A Friend or Foe: Calcineurin across the Gamut of Neurological Disorders

Jackson Saraf,† Pallab Bhattacharya,*†† Kiran Kalia,† Anupom Borah,‡ Deepaneeta Sarmah,† Harpreet Kaur,† Kunjan R Dave,*§ and Dileep R Yavagal§

†Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar, Gujarat 382355, India
‡Cellular and Molecular Neurobiology Laboratory, Department of Life Science and Bioinformatics, Assam University, Silchar, Assam 788011, India
§Department of Neurology, University of Miami Miller School of Medicine, Miami, Florida 33136, United States

ABSTRACT: The serine/threonine phosphatase calcineurin (CaN) is a unique but confounding calcium/calmodulin-mediated enzyme. CaN has shown to play essential roles from regulating calcium homeostasis to being an intricate part of learning and memory formation. Neurological disorders, despite differing in their etiology, share similar pathological outcomes, such as mitochondrial dysfunction and apoptotic signaling brought about by excitotoxic elements. CaN, being deeply integrated in vital neuronal functions, may be implicated in various neurological disorders. Understanding the enzyme and its physiological niche in the nervous system is vital in uncovering its roles in the spectrum of brain disorders. By reviewing the crosstalk in different neurological pathologies, a possible grasp of CaN’s complex signaling may lead to forming better neurotherapy. This Outlook attempts to explore the various neuronal functions of CaN and investigate its pervasive role through the gamut of neurological disorders.

1. CALCINEURIN: A PROLOGUE

Even 40 years after its initial discovery by Wang and Desai as a column fraction, calcineurin (CaN) continues to intrigue the scientific community with its vast biological role.1 In its initial findings, CaN was often referred to as an “affinity-purified phosphodiesterase.”2 Klee and Krinks were the first to purify CaN and presumed it to be a regulatory subunit of a phosphodiesterase based on its ability to inhibit the same.3 Wallace and Lynch further purified CaN from bovine brain extracts using a two-column approach and found its activity to hinder the adenylylate cyclase and nucleotide phosphodiesterases.4,5 Due to similarity in biological activity traced by various research groups, it was therefore speculated that CaN may possibly be a regulatory enzyme. Its abundant allocation in the nervous system prompted Klee et al. to coin the term “calcineurin”.6 Klee’s version of CaN was corroborated by the work of Philip Cohen in which he found a fraction in cellular extracts that dephosphorylated the α-/β-subunits of phosphorylase kinase; the fraction revealed to be nearly identical to the CaN discovered by Klee.7,8

As per consensus, CaN is deemed as a calcium/calmodulin-dependent cyclic nucleotide phosphodiesterase inhibitor to the radical revelation of being the target of immunosuppressant drugs such as cyclosporin A in organ transplants, CaN seems to manifest itself in key biological pathways throughout various tissues in the body and necessitates a deeper investigation in its intricate physiological function.9 Its prevalence in the nervous tissue has recently made it a target of investigation for many neurological disorders, particularly those constituted by calcium excitotoxicity and neurotransmitter imbalance.10,11 It is believed that CaN plays a major role in bridging Ca2+ homeostasis to brain function; studies even point to CaN being the link between aging and brain dysregulation.12

CaN is deemed as a calcium/calmodulin-dependent (Ca2+/CaM) serine-threonine phosphatase, also known as protein phosphatase 2B (PP2B), ubiquitous in most mammalian tissues but predominating in the brain.
Though discussing the vast role of CaN in all the tissues is far beyond the realm of this Outlook, an attempt shall be made to delve into the CaN’s role in the brain and across the spectrum of neurological disorders such as stroke, Alzheimer’s disease, Parkinson’s disease, and other neurodegenerative disorders involving CaN. This crosstalk may clarify the extent of CaN’s involvement in these perplexing disorders.

2. CALCINEURIN: CLASSIFICATION AND STRUCTURE

The classification of serine/threonine phosphatases was established by Cohen in which he proposed two categories: type 1 and type 2 protein phosphatases (PPs). Type 1 PPs dephosphorylate the β-subunit of phosphorylase kinase while type 2 act on the α-subunit. The two PPs can be distinguished by their inhibition study in which phosphopeptide inhibitors act on type 1 but are unable to inhibit type 2 PPs. Cohen also later subclassified type 2 PPs according to the dependence on divalent ions for activity. He designated PP2A, PP2B, and PP2C as having no requirement for divalent ions, requiring Ca2+ (CaN), and requiring Mg2+ for activity, respectively. Evolutionarily speaking, PP1, PP2A, and PP2B (CaN) are some of the most highly conserved PP families throughout nature.

Human CaN is a heterodimeric serine/threonine phosphatase, comprising a unique structure composed of two subunits connected jointly: a 61 kDa catalytic A subunit (though variations between species range from 58 to 64 kDa) and a 19 kDa regulatory B subunit. The B subunit, although smaller in size, consists of a helical B-binding segment, a calmodulin-binding (CaM-binding) segment, and an autoinhibitory domain. The B-binding segment tightly adheres to the A subunit. Half of CaN appears to be cytosolic while the amino terminal glycine of the B subunit is believed to be myristoylated for greater association with the plasma membrane. Furthermore, the B segment consists of four Ca2+-binding sites (EF-motifs). Two Ca2+ sites reside on the N-terminal and have lower affinity (dissociation constant in the micromolar range). These seemingly serve as sensors for the fluctuations in intracellular Ca2+ levels and thereby serve a regulatory role, especially for the binding of CaM to the CaM-binding segment of subunit B. Another two Ca2+ sites reside on the C-terminal of the B subunit. These have much higher affinity (have dissociation constants in the nanomolar range) and act as stabilizers to keep both subunits intact and confer structural stability to the entirety of CaN. The CaM-binding segment relies on Ca2+ to help associate with subunit A, the catalytic site. This structure therefore stands to reason how CaN is able to couple Ca2+ signals to cellular responses. However, the enzyme undergoes complicated conformations to reach the active state. In previous studies, the conformational shift from inactive to active enzymatic states raised several questions. After all, the autoinhibitory site of subunit B (residues 467–486) lies at a relative distance from the B-binding site (341–372) and the CaM-binding segment (391–414). These questions were answered through studies done by Shen et al. in which the group discovered there are a separate sequence of conformational shifts that eventually lead to the fully activated enzyme. They found that, during elevated levels, Ca2+ initially binds to the lower-affinity Ca2+ sites, thereby causing a conformation which exposes the CaM-binding site. This fulfills subunit B’s main role as a regulator. When CaM binds to its respective site, another conformational change stabilizes subunit B by releasing the autoinhibitory peptide and renders the enzyme fully active. In summary, when the enzyme is inactive (resting Ca2+ levels), influx (micromolar) of Ca2+ binding to subunit B causes a conformational change to reveal the CaM-binding site on subunit A (partial activation), which upon binding to CaM displaces the autoinhibitory peptide and stabilizes and fully activates the enzyme (see Figure 1).

3. CALCINEURIN: VITAL HOMEOSTATIC MECHANISMS

3.1. Ca2+ Homeostasis. As CaN is ubiquitous throughout the body (several isoforms exist among different tissues), its roles are accordingly highly diversified. However, this Outlook will focus on CaN’s roles solely in the brain, where the highest levels can be found. CaN is known to constitute slightly more than 1% of total protein in the brain, and it is most densely populated in the caudate putamen and hippocampus but distributed throughout other regions of the brain. CaN is also found at the level of dendrites, axons, neuronal spines, presynaptic terminals, and postsynaptic densities. Magnifying the grandeur of CaN’s role in neuronal homeostasis is its very existence as the sole phosphatase heavily dependent on Ca2+/CaM signaling. It is well-established that calcium and its various calcium channels play a major and intricate role in neuronal function and activity. Calcium is arguably one of the most important secondary messengers capable of initiating pivotal cellular events. Calcium plays an essential role in many biological pathways that may be operating simultaneously within the neuron; drastic changes in its levels are associated with numerous neurological disorders. As Ca2+ relays synaptic signals to the nucleus then to synapse again, it is immensely responsible for turning on and off the various genetic expressions necessary for normal neuronal activity. Therefore, dysregulation (excess and deficient Ca2+ stimulation) in the Ca2+ synapse-to-nucleus signaling can cause breakdown in essential gene expressions that underlie most neurological pathologies, both degenerative and psychiatric in nature.

CaN is an integral member of the Ca2+ signaling pathway on both sides of the synapse. It behaves as a sensor and is vigilant of the intracellular levels of Ca2+ and maintains the proper equilibria necessary for cell maintenance and operation. There are several mechanisms CaN deploys to regulate intracellular Ca2+ levels and the downstream activities that maintain an optimum neuronal microenvironment.

Cal is an integral member of the Ca2+ signaling pathway on both sides of the synapse. It behaves as a sensor and is vigilant of the intracellular levels of Ca2+ and maintains the proper equilibria necessary for cell maintenance and operation.

