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**Review**

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# Mitochondrial $Ca^{2+}$ handling as a cell signaling hub: lessons from astrocyte function

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## Abstract

**Astrocytes are a heterogenous population of macroglial cells spread throughout the central nervous system with diverse functions, expression signatures, and intricate morphologies. Their subcellular compartments contain a distinct range of mitochondria, with functional microdomains exhibiting widespread activities, such as controlling local metabolism and $Ca^{2+}$ signaling. $Ca^{2+}$ is an ion of utmost importance, both physiologically and pathologically, and participates in critical central nervous system processes, including synaptic plasticity, neuron-astrocyte integration, excitotoxicity, and mitochondrial physiology and metabolism. The mitochondrial $Ca^{2+}$ handling system is formed by the mitochondrial $Ca^{2+}$ uniporter complex (MCUc), which mediates $Ca^{2+}$ influx, and the mitochondrial $Na^{+}/Ca^{2+}$ exchanger (NCLX), responsible for most mitochondrial $Ca^{2+}$ efflux, as well as additional components, including the mitochondrial permeability transition pore (mtPTP). Over the last decades, mitochondrial $Ca^{2+}$ handling has been shown to be key for brain homeostasis, acting centrally in physiopathological processes such as astrogliosis, astrocyte-neuron activity integration, energy metabolism control, and neurodegeneration. In this review we discuss the current state of knowledge of the mitochondrial $Ca^{2+}$ handling system molecular composition, highlighting its impact on astrocytic homeostasis.**

## 1. Introduction

Astrocytes are a macroglial cell and one of the most abundant cell types in the brain. They consist of a heterogenous population spread throughout the central nervous system with specific morphologies, functions, and expression signatures (Khakh, Deneen 2019). These star-shaped cells are morphologically intricate, consisting of a cell soma that forms branches, branchlets, leaflets and, ultimately, endfeet (Aboufares El Alaoui et al 2021). Each of which subcellular compartments contributes toward particular cellular functions and interactions with the extracellular space and other cells, and has specific structures and organelle distributions (Aboufares El Alaoui et al 2021). Of note, astrocytes present diverse mitochondrial populations distributed throughout (Aboufares El Alaoui et al 2021), in functional microdomains with distinct activities, such as controlling local metabolism and $Ca^{2+}$ signaling (Agarwal et al 2017) (Fig. 1).

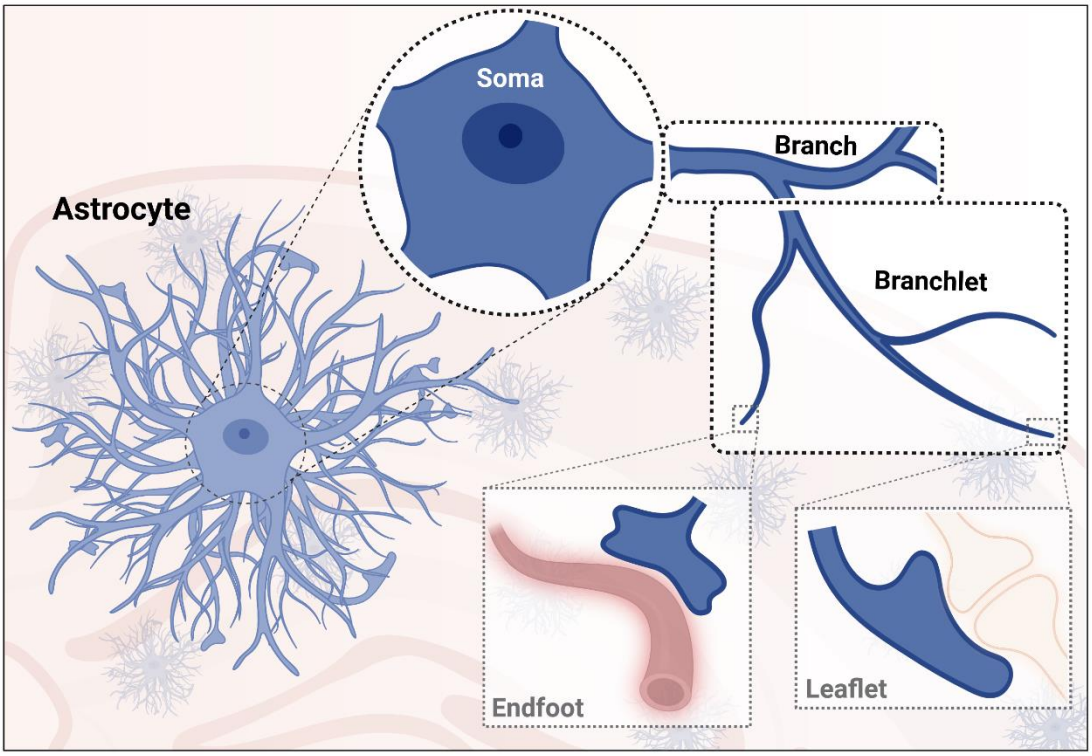


**Figure 1. Astrocyte morphology and subcellular compartments.** General schematic illustration of a protoplasmic astrocyte, depicting its cell body (soma) ramifying into branches, branchlets and, ultimately, endfeet and leaflets in close contact with brain vessels and synapses, respectively (further discussed by Khakh, Deneen 2019).

