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Crosslink between calcium and sodium signalling

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Abstract

Transmembrane ionic gradients, which are an indispensable feature of life, are used for generation of cytosolic ionic signals that regulate a host of cellular functions. Intracellular signalling mediated by Ca^{2+} and Na^{+} is tightly linked through several molecular pathways that generate Ca^{2+} and Na^{+} fluxes and are in turn regulated by both ions. Transient receptor potential (TRP) channels bridge endoplasmic reticulum Ca^{2+} release with generation of Na^{+} and Ca^{2+} currents. The plasmalemmal Na^{+}–Ca^{2+} exchanger (NCX) flickers between forward and reverse mode to co-ordinate the influx and efflux of both ions with membrane polarization and cytosolic ion concentrations. The mitochondrial calcium uniporter channel (MCU) and mitochondrial Na^{+}–Ca^{2+} exchanger (NCLX) mediate Ca^{2+} entry into and release from this organelle and couple cytosolic Ca^{2+} and Na^{+} fluctuations with cellular energetics. Cellular Ca^{2+} and Na^{+} signalling controls numerous functional responses and, in the CNS, provides for fast regulation of astroglial homeostatic cascades that are crucial for maintenance of synaptic transmission.

KEYWORDS

astrocytes, Ca^{2+} signalling, mitochondria, mitochondrial calcium uniporter, Na^{+} signalling, Na^{+}–Ca^{2+} exchanger, neuropathology, transient receptor potential channels

1 | PRINCIPLES OF IONIC SIGNALLING

Transmembrane ionic gradients are an indispensable feature of life; a failure to maintain the intracellular ionic composition invariably instigates cell death. Preservation of transmembrane ion gradients consumes the major part of cellular energy recourses. It has been conjectured that the brain, for example, spends 75% of all ATP for restoration of Na^+–K^+ balance chronically disturbed by synaptic transmission and action potentials (Magistretti, 2009). How transmembrane ion gradients emerged early in evolution remains a matter of conjecture and speculation; most probably, the ionic composition of primeval cells was defined by the concentrations of ions in the primordial ocean (which arguably, contained few free Ca^{2+} ions), by selectivity of membrane aqueous pores (the ancestral ion channels) and by Donnan forces (Case et al., 2007; Chatton, Magistretti, & Barros, 2016; Donnan, 1911; Plattner & Verkhratsky, 2015, 2016).

In this review, we concentrate on the signalling links between Ca^{2+} and Na^+. In both excitable and non-excitable cells, fluxes of Na^+ and Ca^{2+} are co-ordinated by cationic channels and plasmalemmal and mitochondrial Na^+–Ca^{2+} exchangers. Steep transmembrane Na^+ gradients, in addition, provide the energy for plasmalemmal transport of many molecules, including other ions, neurotransmitters and amino acids.

New Findings

- **What is the topic of this review?**
  This paper overviews the links between Ca^{2+} and Na^+ signalling in various types of cells.

- **What advances does it highlight?**
  This paper highlights the general importance of ionic signalling and overviews the molecular mechanisms linking Na^+ and Ca^{2+} dynamics. In particular, the narrative focuses on the molecular physiology of plasmalemmal and mitochondrial Na^+–Ca^{2+} exchangers and plasmalemmal transient receptor potential channels. Functional consequences of Ca^{2+} and Na^+ signalling for co-ordination of neuronal activity with astroglial homeostatic pathways fundamental for synaptic transmission are discussed.

2 | SODIUM–CALCIUM EXCHANGER (NCX): THE KEY ELEMENT OF Na^+–Ca^{2+} SIGNALLING

2.1 | Why is NCX so important for Na^+ and Ca^{2+} signalling?

Although numerous ion channels and transporters affect the cytosolic concentration of Na^+ and Ca^{2+}, the Na^+–Ca^{2+} exchanger (NCX) is the only system that directly couples opposite fluxes of Na^+ and Ca^{2+} across the plasmalemma. This makes the NCX the key molecule integrating Na^+ and Ca^{2+} signalling pathways. The NCX proteins mediate an electrogenic exchange of three Na^+ for one Ca^{2+}, while the net ion flux can occur either in the forward (Ca^{2+} extrusion–Na^+ entry coupling) or in the reverse (Ca^{2+} entry–Na^+ extrusion) mode (Bers, 2002; Blaustein & Lederer, 1999; Khananshvili, 2014). Given that the NCX is electrogenic, membrane depolarization and increase in $[Na^+]_i$ promote the reverse mode of NCX, whereas hyperpolarization or elevated $[Ca^{2+}]_i$ favours the forward mode (Bers, 2002; Blaustein & Lederer, 1999; Kirischuk, Kettenmann, & Verkhratsky, 1997). Thus, from one side, the Na^+ and Ca^{2+} gradients tightly control the kinetics and directionality of NCX operation, whereas any changes in NCX activity dynamically affect Na^+ and Ca^{2+} fluxes. This complex feedback loop involves both thermodynamic and kinetic interactions of Na^+ and Ca^{2+} with ion transport and regulatory domains of NCX (Bers, 2002; Kardos, Héja, Jemnitz, Kovács, & Palkovits, 2017; Kirischuk, Parpura, & Verkhratsky, 2012; Oheim, Schmidt, & Hirrlinger, 2017).

2.2 | Forward and reverse modes of NCX: tightrope walking on the acrobat wire

Dynamic coupling of NCX regulation with Na^+ and Ca^{2+} signalling is particularly relevant in specific cells types (e.g. in astrocytes), where the reversal potential of NCX (E_{NCX}) is close to the resting membrane potential of the cell ($E_m \approx -80$ mV). As the ion-exchange turnover rates of mammalian NCXs are high ($\sim$5000 s^{-1}), even small changes in allosteric regulation of NCX (Bers, 2002; Blaustein & Lederer, 1999; Khananshvili, 2014) might instantly affect the coupled Na^+ and Ca^{2+} signalling (Kardos et al., 2017; Oheim et al., 2017; Robinson & Jackson, 2016). Even small anomalies in allosteric regulation of NCX or coupled ion-transport systems may have devastating outcomes (Boscia et al., 2016; Wagner, Maier, & Bers, 2015). For example, Na^+-driven uptake of neurotransmitters (e.g. GABA or glutamate) elevates $[Na^+]_i$, thus promoting Ca^{2+} entry through NCX operating in the reverse mode and increasing $[Ca^{2+}]_i$, with ensuing excitotoxicity (Boscia et al., 2016; Kirischuk et al., 1997; Rose & Verkhratsky, 2016). Moreover, when E_{NCX} and E_m are close, any small changes in the extracellular $[K^+]$ (for example, released from neurones during repolarization) may change membrane potential, thus affecting the rates and directionality of NCX.

