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**On the special role of NCX in astrocytes:
Translating Na⁺-transients into intracellular Ca²⁺ signals**

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Abstract

As a solute carrier electrogenic transporter, the sodium/calcium exchanger (NCX1-3/SLC8A1-A3) links the trans-plasmalemmal gradients of sodium and calcium ions (Na^+ , Ca^{2+}) to the membrane potential of astrocytes. Classically, NCX is considered to serve the export of Ca^{2+} at the expense of the Na^+ gradient, defined as a “forward mode” operation. Forward mode NCX activity contributes to Ca^{2+} extrusion and thus to the recovery from intracellular Ca^{2+} signals in astrocytes. The reversal potential of the NCX, owing to its transport stoichiometry of 3 Na^+ to 1 Ca^{2+} , is, however, close to the astrocytes’ membrane potential and hence even small elevations in the astrocytic Na^+ concentration or minor depolarisations switch it into the “reverse mode” (Ca^{2+} -import/ Na^+ export). Notably, transient Na^+ elevations in the millimolar range are induced by uptake of glutamate or GABA into astrocytes and/or by the opening of Na^+ -permeable ion channels in response to neuronal activity. Activity-related Na^+ transients result in NCX reversal, which mediates Ca^{2+} influx from the extracellular space, thereby generating astrocyte Ca^{2+} signalling independent from InsP_3 -mediated release from intracellular stores. Under pathological conditions, reverse NCX promotes cytosolic Ca^{2+} overload, while dampening Na^+ elevations of astrocytes. This review provides an overview on our current knowledge about this fascinating transporter and its special functional role in astrocytes. We shall delineate that Na^+ -driven, reverse NCX-mediated astrocyte Ca^{2+} signals are involved neurone-glia interaction. Na^+ transients, translated by the NCX into Ca^{2+} elevations, thereby emerge as a new signalling pathway in astrocytes.

Key words: sodium, calcium, synapse, neurone-glia interaction, astroglial ionic excitability

Highlights

- Astrocytes express all three isoforms of the sodium/calcium exchanger (NCX); the transporters are preferentially located on perisynaptic processes and endfeet.
- NCX contributes to the regulation of Na^+ and Ca^{2+} concentrations in astrocytes at rest; in physiological conditions NCX transport direction fluctuates between forward and reverse mode.
- Transient increases in astrocyte Na^+ concentration resulting from neuronal activity and transmitter release drive the NCX into reverse mode, producing Ca^{2+} influx into astrocytes.
- Na^+ -driven Ca^{2+} signals generated by reverse NCX serve important functional roles in neurone-glia interaction.

Graphical Abstract

Astrocyte NCX couples the plasma membrane gradients of sodium and calcium ions (Na^+ , Ca^{2+}) to the membrane potential of astrocytes. Activity-related intracellular Na^+ transients result in a switch from forward into reverse mode, thereby generating astrocytic Ca^{2+} signals.

1 **1. Introduction**

2 Astrocytes are electrically non-excitable cells, i.e. they do not generate fast regenerative
3 electrical signals like neurones. Instead they rely upon dynamic changes in intracellular
4 concentrations of ions, of which changes in Ca^{2+} and Na^+ are the best studied [1].
5 Intracellular Ca^{2+} signals, which can be transmitted to neighbouring cells, are
6 instrumental in astroglial interaction with neurones [2, 3].

7 A classical route for the generation of astrocyte Ca^{2+} transients is through Ca^{2+}
8 release from intracellular stores. This can happen in response to the binding of
9 transmitters to metabotropic receptors, activation of G_q -proteins coupled to phospholipase
10 C, and the generation of InsP_3 that activates Ca^{2+} channels of the endoplasmic reticulum
11 [4]. Mitochondria represent another intracellular Ca^{2+} store which, in physiological
12 conditions, can release Ca^{2+} through a mitochondrial sodium/calcium exchanger (NLCX)
13 that can also transport lithium [5, 6] (Fig. 1A). Besides its release from intracellular stores,
14 astroglial Ca^{2+} signals can be generated by an influx of Ca^{2+} from the extracellular space
15 following the opening of ionotropic transmitter receptors, following operation of Na^+ -
16 gradient-driven transporters or following Ca^{2+} entry through other ion channels [3].
17 Whereas InsP_3 -mediated signalling seems to be predominant in astrocyte soma, the
18 plasmalemmal entry pathways appear to be specifically relevant for Ca^{2+} signalling in
19 astrocyte processes [7-11].