3.2. Synaptic Exocytosis. Presynaptically, CaN and cyclin-dependent kinase 5 (CDKS) oversee vesicular release of neurotransmitters and prevent excessive exocytosis to maintain an adequate amount of neurotransmitters reaching the postsynaptic sites. These exocytotic processes require an ensemble of proteins that coordinate action potential and Ca2+ signaling to neurotransmitter release. CDKS is able to phosphorylate
proteins, like synapsin 1 which attaches to synaptic vesicles and sequesters them to the release-reluctant resting pool. Studies have shown that CDK5 knockout accelerates synaptic cycling and exocytotic kinetics; similarly, CaN knockout has shown to slow down exocytosis rates. CaN counteracts this effect, dephosphorylates synapsin 1, and is able to reverse the suppression of exocytosis and synaptic cycling induced by CDK5. Therefore, during greater Ca^2+ influx, CaN is able to increase the amount of neurotransmitters released into the synaptic cleft (see Figure 2a).

3.3. Synaptic Endocytosis. CaN also participates in endocytosis as it facilitates clathrin-dependent recycling by targeting different dephosphins, endocytic proteins, one in particular called dynamin 1. The interaction with dynamin 1 is based on CaN’s sensitivity toward Ca^2+ and spurs the depolarization-induced vesicular recycling; disturbance to such an interaction blocks endocytosis. However, such a role has been under controversy as CaN has shown to be implicated at different endocytic sites catalyzing different and opposing events. Regardless, studies continue to confirm that CaN is a key role player in endocytotic processes at both nerve terminals as well as in non-neuronal cells (see Figure 2a).

3.4. Ca^{2+} Influx and Regulation. Postsynaptically, CaN manages the Ca^{2+} homeostasis in the neuron by modulating its influx rates. The entry of Ca^{2+} activates the cell and triggers a cascade of a wide variety of biomolecular events. This intracellular Ca^{2+} influx is either via voltage-gated calcium channels (VGCCs) or ligand-gated channels such as the N-methyl-D-aspartate receptors (NMDARs). For NMDARs, CaN is located in the postsynaptic densities, near the cytoplasm and colocalized with NMDA receptors. The high affinity of CaN for the incoming Ca^{2+} activates it to dephosphorylate the C-terminal of the NR2A subunits of NMDARs, leading to glycine-independent desensitization and weakening of Ca^{2+} currents into the cell by decreasing the average duration of opened Ca^{2+} channels as well as their frequency.

It has also been documented that VGCCs are directly involved in synaptic plasticity and dendritic excitability. VGCCs, specifically the L-type CCs and to some extent the N-type CCs, are phosphorylated. CaN is targeted toward these channels through A-kinase anchoring protein 79/150 (AKAP) when calcium-dependent inactivation (CDI) is stimulated by the Ca^{2+} influx. CDI then further triggers microtubule-associated protein 2B (MAP2B) to guide protein kinase A (PKA) toward the VGCCs for priming and reinitiating Ca^{2+} currents. CaN is able to reduce the Ca^{2+} influx through this negative feedback effect and utilizes it to keep a check on the amount of Ca^{2+} entering the cell. CaN also regulates the Ca^{2+}-induced Ca^{2+} release (CICR), a universal mechanism by which cells are able to couple the inositol triphosphate receptors (IP3Rs) and ryanodine receptors (RyRs) to Ca^{2+} channels to trigger the release of Ca^{2+} from intracellular stores. Intracellular stores of Ca^{2+} reside in the sarcoplasmic reticulum and are mediated by IP3Rs and RyRs. It is believed this is another way CDI can be initiated alternatively to VGCC dephosphorylation due to the intimate association of VGCCs and the CCs opened through RyRs.

Figure 1. Activation of calcineurin (CaN). The phosphatase is activated through the sequence of two subsequent conformations. Initially, the influx of calcium (Ca^{2+}) raises the intracellular calcium load. At the nanomolar range, Ca^{2+} first binds to the two higher-affinity EF motifs (H_x) of subunit B, tightly adhering subunit B to subunit A and stabilizing the enzyme. As Ca^{2+} levels rise to the micromolar threshold, it binds to the lower-affinity EF motifs (L_x), spurring a conformational change that unveils the calmodulin (CaM) site to which CaM binds and initiates a second conformational change responsible for displacing the autoinhibitory peptide. The final conformation stabilizes and fully renders CaN active.
located on the sarcoplasmic reticulum (SR). CaN is able to dephosphorylate a phosphokinase C (PKC) phosphosite on both IP3Rs and RyRs and reduce their sensitivity toward alterations in intracellular Ca²⁺ concentrations and the subsequent affinity for their ligands (IP3 and Ry, respectively), thereby preventing the mechanism of CICR. In a direction that opposes the elevation or reduction in activity. This synaptic scaling often tends to be a negative feedback process and responds to altered synaptic strength to stabilize the neuronal network. CaN has been shown to be an important mediator of synaptic plasticity as its activation or inhibition can block or initiate synaptic scaling which further tends to enhance synaptic strength. CaN is able to regulate this through Ca²⁺ signaling, but at times even Ca²⁺ signaling can be disrupted and in turn can disrupt CaN activity. For the neuronal network to function properly synaptic plasticity must be sustained, and in such cases restoring Ca²⁺ signals becomes imperative. Studies suggest that chronic Ca²⁺-dependent inhibition (CDI) can lead to an attenuation of neuronal firing and

4. SYNAPTIC PLASTICITY

In conjunction to maintaining homeostatic Ca²⁺ levels throughout the neuronal network, CaN also facilitates homeostasis to synaptic plasticity, a term that denotes the flexibility and versatility of the neuronal network to change the synaptic strength in a direction that opposes the elevation or reduction in activity. This synaptic scaling often tends to be a negative feedback process and responds to altered synaptic strength to stabilize the neuronal network. CaN has been shown to be an important mediator of synaptic plasticity as its activation or inhibition can block or initiate synaptic scaling which further tends to enhance synaptic strength. CaN is able to regulate this through Ca²⁺ signaling, but at times even Ca²⁺ signaling can be disrupted and in turn can disrupt CaN activity. For the neuronal network to function properly synaptic plasticity must be sustained, and in such cases restoring Ca²⁺ signals becomes imperative. Studies suggest that chronic Ca²⁺-dependent inhibition (CDI) can lead to an attenuation of neuronal firing and
decrease neuronal activity. As Ca\(^{2+}\) influx decreases, CaN activity also decreases and sets off a compensatory mechanism involving the phosphorylation of serine 845 of GluA1 by PKA bound to AKAP79/150 and the elevated expression of \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) which are more sensitive and permeable to Ca\(^{2+}\), thereby restoring Ca\(^{2+}\) signaling\(^57\) (see Figure 2b). Upon restoration of Ca\(^{2+}\) signals, CaN is reactivated and reverts to facilitating proper neuronal signaling.

CaN has proven to modulate synaptic activity through Ca\(^{2+}\) influx through VGCCs and NMDARs.\(^58\) Furthermore, CaN has proven to confer bidirectional plasticity toward memory and learning while its deficiency has shown to impair working memory.\(^59\) This faculty of CaN to control bidirectional synaptic plasticity has great implications in memory and learning.\(^60\) Both phosphorylation and dephosphorylation play vital roles in memory formation and, respectively, trigger long-term potentiation (LTP) and long-term depression (LTD).\(^61\) Phosphokinases such as CaMKII, PKA, PKC, and MAPK have been involved in producing LTP, a phenomenon marked by both presynaptic and postsynaptic stimulations occurring at higher than normal frequencies or basically an enhanced synaptic transmission, thereby raising the phosphorylation of serine 845 residue of the receptor.\(^82\) Phosphorylation increases greater expressions of the GluA1 on the postsynaptic membrane and the amount of current flowing through the AMPARs, thereby strengthening synaptic transmission.\(^81,82,83,84\) Dephosphorylation of s845 sequesters the receptor and induces LTD.\(^85\) PKA and CaN regulate synaptic plasticity at AMPARs and serve a vital role in maintaining the optimum signaling throughout the neural circuitry.

CaN also serves another regulatory role by dephosphorylating the metabotropic glutamate receptor S (mGluRS) which is responsible for augmenting the responsiveness of neurons to different signals from their microenvironment.\(^86\) In other words, mGluRS elevates LTP. Belonging to group I of mGluRs and located postsynaptically, mGluRS works via the \(G_\text{q}\) pathway: it stimulates phospholipase C to hydrolyze phosphatidinositol biphosphate (PIP2) into IP3 and diacylglycerol (DAG) and further leads to the opening of Ca\(^{2+}\) stores via IP3Rs and RyRs and alteration in activities of various voltage-gated channels.\(^87\) In the process, it triggers a Ca\(^{2+}\) influx through NMDARs and becomes desensitized. CaN, after being activated by the NMDAR-mediated influx of Ca\(^{2+}\), resensitizes mGluR5 by dephosphorylation of its C-terminus and prolongs its activity; this is an important aspect of synaptic plasticity.\(^88\)

5. RECEPTOR SIGNALING

AMPARs are the most abundant excitatory receptors throughout the nervous system when it comes to fast synaptic transmission.\(^60\) Their expansive distribution and association with postsynaptic protein complexes grant them a high-level priority in regards to synaptic transmission and the electrophysiology of neurons.\(^61\) Their dynamic nature is conducive for changes in synaptic plasticity and therefore generates acute responsiveness within cells toward extracellular signals. Similar to metabotropic glutamate receptors and NMDARs, these receptors are also governed by phosphorylation (by PKA bound to AKAP79/150) and dephosphorylation (CaN), both targeting the highly sensitive 845 residue of the receptor.\(^85\) Phosphorylation increases greater expressions of the GluA1 on the postsynaptic membrane and the amount of current flowing through the AMPARs, thereby strengthening synaptic transmission.\(^81,82,83,84\) Dephosphorylation of s845 sequesters the receptor and induces LTD.\(^85\) PKA and CaN regulate synaptic plasticity at AMPARs and serve a vital role in maintaining the optimum signaling throughout the neural circuitry.