2.3 | Regulatory diversity of NCX variants is structurally predefined

In mammals, three gene isoforms (*NCX1*, *NCX2* and *NCX3*) and their splice variants are expressed in a tissue-specific manner, whereas *NCX1* and *NCX3* (but not *NCX2*) undergo alternative splicing (Lytton, 2007; Philipson & Nicoll, 2000). *NCX1* is distributed ubiquitously, *NCX2* is mainly expressed in the brain and in the spinal cord, and

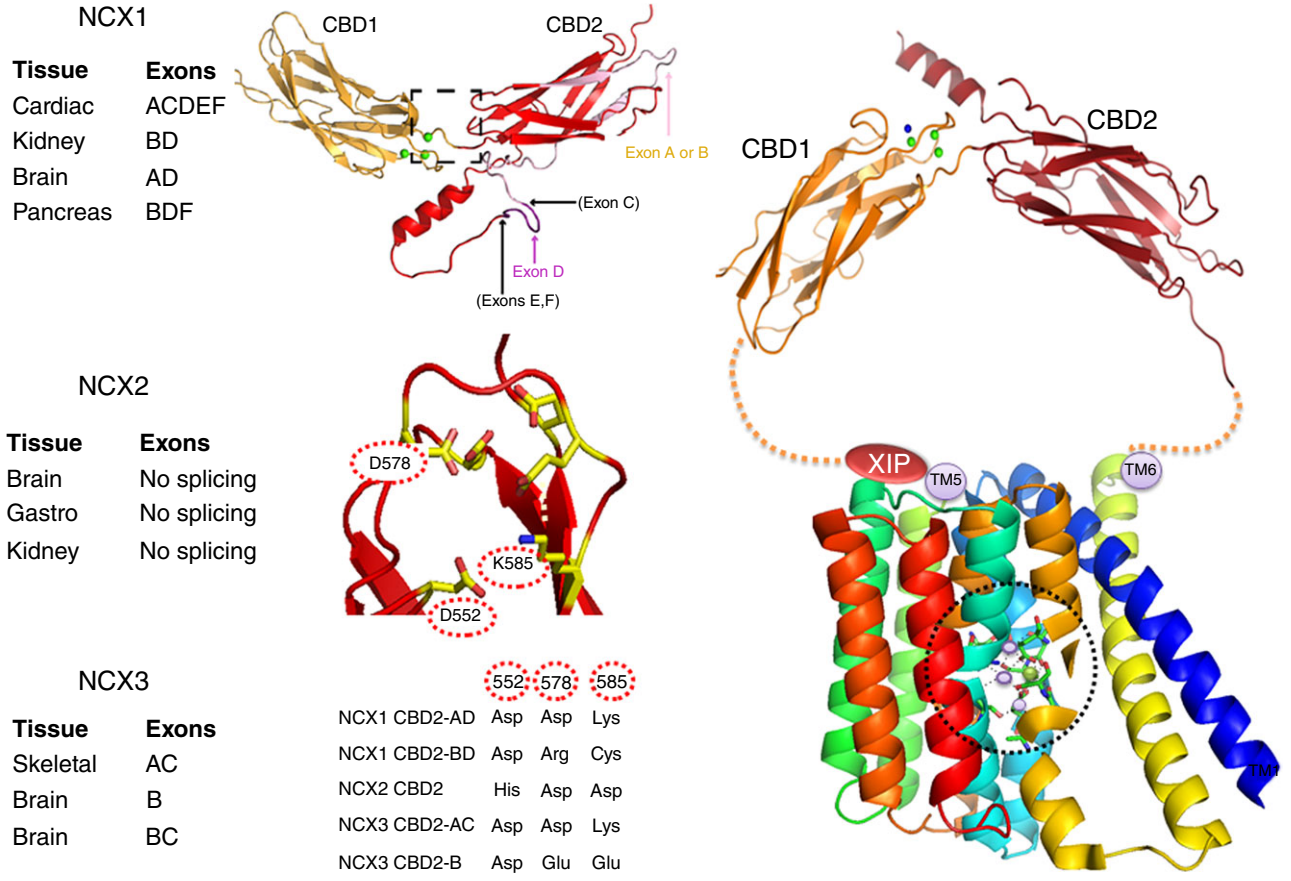

FIGURE 1 Isoform/splice variants of mammalian Na^+–Ca^{2+} exchanger (NCX). Mammalian NCXs contain 10 transmembrane helices (TM1–TM10), where two Ca^{2+}-binding regulatory domains (CBD1 and CBD2) are located between TM5 and TM6. The ion transport sites (encircled by dotted lines) are located ~80 Å away from the regulatory CBD domains. Ca^{2+} binding to CBD1 activates (up to 25-fold) NCXs, whereas Ca^{2+} binding to CBD2 alleviates Na^+-induced inactivation. In NCX1 and NCX3, the splice segment is exclusively located on CBD2, whereas NCX2 does not undergo splicing. In NCX1, splice variants arise from a combination of six small exons (A–F), whereas a mutually exclusive exon (either A or B) appears in every splice variant. Variants of NCX1 containing exon B cannot bind Ca^{2+} to CBD2 and are expressed in non-excitable tissues, whereas variants of NCX1 containing exon A bind two Ca^{2+} ions to CBD2 (allowing Ca^{2+}-dependent alleviation of Na^+-induced inactivation) and are expressed in excitable tissues. NCX3 contains only exons A, B and C. In contrast to NCX1, NCX3 containing exon A (AC) does not bind Ca^{2+} to CBD2 and is expressed in skeletal muscle, whereas variants of NCX3 containing exon B (B and BC) bind three Ca^{2+} ions to CBD2 and are expressed in neurones and glia. Residues in positions 552, 578 and 585 form the Ca^{2+}-binding sites at CBD2, thereby determining the number and affinity of Ca^{2+}-binding sites at CBD2. 'Naturally occurring' single-point substitutions at the three positions indicated diversify the Ca^{2+}-binding capacity at CBD2, thereby resulting in functional differences of exons A and B in NCX1 and NCX3. It would be interesting to test the effects of relevant substitutions of NCX variants in intact cells (e.g. in neurons and glia), with the goal of examining the functional specificity of contributions of NCX variants to Na^+ and Ca^{2+} signalling