20 The recovery from increases in cytosolic Ca^{2+} concentration is mediated by its
21 extrusion into the extracellular space by the plasma membrane Ca^{2+} -ATPase (PCMA), by
22 its transport into the endoplasmic reticulum by the SERCA pump, and by its uptake into
23 mitochondria by a mitochondrial Ca^{2+} uniporter [12, 13]. In addition, astrocytes express
24 the sodium/calcium exchanger (NCX isoforms 1-3) on their plasma membranes [6, 14,

15] (Fig. 1A). NCX contributes to the export of Ca^{2+} from the cytosol - but only at low baseline intracellular Na^+ concentration ($[\text{Na}^+]_i$) as described below.

As a matter of fact, functions of NCX in astrocytes are not fully understood. As mentioned above, and established for many other cell types, the main role initially attributed to the NCX was to assist the PMCA in exporting Ca^{2+} . In this so-called forward mode (Fig. 1A), the energy for Ca^{2+} export by the NCX is provided by the strong inwardly-directed Na^+ gradient given a low baseline $[\text{Na}^+]_i$ ($\leq 10\text{-}12\text{ mM}$) [15]. Based on a wealth of recent experimental findings, however, this view has changed profoundly. Some studies have reported a higher baseline astrocyte $[\text{Na}^+]_i$ ($\sim 15\text{-}17\text{ mM}$) [16, 17], under which condition NCX may fluctuate between forward and reverse mode (or even operate in the reverse mode at rest) (Fig. 1A). Moreover, there is convincing evidence that transient increases in astrocyte $[\text{Na}^+]_i$ which accompany neuronal activity [1, 16, 17] readily drive reverse operation of NCX. This results in an increased influx of Ca^{2+} , directly coupling astrocyte Na^+ transients to Ca^{2+} signalling.

NCX thereby emerges as a dynamic translator of astrocyte Na^+ signals, converting them into influx of Ca^{2+} from the extracellular space. In parallel, NCX shapes cytoplasmic Na^+ signalling by mediating Na^+ entry in the forward mode and extruding Na^+ in the reverse one. This review gives an overview on the special role of NCX in astrocytes with a main emphasis on its putative functions in the healthy brain and a synopsis on its role in ischemic conditions.

2. Expression of NCX in astrocytes

First direct evidence for a functional expression of NCX in astrocytes was obtained in cell cultures from rat brain, where a reduction in the transmembrane Na^+ gradient resulted

1 in a (relatively minor) increase in the $[Ca^{2+}]_i$ [18]. Soon afterwards, NCX mRNA and
2 protein were detected in cultured astrocytes [19, 20]. It is now established that astrocytes
3 express all three subtypes of the NCX gene family, NCX1-3 (SLC8A1-A3) [21-23].
4 Several splicing variants of NCX1 and 3 were also described in astrocytes [24-26].

5 Sharing a moderate sequence identity, the three NCX subtypes exhibit similar
6 functional properties including apparent binding affinities to Na^+ and Ca^{2+} , but display
7 some differences in their requirement for the presence of ATP and their regulation by
8 protein kinases [24, 27, 28]. Expression of NCX subtypes differs between brain regions,
9 indicating specialised functions of these isoforms [29, 30]. The specific roles of the
10 isoforms remain, however, unclear, partly because of a lack of isoform-specific inhibitors.
11 Some general information has been acquired from knock-out animals. NCX3-deficient
12 mice have a higher threshold for LTP induction and show deficits in hippocampus-
13 dependent spatial learning [31]. Mice lacking NCX2, in contrast, show increased LTP
14 and an improvement in memory [32]. General knockout of NCX1 is lethal at embryonic
15 stage [33], making thus an analysis of the effect of its complete removal in the postnatal
16 brain impossible.