6. TRANSCRIPTION REGULATION

One of the major associations of CaN involves the nuclear factor for activated T-cell (NFAT) transcription factor. This family consists of five NFAT downstream targets residing in the cytoplasm in a hyperphosphorylated idle state (NFAT,1-4, etc.)

In conjunction to maintaining homeostatic Ca\(^{2+}\) levels throughout the neuronal network, CaN also facilitates homeostasis to synaptic plasticity, a term that denotes the flexibility and versatility of the neuronal network to change the synaptic strength in a direction that opposes the elevation or reduction in activity.

These channels are highly populated in postsynaptic terminals as well as dendrites.\(^75,74\) Their role is repolarization after the passage of action potentials and preventing their reverse propagation into dendritic regions.\(^75,76\) Overexpressed Kv4.2 channels have shown to shorten action potentials and mitigate LTP; this negative regulation of LTP resonates with that of CaN’s. Increase in NR2B/NR2A subunit ratio has been shown to facilitate synaptic plasticity, and LTP ensues. Overexpressed Kv4.2 channels reduce this ratio and trigger LTD.\(^77\) It has been shown that CaN modulates the activity of these channels in arms with the PKA/AKAP79/150 complex. The dephosphorylation by CaN stabilizes Kv4.2 channels at the postsynaptic terminal, dampening neurotransmission (LTD). PKA counters this activity through phosphorylation of the channels by binding to AKAP79/150 to induce their internalization into the cytoplasm.\(^78\) This presents fewer Kv4.2 channels at the postsynaptic terminal and leads to LTP. Deficits in Kv4.2 channels have shown to produce spatial learning deficits and memory impairment\(^79\) (see Figure 2b).

DOI: 10.1021/acscentsci.8b00230
ACS Cent. Sci. 2018, 4, 805–819
The CaN-NFAT signaling contributes to a wide array of biological developments such as cardiac and lung morphogenesis, differentiation and the growth of skeletal muscle, and immune responses like T-cell activation. As CaN is enriched throughout the brain regions, the tightly regulated signaling makes NFAT as proportionally and deeply integrated in many neuronal responses and activities such as axonal growth, memory formation, and neuronal apoptosis. Furthermore, the pathway is heavily involved in the development of the nervous system (synaptogenesis, corticogenesis), maintenance (myelination, neuronal survival), and nervous system operation (synaptic connectivity, plasticity, and neurotransmission). CaN-NFAT pathway begins when calcium influx activates CaN to dephosphorylate the CaN-phosphosites from the N-terminal of NFAT subunits, exposing their nuclear sites. Once within the nucleus, these NFAT subunits partner with their NFATn counterparts on specific response elements and promote various transcription sequences necessary for the homeostatic functioning of the neuron. However, the Ca2+ that activates CaN may not be sufficient to trigger this pathway. Rather, a greater influx of Ca2+ is provided by unique Ca2+ release activated Ca2+ (CRAC) channels. Some studies also suggest that the NFAT complexes induce a positive feedback loop to increase the intracellular Ca2+ levels via IP3R-RyRs Ca2+ release. Their job is to sustain the Ca2+ threshold necessary to maintain the NFAT−NFATn complexes within the nucleus for extended transcription to fulfill cellular needs at specified time points. A drop-off in intracellular Ca2+ levels signals the rephosphorylation of the NFAT complexes by initial priming by dual-specificity tyrosine-phosphorylation regulated kinases (DYRKs) particularly DYRK1A and DYRK2, recently discovered regulators of NFAT. Once primed, they are further subjected to rephosphorylation for export to the cytoplasm by glycogen synthase kinase 3 β (GSK-3), which acts to fine-tune the NFAT signaling (see Figure 3).

7. CaN–MITOCHONDRIA CONNECTION

The intrinsic and paramount role of mitochondria in orchestrating cell survival and apoptosis has been well-documented. Studies over time have delineated several mechanistic pathways of mitochondria and their tightly regulated control over cell viability. Members of the Bcl-2 family, BAX and BAD, remain in an inactive state after phosphorylation by phosphokinases such as PKA, Akt, and Raf-1. Therefore, phosphorylation promotes cell survival and is employed by various growth factors. CaN has been postulated to be a key element in several of these pathways, particularly those favoring apoptosis. Its ability to dephosphorylate BAD activates the pro-apoptotic factor to bind and complex with the mitochondrial membrane-bound anti-apoptotic factors, Bcl-2 and Bcl-xL.

The dephosphorylation occurs at primarily two serine residues of human BAD, s75 and s99. This dephosphorylation causes BAD to dissociate from the protein 14-3-3 and relocate to the outer mitochondrial membrane (OMM). The dimerization of BAD with Bcl-2 and Bcl-xL (suppression of pro-survival signals) prompt the translocation of BAX to the OMM to initiate the formation of mitochondrial permeability transition pores (mPTP) which exacerbate the apoptotic cascade through the release of cytochrome c from the mitochondria and result in execution of cell death by caspases 3, 6, and 7.

As it has been elucidated that CaN plays a major role in programmed cell death, would the changes in CaN expression affect this role? Such a query arises from the relatively higher concentrations of the PP2B distributed in the brain. What role would a denser population of constitutive CaN play in influencing neuronal survival? Asai et al. went on to address this in a study where they investigated how deeply integrated CaN was in the intrinsic cell death pathway. The group discovered was that CaN predisposes neurons to the cyt c-caspase 3 dependent pathway more frequently in comparison to other cells in the body. The elevated CaN levels (due to Ca2+ overload) render neurons to undergo apoptosis under even normal cell conditions. Thus, Ca2+-induced excitotoxicity through NMDARs increased the probability of inducing cell death due to the greater expression and sensitivity of CaN in neurons.

Despite establishing CaN’s role in apoptosis, its inhibition alone is not enough to prevent the same, and therefore other mechanisms independent of CaN’s dephosphorylation of BAD must exist. This curiosity fueled the search for alternative CaN-linked pathways related to apoptosis. Falling in line with CaN’s role in the intrinsic pathway of apoptosis is another recently discovered component of mitochondrial dysfunction also facilitated by CaN during cellular duress: dynamin-related peptide 1 (DRP1). A cytosolic dynamin GTPase, DRP1 was one of the initial fission proteins to be discovered, but an understanding of its function still remains unclear. Mitochondrial fission is often necessary during biogenesis of new mitochondria, but it may also indicate dysfunction in the organelle and trigger apoptosis. DRP1 is proposed to trigger...
mitochondrial fission by transferring to the OMM surface and forming puncta of DRP1 to form an oligomeric chain that constricts mitochondria to facilitate fission.113 This event resembles to the way dynamin cleaves endocytic vessels.114 Being a GTPase in nature, DRP1 uses GTP-hydrolysis to cause scission of mitochondria through the twisting movement of the chain.115 CaN mediates the dephosphorylation at s637 required to activate DRP1 and translocate it to the OMM to initiate fission.116 However, controversy surrounds whether it is BAD-Bcl-xL dimerization or mitochondrial fission that transpires prior to eliciting cell death.117 Some studies suggest that BAX activation and cytochrome c release follow fission in mammalian cells.118 Other studies point to the overexpression of Bik, triggering CICR that fission occurs following Ca2+.119 The lack of evidence of elongated mitochondrial void of cytochrome c resulting from mitochondrial fusion suggests that fission, not fusion, is more involved in apoptosis.120 This makes sense as healthy cells require the fusion process to create a dynamic interconnected network and therefore take on an elongated morphology in contrast to the spherical shapes encountered in mitochondrial fission (see Figure 4).

This pivotal role of CaN in the intrinsic apoptotic signaling pathway further emphasizes the importance of the balance between phosphorylating and dephosphorylating events that dictate the fate of a cell.

8. CALCINEURIN: ROLE IN NEUROLOGICAL DISORDERS

The complexity of neurological disorders makes it difficult to dissect and diagnose the underlying etiology. However, certain etiologies ranging from dysfunctional VGCCs to disturbed mitochondrial dynamics have been common hallmarks of several neurological disorders.122,123 Keeping in perspective that Ca2+ is the engine that drives nearly all neuronal processes it is therefore worth investigating mediators that are intimately and heavily dependent on Ca2+ signaling, such as CaN.41 CaN, being an integral sensor and regulator of Ca2+ flux, may be deeply involved in many neurodegenerative disorders. The following sections explore the involvement of CaN through the spectrum of neurological disorders.