$Ca^{2+}$ is an ion of seminal importance in cellular homeostasis, both physiologically (Kawamoto et al 2012) and pathologically (Cabral-Costa, Kowaltowski 2020). It is a critical second messenger of key processes in the central nervous system, such as neurovascular coupling (Lourenço, Laranjinha 2021), synaptic plasticity (Kawamoto et al 2012), and neuron-astrocyte integration between excitability and function (Oliveira, Araque 2022). $Ca^{2+}$ is also involved in mitochondrial redox balance and the development of mitochondrial permeability transition (Vercesi et al 2018), participating in the induction of excitotoxicity (Amigo et al 2017) and cell death (Vercesi et al 2018).

$Ca^{2+}$ also evolved as an indicator for energetic demands. Increases in intramitochondrial $Ca^{2+}$ levels can boost tricarboxylic acid cycle dehydrogenase affinity, regulate the activity of oxidative phosphorylation complexes, and, indirectly, activate pyruvate dehydrogenase (Llorente-Folch et al 2015). In addition, cytosolic $Ca^{2+}$ also positively modulates mitochondrial energy metabolism through activation of malate-aspartate and glycerol-phosphate shuttles (Juaristi et al 2019a). Globally, these $Ca^{2+}$ effects modulate mitochondrial metabolism, coupling metabolic needs dictated by cellular activity with ATP production.

This interaction between mitochondria and $Ca^{2+}$ is not passive, as mitochondrial and cytosolic $Ca^{2+}$ levels are tightly coupled, well controlled (Nicholls 2017), and prone to be regulated by brain activity, influencing both information processing and bioenergetic output (Lin et al 2019), with great relevance for the pathophysiology of neurodegenerative diseases (Cabral-Costa, Kowaltowski 2020). In this review, we discuss the importance of the mitochondrial $Ca^{2+}$ handling system, highlighting its impact on astrocytic homeostasis and current questions and gaps in the literature in this field.

## 2. Mitochondrial $Ca^{2+}$ homeostasis

The first descriptions of mitochondrial $Ca^{2+}$ uptake were from experiments conducted more than 6 decades ago in isolated kidney and liver mitochondria (DeLuca and Engstrom 1961; Lehninger et al 1963; Vasington and Murphy 1962). In 1965, Drahota and Lehninger (1965) described what appeared then to be a minor effect of $Na^+$ on mitochondrial $Ca^{2+}$ homeostasis, which may have been the first description of the equilibrium between mitochondrial $Ca^{2+}$ influx and its $Na^+$-dependent efflux. Other phenomena associated with mitochondrial $Ca^{2+}$ homeostasis were described in the following years, including an extensive and time-tested description of the mitochondrial permeability transition (Haworth, Hunter 1979; Hunter, Haworth 1979a, 1979b), characterizing this form of non-selective inner membrane permeabilization caused by high $Ca^{2+}$ loads.

However, at that time the endoplasmic reticulum (ER) was identified as a major $Ca^{2+}$-storage hub in skeletal muscle contraction/relaxation (Endo et al 1970), effectively overshadowing the role of mitochondrial $Ca^{2+}$ handling in cellular homeostasis. Indeed, mitochondrial $Ca^{2+}$ uptake was thought to be irrelevant physiologically until the 1990´s, due to the low affinity of mitochondria for this cation, below the average intracellular concentration range. This view changed in 1991, when mitochondrially-targeted apoaequorin was used to measure the ion in this organelle *in situ*, and membrane-potential dependent mitochondrial $Ca^{2+}$ transients were observed in parallel to cytosolic increases in $Ca^{2+}$ (Rizzuto et al 1992). This was later shown to be possible due to specific increases in $Ca^{2+}$ concentrations in the microdomain around these organelles (Rizzuto et al 1993). Subsequent studies demonstrated that mitochondrial $Ca^{2+}$ uptake and release indeed participated in a myriad of physiological and biological phenomena (Arieli et al 2004; Arnaudeau et al 2001; Chinopoulos et al 2005; Collins et al 2001; Doczi et al 2011; Kowaltowski et al 1996; Murphy et al 1996; Rudolf et al 2004), although the field exhibited experimental difficulties due to the challenge of identifying the molecular composition of mitochondrial transport pathways. This was achieved beginning in 2010, when the genes for the major components of the mitochondrial $Ca^{2+}$ handling system began to be identified with the characterization of the mitochondrial $Na^+/Ca^{2+}$ exchanger

(NCLX) and the mitochondrial $Ca^{2+}$ uniporter (MCU) (Baughman et al 2011; De Stefani et al 2011; Palty et al 2010), allowing for a new era of mechanistic discoveries related to mitochondrial $Ca^{2+}$ homeostasis.