17 In contrast to the PMCA, which seems to be relatively uniformly expressed over
18 the cell surface of astrocytes [34], NCX appears to be targeted to specialized areas of the
19 cell. There is evidence from astrocytes in culture that NCX is preferentially expressed in
20 regions of the plasma membrane in a close vicinity of the endoplasmic reticulum. These
21 regions also contain an $\alpha 2$ -containing Na^+/K^+ -ATPase (NKA), and together with the
22 latter, the NCX has been proposed to generate a local signalling compartment for Ca^{2+}
23 and Na^+ , separated from the bulk of the cytosol [34-36]. In astrocytes in the rat brain

tissue slices, preferential localization of NCX on astrocyte processes contacting glutamatergic synapses was similarly noted [21] (Fig. 1A, B). In addition, the latter study found NCX to be concentrated in astroglial endfeet plastering blood vessels (Fig. 1B). The perisynaptic processes of astrocytes are also rich in Na^+ -dependent glutamate transporters, which generate substantial Na^+ influx during neuronal activity [37, 38]. Based on these anatomical findings, it seems that reverse-mode NCX might serve to provide Ca^{2+} influx into perisynaptic astrocyte processes in response to excitatory synaptic activity [21, 39]. The latter suggestion is in line with NCX' proposed function translating activity-related astrocyte Na^+ transients into intracellular Ca^{2+} signals as detailed below.

3. Properties of NCX under physiological conditions

3.1. Transport direction and involvement of NCX to ion homeostasis at resting conditions

Based on a transport stoichiometry of 3 Na^+ :1 Ca^{2+} , the equilibrium potential of the NCX can be calculated from the Nernst-equilibrium potentials of Na^+ (E_{Na}) and Ca^{2+} (E_{Ca}) according to the following equation: $E_{\text{NCX}} = 3 E_{\text{Na}} - 2 E_{\text{Ca}}$ [14, 15]. In astrocytes, E_{NCX} and NCX operational modality are thus largely determined by the $[\text{Na}^+]_i$. For extracellular concentrations of 2 mM Ca^{2+} and 150 mM Na^+ , and at baseline $[\text{Na}^+]_i$ of 12 mM and $[\text{Ca}^{2+}]_i$ of 80 nM (values taken from [40]), E_{NCX} equals -64 mV at room temperature. Because the latter value is more positive than the typical membrane potential of astrocytes (-85 mV; see [40]), NCX will operate in the forward mode (Ca^{2+} export/ Na^+ import), mediating a net influx of positive charge under these conditions. This is

illustrated schematically in Fig. 1C, which depicts the calculated current of the NCX of neocortical astrocytes in dependence of $[\text{Na}^+]_i$ and $[\text{Ca}^{2+}]_i$ at -85 mV (green dot) [40]. However at slightly higher baseline $[\text{Na}^+]_i$, for example at 17 mM (red dot), E_{NCX} shifts to a more negative value of -91 mV. This results in a driving force favouring its operation in the reverse mode, which is further augmented by plasma membrane depolarization (Fig. 1C; right). Hence in physiological conditions, NCX operates close to its reversal potential and, depending on the exact baseline $[\text{Na}^+]_i$ and membrane potential fluctuates between forward and reverse mode.

These calculations illustrate the intriguing dependence of NCX transport direction on $[\text{Na}^+]_i$ of astrocytes. Notably, baseline $[\text{Na}^+]_i$ of astrocytes of mouse hippocampus and neocortex as determined in different studies does cover this range: values between 12 and 17 mM were reported for cells in acute tissue slices as well as for cells in primary cultures (e. g. [40-48]). These measurements further corroborate the dynamic nature of NCX operation between two modes.

