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REVIEW

Neuronal calcium signaling: function and dysfunction

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Abstract Calcium (Ca^{2+}) is an universal second messenger that regulates the most important activities of all eukaryotic cells. It is of critical importance to neurons as it participates in the transmission of the depolarizing signal and contributes to synaptic activity. Neurons have thus developed extensive and intricate Ca^{2+} signaling pathways to couple the Ca^{2+} signal to their biochemical machinery. Ca^{2+} influx into neurons occurs through plasma membrane receptors and voltage-dependent ion channels. The release of Ca^{2+} from the intracellular stores, such as the endoplasmic reticulum, by intracellular channels also contributes to the elevation of cytosolic Ca^{2+}. Inside the cell, Ca^{2+} is controlled by the buffering action of cytosolic Ca^{2+}-binding proteins and by its uptake and release by mitochondria. The uptake of Ca^{2+} in the mitochondrial matrix stimulates the citric acid cycle, thus enhancing ATP production and the removal of Ca^{2+} from the cytosol by the ATP-driven pumps in the endoplasmic reticulum and the plasma membrane. A Na^{+}/Ca^{2+} exchanger in the plasma membrane also participates in the control of neuronal Ca^{2+}. The impaired ability of neurons to maintain an adequate energy level may impact Ca^{2+} signaling: this occurs during aging and in neurodegenerative disease processes. The focus of this review is on neuronal Ca^{2+} signaling and its involvement in synaptic signaling processes, neuronal energy metabolism, and neurotransmission. The contribution of altered Ca^{2+} signaling in the most important neurological disorders will then be considered.

Keywords Calcium signaling · Calcium channels · Calcium pumps · Neurons · Neurodegenerative disorders · Migraine

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Introduction: general principle of Ca^{2+} signaling

Cell life would not be possible without the information conveyed by calcium (Ca^{2+}) to most of its essential processes. In essence, Ca^{2+} controls virtually all aspects of cell life—but does so only after its signal is suitably processed [1, 2]. The processing is performed by sensor proteins endowed with the ability to specifically complex Ca^{2+} and consists essentially in the conformational change of the complexing protein induced by the binding of Ca^{2+}. Since the regulation of cellular functions is by definition a reversible process, the binding of Ca^{2+} also occurs reversibly. Sensor proteins must bind Ca^{2+} within cells in the presence of much larger concentrations of other potentially competing cations. They do so thanks to the peculiar flexibility of Ca^{2+} as a ligand. The coordination chemistry of Ca^{2+} indeed allows it to be accommodated within binding sites of irregular geometry, such as those that can be expected within proteins. In contrast, Mg^{2+} coordination, given its stricter requirement for a perfectly octahedral geometry of the binding sites, is hard to satisfy within the structure of proteins [2]. Once Ca^{2+} was chosen as a carrier of cellular information, its background concentration within cells had to be maintained at a level low enough to permit it to be significantly changed without prohibitive energy costs. In the course of evolution, systems were thus developed which maintain the concentration of Ca^{2+} within cells

at adequately low levels. Families of soluble proteins are found in all eukaryotic cells whose task is to reversibly bind Ca^{2+}: these proteins simply buffer Ca^{2+} to maintain its background concentration within the appropriately low range without decoding its message. However, the overall task of controlling the concentration of Ca^{2+} within cells is performed by families of proteins intrinsic to membranes that transport Ca^{2+} across membrane boundaries and/or mediate its passage across them down the steep concentration gradient (about 10,000-fold) between the extracellular ambient and the cell interior, and between the lumen of intracellular Ca^{2+}-storing organelles and the cytosol. These proteinaceous systems are channels of various types, ATPases (colloquially called pumps), exchangers [mostly Na^+/Ca^{2+} exchangers (NCXs)], and a complex electrophoretic system that mediates the import of Ca^{2+} across the inner mitochondrial membrane into the matrix. They differ in transport mechanism, affinity for Ca^{2+}, and total transport capacity, and thus offer/provide the multiplicity of responses necessary for the efficient operation of the Ca^{2+} signaling system.

Neuronal Ca^{2+} signaling

Eukaryotic cells use Ca^{2+} as a signal carrier to regulate functions that are common to all of them: they include the control of metabolism by the process of enzyme phosphorylation and dephosphorylation, motility processes like those involving the cytoskeleton, the secretion of various molecules in the process of exocytosis, the transcription of numerous genes, the process of programmed cell death. Interestingly, Ca^{2+} signals have the peculiar property, which is distinctive among second messengers, of autoregulation: i.e., protein systems that control cellular Ca^{2+} homeostasis may be regulated by Ca^{2+} itself, both at the transcriptional and post-transcriptional level [3, 4]. Other important properties that are controlled by Ca^{2+} are cell specific, as for instance, the origin of life in the process of egg fertilization, and the contraction and relaxation of muscles. The signaling function of Ca^{2+} also has aspects that are peculiar and/or particularly important to neurons. Synaptic transmission, which is the form of secretion that leads to the release of neurotransmitters, the process of learning and the formation and consolidation of memory, also through the regulation of specific gene pools, the long-term potentiation (LTP) or depression of synaptic transmission, the direct coupling between the depolarization of the plasma membrane and the increase of intracellular Ca^{2+} are all specific neuronal processes that are under the control of Ca^{2+} signals. Neuronal life depends on the correct functioning of all these processes, and thus depends, to a degree that is perhaps greater than in most other eukaryotic cells, on the precise temporal and spatial regulation of the Ca^{2+} signals.

This review will concentrate on neuronal Ca^{2+} signaling, specifically mentioning the causative role of Ca^{2+} dysfunction in some of the most important neuronal pathologies.

The neuronal Ca^{2+} signaling toolkit

Ca^{2+} fluxes across the plasma membrane and between intracellular organelles integrate diverse cellular functions and support complex signaling processes that are of special importance to the activity of neuronal cells. Both the coordinated action of the systems that handle Ca^{2+} movements and the activity of a vast array of checkpoints that control them are essential to brain physiology.

Ion channels, exchangers, and pumps both in the plasma membrane and the membranes of mitochondria, endoplasmic reticulum (ER), Golgi apparatus, and nucleus (possibly in the acidic compartments as well) contribute to the Ca^{2+} toolkit that, together with the action of G protein-coupled receptors, Ca^{2+} binding proteins, and transcriptional networks, orchestrate neuronal Ca^{2+}-regulated processes. They are important for neuronal plasticity (see below) and underlie critical neuronal functions, such as learning and memory. They also play a critical role in neuronal survival.

Figure 1 shows a cartoon representing the neuronal Ca^{2+} signaling toolkit that will be briefly reviewed in the following paragraphs.

Plasma membrane Ca^{2+} channels

The plasma membrane Ca^{2+} channels are traditionally divided in three major groups according to their mechanism of opening: the voltage-gated Ca^{2+} channels (VOC), the receptor-operated Ca^{2+} channels (ROC), and the store-operated Ca^{2+} entry channels (SOC) which become activated by the emptying of the cellular Ca^{2+} stores. The first two channel types have been known about for a long time, but the molecular identity and the mechanism of action of the last channels were only resolved in 2006, when their pore-forming subunit ORAI was cloned [5]. The transient receptor potential (TRP) channels and the arachidonic acid-regulated Ca^{2+} (ARC) channels, even if not direct components of the SOC, interact with them. The SOC were initially observed in non-excitable cells, but numerous studies have now established their existence also in neuronal cells as well. The main Ca^{2+} channels types are schematically represented in Fig. 2.

The VOC transduce the electrical signals occurring at the cell surface membrane due to local rises of intracellular Ca^{2+}. In neurons, VOC play a role in the generation and propagation of the nerve impulse and in cell homeostasis. The VOC are formed of five distinct subunits (α1,

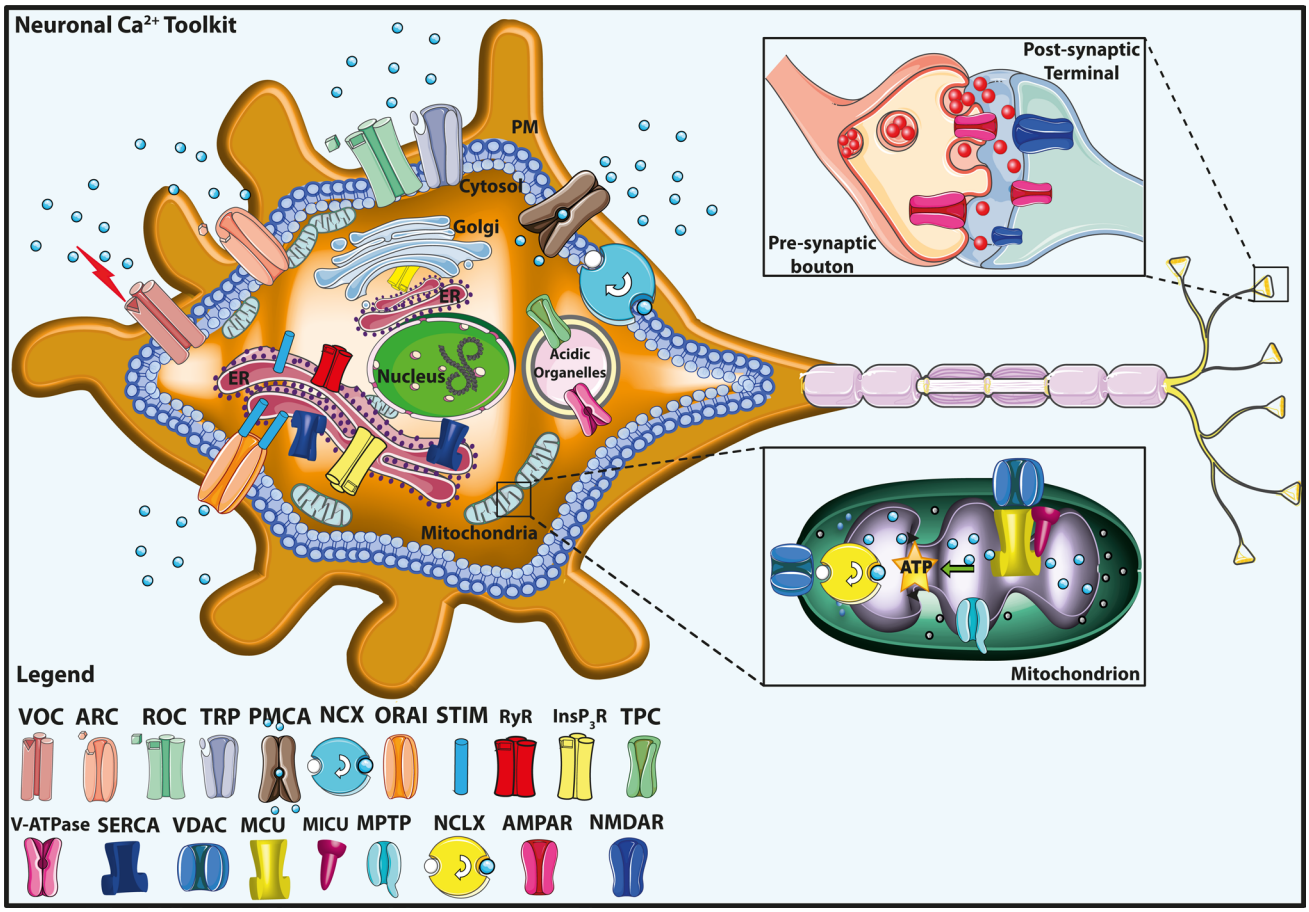

Fig. 1 Neuronal calcium (Ca^{2+}) signaling toolkit. The Ca^{2+} transport proteins, the receptors of the plasma membrane (*PM*), and the intracellular organelles, including mitochondria, endoplasmic reticulum (ER), Golgi apparatus, and acidic organelles, are indicated. The mitochondrial Ca^{2+} handling systems (proteins) are shown in greater detail in the *bottom right inset*. The *top right inset* shows a schematic view of the pre-synaptic bouton and the post-synaptic termination. The legend on the *bottom left* indicates the Ca^{2+} transporter proteins. *VOC* Voltage-gated Ca^{2+} channel, *ROC* receptor-operated Ca^{2+} channel, *ORAI* the pore-forming subunit of store-operated Ca^{2+} entry channel (*SOC*), *STIM* the Ca^{2+} sensor, *TPC* two-pore channel, *ARC* arachidonic acid-regulated Ca^{2+} channel, *TRP* transient receptor potential channel, *PMCA* plasma membrane Ca^{2+} ATPase, *V-ATPase* vacuolar H^+ ATPase, *InsP3R* inositol 1,4,5 tris–phosphate receptors, *RyR* ryanodine receptor, *NCX* plasma membrane Na^+/Ca^{2+} exchanger, *SERCA* sarco-/endoplasmic reticulum Ca^{2+} ATPase, *MCU* mitochondrial Ca^{2+} uniporter, *MICU* mitochondrial Ca^{2+} uniporter regulator, *NCLX* mitochondrial Na^+/Ca^{2+} exchanger, *VDAC* voltage-dependent anion channels, *MPTP* mitochondrial permeability transition pore, *AMPAR* 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, *NMDAR* *N*-methyl-D-aspartate receptor

