Mitochondrial calcium cycling in neuronal function and neurodegeneration

Grant C. Walters* and Yuriy M. Usachev*

Department of Neuroscience and Pharmacology, Iowa Neuroscience Institute, University of Iowa, Iowa City, IA, United States

Mitochondria are essential for proper cellular function through their critical roles in ATP synthesis, reactive oxygen species production, calcium (Ca^{2+}) buffering, and apoptotic signaling. In neurons, Ca^{2+} buffering is particularly important as it helps to shape Ca^{2+} signals and to regulate numerous Ca^{2+}-dependent functions including neuronal excitability, synaptic transmission, gene expression, and neuronal toxicity. Over the past decade, identification of the mitochondrial Ca^{2+} uniporter (MCU) and other molecular components of mitochondrial Ca^{2+} transport has provided insight into the roles that mitochondrial Ca^{2+} regulation plays in neuronal function in health and disease. In this review, we discuss the many roles of mitochondrial Ca^{2+} uptake and release mechanisms in normal neuronal function and highlight new insights into the Ca^{2+}-dependent mechanisms that drive mitochondrial dysfunction in neurologic diseases including epilepsy, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. We also consider how targeting Ca^{2+} uptake and release mechanisms could facilitate the development of novel therapeutic strategies for neurological diseases.

Introduction

Mitochondria are colloquially referred to as the powerhouse of the cell because of their critical role in the production of ATP through oxidative phosphorylation (OXPHOS) (Brookes et al., 2004; Nunnari and Suomalainen, 2012). The production of ATP through OXPHOS is especially important within the brain, which accounts for nearly 20% of the body's resting metabolism (Attwell and Laughlin, 2001; Howarth et al., 2012). Due to the high energy demand of neurons, they are very susceptible to mtDNA mutations that disrupt the electron transport chain; these mutations can result in a variety of neurological dysfunctions including seizures, blindness, and intellectual disabilities (DiMauro and Schon, 2003). In addition to producing ATP, reactive oxygen species (ROS) are produced as a byproduct of OXPHOS.

Mitochondrial ROS production is associated with damage to lipids, proteins, and nucleic acids, and excessive ROS are implicated in neurodegeneration (Nunnari and Suomalainen, 2012; Beckhauser et al., 2016). Super oxide free radicals (O_2^-) produced through OXPHOS are converted to H_2O_2 by enzymes such as superoxide dismutase and subsequently broken down into harmless byproducts (Beckhauser et al., 2016; Angelova and Abramov, 2018). When ROS are not broken down, the resulting oxidative stress can lead to activation of apoptotic signaling cascades and thereby to cell death (Fleury et al., 2002; Ueda et al., 2002). However, ROS also play an important physiological role, regulating signaling pathways that are critical for a wide variety of cellular functions. In neurons, ROS signaling is critical for axon development (Beckhauser et al., 2016), it modulates expression of AMPA and NMDA glutamate receptors (Esteras et al., 2022), and regulates various forms of synaptic plasticity including long-term potentiation (LTP) (Klann,
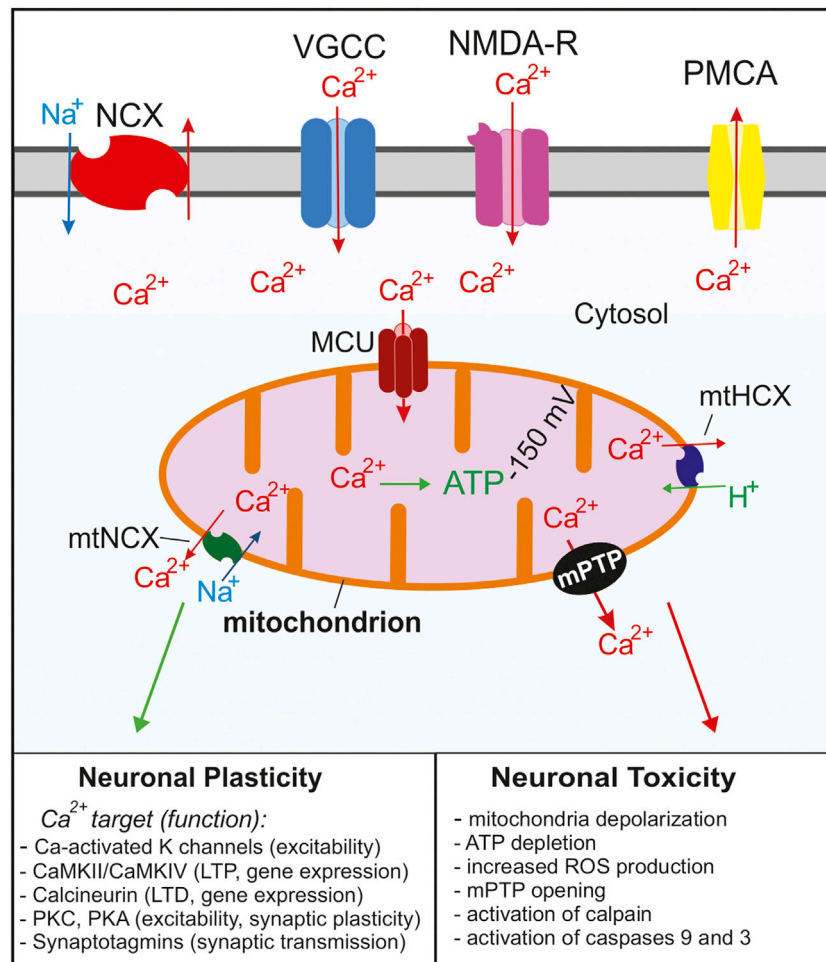
FIGURE 1
Summary of the Ca^{2+} signaling mechanisms in neurons that regulate cytosolic and mitochondrial Ca^{2+} concentrations. Ca^{2+} enters the cell through voltage gated Ca^{2+} channels (VGCC) following depolarization or through glutamate NMDA receptors. As the cytosolic Ca^{2+} concentration increases, the MCU complex opens and Ca^{2+} enters the mitochondria. Ca^{2+} is rapidly removed from the cytosol via Na$^+$/Ca^{2+} exchangers (NCX) and plasma membrane Ca^{2+} ATPases (PMCA). As the cytosolic Ca^{2+} concentration decreases Ca^{2+} is slowly released from the mitochondria through Na$^+$/Ca^{2+} (mtNCX) exchangers and H$^+$/Ca^{2+} exchangers (mtHCX). Proper Ca^{2+} buffering is critical for normal neuronal functions and neuronal plasticity. Impaired buffering and Ca^{2+} overload leads to the opening of the mPTP and activation of apoptotic pathways and is implicated in many neurological diseases. For simplicity, the ER and other Ca^{2+} organelles are omitted from the model.

1998; Beckhauser et al., 2016). Despite the importance of ROS for normal neuronal function, proper regulation of their production is critical, because overproduction and subsequent oxidative stress are implicated in multiple pathologies. These include Alzheimer's disease, Parkinson's disease, Huntington's disease, Epilepsy, and schizophrenia (Bezprozvanny, 2009; Liochev, 2013; Puttachary et al., 2015; Beckhauser et al., 2016; Angelova and Abramov, 2018; Dagda, 2018).

In addition to ATP synthesis and ROS generation, mitochondria also play a critical role in Ca^{2+} signaling. Mitochondria efficiently buffer Ca^{2+} entering the cell during neuronal excitation and then slowly release accumulated Ca^{2+} back to the cytosol; this limits the amplitude and prolongs the duration of cytosolic Ca^{2+} responses. Consequently, mitochondrial have a tremendous ability to control and modulate many Ca^{2+}-dependent functions in neurons, including neuronal excitability, neurotransmitter release, synaptic plasticity, gene expression, and cell survival (Mattson et al., 2008; Pivovarova and Andrews, 2010; Brini et al., 2014; Devine and Kittler, 2018; Jung et al., 2020) (Figure 1). Ca^{2+} uptake into the mitochondrial matrix regulates ATP synthesis by activating multiple components of the OXPHOS pathway, including ATP synthase,

α-ketoglutarate dehydrogenase, isocitrate dehydrogenase, and pyruvate dehydrogenase (Denton et al., 1972; Brookes et al., 2004; Denton, 2009; Tarasov et al., 2012). ATP production depends on the concentration of Ca^{2+} within the mitochondrial matrix, with submicromolar mitochondrial Ca^{2+} concentrations stimulating ATP synthesis, and mitochondrial Ca^{2+} concentrations above 10 μM suppressing ATP production (Fink et al., 2017). While the Ca^{2+} dependent production of ATP is conserved across most cells within the body, neurons have unique functions that are dependent upon Ca^{2+} signaling as discussed below.

Mitochondrial Ca^{2+} uptake and release mechanisms

General characteristics

In neurons at rest, total calcium concentration in the matrix is very low, usually <100 nM (Pivovarova et al., 1999; Stanika et al., 2012). During electrical or synaptic stimulation, total mitochondrial calcium

concentration can increase to millimolar levels within seconds (Pivovarova et al., 1999; Pivovarova et al., 2002). In the case of modest to strong stimulation, the rate of mitochondrial Ca^{2+} uptake in intact neurons can be as high as 200 μM/s relative to the mitochondrial volume (Pivovarova et al., 1999; Colegrove et al., 2000a). Ca^{2+} transport from the cytosol into the mitochondrial matrix requires crossing the outer and inner mitochondrial membranes (OMM and IMM, respectively). The OMM is permeable to molecules smaller than 5 kDa, including Ca^{2+}. This permeability is ascribed to voltage-dependent anion channels (VDACs) also known as mitochondrial porins that are highly expressed in the OMM (Spat et al., 2008; Shoshan-Barmatz et al., 2010; Rizzuto et al., 2012). It is generally assumed that Ca^{2+} concentration in the intermembrane space is the same as in the cytosol. Ca^{2+} transport across the IMM is mediated by a designated transporter, mitochondrial Ca^{2+} uniporter, and is driven by a steep electrical potential across the IMM ($\Delta\Psi_{mt} \sim -150$ mV) (Friel, 2000; Thayer et al., 2002; Nicholls, 2005). Recent electrophysiological and molecular studies strongly suggest that the mitochondrial Ca^{2+} uniporter functions as a Ca^{2+}-selective ion channel, which is consistent with the very high rate of Ca^{2+} uptake by mitochondria (Kirichok et al., 2004; De Stefani et al., 2011; Fieni et al., 2012; Chaudhuri et al., 2013). The mitochondrial Ca^{2+} uniporter channel is gated by Ca^{2+}, and the rate of mitochondrial Ca^{2+} uptake depends on the cytosolic Ca^{2+} concentration $[Ca^{2+}]_i$ as a power function of ~2–2.5 (Gunter and Pfeiffer, 1990; Colegrove et al., 2000a; Nicholls, 2012; Shutov et al., 2013). Earlier studies in isolated mitochondria suggested that the EC_{50} of the $[Ca^{2+}]_i$ for uniporter activation is ~10–20 μM (Gunter and Pfeiffer, 1990). Such a requirement implied that mitochondria are involved in Ca^{2+} transport only in a setting of intense stimulation or pathological $[Ca^{2+}]_i$ elevations caused by toxic conditions. However, most recent studies using intact neurons indicate that mitochondrial Ca^{2+} uptake can be induced by $[Ca^{2+}]_i$ elevations as low as 200–300 nM, and that mitochondrial Ca^{2+} transport contributes to many aspects of Ca^{2+} signaling in neurons, both under physiological and pathological conditions (Figure 1) (Werth and Thayer, 1994; Stout et al., 1998; David, 1999; Pivovarova et al., 1999; Colegrove et al., 2000a; Pivovarova et al., 2004; Medvedeva et al., 2008; Kim and Usachev, 2009; Chouhan et al., 2010; Shutov et al., 2013).

Within the matrix, free Ca^{2+} is rapidly buffered by its incorporation into Ca-phosphate complexes, primarily in the forms of $Ca_3(PO_4)_2$ and $CaHPO_4$ (Chalmers and Nicholls, 2003; Kristian et al., 2007). Heavy Ca^{2+} buffering in the matrix helps to explain why mitochondrial Ca^{2+} concentration typically does not exceed 1–5 μM in neurons even after strong stimulation (David, 1999; Billups and Forsythe, 2002; Chalmers and Nicholls, 2003; David et al., 2003). The ratio of bound:free Ca is a dynamic value that increases as the Ca load of mitochondria increases (Chalmers and Nicholls, 2003). In neurons at rest or with a low mitochondrial Ca load, the ratio is estimated to be ~4,000:1, and as mitochondrial Ca approaches toxic levels the ratio increases to as much as 150,000:1 (Babcock et al., 1997; Chalmers and Nicholls, 2003; Pivovarova et al., 2004; Stanika et al., 2012).

Ca^{2+} accumulated in mitochondria can be released via at least three mechanisms, Na^+/Ca^{2+} exchanger, H^+/Ca^{2+} exchanger, and the mitochondrial permeability transition pore (mPTP; Figure 1) (Bernardi, 1999; Thayer et al., 2002; Nicholls, 2005; Pivovarova and Andrews, 2010; Jung et al., 2020). It is generally agreed that under physiological conditions the mitochondrial Na^+/Ca^{2+} exchanger (mtNCX) is the main pathway mediating Ca^{2+} efflux from mitochondria in neurons (Friel, 2000; Thayer et al., 2002; Nicholls, 2005; Jung et al., 2020; Rysted et al., 2021; Yang et al., 2021). Because the rate of Ca^{2+} efflux from mitochondria is significantly slower than that of Ca^{2+} uptake, Ca^{2+} rapidly accumulates in mitochondria following its entry into the cell during stimulation. Using an approach combining pharmacological tools and mathematical modeling, Friel and colleagues estimated that Ca^{2+} is removed from neuronal mitochondria at 35–75 nM/s, a rate ~1000-fold slower than the rate of mitochondrial Ca^{2+} uptake (Colegrove et al., 2000a; b). However, EPMA-based examination of sympathetic neurons showed that total matrix Ca^{2+} recovered from depolarization-induced peak value of 22 mM–0.9 mM within 15 min, which yields a much faster rate value of ~23 μM/s (Pivovarova et al., 1999). Removal of 1 Ca^{2+} by mtNCX is coupled to mitochondrial uptake of 3 Na^+ (Bernardi, 1999; Palty and Sekler, 2012). Thus, mtNCX is electrogenic, and its activity is predicted to be reduced by mitochondrial depolarization.

While uptake of Ca^{2+} ions into the mitochondria has been studied for many decades, the molecular mechanisms by which Ca^{2+} enters and exits the mitochondria have been discovered only over the past 10 years and are discussed below (Drago et al., 2011; Kamer and Mootha, 2015; De Stefani et al., 2016; Garbincius and Elrod, 2022).

The mitochondrial Ca^{2+} uniporter (MCU)

In 2004, Kirichok and Clapham demonstrated that mitochondrial Ca^{2+} uniporter functions as a Ca^{2+}-selective ion channel (Kirichok et al., 2004). In 2011, two groups identified MCU (mitochondrial Ca^{2+} uniporter) as a pore-forming subunit of the greater uniporter complex (Baughman et al., 2011; De Stefani et al., 2011), and two years later, a MCU paralog and negative regulator of the complex, MCUb, was identified (Raffaello et al., 2013). MCU heteromerizes with MCUb to form a tetrameric pore structure within the inner mitochondrial membrane (Raffaello et al., 2013; Baradaran et al., 2018; Wang et al., 2019) (Figure 2). Several additional components essential for the function of the MCU complex were recently discovered. These include: mitochondria Ca^{2+} uptake isoforms 1, 2, and 3 (MICU1, MICU2, and MICU3), which are auxiliary subunits that regulate Ca^{2+}-dependent gating of the channel; the mitochondrial Ca^{2+} uniporter regulator 1 (MCUR1), and an essential MCU regulator (EMRE), the protein critical for the assembly of MCU complex (Mallilankaraman et al., 2012a; Mallilankaraman et al., 2012b; Csordás et al., 2013; Sancak et al., 2013; Tomar et al., 2016; Patron et al., 2019; Garg et al., 2021). The functions of these subunits are discussed in greater detail later.

In addition to determining tertiary structure of the MCU pore complex, recent studies explored the ion selectivity regions that are conserved across multiple species, with the aim of identifying the selectivity filters within MCU and MCUb. These studies revealed that the cation selectivity of the MCU subunits is determined by the critical residues Asp240 (D) and Glu243 (E) within the carboxylate rings at the mouth of the channel, which form a so-called DXXE motif that is critical for Ca^{2+} selectivity (Oxenoid et al., 2016; Cao et al., 2017). Notably, whereas the substitution of Asp240 to Glu results in a partially functional channel, the substitution of Glu243 to Asp completely inhibits Ca^{2+} uptake (Oxenoid et al., 2016). Previous work also found that mutation of Asp240 or Glu243 to alanine (A)
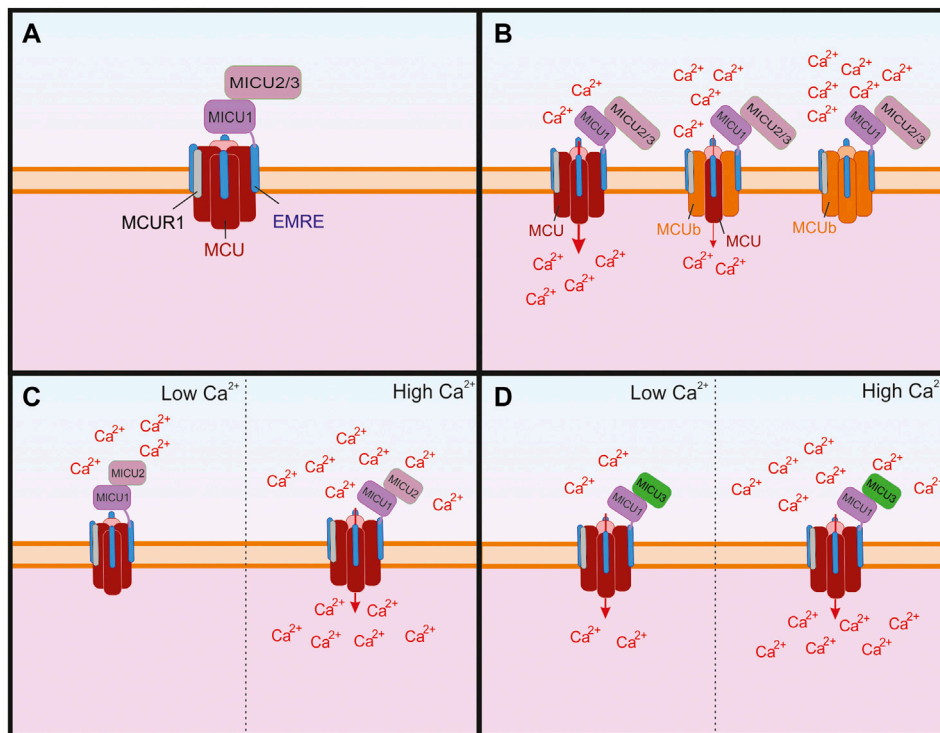
FIGURE 2
The structure of the greater mitochondrial Ca^{2+} uniporter (MCU) complex and its subunits that can regulate its permeability. **(A)** The assembled complex containing a tetramer of pore forming MCU and four subunits of the essential MCU regulator (EMRE), which are critical for the proper assembly of the transmembrane MCU to the mitochondrial Ca^{2+} uptake isoforms 1, 2, and 3 (MICU 1–3). The MCU complex also contains the MCU regulator 1 (MCUR1), which is thought to bind to EMRE. **(B)** The MCU paralog MCUb has been shown to replace subunits of pore forming MCU, which leads to reduced Ca^{2+} through the channel and as more MCUb subunits replace MCU the Ca^{2+} conductivity is reduced. **(C)** MICU1 and MICU two are critical for the Ca^{2+} sensing function of the channel and MICU2 has been shown to regulate Ca^{2+} sensitivity. When Ca^{2+} is low, MICU1 blocks the pore, but in the presence of high Ca^{2+} the conformational changes in MICU1 and MICU2 result in opening of the channel and allow Ca^{2+} to enter the mitochondria. **(D)** In neurons, MICU3 is more abundant and has been shown to replace MICU2 in dimers with MICU1. This replacement leads to increased sensitivity to cytosolic Ca^{2+} of the MCU complex and results in MCU channel opening to lower concentrations of Ca^{2+} than compared to MICU2.

completely inhibited Ca^{2+} uptake through MCU (Baughman et al., 2011).

Defining the roles of MCU in the central and peripheral nervous systems *in vivo* remains a critical task in the field of mitochondrial biology. Although many *in vitro* studies (e.g., in cultured neurons or brain slices) suggested that mitochondrial Ca^{2+} transport is critical to neuronal function, studies in MCU knockout (KO) mice revealed that MCU deletion had surprisingly limited effects *in vivo* (Pan et al., 2013). The development of viable MCU KO mice was a surprise due to the importance of MCU for Ca^{2+} passage into the mitochondria. MCU KO mice were only viable on a CD1 background and KO offspring were not born at Mendelian ratios, but C57BL/6 mice were not viable due to embryonic lethality between E11.5-E13.5 (Pan et al., 2013; Murphy et al., 2014; Murphy and Steenbergen, 2021). The exact cause of embryonic lethality is not known, but it is hypothesized to be due to myocardial development issues (Murphy et al., 2014). Mitochondria from MCU KO mice did not uptake Ca^{2+}, but it was not protective from cardiac ischemic reperfusion injuries (Pan et al., 2013; Murphy and Steenbergen, 2021). However, deletion of MCU from adult mice using a tamoxifen inducible model results in protection from cardiac ischemic reperfusion injuries (Kwong et al., 2015; Luongo et al., 2015). Mitochondria from global MCU KO mice have been shown to have MCU independent Ca^{2+} uptake, which could explain its therapeutic

limitations in the ischemic reperfusion model (Hamilton et al., 2018). MCU independent Ca^{2+} uptake is blocked by the MCU inhibitor Ru360, and therefore it is hypothesized that the other pore forming component MCUb may be capable of compensating for the loss of MCU (Hamilton et al., 2018). MCU independent uptake could also possibly occur through mtNCX functioning in reverse mode and Letm1 (Palty et al., 2010; Jiang et al., 2013). Interestingly, MCU KO mice did not show any deficit in motor function or cognitive performance (Szibor et al., 2020), suggesting that MCU is not critical for baseline neuronal function, but plays a more important role during states of high neuronal activity, stress, neuronal injury, or in disease states. Indeed, a recent study using a mouse high-fat diet model of peripheral diabetic neuropathy showed that the deletion of MCU from peripheral neurons prevented axonal degeneration. Specifically, the deletion of MCU prevented the accumulation of Ca^{2+} in the mitochondria, subsequent Ca^{2+} induced mitochondrial disruption, and eventual loss of nociceptive fibers (George et al., 2021). Inducible neuron-specific MCU KO also protected neurons from hypoxic-ischemic injury (Nichols et al., 2018), in contrast to findings in mice with global deletion of MCU (Pan et al., 2013). The exact mechanisms by which global and inducible MCU KO differ are not known, but compensatory mechanisms, which were discussed above, may occur over time and lead to the differences observed in

these studies. In another study, deletion of MCU from mice carrying the Cdh23[ahl] allele, which causes hearing loss, led to earlier onset of high frequency hearing loss and subsequent significant hearing loss when compared to controls. By 3 months of age, there was also a loss of close to 18% of outer hair cells, but the development of the cochlea was normal (Manikandan et al., 2021). Collectively, these studies suggest that MCU either promote or reduce neuronal survival, with the outcome depending on the developmental state, cellular context, and disease conditions.