Imaging studies using pharmacological inhibitors of NCX [49] add to this reasoning. An established inhibitor of NCX is 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea (KB-R7943) [50, 51]. Exposure to KB-R7943 induced an increase in $[\text{Ca}^{2+}]_i$ in astrocytes of the olfactory bulb, hippocampus and in glioblastoma cells, suggesting that NCX worked in the forward mode extruding Ca^{2+} [52-54]. Conversely, astrocytes cultured from rat visual cortex and cerebellum responded with a decrease in $[\text{Ca}^{2+}]_i$ following administration of KB-R7943, indicating its operation in the reverse mode [44, 46]. The same phenomenon was observed in astrocytes in organotypic cell culture treated with the NCX inhibitor YM-244769 [55] (Fig. 2C).

1 Finally, others reported only a minor influence of NCX inhibition on baseline $[Ca^{2+}]_i$ in
 2 astrocytes [56]; this most likely happens when resting membrane potential roughly equals
 3 E_{NCX} . These conflicting (and yet internally consistent) results emphasize the notion that
 4 NCX in astrocytes at rest fluctuates between forward and reverse mode depending on
 5 $[Ca^{2+}]_i$, $[Na^+]_i$ and V_m .

6 A correct prediction of NCX transport direction in astrocytes is further
 7 complicated by the fact that the above-mentioned values for $[Na^+]_i$ represent bulk
 8 measurements from cell bodies. A recent study on HEK cells employing fluorescence
 9 lifetime imaging (FLIM) provided evidence that the Na^+ concentration ($[Na^+]$) of nuclei
 10 is significantly lower than that of the surrounding cytosol [57]. Moreover, a region with
 11 significantly higher 12 was detected around the nucleus, presumably reflecting higher
 12 $[Na^+]$ in peri-nuclear mitochondria [57]. While such information is not available for
 13 astrocytes yet, a former study is in line with such heterogeneity reporting that
 14 mitochondria in astrocytes have a significantly higher $[Na^+]$ than the cytosol (19 mM
 15 versus 13 mM) [43].

16 Furthermore, it is also unclear whether somatic $[Na^+]$ accurately reflects $[Na^+]$ in
 17 small processes or if perisynaptic processes close to synapses or perivascular endfeet may
 18 exhibit a different (probably higher?) $[Na^+]$. Experiments *in silico* indicate that
 19 perisynaptic microdomains represent distinct signalling compartments of astrocytes that
 20 favour Ca^{2+} influx through reverse NCX [58-61]. Similarly, endfeet were shown to
 21 exhibit slower diffusion dynamics than other astrocyte processes [62] and might maintain
 22 a $[Na^+]$ different from other parts of the cell.

Finally, earlier work also suggested the presence of a local ionic signalling compartment between the plasma membrane and the ER of astrocytes [34-36]. These findings imply that different sub-cellular astrocyte compartments may differ in their $[Na^+]_i$. This is even more so because Na^+ flux across the plasma membrane may predominately occur in nanodomains close to the plasma membrane [34] and thus escape conventional approaches for Na^+ detection such as fluorescence imaging with soluble chemical indicator dyes. As illustrated above, small differences in $[Na^+]_i$ in the mM range will significantly shift E_{NCX} , resulting in different modes of NCX operation (Fig. 1C).

In conclusion these considerations show that the transport direction of plasma membrane NCX in astrocytes at rest has a dynamic behaviour. This is largely due to the fact that it strongly depends on the $[Na^+]_i$, and switches between forward and reverse mode within a narrow range of $[Na^+]_i$ (12-17 mM). It is probably safe to assume that baseline $[Na^+]_i$ of astrocytes, and thus NCX transport direction, may vary in this range depending on the brain region or among different cells and/or cellular sub-compartments of one particular region. Of note, similar conclusion was made for cultured neocortical neurones, in which NCX “might concurrently operate in both the forward and the reverse direction, perhaps in different subcellular location” [63]. Therefore in astrocytes, NCX does fluctuate between transport directions driven by small fluctuations in $[Na^+]_i$ within distinct sub-cellular compartments. While at present there are no reports suggesting “spontaneous” $[Na^+]_i$ oscillations in astrocytes, neuronal activity triggers transient $[Na^+]_i$ increases thereby affecting NCX reversal as described below.

