, energy production and energy distribution. Sodium homeostasis is directly linked to energy metabolism through the activity of the NKA, and thus the understanding of sodium dysregulation is necessary for understanding differential effects of energy failure within neurons and astrocytes.

4. Aim of this study

As already emphasized in the introduction, sodium influx through voltage-gated sodium channels and ionotropic glutamate receptors is the basis for the generation and propagation of action potentials in neurons. Moreover, the steep inwardly directed sodium gradient across membranes provides the energy for the uptake of neurotransmitters (especially into astrocytes), and mediates homeostasis of other ions through sodium-dependent transporter mechanisms. Sodium increases have to be restored by the ATP-consuming NKA, which is the main extrusion mechanisms for sodium ions and the only effective way to maintain the sodium gradient. By the consumption of ATP, sodium homeostasis is directly linked to the cellular energy metabolism. Therefore, a failure of the cellular energy supply has a direct effect on the intracellular sodium homeostasis. Illustrating this phenomenon, the here presented work is complemented by a comprehensive review addressing sodium signaling in neurons and astrocytes under resting conditions and under conditions of an energy limitation (Gerkau et al., 2017b).

Previous studies could show considerable intracellular sodium changes in cell culture and tissue slice preperations under physiological and pathophysiological conditions, but still, there is a huge lack of knowledge on sodium regulation in different brain areas. Albeit, sodium homeostasis and the cellular metabolism are obviously tightly connected, it is still unclear how both are depending on each other. Moreover, there is no quantitative data on sodium measurements in mouse brain *in vivo*.

The goal of the present work was to elucidate and characterize relevant sodium influx pathways in different cell types and compartments under physiological conditions and under conditions of an energy shortage in slices and *in vivo*. To this end, physiological stimulation techniques and pharmacological approaches were used to induce sodium transients. In addition, to mimic energy shortage and to measure the dependence of sodium homeostasis on the cellular metabolism, either stroke *in vivo* or chemical ischemia in tissue slices were introduced. To elucidate intracellular ion changes, quantitative, fluorescence-based wide-field and two-photon imaging with ion-sensitive fluorescent dyes was employed. These were complemented with intracellular calcium changes, extracellular ion dynamics with ion-sensitive microelectrodes, and whole-cell patch-clamp recordings.

5. Summary of Results and Discussion

Around ten years ago, the first intracellular sodium transients were shown in astrocytes of the hippocampus in acute tissue slices (Langer and Rose, 2009); even ten years before that, sodium signals were detected in cell culture preperations (amongst others: Rose and Ransom, 1996). Since this work, several studies addressed sodium influx into especially neurons and astrocytes of well-studied grey matter structures such as hippocampus and cortex, but also in cerebellum. Those signals were mainly generated by the activation of ionotropic glutamate receptors, glutamate transporters, and voltage-gated sodium channels (Fleidervish et al., 2010; Karus et al., 2015; Langer and Rose, 2009; Langer et al., 2012; Mondragao et al., 2016; Rose and Ransom, 1996; Rose and Ransom, 1997). But still, there are a lot of open questions regarding sodium influx pathways and sodium-dependent secondary active transport mechanisms in other cell types of different brain regions and under different conditions. Moreover, and for decades, energy metabolism and the energy budget of the brain has been in the focus of research (Mink et al., 1981; Pellerin and Magistretti, 1994). It has established for years that the NKA is the most energy consuming mechanism in the brain, which obviously links sodium homeostasis and cellular energy metabolism. Therefore, many studies have addressed the functionality of this pump under resting conditions and especially under conditions of limitations in its working process, such as energy shortage during stroke. Around twenty years ago, it was shown that intracellular sodium is increased under ischemic conditions both in cell culture and also in acute slices (Friedman and Haddad, 1994b; Rose et al., 1998). The recovery of those sodium loads was used as an indirect tool to measure the recovery from energy deprivation, since the NKA is the only effective mechanism restoring sodium back to baseline.