8.1. Ischemic Stroke.

The global burden of stroke is a major medical concern that continues to rise in the oncoming years.124 It currently ranks as the second highest cause of mortality worldwide for the elderly population aged 60 years or older.125 Ischemic stroke accounts for nearly 85% of strokes (the rest being hemorrhagic and transient in nature).126 Needless to say, more efficient therapy is in dire need to treat patients facing a narrow therapeutic window. A glance at the etiology shows that stroke, particularly ischemic stroke, is encompassed by excitotoxicity, oxidative and nitrosative stress, inflammation, and edema.127–129 Cerebral ischemia expresses a cumbersome

---

Figure 4. Calcineurin–mitochondrial (CaN–mitochondrial) connection. (A) At abnormally high calcium (Ca2+) influx rates, such as during elevated glutamate signaling, calcineurin (CaN) becomes highly active and triggers the pathway of apoptosis by dephosphorylating the pro-apoptotic factor Bcl-2-associated death promoter (BAD) which translocates to the outer mitochondrial membrane (OMM) and dimerizes with the anti-apoptotic factors B-cell lymphoma-2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xL). The event spurs another pro-apoptotic factor, Bcl-2-associated-X (BAX), to transition to the OMM and catalyze the formation of the mitochondrial permeability transition pore (mPTP). The sustained high Ca2+ load continues to maintain the opening of the mPTP, leading to the release of cytochrome c and formation of the apoptosome, culminating in the caspase cascade leading to apoptosis. (B) CaN has also shown to be involved in triggering mitochondrial fission by dephosphorylating the dynamin-related protein 1 (DRP1). DRP1 translocates to the OMM, forms puncta, and constricts the mitochondria with an oligomeric chain and splices the organelle similar to vesicular cleavage by dynamin. The fission of mitochondria is thought to come before the release of cytochrome c, leading to dysfunction and triggering apoptosis or necrosis based on the rate and severity of the event. Evidence has shown that mitochondrial fission is indicative of mitochondrial dysfunction, and the spherical mitochondrial debris void of cytochrome c implicates the occurrence of cell death pathways.

---
pathology that threatens neuronal viability not only during ischemia but after reperfusion as well; the underlying mechanisms have been well-delineated over the years.\textsuperscript{127,130-133} The ischemic phase sets the stage for cell damage as acidic conditions trigger various membrane pumps to dysfunction and thereby elicit heightened depolarization (Na\(^+-\)H\(^+\) exchanger pumps more Na\(^+\), the Na\(^+\)/K\(^+\) ATPase slows down, while Na\(^+\)/Ca\(^{2+}\) exchanger reverses and raises intracellular Ca\(^{2+}\) levels). Due to the elevated intracellular Ca\(^{2+}\) load, CaN has been reported to be elevated during cerebral ischemia.\textsuperscript{210} Reperfusion also entails Ca\(^{2+}\) overload (by reduction of extracellular H\(^+\) levels and therefore accelerating Na\(^+\)/H\(^+\) exchange and in turn more Ca\(^{2+}\) loading via Na\(^+\)/Ca\(^{2+}\) exchange) and consequently further elevates CaN activation. There is also greater Ca\(^{2+}\) uptake by the mitochondria beyond the capacity via the mitochondrial Ca\(^{2+}\) uniporter (MCU) due to restoration of the electrochemical potential.\textsuperscript{133} Often referred to as the “oxygen paradox”, this oxidative burst has been believed to be the culprit behind mPTP formation, and reperfusion often sends the cell mainly through necrotic/necroptotic signaling along with apoptotic signaling contingent on the extent of Ca\(^{2+}\) loading along with duration and severity of the ischemic milieu.\textsuperscript{134}

During the reperfusion phase, studies have discerned CaN’s ability to dephosphorylate BAD and induce apoptosis.\textsuperscript{107} Furthermore, CaN also dephosphorylates DRP-1 to induce mitochondrial fission and trigger apoptosis (see Table 1).\textsuperscript{116} Many of the mechanistic pathways still remain cryptic, and an understanding of CaN’s behavior throughout not only cerebral ischemia but also other neurodegenerative pathologies is difficult to pinpoint. However, a crosstalk of CaN with other biological molecules during cerebral ischemia may be proposed. In summary, CaN seems to play an integral role in ischemic stroke through the elevated Ca\(^{2+}\) influx, by virtue of activating several markers involved in mitochondrial and subsequent apoptotic signaling.

CaN seems to play an integral role in ischemic stroke through the elevated Ca\(^{2+}\) influx, by virtue of activating several markers involved in mitochondrial and subsequent apoptotic signaling.

### 8.2. Alzheimer’s Disease (AD). The global burden of Alzheimer’s disease (AD) is a major concern as 47 million AD patients have been diagnosed worldwide.\textsuperscript{135} The most prevalent neurodegenerative disease (and predominant cause of dementia) is projected to grow to 76 million cases by 2030. Often reported to impair brain histology, cognitive functions, and behavior as well as deeply impacting the physical, emotional, and financial lifestyle of patients and their close ones, AD is the most lethal and complicated cause of dementia.\textsuperscript{136} The ever-controversial pathology of AD has been hallmark by accumulative lesions of two degenerate proteins, amyloid-β (Aβ) and τ, and have been extensively studied.\textsuperscript{137,138} As AD is a disease where memories become lost, and CaN is a crucial protein deeply associated with learning and memory, this link therefore warrants a deeper investigation. Several studies honing on amyloidosis have detected abnormally high levels of CaN localized around the Aβ lesions and furthermore have detected the same in astrocytic processes enveloping amyloid β-protein (Aβ) plaques, neurofibrillary tangles, and neuritic dystrophy.

### 8.3. Parkinson’s Disease (PD).

PD is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra. The disease affects around 1% of the population older than 65 years old and is characterized by motor symptoms such as tremor, rigidity, and bradykinesia. PD is caused by the accumulation of Lewy bodies, which are aggregates of α-synuclein, a protein that is important in synaptic vesicle transport. The exact mechanism of how α-synuclein accumulates is not fully understood, but it is believed to be involved in mitochondrial dysfunction and oxidative stress.

### 8.4. Huntington’s Disease (HD).

HD is a hereditary neurodegenerative disease characterized by the presence of a trinucleotide repeat expansion in the Huntington’s disease protein (Huntingtin). This expansion leads to the production of a polyglutamine tract that is toxic to neurons in the basal ganglia. The disease is characterized by progressive movement disorders, cognitive impairment, and psychiatric symptoms. The pathogenesis of HD involves the accumulation of huntingtin as well as other proteins, including CaN.

### 8.5. Multiple Sclerosis (MS).

MS is an autoimmune disease that affects the nervous system. The disease is characterized by the development of lesions in the white matter of the brain and spinal cord, leading to a variety of symptoms such as numbness, weakness, and vision problems. The exact cause of MS is not known, but it is believed to involve an immune response against myelin, which is the insulating layer of nerve fibers. CaN expression has been detected in the astrocytes and microglia of MS patients, which suggests that it may play a role in the pathogenesis of the disease.

### 8.6. Epilepsy.

Epilepsy is a neurological disorder characterized by recurrent seizures. Seizures are caused by abnormal electrical discharges in the brain, which can lead to a variety of symptoms such as convulsions, altered consciousness, and behavioral changes. The exact cause of epilepsy is not known, but it is believed to involve a number of factors, including genetic predispositions, brain injury, and autoimmune processes. CaN has been implicated in the pathogenesis of epilepsy, as it has been detected in astrocytes and microglia in the brains of patients with epilepsy.

### 8.7. Schizophrenia.

Schizophrenia is a severe mental illness characterized by hallucinations, delusions, and disorganized thinking. The exact cause of schizophrenia is not known, but it is believed to involve a combination of genetic and environmental factors. CaN has been detected in the brains of patients with schizophrenia, which suggests that it may play a role in the pathogenesis of the disease. CaN has been implicated in the modulation of synaptic transmission, which is thought to be abnormal in schizophrenia.

### 8.8. Traumatic Brain Injury (TBI).

TBI is a common cause of neurological injury that can result in a wide range of symptoms, including cognitive impairment, memory loss, and behavioral changes. The exact cause of TBI is not known, but it is believed to involve a number of factors, including trauma, neuroinflammation, and oxidative stress. CaN has been detected in the brains of patients with TBI, which suggests that it may play a role in the pathogenesis of the disease. CaN has been implicated in the modulation of synaptic transmission, which is thought to be abnormal in TBI.

### Table 1. Summary of Calcineurin’s Role in Neurodegenerative Disorders

| Neurodegenerative Disorder | Pathology Involved | Potential Role of Calcineurin (CaN) |
|----------------------------|--------------------|-----------------------------------|
| Alzheimer’s disease (AD)   | Ischemia/reperfusion injury, excitotoxicity, oxidative stress, inflammation, mitochondrial dysfunction | CaN initiates astrocyte activation by NFAT transcription and astrocytic glutamate release and NFAT transcription of cytokines and oxidative and nitrative stress |
deposits. Certain studies report CaN to become aberrant due to the calcium overload triggered by \( \beta \) oligomer manipulation of glutamate-dependent cascades, though the mechanisms remain unclear. CaN seems to become dysregulated, upregulating the NFAT translocation and transcription, causing dendritic spine loss and neuritic dystrophy, changes that lie in complete contrast to homeostatic CaN-dependent NFAT that underlies axonal growth, elevated dendritic branching, and overall neuronal survival. Deregressed CaN also increases CaMKII dephosphorylation, which downregulates AMPAR-dependent LTD. This imbalance between the two enzymes results in greater LTD, synapse weakening, and memory loss (see Table 1). Thus, deregressed CaN may spur dendritic spine loss, dysregulate AMPAR-mediated LTD, and trigger apoptosis through mitochondrial-mediated pathways.