3.2. Activation of NCX in response to ionic signalling

3.2.1 Contribution of forward NCX to recovery of $[Ca^{2+}]_i$ transients

As elaborated above and schematically illustrated in Fig. 1C, the transport direction of NCX depends on both, Na^+ and the Ca^{2+} transmembrane gradients, as well as on the membrane potential of astrocytes. Fig. 1C also predicts that activity-related increases in $[\text{Ca}^{2+}]_i$ without concomitant increases in $[\text{Na}^+]_i$ result in an increase in the NCX inward current (and Na^+ influx) resulting from enhanced forward mode activity. At low $[\text{Na}^+]_i$, NCX therefore extrudes Ca^{2+} , contributing to the Ca^{2+} signals' recovery (Fig. 1A, 4A) [64]. In other words, NCX activity dampens astrocyte $[\text{Ca}^{2+}]_i$ transients, if these are not accompanied by substantial rise in $[\text{Na}^+]_i$.

Forward operation of NCX is indeed required for Ca^{2+} homeostasis in glioblastoma cells, which undergo increased Ca^{2+} -mediated cell death when treated with NCX inhibitors [52]. Such a role is similar to the proposed function of NCX in other tissues including skeletal muscle and heart as a “low-affinity-high-capacity” Ca^{2+} exporter [65].

3.2.2 Ca^{2+} influx by reverse NCX contributes to astrocyte $[\text{Ca}^{2+}]_i$ signalling

There is a wealth of experimental evidence obtained from astrocytes of different brain regions, both in culture and in acutely isolated tissue slices, demonstrating that neuronal activity, application of exogenous glutamate, agonists of ionotropic glutamate receptors or glutamate transporters result in a transient rise of astrocytic $[\text{Na}^+]_i$ [1, 66] (Fig. 2A). Moreover, it is established that increases in astrocyte $[\text{Na}^+]_i$ (with or without concomitant $[\text{Ca}^{2+}]_i$ signalling) switch NCX into the reverse mode, generating Ca^{2+} influx.

Na^+ -driven reversal of NCX occurs in response to Na^+ influx through various plasmalemmal ion channels. Reverse NCX for example contributed to astrocytic Ca^{2+}

elevations following mechanical stimulation [46, 67, 68] and augmented Ca^{2+} signals induced by application of low doses of glutamate and ATP [69]. Ca^{2+} influx through reverse NCX triggered glutamate release in cultured cortical astrocytes and was potentiated by their depolarization [70]. In Bergmann glial cells [71] and in astrocytes in culture [72], Ca^{2+} influx mediated by reverse NCX was demonstrated following the opening of AMPA receptor channels. In astrocytes in acute neocortical tissue slices, Na^+ entry through NMDA-receptors promoted NCX reversal, significantly prolonging accompanying $[\text{Ca}^{2+}]_i$ transients [40] (Fig. 2B). Finally, $[\text{Ca}^{2+}]_i$ elevations induced by the opening of channelrhodopsin-2 expressed in astrocytes were reported to be mainly due to Na^+ -dependent reversal of NCX [73].

Besides Na^+ influx through ion channels, significant $[\text{Na}^+]_i$ increases in astrocytes are induced by operation of Na^+ -dependent transporters. Particularly prominent Na^+ influx is mediated by the high-affinity-glutamate transporters EAAT1/SLC1A6 and EAAT2/SLC1A2 (known as GLAST and GLT-1 in experiments in rodents), which import 3 Na^+ together with glutamate [74, 75]. Activation of Na^+ -dependent glutamate uptake and accompanying $[\text{Na}^+]_i$ increases resulted in reversal of NCX and Ca^{2+} influx into astrocytes; the NCX-mediated Ca^{2+} influx was further amplified by Ca^{2+} -induced Ca^{2+} release from the endoplasmatic reticulum [76]. Ca^{2+} influx through reverse NCX following a $[\text{Na}^+]_i$ increase in response to activation of glutamate transporters was suggested to be a main source of $[\text{Ca}^{2+}]_i$ transients in fine astrocyte processes (Fig. 2C) [55, 77]. This NCX-mediated interplay between glutamate-transport-induced $[\text{Na}^+]_i$ increases and $[\text{Ca}^{2+}]_i$ signalling was proposed to result in the Ca^{2+} -dependent arrest of mitochondria in perisynaptic astrocyte processes close to active synapses [78, 79]. The

latter mechanism couples the synaptic release of glutamate and resulting astroglial Na^+ signals to the availability of ATP in astrocyte processes, and may, therefore, represent a key process in the neuro-glial metabolic coupling [80].