However, detailed analysis of sodium dynamics and influx pathways on a single cell level are still rare in acute tissue slices and not existent in living animals. In addition, studies of sodium homeostasis under different energy conditions are also missing. To study sodium dynamics, we made use of sodium-sensitive fluorescent dyes to measure fluorescence changes indicative for sodium changes on a single cell level, employing both wide-field and two-photon imaging.

As reported before, sodium enters neurons through voltage-gated sodium channels that generates action potentials (Fleidervish et al., 2010; Rose and Konnerth, 2001). This study shows, for the first time, action potential-induced sodium transients in

axons and dendrites of mitral and granule cells of the olfactory bulb (Ona-Jodar et al., 2017). The somatically induced back-propagating action potentials revealed sodium transients that were mediated by the opening of voltage-gated sodium channels. Those transients are comparable in terms of amplitude and kinetics to sodium signals measured in hippocampal CA1 neurons (Jaffe et al., 1992; Rose and Konnerth, 2001).

As described above, sodium transients are not only detectable in neurons upon action potential generation, but can be detected in astrocytes upon synaptic activity as well (Langer and Rose, 2009; Langer et al., 2012). During synaptic transmission, neurons release glutamate that in turn is taken up by astrocytes. The uptake of glutamate is mediated by glutamate transporters that are expressed in astrocytes in an age-dependent manner (Schreiner et al., 2012). This study shows, that network activity induced sodium transients as introduced by Karus and colleagues (2015) were mediated by the glutamate transporter GLAST in neonatal brains, whereas GLT-1 mediates glutamate uptake in older brains (Karus et al., 2017). This is well in line with expression profiles of both transporter in the hippocampal CA1 region (Schreiner et al., 2012). Moreover, this study shows, for the first time, that sodium transients can not only be evoked at astrocytic somata (Langer and Rose, 2009; Langer et al., 2012, Karus et al., 2015), but also directly at perivascular endfeet enwrapping blood vessels (Langer et al., 2017). Those glutamate transporter-mediated sodium signals were comparable in terms of amplitude and kinetics to somatic sodium signals. Immunohistochemical expression profiles revealed strong expression of both GLAST and GLT-1 at perivascular endfeet and thus confirming the generation of sodium signals at this compartments. Those sodium signals were able to spread within the astrocytic syncytium via gap junctional coupling. Measuring magnesium transients, which is an indirect method to determine intracellular ATP content, this study shows an increasing magnesium level indicative for a decrease in ATP. Therefore, this study shows that intracellular sodium concentration in perivascular endfeet is maintained by the ATP-consuming NKA (Langer et al., 2017). The newly discovered endfeet sodium signals in this study represents an intercellular acting indicator for synaptic activity at the blood brain barrier adding further complexity to neuro-metabolic coupling.

Besides the interrelation of the sodium homeostasis and the cellular ATP content under physiological conditions, the sodium gradient itself provides energy for sodium-dependent secondary active transporters like the NHE, NKCC1, NCX, and NBC. Therefore, this study shows that the working direction of transporters like the NKCC1 in

astroglial cells is strongly dependent on the sodium gradient. In Bergmann glia cells of the cerebellum, where the sodium concentration is comparable to astrocytes of the hippocampal CA1 region and the chloride concentration is relatively high, the NKCC1 is constantly working in the sodium import mode (Untiet et al., 2017). Pharmacological inhibition of the NKCC1 with bumetanide results in a constant decrease in the intracellular sodium concentration. In contrast, NKCC1 expressed in the choroid plexus is working in the other way around (Steffensen et al., 2018). The relatively high intracellular sodium concentration (~35 mM) completely changed the sodium gradient across the plasma membrane. Therefore, inhibition of the NKCC1 results in a strong increase in the intracellular sodium concentration. Thereby, this study shows that sodium-dependent secondary active transport mechanisms are highly dependent on the distribution of sodium ions in the intra- and extracellular compartments and the resulting sodium gradient. Another sodium influx pathway that is revealed in this study, is the sodium-permeable channel ENaC, which is expressed e.g. in neural stem cells of the subventricular zone (Petrik et al., 2018). This study shows, that mechanical activation of this channel through increased liquid flow in the ventricles results in sodium influx, which is absent in conditional ENaC knock-out animals.