Mitochondrial Ca²⁺ uniporter paralog (MCUb)

The pore forming subunit of MCU is surrounded by multiple proteins that make up the greater MCU complex (Figure 2). One of these is MCUb, another pore-forming subunit sharing many structural similarities with MCU (Raffaello et al., 2013). Like MCU, it contains two transmembrane domains. Its protein sequence shares 50% similarity with that of MCU, and it can form hetero-oligomers with MCU. A substitution within the DXXE motif of MCUb is hypothesized to affect its Ca²⁺ permeability. Therefore, MCUb has been proposed to function as a dominant negative regulator of the MCU complex (Raffaello et al., 2013). A recent study revealed a potential new structural role for MCUb in the greater MCU complex (Lambert et al., 2019). Specifically, it showed that the presence of MCUb disrupts the tertiary structure of the complex by competitively displacing MCU from the greater complex and reducing the association of MCU with regulatory proteins MICU1/2. This coincided with reduced function of the complex and disrupted Ca²⁺ uptake into the mitochondria (Lambert et al., 2019; Garbincius and Elrod, 2022). A recent *in vivo* study provided additional support for MCUb as a negative regulator of Ca²⁺ uptake into mitochondria (Huo et al., 2020). In this study, the deletion of MCUb in mice led to increased susceptibility to damage from ischemic-reperfusion injury in the heart, whereas overexpression of MCUb conferred some protection from cardiac remodeling and damage from the infarct (Huo et al., 2020). Studies also revealed that MCUb expression increases following cardiac injury. This led to the hypothesis that the upregulation of MCUb has evolved to prevent mitochondrial Ca²⁺ overload in response to ischemic damage (Lambert et al., 2019; Huo et al., 2020). In contrast to these findings, MCUb is upregulated in a type 2 diabetic mouse heart, and the introduction of a dominant-negative MCUb transgene into the type 2 diabetes mouse model reversed the cardiac defects, as well as the metabolic aspect of the phenotype suggesting detrimental effects of MCUb expression in this model (Cividini et al., 2021). These results are somewhat contrary to the suggestions in previous MCUb studies that the overexpression of MCUb is cytoprotective. It could be that the overexpression of MCUb is initially protective, but that chronic overexpression results in deficits in energy production leading to negative outcomes.

MCUb is also found in brain mitochondria (Hamilton et al., 2018), although its role in neurons remains unknown. Given the protective function of MCUb in the heart (Lambert et al., 2019; Huo et al., 2020) and the detrimental role of mitochondrial Ca²⁺ overload in neurons (Mattson et al., 2008; Briston et al., 2019; Jung et al., 2020; Modesti et al., 2021), it is logical to predict that MCUb plays neuroprotective roles in stroke and neurodegenerative diseases. Future work will determine if this is the case.

MICU1-3

One of the regulatory subunits of the MCU complex was described before MCU was discovered and is encoded by the gene *CBARA1,* which was renamed to mitochondrial Ca²⁺ uptake 1 (MICU1). It is ubiquitously expressed throughout the body including the brain (Plovanich et al., 2013; Patron et al., 2019). MICU1 interacts with MCU *via* EMRE and has two EF hand domains that are critical for its Ca²⁺ sensing function (Perocchi et al., 2010; Baughman et al., 2011). Knockdown of MICU1 leads to inhibition of mitochondrial Ca²⁺ uptake in response to stimulation, yet deletion of MICU1 results in chronically elevated Ca²⁺ concentrations within the mitochondrial matrix (Mallilankaraman et al., 2012b; Csordás et al., 2013). The physiological importance of MICU1 has been demonstrated in both humans and mice. Indeed, loss-of-function mutations in MICU1 are associated with learning and motor deficits in humans (Logan et al., 2014). Similarly, neuron-specific deletion of MICU1 in mice leads to abnormal motor and cognitive phenotypes, which are likely caused by neuronal degeneration in the spinal cord and the brain (Liu et al., 2016; Singh et al., 2022). Mechanistically, the loss or deficit in MICU1 function results in chronic elevation of mitochondrial Ca²⁺ concentration and impairment of mitochondrial Ca²⁺ signaling, which leads to altered neuronal excitability, increased susceptibility to excitotoxic stress and neuronal death dependent on mitochondrial permeability transition pore (Logan et al., 2014; Singh et al., 2022). Thus, MICU1 represents a critical component of mitochondrial Ca²⁺ signaling in neurons.

MICU1 has two paralogs, MICU2 and MICU3 (Plovanich et al., 2013). MICU2 is ubiquitously expressed throughout the body, whereas MICU3 is found primarily in the nervous system (Plovanich et al., 2013; Patron et al., 2019; Ashrafi et al., 2020). Both proteins interact with MICU1 to control Ca²⁺-dependent gating of the MCU channel complex (Kamer and Mootha, 2015; De Stefani et al., 2016; Garbincius and Elrod, 2022) (Figure 2).

MICU1 and MICU2 exist as a heterodimer and have opposing regulatory effects on MCU. When the cytosolic Ca²⁺ concentration is low, MICU2 reduces MCU activity, but when the Ca²⁺ concentration is higher MICU1 increases MCU activity (Patron et al., 2014). Notably, the presence of a disulfide bond between MICU1 and MICU2 is critical for its biochemical behavior, enabling them to physically adjust their positions in response to changes in Ca²⁺ levels; when the disulfide bond is removed mitochondrial Ca²⁺ uptake increases (Petrungaro et al., 2015). A recent structural analysis of the MCU complex showed that at low Ca²⁺ concentrations MICU1 interacts closely with MCU to seal the pore, and that MICU2 is bound to MICU1 but does not directly interact with MCU (Fan et al., 2020). The same study showed that at high levels of Ca²⁺ (2 mM) the MCU complex forms a dimer with a second MCU complex and in this arrangement the MICU2 subunits interact directly. This also results in MICU1 moving away from the pore of the MCU complex, allowing for Ca²⁺ uptake (Fan et al., 2020). In addition to playing a critical role in the function of the MCU complex, MICU1 is critical for directing the assembly of the MCU complex in the presence of Ca²⁺ (Gottschalk et al., 2019). Interestingly, MICU1 is also critical for the induction of cold-induced cell death. In a cold-induced stress model, the deletion of MICU1 prevents hyperpolarization of the mitochondria, which in turn reduces lipid peroxidation and prevents subsequent ferroptosis (Nakamura et al., 2021).

In contrast to MICU1 and MICU2, MICU3 received little attention until recently, when it was found to modulate

mitochondria specifically in brain tissue (Patron et al., 2019). Interestingly, the authors found that it interacted with MICU1 and formed disulfide bonds, which were similar to those between MICU1 and MICU2. However, unlike the case with MICU2, the expression of MICU3 was shown to lead to enhanced uptake of Ca^{2+} into the mitochondria during intensive neuronal activity, which is explained by a higher Ca^{2+} affinity of MICU3 than that of MICU2 (Figure 2). These findings led to the proposal that MICU3 functions similarly to MICU2 by forming heterodimers with MICU1 to control gating of the MCU complex, and that the MICU2/MICU3 ratio determines the Ca^{2+} sensitivity of the MCU complex (Patron et al., 2019; Garbincius and Elrod, 2022). Recent experiments showed that MICU3 is responsible for a higher sensitivity of mitochondrial Ca^{2+} uptake to cytosolic Ca^{2+} in neurons as compared to non-neuronal cells such e.g., HEK293 cells that likely lack MICU3 and rely on the MICU1-MICU2 complex to gate the MCU channel (Ashrafi et al., 2020). Notably, this MICU3-dependent sensitization of mitochondrial Ca^{2+} uptake plays a critical role as the synapses by boosting Ca^{2+}-dependent ATP synthesis in presynaptic terminals (Ashrafi et al., 2020). Additional knowledge of the role of MICU3 within the MCU complex will be important for understanding the mechanism by which it influences neuronal activity, but the available data show that it is critical for normal neuronal activity.

EMRE

Another important component of the uniporter complex is EMRE, which was named for its role as an essential MCU regulator (Sancak et al., 2013; Tsai et al., 2016). This protein is critical for normal uniporter function. It is a 10 kD protein with only one transmembrane domain. When EMRE is deleted the MCU pore forms, but it is not functional and it fails to interact with MICU1 and MICU2 (Sancak et al., 2013; Tsai et al., 2016). EMRE is encoded only in metazoan genomes with no homologs in other organisms (Sancak et al., 2013; Tsai et al., 2016; Liu et al., 2017). The MCU/EMRE complex assembles at a 1:1 stoichiometry, and it does so on the membrane facing side of the MCU tetramer within the transmembrane domains (Wang et al., 2019). This stoichiometry was further supported by recent identification of the assembled MCU complex with EMRE, MICU1, and MICU2 all present. Interestingly, the structure of the EMRE containing MCU complex is similar regardless of whether or not MICU proteins are bound (Fan et al., 2020). This shows that proper MCU/EMRE assembly is not dependent upon MICUs. Recently, EMRE KO mice were characterized and found to be viable and to have normal metabolism, however, the birth rates were low. The EMRE KO mice had reduced mitochondrial Ca^{2+} uptake and were resistant to Ca^{2+} driven mitochondrial dysfunction, but they were not protected from ischemic reperfusion injury (Liu et al., 2020). These mice will be a useful model for further determining the role of Ca^{2+} uptake *via* EMRE/MCU complex formation in other diseases in which mitochondrial Ca^{2+} overload occurs.

Mitochondrial Ca^{2+} extrusion

Under physiological conditions, mitochondrial Na^+/Ca^{2+} (mtNCX) and H^+/Ca^{2+} (mtHCX) exchangers mediate Ca^{2+} extrusion from mitochondria (De Stefani et al., 2016). In excitable cells, such as neurons and muscles, mtNCX is the main contributor to this transport (Crompton et al., 1978; Thayer et al., 2002; Medvedeva et al., 2008; Jadiya et al., 2019; Rysted et al., 2021; Yang et al., 2021; Hagenston et al., 2022). It was discovered that when Ca^{2+} uptake was blocked, Ca^{2+} extrusion from the mitochondria occurs in a Na^+ dependent manner (Nicholls, 1978). Interestingly, the release of Ca^{2+} by mitochondria was not inhibited by the inhibitor of Ca^{2+} uptake ruthenium red, suggesting that a reversal of the uptake mechanism is not responsible for the Ca^{2+} release (Rossi et al., 1973; Nicholls, 1978). An early study, performed in isolated mitochondria, identified the exchanger responsible for Ca^{2+} extrusion as 110 kD in size (Li et al., 1992).

In 2010, Sekler and colleagues proposed that a so-called $Na^+/Ca^{2+}/Li^+$ exchanger (NCLX), a product of gene *Slc8b1*, represents an essential component of the mitochondrial Na^+/Ca^{2+} exchanger (Palty et al., 2010). Historically, NCLX was first described as the sixth member of the family of $Na^+/Ca^{2+}/K^+$ exchangers, and it was named NCKX6 (Cai and Lytton, 2004b). It was found to express as two splice isoforms, with the short isoform being localized to the plasma membrane and the full-length isoform being localized to the ER (Cai and Lytton, 2004b). At about the same time it was shown that NCKX6 was able to exchange Li^+ for Ca^{2+} across the plasma membrane as effectively as it could exchange Na^+ for Ca^{2+}, and the protein was renamed to NCLX (Palty et al., 2004; Palty et al., 2006). Later, the same group showed that in addition to the plasma membrane and ER, NCLX was also enriched in the mitochondrial fraction (Palty et al., 2010). It was also found to partially localize to the mitochondria using gold-particle transmission electron microscopy, although some particles were located outside of the mitochondria as well. In addition to the localization data, these functional assays revealed that mitochondrial Ca^{2+} efflux increased when NCLX was overexpressed and that it decreased when NCLX was silenced using an shRNA approach (Palty et al., 2010). All this led to the proposal that NCLX is an essential contributor to the mitochondrial Na^+/Ca^{2+} exchange (Palty et al., 2010).

NCLX is broadly expressed throughout the body including the brain and spinal cord (Palty et al., 2010; Jadiya et al., 2019; Patron et al., 2019). The *in vivo* role of NCLX was first demonstrated in the cardiomyocytes of adult mice (Luongo et al., 2017). Cardiomyocyte-specific deletion of NCLX resulted in severe lethality (87%) within two weeks, due to heart failure; whereas overexpression of NCLX resulted in protection from ischemic heart damage (Luongo et al., 2017). The same group also provided evidence for the role of NCLX in aging brain (Jadiya et al., 2019). They showed that in the 3xTg-AD mouse model of Alzheimer's disease, the neuron-restricted deletion of NCLX increased tau and amyloid pathology and increased the rate of cognitive decline in mice. Restoration of NCLX expression reduced the acceleration of Alzheimer's pathology (Jadiya et al., 2019). Similarly, disruption of NCLX expression was shown to sensitize hippocampal neurons to excitotoxic stress (Hagenston et al., 2022). A recent study has reported that a human recessive missense mutation in the gene encoding NCLX, *SLC8B1*, impairs its function and is associated with severe intellectual disability (Stavsky et al., 2021). The same study also demonstrated that deletion of NCLX in mice impaired synaptic transmission and long-term synaptic plasticity (Stavsky et al., 2021). Collectively, these studies highlight a key role of NCLX in regulating synaptic function and neuronal survival.

Despite the appreciation of NCLX as a mediator of Ca^{2+} extrusion from mitochondria, several studies showed that NCLX is also localized

on the plasma membrane, endoplasmic reticulum (ER), and possibly other organelles (Haworth and Hunter, 1979; Bernardi, 1999; Cai and Lytton, 2004a; Palty et al., 2004; Palty et al., 2006; Han et al., 2015; Rysted et al., 2021). There is also inconsistency between the ability of NCLX to exchange Ca^{2+} for Li^+ nearly as effectively as for Na^+ (Palty et al., 2004; Roy et al., 2017) and the observations that Li^+-driven mitochondrial Ca^{2+} efflux is only 10–25% of that driven by Na^+ (Crompton et al., 1977; Tokunaga et al., 2000; Rysted et al., 2021). In addition, recent studies showed that NCLX knockdown or knockout had little effect on mitochondrial Ca^{2+} extrusion in airways smooth muscle and B lymphocytes, respectively (Emrich et al., 2022; Johnson et al., 2022). These findings raise several important questions for future research regarding the mechanisms of mitochondrial Ca^{2+} extrusion and the roles of NCLX, such as: 1) What are the mechanisms of cellular trafficking of NCLX? 2) What are the functions of NCLX on the plasma membrane, ER and possibly other organelles? 3) Are there additional yet to be identified molecules that mediate Na^+/Ca^{2+} exchange across the mitochondrial membranes?

Molecular identity of the mPTP

Although Ca^{2+} uptake into the mitochondria is critical for normal cellular function, the entry of excessive amount of Ca^{2+} into the mitochondria can result in Ca^{2+} overload and lead to activation of the mitochondrial permeability transition pore (mPTP); this phenomenon has been associated with many different disease states, and thus the mPTP is considered a promising target for therapeutic intervention (Duchen et al., 1993; Irwin et al., 2003; Du et al., 2008; Martin et al., 2009; Martin et al., 2014). Ca^{2+} dependent activation of the mPTP, in isolated mitochondria, results in the formation of a large non-selective ion channel that is permeable to solutes up to approximately 1,500 Da in size, which allows for the passage of internalized Ca^{2+} and many proapoptotic proteins out of the mitochondria (Haworth and Hunter, 1979; Bernardi, 1999; Pivovarova and Andrews, 2010; Jung et al., 2020). Prolonged opening of the mPTP results in the loss of $\Delta\Psi_{mt}$, which in turn prevents the production of ATP within the mitochondria and leads to cell death (Bernardi, 1999; Briston et al., 2019). Transient, low-conductance opening or "flickering" of the mPTP has been shown to occur in response to increased $[Ca^{2+}]_{mt}$ and helps to reduce the accumulation of Ca^{2+} into the mitochondria (Bernardi and Petronilli, 1996; Ichas et al., 1997; Bernardi, 1999; Loupatatzis et al., 2002; Rasola and Bernardi, 2011; Garbincius and Elrod, 2022). In further support of the Ca^{2+} dependent activation of mPTP, preventing Ca^{2+} uptake into the mitochondria by deleting MCU reduces mPTP activation in response to high levels of Ca^{2+} (Hamilton et al., 2021). Despite the presence of the mPTP being known for many years, the identity of the proteins that make up the mPTP remains highly debated.

Early hypotheses proposed that the mPTP could form as the result of contact between inner and outer mitochondrial proteins, specifically between voltage-dependent anion-selective channels (VDAC) on the outer membrane and adenine nucleotide translocators (ANT) on the inner mitochondrial membrane (Zamzami and Kroemer, 2001). Although, deletion of each of these proteins failed to inhibit formation of the mPTP (Giorgio et al., 2018). More recently, compelling evidence has pointed towards a role for the F_0F_1 ATP synthase in forming the mPTP (Giorgio et al., 2013; Alavian

et al., 2014; Giorgio et al., 2018; Mnatsakanyan et al., 2019; Neginskaya et al., 2019; Carrer et al., 2021). Using blue native gel electrophoresis and immunoprecipitation, Giorgio and colleagues found that the essential mPTP regulator cyclophilin D interacts with F_0F_1 ATP synthase (Giorgio et al., 2009). Subsequent immunoprecipitation experiments identified that the oligomycin sensitivity conferring protein (OSCP) subunit of ATP synthase specifically interacts with cyclophilin D through the same binding site as the ATP synthase inhibitor benzodiazepine 423 (Stelzer et al., 2010; Giorgio et al., 2013). Following these findings, experiments suggested that the c-subunit of F_0 was able to form a voltage-sensitive channel that also appeared to be sensitive to Ca^{2+} exposure (Alavian et al., 2014), and had conductance consistent with activated mPTP (Mnatsakanyan et al., 2022). When c-subunit expression was reduced using an shRNA, mPTP activation was reduced and the survival of HeLa cells increased (Alavian et al., 2014). The deletion of the c-subunit from HeLa cells prevented mPTP activation in response to ionomycin as efficiently as the pre-application of cyclosporin A and overexpression of the c-subunit increased the rate of mPTP transition (Bonora et al., 2013). In HAP1 cells, knocking out the c-subunit of F_0 was also shown to significantly alter, but not eliminate, mPTP activity. In this context, channels formed and remained sensitive to the mPTP inhibitor cyclosporine A and to the ANT inhibitor, bongkrekate (Neginskaya et al., 2019). Deletion of specific subunits of the ATPase impaired the formation of the mPTP. Specifically, the deletion of the g subunit, which also reduced subunit e expression, impaired mPTP channel opening in both mitoplasts and in HeLa cells (Carrer et al., 2021). However, some studies suggested that the mPTP is not ATP synthase, by demonstrating mPTP activation even after the deletion of ATP synthase subunits (He et al., 2017a; He et al., 2017b; Carroll et al., 2019). In support of these findings, the deletion of the b and OSCP subunits in HAP1 cells did not interfere with functional mPTP currents that were not blocked by cyclosporin A (Carrer et al., 2021). It is possible that in the absence of ATPase subunits, the ANT could form a functional mPTP, consistent with the finding that addition of bongkrekate impaired mPTP opening in these cells (Neginskaya et al., 2019; Carrer et al., 2021). However, a recent study using intact HAP1 cells suggests that both ANT and ATPase are required for mPTP activation, but that mitochondrial depolarization occurs through low-conductance, cyclosporin A sensitive permeability transition (Neginskaya et al., 2022). These findings suggest that while mPTP activation requires both ANT and ATPase, the low-conductance or "flickering" activity may occur through either of these molecules or through different mechanisms altogether.

Collectively, the available data on the mPTP represent compelling evidence that the F_0F_1 ATPase plays a critical role in its formation, but also suggest that certain subunits of the ATPase can be compensated for by other mechanisms. The combination of the results for and against the notion that F_0F_1 ATPase is the mPTP suggest that cell-type specific differences exist and that the mPTP can form *via* several mechanisms (He et al., 2017a; He et al., 2017b; Carrer et al., 2021). There are also other proposed Ca^{2+} sensitive, pore-forming components of mPTP including the mitochondrial phosphate carrier (PiC) (Leung et al., 2008) and spastic paraplegia 7 (SPG7) (Shanmughapriya et al., 2015). However other studies suggest these proteins may have a regulatory role as opposed to a role in forming the pore (Briston et al., 2019; Hurst et al., 2019; Boyenle et al., 2022). The key cytotoxic role of mPTP in many diseases, makes this molecular

complex an attractive therapeutic target for neuroprotection, and more generally, cytoprotection. However, the existence of compensatory mechanisms helps explain why targeting this system for the development of therapeutics is so challenging. Even if additional potential inhibitors are identified, they may have significant side effects given the importance of the ATPase for normal cellular function.

Mitochondrial Ca²⁺ cycling in neuronal function

Regulation of synaptic transmission

Ca²⁺ signaling plays an especially important role in neurons because it regulates many aspects of neurotransmitter release and synaptic function (Neher and Sakaba, 2008) (Figure 1). For example, Ca²⁺ triggers synchronous transmitter release *via* the low-affinity presynaptic Ca²⁺ sensors synaptotagmin 1, 2, and 9 (Xu et al., 2007; Bacaj et al., 2013). In addition, the subsequent endocytotic retrieval of synaptic vesicles is regulated by Ca²⁺- and calcineurin-dependent dephosphorylation of dynamin-1 and other endocytotic proteins (Cousin and Robinson, 2001; Clayton and Cousin, 2009). Presynaptic Ca²⁺ also regulates short-term synaptic plasticity by controlling the size of the readily releasable pool (RRP) of synaptic vesicles.

Presynaptic terminals are enriched for mitochondria, an observation that reflects high energy demand at the site of transmitter release (De Camilli et al., 2001; Hollenbeck, 2005; Wimmer et al., 2006). Ca²⁺ buffering by mitochondria limits the amplitude of the activity-driven elevation of presynaptic [Ca²⁺]ᵢ and regulates synaptic strength at many different synapses, including central synapses, nerve-muscular junction, and the first sensory synapse (Tang and Zucker, 1997; David, 1999; David and Barrett, 2000; Billups and Forsythe, 2002; David and Barrett, 2003; Lee et al., 2007; Shutov et al., 2013; Kwon et al., 2016). Indeed, inhibition of mitochondrial Ca²⁺ uptake resulted in a larger presynaptic [Ca²⁺]ᵢ elevation, an increase in the frequency of asynchronous release, and accelerated synaptic depression at the neuro-muscular junction (David, 1999; David and Barrett, 2000; 2003). Similar effects were described at the central synapses (Billups and Forsythe, 2002; Lee et al., 2007). In the case of GABAergic synaptic transmission between retinal amacrine cells, mitochondrial depolarization led to a reduction in evoked transmitter release that was attributed to an increase in Ca²⁺-dependent inactivation of presynaptic voltage-gated Ca²⁺ channels in the absence of Ca²⁺ buffering by mitochondria (Medler and Gleason, 2002).