## 2.1. Mitochondrial $Ca^{2+}$ influx

An instructive way to describe the mitochondrial $Ca^{2+}$ handling system is to separately focus on its influx and efflux components (Fig. 2). Virtually all mitochondrial $Ca^{2+}$ influx is mediated by the inner mitochondrial membrane mitochondrial calcium uniporter complex (MCUc), thoroughly reviewed by Feno et al (2021). In metazoans, the MCUc has the $Ca^{2+}$-selective pore-forming component MCU (Baughman et al 2011; De Stefani et al 2011) arranged in tetramers, intercalated and stabilized by the essential MCU regulator (EMRE) (Sancak et al 2013), and regulatory subunits containing EF-hand $Ca^{2+}$-binding domains – the mitochondrial calcium uptake proteins (MICUs). Additionally, MCUb, a protein with extensive sequence similarity to MCU, may form heteromers with MCU, acting as a dominant-negative agent and suppressing mitochondrial $Ca^{2+}$ uptake (Feno et al 2021; Raffaello et al 2013). MCUc activity may also rely on the putative assembly factor MCU regulator 1 (MCUR1), although this is still under dispute (Giorgi et al 2018). Apart from the composition of the MCUc itself and the direct influence of its regulatory components, post-translational modifications (phosphorylation, Joiner et al 2012; and oxidation, Dong et al 2017) may also be key in modulating the activity of the MCU.

Regarding the regulatory subunits, MICU-1 (Perocchi et al 2010) acts as a seal, directly interacting with the MCU tetramer at the region facing the intermembrane space and restricting ion movement through the channel pore when in its closed state. MICU-2 and MICU-3 (Plovanich et al 2013) have a gatekeeping function and may act as $Ca^{2+}$ sensors, contributing toward the cooperativity observed in MCUc activity. Interestingly enough, MICU-1 may also mediate the cooperative activation of the channel independently (Payne et al 2017), and sense extramitochondrial $Ca^{2+}$ levels (Kamer, Mootha 2014). The stoichiometry of MICU-1 to MCU alone appears to be sufficient to influence mitochondrial $Ca^{2+}$ uptake (Paillard et al 2017). This suggests potential non-redundant functions of MICU-1 that may be related to the origins of its paralogs (Feno et al 2021). The expression profile of MICU-2 and MICU-3 also reinforces their intrinsic regulatory role as $Ca^{2+}$ sensors. While MICU-2 is more ubiquitous, MICU-3 is highly expressed in the brain, specifically in neurons (Patron et al 2019). Removal of MICU-3 from neurons significantly decreases their mitochondrial response to lower $Ca^{2+}$ levels, whereas expression of MICU-3 in non-neuronal cells is sufficient to increase mitochondrial sensitivity to $Ca^{2+}$ (Ashrafi et al 2020). Therefore, MICU-3 grants axonal mitochondria greater $Ca^{2+}$ sensitivity, triggering pre-synaptic $Ca^{2+}$ uptake by mitochondria at a lower $Ca^{2+}$ threshold, which may justify its crucial importance in the maintenance of neuronal function and homeostasis (Patron et al 2019).

## 2.2. Mitochondrial $Ca^{2+}$ efflux

The major player in mitochondrial $Ca^{2+}$ efflux activity is the NCLX (Palty et al 2010), which can exchange either $Na^+$ or $Li^+$ for $Ca^{2+}$. $Li^+$ exchange is used to experimentally confirm activity as mediated by this exchanger, but $Na^+/Ca^{2+}$ activity is evidently predominant *in vivo* (Katoshevski et al 2021; Serna et al 2022). NCLX transport culminates in $Ca^{2+}$ efflux from the mitochondrial matrix in exchange for the entrance of

$Na^+$ from the intermembrane space, a direction which was only shown to be reversible under non-physiological conditions (Samanta et al 2018). NCLX activity follows an electroneutral (2 $Na^+$:1 $Ca^{2+}$) or electrophoretic (3-4 $Na^+$:1 $Ca^{2+}$) stoichiometry that is still under debate (Giorgi et al 2018; Katoshevski et al 2021). Either way, NCLX activity can be allosterically inhibited by mild mitochondrial membrane depolarization, unless when protected by PKA phosphorylation of its regulatory site (Kostic et al 2015). Therefore, mitochondrial $Ca^{2+}$ efflux through NCLX may be controlled by intra- and extra-mitochondrial $Na^+$ and $Ca^{2+}$ levels, and the mitochondrial membrane potential, while also being influenced by PKA-dependent signaling.