As already described before, this study shows that an increase in the intracellular sodium concentration results in ATP consumption to recover sodium back to baseline. This phenomenon describes the relationship between sodium homeostasis and the cellular energy level under physiological conditions (Langer et al., 2017). In order to analyze this relationship further, we addressed this matter under pathophysiological conditions. Therefore, we induced a permanent middle cerebral artery occlusion (MCAO) in living animals to replicate a stroke and also applied blockers for oxidative respiration and glycolysis to mimic ischemic conditions in slices. In order to do so, we applied sodium azide to block complex IV in the respiratory chain and 2-deoxyglucose to block production of glucose-6-phosphate during glycolysis. Both inhibitors and comparable blockers are established protocols used to induce ischemic conditions *in situ* (Breier et al., 1993; Cavallini et al., 2005; Dubinsky and Rothman, 1991; Englund et al., 2001; Gores et al., 1989; Rose et al., 1998).

This study shows, for the first time, intracellular sodium increases in neurons and astrocytes of the somatosensory cortex in the living animals *in vivo* during PIDs after induction of a stroke. Those sodium transients could be reproduced in an acute slice system for further pharmacological characterization (Gerkau et al., 2017a). In addition,

they reveal the direct relationship between sodium and calcium dynamics, combined through the NCX under these conditions.

Intracellular sodium changes induced by PIDs (as detected in the present study) in the penumbral region amounted to ~20 mM in astrocytes and by ~24 mM in neurons in the somatosensory cortex *in vivo*. These changes are in the same range as those induced by metabolic inhibition in reduced culture systems. It has been shown before, that the intracellular sodium concentration increases by ~27 mM after a period of anoxia in cultured hippocampal neurons (Friedman and Haddad, 1994b). Similarly, metabolic inhibition by combined removal of glucose and application of sodium azide increased the intracellular sodium concentration in cultured hippocampal astrocytes by ~20 mM (Rose et al., 1998). The sodium concentration rose monotonically and peaked within 20-25 seconds after which the intracellular sodium concentration recovered back to baseline. Interestingly, and in contrast to former calcium imaging experiments (Rakers and Petzold, 2017), the onset of the sodium increases was indistinguishable between neighboring cells, indicative for a direct measurement of the propagating wave front. Well in line with former reports on the PID wave propagation measured via calcium (Chuquet et al., 2007; Rakers and Petzold, 2017) and with light scattering looking at depolarization waves (Murphy and Corbett, 2009), the propagation speed of sodium signals was in the range of ~30 µm/s. In the penumbra, surrounding the ischemic core, the blood flow is reduced to 50% below normal values, putting an enormous metabolic stress on the cells (Hossmann, 1994; Obrenovitch, 1995). Therefore, pumps like the NKA should have enough energy to counteract strong ionic movements like during PIDs. In this study the sodium changes always recovered back to baseline after the first PIDs, which is an indication for an essentially functional NKA and an intact energy metabolism system. In addition, the recovery of the sodium increases made it is quite clear that the measurements were done in the penumbra and not in the ischemic core, where massive cell death occurs due to excitotoxicity and long-term depolarization. Intracellular calcium waves are well established during PID propagation after stroke induction (Chuquet et al., 2007; Rakers and Petzold, 2017), and also as spontaneous activity in neurons and astrocytes *in vivo* (Grewe et al., 2010; Helmchen et al., 1999; Stosiek et al., 2003). This study shows that in addition to calcium signals, long lasting sodium signals also propagate in a comparable manner, whereas no spontaneous sodium activity was measured.