α2, β, γ, δ) encoded by different genes. Depending on the type of α1 pore-forming subunit, they are divided into three subfamilies, namely, Cav1, Cav2, and Cav3, and into six further classes, termed L, N, P, Q, R, and T, based on the physiological and pharmacological properties of the type of current they carry (Fig. 2a). Each of these channel types is indeed inhibited by specific toxins that have been used to characterize them. The α1 subunit is a 190- to 250-kDa transmembrane protein organized in four repeat domains (I–IV), each containing six transmembrane segments (S1–S6). The S4 segments comprise some positively charged residues and serve as the voltage sensor in the pore-containing subunit. The associated α2, β, γ, δ subunits have auxiliary functions, including the control of channel expression and the modulation of current kinetics. The β subunit is a hydrophilic protein of 50–65 kDa that binds to the intracellular loop connecting domains I and II of the α1 subunit. The α2 and δ subunits are encoded as pre-polypeptides by a single gene and undergo post-translational cleavage to yield two disulfide-bonded and lipid-anchored distinct subunits. The γ subunit has four transmembrane segments: it is an essential component of skeletal muscle Ca^{2+} channels, but it may not be included in the assembly of Ca^{2+} channels in the brain. The β subunits greatly enhance cell-surface expression of the α1 subunits and shift their kinetics and voltage dependence [6, 7]. The α2δ subunits enhance cell-surface expression of α1 subunits [8] and are important for efficiently coupling the

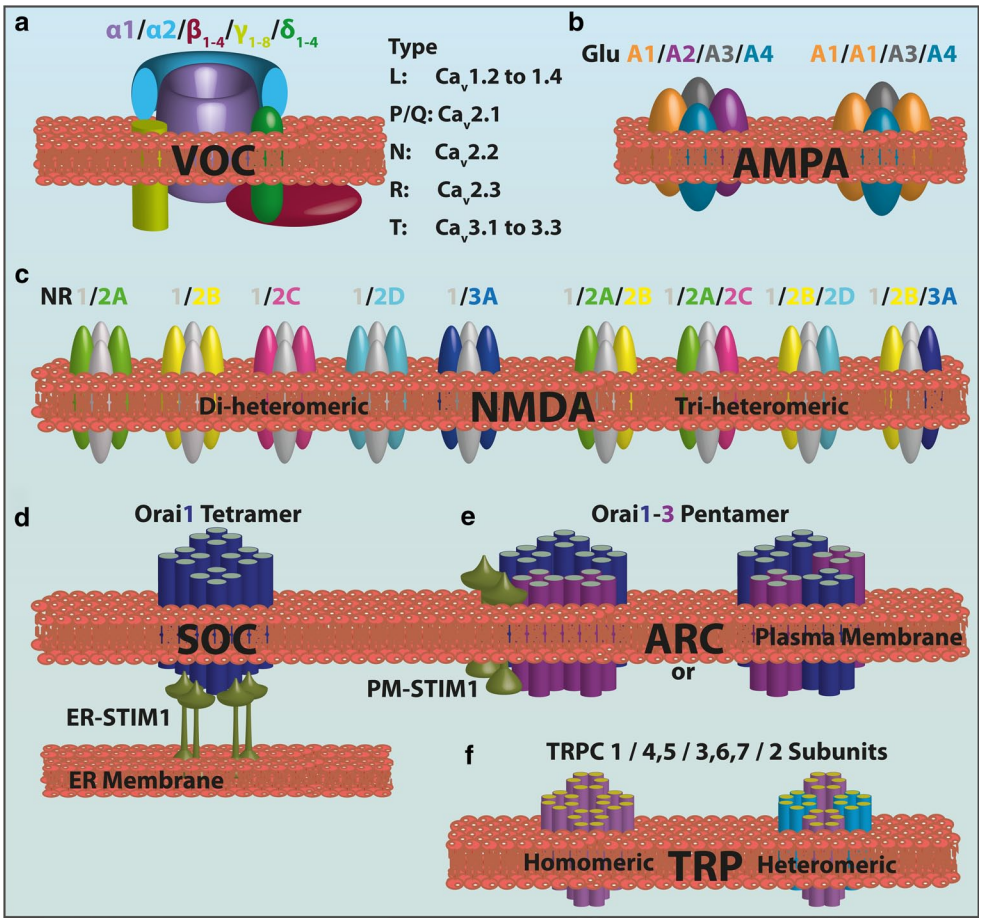

Fig. 2 Assembly and subtypes of the subunits of the main neuronal Ca^{2+} channels. **a** Graphic representation of the VOC complex consisting of the main pore forming α1-subunit plus ancillary β-, γ-, and α2-δ-subunits. Different neuronal α1-subunits correspond to the different types of Ca^{2+} channels identified in native neurons. **b** The AMPAR is a ionotropic transmembrane receptor for glutamate assembled from a pool of four subunits (GluA1–A4) which share a high degree of sequence identity. The number of subunits in the functional channels is unclear. The permeability to Ca^{2+} and other cations, such as sodium (Na^+) and potassium (K^+), is governed by the GluA2 subunit. An AMPAR lacking a GluA2 subunit will be permeable to Na^+, K^+, and Ca^{2+}, while the presence of the GluA2 subunit will render the channel impermeable to Ca^{2+}. **c** The NMDAR is a ionotropic glutamate receptor that allows the flow of Na^+, and of smaller amounts of Ca^{2+}, into the cell. Seven NMDAR subunits have been identified: GluNR1, GluNR2A–D, GluNR3A, and GluN3B (not shown). The various populations of di-heteromeric and tri-heteromeric NMDARs that are thought to exist in the central nervous system are shown. **d**, **e** The basic structure of ORAI1 and ORAI3 proteins. Each has four transmembrane-spanning domains: the different stoichiometry between SOC and ARCs is shown. The SOC pore is formed by a tetramer of ORAI1 subunits, while a pentameric assembly of three ORAI1 subunits and two ORAI3 subunits arranged in two possible conformations forms the ARC channel. Both SOC and ARC are regulated by STIM1; however, SOC activation is regulated by STIM1 in the endoplasmic reticulum (*ER*), while the pool of STIM1 residing in the plasma membrane regulates the ARC. **f** Transient receptor potential (*TRP*) channels (*TRPC*) are relatively non-selectively permeable to cations, including Na^+, Ca^{2+}, and Mg^{2+}. They belong to a large superfamily of cation channels having six transmembrane-spanning segments, which presumably assemble in a ring-like structure with fourfold symmetry. The TRP protein assembles into homo-tetramers or hetero-tetramers. Here, only the "common" family of these TRPC, with its relative subunits, is shown for simplicity

entry of Ca^{2+} to exocytosis at active zones in nerve terminals [9].

The Cav1 subfamily mediates the L-type currents and initiates excitation–contraction coupling in skeletal and cardiac muscle. In neuronal cells, its activity generates Ca^{2+} transients in cell bodies and dendrites that in turn control processes like secretion and gene expression. Cav2 channels generate N-, P/Q-, and R-type currents and are mainly responsible for the initiation of synaptic transmission, neurotransmitter release, and the generation of dendritic Ca^{2+} transients. The Cav3 subfamily is responsible for the T-type current and is important for the pacemaking and repetitive firing of action potentials in cardiac myocytes and thalamic neurons [10].

The ROC are activated by the binding of specific ligands, such as neurotransmitters, to their extracellular

domain. L-Glutamate is the main excitatory neurotransmitter in the mammalian brain, and it activates two classes of receptors, the ionotropic receptors (iGluRs), and the metabotropic receptors (mGluRs). Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-sensitive receptors (AMPARs) and *N*-methyl-D-aspartate sensitive receptors (NMDARs) are the two principal types of ionotropic glutamate receptors. AMPARs mediate fast excitatory synaptic transmission in the mammalian central nervous system (CNS) and are primarily permeable to Na^+ and K^+, but may also become permeable to Ca^{2+}. NMDARs are permeable to both Na^+, which contributes to postsynaptic depolarization, and Ca^{2+}, which generates Ca^{2+} transients and determines intracellular physiological responses. NMDARs respond to glutamate more slowly than AMPARs. Their activation requires not only glutamate (ligand-gating), but also membrane depolarization (voltage dependence), which removes internal Mg^{2+} that normally blocks the channel. Interestingly, the coincidence detection required for the opening of NMDAR channels is particularly important in learning and memory, since the removal of the Mg^{2+} block of NMDARs is crucial to CREB (cAMP response element-binding protein)-dependent gene expression upon long-term memory induction. [11]. Both AMPARs and NMDARs are tetrameric structures. AMPARs are homo- or heterotetramers assembled from GluA1 to A4 subunits (Fig. 2b). The four subunits can combine in various stoichiometries to form receptor subtypes with distinct channel properties [12]. Almost all (99 %) GluA2s in the adult brain contain a positively charged arginine (R) in the M2 channel-forming segment at position 607 (Q/R site), while the other AMPAR subunits have a glutamine (Q) at this position [13]. This is dependent on a site-selective deamination of adenosine to inosine on the pre-mRNA by RNA-editing enzymes [14]. Edited R-containing subunits remain largely unassembled and are retained in the ER. The presence of a positively charged amino acid (R) in the channel-forming segment effectively blocks Ca^{2+} entry and results in AMPARs with low conductance. Unedited Q-containing subunits readily tetramerize and traffic to synapses where they generate high conductance channels which are Ca^{2+} permeable [15]. NMDARs are also assembled as heterotetramers of different subunits in specific brain areas and during development. Seven subunits encoded by different genes have been identified to date (Fig. 2c), and these are grouped into three subfamilies according to sequence homology: the GluN1 subunit, four distinct GluN2 subunits (GluN2A, GluN2B, GluN2C, GluN2D), and two GluN3 subunits (GluN3A and GluN3B). The size of each subunit varies from 900 to 1,480 amino acids due to differences in the length of the intracellular carboxyl-terminal domain, a region that is involved in receptor trafficking and in coupling receptors to signaling cascades. NMDARs typically associate GluN1 subunits with GluN2 subunits or a mixture of GluN2 and GluN3 subunits. The composition of the subunits of NMDARs is plastic, resulting in a large number of receptor subtypes with distinct biophysical, pharmacological, and signaling properties [16]. NMDARs are typically found at extra- and postsynaptic sites. In particular, NMDA-dependent Ca^{2+} influxes are known to regulate CREB-dependent gene transcription [17–21], which is important for the establishment of long-term synaptic plasticity, learning, and memory [22]. NMDARs have also been found in the presynaptic compartment, but their exact roles there are still being debated, although they may facilitate glutamatergic release by increasing both spontaneous and evoked excitatory postsynaptic currents. Their activation is also necessary for the induction of long-term depression (LTD) at both cerebellar parallel fiber–Purkinje cell synapses and neocortical synapses [23].

The mGluRs are coupled to G proteins and are organized with the canonical seven transmembrane domains. They are encoded by eight genes (mGluR1–8) and exist as homodimers that generate Ca^{2+} signals through the activation of distinct downstream signaling cascades that activate phospholipase C and activate, or inhibit, adenylyl cyclase. They are expressed in neuronal and glial cells in the brain, spinal cord, and peripheral neurons. mGluR1 is the most abundantly expressed metabotropic receptor in the mammalian CNS, with its highest expression in the Purkinje cells of the cerebellum. It produces two type of neuronal depolarization, a rapid transient depolarization related to the release of Ca^{2+} from intracellular stores and a prolonged and larger depolarization resulting from the activation of TRP channels (see below).

The purinergic signaling system has many physiological and pathological roles [24–27]. In the CNS, extracellular ATP released by neurons and glial cells (astrocytes) and the diversity of purinergic receptors allow the regulation of several neuronal relevant processes. In addition, different neuronal insults may result in the massive exit of ATP from damaged neural cells, and its consequent increase in the extracellular space may assume cytotoxic effects, ultimately leading to cell death [26]. The ionotropic receptors responding to extracellular ATP to induce membrane depolarization and Ca^{2+} influx [the so-called ionotropic P2X receptors (P2XRs)] deserve a special mention. Their activation has multiple modulatory effects on synaptic plasticity, either inhibiting or facilitating the long-term changes of synaptic strength depending on the physiological context [28]. ATP released during synaptic transmission activates astrocytic receptors, which in turn initiate Ca^{2+} signals and propagate Ca^{2+} waves in the astroglial networks through the activation of metabotropic P2Y receptors (P2YRs) and the diffusion of inositol 1,4,5 tris–phosphate through the gap-junctions [29]. The presence of both P2XRs

and P2YRs at the same astrocyte permits a coordinated action of Ca^{2+} signaling: ionotropic P2XRs are responsible for rapid astrocytic signaling, whereas G protein-coupled P2YRs mediate long-term effects, including trophic responses, through a variety of intracellular pathways, among which gene activation [30, 31].