Apart from NCLX, there are additional putative $Ca^{2+}$ efflux components. Leucine zipper EF-hand containing transmembrane 1 protein (LETM1) was initially proposed to be a $K^+/H^+$ transporter (Dimmer et al 2007), but later pointed out to be a possible $Ca^{2+}/H^+$ exchanger (Jiang et al 2009; Tsai et al 2014), although this is still intensely debated (De Marchi et al 2014; Giorgi et al 2018). More recently, the transmembrane BAX Inhibitor-1 Motif 5 (TMBIM5, also known as MICS1) was suggested to be the mitochondrial $Ca^{2+}/H^+$ exchanger (Austin et al 2021; Patron et al 2022), in addition to presenting a regulatory role in mitochondrial proteostasis (Patron et al 2022). However, we still require strong

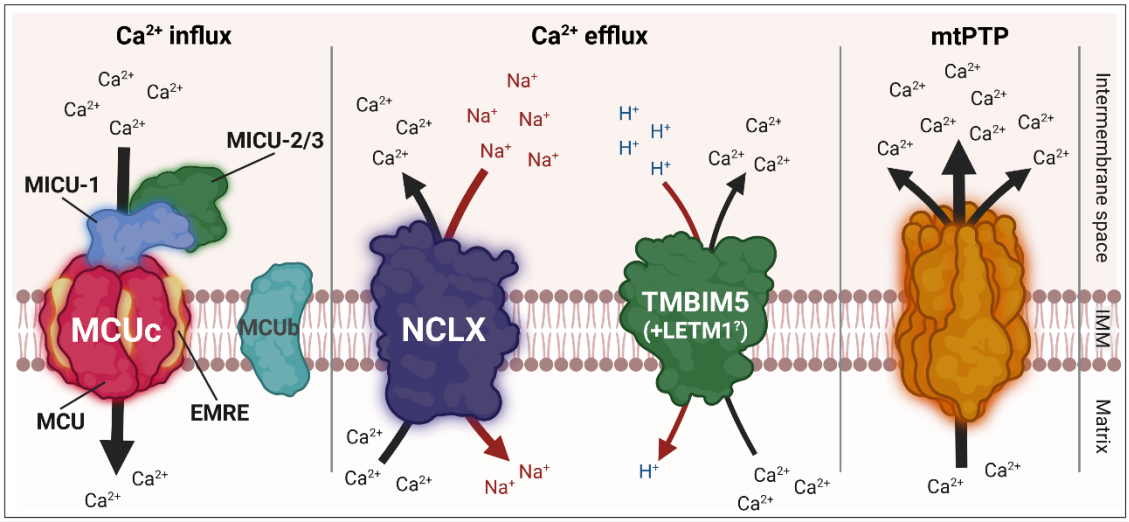


**Figure 2. Mitochondrial $Ca^{2+}$ handling system.** Mitochondrial $Ca^{2+}$ influx is mediated by the mitochondrial calcium uniporter (MCU) complex (MCUc), formed by a tetramer of MCU subunits intercalated by the essential MCU regulator (EMRE). The channel is gated by the mitochondrial calcium uptake protein (MICU-)1, which is bound to MICU-2 or MICU-3, acting as an extramitochondrial $Ca^{2+}$ sensor; MCUb is a regulatory dominant-negative agent that can suppress MCUc activity. Most mitochondrial $Ca^{2+}$ efflux activity is mediated by the mitochondrial $Na^+/Ca^{2+}$ exchanger (NCLX), which moves $Ca^{2+}$ from the mitochondrial matrix out to the intermembrane space in exchange for extramitochondrial $Na^+$. Alternatively, a $Ca^{2+}/H^+$ exchanger may also contribute to a minor, slower mitochondrial $Ca^{2+}$ efflux; it has been recently identified as the transmembrane BAX Inhibitor-1 Motif 5 (TMBIM5), although the leucine zipper EF-hand containing transmembrane 1 protein (LETM1) has previously been associated with this activity, which is still under dispute. Besides $Ca^{2+}$ cycling through these transporters, the opening of the mitochondrial permeability transition pore (mtPTP) can also contribute to a significant release of $Ca^{2+}$ from the mitochondrial matrix, although through a less specific pathway. (IMM: inner mitochondrial membrane).

data to confirm and validate the molecular identity of the mitochondrial $Ca^{2+}/H^{+}$ exchanger.