NCX3 is found in the brain and in the skeletal muscle. NCX proteins comprise 10 transmembrane helices (TM1–TM10; Liao et al., 2012), where a cytoplasmic f-loop (between TM5 and TM6) contains two Ca^{2+}-binding regulatory domains, CBD1 and CBD2 (Hilge, Aelen, & Vuister, 2006; Figure 1). The CBD1 of all isoform/splice variants contains a high-affinity allosteric sensor ($K_d \approx 0.2\ \mu M$), with four Ca^{2+}-binding sites. The CBD2 domain contains a splicing segment, which controls the affinity (K_d = 2–200 μM) and the number (from zero to three) of Ca^{2+}-binding sites at CBD2 (Boyman, Mikhasenko, Hiller, & Khananshvili, 2009; Giladi et al., 2012, 2017; Giladi, Lee, Hiller, Chung, & Khananshvili, 2015; Lee et al., 2016; Tal, Kozlovsky, Brisker, Giladi, & Khananshvili, 2016). Cytosolic Na^+ inactivates NCX1 and NCX3 (but not NCX2) by interacting with yet unknown sites, located outside the CBDs (Giladi, Shor, Lisnyansky, & Khananshvili, 2016a; Giladi, Tal, & Khananshvili, 2016b; Khananshvili, 2016). Cytosolic Ca^{2+} binding to CBD1 activates (up to 25-fold) all mammalian NCXs, whereas Ca^{2+} binding to CBD2 alleviates Na^+-induced inactivation in NCX1 and NCX3 (Boyman et al., 2009, 2011; Giladi et al., 2012; Hilge et al., 2006). Notably, a lack of substantial and long-lasting Na^+ transients emphasizes that there is no need for Ca^{2+}-dependent alleviation of Na^+-dependent inactivation of NCX variants in neurones. In contrast, in glia the NCX1 and NCX3 variants undergo Na^+-induced inactivation, and only Ca^{2+} binding to CBD2 in specific variants can alleviate the activity. Given that NCX2 is insensitive to regulatory Na^+, this isoform (forming no splice variants) is capable of retaining its activity in glia during high-amplitude and prolonged Na^+ transients (Giladi et al., 2012, 2015, 2016a, 2016b; Khananshvili, 2016; Lytton, 2007; Philipson & Nicoll, 2000; Tal et al., 2016).

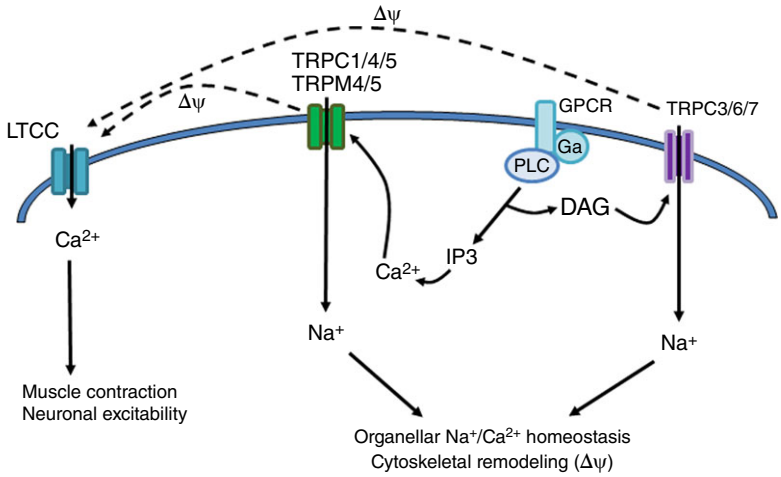

FIGURE 2 Transient receptor potential (TRP) channels mediate Ca^{2+} and Na^{+} entry. The TRP proteins mostly form non-selective channels that are activated by a wide range of intracellular mechanisms and contribute substantially to Na^{+} entry into the cytosol. Through membrane depolarization, TRP channels can control the activation of voltage-gated L-type Ca^{2+} channels and thus regulate physiological processes such as neuronal excitability and muscle contraction. Cytosolic signals contributed by TRP channels are likely to affect ionic homeostasis and the function of various internal organelles, such as mitochondria and lysosomes. Cell depolarization was proposed to regulate Rho proteins, which control cytoskeletal rearrangements that occur during cell migration or during intercellular permeability. Abbreviations: DAG, diacylglycerol; GPCR, G protein-coupled receptor; PLC, phospholipase C; TRPC, TRP canonical channels; LTCC, L-type Ca^{2+} channels; IP3, 1,4,5–trisphosphate; TRPM, TRP melastatin channels

2.4 | So many isoform/splice variants of NCX: are they functionally relevant?

In NCX1, alternative splicing arises from mixing of six small exons (A–F), where the mutually exclusive exons, A and B, are expressed in excitable and non-excitable tissues, respectively (Lytton, 2007; Philipson & Nicoll, 2000). In NCX3, alternative splicing involves only three exons (A, B and C), whereas both mutually exclusive exons, A and B, are expressed in neurones and glia (Giladi et al., 2015, 2017; Lee et al., 2016; Tal et al., 2016). Exons A and B differentially affect Ca^{2+} binding to CBD2 in NCX1 and NCX3 variants, which is controlled by single-point mutations at positions 552, 578 and 585 (Figure 1). More specifically, in NCX1 exon B prevents Ca^{2+} binding to CBD2, whereas in NCX3, exon A precludes Ca^{2+} binding to CBD2 (Tal et al., 2016). This also applies to NCX1 splice variants expressed in non-excitable tissues. For example, in the kidney (BD) and in the pancreas (BDF), splice variants of NCX1 do not bind Ca^{2+} at CBD2, because Ca^{2+}-dependent alleviation of Na^{+}-induced inactivation is not functionally relevant for these NCX variants (Giladi et al., 2012, 2016b).

2.5 | NCX variants differentially shape Na^{+} and Ca^{2+} signalling

In general, Na^{+}-dependent inactivation can transiently inactivate NCX to prevent a toxic amount of Ca^{2+} entry via NCX operating in the reverse mode (Giladi et al., 2016b; Khananshvili, 2016). For example, a massive and rapid entry of Na^{+} through Na^{+} channels and Na^{+}-dependent transporters can transiently elevate subplasmalemmal $[Na^{+}]_i$, which can instantly promote Ca^{2+} entry through the reverse mode of the NCX. This may occur if a given NCX variant lacks Na^{+}-dependent inactivation (e.g. NCX2 or NCX3-AC). This mechanism could be beneficial when a parallel increase in both $[Na^{+}]_i$ and $[Ca^{2+}]_i$ is required. At the same time, this may lead to Ca^{2+} and Na^{+} overload if the relevant regulatory systems fail to terminate this process. Different modes of regulation are expected for NCX1-AD, NCX3-B and NCX3-BC variants, because they are sensitive to Na^{+}-induced inactivation and thus can prevent substantial Ca^{2+} entry through NCX. Of note, NCX3-B and NCX3-BC can recover from Na^{+}-induced inactivation at much lower concentrations of cytosolic Ca^{2+} than NCX1-AD, because NCX3-B and NCX3-BC bind Ca^{2+} to CBD2 with much higher affinity than NCX1-AD (Giladi et al., 2012; Tal et al., 2016). Thus, four isoform/splice variants (NCX1-AD, NCX2, NCX3-B and NCX3-BC) expressed in neurones and glia have diverse and distinct regulatory properties, which are specifically suitable for dynamic coupling of Na^{+} and Ca^{2+} movements.