Ionotropic P2XRs are represented by seven different subtypes/subunits (P2X1–7) that contribute to the generation of heterotrimers or, with the exception of P2X6, homotrimers [32]. These subunits are widely distributed pre-and postsynaptically in different cell types in the brain and spinal cord [33]. They are the main postsynaptic Ca^{2+} entry channels at resting potential when NMDARs are blocked by Mg^{2+} [34]. P2XRs display very high Ca^{2+} permeability, the level of which depends upon the composition of the subunit. Importantly, the permeability of P2X2, P2X4, and P2X7Rs to larger cations increases following repetitive or longer lasting exposure to ATP and (particularly in the case of P2X7Rs) may result in the opening of large pores in the cell membrane and ultimately cell death by either apoptotic or necrotic pathways [35].

The SOC are activated by the release of Ca^{2+} from the ER. As mentioned, they were initially described in non-excitable cells, but they have now been documented in other cell types, such as neurons and skeletal muscle cells. Store-operated Ca^{2+} entry (SOCE) was originally proposed as a mechanism for assuring the refilling of intracellular stores following Ca^{2+} release from these [36]. More recently, the notion has emerged that the influx through this pathway may provide direct Ca^{2+} signals to targets localized to spatially restricted areas close to the sites of Ca^{2+} entry, thus initiating specific signaling pathways; for example, hereditary defects in SOCE lead to severe immunodeficiency [37] due to a perturbation of Ca^{2+} microdomains and to their effect on the generation of Ca^{2+} oscillations and nuclear factor of activated T-cell-regulated gene transcription [38]. The molecules constituting the SOCE pathway were identified only recently as Ca^{2+}-binding transmembrane proteins of the EF-hand family; they are present in two different isoforms. The transmembrane stromal interaction molecules STIM (STIM 1 and 2 proteins) serve as sensors of Ca^{2+} within the ER. STIM communicates with the plasma membrane SOC that is composed of ORAI subunits (Fig. 2d). The ORAI protein family includes three isoforms [39], among which ORAI1 is the pore-forming subunit of the channels [5]. Evidence for the presence and function of ORAI/STIM in neurons has come from recent work in which their expression was abrogated by siRNA in *Drosophila melanogaster*. The abrogation impaired rhythmic firing of the flight motor neurons, resulting in defective flight [40]. By contrast with other tissues, STIM2 is the predominant isoform in mice brain. Cortical neurons isolated from STIM1- and STIM2-deficient mice showed no defects in STIM1-deficient mice, whereas the increase in intracellular Ca^{2+} following the induction of in vitro ischemia was slower, and neuronal recovery faster, in STIM2-deficient mice, suggesting a neuroprotective effect of STIM2 deficiency, which also correlated with similar effects in vivo [41].

The ARC channels are small conductance, highly Ca^{2+}-selective ion channels [42]. They are widely distributed in different cell types (including neurons) where they provide an alternative, store-independent pathway for agonist-activated Ca^{2+} entry. Their activation is specifically dependent on low concentrations of arachidonic acid generated on the intracellular side after physiologically relevant activation of the appropriate receptors on the plasma membrane. Although biophysically similar to the SOC channels, ARC channels function as the predominant route of Ca^{2+} entry during the oscillatory signals generated at low agonist concentrations. Like the SOC, their activation is dependent on STIM1 protein. However, in their case the pool of STIM1 constitutively localized in the plasma membrane, rather than the STIM of the ER membranes, is responsible for the activation. These channels are also formed by ORAI proteins but, at variance with the SOC which are homotetramers of ORAI1 subunits, the pore is formed by a heteropentameric assembly of three ORAI1 subunits and two ORAI3 subunits (Fig. 2e).

The TRP channels are a class of channels that can generate changes in intracellular Ca^{2+} concentration either directly by acting as a Ca^{2+} entry pathway (even if their selectivity for Ca^{2+} changes in the different channels subtypes) or indirectly by causing cell depolarization that triggers the activation of the voltage-dependent ion channels. They are expressed in a large number of tissues and cell types (excitable and non-excitable) and may be activated by phosphatidylinositol phosphates, such as phosphatidylinositol 4,5 bisphosphate (PIP2) [43], and modulated by Ca^{2+}. They are assembled as a tetramer, with each subunit containing six transmembrane domains (Fig. 2f). In mammals, 28 TRP channels have been described to date; these have been classified according to their homology into six different subtypes: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPML (mucolipin), and TRPP (polycistin). The TRPCs have a special relationship with the SOC since ORAI1 has been proposed to interact with TRPCs and to act as regulatory subunit that confers STIM1-mediated store depletion sensitivity to them [44–46]. However, in comparison to SOC, TRPC channels show high Ca^{2+} selectivity, very small single channel conductance and different Ca^{2+} modulation.

Finally, it must be mentioned that most of the TRP channels are also localized in the ER and Golgi membranes and that TRPMLs, also defined as TPC (two-pore channels), have been proposed to mediate the NAADP-activated

intracellular Ca^{2+} release from endosomes and lysosomes [47]. This proposal, however, is controversial [48].

Na^+/Ca^{2+} exchanger and plasma membrane Ca^{2+} ATPase

The plasma membrane Ca^{2+} ATPase (PMCA) and the plasma membrane Na^+/Ca^{2+} exchanger (NCX) are the two systems responsible for Ca^{2+} extrusion to the extracellular environment. The NCX has a low Ca^{2+} affinity but a high capacity for Ca^{2+} transport, whereas the PMCA has the opposite properties. Thus, traditionally, a housekeeping role in maintaining cytosolic Ca^{2+} has been attributed to the PMCA pump, and a dynamic role of counteracting large cytosolic Ca^{2+} variations (especially in excitable cells) to the NCX. However, numerous studies have now provided evidence that the two systems are co-expressed in most cells, including neurons and astrocytes, but also that they may play different signaling role [49, 50].

The Ca^{2+} ATPase of the sarco-/endoplasmic reticulum (SERCA pump) and the electrophoretic uniporter in the mitochondria (MCU) also play important roles in removing excess cytosolic Ca^{2+}, thereby restoring its basal levels.

NCX accomplishes Ca^{2+} extrusion by using the electrochemical gradient of Na^+: during each cycle three Na^+ ions enter the cell and one Ca^{2+} ion is extruded against its gradient. NCX can also work in the reverse mode: the direction of the movement of the transported ions depends entirely upon the electrochemical gradients of Na^+ and Ca^{2+} and on the number of ions that bind to the molecule and are transported. Under the resting cellular ionic condition and membrane potential, the NCX extrudes Ca^{2+} from the cytoplasm. However, in the heart, when the plasma membrane becomes depolarized during systole and the Na^+ levels rise as a consequence of the opening of the plasma membrane voltage-operated Na^+ channels, the exchanger reverses its operation and mediates Ca^{2+} entry. The influx of Ca^{2+} through the NCX may play an important regulatory role in the excitation–contraction process as it now influences the gating of voltage-operated Ca^+ channels and alters the sarcoplasmic reticulum Ca^{2+} load. This mechanism has also been proposed to occur in astrocytes and neuronal cells where NCX could regulate the state of Ca^{2+} refilling of the intracellular stores by acting at the plasma membrane–ER junctions [51]. Three different genes encoding NCX isoforms, NCX1, -2, and -3, have been identified. NCX1 was originally characterized and cloned from the heart, but its expression is widespread, with high levels in the brain and kidney. NCX2 and NCX3 are selectively expressed in different neuronal populations of the brain and in skeletal muscle. All three basic NCX isoforms are essentially co-expressed in brain neurons, thus making it difficult to attribute specific functions to each of them. However, the NCX2 isoform is particularly abundant and widespread, thus suggesting that it plays a key role in neuronal Ca^{2+} homeostasis. NCX3 also appears to be important to neurons: its specific cleavage during brain ischemia and in primary neurons undergoing excitotoxicity has shown that it plays a critical role in ischemic insults [52, 53].

PMCA pumps belong to the family of P-type ATPases, which are characterized by the temporary conservation of ATP energy in the form of a phosphorylated enzyme intermediate (hence P-type) formed between the γ-phosphate of hydrolyzed ATP and an invariant D-residue in a highly conserved sequence of the pump molecules. These pumps are ubiquitously expressed; however, the four basic isoforms (PMCA1, -2, -3, and -4) are specifically distributed among the different tissues, with isoforms 2 and 3 being particularly abundant in neurons.

The PMCA pump is a classical target of calmodulin (CaM) regulation. CaM interacts with a C-terminal domain of the pump [54], with a dissociation constant (K_d) in the nanomolar range [55]. In the absence of CaM, the C-terminal tail of the pump folds over, binding to two sites in the main body of the enzyme and thus keeping it auto-inhibited. CaM binding to the C-terminal domain of the pump removes the C-terminal tail from the binding sites, thereby relieving the auto-inhibition [56, 57]. An important aspect of the regulation of the PMCA pump activity is the stimulation by acidic phospholipids, which decrease the Ca^{2+} affinity of the pump to values even lower than those achieved with optimal CaM (about 0.2 μM [58]), thus presumably contributing to pump regulation, possibly in a CaM-independent way [59, 60].

PMCA pumps have been traditionally considered to be housekeeping enzymes. However, the possibility that they may also have important signaling roles in specific cell types is now emerging. The first and well-characterized evidence comes from the action of isoform 4 in the heart. Studies on the phenotypes of transgenic mice in which PMCA4 was selectively overexpressed or totally ablated in the heart revealed that PMCA4 contributes to the modulation of heart contractility. Specifically, PMCA4 acts to tether membrane-associated neuronal nitric-oxide synthase (nNOS) to a highly compartmentalized PMCA4-associated microdomain formed by the cardiac cell membrane. Thus, in addition to the obvious role in extracellular Ca^{2+} transport, the PMCA4 pump may control heart contractility by regulating the activity of nNOS through the control of Ca^{2+} concentration in a restricted microdomain where it co-localizes with nNOS. PMCA4 overexpression reduces Ca^{2+} concentration in the proximity of the nNOS enzyme and thus reduces the contractility response to β-adrenergic stimulation [61]. Its ablation, perhaps not surprisingly, does not affect the beat-to-beat Ca^{2+} transport across the plasma membrane which is controlled mainly by NCX activity, but instead causes a delocalization of the nNOS protein to the

cytosol, thus causing a significant decrease in the microdomain cGMP, which leads to a significant elevation in local cAMP levels. This increases L-type Ca^{2+} channel activity and ryanodine receptor phosphorylation (see below) and hence enhances contractility [62].

The identification of specific mutations in neuronal PMCA isoforms 2 and 3 and their association with neurodegenerative conditions indicates that they may also have relevance in the regulation of Ca^{2+} signaling in the brain. The PMCA2 isoform is abundant in the apical (stereociliar) membrane of the inner ear hair cells, and the PMCA3 pump is particularly abundant in the choroid plexus and some other brain regions. Genetic hearing loss associated with PMCA2 mutations has been reported both in mice and humans [63–68], and a point mutation in the PMCA3 pump gene has very recently been associated with X-linked congenital cerebellar ataxia in humans [69]. The study of the activity of recombinant PMCA2 and three mutants at the cellular level has revealed that in both cases the Ca^{2+} handling ability of the cells containing the mutated pumps was impaired and that the time necessary to restore basal Ca^{2+} levels after cell stimulation was compromised. The defect is not dramatic in terms of Ca^{2+} handling, thus leaving the possibility open that the disease phenotype may be linked to signaling defects rather than to general Ca^{2+} dyshomeostasis.

Endoplasmic reticulum and mitochondria

The release of Ca^{2+} from the ER occurs in neurons via two types of Ca^{2+} channels/receptors: ryanodine receptors (RyRs) and inositol-1,4,5-tris-phosphate receptors (InsP3Rs). InsP3Rs are ubiquitously expressed in many cell types, whereas RyRs are more characteristic of neurons and muscle cells. Ca^{2+} release through InsP3Rs requires the binding of the second messenger InsP3 in response to the activation of various G-protein-coupled receptors on the cell membrane. Increased cytoplasmic Ca^{2+} concentration is a major trigger for Ca^{2+} release via RyRs, the phenomenon known as Ca^{2+}-induced Ca^{2+} release (CICR). RyRs are also regulated by other intraneuronal messengers, such as cyclic adenosine diphosphate ribose (cADP-ribose). They are only relatively selective for Ca^{2+}, which is at variance with the voltage-gated and store-operated plasma membrane Ca^{2+} channels that are more selective. However, considering that Ca^{2+} is probably the only cation with an appreciable electrochemical gradient across the ER/SR membrane, the lack of selectivity is not detrimental to the cell.