### 2.3. Mitochondrial permeability transition pore (mtPTP)

In addition to canonical mitochondrial $Ca^{2+}$ influx and efflux pathways that act similarly to other membrane uniporters and exchangers, we must also emphasize the importance of the mitochondrial membrane permeability transition, a complex and variable process that leads to the opening of the mitochondrial permeability transition pore (mtPTP), a non-selective membrane pathway that allows movement of ions and small molecules (please refer to Vercesi et al 2018 for further details). While its structure and activation mechanisms are diverse and still debated (Bernardi et al 2021; Vercesi et al 2018), mitochondrial $Ca^{2+}$ overload and oxidative imbalance are undoubtfully strong triggers for mPTP opening (Vercesi et al 2018). In addition, while mtPTP activation mostly culminates in an irreversible activation of the pore – leading to mitochondrial swelling, $Ca^{2+}$ extrusion, and possible cell death (Vercesi et al 2018) – it may also present a fast reversible opening state, called flickering (Bernardi et al 2021), that can shape cytosolic $Ca^{2+}$ signaling by promoting intermittent mitochondrial $Ca^{2+}$ release.

## 3. Astrocytic $Ca^{2+}$ signaling

In 1986, Pearce et al. (Pearce et al 1986) presented one of the first descriptions in astrocytes of functional glutamate receptors, which relied on $Ca^{2+}$ as a signal transducer. Cornell-Bell and colleagues soon after observed that this astrocytic activation induced $Ca^{2+}$ waves, which not only shaped intracellular $Ca^{2+}$ levels over time within a given astrocyte, but also had the ability to propagate from cell to cell (Cornell-Bell et al 1990; Cornell-Bell, Finkbeiner 1991). These seminal works paved the way for investigations of the functional role of astrocytic $Ca^{2+}$ signaling (Fig. 3).

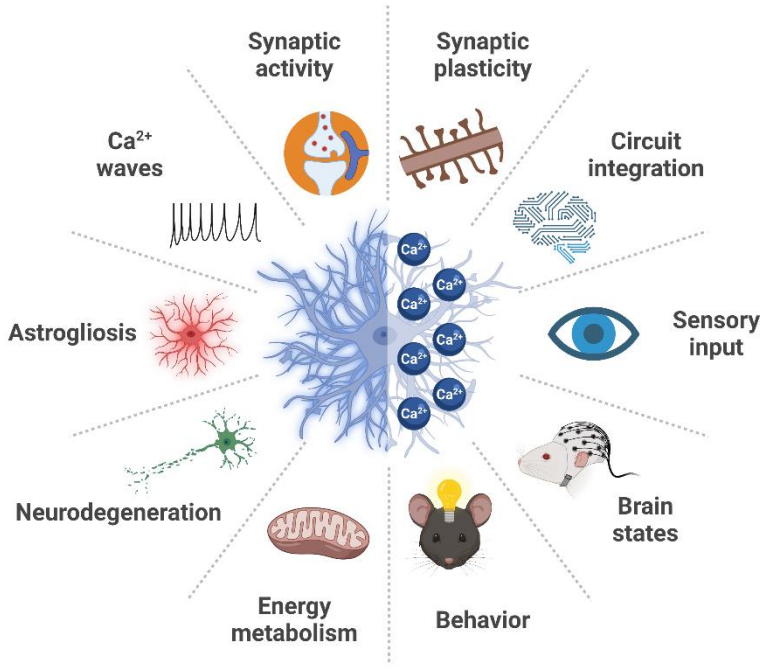


**Figure 3. Astrocytic $Ca^{2+}$ signaling and brain function.** Astrocytes are one of the most abundant cell types in the brain, integrated in a plethora of physiopathological processes, many of them relying on astrocytic $Ca^{2+}$ signaling. By controlling neuronal and astrocyte survival, synaptic plasticity, neuronal coupling and circuit integration, astrocytic $Ca^{2+}$ signaling culminates in the control of superior brain function, including brain states and animal behavior.

Since then, temporal and spatial properties of astrocytic $Ca^{2+}$ signaling have been linked to many brain processes (thoroughly reviewed and discussed by Guerra-Gomes et al 2018 and Oliveira and Araque 2022), including integration of sensory inputs and synaptic activity, synaptic plasticity, regulation of neuronal rhythmical activity and brain states, and several behavioral parameters (Guerra-Gomes et al 2018; Oliveira, Araque 2022). Interestingly, astrocytes may act as a redundant layer in brain circuit integration by activation by neurotransmitters – either inhibitory or excitatory – and release of modulatory gliotransmitters that exert a feedback control over synapses (Guerra-Gomes et al 2018).

All these $Ca^{2+}$-dependent effects may arise from a plethora of cytosolic $Ca^{2+}$ control mechanisms, including G-protein-coupled receptors (GPCRs, mostly Gq-, but also Gi-mediated), transient receptor potential (TRP) channels, store-operated $Ca^{2+}$ entry (SOCE), and, particularly, mitochondrial $Ca^{2+}$ handling (Guerra-Gomes et al 2018).