Chemical ischemia was used several times by several groups in order to replicate ischemic conditions. This was mainly done in cultured neurons and astrocytes (Friedman and Haddad, 1994b; Rose et al., 1998) but also in acute tissue slices (Cavallini et al., 2005). In this study, we show for the first time strong sodium loads in astrocytes and neurons in acute cortical slices during short periods of chemical ischemia. In addition, and well in line with other studies, the extracellular sodium concentration dropped by ~5 mM and the extracellular potassium increased by ~2-3 mM (Erecinska and Silver, 1994). The recovery from sodium loads shown by cells *in vivo* was also seen in acute slices, indicative for properly functional working NKA and a not fully disrupted metabolism (Leis et al., 2005). Long lasting periods of chemical ischemia in contrast, resulted in sustained and large intracellular sodium increases as well as cell swelling, as it would be expected for the ischemic core region *in vivo*. On the other hand, only short exposure to chemical ischemia resulted in small and hardly analyzable sodium transients.

The main mechanisms for sodium influx into neurons and astrocytes under these conditions differ between cell types, as it is reported under physiological conditions. For neurons, the influx after physiological stimulations is mainly mediated by opening of voltage-gated and ligand-gated sodium channels (Muller and Somjen, 2000), primarily NMDA-receptors (Mondragao et al., 2016; Rose and Konnerth, 2001). In this study, we could show that under pathophysiological conditions the main influx pathway for sodium ions into neurons are also NMDA receptors, since their inhibition also reduced the sodium loads. This is not true for astrocytes, even though cortical astrocytes are suggested to express NMDA receptors as well (Lalo et al., 2006; Schipke et al., 2001), there is only a small decreasing trend observed when these are blocked. Astrocytes instead, as reported in other studies in hippocampus and cortex under physiological conditions, prefer glutamate transporter-mediated sodium influx as the major influx pathway (Karus et al., 2017; Karus et al., 2015; Kirischuk et al., 2012; Rose and Chatton, 2016). Glutamate uptake is not only relevant under physiological conditions, but becomes especially relevant under conditions of high glutamate release like during ischemia. In this study, and for the first time, we showed the relevance of glutamate uptake for intracellular sodium signaling during ischemia, since inhibition of glutamate transporters drastically reduced sodium loading in astrocytes. On the other hand, inhibition of glutamate uptake by astrocytes and therefore disturbance of clearance of glutamate from the synaptic cleft reduces protection from excitotoxicity and therefore

increases neuronal sodium loads. This is in accordance with other studies showing that inhibition of glutamate uptake leads to a complete failure of neuronal sodium homeostasis (Karus et al., 2015; Langer and Rose, 2009; Langer et al., 2017) mainly due to increased extracellular glutamate (Jabaudon et al., 1999). Less than 10% of the neurons were able to recover to baseline sodium concentrations when glutamate transporters were blocked.

As already mentioned above, former studies showed that PIDs induced prominent calcium waves in both neurons and astrocytes (Chuquet et al., 2007; Rakers and Petzold, 2017). In this study, and in addition to the already described sodium experiments, calcium oscillations could be induced during a period of chemical ischemia. These oscillations were preceded by an initial phase of a slowly rising intracellular calcium concentration that commenced within a few seconds of metabolism blockage. After ~30 seconds, the initial phase was succeeded by a surge in the intracellular calcium concentration, which resulted in strong oscillations that were even larger in astrocytes than in neurons.