The InsP3Rs are encoded by three different genes that have distinct patterns of tissue expression (however, some overlapping occurs, especially during differentiation). InsP3R1 is the predominant isoform and is widely expressed in tissues, particularly the brain and smooth muscle. The highest expression level of InsP3R1 among neurons occurs in the Purkinje cells of the cerebellum, where it localizes in the cell body, axons, and dendrites, especially in dendritic spines. InsP3R2 expression is lower than that of InsP3R1: its specific localization in the brain remains elusive, but in cerebellar granule cells it is expressed to the same extent as InsP3R1. InsP3R3 is highly expressed in the brain and gastrointestinal tract. The InsP3 channels consist of homo- or heterotetramers of a large (2,700 residues) protein spanning the membrane with a hydrophobic region containing six helices. Several molecules interact with the InsP3R and modulate its activity. Ca^{2+} itself has both stimulatory and inhibitory effects [70] depending on its concentration. Cytosolic Ca^{2+} is a co-agonist of the InsP3Rs, strongly increasing the activity of the latter at concentrations of up to about 300 nM. By contrast, at higher concentrations Ca^{2+} inhibits the receptor. Luminal Ca^{2+} also sensitizes the InsP3Rs, possibly by tuning its sensitivity to cytosolic InsP3. A Ca^{2+}-mediated inhibition of the receptor is assumed to contribute to the termination of local cytosolic Ca^{2+} signals. However, the precise mechanism of the inhibition is not clear [71].

The RyRs are also encoded by three distinct genes with different tissue expression patterns: RyR1 is expressed in skeletal muscles and in the brain with an almost exclusive localization to cerebellar Purkinje cells; RyR2 is the predominant type in the brain (particularly in the cerebellum), and it is also expressed in the heart and in the cerebral cortex. RyR3 is expressed in various brain regions, albeit with low levels of expression, being most predominant in the hippocampus, striatum, and diencephalon. The RyR is also formed by homo-tetramers that associate to form the largest channel known to date (>2 MDa). The C-terminal portion of the protein forms the pore, and the large cytoplasmic region contains the sites where most RyR modulators interact. Excitation–contraction-mediated coupling with the voltage-dependent Ca^{2+} channel dihydropyridine receptor (DHPR; so defined from the inhibitor dihydropyridine) located in the T-tubules of the plasma membrane of muscle cells represents the major gating mechanism. The molecular gating mechanism differs between skeletal and cardiac muscle [72]. In skeletal muscles, a physical interaction (electromechanical coupling) between the Cav1.1 DHPR and RyR1 is required; in cardiac muscle, Ca^{2+} release by the RyR2 is initiated by Ca^{2+} influx via Cav1.2 (i.e., the CICR). cADPR, generated by ADP-ribosyl cyclases, in particular by the ectoenzyme [73], can also gate RyR2 and RyR3. Similar to the InsP3R, the channel activity of the RyR is also modulated by a number of molecules, such as PKA, FK506 binding proteins (FKBP12 and 12.6), CaM, Ca^{2+}/CaM-dependent protein kinase II, calsequestrin, triadin, junctin, Mg^{2+}, ATP, and Ca^{2+} itself.

Ca^{2+} reuptake in the ER/SR is mediated by the P-type Ca^{2+} ATPase SERCA pump which replenishes the ER/SR stores, and thus, together with the PMCA and the NCX, re-establishes resting cytosolic Ca^{2+} values after the Ca^{2+} transient induced by cell stimulation. The SERCA pump is encoded by three independent genes which generate primary transcripts that undergo processes of alternative splicing to generate additional isoforms. Since the pump is ubiquitously distributed in tissues and has no distinctive characteristics in neurons, it falls outside the scope of this review and will not be discussed here. Details on its structure and function can be found in a number of comprehensive reviews (e.g. [74]).

Mitochondria are the other major player in the regulation of Ca^{2+} signals. They participate both in Ca^{2+} buffering and in the formation of special communications with the ER, that permit them to take advantage of localized Ca^{2+} release by vicinal ER channels to accumulate Ca^{2+} into the matrix. Ca^{2+} regulates the ability of mitochondria to provide ATP to energy-demanding processes, including pumps that mediate its removal from the cytosol. Increases of Ca^{2+} concentration in the mitochondrial matrix boost the activity of three enzymes of the tricarboxylic acid cycle: pyruvate dehydrogenase (indirectly via its phosphatase activation), isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase (by binding directly to the enzymes). They are thus essential to the delivery of reducing equivalents to the respiratory chain and thus to the production of ATP. However, matrix Ca^{2+} also directly regulates the F1F0-ATPase [75–77]. In addition to regulating mitochondrial function, mitochondrial Ca^{2+} handling has an important signaling role. By controlling the Ca^{2+} microenvironment in the proximity of the ER or plasma membrane Ca^{2+} channels, mitochondria can positively or negatively control the feedback effect of Ca^{2+} on the channels [78, 79] with functional consequences on several biological processes.

MCU mediates the entry of Ca^{2+} into the mitochondrial matrix [80, 81] which is driven by the negative membrane potential generated by the activity of the respiratory chain components of the inner membrane. The MCU is formed by a 40-kDa pore-forming protein which contains two transmembrane domains and possibly assembles as oligomers. Recently, a second MCU isoform, MCUb, has been identified, which has been shown to act as an inhibitory subunit in the formation of MCU hetero-oligomers [82]. MCU associates with several regulators, among which MICU1 is the best characterized. This is an EF-hand protein that acts as MCU gatekeeper by regulating the threshold of MCU channel opening and allowing its cooperative activation [83, 84], Two paralogs of MICU1, namely, MICU2 and MICU3, and EMRE, an essential single transmembrane protein, have been recently identified; it has been suggested that these form a complex. MICU1 and MICU2 stabilize each other, and EMRE is required for the interaction of MCU with both of them [85, 86].

A mitochondrial NCX (NCLX) [87, 88] mediates the efflux of Ca^{2+} from mitochondria, thus preventing it from reaching thermodynamic equilibrium. Ca^{2+} efflux can be also mediated by the transient opening of the mitochondrial permeability transition pore (mPTP) [89, 90]. Its recent molecular identification has revealed that it is part of the ATP synthase complex, thus further strengthening the link between mitochondrial Ca^{2+} and energy balance [91, 92].

Ca^{2+} binding proteins: Ca^{2+} buffers and Ca^{2+} sensors

Several Ca^{2+} binding proteins are expressed in the nervous system. Their localization and their affinity for Ca^{2+} differ according to their specific role. Functionally, they can be divided into two groups: the Ca^{2+} buffers, which together with the Ca^{2+} clearance mechanisms control the duration and the spread of Ca^{2+} signals, and the Ca^{2+} sensors, which also translate the changes in Ca^{2+} concentration into specific signals. Both Ca^{2+} buffers and sensor proteins belong to various Ca^{2+}-binding protein subtypes. The EF-hand proteins and C2 domain proteins are the most important. CaM is the best known among them: it has four EF-hand binding motifs, and the molecular mechanism by which it decodes the Ca^{2+} signal has been clarified. Fluctuations in intracellular Ca^{2+} concentration not only change its subcellular distribution but also induce different conformational states that result in target-specific activation [93]. Other major cytosolic EF-hand Ca^{2+} binding proteins are calbindin D-28k (CB-D28k), calretinin (CR), and parvalbumin (PV); all of these undergo conformational changes upon Ca^{2+} binding to conserved domains that permit the interaction with target proteins in a Ca^{2+}-regulated manner. Proteins such as CaM thus do not act as pure Ca^{2+} chelators, but they do exert an important modulatory role by regulating the delivery of the Ca^{2+} signal to different substrates. However, a clear distinction between Ca^{2+} buffer and Ca^{2+} sensor function is not simple, since Ca^{2+} sensor proteins obviously also buffer Ca^{2+}. CaM is perhaps the best example of this dual function, as it is present in the brain at high concentrations (up to 100 μM). It has numerous specific roles in neurons, among them the control of glutamate receptors, of ion channels, and of the NO synthase. One important direct function of CaM is possibly the control of VOCs through binding to channel subunits [94]. The major CaM target proteins are Ca^{2+}/CaM-dependent kinases (CaMKs) and the phosphatase calcineurin; these contribute to a number of neuronal regulatory pathways, from synaptic plasticity to gene transcription.

The spatiotemporal aspects of Ca^{2+} signals depend on the kinetics of various Ca^{2+} buffers—in particular, in excitable cells [95–97]. The expression of Ca^{2+}-buffering

proteins varies according to cell types, with the highest Ca^{2+}-buffering capacity being that of cerebellar Purkinje cells which express high levels of CB-D28k and PV [98]. The majority of Ca^{2+} buffers have dissociation constants in the low micromolar range. Thus, in a resting cell they are mostly in the Ca^{2+}-free form. CR is defined as a fast Ca^{2+} buffer; in contrast, PV and CB-D28k are instead considered to be slow Ca^{2+} buffers. Several studies in knockout mice for different Ca^{2+} buffers have revealed that the deletion of one Ca^{2+} binding protein is not compensated by the overexpression of others, underlining the importance of each protein in the cell type in which it is particularly abundant.

Purkinje, stellate, and basket cells in the cerebellum can be defined as "PV-ergic" neurons: [99] the ablation of PV in these cells is compensated by an increase of mitochondrial mass and by a remodeling of mitochondria distribution that results in the accumulation of mitochondria beneath the plasma membrane. These mitochondria may specifically prevent or restrict the diffusion of Ca^{2+} signals from the plasma membrane to the center of the cell, thus acting as a "firewall." CR- or CB-D28k-immunoreactive neurons are instead those of the cortex, hippocampus, and specific peripheral nervous systems. Cerebellar granule cells also express CR, and, as mentioned, Purkinje cells present high amounts of CB-D28k. The loss of CR affects the electron-responsiveness of cerebellar granule cells, and the decrease in Ca^{2+} buffering results in increased excitability: cerebellar granule cells have faster action potentials and generate repetitive spikes with enhanced frequency [100]. The loss of CB-D28k increases the Ca^{2+}-dependent inactivation of high voltage-gated channels, thus reducing Ca^{2+} entry during the prolonged action potential trains that occur, for example, during epileptic seizures. The absence of CB-D28k induces alterations in the morphology of the spines in cerebellar cells, possibly accounting for the motor coordination impairment observed in KO mice [101].

Neuronal Ca^{2+} sensors (NCSs) are a family of EF-hand proteins which have an important role in synaptic function and in neuronal diseases. The NCSs show little sequence homology with CaM (<20 %). In mammals they are encoded by 14 different genes. Their restricted expression in particular classes of neurons and their high Ca^{2+} sensitivity contribute to their non-redundancy with respect to CaM. Additionally, posttranslational myristoylation or palmitoylation permits their alternative Ca^{2+}-dependent membrane association, thus allowing changes in their subcellular locations and in binding specific targets for regulation. Unlike CaM, not all EF-hands of NCS proteins are functional, and unlike the dumbbell-like structure of CaM that undergoes conformational changes to bind the target, NCSs are compact and globular proteins that do not significantly change conformation upon Ca^{2+} and substrate binding.

Only two of the NCSs, namely, NCS-1 and calsenilin/DREAM/KchIP-3, will be briefly discussed in this review. NCS-1 is the most widely expressed and characterized NCS. It has a higher Ca^{2+} affinity than CaM, and therefore it may preferentially interact with some partners when the Ca^{2+}-signals are below the threshold required for CaM activation. NCS-1 plays a role in the regulation of several neuronal activities, such as neurotransmitter release [102–104], voltage-gated Ca^{+} channel activity [105, 106], short-term synaptic plasticity [107], and the LTD [108]. A study in *Drosophila* has shown that the fly NCS-1 ortholog frequenin interacts with a VOC [109], equivalent to the mammalian P/Q-like Cav2.1 channel which is regulated by CaM [110]. Interestingly, NCS-1 expression is not confined to a specific neuronal population, and many of its regulated functions are evolutionary conserved. However, the appearance of other NCS members has revealed a more specialized function in specific neurons, suggesting that these proteins may have evolved to carry out separate roles with respect to CaM.