### 3.1. Astrocytic mitochondrial $Ca^{2+}$ handling

Astrocytic $Ca^{2+}$ waves are spatially controlled by mitochondria (Simpson et al 1998), which, apart from the soma, are distributed through most of the astrocytic subcompartments, although found at higher density in branches (Aboufares El Alaoui et al 2021). Mitochondria are frequently found in association with the ER (Aboufares El Alaoui et al 2021) which may tightly interact with mitochondria through $IP_3$ receptor-mediated (Wu et al 2007; Zheng et al 2013) or independent $Ca^{2+}$ exchanges in local microdomains (Okubo et al 2019). While $Ca^{2+}$ is a crucial messenger during astrocytic activation, only 45% of perisynaptic astrocytic processes – as the astrocytic structures are called when wrapping around a synaptic region – displayed ER content. This suggests a strong importance of alternative pools for $Ca^{2+}$ exchange in these cells (Aboufares El Alaoui et al 2021) to achieve physiological mitochondrial $Ca^{2+}$ handling. In line with this, astrocytic mitochondria were shown to be recruited to and confined in regions near to active synaptic signaling, in a $Ca^{2+}$-dependent manner (Jackson et al 2014; Jackson, Robinson 2015; Stephen et al 2015).

Astrocytes show a greater mitochondrial $Ca^{2+}$ buffering capacity when compared to neurons (Oliveira, Gonçalves 2009), in keeping with the expected resilience of this cell type when facing diverse stimuli and stresses. Interestingly however, astrocytes and neurons of cortical origin have increased $Ca^{2+}$ buffering capacity in comparison to the same cell types in the striatum (Oliveira, Gonçalves 2009). In this line, striatal astrocytes show increased mitochondrial $Ca^{2+}$ influx compared to hippocampal astrocytes (Huntington, Srinivasan 2021). Although this may be due to a difference in local baseline activity and, consequently, in $Ca^{2+}$ transients and energetic demand (Huntington, Srinivasan 2021), it could also indicate that components and/or regulation of the mitochondrial $Ca^{2+}$ handling system may be tightly controlled not only in a cell-specific manner, but also according to tissue region. Some brain areas are more prone to mitochondrial damage, and, consequently, associated with neurodegenerative processes (Cabral-Costa, Kowaltowski 2020). Therefore, many pathological models in which a general susceptibility to insult of a brain area is observed may not be related only to neuronal-specific mechanisms, but also rely on astrocytic targeting (Oliveira, Gonçalves 2009).

In fact, incubation of astrocytes with tau protein was able to inhibit mitochondrial $Ca^{2+}$ efflux (Britti et al 2020), which points to an interesting potential mechanism involved

in tauopathies-associated neurodegeneration. In addition, in an amyotrophic lateral sclerosis (ALS) mouse model, astrocytes displayed increased $Ca^{2+}$ transients in astrocytic microdomains, which were formed independently of neurotransmitter release and the ER, and relied on mitochondrial $Ca^{2+}$ handling and mPTP flickering (Agarwal et al 2017). Furthermore, astrocyte-derived extracellular vesicles in the plasma derived from patients with resolved acute COVID-19 showed an increase in MCU and NCLX levels, also pointing to a potential effect of SARS-CoV-2 infection over astrocytic mitochondrial $Ca^{2+}$ handling (Peluso et al 2022).

Mitochondrial $Ca^{2+}$ uptake is relevant in physiological protective contexts as well. Cerebrovascular damage-induced neovascularization requires mitochondrial-ER interaction in astrocytic perivascular processes, in a mechanism that depends on mitochondrial $Ca^{2+}$ uptake to allow for the control of $Ca^{2+}$ transients (Göbel et al 2020). In addition, MCUc $Ca^{2+}$ uptake was demonstrated to be seminal for mitochondrial-associated type-1 cannabinoid receptor (mtCB1) signaling, independently of cytosolic $Ca^{2+}$ (Serrat et al 2021). Astrocytic mitochondrial $Ca^{2+}$ uptake was also shown to contribute toward protective effects in brain damage (Zheng et al 2013), hypoxia (Smith et al 2004), and aging (Wu et al 2007).