### 8.3. Parkinson’s Disease (PD)

PD follows AD as the second most burdensome neurodegenerative disease worldwide affecting a wide range of age groups: from 41 per 100,000 in the fourth decade to over 1900 per 100,000 for elderly above 80 years of age. PD is the most notorious movement disorder plagued by inclusions of aberrant \( \alpha \)-synuclein in both the perikaryon and the processes of dopaminergic distributed throughout the substantia nigra pars compacta region. Studies have reported altered and abnormal Ca\(^{2+}\) signaling induced by the synucleinopathy en route to weakening of LTP and memory impairment. CaN, deeply involved with LTP and memory formation, compellingly points to a link in PD. A study elicited CaN’s confounding switch between protective Ca\(^{2+}\) regulation to a cascade of downstream sequences that exacerbate the toxicity perpetuated by \( \alpha \)-synuclein. The study also witnessed enhanced nuclear localization of NFAT, in the brains of PD patients. This association of NFAT to PD further bolsters elevated cytosolic Ca\(^{2+}\) load and the failure of CaN. This may be due to several mechanisms unexplored at the time. However, it may be proposed that elevated Ca\(^{2+}\) due to \( \alpha \)-synuclein may be responsible for overactivating CaN which drives it toward LTP weakening. Meanwhile, sustained Ca\(^{2+}\) load prevents the rephosphorylation of NFAT and may explain its retention in the nucleus. The consequent inflammatory signaling interferes with synaptic connectivity and neurotransmission, exacerbating neuronal survival (see Table 1). Thus, CaN seems to entail LTP weakening, prolonged NFAT-mediated inflammation, and the consequential breakdown of neurotransmission.

### 8.4. Huntington’s Disease (HD)

Hallmarked by the death of medium spiny neurons of the striatum and the consequential aggressive progression of motor, cognitive, and behavioral decline, HD is now the most common monogenic brain disorder in developed nations (Europe and North America) with a prevalence of nearly 10 times more than that of Asian nations. The CAG expansion (beyond 35 repeats) in the gene responsible for the production of huntingtin (HTT) protein has been discovered in nearly 90% of HD patients. Pathology manifests through the aberrant splicing pattern of HTT fragments, particularly those accumulating in HTT exon 1 protein. However, the pathology comprises proteins involved in a vast array of neuronal functions, from regulating the cell cycle to cell signaling, emphasizing the complexity of HD and the quest for more promising therapeutic targets. A link between CaN and HD, associating elevated levels of the phosphatase in the disease after NMDAR activation triggering the intrinsic apoptotic pathway, has been reported. Studies have pointed to the phosphorylation of s421 evincing an inverse relationship with disease susceptibility. Greater dephosphorylation of s421 seems to disrupt the axonal transport and diminished BDNF transport, promoting neurodegeneration; the inhibition of CaN increased phosphorylation of s421 thereby restoring antero- and retrograde axonal transport in mutant HTT. Mutant HTT has also shown to downregulate CaN and trigger hyperphosphorylation of \( \tau \) protein in another study without any noticeable changes in \( \tau \) kinases (see Table 1). It can therefore be proposed that elevated CaN, due to hyperactivation of NMDARs (induced by mutant HTT), seems to disrupt axonal and BDNF transport and triggers mitochondrial-mediated apoptotic pathways.

### 8.5. Amyotrophic Lateral Sclerosis (ALS)

ALS is an age-dependent insidious and fatal motor neuron (MN) disease of the cortex, brain stem, and spinal cord, encompassing damage to both upper and lower motoneurons. It has a current global prevalence of 1.9 per 100,000 that threatens to amass by 69% by 2040. Along with the recent breakthrough discovery of a genetic culprit of ALS (an intrinsic hexanucleotide expansion in the C9orf72 gene), the predominance of pathological studies revolve around proteinopathies and Ca\(^{2+}\) overload excitotoxicity brought about hyperactivated glutamatergic signaling, mainly a ramification of the lack of GluR2 subunits and heightened permeability in AMPARs. The altered intracellular Ca\(^{2+}\) milieu sets off a Ca\(^{2+}\)-dependent cascade, still not clearly understood, which culminates in cell death. The affected neurons eventually lose their Ca\(^{2+}\) buffering capacities, and therefore, mitochondrial dysfunction and apoptosis via the intrinsic pathway have been reported. CaN has shown to be highly involved in triggering this pathway during Ca\(^{2+}\) overload. Plaques of trans-activating response region DNA binding protein (TDP-43) have been depicted in nearly 90% of ALS cases, and Liachko et al. have found that dephosphorylation of TDP-43 at sites s409/410 by CaN helps to limit toxic protein accumulation. Kim et al. shed further light on the interaction between TDP-43 and CaN when the group observed weakened dephosphorylation of TDP-43 by CaN after its poor binding with mutated superoxide dismutase 1 (SOD1). Furthermore, TDP-43 pathology is now believed to drive ALS progression along with being a factor in other proteinopathies like AD and PD (see Table 1). CaN seems to therefore exacerbate neuronal damage through its role in mitochondrial dysfunction as well through its failure of limiting the intracellular toxic TDP-43 levels due to interfering binding by SOD1.

### 8.6. Multiple Sclerosis (MS)

MS is a chronic, autoimmune inflammatory neurodegenerative disease that plagues about 2.5 million people worldwide. Glutamate-induced excitotoxicity has been implicated to be a driving force in neuronal death. Studies report that inflammatory cells in foci ensuing autoimmune inflammatory insult are a major source of extracellular glutamate, releasing it via cysteine/glutamate transporters Xc\(^{-}\). The surplus glutamate not only further activates more T-cells via their upregulated expression of NMDARs, but it also triggers the rapid influx of Ca\(^{2+}\) in neuronal cells that accelerates pro-apoptotic signaling. CaN is speculated to play a role in MS by virtue of the large intracellular Ca\(^{2+}\) load driving it to facilitate the intrinsic pathway and mitochondrial dysfunction. It also can be postulated that excitotoxicity drives the exocytotic kinetics through the activated CaN and synapsin 1 complex to release greater glutamate, which fuels the vicious T-cell-mediated autoimmunity and exacerbates excitotoxic milieu.
in neighboring neurons (see Table 1).\(^{36,59}\) CaN, by virtue of Ca\(^{2+}\) overload, seems to accelerate pro-apoptotic signaling as well as drive the excess exocytosis of glutamate into the synapse, exacerbating inflammation-induced neurodegeneration. Such a cascade may deserve deeper investigation in future studies concerning MS and the role of CaN.

### 9. OTHER NEURODEGENERATIVE DISORDERS

CaN is involved in other intricate neurological pathologies (Table 1). Knockdown of the CaN\(\gamma\) subunit has shown to alter synaptic vesicle cycling and has shown to cause psychiatric disorders like schizophrenia.\(^{37}\) In an epileptic study, CaN has shown to augment neuronal excitability by greater endocytosis of GABA\(_{A}\) receptors, disturbing the excitatory/inhibitory equilibrium to prevent inhibitory actions of GABA that mitigate the synchronous neuronal discharge responsible for seizure activity.\(^{160}\) The same study also showed the prevention of mitochondrial dysfunction by inhibition of CaN with cyclosporin A (CsA), a cyclophilin D binding drug that complexes with CaN to render it inactive and thereby preventing the opening of mPTP. A study based on traumatic brain injury (TBI) showed how the CaN/NFAT pathway following TBI induced astrocyte activation that further compounded the inflammatory environment and escalated neuronal damage.\(^{161}\) Neuroinflammation is a major component in CNS disorders and acute and chronic brain injury. Inhibition of CaN mitigated the detrimental activation of NFAT that transcribes inflammatory markers and recruits astrocytes and glial cells to the site of injury. In neuroinflammation studies, CaN plays both anti- and pro-inflammatory roles based on its interactions with transcription factors and displays a complex but unique dichotomy in mediating inflammatory milieu. For instance, it can associate with NFAT and activator protein 1 (AP1) to activate T-cells while inducing their anergy and tolerance by associating NFAT with forkhead box P3 (FOXP3).\(^{9}\) In conjunction, activation of Nuclear Factor \(\kappa B\) (NF\(\kappa B\)) and NFAT triggers the transcription of cytokines, activating astrocytes and microglia while promoting T-cell expansion in the CNS tissue, ultimately leading to inflammation-mediated neuronal damage and cell death.\(^{162}\)

### 10. IMMUNOSUPPRESSION OF CaN AND ITS EFFECTS ON THE BRAIN

CaN inhibitors (CNIs), namely, cyclosporine A and tacrolimus, have been immensely utilized in the field of organ transplantation, becoming the cornerstone therapy for preventing immune rejections.\(^{163}\) However, their effects on the brain remain a concern. CNI therapy used in kidney transplant patients has elicited neurological symptoms such as recurrent headaches, tremors, seizures, extrapyramidal syndrome, encephalopathy, and posterior encephalopathy syndrome.\(^{164}\)

Other clinical studies have shown neurotoxicity with the initiation of CNI therapy.\(^{165,166}\) A recent study focusing on the neural effects of CNIs in orthotopic liver transplant patients by Pfllgrat et al. showcased cognitive decline and altered brain structure.\(^{65}\) The neurological adverse reactions observed in CNI therapy further emphasize the complex role of CaN in the brain. Inhibiting CaN with CNIs alone to treat neurological disorders is still up for debate and requires extensive investigation to overcome the associated neurotoxicity. Furthermore, safer drugs void of neurotoxic side effects are desperately required in organ transplantation.