Another important factor that has to be taken into account for dynamic coupling of NCX regulation with Na^{+} and Ca^{2+} fluxes is that mammalian NCX variants show nearly 100-fold differences in slow dissociation of 'occluded' Ca^{2+} from the allosteric sensor that has a comparable affinity for Ca^{2+} binding (K_d = 0.2–0.5 μM) among NCX variants (Boyman et al., 2009; Giladi et al., 2012; Tal et al., 2016). Moreover, the observed differences in the dissociation kinetics of regulatory Ca^{2+} match the inactivation kinetics of NCX variants upon removal of cytosolic Ca^{2+} (Giladi et al., 2012, 2016b; Khananshvili, 2016; Tal et al., 2016). Only one isoform/splice variant is expressed in cardiac (NCX1-ACDEF) or skeletal (NCX3-AC) muscle, whereas a number of variants (NCX1-AD, NCX2, NCX3-B and NCX3-BC) coexist in neurones and neuroglia (Giladi et al., 2012, 2015; Tal et al., 2016). Moreover, the rate of regulatory Ca^{2+} dissociation (and thus, the rate of NCX inactivation) is ~10 times slower in the cardiac than in the skeletal variant (Giladi et al., 2015; Tal et al., 2016) and is ~10 times slower in the skeletal variant than in neuronal NCX variants (Tal et al., 2016). These 100-fold differences among NCX variants, expressed in

distinct excitable tissues, might have physiological relevance, because NCX in the cardiac and skeletal myocytes must clear up (extrude) very much more cytosolic Ca^{2+} [released from the sarcoplasmic reticulum (SR)] than in neurones (Giladi et al., 2016b; Khananshvili, 2016).

2.6 | Subcellular allocation of NCX variants: a crucial factor for integrating global events?

Computer-aided modelling reveals that NCX can significantly contribute to Ca^{2+}-induced Ca^{2+} release (CICR) in cardiomyocytes (Chu et al., 2016), when taking into account experimentally obtained parameters of allosteric regulation of cardiac NCX and high-resolution cell imaging of the subcellular NCX location in cardiomyocytes (Chu et al., 2016). Increasing the fraction of NCX in the dyad and peri-dyadic (PD) domains decreases the frequency and fidelity of Ca^{2+} sparks, as well as diastolic $[Ca^{2+}]_i$, although the amplitude and duration of Ca^{2+} sparks are less sensitive to NCX spatial redistribution. Moreover, NCX can promote Ca^{2+} entry into the dyad to trigger the SR Ca^{2+} release through ryanodine receptors at depolarized membrane potentials and with elevated local Na^+ concentrations. A relatively high fraction of NCX (~48%) in the dyadic and PD spaces (with a dyad-to-PD ratio of roughly 1:2) promotes the Ca^{2+}-dependent allosteric activation of NCX compared with the NCX molecules bordering the bulk cytosol. This site-confined allosteric activation of NCX can 'functionally localize' exchanger activity to the dyad and PD areas to reduce NCX-mediated Ca^{2+} extrusion from the bulk of the cytosol. This may protect the cell from acute loss of Ca^{2+} during diastole (and thus, from arrhythmia), because NCX can generate depolarizing currents through the forward mode in response to 'spontaneous' Ca^{2+} release from the SR.

3 | TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS: MAJOR CONTRIBUTORS TO CYTOSOLIC Na^+ AND Ca^{2+} SIGNALS

As a result of their unique ability to conduct both Na^+ and Ca^{2+}, TRP channels are particularly important for co-ordinating the signalling between the cell membrane and intracellular organelles. The TRP superfamily includes 28 members, which can be classified into six different families based on sequence homology (Earley & Brayden, 2015; Nilius, Voets, & Peters, 2005b). These families are as follows: seven canonical (TRPC), eight melastatin (TRPM), six vanilloid (TRPV), three mucolipin (TRPML), three polycystin (TRPP) and one ankyrin (TRPA) members. The seven TRPCs are called canonical because of their close homology to the founding member, *Drosophila* TRP (Trebak, Lemonnier, Smyth, Vazquez, & Putney, 2007; Vazquez, Wedel, Aziz, Trebak, & Putney, 2004). All TRP proteins have six transmembrane domains (S1–S6), with intracellular N- and C-termini. They form functional tetrameric channels in humans, with the exception of TRPC2, which is a pseudogene. However, TRPC2 is functionally expressed in rodents and plays a crucial role in pheromone sensing (Liman, Corey, & Dulac, 1999). Substantial controversy surrounds the role of TRP channels (and TRPC in particular) in encoding molecular components of the store-operated Ca^{2+} entry (SOCE) pathway, which is a ubiquitous Ca^{2+} entry route into cells activated downstream of phospholipase C-coupled receptors (Trebak & Putney, 2017). Store-operated Ca^{2+} entry is activated upon endoplasmic reticulum (ER) Ca^{2+} store depletion following inositol-1,4,5-trisphophate receptor ($InsP_3R$)-mediated Ca^{2+} release (Trebak & Putney, 2017). The discovery of Stromal Interacting Molecule (STIM) and ORAI proteins as the *bona fide* SOCE molecular components has cast a shadow of doubt on the role of TRPC proteins in SOCE (Feske et al., 2006; Liou et al., 2005; Roos et al., 2005; Vig et al., 2006; Zhang et al., 2006). STIM proteins act as Ca^{2+} sensors in the ER that perceive store depletion and link this depletion through direct protein–protein interactions to ORAI channels at the plasma membrane, thus instigating Ca^{2+} influx. TRPCs appear to be either concomitantly activated through phospholipase C-mediated production of second messengers, such as diacylglycerol-mediated activation of TRPC3/6/7 (Trebak, Vazquez, Bird, & Putney, 2003) or subsequently activated by Ca^{2+} microdomains generated by ORAI Ca^{2+}-selective channels (e.g. TRPC1/4/5; Trebak & Putney, 2017). At the same time, TRPC1/3/5 channels seem to be the main molecular component of SOCE in astroglia (Reyes, Verkhratsky, & Parpura, 2013; Verkhratsky & Parpura, 2014).