The calsenilin/DREAM/KChIP3 protein is not confined to neuronal cells, even if it is expressed predominantly in the brain. KChIP3 is one of the KChIP proteins that have been found to be associated with voltage-gated K^{+} channels [111]. KChIP3 is also known as DREAM or calsenilin and has a role both in the regulation of gene transcription [112] and in the binding and processing of presenilin [113], a component of the γ-secretase enzyme involved in amyloid precursor protein (APP) processing and in familial Alzheimer's disease [114]. Interestingly, the expression of two genes encoding two Ca^{2+}-regulating proteins, i.e., the NCX and one subunit of the L-type channels, is a target of Ca^{2+}-dependent DREAM regulation [115, 116].

Analysis of DREAM knockout transgenic mice or of mice overexpressing a DREAM dominant negative mutant insensitive to Ca^{2+} regulation has resulted in the identification of additional physiological neuronal roles for KChIP3. There is evidence for an enhancement of learning (contextual fear memory) in the absence of KChIP3 [117, 118] and of an impairment of LTD in knockout mouse studies [119]. The effect of DREAM on LTD was suggested to be due to direct or indirect interaction with NMDAR function [119, 120]. Another important role for KChIP3 in neuronal physiology has been identified in mice expressing the Ca^{2+}-insensitive constitutively active mutant. KChIP3 is involved in the mechanisms of pain sensation by acting on the expression of several genes related to pain, including prodynorphin and brain-derived neurotrophic factor receptor [121].

Why is Ca^{2+} especially important to neurons?

As mentioned, neurons depend on Ca^{2+} for the control of processes that are not common to all eukaryotic cells.

One of these, the release of neurotransmitter(s), is the neuronal variant of the process of secretion that operates in the release of hormones by other cell types. The molecular mechanism of the process is best understood in neurons. Neurotransmitters convey signals from the axon terminal across the synapse to the next neuron or cell. They are packaged into synaptic vesicles which fuse with the presynaptic portion of the plasma membrane of the terminal, discharging their contents into the synaptic cleft to reach specific receptors on the postsynaptic membrane of the next neuron. Once fusing with the plasma membrane and releasing the neurotransmitter, the membrane is recycled to reform the synaptic vesicles. Ca^{2+} has a dual role in the process of neurotransmitter release: it penetrates into the axonal terminal as a result of the depolarization of its plasma membrane that opens VOC. Once in the terminal, it promotes the fusion of the synaptic vesicles with the presynaptic plasma membrane, in a process that involves a number of proteins of the large SNARE superfamily (see [122] for a comprehensive coverage of the topic). The Ca^{2+} sensor in the membrane of the synaptic vesicle is synaptotagmin, a protein that contains two cytoplasmic C2 Ca^{2+}-binding domains: upon the binding of Ca^{2+} to these domains, synaptotagmin docks to the presynaptic membrane via interaction with the SNARE proteins synaptobrevin and SNAP 25, eventually discharging the content of the synaptic vesicle into the synaptic cleft.

LTP and LTD also are neuron-specific processes that make use of Ca^{2+} signals. LTP defines the long-lasting increase in synaptic transmission following high-frequency stimulation. Synapses have the ability to modify their strength, i.e., they display plasticity. Since learning and memory are generally related to modifications of the synaptic strength, LTP is widely assumed to be involved in these processes. LTP was originally discovered in the hippocampus, subsequently to be also found in a number of other neuronal types. The liberation of glutamate by the presynaptic terminal triggers its release. This neurotransmitter interacts first with postsynaptic AMPARs, inducing the penetration of Na^{+} into the postsynaptic neuron. Its depolarization removes the Mg^{2+} block of the postsynaptic NMDA receptors, permitting the influx of Ca^{2+} into the neuron and the activation of a number of kinases and other enzymes, such as the NO synthase. The role of CaM kinase II in the molecular mechanism of memory has been repeatedly discussed [123], and the activity of (nuclear) CaM kinase IV appears to be important for memory consolidation.

LTD is the opposite of LTP. It defines the persistent weakening of synaptic strength that, depending on the brain areas (e.g., cerebellum or hippocampus), may result from strong synaptic stimulation or from protracted weak stimulation. As for LTP, the neurotransmitter most commonly involved in LTD is glutamate. LTD appears to be linked to the reduced density of the postsynaptic AMPARs and NMDARs. It cooperates with LTP so that the ability to fine-tune their opposite functions will reflect synaptic plasticity.

One on-going problem in the general area of Ca^{2+} signaling is that of the transfer of Ca^{2+} from the cytosol to the nucleus: or, more specifically, of the transfer of Ca^{2+} signals from the plasma membrane to the nucleoplasm. The large pores of the nuclear envelope would logically suggest that cytoplasmic and nuclear Ca^{2+} are in continuous passive equilibrium. Yet a number of indications suggest that some form of gating may operate in the transmission of Ca^{2+} signals through the nuclear envelope. These indications are particularly convincing in neurons [124]. Ca^{2+} signals initiated by synaptic activity specifically propagate to the nucleus to switch on genetic programs: they are perhaps the most important path in the communication between the synapse and the nucleus. In the nucleus, Ca^{2+} targets the transcription factor CREB, but also the CREB binding protein (CBP), which is critical to the transcriptional activity of CREB. CBP also interacts with other transcription factors, thus conferring Ca^{2+} control to a number of target genes. Nuclear Ca^{2+} also targets the multifunctional transcriptional repressor DREAM (see above), causing its dissociation from DRE sites in DNA and reactivating the transcription of a number of genes. Finally, the path of Ca^{2+} transfer from the plasma membrane to the nucleus may trigger functionally different processes: Ca^{2+} coming specifically from extrasynaptic NMDARs may modulate death processes in hippocampal neurons by promoting the translocation of the Forkhead transcription factor FoxO3a into the nucleus [125]. This neuronal death program is counteracted by the activation of nuclear CaMKIV triggered by synaptic activity.

Dysregulation of Ca^{2+} signaling in the brain and neuronal degeneration

Considering the special importance of Ca^{2+} signaling to neuronal function, the involvement of its defects in neuronal diseases is hardly surprising. The causative role of the malfunction of Ca^{2+} regulation is now being increasingly documented in a vast array of important neuronal pathologies, particularly, but not exclusively, those characterized by neurodegenerative processes. A number of comprehensive reviews on the topic have appeared [126–129]: here, we will succinctly discuss the most important pathologies. Table 1 and the cartoon in Fig. 3 summarize the main pathways involved in the generation of the Ca^{2+} dyshomeostasis that eventually give rise the neuronal pathologies.

Table 1 Summary of the information presented in this review

Disease/pathology	Type of neuron/brain area principally affected by the specific pathology	Protein/organelle[a]	Location	Effect on Ca^{2+} homeostasis or putative affected Ca^{2+} pathway in the pathology[b]
Amyotrophic lateral sclerosis (ALS)	Upper and lower motor neurons of the motor cortex, brain stem and spinal cord	AMPAR	Plasma membrane	Excitotoxicity
		GluR2 subunit (AMPAR)	Plasma membrane	
		Calbindin D-28k	Cytosol	Decreased Ca^{2+}-buffering capacity
		Paravalbumin	Cytosol	
		Calreticulin	ER	
		Mitochondria		
Huntington's disease (HD)	Spiny neurons of striatum	Calbindin D-28k	Cytosol	Decreased Ca^{2+}-buffering capacity
		Calretinin	Cytosol	
		NMDAR	Plasma membrane	Excitotoxicity
		Postsynaptic density 95	Plasma membrane	Loss of NMDAR/AMPAR inhibition
		Mitochondria		Ca^{2+} handling defects
		ER		Increased release of Ca^{2+}
Cerebellar ataxia	Purkinje neurons of cerebellum. The spinal cord can also be affected	Calbindin D-28k	Cytosol	Decreased Ca^{2+}-buffering capacity
		Cav2.1 (SCA6)	Plasma membrane	Accumulation of mutant channel
		Cav2.1 (EA2)	Plasma membrane	Loss of channel function
		InsP3R (SCA2/3)	Endoplasmic reticulum	Increased InsP3 sensitivity
		PMCA2	Plasma membrane	Loss of function (ataxic phenotype)
		PMCA3 (X-linked ataxia)	Plasma membrane	Loss of function
Alzheimer's disease (AD)	Cortical neurons	CALHM1	Plasma membrane	Regulation of amyloid metabolism
		NMDAR	Plasma membrane	Aβ42-PP mediated increased Ca^{2+} entry
		Aβ42	Extracellular space	Increased membrane Ca^{2+} permeability
		N-type Ca^{2+} channels	Plasma membrane	Aβ42 mediated increased Ca^{2+} entry
		PS	ER	Altered ER Ca^{2+} level
		InsP3R	ER	Postsynaptic-mediated altered activation
		RyR	ER	
		SERCA	ER	
		PS/Aβ42		Altered ER–mitochondria Ca^{2+} transfer
Parkinson's disease (PD)	Dopaminergic neurons of the substantia nigra pars compacta	α-Synuclein	Cytosol/mitochondria	Modulation of ER–mitochondria Ca^{2+} transfer/increased membrane Ca^{2+} permeability
		Parkin	Cytosol/mitochondria	Mitophagy/modulation of ER–mitochondria Ca^{2+} transfer
		DJ-1	Cytosol/mitochondria	Oxidative stress defense/modulation of ER–mitochondria Ca^{2+} transfer
		Cav1.3	Plasma membrane	Increased Ca^{2+}-induced oxidative stress in SNPC neurons
		PINK1	Mitochondria	Mitochondrial dynamics/mitophagy/Ca^{2+} modulation (MCU, Na^+/Ca^{2+} exchanger, mPTP)
Familial hemiplegic migraine (FHM)	Cortical neurons	Cav2.1 (FHM1)	Plasma membrane	Increased open probability
		ATP1A2 (FHM2)	Plasma membrane	Glutamate clearance/regulation of Na^+/Ca^{2+} exchanger

ER Endoplasmic reticulum, *AMPAR* 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, *NMDAR* *N*-methyl-D-aspartate receptor; for other abbreviations, see text

[a] Proteins or organelles involved in the process of Ca^{2+} homeostasis and in the pathogenesis of the specified disease/pathology. For the proteins (mostly, but not exclusively, Ca^{2+} channels or pumps) the principal location within the cells is indicated

[b] Possible effects on Ca^{2+} signaling described by the papers quoted in this review