Similar to glutamate transport, uptake of GABA by astrocytes is coupled to the influx of Na^+ [81]. Because GABA is co-transported with only 2 Na^+ , activation of GABA transport produces smaller elevations in astrocyte $[\text{Na}^+]_i$ as compared to glutamate transporters [82, 83]. Nonetheless, experiments on hippocampal tissue slices provide convincing evidence that a $[\text{Na}^+]_i$ rise generated by operation of the astrocytic GABA transporter GAT-3/SLC6A11 reverses NCX, thus producing a $[\text{Ca}^{2+}]_i$ increase (Fig. 2D). This $[\text{Ca}^{2+}]_i$ elevation was claimed to trigger the release of ATP and adenosine from astrocytes, which activated adenosine receptors on nearby presynaptic terminals, resulting in a depression of glutamatergic synaptic input [53]. In the olfactory bulb, GABAergic signalling activates astrocyte GABA uptake and resulting $[\text{Na}^+]_i$ increases led to a NCX-mediated $[\text{Ca}^{2+}]_i$ influx sufficient to trigger Ca^{2+} -induced Ca^{2+} release [54]. GABA-related transient Na^+ elevations are thus directly translated into astrocyte Ca^{2+} signals in these two different systems.

Astroglial Na^+ -bicarbonate co-transporter NBCe1/SLC4A4 is another pathway generating $[\text{Na}^+]_i$ elevations [84, 85]. In the brainstem, NBCe1 is activated by extracellular acidification. In response to the resulting Na^+ entry, $[\text{Na}^+]_i$ increases switch NCX to reverse mode, generating Ca^{2+} signalling that facilitates the release of ATP from astrocytes. This Na^+ -triggered, Ca^{2+} -dependent release of ATP is involved in the adaptive response of the neuronal network to changes in the P_{CO_2} [86].

1 In addition to the above-mentioned transporters and channels, a multitude of other
2 pathways mediate Na^+ influx into astrocytes in response to neuronal activity [1, 87]. Na^+ -
3 influx through these channels/transporters results in accumulation of Na^+ , which is
4 prominent below the membrane and/or in sub-cellular compartments that exhibit a high
5 surface-to-volume-ratio [11]. Because of the intricate dependence of NCX operation on
6 $[\text{Na}^+]_i$, it is safe to assume that these Na^+ influx pathways will trigger secondary Ca^{2+}
7 influx through reverse NCX.

9 **4. Role of NCX under ischemic conditions**

10 The role of NCX under pathological conditions has been addressed in the context of
11 various neurological diseases including those related to glutamate excitotoxicity and
12 ischemic stroke [15, 88-90]. The specific involvement of the three isoforms in ischemic
13 injury was studied using different animal models.

14 Manipulations with expression of NCX1 clearly indicated that it serves a
15 protective role in brain ischemia [91-93]. The same is true for NCX2, because mice
16 lacking this isoform exhibit an increased vulnerability to cerebral ischemia [94] (Fig. 3A).
17 Suppression of the NCX3 gene in mice results in massive neuronal death and aggravates
18 brain damage after ischemia [93, 95] (Fig. 3B). In cerebellar granule neurones, Ca^{2+}
19 overload and excitotoxicity were increased by RNA interference to silence NCX3,
20 indicating an increased vulnerability in the absence of this transporter related to a
21 disturbance of intracellular ion homeostasis [96].