Mitochondrial Ca²⁺ transport also contributes to post-tetanic potentiation (PTP) at neuro-muscular and central synapses (Tang and Zucker, 1997; Zhong et al., 2001; Storozhuk et al., 2005; García-Chacón et al., 2006; Lee et al., 2007). PTP is a form of short-term synaptic plasticity that is induced by high-frequency (tetanic) stimulation of the presynaptic terminal, and is characterized by an enhancement of synaptic strength that lasts for many minutes (Zucker and Regehr, 2002). This phenomenon relies on a prolonged presynaptic [Ca²⁺]ᵢ elevation (residual [Ca²⁺]ᵢ or [Ca²⁺]ᵢ plateau) following the initial [Ca²⁺]ᵢ rise to the micromolar levels in response to intense stimulation (Zucker and Regehr, 2002; Neher and Sakaba, 2008). In many instances prolonged Ca²⁺ release from presynaptic mitochondria accounts for the residual [Ca²⁺]ᵢ (Tang and Zucker, 1997; Zhong et al., 2001; García-Chacón et al., 2006; Lee et al., 2007; Medvedeva et al., 2008). For example, high-frequency

presynaptic stimulation produced a prolonged residual presynaptic [Ca²⁺]ᵢ elevation and PTP in nerve muscular junctions of crayfish and mouse, as well at mossy fiber synapses of the rat hippocampus that were blocked by inhibitors of mitochondrial Ca²⁺ transport (Tang and Zucker, 1997; Zhong et al., 2001; García-Chacón et al., 2006; Lee et al., 2007). A similar mechanism was shown to contribute to short-term facilitation at the calyx of Held synapse (Yang et al., 2021). Mitochondria-dependent PTP was also reported for GABAergic synaptic transmission between neocortical neurons, although presynaptic [Ca²⁺]ᵢ was not measured in this study (Storozhuk et al., 2005). It is most likely that Ca²⁺ release from presynaptic mitochondria is mediated by the mitochondrial Na⁺/Ca²⁺ exchanger (García-Chacón et al., 2006; Lee et al., 2007; Medvedeva et al., 2008). However alternative mechanisms may also be involved at some synapses (Zhong et al., 2001).

Findings from recent studies in *Drosophila* motor terminals suggest that mitochondrial Ca²⁺ uptake enhances energy metabolism by presynaptic mitochondria (Chouhan et al., 2012; Ivannikov and Macleod, 2013). These observations are consistent with the ability of mitochondrial Ca²⁺ to stimulate Ca²⁺-dependent dehydrogenases, pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, thereby increasing the input of critical substrates into the oxidative phosphorylation reaction and ATP synthesis (Denton, 2009; Glancy and Balaban, 2012). Given that [Ca²⁺]ₘₜ rarely exceeds 5 μM, it is likely that pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, each of which has Ca²⁺ Kd of ~0.8 μM (for comparison, isocitrate dehydrogenase has a Ca²⁺ Kd of~40 μM) (McCormack et al., 1990), are the primary targets of matrix Ca²⁺. Such a mechanism may be an important coordinator of ATP supply and demand at the synapse. Indeed, increased synaptic activity is associated with larger amounts of Ca²⁺ entering the presynaptic terminal, which in turn increases Ca²⁺ flux into the mitochondria and stimulates ATP-synthesis. Thus, mitochondrial Ca²⁺ transport regulates synaptic transmission through at least two general mechanisms, with one contributing to the shaping of presynaptic [Ca²⁺]ᵢ signals, and the other impacting the ATP synthesis at the synapse.

Recent work started exploring the roles of specific molecular components of the mitochondrial Ca²⁺ transport in synaptic transmission. Inhibiting MCU in the *Drosophila* mushroom body neurons caused impairment of synaptic function and olfactory memory (Drago and Davis, 2016). In rat hippocampal neurons, MCU and MICU3 were found to contribute to presynaptic ATP synthesis during synaptic activity (Ashrafi et al., 2020). The same study also found that MCU silencing slowed the rate of endocytosis of synaptic vesicles (Ashrafi et al., 2020). However, an opposite effect of MCU silencing on the rate of synaptic vesicle endocytosis in hippocampal neurons was also reported (Marland et al., 2016). Thus, despite recent progress many questions remain about the role of MCU and other components of the MCU complex at synapses, including their specific roles in regulating synaptic transmission and synaptic plasticity at various excitatory and inhibitory synapses.

Regulation of intrinsic excitability in neurons

One of the essential roles of Ca²⁺ as a second messenger is to regulate neuronal excitability (Figure 1). This is achieved through the gating of three types of ion channels: Ca²⁺-activated K⁺, Ca²⁺-activated

Cl⁻ and Ca²⁺-activated cation channels (Partridge and Swandulla, 1988; Marty, 1989; Scott et al., 1995; Sah, 1996; Hartzell et al., 2005). The small- and large-conductance Ca²⁺-activated K⁺ channels, SK and BK, respectively, are widely expressed in neurons and play important roles in regulating their excitability (Sah, 1996; Wei et al., 2005). Ca²⁺-activated cation and Cl⁻ channels require $[Ca^{2+}]_i$ elevations in the 0.1–1 μM range to open, and the former channels contribute to neuronal depolarization, whereas the latter can either depolarize or hyperpolarize the membrane depending on the transmembrane Cl⁻ gradient and membrane potential (Partridge and Swandulla, 1988; Scott et al., 1995; Hartzell et al., 2005). Ca²⁺ also modulates many other channels and receptors including voltage-gated Ca²⁺ channels, TRP channels, and NMDA glutamate receptors (McBain and Mayer, 1994; Catterall, 2000; Caterina and Julius, 2001; Budde et al., 2002; Clapham, 2003; Chuang et al., 2004; Catterall et al., 2013).

Mitochondria buffering and the release of Ca²⁺ have complex effects on Ca²⁺-activated and -modulated conductances and neuronal excitability. For example, blocking mitochondrial Ca²⁺ uptake by applying protonophores or inhibitors of electron transport resulted in hyperpolarization mediated by Ca²⁺-activated K⁺ channels in hippocampal and myenteric neurons (Nowicky and Duchen, 1998; Vanden Berghe et al., 2002). Similarly, intracellular application of the MCU selective inhibitor Ru360 facilitated slow afterhyperpolarization (AHP) in CA1 hippocampal pyramidal neurons (Groten and MacVicar, 2022). Several reports also showed that blocking mitochondrial Ca²⁺ uptake reduced voltage-gated Ca²⁺ currents, likely due to an increase in Ca²⁺-dependent inactivation of the Ca²⁺ channels (Hernandez-Guijo et al., 2001; Medler and Gleason, 2002; Shutov et al., 2013). Thus, under normal conditions, mitochondrial Ca²⁺ uptake limits Ca²⁺-dependent activation of BK and SK channels, and minimizes Ca²⁺-dependent inactivation of voltage-gated Ca²⁺ channels. The latter effect is especially critical for the synapse, where Ca²⁺ flux through these channels trigger transmitter release.

A recent study has demonstrated a specific role of MCU in regulating so-called Ca²⁺-release activated Ca²⁺ (CRAC) channels and Ca²⁺ signaling (Yoast et al., 2021). CRAC channels are activated in response to agonist stimulation and inositol-1,4,5-trisphosphate-induced Ca²⁺ release from ER stores and play important roles in Ca²⁺ signaling in a broad variety of non-excitable and excitable cells (Prakriya and Lewis, 2015; Emrich et al., 2021). In neurons, CRAC channels can control both excitability and synaptic transmission (Gemes et al., 2011; Ureshino et al., 2019; Hori et al., 2020; Maneshi et al., 2020). In their work, Yoast et al., found that MCU deletion led to increased Ca²⁺-dependent inactivation of CRAC channels and reduced Ca²⁺ currents through the channels. Yet, the net result was an overall amplification of cytosolic Ca²⁺ signaling caused by elimination of mitochondrial Ca²⁺ buffering in the absence of MCU (Yoast et al., 2021; Walters and Usachev, 2022). Although this effect was documented in non-excitable cell lines, given that CRAC channels and MCU are present in the nervous tissues, it is predicted that a similar mechanism could contribute to the regulation of neuronal excitability (Walters and Usachev, 2022).

Mitochondrial Ca²⁺ cycling in neurological diseases

Mitochondrial Ca²⁺ regulation in epilepsy

Epilepsy is one of the most common neurologic conditions worldwide, with close to 70 million people currently diagnosed and approximately 1 in 26 people expected to develop epilepsy in their lifetime (Hesdorffer et al., 2011; Friedman et al., 2018). For many of these patients, available pharmaceutical interventions reduce the prevalence of seizures. Unfortunately, 30% of patients have epilepsy that is poorly controlled by current pharmaceuticals, a condition referred to as refractory epilepsy (Kwan and Brodie, 2000). Patients with refractory epilepsy are at a much greater risk of experiencing sudden unexpected death in epilepsy (SUDEP, 1/150) than those with non-refractory epilepsy (1/1,000) (Thurman et al., 2014; Friedman et al., 2018). The increased risk of mortality associated with refractory epilepsy justifies further research of mechanisms underlying epilepsy; such knowledge will be required to identify novel therapeutic targets for epilepsy.

The changes in neuronal networks that result in epilepsy can have multiple causes. For example, lesions that occur following strokes or traumatic brain injuries result in structural changes that increase the risk of epilepsy. Genetic mutations are another common cause for epilepsy. In many cases, however, epilepsy is of unknown etiology, i.e., no clear underlying cause can be identified (Berg et al., 2010). Multiple forms of epilepsy have been associated with genetic mutations that lead to metabolic and mitochondrial dysfunction; mutations in both mitochondrial and nuclear DNA have been implicated in these cases (Bindoff and Engelsen, 2012; Zsurka and Kunz, 2015; Pearson-Smith and Patel, 2017). One such example is myoclonic epilepsy and ragged-red fibers (MERRF) syndrome, for which myoclonus is a defining feature that presents along with other neurologic defects. Studies of cultured cells into which the patient mitochondrial DNA mutation had been introduced showed that this resulted in decreased ATP-dependent protease activity and increased ROS production (Wu et al., 2010). MERRF mutations also resulted in disrupted mitochondrial Ca²⁺ handling but did not alter cytosolic Ca²⁺ regulation. The authors observed reduced ATP production in these cells following stimulation with histamine. They hypothesized that the reduction in ATP production resulted from a reduction in Ca²⁺ entry into the mitochondria, and that this led to reduced activation of Ca²⁺-dependent enzymes (Brini et al., 1999). Other examples of genetic diseases that lead to epilepsy by interfering with metabolic function include Alpers-Huttenlocher syndrome (AHS) and Leigh syndrome; in each, mutations lead to reduced ATP production and refractory epilepsy (Bindoff and Engelsen, 2012; Zsurka and Kunz, 2015; Pearson-Smith and Patel, 2017). Although, disrupted energy production results in many different forms of epilepsy, a loss-of-function mutation in MICU1 in humans instead results in myopathy, movement disorders, and learning disabilities (Logan et al., 2014). The propensity for mitochondrial diseases to result in epilepsy identify the mitochondria as targets for new therapies for epilepsy.

Epilepsy occurs when brain networks transition into a state of hyperexcitability, a process known as epileptogenesis (Scharfman, 2007). Hyperexcitability of neuronal networks is a key change that occurs in individuals who develop epilepsy. It can be caused by changes in gene expression, posttranslational modification, or synaptic circuits (Kinjo et al., 2018). These processes are frequently Ca²⁺ dependent. For example, dendritic arborization and synaptogenesis are dependent upon changes in gene expression following increases in intracellular Ca²⁺ levels (Lohmann and Wong, 2005; Redmond and Ghosh, 2005). Mitochondrial Ca²⁺ regulation has been shown to be important for neurotransmission and altered mitochondrial function can disrupt synaptogenesis (MacAskill et al., 2010; Sun et al., 2013). Proper Ca²⁺ regulation by

FIGURE 3
Mitochondrial Ca^{2+} overload leading to activation of the mPTP is a common pathway in many neurological diseases. **(A)** In epilepsy, seizures lead to buildup of cytosolic and mitochondrial Ca^{2+} through NMDA receptors, Ca^{2+} permeable AMPA receptors and Ca$_v$2 and Ca$_v$3 voltage-gated Ca^{2+} channels (VGCCs) in neurons that are in a state of hyperexcitability. Long seizures or repeated seizures can lead to Ca^{2+} overload in the mitochondria and trigger activation of the mPTP, ultimately leading to neuronal toxicity. Commonly prescribed antiepileptic drugs target these channels including: ethosuximide, which is a Ca$_v$3 VGCC inhibitor, as well as gabapentin and pregabalin, which act by binding to the with α2δ-1 subunit of voltage-gated Ca^{2+} channels, which could disrupt plasma membrane expression and function of Ca$_v$2 VGCCs, NMDA receptors, and Ca^{2+} permeable AMPA receptors. **(B)** In Alzheimer's disease, Ca^{2+} overload in the mitochondria occurs through multiple mechanisms. Ca^{2+} entry into the cytosol can be enhanced by binding of Aβ to NMDA receptors. Internal release of Ca^{2+} from the ER through activation of metabotropic glutamate receptors (mGluR) also increases the accumulation of Ca^{2+} into the mitochondria. Enhanced Ca^{2+} extrusion from the ER has also been shown to occur through interactions between presenilin 1/2 (PS1/2) and inositol trisphosphate receptors (IP$_3$R) and ryanodine receptors (RyR). This interaction seems to occur at mitochondria associated membranes (MAMs), which due to the proximity to the mitochondria, drives Ca^{2+} uptake into the mitochondria. Aβ oligomers also can form a pore in the mitochondria that can lead to increases in Ca^{2+} in the mitochondria. Ca^{2+} extrusion through the mitochondrial Na$^+$/Ca^{2+} exchanger (mtNCX) has been shown to be impaired by tau accumulation leading to excessive Ca^{2+} accumulation in the mitochondria. Memantine, one of the FDA approved drugs for AD, inhibits NMDA receptors, which could block excessive Ca^{2+} uptake and to reduce glutamate induced excitotoxicity. **(C)** In Parkinson's disease (PD), substantia nigra dopaminergic neurons are at risk of Ca^{2+} overload due to the presence of voltage-gated Ca$_v$1.3 channels, which are critical for the pacemaking activity of these neurons. One common form of autosomal recessive familial PD occurs through mutations in *PARK2*, the gene for Parkin. Parkin is responsible for regulating mitochondrial Ca^{2+} uptake isoform 1 (MICU1) degradation and may lead to disrupted MCU assembly altering mitochondrial Ca^{2+} uptake. Another form of autosomal recessive PD is caused by mutations in *PINK1*, which can impair mtNCX function and increase Ca^{2+} accumulation into the mitochondria. In astrocytes, ER Ca^{2+} release at MAMs may be enhanced in PD due to mutations in leucine-rich repeat kinase 2 (LRRK2), which enhance Ca^{2+} uptake into the ER through activation of sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA). The NMDA receptor antagonist amantadine is used to prevent dyskinesia in PD patients. **(D)** In amyotrophic lateral sclerosis (ALS), motor neurons of the spinal cord and brain begin to degenerate. These neurons have been shown to have increased Ca^{2+} uptake through glutamate NMDA and Ca^{2+} permeable AMPA receptors, which enhances their susceptibility to mitochondrial Ca^{2+} overload. Excitotoxic glutamate, which signals through NMDA and GluA2-lacking Ca^{2+} permeable AMPA receptors, is the target of Riluzole, one of the few FDA approved drugs for ALS.

mitochondria is essential for normal neuronal processes including synaptogenesis, excitability, neurotransmission, and ATP synthesis (Billups and Forsythe, 2002; Thayer et al., 2002; Mattson et al., 2008; Usachev, 2015). During seizures, excess Ca^{2+} can build up within the cytosol and eventually lead to mitochondrial Ca^{2+} overload, which is known to lead to cell death (Figure 3).

Indeed, hippocampal sclerosis and neuronal loss in the hippocampus and other limbic and mesial cortical structures is the very common pathological finding in patients with temporal lobe epilepsy, the most prevalent form of epilepsy in humans (Levesque and Avoli, 2013; Tai et al., 2018). In fact, frequent generalized convulsive seizures are a strong predictor of cognitive decline as well as

progressive development of behavioral and psychiatric abnormalities in patients with epilepsy, all of which is linked to neuronal damage in the limbic and mesial cortical regions (Kovac et al., 2017; Tai et al., 2018; Farrell et al., 2019). Cell death is particularly apparent following status epilepticus, sustained generalized convulsive seizures lasting 5 min or more (Walker, 2018). These prolonged seizures result in Ca^{2+} overload in neurons and astrocytes (Tian et al., 2005; Fellin et al., 2006; Ding et al., 2007). Neurotoxic build-up of glutamate released from firing neurons and astrocytes is thought to be the major trigger of neuronal damage (i.e., excitotoxicity) caused by prolonged seizures, as well as in other pathological conditions (e.g., stroke, and neurodegenerative diseases)

(Nicholls and Ward, 2000; Mattson et al., 2008; Moskowitz et al., 2010; Kovac et al., 2017). Glutamate-induced Ca^{2+} entry, largely *via* NMDA as well as Ca^{2+}-permeable AMPA glutamate receptors, mitochondrial Ca^{2+} overload and Ca^{2+} deregulation initiate neurotoxic cascades through many mechanisms, including rupture of mitochondrial membranes, mitochondrial depolarization, ROS generation and opening of mitochondrial permeability transition pore (mPTP) and release of cytochrome c and other proapoptotic factors (Figure 3) (Nicholls and Ward, 2000; Newmeyer and Ferguson-Miller, 2003; Orrenius et al., 2003; Munoz-Pinedo et al., 2006; Mattson et al., 2008; Moskowitz et al., 2010; Guo and Ma, 2021). Accordingly, selective antagonists of glutamate NMDA or Ca^{2+}-permeable AMPA receptors reduced seizure-induced neuronal toxicity in animal models of status epilepticus and temporal lobe epilepsy (Ding et al., 2007; Kovac et al., 2017; Walker, 2018; Konen et al., 2020).

Antiepileptic drugs such as gabapentin and pregabalin were also shown to reduce hippocampal neuronal loss in the rodent models of temporal lobe epilepsy and to improve mitochondrial function (Huang et al., 2013; Rossi et al., 2013; Finsterer and Scorza, 2017). Although originally designed as GABAmimetics, it is now well accepted that the anticonvulsant action of these gabapentinoids is mediated by mechanisms distinct from GABAergic activity. Instead, it has been proposed that these drugs interact with α2δ-1 subunit of voltage-gated Ca^{2+} channels, which in turn interferes with α2δ-1-dependent insertion and retention of α1 subunits of the channels (Dolphin, 2012; Sills and Rogawski, 2020) (Figure 3). An additional mechanism likely involves interaction of α2δ-1 subunit with glutamate NMDA receptor (Sills and Rogawski, 2020), and disrupting this interaction by gabapentin was shown to inhibit neuronal toxicity caused by ischemic stroke (Luo et al., 2018). The α2δ-1 subunit can also interact with the GluA1 and GluA2 subunits of glutamate AMPA receptors, which promotes expression of GluA2-lacking Ca^{2+}-permeable AMPA receptors, the process that can be disrupted by gabapentin (Li et al., 2021). Thus, it is plausible that the described neuroprotective effects of gabapentin and pregabalin are mediated by their inhibitory action on NMDA and Ca^{2+}-permeable GluA2-lacking AMPA receptors (Figure 3).

The mitochondrial Ca^{2+} overload due to mitochondrial Ca^{2+} uptake is a major trigger of excitotoxicity and neuronal death downstream of Ca^{2+} entry into neurons *via* glutamate NMDA receptors and other Ca^{2+} channels (Nicholls and Ward, 2000; Mattson et al., 2008; Kovac et al., 2017). Consequently, it is predicted that inhibiting mitochondrial Ca^{2+} uptake is neuroprotective. To this end, a recent study reported that systemically administered MCU inhibitor Ru360 reduced hippocampal neuronal death in a rat model of pilocarpine-induced status epilepticus (Wang et al., 2015). The findings of this work however, should be interpreted with caution because Ru360 poorly penetrates through the plasma membrane, which is required for inhibiting MCU (Hajnoczky et al., 2006; Woods and Wilson, 2020; Marta et al., 2021). In addition, Ru360 is very unstable for *in vivo* work, and can affect various ion channels of the plasma membrane when applied extracellularly (Hajnoczky et al., 2006; Woods and Wilson, 2020; Marta et al., 2021). Thus, additional studies are needed to test the specific roles of the mitochondrial Ca^{2+} uptake and release mechanisms transport in neurotoxicity associated with seizures and epilepsy.

In summary, mitochondrial Ca^{2+} cycling can affect neural activity and susceptibility to seizures and epilepsy *via* several mechanisms,

including regulation of neuronal excitability, synaptic transmission, bioenergetics, and excitotoxicity (Figures 1, 3). A better understanding of neuronal Ca^{2+} regulation may prove essential for further elucidation of the mechanisms that underlie epilepsy, and thus for identifying potential therapies.

Mitochondrial Ca^{2+} regulation in Alzheimer's disease

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder and the most common form of dementia in the elderly. It is characterized by progressive impairment of memory and cognitive functions and may lead to a completely vegetative state and early death (Querfurth and LaFerla, 2010; Scheltens et al., 2016; Corriveau et al., 2017). AD currently affects approximately 5.8 million Americans who are 65 years of age and older and current estimates predict that this number will increase to 13.8 million by 2050 (Alzheimer's and Dementia, 2020a).

AD pathology is characterized by an accumulation of β-amyloid (Aβ) and the formation of neurofibrillary tangles from accumulated tau protein (Hyman et al., 2012). According to the amyloid hypothesis of AD, progressive accumulation and deposition of Aβ42 plaques leads to a cascade of pathological changes in the brain that ultimately lead to the synaptic and neuronal loss and progressing dementia (Selkoe and Hardy, 2016; Golde et al., 2018). Such changes include inflammation, the activation of microglia and astrocytes, an accumulation of hyperphosphorylated tau and neurofibrillary tangles, altered ionic homeostasis, and oxidative injury (Selkoe and Hardy, 2016; Golde et al., 2018). Neuronal Ca^{2+} deregulation is thought to play critical roles during various phases of AD and can manifest in many forms, including chronic elevation of the cytosolic and mitochondrial Ca^{2+} concentrations, enhanced Ca^{2+} release from intracellular Ca^{2+} stores, spontaneous Ca^{2+} spikes, and enhanced activity of glutamate NMDA receptors (Bezprozvanny and Mattson, 2008; Mattson et al., 2008; Querfurth and LaFerla, 2010; Workgroup and Khachaturian, 2017) (Figure 3). Although various mechanisms contribute to Ca^{2+} deregulation in AD, most of them ultimately lead to a common outcome–mitochondrial Ca^{2+} overload. This in turn, is thought to trigger neuronal demise through a range of neurotoxic mechanisms, including mPTP opening and release of proapoptotic factors, mitochondrial depolarization and ROS generation (Bezprozvanny and Mattson, 2008; Mattson et al., 2008; Querfurth and LaFerla, 2010).