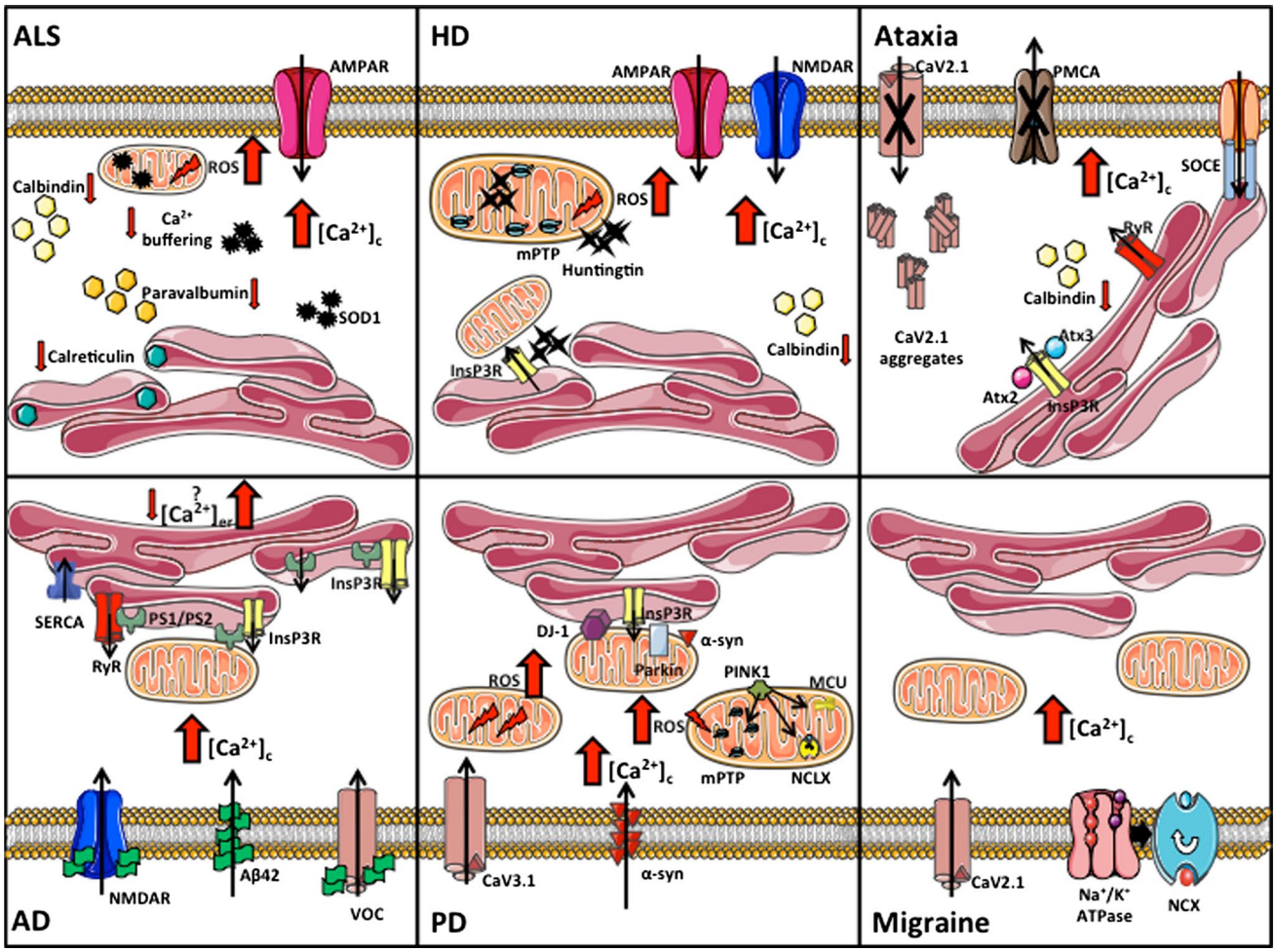

Fig. 3 Perturbed Ca^{2+} homeostasis in neuronal disorders. The cartoon summarizes the main pathways involved in the Ca^{2+} dyshomeostasis processes linked to the neuronal pathologies described in the text. *ALS* Amyotrophic lateral sclerosis, *HD* Huntington's disease, *AD* Alzheimer's disease, *PD* Parkinson's disease, *ROS* reactive oxygen species, *PS* presenilin, *SOD* superoxide dismutase, *mPTP* mitochondrial permeability transition pore, *ATX* ataxin, *PINK1* PTEN-induced kinase, *α-syn* alpha-synuclein

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS; also called Lou Gehrig's disease from the name of a prominent USA baseball player who died of it) is a neurodegenerative disorder caused by the progressive and selective loss of motor neurons in the spinal cord and the brain that leads to paralysis of the voluntary muscles and eventual death. The disease typically affects men and women aged 50–60 years: the majority of cases are sporadic, but about 10 % are inherited. The clinical and pathological phenotype is the same in the sporadic and the inherited ALS: at the molecular level, mutations in the Zn–Cu superoxidase dismutase (SOD1) (more than 100 have been described) are present in about 20 % of the familial ALS cases [130], whereas autoimmunity and excitotoxicity processes are suggested to be causative of sporadic ALS. SOD1 is an ubiquitous enzyme that protects cells against oxidative stress. Interestingly, the mutations do not cause a defect of SOD1, but rather its gain-of-function. The mechanism by which its mutations induce the ALS phenotype has not yet been elucidated, but it is assumed to involve a number of phenomena including mitochondrial dysfunction, excess production of radical species, protein misfolding, and glutamate excitotoxicity. It has been suggested that the vulnerability of motor neurons in ALS may be linked to their selective susceptibility to excitotoxicity mediated by glutamate AMPARs [131]. The perturbation of cellular Ca^{2+} homeostasis is now increasingly considered to be important in the pathogenesis of ALS [132, 133]: it may involve malfunctions of membrane channels and/or of the mitochondrial Ca^{2+} controlling systems, as well as alterations in the Ca^{2+}-buffering capacity of proteins. The expression of CB-D28k and of PV is significantly downregulated in the motor neurons that are

lost early in ALS (e.g., spinal and cranial motor neurons), while it is normal in those that are damaged only late in the disease process [134]. A dramatic reduction in the level of CB-D28k has also been detected in several brain domains of ALS patients [135]. The importance of Ca^{2+}-buffering proteins is also underlined by studies on animal models of ALS. The loss of motor neurons was found to be significantly lower in PV transgenic mice interbred with mutant SOD1 transgenic mice than in mutant SOD1 transgenic mice [136]. Expression of the ER Ca^{2+}-buffering protein calreticulin has also been found to be decreased in neuronal models of ALS [137].

Mitochondrial alterations, both functional and ultrastructural, have been frequently described in both ALS patients and in mice models of ALS [138]. Decreased mitochondrial Ca^{2+}-buffering capacity has been observed in the brain and spinal cord of mutant SOD1 transgenic mice [139], and the transfection of cultured neurons with mutant SOD1 has been found to disrupt the ability of mitochondria to buffer Ca^{2+} [140]. SOD1 is predominantly cytosolic, but it also localizes to mitochondria, and the intramitochondrial localization of mutant SOD1 has been observed to be correlated with damaged mitochondrial function [141, 142]. Defects in complex IV of the respiratory chain have been consistently observed [142–145], but mutant SOD 1 could also damage mitochondria from outside. Various aspects of mitochondrial bioenergetics can be affected in ALS, but Ca^{2+} handling has a special place, as its malfunction has a direct role on cytosolic Ca^{2+} overload and cell death. Defects of mitochondrial Ca^{2+} uptake have been observed in mitochondria isolated from the brain and spinal cord of ALS mice, but not from unaffected liver [139], in organotypic brainstem slices [146], or in cultured neurons [147].

Intracellular Ca^{2+} increases in motor neurons are mediated by VOC and ionotropic receptors. Hyperactivation of glutamate receptors is a leading cause of the overload of Ca^{2+} in ALS motor neurons [148]. Motor neurons are particularly vulnerable to the excitotoxity of AMPARs [149]: the defective editing of the GluR2 subunit specifically increases Ca^{2+} influx through the receptors in susceptible motor neurons, e.g., in the spinal cord of ALS patients [150]. The AMPA receptor-mediated excitotoxicity may be exacerbated by the lower cytosolic Ca^{2+} clearance that has been observed in mutant SOD1 motor neurons [151].

Huntington's disease

Huntington's disease (HD) is a genetic, autosomal-dominant neurodegenerative disorder that terminates with death within 15–20 years after its onset. Its phenotype is causally related to the loss of striatal GABAergic neurons [the medium spiny neurons which produce gamma-aminobutyric-acid (GABA)] that control brain development, movements, and psychiatric functions such as memory. Its symptoms thus include neuropsychiatric defects, such as chorea, motility impairments, and dementia. HD is one of the nine polyglutamine diseases caused by the expansion of CAG repeats encoding a polyglutamine tract in the genes of different proteins. In HD, the polyQ expansion occurs at the N-terminal portion of huntingtin (Htt), a ubiquitous protein that normally contains up to 35 Qs in the N-terminal portion: in HD, Htt may number more than 100 Qs, with their number being inversely correlated with the age of disease onset and its severity. The function of Htt is not fully understood, but one suggestion is that it acts as a scaffold in the control of numerous cellular processes, including gene transcription, vesicular and organellar trafficking, mitochondrial functions (including the handling of Ca^{2+} and the expression of respiratory chain components), the production/scavenging balance of reactive oxygen species, the balance of Ca^{2+} in the ER, and apoptosis [152]. The polyQ expansion in mutant Htt renders it susceptible to proteolytic cleavage, which appears to be executed by caspase 6 [153], producing N-terminal fragments that accumulate in the neuron as toxic mono/oligomers that could translocate to the nucleus to impair transcription and/or grow to aggregates that sequester important components such as transcription factors and CaM [154–156]. It has also been claimed that the toxic fragments associate with organelles, for example, the mitochondria, altering their function. The cellular/molecular etiology of the disease still has numerous unclear aspects, including the reason for the late onset even if the genetic defect is present at birth and the trigger leading neurons to die only after decades of coexistence with the anomalous protein.

Ca^{2+} dyshomeostasis as a causative factor in the cellular phenotype of HD is supported by several lines of evidence [157–159]. It may involve diverse mechanisms, including alterations of buffering capacity by Ca^{2+} binding proteins, malfunction of Ca^{2+} channels, particularly those involved in glutamate excitotoxicity, and disruption of the mitochondrial Ca^{2+} handling system. A specific loss of neurons containing CB-D28k has been observed in the brains of HD patients [160], which is in line with the decrease of Ca^{2+}-buffering capacity in the specific brain areas that house these neurons. It has also been shown that CR interacts with Htt, with a slight preference for the protein that contains an expanded polyQ tract [161]. The overexpression of CR reduces the cytotoxicity caused by the mutant Htt in both neuronal and non-neuronal cells, whereas the downregulation of CR increases neuronal death caused by the mutant protein.

In terms of excitotoxicity, ample evidence has documented the excessive activation of glutamate-gated Ca^{2+} channels and its detrimental effects on neurons of HD patients, including the early loss of striatal neurons that

express high levels of NMDARs [157] and the promotion of glutamate excitotoxicity by mutant Htt [162]. The latter disturbs the interaction of wild-type Htt with the postsynaptic density 95, which normally inhibits the activity of NMDARs and AMPARs.

Mitochondrial dysfunction is perhaps the most important factor in cellular HD etiology. As mentioned above, components of the respiratory chain, most notably complex II, are downregulated in cellular models of HD and in the brains of HD patients [163]. The HD phenotype has actually been reproduced in mice, and in cellular models, by the complex II inhibitor 3-nitropropionic acid [164]. The decreased efficiency of the respiratory chain is naturally expected to affect energy-linked mitochondrial reactions, including, and most importantly, Ca^{2+} uptake, and thus the ability of the organelles to control Ca^{2+} homeostasis in the cytosol. Defects in the handling of Ca^{2+} by mitochondria have indeed been repeatedly observed in cellular models of HD [159, 165–167] and in isolated mitochondria exposed to polyglutamine tracts. The permeability transition pore, which permits the exit of pro-apoptotic factors from mitochondria, appears to open at a lower Ca^{2+} concentration threshold in isolated mitochondria [167] and in mitochondria in HD model cells [159]. The mitochondrial Ca^{2+} dysfunction has been attributed to the direct interaction of mutant Htt with mitochondria [165], but could also be mediated by the Htt-promoted release of Ca^{2+} from neighboring ER [168], which would expose mitochondria to abnormally high, and thus harmful, concentrations of Ca^{2+}.

Alzheimer's disease

Alzheimer's disease (AD) is a devastating neurological disorder characterized by the progressive decline of cognitive function and increased neuronal cell death. At the morphological level, the accumulation of abnormal fibers in neuronal bodies and the presence of senile plaques in their extracellular space represent the main hallmarks. These structures are formed by a hyperphosphorylated form of the microtubular protein tau and by peptide β-amyloid (Aβ) (in its most frequent forms, Aβ40 and Aβ42). Aβ40 and Aβ42 derive from the transmembrane protein amyloid precursor protein (APP) that is alternatively processed by α, β, and γ secretases. Although the majority of AD cases are sporadic, mutations in the genes encoding for APP and for presenilin-1 and -2 (PS1 and PS2), two proteins belonging to the γ-secretase enzymatic complex, have been linked to the autosomal-dominant familial form of AD (FAD). Since the majority of FAD mutations are associated with enhanced fibrillization of the amyloidogenic Aβ42 fragment, an increase in amyloid metabolism is generally considered to be the major mechanism in the pathogenesis of AD. However, the finding that the Ca^{2+} homeostasis modulator 1 (CALHM1), a voltage-gated ion channel which promotes Ca^{2+} entry from the extracellular ambient, may regulate amyloid metabolism [169], as well as the advances in the functional characterization of presenilins have led to the "calcium hypothesis" of AD [170–172]. A number of studies on cell or animal models of FAD have suggested that a dysregulation of Ca^{2+} release from the ER could be involved in the pathogenic mechanism of AD. However, the molecular details and the effectors of this dysregulation are still controversial. The different aspects of the Ca^{2+} hypothesis can be summarized as follows: Ca^{2+} concentration measurements in cortical neurons of 3xTg-AD animals [173] (a triple transgenic model for AD, having a homozygous mutation for PS1 and homozygous APPSwe and tauP301L transgenes) and in the spines or dendrites of cortical neurons located close to amyloid deposits [174] have revealed higher basal Ca^{2+} levels than in corresponding non-pathological neurons. Alternative mechanisms for the generation of the neuronal Ca^{2+} overload in AD neurons have been proposed. Some have focused on the Ca^{2+} entry pathway showing that Aβ42 oligomers, by binding to the cellular prion protein that may function as the receptor [175], may enhance Ca^{2+} entry through the ROC (e.g., the NMDAR [176]), increase the Ca^{2+} permeability of the membrane [177, 178], or increase the sensitivity of the VOC [179]. Other proposals have focused on the release of Ca^{2+} from internal stores, suggesting that presenilin (PS) may directly regulate the Ca^{2+} leakiness of the ER membrane by forming a novel Ca^{2+} permeable channel [180] or by interfering with the activity of the resident ER Ca^{2+} InsP3Rs and RyRs channels [181, 182]. These channels could in turn even interfere with the activity of the SERCA pump [183, 184] and with the ER–mitochondria relationship [185]. PS mutants have been shown to alter both the expression and the sensitivity of the ER Ca^{2+} release channels, i.e., RyRs and InsP3Rs, in different AD cell models [186–189] and in neurons from transgenic AD mice [190]. Several FAD-causing PS mutants indeed enhance the response of InsP3Rs to saturating and suboptimal levels of InsP3 [182, 191]. Similarly, neurons from 3xTg-AD mice have been shown to display enhanced caffeine-induced Ca^{2+} release from the RyRs [192] and to undergo changes in their expression (especially of the RyR3 isoform) [193]. In the latter study, RyR-mediated Ca^{2+} upregulation within synaptic compartments was associated with altered synaptic homeostasis and network depression at early (presymptomatic) AD stages [193]. RyRs have recently been proposed as targets to prevent disease progression: short-term sub-chronic treatment of AD mouse models with the RyR inhibitor dantrolene fully normalized ER Ca^{2+} signaling within somatic and dendritic compartments in hippocampal slices from mice with early and later stages of AD. In

addition, the elevated RyR2 levels and the altered synaptic transmission and plasticity observed in the AD mice were restored to control levels by dantrolene. Aβ deposition within the cortex and hippocampus has also been found to be reduced by the drug [194].