As reported in previous studies, inhibition of NMDA receptors reduced calcium signals in neurons (Lee et al., 1999) in our experiments, whereas astrocytic calcium signals remained unaltered. This is in accordance with other reports showing that large amounts of calcium are released mainly out of internal stores as well as through the activation of TRPV4 channels (Rakers and Petzold, 2017; Rakers et al., 2017). In addition to these pathways, inhibition of another mechanism revealed a huge impact on intracellular calcium levels under ischemic conditions. Blocking the NCX, in our experiments, reduced the intracellular calcium loading dramatically, suggesting that the NCX contributes significantly to the shape of the calcium signals under this condition. The NCX is seen as a calcium exporter under physiological conditions (Carafoli and Longoni, 1987), but can reverse upon intracellular sodium concentration increases, and thereafter work as a calcium loader (Boscia et al., 2016; Gerkau et al., 2017; Khananshvili, 2014; Kirischuk et al., 1997). Furthermore, this study showed for the first time that in addition to reduced calcium loads, sodium is increased, when the NCX is inhibited under ischemic conditions, supporting the proposed reverse working model. This is again in line with other reports also looking at calcium extrusion while intracellular sodium is increased in cultured neurons (Kiedrowski et al., 1994; Song et al., 2013). Both sets of experiments strongly suggest that the reverse mode of NCX causes

calcium influx and at the same time mediates sodium efflux, reducing sodium loads and accelerating the recovery to baseline under ischemic conditions.

This study establishes that synaptic transmission in olfactory bulb, cortex and hippocampus results in long lasting sodium transients in neurons and astrocytes. These transients are mediated through voltage-gated sodium channels and glutamate transporters and are restored by the consumption of ATP. Furthermore, the sodium gradient affect sodium-dependent secondary active transporters as well as sodium-permeable channels and might even reverse working modes by a decrease in its driving force. In addition, this study establishes that ischemic conditions both *in vivo* and *in situ* result in strong and long lasting sodium transients accompanied by calcium increases as well as ATP decreases. These transients are predominantly mediated by NMDA receptors in neurons and glutamate transporters in astrocytes. Furthermore, this study highlights an importance of the downstream activated sodium-dependent secondary transporter, the NCX, which is strongly dependent on the driving force of sodium and changes its operation mode with shifts in the membrane potential. Taking into account this multitude of effects, a decrease in the sodium driving force might exert on cellular functions, sodium transients represent a signal for increasing metabolic needs. The results thus support that sodium homeostasis is directly linked to energy metabolism through the activity of the NKA.

6. Publications and Manuscripts

Publications are sorted chronologically, starting by the first publication.
For reasons of copyright protection, the published version of this dissertation does not contain reprints of the articles.

6.1. Published manuscripts

Pages 44-55:

Roles of the astrocytic Na(+)/K(+)-ATPase and glycogenolysis for K(+) homeostasis in mammalian brain

Hertz L, **Gerkau NJ**, Xu J, Durry S, Song D, Rose CR, Peng L

Journal of Neuroscience Research 93(7):1019-30 (2015) Review

Impact factor 2015: 2.689

I performed

- Analysis of ion-sensitive microelectrodes measurements

I contributed to

- Interpretation of data
- Drafting and revision of manuscript and figures

Pages 57-72:

Rapid sodium signaling couples glutamate uptake to breakdown of ATP in perivascular astrocyte endfeet

Langer J, **Gerkau NJ**, Derouiche A, Kleinhans C, Moshrefi-Ravasdjani B, Fredrich M, Kafitz KW, Seifert G, Steinhäuser C, Rose CR

Glia 65(2):293-308 (2017)

Impact factor 2017: 6.2

I performed

- About 10% of experiments and analysis, illustrated in Figure 2 and 7, and in the supplement Figure 2

I contributed to

- The experimental design
- Interpretation of data
- Drafting and revision of manuscript and figure

Pages 74-86:

Glutamate transporter-associated anion channels adjust intracellular chloride concentrations during glial maturation

Untiet V, Kovermann P, **Gerkau NJ**, Gensch T, Rose CR, Fahlke C

Glia 65(2):388-400 (2017)

Impact factor 2017: 6.2

I performed

- All sodium experiments and analysis, illustrated in Figure 3

I contributed to

- The experimental design
- Interpretation of data
- Drafting and revision of manuscript and figures

Pages 88-98:

Differential effects of energy deprivation on intracellular sodium homeostasis in neurons and astrocytes