Mitochondrial dysfunction in AD may be driven by multiple factors, but in this review we focus on the role of Ca^{2+} dysregulation in the progression of AD. Ca^{2+} dysregulation by mitochondria in the context of AD pathology was proposed to be a consequence of the aggregation of Aβ within the mitochondria by forming functional channels that have a high affinity for Ca^{2+} ions (Figure 3) (Arispe et al., 1993; Jang et al., 2007; Hansson Petersen et al., 2008; Rosales-Corral et al., 2012). Direct channel formation into the mitochondria has received limited attention since, but Aβ induced Ca^{2+} release from ER has been proposed to contribute to mitochondrial Ca^{2+} overload (Jensen et al., 2013). In both *Xenopus* oocytes and cultured SH-SY5Y cells, ER Ca^{2+} release was triggered by soluble Aβ *via* generation of inositol triphosphate (IP_3) (Demuro and Parker, 2013; Jensen et al., 2013). Interestingly, in SH-SY5Y cells an increase in ER Ca^{2+} was also observed in the absence of IP_3 receptors, suggesting that a more direct effect of Aβ on the ER accounts for the

increased cytotoxicity of Aβ oligomers (Jensen et al., 2013). ER Ca^{2+} regulation is also disrupted by mutations in presenilin 1 (PS1) and presenilin 2 (PS2). Mutated PS1 and PS2 modulate IP_3R activity and increase Ca^{2+} release (Cheung et al., 2008; Modesti et al., 2021). PS1 mutants have also been shown to interact with ryanodine receptors and increase their activity (Rybalchenko et al., 2008; Chakroborty et al., 2009; Modesti et al., 2021), which combined with increased release from IP3-sensitive Ca^{2+} stores might contribute to elevated cytosolic Ca^{2+}, excessive mitochondrial Ca^{2+} load, and Ca^{2+} dysregulation associated with AD (Figure 3). The store-operated Ca^{2+} entry into the ER is also disrupted in "aging" neurons and is further deregulated by the presence of Aβ oligomers, which drives increases in cytosolic Ca^{2+} concentration (Calvo-Rodriguez et al., 2019). Injections of Aβ oligomers into the rat hippocampus leads to increased expression of type 2 ryanodine receptors at mitochondria associated membranes, which leads to increased Ca^{2+} release from the ER in response to Ca^{2+} signals and drives increased cytosolic Ca^{2+} concentrations (More et al., 2018). In addition to its potential direct actions on internal Ca^{2+} stores, Aβ is thought to modulate NMDA receptor activity and has been shown to contribute to Ca^{2+}-induced cytotoxicity (Popugaeva et al., 2018). Glutamate induced excitotoxicity through NMDA receptors is also the target of one of the few FDA approved therapies for AD, memantine (Sonkusare et al., 2005). However, memantine's effectiveness has been limited to symptomatic improvements in cognition of patients with moderate-to-severe AD (Areosa et al., 2005; Li et al., 2019).

It has been proposed that many forms of Ca^{2+} dysregulation in AD and other neurodegenerative disorders ultimately converge on mitochondrial Ca^{2+} overload, a crucial event that triggers mPTP opening, release of proapoptotic factors, ROS generation, and cell death (Bezprozvanny and Mattson, 2008; Mattson et al., 2008; Plotegher et al., 2021). Consequently, inhibiting MCU is predicted to be neuroprotective. Recent studies support this idea. Indeed, MCU was shown to be upregulated in the brain of 3xTg-AD (APP/PS1/tau model of AD) mice, whereas knocking down MCU led to reduced synaptic loss, increased mitochondrial mass and improved behavioral performance in memory tasks in 3xTg-AD mice (Cai et al., 2022). Another group recently reported that application of soluble Aβ peptides onto neurons increased mitochondrial Ca^{2+} concentration and promoted neuronal death, which could be reversed by extracellularly applied MCU inhibitor Ru360 (Calvo-Rodriguez et al., 2020). Complicating the interpretation of these findings is the observations that Ru360 poorly penetrates through the plasma membrane, which is required for inhibiting MCU, and can also affect various ion channels on the plasma membrane (Hajnoczky et al., 2006; Woods and Wilson, 2020; Marta et al., 2021). Thus, additional studies using more suitable pharmacological tools and genetic approaches will be needed to thoroughly test the role of MCU and other components of the MCU complex in AD models.

Along with Ca^{2+} uptake machinery, mitochondrial Ca^{2+} extrusion mechanisms are also potentially critical in AD progression. Recently, tau has been shown to impair Ca^{2+} extrusion out of the mitochondria through mtNCX and resulted in faster mitochondrial depolarization in response to repeated stimulations (Britti et al., 2020; Esteras and Abramov, 2020). NCLX, which has been proposed to be molecular determinant of the mtNCX transport, has reduced expression in the frontal cortex of patients with AD and overexpression of NCLX in animal models of AD slows disease progression (Jadiya et al., 2019; Garbincius and Elrod, 2022). While Ca^{2+} cycling within the cytosol,

ER, and mitochondria are key factors in AD progression (Figure 3), further investigation is required to identify how the relationship between these cellular machineries is driving Ca^{2+} overload, mitochondrial dysfunction, and apoptotic signaling.

Mitochondrial Ca^{2+} regulation in Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder that is defined by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Braak et al., 2003; Bezprozvanny, 2009; Surmeier et al., 2017; Fu et al., 2018). Before the substantia nigra deteriorates, neurons in multiple brainstem nuclei (including the dorsal motor nucleus, the raphe nucleus, and the locus coeruleus) begin to die (Braak et al., 2003; Fu et al., 2018). Another common hallmark of PD is Lewy body pathology, which spreads to some common brain regions as the disease progresses (Surmeier et al., 2017). Despite the variability of Lewy pathology, one common trend occurs, which is the neuronal loss within the SNpc (Surmeier, 2007). Dopaminergic neurons of the SNpc are uniquely susceptible to perturbations that lead to the development of PD, when compared to dopaminergic neurons of the ventral tegmental area (VTA), which has been shown due to the dependence of SNpc neurons on $Ca_v1.3$ for their rhythmicity (Chan et al., 2007; Surmeier, 2007; Chan et al., 2009; Fu et al., 2018). This unique dependence on $Ca_v1.3$ likely leads to greater accumulation of Ca^{2+} into the mitochondria and subsequent Ca^{2+} overload when combined with other challenges such as genetic mutations or environmental toxins (Figure 3).

Many genetic causes of PD point to mitochondrial Ca^{2+}-overload as a key target in the treatment of PD (Verma et al., 2018; Jung et al., 2020; Lim et al., 2021a; Modesti et al., 2021; Soman and Dagda, 2021). PINK1, which is encoded by a gene whose mutation causes autosomal recessive PD, is a mitochondrially targeted protein that was shown to regulate Ca^{2+} efflux from the mitochondria (Gandhi et al., 2009). Genetic knockdown or knockout of PINK1 resulted in impairment of Ca^{2+} extrusion from the mitochondria in neurons, coupled with increases in ROS production (Gandhi et al., 2009) (Figure 3). Mitochondria purified from PINK1 knockout mice had reduced Ca^{2+} loading capacities and enhanced mPTP activation (Akundi et al., 2011). PINK1 KO midbrain neurons are susceptible to excitotoxic dopamine treatment, but were shown to be protected by NCLX overexpression *in vitro* (Kostic et al., 2015). Deletion of MCU in zebrafish prevented loss of DA neurons in the PINK1 and MPTP models of PD, supporting the potential of targeting MCU for the prevention of PD progression (Soman et al., 2017; Soman et al., 2019). Further support of the role of MCU and mitochondrial Ca^{2+} regulation in PD comes from recent work reporting that MICU1 is regulated by the E3 ubiquitin ligase Parkin, whose mutation leads to another form of familial PD (Matteucci et al., 2018). Overexpression of Parkin reduces MICU1 expression and leads to reduced MICU2 expression due to its dependence on forming dimers with MICU1. Given the critical role of Parkin in regulating MICU1 expression, its mutations could lead to disrupted assembly of the MCU complex and abnormal handling of mitochondrial Ca^{2+} (Matteucci et al., 2018) (Figure 3).

The most common familial form of PD is caused by mutations in leucine-rich repeat kinase 2 (LRRK2) (Lee et al., 2019; Kovacs et al., 2021). Experiments in astrocytes identified that mutant

LRRK2 modulates sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) function and reduces ER Ca^{2+} uptake (Lee et al., 2019). The reduced uptake of Ca^{2+} into the ER increases ER stress and leads to mitochondrial Ca^{2+} overload (Figure 3) (Lee et al., 2019; Kovacs et al., 2021; Modesti et al., 2021). Human fibroblasts from patients with LRRK2 mutations show upregulation of MCU and MICU1, and mitochondria have increased Ca^{2+} uptake (Verma et al., 2017; Modesti et al., 2021). In SH-SY5Y cells with mutant LRRK2, targeting MCU with inhibitors or genetic knockdown reduced Ca^{2+} uptake and improved neurite growth and stability (Verma et al., 2017). These studies suggest that targeting mitochondrial Ca^{2+} uptake or release may be beneficial in delaying the onset or slowing the progression of PD in humans.

The above-described experiments show the importance of Ca^{2+} regulation and the impact that its dysfunction can have in the progression of PD (Figure 3). Although targeting mechanisms of Ca^{2+} regulation to inhibit PD progression holds promise, additional work is needed to determine how effective this would be in preventing the PD progression. The $Ca_v1.3$ inhibitor isradipine has shown to prevent the onset and progression of PD in rodent models of PD (Chan et al., 2009), but has failed to delay clinical progression and onset in clinical trials (2020b; Venuto et al., 2021). Studies have suggested that certain L-type Ca^{2+}-channel inhibitors used in patients with hypertension to some extent reduce the risk of PD, but currently no FDA approved drugs available to selectively target $Ca_v1.3$ in humans (Chan et al., 2009). Another way of preventing Ca^{2+} overload and excitotoxicity is by reducing NMDA receptor activity. The drug amantadine is a NMDA receptor antagonist that is approved for the treatment of PD induced dyskinesia (Schwab et al., 1969; Olanow et al., 2009). While the clinical use of amantadine is to help reducing symptoms of PD, it could aid in delaying disease progression due to its potential effect on reducing excitotoxic Ca^{2+} signaling through NMDA receptors (Olanow et al., 2009). It is also possible that targeting the MCU complex would be useful in the prevention of PD progression, the approach that showed efficacy in zebrafish models of PD (Soman et al., 2017; Soman et al., 2019). Drug targets of MCU and their efficacy in PD is also discussed in recent reviews (Dey et al., 2020; Woods and Wilson, 2020). However, further testing of these hypotheses in mouse models of PD and humans will require more selective inhibitors of Ca^{2+} regulating proteins.

Mitochondrial Ca^{2+} regulation in amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that results in progressive loss of motor neurons throughout the brain and spinal cord, leading to death typically within 2.5–3 years after its initial diagnosis (Rosen et al., 1993; Bezprozvanny, 2009; Fu et al., 2018). Although most cases of ALS occur sporadically, approximately 5–10% cases of ALS are familial, with mutations in the Cu/Zn superoxide dismutase (SOD1) gene accounting for approximately 20% of familial ALS cases (Rosen et al., 1993; Cook and Petrucelli, 2019). These mutations lead to toxic accumulation of SOD1 on the mitochondria leading to cell death (Van Den Bosch et al., 2006; Bezprozvanny, 2009). Notably, in mice and rats mutations in SOD1 lead to motor neuron disease similar to familial ALS (Gurney et al., 1994; Howland et al., 2002). Distinct populations of motor neurons are vulnerable in ALS. The neurons most affected are fast-

fatigable motor neurons; the most resistant are slow motor neurons, and distinct brainstem motor nuclei populations also remain mostly unaffected (Fu et al., 2018). These findings suggest that, as is the case for PD, a unique neuronal physiology may lead to vulnerability to Ca^{2+} overload in ALS. Spinal motor neurons express lower levels of the Ca^{2+} binding proteins calbindin D_{28K} and parvalbumin (Shaw and Eggett, 2000; Appel et al., 2001). Consistent with this notion, the motor neurons of the brainstem nuclei that are spared from ALS degeneration have higher levels of Ca^{2+} binding proteins (Van Den Bosch et al., 2006; Bezprozvanny, 2009). Further support for the importance of Ca^{2+} binding proteins in motor neurons is that the overexpression of rat parvalbumin in SOD1 mutant mice both delayed the onset of symptoms and prolonged survival of a mouse model of familial ALS (Beers et al., 2001). Increasing the expression of calbindin D_{28K}, with glia derived neurotrophic factors, protected motor neurons of spinal cord slices from glutamate induced toxicity (Spruill and Kuncl, 2015).

In addition to the differences in the expression of Ca^{2+}-binding proteins, motor neurons have been shown to contain glutamate AMPA receptors that are more permeable to Ca^{2+}, thereby adding to their susceptibility to excitotoxicity (Van Den Bosch et al., 2006). This was found to be due to a reduction in the number of GluA2 receptor subunits present within the motor neurons (Shaw and Eggett, 2000; Van Den Bosch et al., 2006). The level of GluA2 subunit expression has been shown to alter lifespan in SOD1 ALS mice. Crossing GluA2 knockout mice with SOD1 mice shortens their lifespan and overexpressing GluA2 increases their lifespan (Van Den Bosch et al., 2006). Along with decreases in the expression of the GluA2 subunits, iPSC derived motor neurons containing the familial *C9orf72* mutation showed increased expression of GluA1 subunits, further contributing to a reduced GluA2/GluA1 ratio and increased Ca^{2+} permeability of AMPA receptors (Selvaraj et al., 2018). A recent assessment of post-mortem tissue from ALS patients found that AMPA receptor subunits were disrupted in both familial and sporadic ALS cases (Gregory et al., 2020). Familial *C9orf72* mutations and sporadic cases showed increased GluA1 expression, whereas SOD1 mutation cases showed reduced GluA2 expression (Gregory et al., 2020). While there appear to be differences in the AMPA receptor subunits expressed in ALS patients, the common denominator is a reduced GluA2/GluA1 ratio that leads to enhanced Ca^{2+} permeability of the receptors (Figure 3).

The excessive Ca^{2+} influx associated with ALS combined with lower expression of Ca^{2+}-binding proteins in motor neurons, leave mitochondria in these neurons at high risk of Ca^{2+} overload (Figure 3). Mitochondria Ca^{2+} buffering is dysfunctional in SOD1 mutant mice (Damiano et al., 2006). In mitochondria of the brain and spinal cord, dysfunction in the form of decreased Ca^{2+} loading capacity occurs much earlier than the onset of symptoms and significant motor neuron loss (Damiano et al., 2006). This was supported by a subsequent study that found that mitochondria of motor neurons of pre-symptomatic SOD1 mutant mice became more depolarized from Ca^{2+} entry in response to repeated stimulations (Nguyen et al., 2009). Using motor neurons derived from induced pluripotent stem cells of patients with two different ALS causing mutations (*C9orf72* and *TARDBP*), it was determined that the neurons had enhanced Ca^{2+} release following stimulation and both mutations also led to upregulation of Ca^{2+} permeable AMPA and NMDA receptors (Dafinca et al., 2020). Interestingly, both mutations resulted in

higher levels of MICU1 compared to MICU2, with *C9orf72* mutations reducing MCU and MICU2 expression, whereas *TARDBP* increasing MICU1 expression, altogether resulting in reduced uptake of Ca^{2+} into the mitochondria (Dafinca et al., 2020). In hypoglossal motor neurons, mitochondrial Ca^{2+} uptake is impaired later in disease progression, and cytosolic Ca^{2+} clearance is enhanced relative to that in neurons resistant to ALS pathology (Fuchs et al., 2013). An additional analysis showed that in late stage SOD1 mutant mouse hypoglossal motor neurons, not only are MCU and MICU1 upregulated, but so are Letm1, UCP2, and NCX1, one of the plasma membrane Na^+/Ca^{2+} exchangers (Mühling et al., 2014). As the disease progresses motor neurons attempt to compensate for increased cytosolic Ca^{2+} concentrations by upregulating mechanisms to improve mitochondrial buffering, but also extrusion across the plasma membrane. However, excessive mitochondrial Ca^{2+} load can lead to activation of the mPTP. Notably, in SOD1 mutant mice the mPTP-promoting protein cyclophilin D is upregulated, the mitochondria are swollen, and mPTP can be activated. Deleting a single copy of cyclophilin D gene in SOD1 mutant mice led to delayed disease onset and prolonged lifespan when compared SOD1 mutant mice alone (Martin et al., 2009). These latter findings suggest that inhibiting mitochondrial Ca^{2+} uptake and mPTP activation may be beneficial in preventing the onset and progression of ALS.

The challenge with ALS is that many potential treatments have failed in clinical trials. For example, the FDA approved drug Riluzole has led to only a modest increase in lifespan (about 3 months) (Bezprozvanny, 2009; Smith et al., 2019). The mechanism of action of this drug is not completely clear, but it appears to inhibit glutamate NMDA receptors, an effect that can reduce the overall influx of Ca^{2+} into the cell (Bezprozvanny, 2009; Smith et al., 2019). Many other approaches targeting mitochondrial function and ROS production have failed in clinical trials (Smith et al., 2019). The importance of Ca^{2+} overload in ALS has been extensively discussed, and while studies have shown changes in MCU/MICU1/MICU2 expression in ALS (Fuchs et al., 2013; Mühling et al., 2014; Dafinca et al., 2020), studies targeting mitochondrial Ca^{2+} uptake and release mechanisms have been limited (Tadić et al., 2019). One of the only studies targeting MCU in SOD1 mutant mouse neurons showed that MCU expression is higher in cultured SOD1 mutant motor neurons vs. control motor neurons, but MCU is downregulated in adulthood (Tadić et al., 2019). The application of KN-62, a selective inhibitor of the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), protected against kainic acid-induced excitotoxity *in vitro*, and this appeared to involve a reduction in MCU expression (Tadić et al., 2019). Although this study holds promise for targeting the MCU complex in ALS, further studies are needed to determine its potential as a therapeutic target.

Summary and perspectives

Mitochondrial Ca^{2+} buffering is essential for normal neuronal function and dysfunctional Ca^{2+} buffering and subsequent Ca^{2+} overload is common to many neurological diseases. Thanks to the recent identification of the components of the MCU complex, targeting the mitochondrial Ca^{2+} uptake and release machinery has become possible and has helped to refine the roles of these processes in neuronal function, as well as enable investigation of the effectiveness of targeting these proteins in disease prevention. Despite progress, many major questions remain unanswered in defining the roles of specific

components of mitochondrial Ca^{2+} uptake and release systems in neurodegenerative disorders, epilepsy, stroke and other neurological conditions. Recent advancements in genetic targeting of all the major components of mitochondrial Ca^{2+} transport have provided important tools for addressing these questions. Thus, an important task for future research would be to systematically examine the impact of deletion or overexpression of MCU, MCUb, MICU3 and other components of mitochondrial Ca^{2+} transport in animal models of AD, PD, ALS, epilepsy, and other neurological conditions.

Even though this review focused primarily on neurons, it is important to point out that glial cells, such as astrocytes and microglia, also play crucial roles in epilepsy and neurodegenerative diseases as highlighted in many excellent reviews on this topic (Salter and Stevens, 2017; Patel et al., 2019; Santello et al., 2019; Escartin et al., 2021). Equally important is that Ca^{2+} signaling and mitochondria Ca^{2+} transport play crucial roles in these cells in the context of many neurological diseases (Shigetomi et al., 2019; Lim et al., 2021b; Heuser and Enger, 2021; Sobolczyk and Boczek, 2022). Thus, another task for future studies would be to define cell-type specific roles of MCU and other molecular components of mitochondrial Ca^{2+} transport in neurological diseases by genetically targeting these molecules in neurons, glia, and immune cells.

Another important line of future research would be development of potent and selective pharmacological tools that target MCU. Although a commonly used MCU inhibitor Ru360 is both potent and selective in *in vitro* assays, its poor plasma membrane permeability and low stability in aqueous solutions limit its applicability for *in vivo* research (Hajnoczky et al., 2006; Woods and Wilson, 2020; Marta et al., 2021). A recently developed analog of Ru360, Ru265, shows improved potency and much more readily penetrates through plasma membrane (Woods and Wilson, 2020). The latter property is mediated by a ubiquitously expressed organic cation transporter 3 (OCT3) (Woods et al., 2020). Notably, administration of Ru265 provided a neuroprotective effect *in vivo* in mice subjected to hypoxic/ischemic brain injury (Novorolsky et al., 2020). Currently, only a few of the available pharmacological inhibitors of MCU are translatable to human medicine. One FDA-approved drug has been shown to inhibit MCU is mitoxantrone (Arduino et al., 2017), but it is also an inhibitor of topoisomerase II and chemotherapeutic with high cytotoxicity (Woods and Wilson, 2020). Mitoxantrone is approved as a treatment for relapsing-remitting multiple sclerosis but may carry some risk for cardiotoxicity as well as risk for the development of cancers (Scott and Figgitt, 2004; Buttmann et al., 2016; Chartier et al., 2018). Interestingly, mitoxantrone improved neurological symptoms and delayed the progression of multiple sclerosis in patients while also being reasonably well tolerated (Scott and Figgitt, 2004). Given its ability to inhibit MCU, it could be an interesting candidate to test in ALS due to its similarities with multiple sclerosis. Another recent screening of FDA-approved compounds has identified two drugs that modulate MCU activity: amorolfine, which promotes mitochondrial uptake of Ca^{2+}, and benzethonium, which inhibits this process (De Mario et al., 2021). Since mitochondrial Ca^{2+} overload is a common neurotoxic mechanism implicated AD, PD, ALS, and epilepsy (Figure 3), it will be critical for future experiments to test the efficacy of mitoxantrone, amorolfine, and benzethonium in animal models of these neurological diseases. The major bottleneck in investigating the efficacy of targeting Ca^{2+} uptake in human disease is that only a few drugs and small-molecule inhibitors of the uptake and release mechanisms are currently available. Thus, there is a critical

need to screen for such agents, and to develop small-molecule inhibitors that could potentially be effective in treating neurologic diseases in which Ca^{2+} overload is a key problem.

Author contributions

GW and YU wrote and edited the manuscript.