There are certain risk factors that can be assessed prior to organ transplantation that may help avoid possible neurologic complications. Encephalopathy, although often indicated in solid organ transplant patients, can be assessed by varying characteristic abnormalities observed in magnetic resonance imaging (MRI). For instance, altered signal changes can be observed in the insular and cingulated cortices with relatively lesser changes in occipital and perirolandic cortex may indicate possible neurotoxicity.\(^{166}\) Other diagnostic suggestions involve the requirement for more sensitive Doppler devices to monitor intracranial pressure noninvasively, monitoring of EEG levels post-transplant, and impaired verbal and motor responsiveness without electrolyte imbalance, tinnitus, or hearing loss (improved after discontinuation of tacrolimus).\(^{167}\) Alternative therapies to CNI involve monoclonal antibodies (belatacept) along with newer CNI-free therapies in the pipeline.\(^{170}\) CNI-free immunosuppressant therapy may prove to be beneficial in organ transplant patients in the future.\(^{171}\)

### 11. CONCLUSION

Neurodegenerative disorders present a major conundrum in medical science. The complex neuropathologies, despite sharing common pathological manifestations, arise from dysregulated crosstalk and genetic expressions that are difficult to trace. Some biological molecules involved in regulating much of the neuronal functions may be honed in on as potential therapeutic targets. CaN is one such target that is invertebrate to running essential neuronal components such as regulating Ca\(^{2+}\) homeostasis and Ca\(^{2+}\)-dependent downstream processes, synaptic plasticity, neurotransmission release, and synaptic cycling. Furthermore, it is involved in regulating transcription processes and the overall neuronal survival, operation, and maintenance. Due to such a diversified and crucial biological role, CaN may lie at the crux of many neurodegenerative disorders. From stroke to proteinopathies to inflammatory pathologies, CaN has been reported in underlying mechanistic pathways. However, it is rather perplexing to pinpoint whether CaN serves to protect and salvage neurons during neurological disorders or whether it perpetuates neuronal damage. The convoluted pathways of CaN exhibit its rescue of one aspect of the cell while deteriorating another during neuropathic conditions. Furthermore, some pathways are not truly delineated due to their erratic and volatile dispositions. Retentive in mind should also be that simple inhibition of an endogenous molecule as vital as CaN is to neuronal function alone may bring more harm than good in neurotherapy. Although many of the signaling cascades remain to be elucidated, and manipulating CaN may seem daunting, CaN warrants a deeper investigation. By bringing a greater focus to understanding its roles, it may be possible to strategize methods to modulate CaN as a potential therapeutic target to alleviate neurological disorders.

### AUTHOR INFORMATION

**Corresponding Author**  
Phone: +91-79 66745555. Fax: +91-79 66745560. E-mail: pallab.bhu@gmail.com; pallab.bhattacharya@niperahm.ac.in.

**ORCID**  
Pallab Bhattacharya: 0000-0003-2867-1650

**Author Contributions**  
J.S., P.B., K.R.D., and D.R.Y. conceived and designed the study. J.S., D.S., H.K., K.R.D., and P.B. outlined the performed...
rigorous literature search. J.S., D.S., H.K., K.K., and P.B. conceived and designed the figures and images. J.S., P.B., K.R.D., K.K., and D.R.Y. wrote the manuscript.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
The authors acknowledge Department of Science and Technology (DST), Government of India, for their financial support through Grant (SB/YS/LS-196/2014), International Society for Neurochemistry (ISN) Return Home Grant, Department of Pharmaceuticals, Ministry of Chemical and Fertilizers, Government of India, and National Institute of Pharmaceutical Education and Research (NIPER) Ahmedabad, Gandhinagar, India. The authors also want to express their thanks to Prof. Larry Benowitz, Boston Children’s Hospital, Harvard Medical School, Boston, MA, United States, and the Director, NIPER Ahmedabad, for providing necessary support.

REFERENCES
(1) Wang, J. H.; Desai, R. A brain protein and its effect on the Ca2+-and protein modulator-activated cyclic nucleotide phosphodiesterase. Biochim. Biophys. Acta. 1976, 72 (3), 926.
(2) Watterson, D. M.; Vanaman, T. C. Affinity chromatography purification of a cyclic nucleotide phosphodiesterase using immobilized modulator protein, a troponin C-like protein from brain. Biochim. Biophys. Acta. 1976, 73 (1), 40.
(3) Klee, C.; Krinks, M. Purification of cyclic 3’ 5’-nucleotide phosphodiesterase inhibitory protein by affinity chromatography on activator protein coupled to Sepharose. Biochemistry 1978, 17 (1), 120.
(4) Wallace, R. W.; Lynch, T. J.; Tallant, E. A.; Cheung, W. Y. An endogenous inhibitor protein of brain adenylylcyclase and cyclic nucleotide phosphodiesterase. Arch. Biochem. Biophys. 1978, 187 (2), 328.
(5) Wallace, R. W.; Lynch, T. J.; Tallant, E. A.; Cheung, W. Y. Purification and characterization of an inhibitor protein of brain adenylylcyclase and cyclic nucleotide phosphodiesterase. J. Biol. Chem. 1979, 254 (2), 377.
(6) Klee, C.; Crouch, T.; Krinks, M. Calcineurin: a calcium-and calmodulin-binding protein of the nervous system. Proc. Natl. Acad. Sci. U. S. A. 1979, 76 (12), 6270.
(7) Stewart, A. A.; Ingels, T. S.; Manalan, A.; Klee, C. B.; Cohen, P. Discovery of A Ca2+- and calmodulin-dependent protein phosphatase. FEBS Lett. 1982, 137 (1), 80.
(8) INGELSTOFF, T. S.; COHEN, P. The protein phosphatases involved in cellular regulation. Eur. J. Biochem. 1983, 132 (2), 255.
(9) Furman, J. L.; Norris, C. M. Calcineurin and glial signaling: neuroinflammation and beyond. J. Neuroinflammation 2014, 11 (1), 18.
(10) Liu, J.; Farmer, J. D.; Jr.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell 1991, 66 (4), 807.
(11) Carriedo, S. G.; Yin, H. Z.; Sensi, S. L.; Weiss, J. H. Rapid Ca2+ entry through Ca2+-permeable AMPA/Kainate channels triggers marked intracellular Ca2+ rises and consequent oxygen radical production. J. Neurosci. 1998, 18 (19), 7727.
(12) Cheng, C.; Fass, D. M.; Reynolds, I. J. Emergence of excitotoxicity in cultured forebrain neurons coincides with larger glutamate-stimulated [Ca2+]i increases and NMDA receptor mRNA levels. Brain Res. 1999, 849 (1–2), 97.
(13) Foster, T. C.; Sharrow, K. M.; Kato, J. B.; Norris, C. M.; Kumar, A. Calcineurin links Ca2+-disregulation with brain aging. J. Neurosci. 2001, 21 (11), 4066.
(14) Ingels, T. S.; Cohen, P. Protein phosphatases: properties and role in cellular regulation. Science 1983, 221 (4608), 331.