How does Na^+ conductance by TRP channels shape Ca^{2+} signalling in cell microdomains? For example, TRPM4 and TRPM5 channels are essentially impermeant to Ca^{2+} ions (Liman, 2007). These channels have relatively higher permeability to Na^+ compared with K^+ and mediate large inward currents at physiological membrane potentials and buffering conditions. Indeed, TRPM4 has profound depolarizing effects and, as such, can greatly alter Ca^{2+} influx. TRPM4 activity is regulated by micromolar concentrations of cytosolic Ca^{2+}, and this regulation is mediated by direct interaction of the Ca^{2+}–calmodulin complex with the C-terminal region of TRPM4. Therefore, these interactions are likely to occur at microdomains of high $[Ca^{2+}]_i$ near the plasma membrane or at the ER–plasma membrane junctions. TRPM4 channels were shown to be activated as a result of local Ca^{2+} release from the ER via the $InsP_3R$ (Gonzales & Earley, 2012; Gonzales, Garcia, Amberg, & Earley, 2010). TRPM4-mediated phosphorylation by protein kinase C was also shown to enhance the sensitivity of TRPM4 to activation by Ca^{2+} (Nilius et al., 2005a). Na^+ signals generated by TRPM4 channels have been shown to impinge on a wide range of physiological functions (Fig. 2), including the membrane excitability of neurones, pancreatic β-cells, smooth muscle cells from bladder and different vascular beds, as well as atrial cardiomyocytes (Mathar et al., 2014). Furthermore, TRPM4 is crucial for the maintenance of membrane potential in non-excitable cells, such as dendritic and haematopoietic cells. Dendritic and mast cells from TRPM4 knockout mice show membrane hyperpolarization owing to a lack of depolarizing TRPM4 Na^+ currents, which enhances the driving force for Ca^{2+} entry into the cytosol, causes Ca^{2+} overload and alters cellular function (Mathar et al., 2014).

Another example is endothelial permeability to G protein-coupled receptor agonists, which depends on TRPC channels, mainly TRPC1, TRPC4 and TRPC6 (Shinde et al., 2013). An increase in $[Ca^{2+}]_i$ was proposed to activate the endothelial actomyosin cytoskeleton to induce stress fibre formation and inter-endothelial cell gaps. However,

subsequent studies showed that endothelial SOCE is mediated by STIM1 and ORAI1 independently of TRPC1, TRPC4 or TRPC6 (Abdullaev et al., 2008). Knockdown of ORAI1 or pharmacological abrogation of plasmalemmal Ca^{2+} entry and Ca^{2+} release from the ER did not affect the ability of G protein-coupled receptor agonists to modulate the permeability of human microvascular endothelial cells (Stolwijk et al., 2016; Stolwijk, Matrougui, Renken, & Trebak, 2015). These findings suggest that the role of TRPC in modulating endothelial permeability could be mediated by Na^+ entry through these channels. Therefore, although it is evident that TRP channels contribute to Ca^{2+} signals that control a wide variety of physiological functions, and although it remains challenging to separate the contributions of Ca^{2+} *versus* Na^+ signals generated by TRP channels, Na^+ entry through TRP channels is likely to be a major contributor to many, if not all, of these functions. Unfortunately, dyes and protocols for Na^+ measurements within cellular compartments with high temporal and spatial resolution are, as yet, not available. Achieving such measurements will shed light on the contribution of Na^+ to cell signalling and function and would enhance our knowledge of the mechanisms of physiology and disease.

4 | Na^+ AND Ca^{2+} SIGNALLING IN REGULATION OF MITOCHONDRIAL FUNCTION

4.1 | Molecular physiology of mitochondrial Ca^{2+} and Na^+ signalling

Mitochondrial Ca^{2+} cycles are linked to several crucial processes. During physiological cell stimulation, Ca^{2+}-binding proteins located at the outer mitochondrial membrane sense cytosolic gradients of Ca^{2+} and move the organelle on the cytoskeletal network rail towards cell regions of intense Ca^{2+} signalling, the so-called Ca^{2+} hotspots near the ER and plasma membrane (Pizzo, Drago, Filadi, & Pozzan, 2012; Rizzuto & Pozzan, 2006). By regulating local Ca^{2+} concentrations at these regions, mitochondria can control the activity of multiple ER and plasma membrane channels that are strongly and allosterically modulated by Ca^{2+} (Giacomello et al., 2010) and thereby contribute to global cellular Ca^{2+} signals (Tinel et al., 1999). Mitochondria also link cellular Ca^{2+} signalling to energy production, triggered by the stimulatory actions of Ca^{2+} on at least three enzymes of the Krebs cycle and F_0F_1 ATPase (Szabadkai & Duchen, 2008, 2009). Such 'coupling' by Ca^{2+} represents an effective feedforward mechanism to meet rising energy demand in signalling cells (Hajnoczky, Robb-Gaspers, Seitz, & Thomas, 1995; Jouaville, Pinton, Bastianutto, Rutter, & Rizzuto, 1999). However, mitochondrial Ca^{2+} signalling also has a dark side. Although the elevation of Ca^{2+} in the mitochondrial matrix plays essential physiological roles if transient, when sustained it leads to Ca^{2+} overload that, in conjunction with oxidative stress, represents a primary trigger of cell death (Duchen, 2000). Mitochondrial Ca^{2+} overload is often linked to impaired mitochondrial Ca^{2+} handling by both Ca^{2+} influx and efflux machinery and represents a hallmark event of ischaemic and neurodegenerative disorders (Gandhi et al., 2009). A major breakthrough in understanding the link between mitochondrial Na^+ and Ca^{2+} signalling came in 2010, when the gene for the mitochondrial Na^+–Ca^{2+} exchanger was discovered. It was found to be a member of the NCX superfamily and was termed NCLX (Li^+-permeable Na^+–Ca^{2+} exchanger; Palty et al., 2010). In parallel, the components of the mitochondrial Ca^{2+} uniporter complex (the MCU) were identified (Perocchi et al., 2010; Baughman et al., 2011; De Stefani, Raffaello, Teardo, Szabo & Rizzuto, 2011).

4.2 | The *in vivo* pathophysiological role of MCU and NCLX-dependent Ca^{2+} signalling

Given that mitochondrial Ca^{2+} homeostasis regulates many essential aspects of cellular physiology, one could easily expect that loss-of-function mutations or genetic ablation of Ca^{2+} influx and efflux machineries would be incompatible with life. Knockout mouse models of two essential subunits of the uniporter, MCU and essential MCU regulator (EMRE), generated by the International Mouse Phenotype Consortium, confirm that a complete block of mitochondrial Ca^{2+} uptake in the C57BL/6 strain leads to embryonic lethality. Surprisingly, $MCU^{+/-}$ and $EMRE^{+/-}$ heterozygous mice show different phenotypes. The former manifests a decreased cardiac stroke volume, abnormal fasting glucose concentration and amount of fat, whereas the latter shows neurological defects and impairment of blood glucose homeostasis. In contrast, $MCUB^{-/-}$ homozygous mice, which lose dominant-negative regulation of MCU, are viable but show vestibular impairment and ECG defects. Unexpectedly, the constitutive knockout of MCU in a mixed C57BL/6 × CD1 mouse background resulted in viable embryos and, with the exception of mild muscle weakness, the mice lacked any significant phenotype (Herzig, Maundrell, & Martinou, 2013). It remains unclear how the genetic background has such a profound impact, but it is possible that some type of compensation (e.g. the activation of alternative routes for mitochondrial Ca^{2+} uptake) could take place. This concept is supported by results obtained in a heart-specific transgenic mouse model constitutively expressing a dominant-negative MCU isoform (DN-MCU; Rasmussen et al., 2015; Wu et al., 2015). Although cardiac mitochondria from DN-MCU mice are unable to take up Ca^{2+}, MCU appears to be dispensable for normal heart function and comes into play only during physiological stress, such as during an isoprenaline-induced fight-or-flight response. Conversely, a conditional MCU knockout model in adult mice lacked any fight-or-flight response and had no major defects both in normal conditions and after cardiac pressure overload induced by transverse aortic constriction (Kwong et al., 2015; Luongo et al., 2015).