22 These and other studies suggest a generally neuroprotective role of NCX. Notably,
23 however, the neuroprotective effects do not seem to involve counteracting excitotoxic

cellular Ca^{2+} overloading. To the contrary, there is ample evidence for Na^+ -driven reversal of NCX, generating $[\text{Ca}^{2+}]_i$ elevations under ischemic conditions both *in situ* as well as *in vivo* [45, 76, 97-101]. Notably, while generating substantial Ca^{2+} influx during metabolic inhibition, reverse NCX also dampened Na^+ influx into astrocytes in tissue slices of mouse cortex [101] (Fig. 3C).

The reported beneficial effects of NCX in different models for ischemic stroke are thus likely to be related to its ability to export Na^+ and to counteract the cellular Na^+ loading and depolarization accompanying ischemia. Further studies, however, are required to address the specific role of astrocyte NCX under pathological conditions, using animal models with an astrocyte-specific deletion of NCX isoforms.

5. Conclusions

There is firm evidence for expression of NCX in astrocytes. Upon generation of astrocyte $[\text{Ca}^{2+}]_i$ signalling, NCX can serve as a Ca^{2+} exporter, but only if baseline $[\text{Na}^+]_i$ is low and in the absence of additional cellular Na^+ elevations (Fig. 4A). Because of its stoichiometry of 3 Na^+ to 1 Ca^{2+} , NCX reversal potential is rather close to the membrane potential and its transport direction largely governed by subtle changes in $[\text{Na}^+]_i$ and V_m . Indeed, small fluctuations (several mM) in $[\text{Na}^+]_i$ rapidly switch NCX between forward and reverse mode (or silence transport altogether when E_{NCX} equals V_m). Neuronal activity is accompanied by transient increases in astrocyte $[\text{Na}^+]_i$ resulting from activation of Na^+ -dependent transporters and/or channel-mediated Na^+ influx. There is overwhelming evidence that these $[\text{Na}^+]_i$ transients switch NCX into reverse mode, generating thus Ca^{2+} influx (Fig. 4B). In the light of this direct functional coupling

1 between the two ions, it seems appropriate to conclude that $[\text{Na}^+]_i$ transients in astrocytes,
2 directly translated by reverse NCX into $[\text{Ca}^{2+}]_i$ elevations, represent a new form of
3 cellular signalling, influencing astrocyte properties and playing a role in neuron-glia
4 interaction.

5

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Conflict of interest

The authors declare no conflict of interest and no competing financial interest.

Author contributions

All authors have contributed to the first version of the manuscript and approved the final article.

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Figure Legends

Figure 1. A: Schematic representation of an astrocyte expressing sodium/calcium-exchanger (NCX) predominantly on distal processes and endfeet. Two transport directions are highlighted: green represents the *forward* (Ca^{2+} export/ Na^+ import), red the *reverse* mode (Ca^{2+} import/ Na^+ export). The mitochondrial sodium/calcium/lithium-exchanger (NLCX) is highlighted in purple. **B:** Top: NCX1 positive astrocytes in subcortical layers (scale bar, 30 μm). Bottom left: NCX3 labelling of two astrocyte processes (asp; scale bar, 0.2 μm). Bottom right: NCX3 labelling in astrocytes of the CA1 region of the hippocampus at perivascular astrocyte processes (asp) opposed to a blood vessel (bv) (scale bar, 0.5 μm). **C:** Dependence of NCX current density on $[\text{Na}^+]_i$ and $[\text{Ca}^{2+}]_i$ at a resting membrane potential of -85 mV (left) and at -75 mV (right). White line indicates the reversal potential of NCX, defining the border between the *reverse* and *forward mode* of the exchanger. Green dots represent Na^+ concentration of 12 mM and Ca^{2+} concentration of 80 nM, red dots mark a sodium concentration of 17 mM. The colour scheme encodes the current amplitude per μm^2 . Taken from (with permission): B: [21] and C: modified from [40].