Funding

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Akundi, R. S., Huang, Z., Eason, J., Pandya, J. D., Zhi, L., Cass, W. A., et al. (2011). Increased mitochondrial calcium sensitivity and abnormal expression of innate immunity genes precede dopaminergic defects in Pink1-deficient mice. *PLoS One* 6 (1), e16038. doi:10.1371/journal.pone.0016038

Alavian, K. N., Beutner, G., Lazrove, E., Sacchetti, S., Park, H.-A., Licznerski, P., et al. (2014). An uncoupling channel within the c-subunit ring of the F_1F_O ATP synthase is the mitochondrial permeability transition pore. *Proc. Natl. Acad. Sci.* 111(29), 10580–10585. doi:10.1073/pnas.1401591111

Alzheimer's and Dementia (2020a). Alzheimer's disease facts and figures. *Alzheimer's Dementia* 16 (3), 391–460. doi:10.1002/alz.12068

Angelova, P. R., and Abramov, A. Y. (2018). Role of mitochondrial ROS in the brain: From physiology to neurodegeneration. *FEBS Lett.* 592 (5), 692–702. doi:10.1002/1873-3468.12964

Appel, S. H., Beers, D., Siklos, L., Engelhardt, J. I., and Mosier, D. R. (2001). Calcium: The darth vader of ALS. *Amyotroph. Lateral Scler. Other Mot. Neuron Disord.* 2 (1), 47–54. doi:10.1080/146608201300079418

Arduino, D. M., Wettmarshausen, J., Vais, H., Navas-Navarro, P., Cheng, Y., Leimpek, A., et al. (2017). Systematic identification of MCU modulators by orthogonal interspecies chemical screening. *Mol. Cell* 67 (4), 711–723. e717. doi:10.1016/j.molcel.2017.07.019

Areosa, S. A., Sherriff, F., and McShane, R. (2005). Memantine for dementia. *Cochrane database Syst. Rev.* 2005, CD003154. doi:10.1002/14651858.CD003154.pub4

Arispe, N., Pollard, H. B., and Rojas, E. (1993). Giant multilevel cation channels formed by Alzheimer disease amyloid beta-protein [A beta P-(1-40)] in bilayer membranes. *Proc. Natl. Acad. Sci.* 90 (22), 10573–10577. doi:10.1073/pnas.90.22.10573

Ashrafi, G., de Juan-Sanz, J., Farrell, R. J., and Ryan, T. A. (2020). Molecular tuning of the axonal mitochondrial Ca2+ uniporter ensures metabolic flexibility of neurotransmission. *Neuron* 105 (4), 678–687. e675. doi:10.1016/j.neuron.2019.11.020

Attwell, D., and Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *J. Cereb. Blood Flow Metabolism* 21 (10), 1133–1145. doi:10.1097/00004647-200110000-00001

Babcock, D. F., Herrington, J., Goodwin, P. C., Park, Y. B., and Hille, B. (1997). Mitochondrial participation in the intracellular Ca^{2+} network. *J. Cell Biol.* 136 (4), 833–844. doi:10.1083/jcb.136.4.833

Bacaj, T., Wu, D., Yang, X., Morishita, W., Zhou, P., Xu, W., et al. (2013). Synaptotagmin-1 and synaptotagmin-7 trigger synchronous and asynchronous phases of neurotransmitter release. *Neuron* 80 (4), 947–959. doi:10.1016/j.neuron.2013.10.026

Baradaran, R., Wang, C., Siliciano, A. F., and Long, S. B. (2018). Cryo-EM structures of fungal and metazoan mitochondrial calcium uniporters. *Nature* 559 (7715), 580–584. doi:10.1038/s41586-018-0331-8

Baughman, J. M., Perocchi, F., Girgis, H. S., Plovanich, M., Belcher-Timme, C. A., Sancak, Y., et al. (2011). Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature* 476 (7360), 341–345. doi:10.1038/nature10234

Beckhauser, T. F., Francis-Oliveira, J., and De Pasquale, R. (2016). Reactive oxygen species: Physiological and physiopathological effects on synaptic plasticity. *J. Exp. Neurosci.* 10 (1), 23–48. doi:10.4137/JEN.S39887

Beers, D. R., Ho, B.-K., Siklós, L., Alexianu, M. E., Mosier, D. R., Mohamed, A. H., et al. (2001). Parvalbumin overexpression alters immune-mediated increases in intracellular calcium, and delays disease onset in a transgenic model of familial amyotrophic lateral sclerosis. *J. Neurochem.* 79 (3), 499–509. doi:10.1046/j.1471-4159.2001.00582.x

Berg, A. T., Berkovic, S. F., Brodie, M. J., Buchhalter, J., Cross, J. H., Van Emde Boas, W., et al. (2010). Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia* 51 (4), 676–685. doi:10.1111/j.1528-1167.2010.02522.x

Bernardi, P. (1999). Mitochondrial transport of cations: Channels, exchangers, and permeability transition. *Physiol. Rev.* 79 (4), 1127–1155. doi:10.1152/physrev.1999.79.4.1127

Bernardi, P., and Petronilli, V. (1996). The permeability transition pore as a mitochondrial calcium release channel: A critical appraisal. *J. Bioenergetics Biomembr.* 28 (2), 131–138. doi:10.1007/BF02110643

Bezprozvanny, I. (2009). Calcium signaling and neurodegenerative diseases. *Trends Mol. Med.* 15 (3), 89–100. doi:10.1016/j.molmed.2009.01.001

Bezprozvanny, I., and Mattson, M. P. (2008). Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci.* 31 (9), 454–463. doi:10.1016/j.tins.2008.06.005

Billups, B., and Forsythe, I. D. (2002). Presynaptic mitochondrial calcium sequestration influences transmission at mammalian central synapses. *J. Neurosci.* 22 (14), 5840–5847. doi:10.1523/JNEUROSCI.22-14-05840.2002

Bindoff, L. A., and Engelsen, B. A. (2012). Mitochondrial diseases and epilepsy. *Epilepsia* 53 (4), 92–97. doi:10.1111/j.1528-1167.2012.03618.x

Bonora, M., Bononi, A., De Marchi, E., Giorgi, C., Lebiedzinska, M., Marchi, S., et al. (2013). Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition. *Cell Cycle* 12 (4), 674–683. doi:10.4161/cc.23599

Boyenle, I. D., Oyedele, A. K., Ogunlana, A. T., Adeyemo, A. F., Oyelere, F. S., Akinola, O. B., et al. (2022). Targeting the mitochondrial permeability transition pore for drug discovery: Challenges and opportunities. *Mitochondrion* 63, 57–71. doi:10.1016/j.mito.2022.01.006

Braak, H., Tredici, K. D., Rüb, U., de Vos, R. A. I., Jansen Steur, E. N. H., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24 (2), 197–211. doi:10.1016/S0197-4580(02)00065-9

Brini, M., Calì, T., Ottolini, D., and Carafoli, E. (2014). Neuronal calcium signaling: Function and dysfunction. *Cell. Mol. Life Sci.* 71 (15), 2787–2814. doi:10.1007/s00018-013-1550-7

Brini, M., Pinton, P., King, M. P., Davidson, M., Schon, E. A., and Rizzuto, R. (1999). A calcium signaling defect in the pathogenesis of a mitochondrial DNA inherited oxidative phosphorylation deficiency. *Nat. Med.* 5 (8), 951–954. doi:10.1038/11396

Briston, T., Selwood, D. L., Szabadkai, G., and Duchen, M. R. (2019). Mitochondrial permeability transition: A molecular lesion with multiple drug targets. *Trends Pharmacol. Sci.* 40 (1), 50–70. doi:10.1016/j.tips.2018.11.004

Britti, E., Ros, J., Esteras, N., and Abramov, A. Y. (2020). Tau inhibits mitochondrial calcium efflux and makes neurons vulnerable to calcium-induced cell death. *Cell Calcium* 86, 102150. doi:10.1016/j.ceca.2019.102150

Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W., and Sheu, S.-S. (2004). Calcium, ATP, and ROS: A mitochondrial love-hate triangle. *Am. J. Physiology - Cell Physiology* 287 (4), C817–C833. doi:10.1152/ajpcell.00139.2004

Budde, T., Meuth, S., and Pape, H. C. (2002). Calcium-dependent inactivation of neuronal calcium channels. *Nat. Rev. Neurosci.* 3 (11), 873–883. doi:10.1038/nrn959

Buttmann, M., Seuffert, L., Mäder, U., and Toyka, K. V. (2016). Malignancies after mitoxantrone for multiple sclerosis: A retrospective cohort study. *A Retrosp. cohort study* 86 (23), 2203–2207. doi:10.1212/wnl.0000000000002745

Cai, H., Qiao, J., Chen, S., Yang, J., Hölscher, C., Wang, Z., et al. (2022). *MCU knockdown in hippocampal neurons improves memory performance of an Alzheimer's disease mouse model. Acta Biochimica Biophysica Sinica* 54 (1672-9145), 1528–1539. doi:10.3724/abbs.2022138

Cai, X., and Lytton, J. (2004b). Molecular cloning of a sixth member of the K+-dependent Na+/Ca2+ exchanger gene family, NCKX6. *J. Biol. Chem.* 279 (7), 5867–5876. doi:10.1074/jbc.M310908200

Cai, X., and Lytton, J. (2004a). The cation/Ca2+ exchanger superfamily: Phylogenetic analysis and structural implications. *Mol. Biol. Evol.* 21 (9), 1692–1703. doi:10.1093/molbev/msh177

Calvo-Rodriguez, M., Hernando-Perez, E., Nuñez, L., and Villalobos, C. (2019). Amyloid β oligomers increase ER-mitochondria Ca2+ cross talk in young hippocampal neurons and exacerbate aging-induced intracellular Ca2+ remodeling. *Front. Cell. Neurosci.* 13, 22. doi:10.3389/fncel.2019.00022

Calvo-Rodriguez, M., Hou, S. S., Snyder, A. C., Kharitonova, E. K., Russ, A. N., Das, S., et al. (2020). Increased mitochondrial calcium levels associated with neuronal death in a mouse model of Alzheimer's disease. *Nat. Commun.* 11 (1), 2146. doi:10.1038/s41467-020-16074-2

Cao, C., Wang, S., Cui, T., Su, X.-C., and Chou, J. J. (2017). Ion and inhibitor binding of the double-ring ion selectivity filter of the mitochondrial calcium uniporter. *Proc. Natl. Acad. Sci.* 114 (14), E2846–E2851. doi:10.1073/pnas.1620316114

Carrer, A., Tommasin, L., Šileikytė, J., Ciscato, F., Filadi, R., Urbani, A., et al. (2021). Defining the molecular mechanisms of the mitochondrial permeability transition through genetic manipulation of F-ATP synthase. *Nat. Commun.* 12 (1), 4835. doi:10.1038/s41467-021-25161-x

Carroll, J., He, J., Ding, S., Fearnley, I. M., and Walker, J. E. (2019). Persistence of the permeability transition pore in human mitochondria devoid of an assembled ATP synthase. *Proc. Natl. Acad. Sci.* 116(26), 12816–12821. doi:10.1073/pnas.1904005116

Caterina, M. J., and Julius, D. (2001). The vanilloid receptor: A molecular gateway to the pain pathway. *Annu. Rev. Neurosci.* 24, 487–517. doi:10.1146/annurev.neuro.24.1.487

Catterall, W. A., Leal, K., and Nanou, E. (2013). Calcium channels and short-term synaptic plasticity. *J. Biol. Chem.* 288 (15), 10742–10749. doi:10.1074/jbc.R112.411645

Catterall, W. A. (2000). Structure and regulation of voltage-gated Ca2+ channels. *Annu. Rev. Cell Dev. Biol.* 16, 521–555. doi:10.1146/annurev.cellbio.16.1.521

Chakroborty, S., Goussakov, I., Miller, M. B., and Stutzmann, G. E. (2009). Deviant ryanodine receptor-mediated calcium release resets synaptic homeostasis in presymptomatic 3xTg-AD mice. *J. Neurosci.* 29 (30), 9458–9470. doi:10.1523/jneurosci.2047-09.2009

Chalmers, S., and Nicholls, D. G. (2003). The relationship between free and total calcium concentrations in the matrix of liver and brain mitochondria. *J. Biol. Chem.* 278 (21), 19062–19070. doi:10.1074/jbc.M212661200

Chan, C. S., Gertler, T. S., and Surmeier, D. J. (2009). Calcium homeostasis, selective vulnerability and Parkinson's disease. *Trends Neurosci.* 32 (5), 249–256. doi:10.1016/j.tins.2009.01.006

Chan, C. S., Guzman, J. N., Ilijic, E., Mercer, J. N., Rick, C., Tkatch, T., et al. (2007). 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature* 447 (7148), 1081–1086. doi:10.1038/nature05865

Chartier, N., Epstein, J., Soudant, M., Dahan, C., Michaud, M., Pittion-Vouyovitch, S., et al. (2018). Clinical follow-up of 411 patients with relapsing and progressive multiple sclerosis 10 years after discontinuing mitoxantrone treatment: A real-life cohort study. *Eur. J. Neurology* 25 (12), 1439–1445. doi:10.1111/ene.13748

Chaudhuri, D., Sancak, Y., Mootha, V. K., and Clapham, D. E. (2013). MCU encodes the pore conducting mitochondrial calcium currents. *eLife* 2, e00704. doi:10.7554/eLife.00704

Cheung, K.-H., Shineman, D., Müller, M., Cárdenas, C., Mei, L., Yang, J., et al. (2008). Mechanism of Ca2+ disruption in alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. *Neuron* 58 (6), 871–883. doi:10.1016/j.neuron.2008.04.015

Chouhan, A. K., Ivannikov, M. V., Lu, Z., Sugimori, M., Llinas, R. R., and Macleod, G. T. (2012). Cytosolic calcium coordinates mitochondrial energy metabolism with presynaptic activity. *J. Neurosci.* 32 (4), 1233–1243. doi:10.1523/JNEUROSCI.1301-11.2012

Chouhan, A. K., Zhang, J., Zinsmaier, K. E., and Macleod, G. T. (2010). Presynaptic mitochondria in functionally different motor neurons exhibit similar affinities for Ca2+ but exert little influence as Ca2+ buffers at nerve firing rates *in situ*. *J. Neurosci.* 30 (5), 1869–1881. doi:10.1523/JNEUROSCI.4701-09.2010

Chuang, H. H., Neuhausser, W. M., and Julius, D. (2004). The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. *Neuron* 43 (6), 859–869. doi:10.1016/j.neuron.2004.08.038

Cividini, F., Scott, B. T., Suarez, J., Casteel, D. E., Heinz, S., Dai, A., et al. (2021). Ncor2/PPARα-Dependent upregulation of MCUb in the type 2 diabetic heart impacts cardiac metabolic flexibility and function. *Diabetes* 70 (3), 665–679. doi:10.2337/db20-0779

Clapham, D. E. (2003). TRP channels as cellular sensors. *Nature* 426 (6966), 517–524. doi:10.1038/nature02196

Clayton, E. L., and Cousin, M. A. (2009). Quantitative monitoring of activity-dependent bulk endocytosis of synaptic vesicle membrane by fluorescent dextran imaging. *J. Neurosci. Methods* 185 (1), 76–81. doi:10.1016/j.jneumeth.2009.09.016

Colegrove, S. L., Albrecht, M. A., and Friel, D. D. (2000a). Dissection of mitochondrial Ca2+ uptake and release fluxes *in situ* after depolarization-evoked [Ca2+]i elevations in sympathetic neurons. *J. Gen. Physiol.* 115 (3), 351–370. doi:10.1085/jgp.115.3.351

Colegrove, S. L., Albrecht, M. A., and Friel, D. D. (2000b). Quantitative analysis of mitochondrial Ca2+ uptake and release pathways in sympathetic neurons - reconstruction of the recovery after depolarization-evoked [Ca2+](i) elevations. *J. General Physiology* 115 (3), 371–388. doi:10.1085/jgp.115.3.371

Cook, C., and Petrucelli, L. (2019). Genetic convergence brings clarity to the enigmatic red line in ALS. *Neuron* 101 (6), 1057–1069. doi:10.1016/j.neuron.2019.02.032

Corriveau, R. A., Koroshetz, W. J., Gladman, J. T., Jeon, S., Babcock, D., Bennett, D. A., et al. (2017). Alzheimer's disease–related dementias summit 2016: National research priorities. *Neurology* 89 (23), 2381–2391. doi:10.1212/wnl.0000000000004717

Cousin, M. A., and Robinson, P. J. (2001). The dephosphins: Dephosphorylation by calcineurin triggers synaptic vesicle endocytosis. *Trends Neurosci.* 24 (11), 659–665. doi:10.1016/S0166-2236(00)01930-5

Crompton, M., Künzi, M., and Carafoli, E. (1977). The calcium-induced and sodium-induced effluxes of calcium from heart mitochondria. Evidence for a sodium-calcium carrier. *Eur. J. Biochem.* 79 (2), 549–558. doi:10.1111/j.1432-1033.1977.tb11839.x

Crompton, M., Moser, R., Lüdi, H., and Carafoli, E. (1978). The interrelations between the transport of sodium and calcium in mitochondria of various mammalian tissues. *Eur. J. Biochem.* 82 (1), 25–31. doi:10.1111/j.1432-1033.1978.tb11993.x

Csordás, G., Golenár, T., Seifert, E. L., Kamer, K. J., Sancak, Y., Perocchi, F., et al. (2013). MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca2+ uniporter. *Cell Metab.* 17 (6), 976–987. doi:10.1016/j.cmet.2013.04.020

Dafinca, R., Barbagallo, P., Farrimond, L., Candalija, A., Scaber, J., Ababneh, N. A., et al. (2020). Impairment of mitochondrial calcium buffering links mutations in C9ORF72 and TARDBP in iPS-derived motor neurons from patients with ALS/FTD. *Stem Cell Rep.* 14 (5), 892–908. doi:10.1016/j.stemcr.2020.03.023

Dagda, R. K. (2018). Role of mitochondrial dysfunction in degenerative brain diseases, an overview. *Brain Sci.* 8 (10), 178. doi:10.3390/brainsci8100178

Damiano, M., Starkov, A. A., Petri, S., Kipiani, K., Kiaei, M., Mattiazzi, M., et al. (2006). Neural mitochondrial Ca2+ capacity impairment precedes the onset of motor symptoms in G93A Cu/Zn-superoxide dismutase mutant mice. *J. Neurochem.* 96 (5), 1349–1361. doi:10.1111/j.1471-4159.2006.03619.x

David, G., and Barrett, E. F. (2003). Mitochondrial Ca2+ uptake prevents desynchronization of quantal release and minimizes depletion during repetitive stimulation of mouse motor nerve terminals. *J. Physiol.* 548 (2), 425–438. doi:10.1113/jphysiol.2002.035196

David, G., and Barrett, E. F. (2000). Stimulation-evoked increases in cytosolic [Ca2+] in mouse motor nerve terminals are limited by mitochondrial uptake and are temperature-dependent. *J. Neurosci.* 20 (19), 7290–7296. doi:10.1523/JNEUROSCI.20-19-07290.2000

David, G. (1999). Mitochondrial clearance of cytosolic Ca2+ in stimulated lizard motor nerve terminals proceeds without progressive elevation of mitochondrial matrix [Ca2+]. *J. Neurosci.* 19 (17), 7495–7506. doi:10.1523/JNEUROSCI.19-17-07495.1999

David, G., Talbot, J., and Barrett, E. F. (2003). Quantitative estimate of mitochondrial [Ca2+] in stimulated motor nerve terminals. *Cell Calcium* 33 (3), 197–206. doi:10.1016/s0143-4160(02)00229-4

De Camilli, P., Haucke, V., Takey, K., and Mugnaini, E. (2001). "The structure of synapses," in *Synapses*. Editors M. W. Cowan, T. C. Sudhof, and C. F. Stevens (Baltimore London: The Johns Hopkins University Press), 89–133.