In contrast to the mild phenotype of MCU knockout, a conditional knockout of NCLX led to rapid and fatal heart failure (Luongo et al., 2017). Lethality was correlated with severe myocardial dysfunction. The cardiac pathology was largely attributed to mitochondrial Ca^{2+} overload, leading to enhanced generation of superoxide and necrotic cell death, which was prevented, in part, by genetic inhibition of mitochondrial permeability transition pore activation. In contrast, overexpression of NCLX in the mouse heart augmented mitochondrial Ca^{2+} clearance, and like MCU knockout it conferred protection against ischaemia-induced cardiomyocyte necrosis and heart failure (Luongo et al., 2017).

4.3 | The physiological implication of cytosolic and mitochondrial Na^+ signalling

One consequence of a 3 Na^+:1 Ca^{2+} stoichiometry of NCLX is that NCLX is a high-capacity mitochondrial Na^+ transporter and therefore the major mitochondrial route of Na^+ influx (Maack et al., 2006). Another important functional feature of NCLX, linking it to global cellular Na^+ signalling, is that the apparent K_m to cytosolic Na^+ of ~10 mM is very close to resting cytosolic Na^+ free concentrations. Hence, even a slight change in cytosolic Na^+ will strongly modulate NCLX activity. Thus, NCLX is highly tuned to interact functionally with the plasma membrane Na^+ influx pathways, responding to changes in the cytosolic Na^+ concentration (Figure 3). Several studies underscore the importance of the Na^+ signalling interaction of NCLX and the plasma membrane transporters. In pancreatic β-cells, glucose-dependent depolarization leads to cytosolic Na^+ influx via the voltage-gated Na^+ channel (Sekler, Hershfinkel, & Nita, 2013). This cytosolic Na^+ wave is propagated to the mitochondria by NCLX, which is strongly activated by this cytosolic Na^+ surge and maintains the mitochondrial Ca^{2+} level required for the metabolic activity used for ATP production that controls insulin secretion (Nita et al., 2014). The Na^+,K^+-ATPase that controls the cytosolic Na^+ concentrations participates in this Na^+ crosstalk between the plasma membrane and the mitochondria.

Another example of Na^+ signalling crosstalk is the interaction of NCLX with the nociceptive noxious heat-activated receptor, TRPV1. This receptor evokes membrane Na^+ and Ca^{2+} fluxes, thereby triggering the depolarization required for activation of nociceptive neurones. Recent studies show that TRPV1-dependent Na^+ fluxes are communicated to the mitochondria by NCLX (Kim & Usachev, 2009; Nita et al., 2016). They are required for activation of mitochondrial Ca^{2+} signalling, which in turn controls cytosolic Ca^{2+}, which allosterically regulates TRPV1 conductance (Nita et al., 2016).

Finally, SOCE is the major Ca^{2+} influx pathway in non-excitable cells. It is activated by the ER Ca^{2+} store, which harbours the Ca^{2+} sensor STIM1, which in turn dimerizes, physically interacts and activates the cell membrane Ca^{2+} channel. A recent study suggested that Na^+ influx triggered by Ca^{2+} store depletion controls communication with the mitochondria by activating NCLX. In support of this model, omission of extracellular Na^+, which prevents the rise in cytosolic Na^+, inhibits NCLX activity. Activation of NCLX by Na^+ flux evoked a mitochondrial redox response, required for preventing ORAI1 inactivation by oxidation of its redox-sensitive cysteine residue (Ben-Kasus Nissim et al., 2017).

5 | ASTROGLIAL Na^+ AND Ca^{2+} SIGNALLING AND NEUROTRANSMISSION

Astroglial Ca^{2+} signalling is generally acknowledged to be a substrate for astrocyte excitability (Agulhon et al., 2008; Bazargani

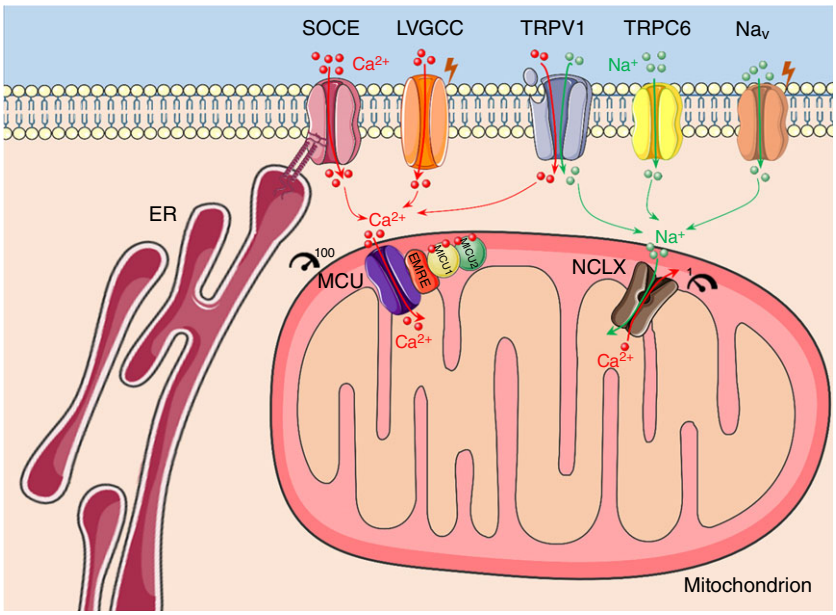

FIGURE 3 Crosstalk of Na^+ and Ca^{2+} signalling between the cell membrane and the mitochondria. Influx of Ca^{2+} into cells is mediated by multiple cell membrane pathways, among which are the store-operated Ca^{2+} pathway (SOCE), the L-type voltage-gated Ca^{2+} channel (LVGCC) and the cationic channel TRPV1. Influx of Ca^{2+} into cells is followed by rapid Ca^{2+} influx into the mitochondria, mediated by the mitochondrial Ca^{2+} uniporter (MCU). Dynamic complexing of the MCU with EMRE MICU1 and MICU2 controls the affinity and intensity of Ca^{2+} influx via the MCU. Mitochondrial Ca^{2+} influx by the MCU is followed by a ~100-fold slower Ca^{2+} efflux through the rate-limiting mitochondrial Na^+–Ca^{2+} exchanger, NCLX. The removal of mitochondrial Ca^{2+} by NCLX is upregulated by (i) an increase in mitochondrial Ca^{2+} triggered by MCU, and (ii) an increase in cytosolic Na^+ mediated by TRP channels or by the voltage-sensitive Na^+ channel (Na_v). As a result of the low affinity of NCLX for cytosolic Na^+, cytosolic Na^+ influx is essential for its activation. This Ca^{2+} and Na^+ signalling between the cell membrane and mitochondria is linked to multiple physiological processes ranging from insulin secretion to pain sensation. Impaired mitochondrial MCU or NCLX activity leads to mitochondrial Ca^{2+} overload, which is a major cause of ischaemic or neurodegenerative damage. Abbreviation: ER, endoplasmic reticulum