But astrocytic $Ca^{2+}$ is not a simple readout of a second messenger. As discussed above, glutamate activation was the background for the characterization of astrocytic $Ca^{2+}$ signaling (Cornell-Bell et al 1990; Cornell-Bell, Finkbeiner 1991; Pearce et al 1986). And although glutamate stimuli are known to enhance ATP production in astrocytes, they induce an increase in ATP production mostly due to cytosolic $Na^+$ uptake: glutamate uptake in astrocytes occurs through co-transport with $Na^+$, thus activating the $Na^+/K^+$-ATPase to reestablish ionic homeostasis (Juaristi et al 2019b). Although this explains the glutamate-induced increase in ATP demand, $Na^+$ signaling may also play an additional role in mitochondrial function, including implications in mitochondrial $Ca^{2+}$ transport. The major mechanism for mitochondrial $Ca^{2+}$ efflux, NCLX, is coupled with mitochondrial $Na^+$ influx, which was previously shown to be central in mitochondrial respiratory chain-linked signaling in hypoxia (Hernansanz-Agustín et al 2020). Thus, glutamate-dependent $Na^+$ effects on metabolism may not be restricted to cytosolic ATP depletion, but may also include mitochondrial $Na^+$ and, indirectly, $Ca^{2+}$-mediated effects. This is an interesting critical point for astrocytic function, as it is tightly coupled – both functionally and energetically – to neuronal activity (Bonvento, Bolaños 2021).

Parnis et al. (Parnis et al 2013) were the first to modulate NCLX activity in astrocytes. NCLX silencing, as expected, increased basal mitochondrial $Ca^{2+}$ influx and total content, while significantly decreasing efflux activity, culminating in increased cytosolic $Ca^{2+}$ clearance after a purinergic-induced $Ca^{2+}$ wave (Parnis et al 2013). This was shown to also decrease store-operated $Ca^{2+}$ entry (SOCE) and $Ca^{2+}$ wave propagation between astrocytes. Functionally, NCLX silencing inhibited astrocytic proliferation.

NCLX knockdown also induced neurodegeneration and a decrease in astrocyte numbers, both *in vitro* and *in vivo* (Hagenston et al 2022). In this experimental design, both neurons and astrocytes were affected by the shRNA, in mixed cultures and in the intact brain, so the authors correlated these results with astrodegeneration induced by NCLX silencing (Hagenston et al 2022). Nonetheless, NCLX silencing could have also hampered astrogliosis induced by the viral stereotaxic injection, as NCLX knockdown may inhibit astrocyte proliferation (Parnis et al 2013). Therefore, even though NCLX-linked neurodegeneration (Jadiya et al 2019) and cognitive impairment (Stavsky et al 2021) may

be primarily associated with an intrinsic neuronal NCLX activity impairment (Hagenston et al 2022), astrocytic NCLX function may also hold a relevant homeostatic role.

In fact, further NCLX activity implications in cellular function have been explored in β-cells (Kostic et al 2018; Nita et al 2015, 2014, 2012), brown adipose tissue (Assali et al 2020), colorectal cancer (Pathak et al 2020), cardiovascular cells (De La Fuente et al 2018; Garbincius et al 2022; Hernansanz-Agustín et al 2020; Luongo et al 2017), and neurons (Britti et al 2021, 2020; Hagenston et al 2022; Jadiya et al 2019; Kostic et al 2015, 2018; Ludtmann et al 2019; Sharma et al 2017; Stavsky et al 2021). However, there little understood regarding NCLX functional roles in astrocytes. Thus, considering the importance of $Ca^{2+}$ signaling for brain activity and astrocyte homeostasis, a deeper comprehension of astrocytic mitochondrial $Ca^{2+}$ handling mechanisms and regulation could shed a light on the pathophysiology and potential pharmacological targets of the central nervous system and respective diseases (Cabral-Costa, Kowaltowski 2020) (Fig. 4).