As mentioned, it has also been proposed that PS can form Ca^{2+} permeable leak channels on the ER membrane and that FAD-linked mutations could impair the Ca^{2+} permeability of the ER [180]. This proposal is still controversial, as other data have instead shown that some FAD-linked PS2 mutations decrease the ER Ca^{2+} content rather than increase it [195–197]. An increase in ER Ca^{2+} leakage was observed in PS1- and PS2-silenced fibroblasts [184] and in different cell models expressing mutant PS2. The effect has been attributed to increased Ca^{2+} leakiness through InsP3 and RyR channels and also to the reduction of SERCA pumping activity by both wild-type and mutant PS [184]. Interestingly, it has recently been shown that nanomolar concentrations of the amyloid β-peptide upregulate the InsP3R and the VDAC and increase the number of ER–mitochondria contact points and the mitochondrial Ca^{2+} concentration. This suggests a role of ER–mitochondria contacts and cross-talk in AD pathology [185, 198], thus adding further complexity to the Ca^{2+} hypothesis of AD.

Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative condition clinically characterized by motor impairment involving resting tremor, bradykinesia, and rigidity. Motor deficits are due to the specific loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). In the large majority of cases, these neurons also display typical cytoplasmic protein inclusions called Lewy bodies [199]. The oral administration of the dopamine precursor L-3,4 dihyroxyphenylalanine (L-DOPA) ameliorates the symptoms, at least in the early stages of the disease, but elevated cytosolic levels of dopamine (DA) and its metabolite are neurotoxic. Many factors are involved in the etiopathogenesis of the disease, age being the greatest risk factor [200], but environmental and genetic factors are also determinants [201]. In particular, exposure to pesticides and substances that are toxic to mitochondria, as well as mutations in several genes which encode proteins related to mitochondrial function, have been causally linked to PD. At the cellular level, PD is widely associated with defects in the respiratory chain complex I [202]. More recently, an important role has been attributed to the dysfunction of the mitochondrial quality control system [203]. This aspect will not be discussed in this review, but a number of recent reviews cover this topic extensively [204–206].

The possible involvement of Ca^{2+} in PD originates from the observation that the expression of the Ca^{2+}-buffering protein CB-D28k is negatively correlated with neuronal vulnerability in PD [207, 208] and from the observation that the cytoplasmic Ca^{2+} concentration was higher in SNc dopaminergic neurons (which express Cav1.3 L-type Ca^{2+} channels that guarantee their autonomous pacemaking activity [209, 210]) than in the neighboring dopaminergic neurons in the ventral tegmental area (VTA). Higher Ca^{2+} levels have been shown to correlate with a two- to three-fold increase in cytosolic levels of DA in SNc neurons with respect to VTA neurons, thus explaining their greater susceptibility to L-DOPA-induced neurotoxicity [210].

Recently, increased Ca^{2+} entry related to spontaneous pacemaking activity has been shown to contribute to the PD risk by increasing mitochondrial oxidative stress. The development of transgenic mice expressing the redox-sensitive variant of the green fluorescent protein with a mitochondrial-matrix targeting sequence under the control of the tyrosine hydroxylase promoter has enabled quantitative monitoring of the oxidation of mitochondrial matrix proteins, resulting in the finding that Ca^{2+} entry through the L-type channels is normally counteracted by a mild transient mitochondrial depolarization that parallels cytosolic Ca^{2+} oscillations. When mitochondrial function is compromised, i.e., by exposure to mitochondrial toxins or by mutations in PD-related genes, this protective role may be affected, selectively compromising SNc neurons [211, 212]. Other studies on model cells that overexpress familial PD-related proteins or which have been silenced for their expression have indicated the possibility that they may be involved in the modulation of Ca^{2+} signals. Our laboratory has recently shown that α-synuclein, parkin, and DJ-1 overexpression enhanced ER-mitochondrial Ca^{2+} transfer by increasing the tethering between the two organelles and that this privileged communication was required to guarantee mitochondrial physiology and morphology (Fig. 4) [213–215]. Other groups have shown the involvement of α-synuclein in the modulation of the plasma membrane Ca^{2+} entry pathway, suggesting that its aberrant expression, or the presence of aggregate forms, may enhance Ca^{2+} influx from the extracellular ambient, thus making the cells more susceptible to Ca^{2+} overload [216–218]. Finally, intriguing data supporting a role for the Ca^{2+}/mitochondria connection in PD pathogenesis have been obtained in cell models in which the expression of the mitochondrial serine/threonine kinase PINK1 was silenced. Further investigations on this aspect are necessary, as the data currently available are still controversial. However, even if a disturbance in mitochondrial Ca^{2+} handling is involved, it is still not known whether it is due to the altered activity of the mitochondrial Ca^{2+} uniporter [219], of the mitochondrial Na/Ca^{2+} exchanger [220], or of the mPTP [221]. It could

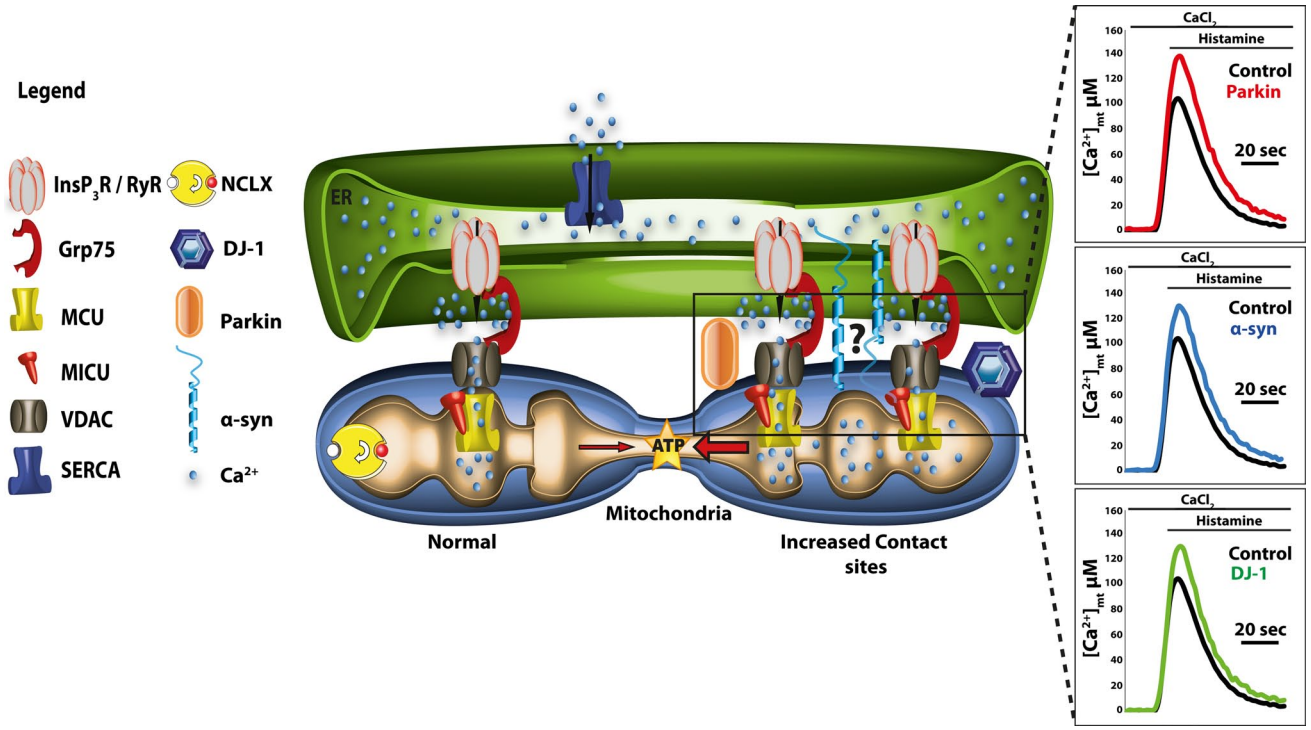

Fig. 4 Three Parkinson's disease (PD)-related proteins, namely, α-synuclein (*α-syn*), parkin, and DJ-1, control mitochondrial Ca^{2+} uptake by modulating ER–mitochondria contact sites. Mitochondria accumulate Ca^{2+} into the matrix via the electrophoretic MCU using the electrochemical gradient generated by the respiratory chain. The VDAC controls Ca^{2+} diffusion through the outer mitochondrial membrane, facilitating Ca^{2+} entry into mitochondria by coupling with the InsP3R through the chaperone protein Grp75. The NCLX mediates Ca^{2+} extrusion from the mitochondria, thus preventing the attainment of the thermodynamic equilibrium. ER–mitochondria tethering has recently emerged as a key element in cell Ca^{2+} metabolism and mitochondrial physiology. Our recent findings provide evidence for a role of α-syn, parkin and DJ-1 in favoring ER–mitochondria Ca^{2+} transfer by enhancing the ER–mitochondria contact sites [213–215]. The traces (*right*) refer to mitochondrial Ca^{2+} transients generated by the stimulation of HeLa cells overexpressing parkin, α-syn, or DJ-1 with an InsP3-linked agonist (histamine). The peak amplitude of the mitochondrial Ca^{2+} transients was higher in overexpressing cells than in control cells

also be causatively linked to the general impairment of the mitochondrial respiratory chain [222].

Ataxias

Ataxias are a large group of neurological disorders (more than 40 in the most recent classifications) characterized by the inability to coordinate muscle activity during voluntary movement. They are most frequently caused by disorders of the cerebellum or the posterior column of the spinal cord, with Purkinje neurons in the cerebellum being predominantly affected. Ataxias may be inherited, the genetic defect being either recessive or dominant, or caused by non-genetic factors/circumstances, such as injuries, surgery, exposure to toxicants, infections, and tumors. Some forms of ataxias, such as spinocerebellar ataxia 2 and 3 (also known as Machado–Joseph disease), belong to the group of polyglutamine diseases mentioned above, i.e., their origin can be traced back to the abnormal expansion of the polyQ tract in the cytosolic proteins ataxin2 in SCA2 (Atx2) and ataxin3 in SCA3 (Atx3), respectively. Ca^{2+} homeostasis dysregulation is common in ataxic neurons: here the most interesting cases will be discussed, as a comprehensive coverage of this topic will not be possible within the scope of this review.

As in the case of other neurodegenerative disorders, dysregulation of Ca^{2+} homeostasis in ataxias may be causatively related to deficient Ca^{2+} buffering by Ca^{2+}-binding proteins. Purkinje neurons are known to contain high amounts of CB-D28k, which acts as a fast, high-affinity Ca^{2+} buffer that helps to control the synaptically evoked Ca^{2+} transients. Disruption of the calbindin gene in mice has been found to result in impaired motor coordination and to alter the fast—but not the slow—component of the postsynaptic Ca^{2+} transient induced in the Purkinje dendritic tree by stimulation of the climbing fibers in cerebellar slices [223]. Other studies in cultured cells have shown reductions in the peak amplitude of slower Ca^{2+} transients with increases in the level of calbindin in cultured cells [224, 225].