(15) Cohen, P. The structure and regulation of protein phosphatases. Annu. Rev. Biochem. 1989, 58 (1), 453.
(16) Cohen, P.; Cohen, P. Protein phosphatases come of age. J. Biol. Chem. 1989, 264 (36), 21435.
(17) Klee, C.; Draetta, G.; Hubbard, M. Calcineurin. Adv. Enzymol. Relat. Areas Mol. Biol. 2006, 61, 149.
(18) Griffith, J. P.; Kim, J. L.; Kim, E. E.; Sintchak, M. D.; Thomson, J. A.; Fleming, M. A.; Fleming, M.; Caron, P. R.; Hsiao, K.; Navia, M. A. X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12-FK506 complex. Cell 1995, 82 (3), 507.
(19) Coghlan, V. M.; Perrino, B. A.; Howard, M.; Langenberg, L. K.; Hicks, J. B.; Gallatin, W. M.; Scott, J. D. Association of protein kinase A and protein phosphate 2B with a common anchoring protein. Science 1995, 267 (5194), 108.
(20) Kissinger, C. R.; Parge, H. E.; Knighton, D. R.; Lewis, C. T.; Pelletier, L. A.; Tempczyk, A.; Kalish, V. J.; Tucker, K. D.; Showalter, R. E.; Moomaw, E. W. Crystal structures of human calcineurin and the human FKBP12–FK506–calcineurin complex. Nature 1995, 378 (6557), 641.
(21) Feng, B.; Stemmer, P. M. Interactions of calcineurin A, calcineurin B, and Ca2+. Biochemistry 1999, 38 (38), 12481.
(22) Gallagher, S. C.; Grace, Z. H.; Li, S.; Dyer, R. B.; Trewella; J.; Klee, C. B. There is communication between all four Ca2+-bindings sites of calcineurin A. Biochemistry 2001, 40 (40), 12094.
(23) Shen, X.; Li, H.; Ou, Y.; Tao, W.; Dong, A.; Kong, J.; Ji, C.; Yu, S. The secondary structure of calcineurin regulatory region and conformational change induced by calcium/calmodulin binding. J. Biol. Chem. 2008, 283 (17), 11407.
(24) Jiang, H.; Xiong, F.; Kong, S.; Ogawa, T.; Kobayashi, M.; Liu, J. O. Distinct tissue and cellular distribution of two major isoforms of calcineurin. Mol. Immunol. 1997, 34 (8–9), 665.
(25) Solà, C.; Tusell, J. M.; Serratosa, J. Comparative study of the distribution of calmodulin kinase II and calcineurin in the mouse brain. J. Neurosci. Res. 1999, 57 (5), 651.
(26) Yakel, J. L. Calcineurin regulation of synaptic function: from ion channels to transmitter release and gene transcription. Trends Pharmacol. Sci. 1997, 18 (4), 124.
(27) Miller, R. J. Multiple calcium channels and neuronal function. Science 1987, 235 (4784), 46.
(28) Kennedy, M. B. Regulation of neuronal function by calcium. Trends Neurosci. 1989, 12 (11), 417.
(29) Berthier, M. J.; Lipp, P.; Bootman, M. D. The versatility and universality of calcium signalling. Nat. Rev. Mol. Cell Biol. 2000, 1 (1), 11.
(30) Bading, H. Nuclear calcium signalling in the regulation of brain function. Nat. Rev. Neurosci. 2013, 14 (9), 593.
(31) Mansuy, I. M. Calcineurin in memory and bidirectional plasticity. Biochem. Biophys. Res. Commun. 2003, 311 (4), 1195.
(32) Verstegen, A. M.; Taglietti; E.; Lignani, G.; Marte, A.; Stolero, T.; Atias, M.; Corradi, A.; Valtorta, F.; Gitler, D.; Onofri, F. phosphorylation of synapsin I by cyclin-dependent kinase-5 sets the ratio between the resting and recycling pools of synaptic vesicles at hippocampal synapses. J. Neurosci. 2014, 34 (21), 7266.
(33) Alabi, A. A.; Tsien, R. W. Synaptic vesicle pools and dynamics. Cold Spring Harbor Perspect. Biol. 2012, 4 (8), a013680.
(34) Cottrell, J. R.; Levenson, J. M.; Kim, S. H.; Gibson, H. E.; Richardson, K. A.; Sivula, M.; Li, B.; Ashford, C. J.; Heindl, K. A.; Babcock, R. J. Working memory impairment in calcineurin knock-out mice is associated with alterations in synaptic vesicle cycling and disruption of high-frequency synaptic and network activity in prefrontal cortex. J. Neurosci. 2013, 33 (27), 10938.
(35) Kim, S. H.; Ryan, T. A. CDK5 serves as a major control point in neurotransmitter release. Neuron 2010, 67 (5), 797.
(36) Kim, S. H.; Ryan, T. A. Balance of calcineurin Aa and CDK5 activities sets release probability at nerve terminals. J. Neurosci. 2013, 33 (21), 8937.
(37) Cottrell, J. R.; Li, B.; Kyung, J. W.; Ashford, C. J.; Mann, J. J.; Horvath, T. L.; Ryan, T. A.; Kim, S. H.; Gerber, D. J. Calcineurin Aγ
is a functional phosphatase that modulates synaptic vesicle endocytosis. J. Biol. Chem. 2016, 291 (4), 1484.

(38) Ferguson, S. M.; De Camilli, P. Dynamin, a membrane-remodelling GTPase. Nat. Rev. Mol. Cell Biol. 2012, 13 (2), 75.

(39) Clayton, E. L.; Anggono, V.; Smillie, K. J.; Chau, N.; Robinson, P. J.; Cousin, M. A. The phospho-dependent dynamin-syndapin interaction triggers activity-dependent bulk endocytosis of synaptic vesicles. J. Neurosci. 2009, 29 (24), 7706.

(40) Wu, X.-S.; Zhang, Z.; Zhao, W.-D.; Wang, D.; Luo, F.; Wu, L.-G. Calcineurin is universally involved in vesicle endocytosis at neuronal and nonneuronal secretory cells. Cell Rep. 2014, 7 (4), 982.

(41) Berridge, M. J. Neuronal calcium signaling. Neuron 1998, 21 (1), 13.

(42) Ross, W. N. Understanding calcium waves and sparks in central neurons. Nat. Rev. Neurosci. 2012, 13 (3), 157.

(43) Wang, L.-Y.; Orser, B. A.; Brautigam, D. L.; MacDonald, J. F. Regulation of NMDA receptors in cultured hippocampal neurons by protein phosphatases 1 and 2A. Nature 1994, 369 (6477), 230.

(44) Krupp, J. J.; Vissel, B.; Thomas, C. G.; Heinemann, S. F.; Westbrook, G. L. Calcineurin acts via the C-terminus of NR2A to modulate desensitization of NMDA receptors. Neuropharmacology 2002, 42 (5), 593.

(45) Fino, E.; Paille, V.; Cui, Y.; Morera-Herreras, T.; Deniau, J. M.; Venance, L. Distinct coincidence detectors govern the corticothalamic spike timing-dependent plasticity. J. Physiol. 2010, 588 (16), 3045.

(46) Plotkin, J. L.; Day, M.; Surmeier, D. J. Synaptically driven state transitions in distal dendrites of striatal spiny neurons. Nat. Neurosci. 2011, 14 (7), 881.

(47) Oliveria, S. P.; Dell’Acqua, M. L.; Sather, W. A. AKAP79/150 anchoring of calcineurin controls neuronal L-type-Ca2+ channel activity and nuclear signaling. Neuron 2007, 55 (2), 261.

(48) Budde, T.; Meuth, S.; Pape, H.-C. Calcium-dependent inactivation of neuronal calcium channels. Nat. Rev. Neurosci. 2002, 3 (11), 873.

(49) Dittmer, P. J.; Dell’Acqua, M. L.; Sather, W. A. Ca2+-calcineurin-dependent inactivation of neuronal L-type Ca2+ channels requires priming by AKAP-anchored protein kinase A. Cell Rep. 2014, 7 (5), 1410.

(50) Cameron, A. M.; Steiner, J. P.; Roskams, A. J.; Ali, S. M.; Ronnett, G. V.; Snyder, S. H. Calcineurin associated with the inositol 1,4,5-trisphosphate receptor-FKBP12 complex modulates Ca2+ flux. Cell 1995, 83 (3), 463.

(51) Sun, H.; Leblanc, N.; Chao, Z.; Chen, L.; Gao, I. A.; Chen, L. Calcineurin mediates homeostatic synaptic plasticity by regulating retinoic acid synthesis. Proc. Natl. Acad. Sci. U. S. A. 1995, 92 (1), 121.

(52) Fabiato, A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. American Journal of Physiology-Cell Physiology 1983, 245 (1), C1.

(53) Arendt, K. L.; Zhang, Z.; Ganase, S.; Hintze, M.; Shin, M. M.; Tang, Y.; Cho, J.; Graef, I. A.; Chen, L. Calcineurin mediates homeostatic synaptic plasticity by regulating retinoic acid synthesis. Proc. Natl. Acad. Sci. U. S. A. 2011, 112 (42), E5744.

(54) Turrigiano, G. Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. Cold Spring Harbor Perspect. Biol. 2012, 4 (1), a005736.

(55) Malleret, G.; Haditsch, U.; Genoux, D.; Jones, M. W.; Bliss, T. V.; Vanhoose, A. M.; Weitlauf, C.; Kandel, E. R.; Winder, D. G.; Mansuy, I. M. Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell 2001, 104 (5), 675.

(56) Giese, K. P.; Mizuno, K. The roles of protein kinases in learning and memory. Learn. Mem. 2013, 20 (10), 540.

(57) Lissman, J.; Yasuda, R.; Raghavachari, S. Mechanisms of CAMKII action in long-term potentiation. Nat. Rev. Neurosci. 2012, 13 (3), 169.

(58) Baumgärtel, K.; Mansuy, I. M. Neural functions of calcineurin in synaptic plasticity and memory. Learn. Mem. 2012, 19 (9), 375.

(59) Beattie, E. C.; Carroll, R. C.; Yu, X.; Morishita, W.; Yasuda, H.; von Zastrow, M.; Malenka, R. C. Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. Nat. Neurosci. 2000, 3 (12), 1291.

(60) Jones, M. V.; Westbrook, G. L. Shaping of IPSCs by endogenous calcineurin activity. J. Neurosci. 1997, 17 (20), 7626.

(61) Lu, Y. M.; Mansuy, I. M.; Kandel, E. R.; Roder, J. Calcineurin-mediated LTD of GABAergic inhibition underlies the increased excitability of CA1 neurons associated with LTD. Neuron 2000, 26 (1), 197.