Figure 2: A: Cytosolic Na^+ signals induced by glutamatergic stimulation in acute cortical brain slices. Left: image of SR101 (top) and SBFI fluorescence (bottom) taken from layer 2/3 (L 2/3). Right: merge of SR101 and SBFI. Scale bars: 25 μm . Bottom: somatic $[\text{Na}^+]_i$ transients induced by synaptic stimulation (10 pulses/50 Hz), by focal pressure application of glutamate (1 mM/100 ms), and by NMDA-iontophoresis (50 mM/50 ms).

B: Top: $[Ca^{2+}]_i$ transients induced by NMDA-iontophoresis (50 mM/50 ms) in astrocyte processes. Bottom: Means $\pm$ S.E.M. of mono-exponential decay time constants (τ) of $[Ca^{2+}]_i$ changes in response to NMDA under control conditions, with KB-R7943 (KBR; left) or with SEA0400 (SEA; right) and after wash-out of the drugs. **: $0.001 \leq p < 0.01$; *: $0.01 \leq p < 0.05$. **C:** Ca^{2+} signalling in astrocyte processes as detected with a genetically-expressed calcium sensor. Effects of the glutamate transport blocker TFB-TBOA on Ca^{2+} signalling are shown in the top trace; the bottom trace illustrates the effect of YM-244769, a blocker of reverse mode NCX. GluT: glutamate transport. **D:** Top: Individual astrocytes loaded with Fluo-2. Centre: $[Ca^{2+}]_i$ transients in astrocytic processes induced by application of GABA. Bottom: in the presence of the NCX blocker KB-R7943, GABA-induced $[Ca^{2+}]_i$ transients are suppressed. Taken from (with permission): A, B: modified from [40]; C: modified from [55]; D: modified from [53].

Figure 3: Protective role of reverse mode NCX in ischemic conditions. **A:** Role of NCX knock-out. Top and left: Brains of NCX2^{-/-} (A) and wild-type mice (B), subjected to a middle cerebral artery occlusion. Right (C): Quantification of infarction volume. **B:** Brain sections of wild-type and congenic NCX3^{+/+}, NCX3^{+/-} and NCX3^{-/-}, subjected to a middle cerebral artery occlusion. The histogram shows the quantification of the infarct volume as compared with the ipsilateral hemisphere. **C:** Changes in $[Na^+]_i$ (**left**) and $[Ca^{2+}]_i$ (**right**) evoked by a 2-min period of chemical ischemia in control (black trace) and in the presence of KB-R7943 (red traces). The dotted blue lines show estimated Na^+ export (left) and Ca^{2+} import (right) through NCX. Taken from (with permission): A: [94]; B: [102], Copyright [2008] Society for Neuroscience; C: modified from [101].

Figure 4: Schematic illustration of NCX operating in *forward* or *reverse mode*. **A:** At low intracellular Na^+ concentrations (~ 12 mM) NCX operates in *forward mode* (indicated by green arrow/arrowheads). When Ca^{2+} rises in the cytosol, NCX expels Ca^{2+} , contributing to the recovery of Ca^{2+} signals. **B:** At higher $[\text{Na}^+]_i$ (~ 17 mM), induced by e.g. Na^+ -driven uptake of neurotransmitters (GluT or GAT), by Na^+ - HCO_3^- -co-transport (NBC), or by channel mediated Na^+ influx through ionotropic receptors (iGluR), acid-sensing ion channels (ASICs), purinoceptors (P2X) or transient receptor potential channels (TRP), NCX will operate in *reverse mode* (indicated by red arrow/arrowheads). This results in export of Na^+ , and import of Ca^{2+} . Reverse NCX thereby contributes to local Ca^{2+} signalling in astrocytes.

Aig.1

B

C

Fig.1

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A Glutamate-related sodium transients

sodium

B NCX reversal driven by NMDAR

C NCX reversal driven by GluT

D NCX reversal driven by GAT

Fig.2

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Fig.3

A NCX2 is neuroprotective

B NCX3 is neuroprotective

C NCX reversal during chemical ischemia
sodium

calcium

Fig.3

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Fig.4

forward mode

Low $[Na^+]_i$

B

reverse mode

increased $[Na^+]_i$

Fig.4

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