Several forms of ataxia are caused by mutations in Ca^{2+} channel genes and thus qualify as Ca^{2+} channelopathies. Among these, spinocerebellar ataxia 6 (SCA6) is both a Ca^{2+} channelopathy and a polyQ disease, as an expansion of the CAG repeat tract occurs in the gene encoding the Cav2.1 subunit of the P/Q voltage-sensitive Ca^{2+} channels which are abundantly expressed in Purkinje neurons, where they mediate up to 90 % of their high-voltage activated Ca^{2+} influx. Since the electrophysiological properties of the P/Q channels are not altered by the SCA6 mutation, the pathogenesis of SCA6 has been suggested to involve the toxic accumulation of the mutant channel subunit [226]. The gene of the Cav2.1 subunit of the P/Q channel is also involved in episodic ataxia type 2 (EA2): a large number of mutations cause truncation of the subunit, causing in this case loss of P/Q channel function.

The abnormal polyQ tract in Atx2 and Atx3 in SCA2 and SCA3 instead interferes with the interaction of the two proteins with isoform 1 of the InsP3R. The interaction of the mutant proteins (but not that of the wild-type proteins) with the receptor increases its sensitivity to InsP3 in lipid bilayer experiments [227, 228], favoring the release of Ca^{2+} from the reticular store, and at the same time promotes the influx of Ca^{2+} into the neuron through the SOC. To validate the physiological significance of the findings, the authors of these studies fed transgenic mice carrying abnormal Atx2 and Atx3 polyQ tracts with dantrolene (the inhibitor of the RyR which is activated by the release of Ca^{2+} from the ER and by Ca^{2+} entering through the SOC, see above) and found age-dependent alleviation of the ataxic symptoms.

Recent findings have revealed that yet another regulator of cellular Ca^{2+} homeostasis, the PMCA pump, is involved in the genesis of ataxias. As mentioned above, PMCA2 and PMCA3 isoforms are predominantly expressed in neurons. Mutations in the PMCA2 gene were originally described as causing hereditary deafness and vestibular abnormalities [229]. The special functional properties of the PMCA2 isoform, which is specifically able to efficiently extrude Ca^{2+} in the absence of the natural activator CaM, are evidently optimally suited to maintain the delicate Ca^{2+} balance requirements between the outer hair cells of the Corti organ and the endolymph [74]. However, PMCA2 is also the dominant pump isoform of the cerebellum and is very abundant in the soma, dendrites, and spines of Purkinje cells. In keeping with the role of Purkinje neurons in motor coordination, the genetic deletion of the pump is associated with an overt ataxic phenotype in mice [230]. The Purkinje neurons of PMCA2 knockout mice are morphologically damaged, with their dendritic tree stunted and disordered. The recovery time of their dendritic Ca^{2+} transients is greatly increased [230, 231], and their overall physiology is disturbed (e.g., hyperpolarized membrane potentials, prolonged Ca^{2+}-dependent outward K^+ current, abnormal action potential firing). Somehow, even in the presence of the severe disturbance of Ca^{2+} homeostasis, Purkinje neurons in the PMCA2 knockout mice nevertheless manage to survive. The compartmentalization of Ca^{2+} homeostasis mechanisms between Purkinje dendrites and soma may possibly play a role [232], and other Ca^{2+} controlling mechanisms could compensate for the lack of PMCA2 function [233, 234]. The PMCA3 pump, which is also expressed in Purkinje neurons [235], could, for example, provide a plausible compensatory mechanism, but it does not appear to be overexpressed in PMCA2 knockout Purkinje neurons [231]. The PMCA3 pump is not as well-known as the other isoforms of the PMCA family, and its role in Ca^{2+} homeostasis in neurons is not yet understood. It is thus of particular interest that genetic screening has recently identified a mutation in PMCA3 in a family with X-linked cerebellar ataxia [69]. The mutation replaces a Gly with an Asp in the C-terminal CaM binding domain of the pump. The speed at which the mutated pump, overexpressed in model cells, clears the Ca^{2+} transients induced by the appropriate agonists is much slower than that of the non-mutated pump, thus creating a situation of cytosolic Ca^{2+} overload. The molecular defect is comparatively modest with respect to the severe ataxic phenotype of the affected patient: work now in progress is using alternative protocols to clarify whether the mutation only affects the Ca^{2+} extrusion function of the PMCA3 pump, or whether, possibly by interfering with the autoinhibitory mechanism of the C-terminal CaM binding domain, the mutation has more dramatic consequences for the local intracellular Ca^{2+} signaling regulation (Fig. 5).

Migraine

Migraine is a common episodic neurological disorder that manifests itself as recurrent attacks of typically throbbing and unilateral, often severe, headache, with associated features such as nausea, phonophobia, and/or photophobia; in one-third of patients the headache is preceded by transient neurological symptoms that are most frequently visual, but may involve other senses [migraine with aura (MA)]. Migraine is a disorder of brain excitability which has complex physiopathological mechanisms [236] that have begun to be understood only recently thanks to the use of mouse models of familial hemiplegic migraine (FHM), a rare genetic autosomal-dominant form of MA. Three genes, all encoding ion channels or transporters, have been identified in FHM [237]. FHM1 is caused by missense mutations in CACNA1A, the gene encoding the pore-forming subunit of neuronal Cav2.1 (P/Q-type) voltage-gated Ca^{2+} channels [238]. These channels are widely expressed in the nervous system and play a dominant role in controlling neurotransmitter release, particularly

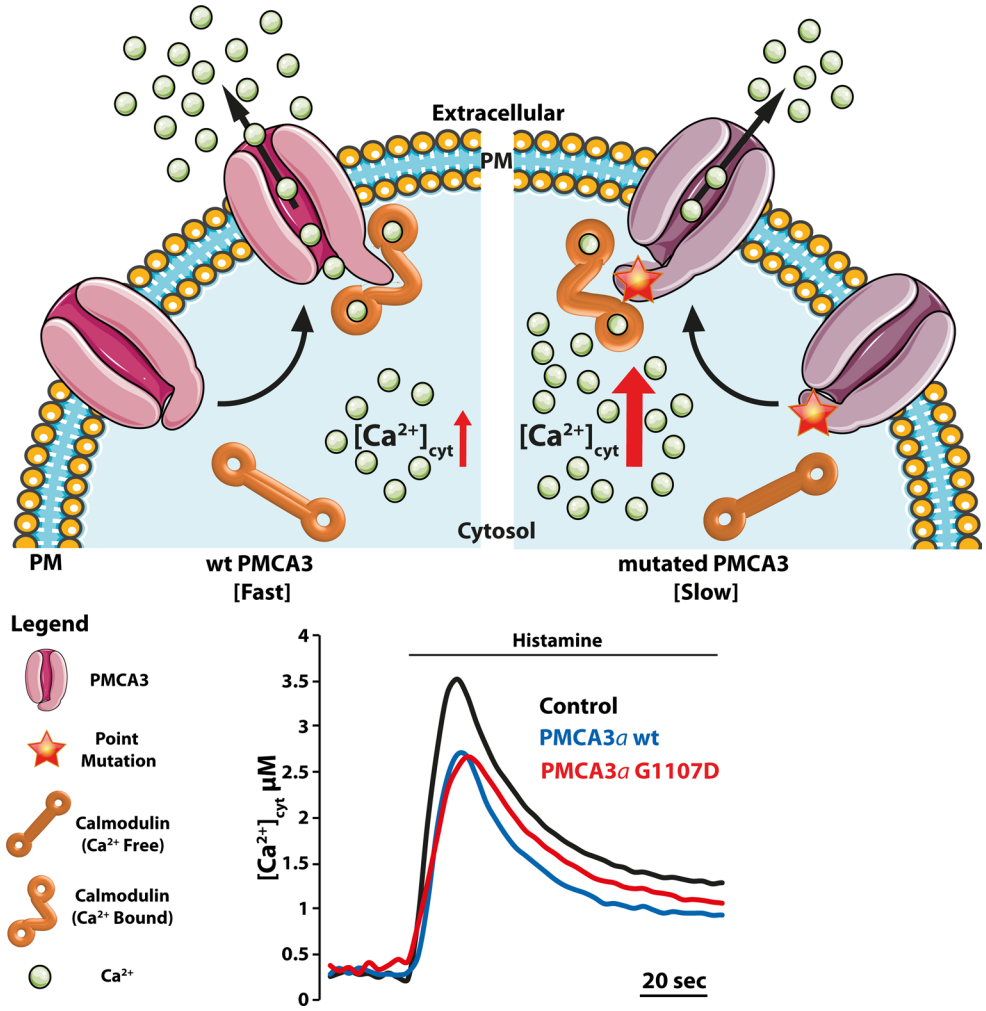

Fig. 5 A mutation in the plasma membrane (*PM*) Ca^{2+} ATPase 3 (*PMCA3*) pump causes X-linked congenital cerebellar ataxia. The PMCA3 pump is highly expressed in the cerebellum, particularly in the presynaptic terminals of parallel fibers–Purkinje neurons. The *cartoon* in the figure summarizes the recent finding that a missense mutation of the X-linked PMCA3 pump gene in a family with congenital cerebellar ataxia impaired the Ca^{2+} extrusion ability of the pump. The figure also shows cytosolic Ca^{2+} measurements in model cells (HeLa) overexpressing the PMCA3a wild-type (*wt*) (*blue trace*) or PMCA3a G1107D mutant pump (*red trace*). Cells were stimulated with a Ca^{2+} mobilizing agonist (histamine). As mentioned in the text, the "a" isoform is a spliced variant of the PMCA3 pump. The *black trace in the bottom panel* shows the Ca^{2+} transient in untransfected control cells. The reduction of the peak amplitude in PMCA3-overexpressing cells is due to the Ca^{2+} extrusion activity of the overexpressed pump. The mutant PMCA3a pump (*red trace*) has a reduced ability to extrude Ca^{2+} from the cells: the decline of the *red trace* is slower than that of the *blue trace*, which corresponds to cells overexpressing the wt PMCA3a pump [69]

at central synapses. Analysis of the properties of mutant recombinant human Cav2.1 channel currents in different neurons (including cortical and trigeminal ganglion neurons) of knock-in mice carrying FHM1 mutations have revealed that the mutations produce gain-of-function of the channels, mainly due to their increased open probability and activation at lower voltages. In the cerebral cortex of FHM1 knock-in mice, excitatory synaptic transmission was found to be enhanced [239].

FHM2 is caused by mutations (mainly missense) in ATP1A2, the gene encoding the α2 subunit of the Na^+/K^+ ATPase [240]. In the brain, this pump isoform is expressed primarily in neurons during embryonic development and at time of birth, but in adults it is almost exclusively expressed in astrocytes. It colocalizes with glutamate transporters in astrocytic processes surrounding glutamatergic synapses, suggesting a specific role in glutamate clearance [241]. It also colocalizes with the NCX in microdomains that overlie sub-plasma membrane ER, suggesting a possible role in the regulation of intracellular Ca^{2+}. FHM2 mutations cause complete or partial loss-of-function of recombinant Na^+/K^+ ATPases due to the loss or reduction of its catalytic activity or to impairment of its delivery to the plasma membrane [242, 243].

FHM3 is caused by missense mutations in SCNA1A, the gene encoding the pore-forming subunit of neuronal NaV1.1 voltage-gated Na^+ channels [244]. These channels are highly expressed in inhibitory inter-neurons where they play an important role in sustaining high-frequency firing [245]. Conflicting findings were obtained from the analysis of mutant recombinant human NaV1.1 channels expressed in non-neuronal cells, pointing to either gain- or loss-of-function effects [246, 247].

Although it is clear that most migraine attacks start in the brain and that they are essentially due to an imbalance between excitatory and inhibitory signals, the nature and mechanisms of the primary brain dysfunctions, as well as of the mechanisms that activate the trigeminovascular pain pathway, are still unsolved questions. FHM1 and -2 share features that suggest, as in other neurological disorders, exaggerated Ca^{2+} signals. Since the symptoms are not extended to all of the nervous system, but rather are confined to specific areas, specific characteristics of different neuronal populations may also play a role.

Concluding remarks

The development of genetic models and the improvement in technologies to image Ca^{2+} signals in vivo have greatly advanced the understanding of the molecular regulation of cellular Ca^{2+} homeostasis and its perturbation in neurological disorders. The dis-homeostasis of Ca^{2+} as a causative pathogenic mechanism of these disorders has now gained wide consensus. The identification of the defects affecting selective Ca^{2+} signaling processes is a necessary step in the road to therapeutic strategies that would correct the disturbances of neuronal Ca^{2+} signaling. Clearly, a number of questions still need to be solved, the foremost one being perhaps the understanding of the reasons for the specific vulnerability of different neuronal populations in the neurodegenerative processes. In this context the "Ca^{2+} hypothesis" of neurodegeneration can be considered to be a general background at the basis of different neuronal pathologies, which must be integrated with specific factors that determine the specificity of each disease process.

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