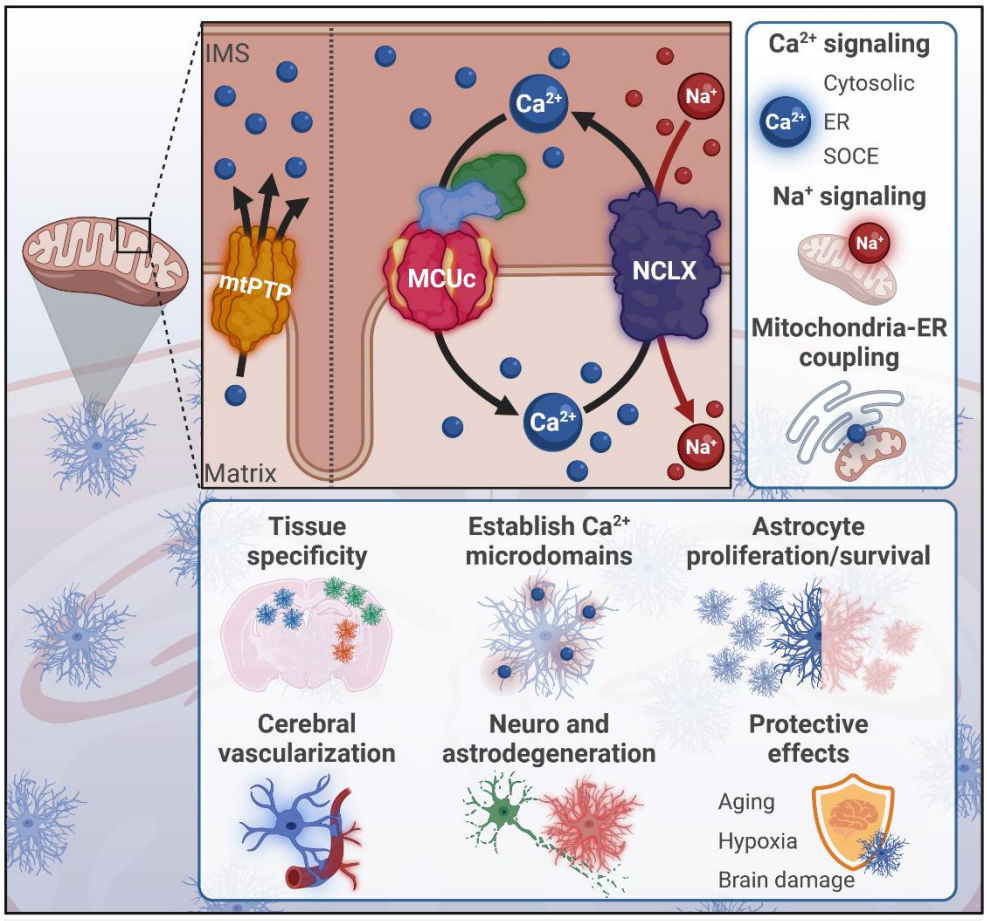


**Figure 4. Astrocytic mitochondrial $Ca^{2+}$ handling.** Apart from directly controlling cellular $Ca^{2+}$ – and, indirectly, $Na^{+}$ – signaling, the mitochondrial $Ca^{2+}$ handling system is tightly coupled with many astrocytic functions, from defining $Ca^{2+}$ microdomains in astrocytic processes through controlling astrocyte proliferation and survival, with distinct tissue-specific profiles. This contributes to key astrocyte outputs in brain physiology, such as neovascularization and survival of brain cells, and protective effects under diverse challenges.

## 4. Open questions

- What are the underlying mechanisms and implications behind the composition and functional heterogeneity of the mitochondrial $Ca^{2+}$ handling system in astrocytes throughout different brain areas?
- How is the mitochondrial $Ca^{2+}$ handling system (mainly MCUc and NCLX activities) physiologically controlled in astrocytes – at transcriptional, assembly, and post-translational levels?
- How does mitochondrial $Ca^{2+}$ efflux through NCLX control astrocytic proliferation and cell death?
- Are there other physiological roles of NCLX activity in astrocytic function?

## 5. Summary

- Astrocytic $Ca^{2+}$ signaling temporally and spatially controls a plethora of brain processes, including synaptic plasticity, electrophysiological brain states, and behavior. $Ca^{2+}$ levels are influenced by many transporters and channels, including the mitochondrial $Ca^{2+}$ handling system, which is key in controlling several pathways, such as cell survival, ionic balance, and metabolism.
- $Ca^{2+}$ signaling events, even those more focused on cytosolic $Ca^{2+}$ transients, must also be interpreted in light of mitochondrial $Ca^{2+}$ handling and its potential consequences.
- Mitochondrial $Ca^{2+}$ handling shapes astrocytic function and is key toward brain homeostasis. Further mechanistic studies, as well as dissection of available single cell omic datasets, may allow for the investigation of the processes behind the heterogeneity of astrocytic mitochondrial $Ca^{2+}$ handling molecular composition.
- There is still much to be understood regarding the role of the mitochondrial $Ca^{2+}$ handling system (especially the mitochondrial $Ca^{2+}$ efflux transporter NCLX), on astrocytic function, which could further advance the understanding of brain pathophysiological mechanisms.

### Abbreviations

| | |
|---|---|
| ALS | amyotrophic lateral sclerosis |
| EMRE | essential MCU regulator |
| ER | endoplasmic reticulum |
| GPCR | G-protein-coupled receptors |
| IMM | inner mitochondrial membrane |
| LETM1 | leucine zipper EF-hand containing transmembrane 1 |
| MCU | mitochondrial $Ca^{2+}$ uniporter |
| MCUc | MCU complex |
| MCUR1 | MCU regulator 1 |
| MICU | mitochondrial calcium uptake protein |
| mtCB1 | mitochondrial-associated type-1 cannabinoid receptor |
| mtPTP | mitochondrial permeability transition pore mitochondrial |
| NCLX | $Na^{+}/Ca^{2+}$ exchanger |
| SOCE | store-operated $Ca^{2+}$ entry |
| TMBIM5 | transmembrane BAX Inhibitor-1 Motif 5 |
| TRP | transient receptor potential |

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