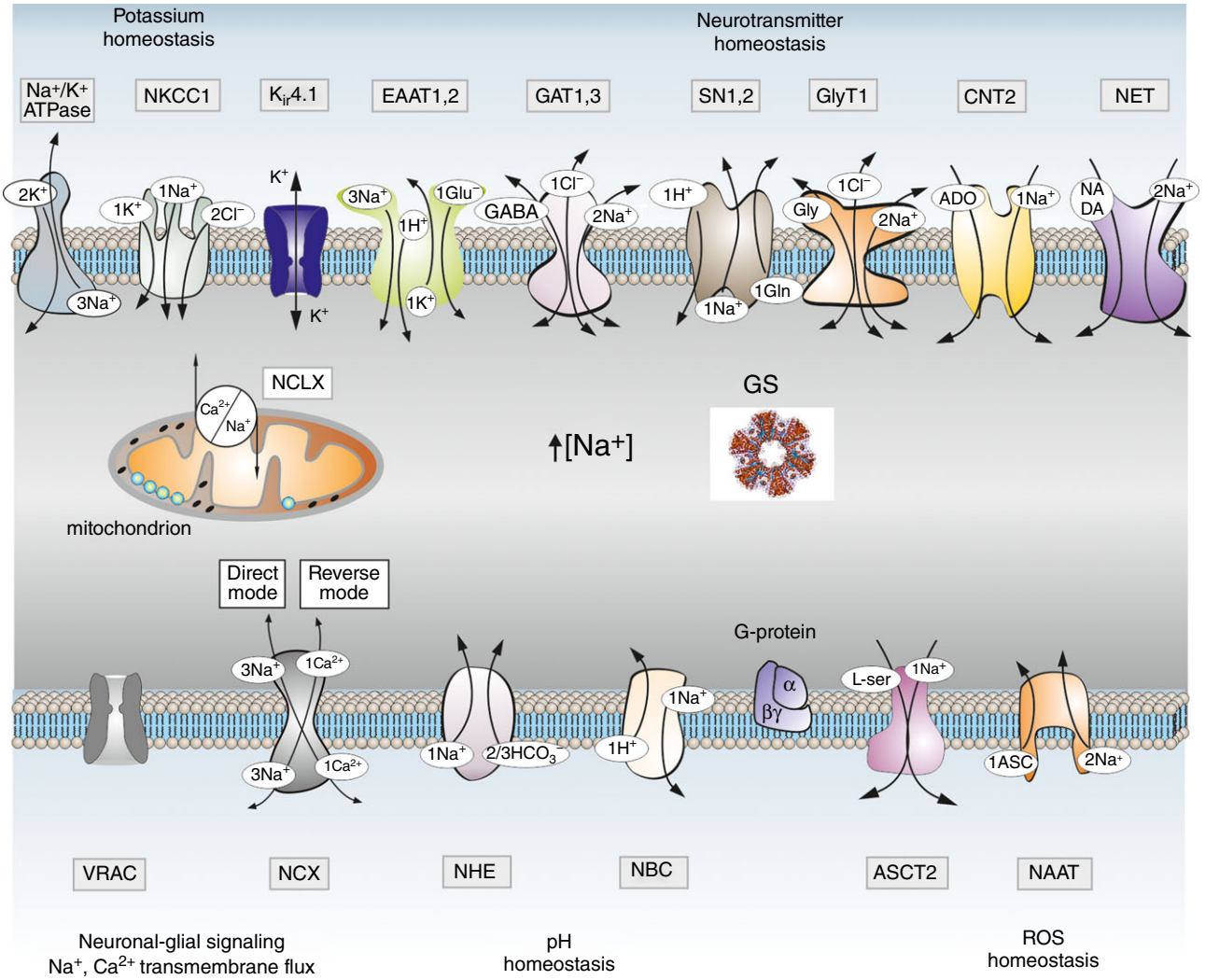

FIGURE 4 Astroglial Na^+ signals regulate homeostatic functions. Abbreviations: ASCT2, alanine–serine–cysteine transporter 2; ASIC, acid-sensing ion channels; CNT2, concentrative nucleoside transporters; EAAT, excitatory amino acid transporters; ENaC, epithelial sodium channel; GAT, GABA transporter; GS, glutamine synthetase, GlyT1, glycine transporter; NAAT, Na^+-dependent ascorbic acid transporter; NBC, Na^+–HCO_3^- cotransporter; NCLX, mitochondrial Na^+–Ca^{2+} exchanger; NCX, Na^+–Ca^{2+} exchanger; NET, noradrenaline transporter; NHE, Na^+–H^+ exchanger; NKCC1, Na^+–K^+–Cl^- cotransporter; SN1,2, sodium-coupled neutral amino acid transporters, which underlie exit of glutamine; VRAC, volume-regulated anion channels

& Attwell, 2016; Cornell-Bell, Finkbeiner, Cooper, & Smith, 1990; Finkbeiner, 1992; Parpura, Grubisic, & Verkhratsky, 2011; Verkhratsky & Kettenmann, 1996; Verkhratsky, Orkand, & Kettenmann, 1998; Verkhratsky, Rodríguez, & Parpura, 2012; Volterra, Liaudet, & Savtchouk, 2014). Organization of astroglial Ca^{2+} signalling is complex and spatially compartmentalized, with both plasmalemmal and intracellular channels and transporters contributing to global and local cytosolic Ca^{2+} dynamics (Rusakov, 2015; Shigetomi, Patel, & Khakh, 2016). In astroglial processes, and especially in distal processes, which form the synaptic cradle (Verkhratsky & Nedergaard, 2014), Ca^{2+} signals occur in a form of localized microdomains, which are sometimes associated with morphological structures, such as 'appendages' in Bergmann glial cells (Grosche et al., 1999) or perisynaptic leaflets (Shigetomi et al., 2016). The molecular pathways underlying these local forms of Ca^{2+} signalling involve ER Ca^{2+} release mediated by all three types of $InsP_3$ receptors ($InsP_3R1/2/3$; Di Castro et al., 2011; Kirischuk, Kirchhoff, Matyash, Kettenmann, & Verkhratsky, 1999; Sherwood et al., 2017) as well as plasmalemmal Ca^{2+} influx. Plasmalemmal Ca^{2+} entry is mediated by ionotropic receptors (NMDA and AMPA glutamate receptors, $\alpha 7$ acetylcholine receptors or $P2X_{1/5}$ or $P2X_7$ purinoceptors (Illes, Verkhratsky, Burnstock, & Franke, 2012; Lalo, Pankratov, Parpura, & Verkhratsky, 2011; Sharma & Vijayaraghavan, 2001; Verkhratsky & Kirchhoff, 2007), TRP channels (Shigetomi, Tong, Kwan, Corey, & Khakh, 2012; Verkhratsky, Reyes, & Parpura, 2014) and NCX operating in reverse mode (Verkhratsky et al., 2012). Plasmalemmal Ca^{2+} entry is particularly important for generation of Ca^{2+} signals in perisynaptic astroglial processes, which are generally devoid of intracellular organelles, including the ER (Verkhratsky & Nedergaard, 2014).