De Mario, A., Tosatto, A., Hill, J. M., Kriston-Vizi, J., Ketteler, R., Vecellio Reane, D., et al. (2021). Identification and functional validation of FDA-approved positive and negative modulators of the mitochondrial calcium uniporter. *Cell Rep.* 35 (12), 109275. doi:10.1016/j.celrep.2021.109275

De Stefani, D., Raffaello, A., Teardo, E., Szabo, I., and Rizzuto, R. (2011). A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. *Nature* 476 (7360), 336–340. doi:10.1038/nature10230

De Stefani, D., Rizzuto, R., and Pozzan, T. (2016). Enjoy the trip: Calcium in mitochondria back and forth. *Annu. Rev. Biochem.* 85, 161–192. doi:10.1146/annurev-biochem-060614-034216

Demuro, A., and Parker, I. (2013). Cytotoxicity of intracellular Aβ42 amyloid oligomers involves Ca2+ release from the endoplasmic reticulum by stimulated production of inositol trisphosphate. *J. Neurosci.* 33 (9), 3824–3833. doi:10.1523/jneurosci.4367-12.2013

Denton, R. M., Randle, P. J., and Martin, B. R. (1972). Stimulation by calcium ions of pyruvate dehydrogenase phosphate phosphatase. *Biochem. J.* 128 (1), 161–163. doi:10.1042/bj1280161

Denton, R. M. (2009). Regulation of mitochondrial dehydrogenases by calcium ions. *Biochim. Biophys. Acta* 1787 (11), 1309–1316. doi:10.1016/j.bbabio.2009.01.005

Devine, M. J., and Kittler, J. T. (2018). Mitochondria at the neuronal presynapse in health and disease. *Nat. Rev. Neurosci.* 19 (2), 63–80. doi:10.1038/nrn.2017.170

Dey, K., Bazala, M. A., and Kuznicki, J. (2020). Targeting mitochondrial calcium pathways as a potential treatment against Parkinson's disease. *Cell Calcium* 89, 102216. doi:10.1016/j.ceca.2020.102216

DiMauro, S., and Schon, E. A. (2003). Mitochondrial respiratory-chain diseases. *N. Engl. J. Med.* 348 (26), 2656–2668. doi:10.1056/NEJMra022567

Ding, S., Fellin, T., Zhu, Y., Lee, S.-Y., Auberson, Y. P., Meaney, D. F., et al. (2007). Enhanced astrocytic Ca²⁺ signals contribute to neuronal excitotoxicity after status epilepticus. *J. Neurosci.* 27 (40), 10674–10684. doi:10.1523/jneurosci.2001-07.2007

Dolphin, A. C. (2012). Calcium channel auxiliary α2δ and β subunits: Trafficking and one step beyond. *Nat. Rev. Neurosci.* 13 (8), 542–555. doi:10.1038/nrn3311

Drago, I., and Davis, R. L. (2016). Inhibiting the mitochondrial calcium uniporter during development impairs memory in adult Drosophila. *Cell Rep.* 16 (10), 2763–2776. doi:10.1016/j.celrep.2016.08.017

Drago, I., Pizzo, P., and Pozzan, T. (2011). After half a century mitochondrial calcium in- and efflux machineries reveal themselves. *EMBO J.* 30 (20), 4119–4125. doi:10.1038/emboj.2011.337

Du, H., Guo, L., Fang, F., Chen, D., A Sosunov, A., M McKhann, G., et al. (2008). Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nat. Med.* 14 (10), 1097–1105. doi:10.1038/nm.1868

Duchen, M. R., McGuinness, O., Brown, L. A., and Crompton, M. (1993). On the involvement of a cyclosporin A sensitive mitochondrial pore in myocardial reperfusion injury. *Cardiovasc. Res.* 27 (10), 1790–1794. doi:10.1093/cvr/27.10.1790

Emrich, S. M., Yoast, R. E., Fike, A. J., Bricker, K. N., Xin, P., Zhang, X., et al. (2022). The mitochondrial sodium/calcium exchanger NCLX (Slc8b1) in B lymphocytes. *Cell Calcium* 108, 102667. doi:10.1016/j.ceca.2022.102667

Emrich, S. M., Yoast, R. E., and Trebak, M. (2021). Physiological functions of CRAC channels. *Annu. Rev. Physiol.* 84, 355–379. doi:10.1146/annurev-physiol-052521-013426

Escartin, C., Galea, E., Lakatos, A., O'Callaghan, J. P., Petzold, G. C., Serrano-Pozo, A., et al. (2021). Reactive astrocyte nomenclature, definitions, and future directions. *Nat. Neurosci.* 24 (3), 312–325. doi:10.1038/s41593-020-00783-4

Esteras, N., and Abramov, A. Y. (2020). Mitochondrial calcium deregulation in the mechanism of beta-amyloid and tau pathology. *Cells* 9 (9), 2135. doi:10.3390/cells9092135

Esteras, N., Kopach, O., Maiolino, M., Lariccia, V., Amoroso, S., Qamar, S., et al. (2022). Mitochondrial ROS control neuronal excitability and cell fate in frontotemporal dementia. *Alzheimer's Dementia* 18 (2), 318–338. doi:10.1002/alz.12394

Fan, M., Zhang, J., Tsai, C.-W., Orlando, B. J., Rodriguez, M., Xu, Y., et al. (2020). Structure and mechanism of the mitochondrial Ca2+ uniporter holocomplex. *Nature* 582 (7810), 129–133. doi:10.1038/s41586-020-2309-6

Farrell, J. S., Nguyen, Q. A., and Soltesz, I. (2019). Resolving the micro-macro disconnect to address core features of seizure networks. *Neuron* 101 (6), 1016–1028. doi:10.1016/j.neuron.2019.01.043

Fellin, T., Gomez-Gonzalo, M., Gobbo, S., Carmignoto, G., and Haydon, P. G. (2006). Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. *J. Neurosci.* 26 (36), 9312–9322. doi:10.1523/jneurosci.2836-06.2006

Fieni, F., Lee, S. B., Jan, Y. N., and Kirichok, Y. (2012). Activity of the mitochondrial calcium uniporter varies greatly between tissues. *Nat. Commun.* 3, 1317. doi:10.1038/ncomms2325

Fink, B. D., Bai, F., Yu, L., and Sivitz, W. I. (2017). Regulation of ATP production: Dependence on calcium concentration and respiratory state. *Am. J. Physiology-Cell Physiology* 313 (2), C146–C153. doi:10.1152/ajpcell.00086.2017

Finsterer, J., and Scorza, F. A. (2017). Effects of antiepileptic drugs on mitochondrial functions, morphology, kinetics, biogenesis, and survival. *Epilepsy Res.* 136, 5–11. doi:10.1016/j.eplepsyres.2017.07.003

Fleury, C., Mignotte, B., and Vayssière, J.-L. (2002). Mitochondrial reactive oxygen species in cell death signaling. *Biochimie* 84 (2), 131–141. doi:10.1016/S0300-9084(02)01369-X

Friedman, D., Kannan, K., Faustin, A., Shroff, S., Thomas, C., Heguy, A., et al. (2018). Cardiac arrhythmia and neuroexcitability gene variants in resected brain tissue from patients with sudden unexpected death in epilepsy (SUDEP). *npj Genomic Med.* 3 (1), 9. doi:10.1038/s41525-018-0048-5

Friel, D. D. (2000). Mitochondria as regulators of stimulus-evoked calcium signals in neurons. *Cell Calcium* 28 (5-6), 307–316. doi:10.1054/ceca.2000.0172

Fu, H., Hardy, J., and Duff, K. E. (2018). Selective vulnerability in neurodegenerative diseases. *Nat. Neurosci.* 21 (10), 1350–1358. doi:10.1038/s41593-018-0221-2

Fuchs, A., Kutterer, S., Mühling, T., Duda, J., Schütz, B., Liss, B., et al. (2013). Selective mitochondrial Ca2+ uptake deficit in disease endstage vulnerable motoneurons of the SOD1G93A mouse model of amyotrophic lateral sclerosis. *J. Physiology* 591 (10), 2723–2745. doi:10.1113/jphysiol.2012.247981

Gandhi, S., Wood-Kaczmar, A., Yao, Z., Plun-Favreau, H., Deas, E., Klupsch, K., et al. (2009). PINK1-Associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol. Cell* 33 (5), 627–638. doi:10.1016/j.molcel.2009.02.013

Garbincius, J. F., and Elrod, J. W. (2022). Mitochondrial calcium exchange in physiology and disease. *Physiol. Rev.* 102 (2), 893–992. doi:10.1152/physrev.00041.2020

García-Chacón, L. E., Nguyen, K. T., David, G., and Barrett, E. F. (2006). Extrusion of Ca(2+) from mouse motor terminal mitochondria via a Na(+)–Ca(2+) exchanger increases post-tetanic evoked release. *J. Physiology* 574 (3), 663–675. doi:10.1113/jphysiol.2006.110841

Garg, V., Suzuki, J., Paranjpe, I., Unsulangi, T., Boyman, L., Milescu, L. S., et al. (2021). The mechanism of MICU-dependent gating of the mitochondrial Ca2+uniporter. *eLife* 10, e69312. doi:10.7554/eLife.69312

Gemes, G., Bangaru, M. L., Wu, H. E., Tang, Q., Weihrauch, D., Koopmeiners, A. S., et al. (2011). Store-operated Ca2+ entry in sensory neurons: Functional role and the effect of painful nerve injury. *J. Neurosci.* 31 (10), 3536–3549. doi:10.1523/JNEUROSCI.5053-10.2011

George, D. S., Hackelberg, S., Jayaraj, N. D., Ren, D., Edassery, S. L., Rathwell, C., et al. (2021). Mitochondrial calcium uniporter deletion prevents painful diabetic neuropathy by restoring mitochondrial morphology and dynamics. *PAIN* 163, 560–578. doi:10.1097/j.pain.0000000000002391

Giorgio, V., Bisetto, E., Soriano, M. E., Dabbeni-Sala, F., Basso, E., Petronilli, V., et al. (2009). Cyclophilin D modulates mitochondrial F0F1-ATP synthase by interacting with the lateral stalk of the complex. *J. Biol. Chem.* 284 (49), 33982–33988. doi:10.1074/jbc.M109.020115

Giorgio, V., Guo, L., Bassot, C., Petronilli, V., and Bernardi, P. (2018). Calcium and regulation of the mitochondrial permeability transition. *Cell Calcium* 70, 56–63. doi:10.1016/j.ceca.2017.05.004

Giorgio, V., von Stockum, S., Antoniel, M., Fabbro, A., Fogolari, F., Forte, M., et al. (2013). Dimers of mitochondrial ATP synthase form the permeability transition pore. *Proc. Natl. Acad. Sci.* 110 (15), 5887–5892. doi:10.1073/pnas.1217823110

Glancy, B., and Balaban, R. S. (2012). Role of mitochondrial Ca2+ in the regulation of cellular energetics. *Biochemistry* 51 (14), 2959–2973. doi:10.1021/bi2018909

Golde, T. E., DeKosky, S. T., and Galasko, D. (2018). Alzheimer's disease: The right drug, the right time. *Science* 362(6420), 1250–1251. doi:10.1126/science.aau0437

Gottschalk, B., Klec, C., Leitinger, G., Bernhart, E., Rost, R., Bischof, H., et al. (2019). MICU1 controls cristae junction and spatially anchors mitochondrial Ca2+ uniporter complex. *Nat. Commun.* 10 (1), 3732. doi:10.1038/s41467-019-11692-x

Gregory, J. M., Livesey, M. R., McDade, K., Selvaraj, B. T., Barton, S. K., Chandran, S., et al. (2020). Dysregulation of AMPA receptor subunit expression in sporadic ALS post-mortem brain. *J. Pathology* 250 (1), 67–78. doi:10.1002/path.5351

Groten, C. J., and MacVicar, B. A. (2022). Mitochondrial Ca2+ uptake by the MCU facilitates neuron excitability and metabolism during action potential firing. *Commun. Biol.* 5 (1), 900. doi:10.1038/s42003-022-03848-1

Gunter, T. E., and Pfeiffer, D. R. (1990). Mechanisms by which mitochondria transport calcium. *Am. J. Physiol.* 258, C755–C786. doi:10.1152/ajpcell.1990.258.5.C755

Guo, C., and Ma, Y. Y. (2021). Calcium permeable-AMPA receptors and excitotoxicity in neurological disorders. *Front. Neural Circuits* 15, 711564. doi:10.3389/fncir.2021.711564

Gurney, M. E., Pu, H., Chiu, A. Y., Canto, M. C. D., Polchow, C. Y., Alexander, D. D., et al. (1994). Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* 264(5166), 1772–1775. doi:10.1126/science.8209258

Hagenston, A. M., Yan, J., Bas-Orth, C., Tan, Y., Sekler, I., and Bading, H. (2022). Disrupted expression of mitochondrial NCLX sensitizes neuroglial networks to excitotoxic stimuli and renders synaptic activity toxic. *J. Biol. Chem.* 298 (2), 101508. doi:10.1016/j.jbc.2021.101508

Hajnoczky, G., Csordas, G., Das, S., Garcia-Perez, C., Saotome, M., Sinha Roy, S., et al. (2006). Mitochondrial calcium signalling and cell death: Approaches for assessing the role of mitochondrial Ca2+ uptake in apoptosis. *Cell Calcium* 40 (5-6), 553–560. doi:10.1016/j.ceca.2006.08.016

Hamilton, J., Brustovetsky, T., and Brustovetsky, N. (2021). The effect of mitochondrial calcium uniporter and cyclophilin D knockout on resistance of brain mitochondria to Ca²⁺-induced damage. *J. Biol. Chem.* 296, 100669. doi:10.1016/j.jbc.2021.100669

Hamilton, J., Brustovetsky, T., Rysted, J. E., Lin, Z., Usachev, Y. M., and Brustovetsky, N. (2018). Deletion of mitochondrial calcium uniporter incompletely inhibits calcium uptake and induction of the permeability transition pore in brain mitochondria. *J. Biol. Chem.* 293 (40), 15652–15663. doi:10.1074/jbc.RA118.002926

Han, Y.-E., Ryu, S.-Y., Park, S.-H., Lee, K.-H., Lee, S.-H., and Ho, W.-K. (2015). Ca2+ clearance by plasmalemmal NCLX, Li+-permeable Na+/Ca2+ exchanger, is required for the sustained exocytosis in rat insulinoma INS-1 cells. *Pflügers Archiv - Eur. J. Physiology* 467 (12), 2461–2472. doi:10.1007/s00424-015-1715-3

Hansson Petersen, C. A., Alikhani, N., Behbahani, H., Wiehager, B., Pavlov, P. F., Alafuzoff, I., et al. (2008). The amyloid β-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae. *Proc. Natl. Acad. Sci.* 105 (35), 13145–13150. doi:10.1073/pnas.0806192105

Hartzell, C., Putzier, I., and Arreola, J. (2005). Calcium-activated chloride channels. *Annu. Rev. Physiology* 67, 719–758. doi:10.1146/annurev.physiol.67.032003.154341

Haworth, R. A., and Hunter, D. R. (1979). The Ca2+-induced membrane transition in mitochondria: II. Nature of the Ca2+ trigger site. *Archives Biochem. Biophysics* 195 (2), 460–467. doi:10.1016/0003-9861(79)90372-2

He, J., Carroll, J., Ding, S., Fearnley, I. M., and Walker, J. E. (2017a). Permeability transition in human mitochondria persists in the absence of peripheral stalk subunits of ATP synthase. *Proc. Natl. Acad. Sci.* 114(34), 9086–9091. doi:10.1073/pnas.1711201114

He, J., Ford, H. C., Carroll, J., Ding, S., Fearnley, I. M., and Walker, J. E. (2017b). Persistence of the mitochondrial permeability transition in the absence of subunit c of human ATP synthase. *Proc. Natl. Acad. Sci.* 114(13), 3409–3414. doi:10.1073/pnas.1702357114

Hernandez-Guijo, J. M., Maneu-Flores, V. E., Ruiz-Nuno, A., Villarroya, M., Garcia, A. G., and Gandia, L. (2001). Calcium-dependent inhibition of L, N, and P/Q Ca2+ channels in chromaffin cells: Role of mitochondria. *J. Neurosci.* 21 (8), 2553–2560. doi:10.1523/JNEUROSCI.21-08-02553.2001

Hesdorffer, D. C., Logroscino, G., Benn, E. K. T., Katri, N., Cascino, G., and Hauser, W. A. (2011). Estimating risk for developing epilepsy: A population-based study in rochester, Minnesota. *A population-based study Rochester, Minn.* 76 (1), 23–27. doi:10.1212/WNL.0b013e318204a36a

Heuser, K., and Enger, R. (2021). Astrocytic Ca(2+) signaling in epilepsy. *Front. Cell Neurosci.* 15, 695380. doi:10.3389/fncel.2021.695380

Hollenbeck, P. J. (2005). Mitochondria and neurotransmission: Evacuating the synapse. *Neuron* 47 (3), 331–333. doi:10.1016/j.neuron.2005.07.017

Hori, K., Tsujikawa, S., Novakovic, M. M., Yamashita, M., and Prakriya, M. (2020). Regulation of chemoconvulsant-induced seizures by store-operated Orai1 channels. *J. Physiol.* 598 (23), 5391–5409. doi:10.1113/JP280119

Howarth, C., Gleeson, P., and Attwell, D. (2012). Updated energy budgets for neural computation in the neocortex and cerebellum. *J. Cereb. Blood Flow Metabolism* 32 (7), 1222–1232. doi:10.1038/jcbfm.2012.35

Howland, D. S., Liu, J., She, Y., Goad, B., Maragakis, N. J., Kim, B., et al. (2002). Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc. Natl. Acad. Sci.* 99(3), 1604–1609. doi:10.1073/pnas.032539299

Huang, C. W., Lai, M. C., Cheng, J. T., Tsai, J. J., Huang, C. C., and Wu, S. N. (2013). Pregabalin attenuates excitotoxicity in diabetes. *PLoS One* 8 (6), e65154. doi:10.1371/journal.pone.0065154

Huo, J., Lu, S., Kwong, J. Q., Bround, M. J., Grimes, K. M., Sargent, M. A., et al. (2020). MCUb induction protects the heart from post-ischemic remodeling. *Circulation Res.* 0, 379. doi:10.1161/CIRCRESAHA.119.316369

Hurst, S., Baggett, A., Csordas, G., and Sheu, S.-S. (2019). SPG7 targets the m-AAA protease complex to process MCU for uniporter assembly, Ca2+ influx, and regulation of mitochondrial permeability transition pore opening. *J. Biol. Chem.* 294 (28), 10807–10818. doi:10.1074/jbc.RA118.006443

Hyman, B. T., Phelps, C. H., Beach, T. G., Bigio, E. H., Cairns, N. J., Carrillo, M. C., et al. (2012). National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimer's Dementia* 8 (1), 1–13. doi:10.1016/j.jalz.2011.10.007

Ichas, F., Jouaville, L. S., and Mazat, J.-P. (1997). Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals. *Cell* 89 (7), 1145–1153. doi:10.1016/S0092-8674(00)80301-3

Irwin, W. A., Bergamin, N., Sabatelli, P., Reggiani, C., Megighian, A., Merlini, L., et al. (2003). Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. *Nat. Genet.* 35 (4), 367–371. doi:10.1038/ng1270

Ivannikov, M. V., and Macleod, G. T. (2013). Mitochondrial free Ca²⁺ levels and their effects on energy metabolism in Drosophila motor nerve terminals. *Biophysical J.* 104 (11), 2353–2361. doi:10.1016/j.bpj.2013.03.064

Jadiya, P., Kolmetzky, D. W., Tomar, D., Di Meco, A., Lombardi, A. A., Lambert, J. P., et al. (2019). Impaired mitochondrial calcium efflux contributes to disease progression in models of Alzheimer's disease. *Nat. Commun.* 10 (1), 3885. doi:10.1038/s41467-019-11813-6

Jang, H., Zheng, J., and Nussinov, R. (2007). Models of β-amyloid ion channels in the membrane suggest that channel formation in the bilayer is a dynamic process. *Biophysical J.* 93 (6), 1938–1949. doi:10.1529/biophysj.107.110148

Jensen, L., Bultynck, G., Luyten, T., Amijee, H., Bootman, M., and Roderick, H. (2013). Alzheimer's disease-associated peptide Aβ42 mobilizes ER Ca 2+ via InsP3R-dependent andindependent mechanisms. *Front. Mol. Neurosci.* 6, 36. doi:10.3389/fnmol.2013.00036

Jiang, D., Zhao, L., Clish, C. B., and Clapham, D. E. (2013). Letm1, the mitochondrial Ca2+/H+ antiporter, is essential for normal glucose metabolism and alters brain function in Wolf–Hirschhorn syndrome. *Proc. Natl. Acad. Sci.* 110 (24), E2249–E2254. doi:10.1073/pnas.1308558110

Johnson, M. T., Benson, J. C., Pathak, T., Xin, P., McKernan, A. S., Emrich, S. M., et al. (2022). The airway smooth muscle sodium/calcium exchanger NCLX is critical for airway remodeling and hyperresponsiveness in asthma. *J. Biol. Chem.* 298 (8), 102259. doi:10.1016/j.jbc.2022.102259

Jung, H., Kim, S. Y., Canbakis Cecen, F. S., Cho, Y., and Kwon, S. K. (2020). Dysfunction of mitochondrial Ca(2+) regulatory machineries in brain aging and neurodegenerative diseases. *Front. Cell Dev. Biol.* 8, 599792. doi:10.3389/fcell.2020.599792

Kamer, K. J., and Mootha, V. K. (2015). The molecular era of the mitochondrial calcium uniporter. *Nat. Rev. Mol. Cell Biol.* 16, 545–553. doi:10.1038/nrm4039

Kim, M. S., and Usachev, Y. M. (2009). Mitochondrial Ca2+ cycling facilitates activation of the transcription factor NFAT in sensory neurons. *J. Neurosci.* 29 (39), 12101–12114. doi:10.1523/JNEUROSCI.3384-09.2009

Kinjo, E. R., Rodríguez, P. X. R., dos Santos, B. A., Higa, G. S. V., Ferraz, M. S. A., Schmeltzer, C., et al. (2018). New insights on temporal lobe epilepsy based on plasticity-related network changes and high-order statistics. *Mol. Neurobiol.* 55 (5), 3990–3998. doi:10.1007/s12035-017-0623-2

Kirichok, Y., Krapivinsky, G., and Clapham, D. E. (2004). The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* 427 (6972), 360–364. doi:10.1038/nature02246

Klann, E. (1998). Cell-permeable scavengers of superoxide prevent long-term potentiation in hippocampal area CA1. *J. Neurophysiology* 80 (1), 452–457. doi:10.1152/jn.1998.80.1.452

Konen, L. M., Wright, A. L., Royle, G. A., Morris, G. P., Lau, B. K., Seow, P. W., et al. (2020). A new mouse line with reduced GluA2 Q/R site RNA editing exhibits loss of dendritic spines, hippocampal CA1-neuron loss, learning and memory impairments and NMDA receptor-independent seizure vulnerability. *Mol. Brain* 13 (1), 27. doi:10.1186/s13041-020-0545-1

Kostic, M., Ludtmann, M. H. R., Bading, H., Hershfinkel, M., Steer, E., Chu, C. T., et al. (2015). PKA phosphorylation of NCLX reverses mitochondrial calcium overload and depolarization, promoting survival of PINK1-deficient dopaminergic neurons. *Cell Rep.* 13 (2), 376–386. doi:10.1016/j.celrep.2015.08.079

Kovac, S., Dinkova Kostova, A., Herrmann, A., Melzer, N., Meuth, S., and Gorji, A. (2017). Metabolic and homeostatic changes in seizures and acquired epilepsy—mitochondria, calcium dynamics and reactive oxygen species. *Int. J. Mol. Sci.* 18 (9), 1935. doi:10.3390/ijms18091935

Kovacs, G., Reimer, L., and Jensen, P. H. (2021). Endoplasmic reticulum-based calcium dysfunctions in synucleinopathies. *Front. Neurology* 12, 742625. doi:10.3389/fneur.2021.742625

Kristian, T., Pivovarova, N. B., Fiskum, G., and Andrews, S. B. (2007). Calcium-induced precipitate formation in brain mitochondria: Composition, calcium capacity, and retention. *J. Neurochem.* 102 (4), 1346–1356. doi:10.1111/j.1471-4159.2007.04626.x

Kwan, P., and Brodie, M. J. (2000). Early identification of refractory epilepsy. *N. Engl. J. Med.* 342 (5), 314–319. doi:10.1056/nejm200002033420503

Kwon, S. K., Sando, R., 3rd, Lewis, T. L., Hirabayashi, Y., Maximov, A., and Polleux, F. (2016). LKB1 regulates mitochondria-dependent presynaptic calcium clearance and neurotransmitter release properties at excitatory synapses along cortical axons. *PLoS Biol.* 14 (7), e1002516. doi:10.1371/journal.pbio.1002516

Kwong, J. Q., Lu, X., Correll, R. N., Schwanekamp, J. A., Vagnozzi, R. J., Sargent, M. A., et al. (2015). The mitochondrial calcium uniporter selectively matches metabolic output to acute contractile stress in the heart. *Cell Rep.* 12 (1), 15–22. doi:10.1016/j.celrep.2015.06.002

Lambert, J. P., Luongo, T. S., Tomar, D., Jadiya, P., Gao, E., Zhang, X., et al. (2019). MCUB regulates the molecular composition of the mitochondrial calcium uniporter channel to limit mitochondrial calcium overload during stress. *Circulation* 140 (21), 1720–1733. doi:10.1161/CIRCULATIONAHA.118.037968

Lee, D., Lee, K. H., Ho, W. K., and Lee, S. H. (2007). Target cell-specific involvement of presynaptic mitochondria in post-tetanic potentiation at hippocampal mossy fiber synapses. *J. Neurosci.* 27 (50), 13603–13613. doi:10.1523/JNEUROSCI.3985-07.2007

Lee, J. H., Han, J.-h., Kim, H., Park, S. M., Joe, E.-h., and Jou, I. (2019). Parkinson's disease-associated LRRK2-G2019S mutant acts through regulation of SERCA activity to control ER stress in astrocytes. *Acta Neuropathol. Commun.* 7 (1), 68. doi:10.1186/s40478-019-0716-4

Leung, A. W. C., Varanyuwatana, P., and Halestrap, A. P. (2008). The mitochondrial phosphate carrier interacts with cyclophilin D and may play a key role in the permeability transition. *J. Biol. Chem.* 283 (39), 26312–26323. doi:10.1074/jbc.M805235200

Levesque, M., and Avoli, M. (2013). The kainic acid model of temporal lobe epilepsy. *Neurosci. Biobehav Rev.* 37, 2887–2899. doi:10.1016/j.neubiorev.2013.10.011

Li, D.-D., Zhang, Y.-H., Zhang, W., and Zhao, P. (2019). Meta-analysis of randomized controlled trials on the efficacy and safety of donepezil, galantamine, rivastigmine, and memantine for the treatment of Alzheimer's disease. *Front. Neurosci.* 13, 472. doi:10.3389/fnins.2019.00472

Li, L., Chen, S. R., Zhou, M. H., Wang, L., Li, D. P., Chen, H., et al. (2021). α2δ-1 switches the phenotype of synaptic AMPA receptors by physically disrupting heteromeric subunit assembly. *Cell Rep.* 36 (3), 109396. doi:10.1016/j.celrep.2021.109396

Li, W., Shariat-Madar, Z., Powers, M., Sun, X., Lane, R. D., and Garlid, K. D. (1992). Reconstitution, identification, purification, and immunological characterization of the 110-kDa Na+/Ca2+ antiporter from beef heart mitochondria. *J. Biol. Chem.* 267 (25), 17983–17989. doi:10.1016/s0021-9258(19)37140-6

Lim, D., Dematteis, G., Tapella, L., Genazzani, A. A., Cali, T., Brini, M., et al. (2021a). Ca(2+) handling at the mitochondria-ER contact sites in neurodegeneration. *Cell Calcium* 98, 102453. doi:10.1016/j.ceca.2021.102453

Lim, D., Semyanov, A., Genazzani, A., and Verkhratsky, A. (2021b). Calcium signaling in neuroglia. *Int. Rev. Cell Mol. Biol.* 362, 1–53. doi:10.1016/bs.ircmb.2021.01.003

Liochev, S. I. (2013). Reactive oxygen species and the free radical theory of aging. *Free Radic. Biol. Med.* 60, 1–4. doi:10.1016/j.freeradbiomed.2013.02.011

Liu, J. C., Liu, J., Holmstrom, K. M., Menazza, S., Parks, R. J., Fergusson, M. M., et al. (2016). MICU1 serves as a molecular gatekeeper to prevent *in vivo* mitochondrial calcium overload. *Cell Rep.* 16 (6), 1561–1573. doi:10.1016/j.celrep.2016.07.011

Liu, J. C., Parks, R. J., Liu, J., Stares, J., Rovira, I. I., Murphy, E., et al. (2017). "The *in vivo* Biology of the mitochondrial calcium uniporter," in *Mitochondrial dynamics in cardiovascular medicine*. Editor G. Santulli (Cham: Springer International Publishing), 49–63.