Gerkau NJ, Rakers C, Petzold GC, Rose CR

Journal of Neuroscience Research 95(11):2275-2285 (2017) Review

Impact factor 2017: 2.481

I performed

- First draft of the manuscript and preparation of all figures

I contributed to

- Drafting and revision of the whole manuscript and figures

Pages 100-111:

Two-Photon Na^+ Imaging Reports Somatically Evoked Action Potentials in Rat Olfactory Bulb Mitral and Granule Cell Neurites

Ona-Jodar T, **Gerkau NJ**, Sara Aghvami S, Rose CR, Egger V

Frontiers in Cellular Neuroscience 11:50 (2017)

Impact factor 2017: 4.555

I performed

- Calibration experiments and analysis, illustrated in Figure 1

I contributed to

- The experimental design
- Interpretation of data
- Drafting and revision of manuscript and figures

Pages 113-129:

Reverse NCX Attenuates Cellular Sodium Loading in Metabolically Compromised Cortex

Gerkau NJ, Rakers C, Durry S, Petzold GC, Rose CR

Cerebral Cortex 1-17 (2017)

Impact factor 2017: 6.559

I performed

- All experiments and analysis, except for the ion-sensitive microelectrode experiments

I contributed to

- The experimental design
- Interpretation of data
- Drafting and revision of manuscript and figures

Pages 131-143:

Differential Contribution of GLAST and GLT-1 to Network Sodium Signalling in the Early Postnatal Hippocampus

Karus C, **Gerkau NJ**, Rose CR

Opera Medica et Physiologica 3(3):71-83 (2017)

Impact factor 2017: data not yet available

I performed

- All two-photon experiments and analysis, illustrated in Figure 4 and 5

I contributed to

- The experimental design
- Interpretation of data
- Drafting and revision of manuscript and figure

Pages 145-157:

Cotransporter-Mediated Water Transport Underlying Cerebrospinal Fluid Formation

Steffensen AB, Oernbol EK, Stoical A, **Gerkau NJ**, Barbuskaite D, Tritsaris K, Rose CR, MacAulay N

Nature Communications 9(1):2167

Impact factor 2016: 12.124

I performed

- All sodium experiments and analysis, illustrated in Figure 4

I contributed to

- The experimental design
- Interpretation of data
- Drafting and revision of manuscript and figures

Pages 159-182:

Epithelial sodium channel regulates adult neural stem cell proliferation in a flow-dependent manner

Petrik D, Myoga MH, Grade S, **Gerkau NJ**, Pusch M, Rose CR, Grothe B, Götz M

Cell Stem Cell 22(6):865-878

Impact factor 2016: 23.394

I performed

- All sodium experiments and analysis, illustrated in Figure 6

I contributed to

- The experimental design
- Interpretation of data
- Drafting and revision of manuscript and figure

6.2. Submitted manuscripts

Pages 184-206:

Imaging of local and global sodium signals in astrocytes

Gerkau NJ, Kafitz KW, Rose CR

Submitted 02/2018 Methods in Molecular Biology

Invited contribution to: “Astrocytes - The Neurovascular unit“ in Methods in Molecular Biology, Springer Nature

I performed

- The first version of the manuscript and figures

I contributed to

Drafting and revision of manuscript and figure

Pages 208-254:

Mechanisms and consequences of sodium signals in astrocytes of the mouse neocortex

Ziemens D, Oschmann F, **Gerkau NJ**, Rose CR

Submitted 08/2018 J Neurosci

Impact factor 2015: 5.924

I contributed to

- Drafting and revision of manuscript and figures

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Eidesstattliche Erklärung

Ich versichere an Eides Statt, dass die vorliegende Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist. Textstellen oder Abbildungen, die wörtlich oder abgewandelt aus anderen Arbeiten stammen, habe ich mit einer Quellenangabe versehen.
Diese Arbeit wurde weder vollständig noch in Teilen einem anderen Prüfungsamt zur Erlangung eines akademischen Grades vorgelegt.

Niklas J. Gerkau Düsseldorf