Incidentally, all these plasmalemmal pathways are associated with generatiof of Na^+ fluxes. All astroglial ionotropic receptors, although having relatively high Ca^{2+} permeability ($P_{Ca}/P_{monovalent}$ varies from

one for AMPA receptors, two to three for NMDA and $P2X_{1/5}$ receptors, approximately six for α7 ACh receptors and >10 for $P2X_7$ receptors; Palygin, Lalo, Verkhratsky, & Pankratov, 2010; Pankratov, Lalo, Krishtal, & Verkhratsky, 2009), mostly produce Na^+ currents (reflecting a much higher concentration of Na^+ in the extracellular space). Likewise, all TRP channels expressed in astrocytes (TRPV1, TRPA1 and PTRC1/3/5) are Na^+–Ca^{2+} channels (Reyes et al., 2013; Verkhratsky et al., 2014). Astroglial TRPC channels are the main part of the store-operated mechanism (Verkhratsky & Parpura, 2014); hence, they link Na^+ influx to the depletion of the ER Ca^{2+} store (Verkhratsky et al., 2014). Another major pathway for Na^+ influx into astrocytes is associated with operation of EAAT1/2 glutamate transporters that couple translocation of single glutamate molecule with influx of three Na^+ and one H^+ ion and countertransport of one K^+ ion; this stoichiometry makes the transporter electrogenic and generates a substantial Na^+ influx that may elevate $[Na^+]_i$ by 10–20 mM (Kirischuk, Kettenmann, & Verkhratsky, 2007; Rose & Verkhratsky, 2016). Astroglial NCX fluctuates between forward and reverse mode (because E_{NCX} is close to astroglial V_m) depending on $[Na^+]_i$ and changes in membrane potential. Finally, the extrusion of Na^+ and maintenance of resting cytosolic Na^+ concentration (which ranges between 15 and 20 mM, thus being almost two times higher than in neurones (Rose & Verkhratsky, 2016)) is mediated predominantly by the sodium-potassium ATPase, NKA. Resting $[Na^+]_i$ may also be influenced by the activity of the Na^+–K^+–Cl^- transporter NKCC1 and the Na^+–bicarbonate (NBC) transporter (Kelly, Kafitz, Roderigo, & Rose, 2009).

Powerful Na^+-transporting machinery expressed in the astroglial membrane underlies the generation of relatively large and long-lasting $[Na^+]_i$ transients upon stimulation. These astroglial Na^+ signals have been observed in astrocytes in cell culture and in brain slices in response to physiological stimulation (application of neurotransmitters or neuronal activity; Kirischuk et al., 2007, 2012; Rose & Karus, 2013; Rose & Ransom, 1996; Rose & Verkhratsky, 2016). Astroglial $[Na^+]_i$ transients last for tens and hundreds of seconds, which may indicate either long-lasting Na^+ entry or dynamic buffering by binding/unbinding to Na^+-dependent transporters abundantly populating perisynaptic astroglial membranes, or reflect other yet uncharacterized mechanisms (Rose & Verkhratsky, 2016). Cytoplasmic astroglial Na^+ signals can also propagate through the astroglial syncytia with a speed of ~60 μm s^{-1}; this propagation occurs through diffusion via connexin30 (CX30) or connexin43 (Cx43) gap junctional channels (Langer, Stephan, Theis, & Rose, 2012).

Sodium signalling regulates astroglial homeostatic function mainly through direct control of plasmalemmal solute carrier (SLC) transporters (Figure 4). Astroglial Na^+-dependent transporters mediate an uptake of glutamate (through EAAT1/2), GABA (GAT1/3), glycine (GlyT1), noradrenaline and dopamine (through the noradrenaline transporter, NET) and adenosine (through concentrative nucleoside transporters, CNT2). Intracellular Na^+ also regulates the main component of glutamate–glutamine shuttle, the glutamine synthetase (which converts glutamate to glutamine) and Na^+-coupled neutral amino acid transporters SN1/2, which export glutamine. Sodium signals also contribute to the regulation of K^+ buffering by affecting NKA transport; cytosolic Na^+ also controls Na^+–H^+ and Na^+–HCO_3^- transporters, thus contributing to the regulation of pH. By directly defining the reversal potential of NCX, astroglial $[Na^+]_i$ controls the operational mode of NCX and contributes to Ca^{2+} signalling. Finally, $[Na^+]_i$ is linked to metabolism, through initiation of glycolysis and lactate production (for detailed account and further references, see Chatton et al., 2016; Kirischuk et al., 2012; Kirischuk, Heja, Kardos, & Billups, 2016; Rose & Verkhratsky, 2016). To conclude, astroglial Na^+ signalling represents the fast co-ordination of neuronal activity with the 'homeostatic' response of astroglia mediated through Na^+-dependent transporters, concentrated in perisynaptic processes.

6 | CONCLUSIONS

Intracellular signalling mediated by Ca^{2+} and Na^+ operates in the majority of cells. Several molecular cascades co-ordinate movements of these two ions to regulate cellular functions. Despite the emerging importance of Na^+ signalling in various cell types, the number of studies describing cellular and subcellular Na^+ dynamics, when compared with Ca^{2+}, is minimal. A major obstacle for further advancement in the field is the lack of good cytosolic and organellar Na^+ reporters. There are no known green fluorescent protein-based Na^+ reporters. Sadly, the production of the only fluorescent mitochondrial Na^+ reporter, Corona Red, was recently discontinued, and the spectral properties of the few available cytosolic Na^+ probes are far from being ideal, posing a serious technical challenge to investigators in the field. It is our hope that the exiting new insights gained on about Na^+ signalling will lead to the development of such tools.

AUTHOR CONTRIBUTIONS

All authors participated equally in writing the paper. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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