Liu, J. C., Syder, N. C., Ghorashi, N. S., Willingham, T. B., Parks, R. J., Sun, J., et al. (2020). EMRE is essential for mitochondrial calcium uniporter activity in a mouse model. *JCI Insight* 5 (4), e134063. doi:10.1172/jci.insight.134063

Logan, C. V., Szabadkai, G., Sharpe, J. A., Parry, D. A., Torelli, S., Childs, A.-M., et al. (2014). Loss-of-function mutations in MICU1 cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling. *Nat. Genet.* 46 (2), 188–193. doi:10.1038/ng.2851

Lohmann, C., and Wong, R. O. L. (2005). Regulation of dendritic growth and plasticity by local and global calcium dynamics. *Cell Calcium* 37 (5), 403–409. doi:10.1016/j.ceca.2005.01.008

Loupatatzis, C., Seitz, G., Schönfeld, P., Lang, F., and Siemen, D. (2002). Single-channel currents of the permeability transition pore from the inner mitochondrial membrane of rat liver and of a human hepatoma cell line. *Cell Physiol. Biochem.* 12 (5-6), 269–278. doi:10.1159/000067897

Luo, Y., Ma, H., Zhou, J. J., Li, L., Chen, S. R., Zhang, J., et al. (2018). Focal cerebral ischemia and reperfusion induce brain injury through α2δ-1-bound NMDA receptors. *Stroke* 49 (10), 2464–2472. doi:10.1161/STROKEAHA.118.022330

Luongo, T. S., Lambert, J. P., Gross, P., Nwokedi, M., Lombardi, A. A., Shanmughapriya, S., et al. (2017). The mitochondrial Na(+)/Ca(2+) exchanger is essential for Ca(2+) homeostasis and viability. *Nature* 545 (7652), 93–97. doi:10.1038/nature22082

Luongo, T. S., Lambert, J. P., Yuan, A., Zhang, X., Gross, P., Song, J., et al. (2015). The mitochondrial calcium uniporter matches energetic supply with cardiac workload during stress and modulates permeability transition. *Cell Rep.* 12 (1), 23–34. doi:10.1016/j.celrep.2015.06.017

MacAskill, A. F., Atkin, T. A., and Kittler, J. T. (2010). Mitochondrial trafficking and the provision of energy and calcium buffering at excitatory synapses. *Eur. J. Neurosci.* 32(2), 231–240. doi:10.1111/j.1460-9568.2010.07345.x

Mallilankaraman, K., Cárdenas, C., Doonan, P. J., Chandramoorthy, H. C., Irrinki, K. M., Golenár, T., et al. (2012a). MCUR1 is an essential component of mitochondrial Ca2+ uptake that regulates cellular metabolism. *Nat. Cell Biol.* 14, 1336. doi:10.1038/ncb2622

Mallilankaraman, K., Doonan, P., Cárdenas, C., Chandramoorthy, H. C., Müller, M., Miller, R., et al. (2012b). MICU1 is an essential gatekeeper for MCU-mediated mitochondrial Ca2+ uptake that regulates cell survival. *Cell* 151 (3), 630–644. doi:10.1016/j.cell.2012.10.011

Maneshi, M. M., Toth, A. B., Ishii, T., Hori, K., Tsujikawa, S., Shum, A. K., et al. (2020). Orai1 channels are essential for amplification of glutamate-evoked Ca(2+) signals in dendritic spines to regulate working and associative memory. *Cell Rep.* 33 (9), 108464. doi:10.1016/j.celrep.2020.108464

Manikandan, M., Walker, S., Deshmukh, A. R., Perea, E., Wang, D., Alagramam, K. N., et al. (2021). Mitochondrial calcium uniporter is essential for hearing and hair cell preservation in congenic FVB/NJ mice. *Sci. Rep.* 11 (1), 9660. doi:10.1038/s41598-021-88841-0

Marland, J. R., Hasel, P., Bonnycastle, K., and Cousin, M. A. (2016). Mitochondrial calcium uptake modulates synaptic vesicle endocytosis in central nerve terminals. *J. Biol. Chem.* 291 (5), 2080–2086. doi:10.1074/jbc.M115.686956

Marta, K., Hasan, P., Rodriguez-Prados, M., Paillard, M., and Hajnoczky, G. (2021). Pharmacological inhibition of the mitochondrial Ca(2+) uniporter: Relevance for pathophysiology and human therapy. *J. Mol. Cell Cardiol.* 151, 135–144. doi:10.1016/j.yjmcc.2020.09.014

Martin, L. J., Gertz, B., Pan, Y., Price, A. C., Molkentin, J. D., and Chang, Q. (2009). The mitochondrial permeability transition pore in motor neurons: Involvement in the pathobiology of ALS mice. *Exp. Neurol.* 218 (2), 333–346. doi:10.1016/j.expneurol.2009.02.015

Martin, L. J., Semenkow, S., Hanaford, A., and Wong, M. (2014). Mitochondrial permeability transition pore regulates Parkinson's disease development in mutant α-synuclein transgenic mice. *Neurobiol. Aging* 35 (5), 1132–1152. doi:10.1016/j.neurobiolaging.2013.11.008

Marty, A. (1989). The physiological role of calcium-dependent channels. *Trends Neurosci.* 12 (11), 420–424. doi:10.1016/0166-2236(89)90090-8

Matteucci, A., Patron, M., Vecellio Reane, D., Gastaldello, S., Amoroso, S., Rizzuto, R., et al. (2018). Parkin-dependent regulation of the MCU complex component MICU1. *Sci. Rep.* 8 (1), 14199. doi:10.1038/s41598-018-32551-7

Mattson, M. P., Gleichmann, M., and Cheng, A. (2008). Mitochondria in neuroplasticity and neurological disorders. *Neuron* 60 (5), 748–766. doi:10.1016/j.neuron.2008.10.010

McBain, C. J., and Mayer, M. L. (1994). N-methyl-D-aspartic acid receptor structure and function. *Physiol. Rev.* 74 (3), 723–760. doi:10.1152/physrev.1994.74.3.723

McCormack, J. G., Halestrap, A. P., and Denton, R. M. (1990). Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiol. Rev.* 70, 391–425. doi:10.1152/physrev.1990.70.2.391

Medler, K., and Gleason, E. L. (2002). Mitochondrial Ca2+ buffering regulates synaptic transmission between retinal amacrine cells. *J. Neurophysiology* 87 (3), 1426–1439. doi:10.1152/jn.00627.2001

Medvedeva, Y. V., Kim, M. S., and Usachev, Y. M. (2008). Mechanisms of prolonged presynaptic Ca2+ signaling and glutamate release induced by TRPV1 activation in rat sensory neurons. *J. Neurosci.* 28 (20), 5295–5311. doi:10.1523/JNEUROSCI.4810-07.2008

Mnatsakanyan, N., Llaguno, M. C., Yang, Y., Yan, Y., Weber, J., Sigworth, F. J., et al. (2019). A mitochondrial megachannel resides in monomeric F1FO ATP synthase. *Nat. Commun.* 10 (1), 5823. doi:10.1038/s41467-019-13766-2

Mnatsakanyan, N., Park, H.-A., Wu, J., He, X., Llaguno, M. C., Latta, M., et al. (2022). Mitochondrial ATP synthase c-subunit leak channel triggers cell death upon loss of its F1 subcomplex. *Cell Death Differ.* 29, 1874–1887. doi:10.1038/s41418-022-00972-7

Modesti, L., Danese, A., Angela Maria Vitto, V., Ramaccini, D., Aguiari, G., Gafà, R., et al. (2021). Mitochondrial Ca2+ signaling in health, disease and therapy. *Cells* 10 (6), 1317. doi:10.3390/cells10061317

More, J., Galusso, N., Veloso, P., Montecinos, L., Finkelstein, J. P., Sanchez, G., et al. (2018). N-acetylcysteine prevents the spatial memory deficits and the redox-dependent RyR2 decrease displayed by an Alzheimer's disease rat model. *Front. Aging Neurosci.* 10, 399. doi:10.3389/fnagi.2018.00399

Moskowitz, M. A., Lo, E. H., and Iadecola, C. (2010). The science of stroke: Mechanisms in search of treatments. *Neuron* 67 (2), 181–198. doi:10.1016/j.neuron.2010.07.002

Mühling, T., Duda, J., Weishaupt, J. H., Ludolph, A. C., and Liss, B. (2014). Elevated mRNA-levels of distinct mitochondrial and plasma membrane Ca2+ transporters in individual hypoglossal motor neurons of endstage SOD1 transgenic mice. *Front. Cell. Neurosci.* 8, 353. doi:10.3389/fncel.2014.00353

Munoz-Pinedo, C., Guio-Carrion, A., Goldstein, J. C., Fitzgerald, P., Newmeyer, D. D., and Green, D. R. (2006). Different mitochondrial intermembrane space proteins are released during apoptosis in a manner that is coordinately initiated but can vary in duration. *Proc. Natl. Acad. Sci. U. S. A.* 103 (31), 11573–11578. doi:10.1073/pnas.0603007103

Murphy, E., Pan, X., Nguyen, T., Liu, J., Holmström, K. M., and Finkel, T. (2014). Unresolved questions from the analysis of mice lacking MCU expression. *Biochem. Biophysical Res. Commun.* 449 (4), 384–385. doi:10.1016/j.bbrc.2014.04.144

Murphy, E., and Steenbergen, C. (2021). Regulation of mitochondrial Ca2+ uptake. *Annu. Rev. Physiology* 83 (1), 107–126. doi:10.1146/annurev-physiol-031920-092419

Nakamura, T., Ogawa, M., Kojima, K., Takayanagi, S., Ishihara, S., Hattori, K., et al. (2021). The mitochondrial Ca2+ uptake regulator, MICU1, is involved in cold stress-induced ferroptosis. *EMBO Rep.* 22 (5), e51532. doi:10.15252/embr.202051532

Neginskaya, M. A., Morris, S. E., and Pavlov, E. V. (2022). Both ANT and ATPase are essential for mitochondrial permeability transition but not depolarization. *iScience* 25 (11), 105447. doi:10.1016/j.isci.2022.105447

Neginskaya, M. A., Solesio, M. E., Berezhnaya, E. V., Amodeo, G. F., Mnatsakanyan, N., Jonas, E. A., et al. (2019). ATP synthase C-Subunit-Deficient mitochondria have a small cyclosporine A-sensitive channel, but lack the permeability transition pore. *Cell Rep.* 26 (1), 11–17. e12. doi:10.1016/j.celrep.2018.12.033

Neher, E., and Sakaba, T. (2008). Multiple roles of calcium ions in the regulation of neurotransmitter release. *Neuron* 59 (6), 861–872. doi:10.1016/j.neuron.2008.08.019

Newmeyer, D. D., and Ferguson-Miller, S. (2003). Mitochondria: Releasing power for life and unleashing the machineries of death. *Cell* 112 (4), 481–490. doi:10.1016/s0092-8674(03)00116-8

Nguyen, K. T., García-Chacón, L. E., Barrett, J. N., Barrett, E. F., and David, G. (2009). The Psi(m) depolarization that accompanies mitochondrial Ca2+ uptake is greater in mutant SOD1 than in wild-type mouse motor terminals. *Proc. Natl. Acad. Sci. U. S. A.* 106 (6), 2007–2011. doi:10.1073/pnas.0810934106

Nicholls, D. G. (1978). Calcium transport and porton electrochemical potential gradient in mitochondria from Guinea-pig cerebral cortex and rat heart. *Biochem. J.* 170 (3), 511–522. doi:10.1042/bj1700511

Nicholls, D. G. (2005). Mitochondria and calcium signaling. *Cell Calcium* 38 (3-4), 311–317. doi:10.1016/j.ceca.2005.06.011

Nicholls, D. G. (2012). "Mitochondria, sodium, and calcium in neuronal dysfunction," in *Mitochondrial dysfunction in neurodegenerative disorders*. Editors A. K. Reeve, K. J. Krishnan, M. R. Duchen, and D. M. Turnbull (London: Springer), 113–125.

Nicholls, D. G., and Ward, M. W. (2000). Mitochondrial membrane potential and neuronal glutamate excitotoxicity: Mortality and millivolts. *Trends Neurosci.* 23 (4), 166–174. doi:10.1016/s0166-2236(99)01534-9

Nichols, M., Pavlov, E., and Robertson, G. S. (2018). Tamoxifen-induced knockdown of the mitochondrial calcium uniporter in Thy1-expressing neurons protects mice from hypoxic/ischemic brain injury. *Cell Death Dis.* 9 (6), 606. doi:10.1038/s41419-018-0607-9

Novorolsky, R. J., Nichols, M., Kim, J. S., Pavlov, E. V., J J W., Wilson, J. J., et al. (2020). The cell-permeable mitochondrial calcium uniporter inhibitor Ru265 preserves cortical neuron respiration after lethal oxygen glucose deprivation and reduces hypoxic/ischemic brain injury. *J. Cereb. Blood Flow. Metab.* 40 (6), 1172–1181. doi:10.1177/0271678x20908523

Nowicky, A. V., and Duchen, M. R. (1998). Changes in [Ca2+]i and membrane currents during impaired mitochondrial metabolism in dissociated rat hippocampal neurons. *J. physiology* 507 (1), 131–145. doi:10.1111/j.1469-7793.1998.131bu.x

Nunnari, J., and Suomalainen, A. (2012). Mitochondria: In sickness and in health. *Cell* 148 (6), 1145–1159. doi:10.1016/j.cell.2012.02.035

Olanow, C. W., Stern, M. B., and Sethi, K. (2009). The scientific and clinical basis for the treatment of Parkinson disease (2009). *Neurology* 72, S1–S136. doi:10.1212/WNL.0b013e3181a1d44c

Orrenius, S., Zhivotovsky, B., and Nicotera, P. (2003). Regulation of cell death: The calcium-apoptosis link. *Nat. Rev. Mol. Cell Biol.* 4 (7), 552–565. doi:10.1038/nrm1150

Oxenoid, K., Dong, Y., Cao, C., Cui, T., Sancak, Y., Markhard, A. L., et al. (2016). Architecture of the mitochondrial calcium uniporter. *Nature* 533, 269–273. doi:10.1038/nature17656

Palty, R., Hershfinkel, M., Yagev, O., Saar, D., Barkalifa, R., Khananshvili, D., et al. (2006). Single α-domain constructs of the Na+/Ca2+ exchanger, NCLX, oligomerize to form a functional exchanger. *Biochemistry* 45 (39), 11856–11866. doi:10.1021/bi060633b

Palty, R., Ohana, E., Hershfinkel, M., Volokita, M., Elgazar, V., Beharier, O., et al. (2004). Lithium-calcium exchange is mediated by a distinct potassium-independent sodium-calcium exchanger. *J. Biol. Chem.* 279 (24), 25234–25240. doi:10.1074/jbc.M401229200

Palty, R., and Sekler, I. (2012). The mitochondrial Na(+)/Ca(2+) exchanger. *Cell Calcium* 52 (1), 9–15. doi:10.1016/j.ceca.2012.02.010

Palty, R., Silverman, W. F., Hershfinkel, M., Caporale, T., Sensi, S. L., Parnis, J., et al. (2010). NCLX is an essential component of mitochondrial Na+/Ca2+ exchange. *Proc. Natl. Acad. Sci.* 107 (1), 436–441. doi:10.1073/pnas.0908099107

Pan, X., Liu, J., Nguyen, T., Liu, C., Sun, J., Teng, Y., et al. (2013). The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. *Nat. Cell Biol.* 15, 1464. doi:10.1038/ncb2868

Parkinson Study Group STEADY-PD III Investigators (2020). Isradipine versus placebo in early Parkinson disease. *Ann. Intern. Med.* 172 (9), 591–598. doi:10.7326/m19-2534./m.32227247

Partridge, L. D., and Swandulla, D. (1988). Calcium-activated non-specific cation channels. *Trends Neurosci.* 11 (2), 69–72. doi:10.1016/0166-2236(88)90167-1

Patel, D. C., Tewari, B. P., Chaunsali, L., and Sontheimer, H. (2019). Neuron-glia interactions in the pathophysiology of epilepsy. *Nat. Rev. Neurosci.* 20 (5), 282–297. doi:10.1038/s41583-019-0126-4

Patron, M., Checchetto, V., Raffaello, A., Teardo, E., Vecellio Reane, D., Mantoan, M., et al. (2014). MICU1 and MICU2 finely tune the mitochondrial Ca^{2+} uniporter by exerting opposite effects on MCU activity. *Mol. Cell* 53 (5), 726–737. doi:10.1016/j.molcel.2014.01.013

Patron, M., Granatiero, V., Espino, J., Rizzuto, R., and De Stefani, D. (2019). MICU3 is a tissue-specific enhancer of mitochondrial calcium uptake. *Cell Death Differ.* 26 (1), 179–195. doi:10.1038/s41418-018-0113-8

Pearson-Smith, J. N., and Patel, M. (2017). Metabolic dysfunction and oxidative stress in epilepsy. *Int. J. Mol. Sci.* 18 (11), 2365. doi:10.3390/ijms18112365

Perocchi, F., Gohil, V. M., Girgis, H. S., Bao, X. R., McCombs, J. E., Palmer, A. E., et al. (2010). MICU1 encodes a mitochondrial EF hand protein required for Ca2+ uptake. *Nature* 467 (7313), 291–296. doi:10.1038/nature09358

Petrungaro, C., Zimmermann, K. M., Küttner, V., Fischer, M., Dengjel, J., Bogeski, I., et al. (2015). The Ca(2+)-dependent release of the mia40-induced MICU1-MICU2 dimer from MCU regulates mitochondrial Ca(2+) uptake. *Cell Metab.* 22 (4), 721–733. doi:10.1016/j.cmet.2015.08.019

Pivovarova, N. B., and Andrews, S. B. (2010). Calcium-dependent mitochondrial function and dysfunction in neurons. *FEBS J.* 277 (18), 3622–3636. doi:10.1111/j.1742-4658.2010.07754.x

Pivovarova, N. B., Hongpaisan, J., Andrews, S. B., and Friel, D. D. (1999). Depolarization-induced mitochondrial Ca accumulation in sympathetic neurons: Spatial and temporal characteristics. *J. Neurosci.* 19 (15), 6372–6384. doi:10.1523/JNEUROSCI.19-15-06372.1999

Pivovarova, N. B., Nguyen, H. V., Winters, C. A., Brantner, C. A., Smith, C. L., and Andrews, S. B. (2004). Excitotoxic calcium overload in a subpopulation of mitochondria triggers delayed death in hippocampal neurons. *J. Neurosci.* 24 (24), 5611–5622. doi:10.1523/JNEUROSCI.0531-04.2004

Pivovarova, N. B., Pozzo-Miller, L. D., Hongpaisan, J., and Andrews, S. B. (2002). Correlated calcium uptake and release by mitochondria and endoplasmic reticulum of CA3 hippocampal dendrites after afferent synaptic stimulation. *J. Neurosci.* 22 (24), 10653–10661. doi:10.1523/JNEUROSCI.22-24-10653.2002

Plotegher, N., Filadi, R., Pizzo, P., and Duchen, M. R. (2021). Excitotoxicity revisited: Mitochondria on the verge of a nervous breakdown. *Trends Neurosci.* 44 (5), 342–351. doi:10.1016/j.tins.2021.01.001

Plovanich, M., Bogorad, R. L., Sancak, Y., Kamer, K. J., Strittmatter, L., Li, A. A., et al. (2013). MICU2, a paralog of MICU1, resides within the mitochondrial uniporter complex to regulate calcium handling. *PLoS ONE* 8 (2), e55785. doi:10.1371/journal.pone.0055785

Popugaeva, E., Pchitskaya, E., and Bezprozvanny, I. (2018). Dysregulation of intracellular calcium signaling in alzheimer's disease. *Antioxidants Redox Signal.* 29 (12), 1176–1188. doi:10.1089/ars.2018.7506

Prakriya, M., and Lewis, R. S. (2015). Store-operated calcium channels. *Physiol. Rev.* 95 (4), 1383–1436. doi:10.1152/physrev.00020.2014

Puttachary, S., Sharma, S., Stark, S., and Thippeswamy, T. (2015). Seizure-induced oxidative stress in temporal lobe epilepsy. *Biomed. Res. Int.* 2015, 745613. doi:10.1155/2015/745613

Querfurth, H. W., and LaFerla, F. M. (2010). Alzheimer's disease. *N. Engl. J. Med.* 362 (4), 329–344. doi:10.1056/NEJMra0909142

Raffaello, A., De Stefani, D., Sabbadin, D., Teardo, E., Merli, G., Picard, A., et al. (2013). The mitochondrial calcium uniporter is a multimer that can include a dominant-negative pore-forming subunit. *EMBO J.* 32 (17), 2362–2376. doi:10.1038/emboj.2013.157

Rasola, A., and Bernardi, P. (2011). Mitochondrial permeability transition in Ca2+-dependent apoptosis and necrosis. *Cell Calcium* 50 (3), 222–233. doi:10.1016/j.ceca.2011.04.007

Redmond, L., and Ghosh, A. (2005). Regulation of dendritic development by calcium signaling. *Cell Calcium* 37 (5), 411–416. doi:10.1016/j.ceca.2005.01.009

Rizzuto, R., De Stefani, D., Raffaello, A., and Mammucari, C. (2012). Mitochondria as sensors and regulators of calcium signalling. *Nat. Rev. Mol. Cell Biol.* 13 (9), 566–578. doi:10.1038/nrm3412

Rosales-Corral, S., Acuna-Castroviejo, D., Tan, D. X., López-Armas, G., Cruz-Ramos, J., Munoz, R., et al. (2012). Accumulation of exogenous amyloid-beta peptide in hippocampal mitochondria causes their dysfunction: A protective role for melatonin. *Oxidative Med. Cell. Longev.* 2012, 843649. doi:10.1155/2012/843649

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., et al. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362 (6415), 59–62. doi:10.1038/362059a0

Rossi, A. R., Angelo, M. F., Villarreal, A., Lukin, J., and Ramos, A. J. (2013). Gabapentin administration reduces reactive gliosis and neurodegeneration after pilocarpine-induced status epilepticus. *PLoS One* 8 (11), e78516. doi:10.1371/journal.pone.0078516

Rossi, C. S., Vasington, F. D., and Carafoli, E. (1973). The effect of ruthenium red on the uptake and release of Ca 2+ by mitochondria. *Biochem. Biophys. Res. Commun.* 50 (3), 846–852. doi:10.1016/0006-291x(73)91322-3

Roy, S., Dey, K., Hershfinkel, M., Ohana, E., and Sekler, I. (2017). Identification of residues that control Li+ versus Na+ dependent Ca2+ exchange at the transport site of the mitochondrial NCLX. *Biochimica Biophysica Acta (BBA) - Mol. Cell Res.* 1864 (6), 997–1008. doi:10.1016/j.bbamcr.2017.01.011

Rybalchenko, V., Hwang, S.-Y., Rybalchenko, N., and Koulen, P. (2008). The cytosolic N-terminus of presenilin-1 potentiates mouse ryanodine receptor single channel activity. *Int. J. Biochem. Cell Biol.* 40 (1), 84–97. doi:10.1016/j.biocel.2007.06.023

Rysted, J. E., Lin, Z., Walters, G. C., Rauckhorst, A. J., Noterman, M., Liu, G., et al. (2021). Distinct properties of Ca2+ efflux from brain, heart and liver mitochondria: The effects of Na+, Li+ and the mitochondrial Na+/Ca2+ exchange inhibitor CGP37157. *Cell Calcium* 96, 102382. doi:10.1016/j.ceca.2021.102382

Sah, P. (1996). Ca^{2+}-activated K$^+$ currents in neurones: Types, physiological roles and modulation. *Trends Neurosci.* 19 (4), 150–154. doi:10.1016/s0166-2236(96)80026-9

Salter, M. W., and Stevens, B. (2017). Microglia emerge as central players in brain disease. *Nat. Med.* 23 (9), 1018–1027. doi:10.1038/nm.4397

Sancak, Y., Markhard, A. L., Kitami, T., Kovács-Bogdán, E., Kamer, K. J., Udeshi, N. D., et al. (2013). EMRE is an essential component of the mitochondrial calcium uniporter complex. *Science* 342 (6164), 1379–1382. doi:10.1126/science.1242993

Santello, M., Toni, N., and Volterra, A. (2019). Astrocyte function from information processing to cognition and cognitive impairment. *Nat. Neurosci.* 22 (2), 154–166. doi:10.1038/s41593-018-0325-8

Scharfman, H. E. (2007). The neurobiology of epilepsy. *Curr. neurology Neurosci. Rep.* 7 (4), 348–354. doi:10.1007/s11910-007-0053-z

Scheltens, P., Blennow, K., Breteler, M. M. B., de Strooper, B., Frisoni, G. B., Salloway, S., et al. (2016). Alzheimer's disease. *Lancet* 388 (10043), 505–517. doi:10.1016/S0140-6736(15)01124-1

Schwab, R. S., England, A. C., Jr., Poskanzer, D. C., and Young, R. R. (1969). Amantadine in the treatment of Parkinson's disease. *JAMA* 208 (7), 1168–1170. doi:10.1001/jama.1969.03160070046011

Scott, L. J., and Figgitt, D. P. (2004). Mitoxantrone: A review of its use in multiple sclerosis. *CNS Drugs* 18 (6), 379–396. doi:10.2165/00023210-200418060-00010

Scott, R. H., Sutton, K. G., Griffin, A., Stapleton, S. R., and Currie, K. P. (1995). Aspects of calcium-activated chloride currents: A neuronal perspective. *Pharmacol. Ther.* 66 (3), 535–565. doi:10.1016/0163-7258(95)00018-c

Selkoe, D. J., and Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* 8 (6), 595–608. doi:10.15252/emmm.201606210

Selvaraj, B. T., Livesey, M. R., Zhao, C., Gregory, J. M., James, O. T., Cleary, E. M., et al. (2018). C9ORF72 repeat expansion causes vulnerability of motor neurons to Ca2+-permeable AMPA receptor-mediated excitotoxicity. *Nat. Commun.* 9 (1), 347. doi:10.1038/s41467-017-02729-0

Shanmughapriya, S., Rajan, S., Hoffman, N. E., Higgins, A. M., Tomar, D., Nemani, N., et al. (2015). SPG7 is an essential and conserved component of the mitochondrial permeability transition pore. *Mol. Cell* 60 (1), 47–62. doi:10.1016/j.molcel.2015.08.009

Shaw, P. J., and Eggett, C. J. (2000). Molecular factors underlying selective vulnerability of motor neurons to neurodegeneration in amyotrophic lateral sclerosis. *J. Neurol.* 247, I17–I27. doi:10.1007/bf03161151

Shigetomi, E., Saito, K., Sano, F., and Koizumi, S. (2019). Aberrant calcium signals in reactive astrocytes: A key process in neurological disorders. *Int. J. Mol. Sci.* 20 (4), 996. doi:10.3390/ijms20040996

Shoshan-Barmatz, V., De Pinto, V., Zweckstetter, M., Raviv, Z., Keinan, N., and Arbel, N. (2010). VDAC, a multi-functional mitochondrial protein regulating cell life and death. *Mol. aspects Med.* 31 (3), 227–285. doi:10.1016/j.mam.2010.03.002

Shutov, L. P., Kim, M. S., Houlihan, P. R., Medvedeva, Y. V., and Usachev, Y. M. (2013). Mitochondria and plasma membrane Ca2+-ATPase control presynaptic Ca2+ clearance in capsaicin-sensitive rat sensory neurons. *J. Physiol.* 591 (10), 2443–2462. doi:10.1113/jphysiol.2012.249219

Sills, G. J., and Rogawski, M. A. (2020). Mechanisms of action of currently used antiseizure drugs. *Neuropharmacology* 168, 107966. doi:10.1016/j.neuropharm.2020.107966

Singh, R., Bartok, A., Paillard, M., Tyburski, A., Elliott, M., and Hajnoczky, G. (2022). Uncontrolled mitochondrial calcium uptake underlies the pathogenesis of neurodegeneration in MICU1-deficient mice and patients. *Sci. Adv.* 8 (11), eabj4716. doi:10.1126/sciadv.abj4716

Smith, E. F., Shaw, P. J., and De Vos, K. J. (2019). The role of mitochondria in amyotrophic lateral sclerosis. *Neurosci. Lett.* 710, 132933. doi:10.1016/j.neulet.2017.06.052

Sobolczyk, M., and Boczek, T. (2022). Astrocytic calcium and cAMP in neurodegenerative diseases. *Front. Cell Neurosci.* 16, 889939. doi:10.3389/fncel.2022.889939

Soman, S. K., Bazała, M., Keatinge, M., Bandmann, O., and Kuznicki, J. (2019). Restriction of mitochondrial calcium overload by mcu inactivation renders a neuroprotective effect in zebrafish models of Parkinson's disease. *Biol. Open* 8 (10), bio044347. doi:10.1242/bio.044347

Soman, S. K., and Dagda, R. K. (2021). Role of cleaved PINK1 in neuronal development, synaptogenesis, and plasticity: Implications for Parkinson's disease. *Front. Neurosci.* 15, 769331. doi:10.3389/fnins.2021.769331

Soman, S., Keatinge, M., Moein, M., Da Costa, M., Mortiboys, H., Skupin, A., et al. (2017). Inhibition of the mitochondrial calcium uniporter rescues dopaminergic neurons in pink1−/− zebrafish. *Eur. J. Neurosci.* 45 (4), 528–535. doi:10.1111/ejn.13473

Sonkusare, S. K., Kaul, C. L., and Ramarao, P. (2005). Dementia of Alzheimer's disease and other neurodegenerative disorders—Memantine, a new hope. *Pharmacol. Res.* 51 (1), 1–17. doi:10.1016/j.phrs.2004.05.005

Spat, A., Szanda, G., Csordas, G., and Hajnoczky, G. (2008). High- and low-calcium-dependent mechanisms of mitochondrial calcium signalling. *Cell Calcium* 44 (1), 51–63. doi:10.1016/j.ceca.2007.11.015

Spruill, M. M., and Kuncl, R. W. (2015). Calbindin-D28K is increased in the ventral horn of spinal cord by neuroprotective factors for motor neurons. *J. Neurosci. Res.* 93 (8), 1184–1191. doi:10.1002/jnr.23562

Stanika, R. I., Villanueva, I., Kazanina, G., Andrews, S. B., and Pivovarova, N. B. (2012). Comparative impact of voltage-gated calcium channels and NMDA receptors on mitochondria-mediated neuronal injury. *J. Neurosci.* 32 (19), 6642–6650. doi:10.1523/JNEUROSCI.6008-11.2012

Stavsky, A., Stoler, O., Kostic, M., Katoshevsky, T., Assali, E. A., Savic, I., et al. (2021). Aberrant activity of mitochondrial NCLX is linked to impaired synaptic transmission and is associated with mental retardation. *Commun. Biol.* 4 (1), 666. doi:10.1038/s42003-021-02114-0

Stelzer, A. C., Frazee, R. W., Van Huis, C., Cleary, J., Opipari, A. W., Jr., Glick, G. D., et al. (2010). NMR studies of an immunomodulatory benzodiazepine binding to its molecular target on the mitochondrial F(1)F(0)-ATPase. *Biopolymers* 93 (1), 85–92. doi:10.1002/bip.21306

Storozhuk, M. V., Ivanova, S. Y., Balaban, P. M., and Kostyuk, P. G. (2005). Possible role of mitochondria in posttetanic potentiation of GABAergic synaptic transmission in rat neocortical cell cultures. *Synapse* 58 (1), 45–52. doi:10.1002/syn.20186

Stout, A. K., Raphael, H. M., Kanterewicz, B. I., Klann, E., and Reynolds, I. J. (1998). Glutamate-induced neuron death requires mitochondrial calcium uptake. *Nat. Neurosci.* 1 (5), 366–373. doi:10.1038/1577

Sun, T., Qiao, H., Pan, P.-Y., Chen, Y., and Sheng, Z.-H. (2013). Motile axonal mitochondria contribute to the variability of presynaptic strength. *Cell Rep.* 4 (3), 413–419. doi:10.1016/j.celrep.2013.06.040

Surmeier, D. J. (2007). Calcium, ageing, and neuronal vulnerability in Parkinson's disease. *Lancet Neurology* 6 (10), 933–938. doi:10.1016/S1474-4422(07)70246-6

Surmeier, D. J., Obeso, J. A., and Halliday, G. M. (2017). Selective neuronal vulnerability in Parkinson disease. *Nat. Rev. Neurosci.* 18 (2), 101–113. doi:10.1038/nrn.2016.178

Szibor, M., Gizatullina, Z., Gainutdinov, T., Endres, T., Debska-Vielhaber, G., Kunz, M., et al. (2020). Cytosolic, but not matrix, calcium is essential for adjustment of mitochondrial pyruvate supply. *J. Biol. Chem.* 295 (14), 4383–4397. doi:10.1074/jbc.RA119.011902

Tadić, V., Adam, A., Goldhammer, N., Lautenschlaeger, J., Oberstadt, M., Malci, A., et al. (2019). Investigation of mitochondrial calcium uniporter role in embryonic and adult motor neurons from G93AhSOD1 mice. *Neurobiol. Aging* 75, 209–222. doi:10.1016/j.neurobiolaging.2018.11.019

Tai, X. Y., Bernhardt, B., Thom, M., Thompson, P., Baxendale, S., Koepp, M., et al. (2018). Review: Neurodegenerative processes in temporal lobe epilepsy with hippocampal sclerosis: Clinical, pathological and neuroimaging evidence. *Neuropathol. Appl. Neurobiol.* 44 (1), 70–90. doi:10.1111/nan.12458

Tang, Y. G., and Zucker, R. S. (1997). Mitochondrial involvement in post-tetanic potentiation of synaptic transmission. *Neuron* 18 (3), 483–491. doi:10.1016/s0896-6273(00)81248-9

Tarasov, A. I., Griffiths, E. J., and Rutter, G. A. (2012). Regulation of ATP production by mitochondrial Ca(2+). *Cell calcium* 52 (1), 28–35. doi:10.1016/j.ceca.2012.03.003

Thayer, S. A., Usachev, Y. M., and Pottorf, W. J. (2002). Modulating Ca2+ clearance from neurons. *Front. Biosci.* 7, D1255–D1279. doi:10.2741/A838

Thurman, D. J., Hesdorffer, D. C., and French, J. A. (2014). Sudden unexpected death in epilepsy: Assessing the public health burden. *Epilepsia* 55(10), 1479–1485. doi:10.1111/epi.12666

Tian, G.-F., Azmi, H., Takano, T., Xu, Q., Peng, W., Lin, J., et al. (2005). An astrocytic basis of epilepsy. *Nat. Med.* 11 (9), 973–981. doi:10.1038/nm1277

Tokunaga, H., Hollenberg, N. K., and Graves, S. W. (2000). Sodium-dependent calcium release from vascular smooth muscle mitochondria. *Hypertens. Res.* 23 (1), 39–45. doi:10.1291/hypres.23.39

Tomar, D., Dong, Z., Shanmughapriya, S., Koch, D. A., Thomas, T., Hoffman, N. E., et al. (2016). MCUR1 is a scaffold factor for the MCU complex function and promotes mitochondrial bioenergetics. *Cell Rep.* 15 (8), 1673–1685. doi:10.1016/j.celrep.2016.04.050

Tsai, M. F., Phillips, C. B., Ranaghan, M., Tsai, C. W., Wu, Y., Willliams, C., et al. (2016). Dual functions of a small regulatory subunit in the mitochondrial calcium uniporter complex. *Elife* 5, e15545. doi:10.7554/eLife.15545

Ueda, S., Masutani, H., Nakamura, H., Tanaka, T., Ueno, M., and Yodoi, J. (2002). Redox control of cell death. *Antioxidants Redox Signal.* 4 (3), 405–414. doi:10.1089/15230860260196209

Ureshino, R. P., Erustes, A. G., Bassani, T. B., Wachilewski, P., Guarache, G. C., Nascimento, A. C., et al. (2019). The interplay between Ca(2+) signaling pathways and neurodegeneration. *Int. J. Mol. Sci.* 20 (23), 6004. doi:10.3390/ijms20236004

Usachev, Y. M. (2015). "Mitochondrial Ca2+ transport in the control of neuronal functions: Molecular and cellular mechanisms," in *The functions, disease-related dysfunctions, and therapeutic targeting of neuronal mitochondria.* Editors V. K. G. J. M. Hardwick and E. A. Jonas (New York: Wiley), 103–129.

Van Den Bosch, L., Van Damme, P., Bogaert, E., and Robberecht, W. (2006). The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochimica Biophysica Acta (BBA) - Mol. Basis Dis.* 1762 (11), 1068–1082. doi:10.1016/j.bbadis.2006.05.002

Vanden Berghe, P., Kenyon, J. L., and Smith, T. K. (2002). Mitochondrial Ca2+ uptake regulates the excitability of myenteric neurons. *J. Neurosci.* 22 (16), 6962–6971. doi:10.1523/JNEUROSCI.22-16-06962.2002

Venuto, C. S., Yang, L., Javidnia, M., Oakes, D., James Surmeier, D., and Simuni, T. (2021). Israpidine plasma pharmacokinetics and exposure–response in early Parkinson's disease. *Ann. Clin. Transl. Neurology* 8 (3), 603–612. doi:10.1002/acn3.51300

Verma, M., Callio, J., Otero, P. A., Sekler, I., Wills, Z. P., and Chu, C. T. (2017). Mitochondrial calcium dysregulation contributes to dendrite degeneration mediated by PD/LBD-Associated LRRK2 mutants. *J. Neurosci.* 37 (46), 11151–11165. doi:10.1523/jneurosci.3791-16.2017

Verma, M., Wills, Z., and Chu, C. T. (2018). Excitatory dendritic mitochondrial calcium toxicity: Implications for Parkinson's and other neurodegenerative diseases. *Front. Neurosci.* 12, 523. doi:10.3389/fnins.2018.00523

Walker, M. C. (2018). Pathophysiology of status epilepticus. *Neurosci. Lett.* 667, 84–91. doi:10.1016/j.neulet.2016.12.044

Walters, G. C., and Usachev, Y. M. (2022). MCU (mitochondrial Ca(2+) uniporter) makes the calcium go round. *J. Biol. Chem.* 298 (2), 101604. doi:10.1016/j.jbc.2022.101604

Wang, C., Xie, N., Wang, Y., Li, Y., Ge, X., and Wang, M. (2015). Role of the mitochondrial calcium uniporter in rat hippocampal neuronal death after pilocarpine-induced status epilepticus. *Neurochem. Res.* 40 (8), 1739–1746. doi:10.1007/s11064-015-1657-3

Wang, Y., Nguyen, N. X., She, J., Zeng, W., Yang, Y., Bai, X.-c., et al. (2019). Structural mechanism of EMRE-dependent gating of the human mitochondrial calcium uniporter. *Cell* 177 (5), 1252–1261. e1213. doi:10.1016/j.cell.2019.03.050

Wei, A. D., Gutman, G. A., Aldrich, R., Chandy, K. G., Grissmer, S., and Wulff, H. (2005). International Union of Pharmacology. LII. Nomenclature and molecular relationships of calcium-activated potassium channels. *Pharmacol. Rev.* 57 (4), 463–472. doi:10.1124/pr.57.4.9

Werth, J. L., and Thayer, S. A. (1994). Mitochondria buffer physiological calcium loads in cultured rat dorsal root ganglion neurons. *J. Neurosci. official J. Soc. Neurosci.* 14 (1), 348–356. doi:10.1523/JNEUROSCI.14-01-00348.1994

Wimmer, V. C., Horstmann, H., Groh, A., and Kuner, T. (2006). Donut-like topology of synaptic vesicles with a central cluster of mitochondria wrapped into membrane protrusions: A novel structure-function module of the adult calyx of Held. *J. Neurosci.* 26 (1), 109–116. doi:10.1523/JNEUROSCI.3268-05.2006

Woods, J. J., Lovett, J., Lai, B., Harris, H. H., and Wilson, J. J. (2020). Redox stability controls the cellular uptake and activity of ruthenium-based inhibitors of the mitochondrial calcium uniporter (MCU). *Angew. Chem. Int. Ed.* 59 (16), 6482–6491. doi:10.1002/anie.202000247

Woods, J. J., and Wilson, J. J. (2020). Inhibitors of the mitochondrial calcium uniporter for the treatment of disease. *Curr. Opin. Chem. Biol.* 55, 9–18. doi:10.1016/j.cbpa.2019.11.006

Workgroup, A. s. A. C. H., and Khachaturian, Z. S. (2017). Calcium hypothesis of alzheimer's disease and brain aging: A framework for integrating new evidence into a comprehensive theory of pathogenesis. *Alzheimer's Dementia* 13 (2), 178–182. e117. doi:10.1016/j.jalz.2016.12.006

Wu, S. B., Ma, Y. S., Wu, Y. T., Chen, Y. C., and Wei, Y. H. (2010). Mitochondrial DNA mutation-elicited oxidative stress, oxidative damage, and altered gene expression in cultured cells of patients with MERRF syndrome. *Mol. Neurobiol.* 41 (2-3), 256–266. doi:10.1007/s12035-010-8123-7

Xu, J., Mashimo, T., and Südhof, T. C. (2007). Synaptotagmin-1, -2, and -9: Ca2+ sensors for fast release that specify distinct presynaptic properties in subsets of neurons. *Neuron* 54 (4), 567–581. doi:10.1016/j.neuron.2007.05.004

Yang, C. H., Lee, K. H., Ho, W. K., and Lee, S. H. (2021). Inter-spike mitochondrial Ca(2+) release enhances high frequency synaptic transmission. *J. Physiol.* 599 (5), 1567–1594. doi:10.1113/JP280351

Yoast, R. E., Emrich, S. M., Zhang, X., Xin, P., Arige, V., Pathak, T., et al. (2021). The Mitochondrial Ca(2+) uniporter is a central regulator of interorganellar Ca(2+) transfer and NFAT activation. *J. Biol. Chem.* 297 (4), 101174. doi:10.1016/j.jbc.2021.101174

Zamzami, N., and Kroemer, G. (2001). The mitochondrion in apoptosis: How pandora's box opens. *Nat. Rev. Mol. Cell Biol.* 2 (1), 67–71. doi:10.1038/35048073

Zhong, N., Beaumont, V., and Zucker, R. S. (2001). Roles for mitochondrial and reverse mode Na^+/Ca^{2+} exchange and the plasmalemma Ca^{2+} ATPase in post-tetanic potentiation at crayfish neuromuscular junctions. *J. Neurosci.* 21 (24), 9598–9607. doi:10.1523/JNEUROSCI.21-24-09598.2001

Zsurka, G., and Kunz, W. S. (2015). Mitochondrial dysfunction and seizures: The neuronal energy crisis. *Lancet Neurology* 14 (9), 956–966. doi:10.1016/S1474-4422(15)00148-9

Zucker, R. S., and Regehr, W. G. (2002). Short-term synaptic plasticity. *Annu. Rev. Physiol.* 64, 355–405. doi:10.1146/annurev.physiol.64.092501.114547