(62) Wang, J.; Liu, S.; Haditsch, U.; Tu, W.; Cochrane, K.; Ahmadian, G.; Tran, L.; Paw, J.; Wang, Y.; Mansuy, I. Interation of calcineurin and type-A GABA receptor y2 subunits produces long-term depression at CA1 inhibitory synapses. J. Neurosci. 2003, 23 (3), 826.

(63) Winder, D. G.; Mansuy, I. M.; Osman, M.; Moslem, T. M.; Kandel, E. R. Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. Cell 1998, 92 (1), 25.

(64) Lüscher, C.; Malenka, R. C. NMDA-receptor-dependent long-term potentiation and long-term depression (LTP/LTD). Cold Spring Harbor Perspect. Biol. 2012, 4 (6), a005710.

(65) Malenka, R. C.; Bear, M. F. LTP and LTD: an embarrassment of riches. Neuron 2004, 44 (1), 5.

(66) Lin, L.; Sun, W.; Kung, F.; Dell’Acqua, M. L.; Hoffman, D. A. AKAP79/150 impacts intrinsic excitability of hippocampal neurons through phospho-regulation of A-type K+ channel trafficking. J. Neurosci. 2011, 31 (4), 1323.

(67) Yao, J.-j.; Zhao, Q.-R.; Liu, D.-D.; Chow, C.-W.; Mei, Y.-A. Neuritun Up-regulates Kv4.2 α-Subunit of Potassium Channel Expression and Affects Neuronal Excitability by Regulating the Calcium-Calcineurin-NFAT,4 Signaling Pathway. J. Biol. Chem. 2016, 291 (33), 17369.

(68) Jinno, S.; Jeromin, A.; Kosaka, T. Postsynaptic and extrasynaptic localization of Kv4.2 channels in the mouse hippocampal region, with special reference to targeted clustering at gabaaergic synapses. Neuroscience 2005, 134 (2), 483.

(69) Alonso, G.; Widmer, H. Clustering of Kv4.2 potassium channels in postsynaptic membrane of rat supraoptic neurons: an ultrastructural study. Neuroscience 1997, 77 (3), 617.

(70) Kim, J.; Wei, D. S.; Hoffman, D. A. Kv4 potassium channel subunits control action potential repolarization and frequency-dependent broadening in rat hippocampal CA1 pyramidal neurones. J. Physiol. 2005, 569 (1), 41.

(71) Hoffman, D. A.; Magee, J. C.; Colbert, C. M.; Johnston, D. K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. Nature 1997, 387 (6636), 869.

(72) Yashiro, K.; Philpot, B. D. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology 2008, 55 (7), 1081.

(73) Schrader, L. A.; Anderson, A. E.; Mayne, A.; Pfaffinger, P. J.; Sweatt, J. D. PKA modulation of Kv4.2-encoded A-type potassium channels requires formation of a supramolecular complex. J. Neurosci. 2002, 22 (23), 10123.
plasticity: AMPA receptor trafficking. ACS Central Science

ACS Central Science

lacking A-type K+ channel subunits. Hippocampus

and function. Pharmacol. Rev.

Dingledine, R. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol. Rev. 2010, 62 (3), 405.

Shepherd, J. D.; Huganir, R. L. The cell biology of synaptic plasticity: AMPA receptor trafficking. Annu. Rev. Cell Dev. Biol. 2007, 23, 613.

Lee, H.-K.; Takamiya, K.; Han, J.-S.; Man; H.; Kim, C.-H.; Rumbaugh, G.; Yu, S.; Ding, L.; He, C.; Petrolea, R. S. Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 2003, 112 (5), 631.

Sekine-Aizawa, Y.; Huganir, R. L. Regulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor trafficking through PKA phosphorylation of the Glu receptor 1 subunit. Proc. Natl. Acad. Sci. U. S. A. 2007, 104 (9), 3579.

Roche, K. W.; O’brien, R. J.; Mammen, A. L.; Bernhardt, J.; Huganir, R. L. Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 1996, 16 (6), 1179.

Huganir, R. L.; Kameyama, K.; Huganir, R. L.; Bear, M. F. NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. Neuron 1998, 21 (5), 1151.

Jong, Y.-J.; Kumar, V.; O’Malley, K. L. Intracellular metabotropic glutamate receptor 5 (mGlur5) activates cascades distinct from cell surface counterparts. J. Biol. Chem. 2009, 284 (51), 35827.

Fagni, L.; Chavis, P.; Ango, F.; Bockaert, J. Complex interactions between mGlRs, intracellular Ca2+ stores and ion channels in neurons. Trends Neurosci. 2000, 23 (2), 80.

Alagarsamy, S.; Saugstad, J.; Warren, L.; Mansuy, I. M.; Gereau IV, R. W.; Conn, P. J. NMDA-induced potentiation of mGlRs is mediated by activation of protein phosphatase 2B/calcineurin. Neuropharmacology 2005, 49, 135.

Crabtree, G. R.; Olson, E. N. NFAT signaling: choreographing the social lives of cells. Cell 2002, 109 (2), S67.

Macian, F. NFAT proteins: key regulators of T-cell development and function. Nat. Rev. Immunol. 2005, 5 (6), 472.

Crabtree, G. R.; Schreiber, S. L. SnapShot: Ca2+-calcineurin-NFAT signaling. Cell 2009, 138 (1), 210.

Shin, S.-Y.; Yang, H. W.; Kim, J.-R.; Do Heo, W.; Cho, K.-H. A hidden incoherent switch regulates RCAN1 in the calcineurin–NFAT signaling network. J. Cell Sci. 2011, 124 (1), 82.

Kipanulya, M. J.; Kimaro, W. H.; Ete, P. F. The emerging roles of the calcineurin-nuclear factor of activated T-lymphocytes pathway in nervous system functions and diseases. Journal of Aging Research 2016, 2016, 5081021.

Shin, S.-Y.; Choo, S.-M.; Kim, D.; Baek, S. J.; Wolkenhauer, O.; Cho, K.-H. Switching feedback mechanisms realize the dual role of MCP in the regulation of calcineurin activity. FEBS Lett. 2006, 580 (25), 5965.

Parekh, A. B.; Putney, J. W., Jr Store-operated calcium channels. Physiol. Rev. 2005, 85 (2), 757.

Nguyen, T.; Di Giovanni, S. NFAT signaling in neural development and axon growth. Int. J. Dev. Neurosci. 2008, 26 (2), 141.

Gwack, Y.; Sharmar, S.; Naradone, J.; Tanasa, B.; Iuga, A.; Srikant, S.; Okamura, H.; Bolton, D.; Feske, S.; Hogan, P. G. A genome-wide Drosophila RNAi screen identifies DYRK-family kinases as regulators of NFAT. Nature 2006, 441 (7093), 646.

Beals, C. R.; Sheridan, C. M.; Turck, C. W.; Gardner, P.; Crabtree, G. R. Nuclear export of NF-ATc enhanced by glycogen synthase kinase-3. Science 1997, 275 (5308), 1930.

Green, D. R.; Reed, J. C. Mitochondria and apoptosis. Science 1998, 281 (5381), 1309–1312.

Rossé, T.; Olivier, R.; Monney, L.; Rager, M.; Consus, S.; Fellay, I.; Jansen, B.; Borner, C. Bcl-2 prolongs cell survival after Bax-induced release of cytochrome c. Nature 1998, 391 (6666), 496.

Nicholls, D. G.; Budd, S. L. Mitochondria and neuronal survival. Physiol. Rev. 2000, 80 (1), 315.

Green, D. R.; Kroemer, G. The pathophysiology of mitochondrial cell death. Science 2004, 305 (5684), 626.

Guyton, K. Z.; Liu, Y.; Goroose, M.; Xu, Q.; Holbrook, N. J. Activation of mitogen-activated protein kinase by ho role in cell survival following oxidant injury. J. Biol. Chem. 1996, 271 (8), 4138.

Philpott, K. L.; McCarthy, M. J.; Klippel, A.; Rubin, L. L. Activated phosphatidylinositol 3-kinase and Akt kinase promote survival of superior cervical neurons. J. Cell Biol. 1997, 139 (3), 809.

Chen, J.; Fujii, K.; Zhang, L.; Roberts, T.; Fu, H. Raf-1 promotes cell survival by antagonizing apoptosis signal-regulating kinase 1 through a MEK–ERK independent mechanism. Proc. Natl. Acad. Sci. U. S. A. 2001, 98 (14), 7783.

Datta, S. R.; Ranger, A. M.; Lin, M. Z.; Sturgill, J. F.; Ma, Y.-C.; Cowan, C. W.; Dikkes, P.; Korsmeyer, S. J.; Greenberg, M. E. Survival factor-mediated BAD phosphorylation raises the mitochondrial threshold for apoptosis. Dev. Cell. 2002, 3 (5), 631.

Wang, H.-G.; Pathan, N.; Ethell, I. M.; Krajewski, S.; Yamaguchi, Y.; Shibasaki, F.; McKeon, F.; Bobo, T.; Franke, T. F.; Reed, J. C. Ca2+-induced apoptosis through calcineurin dephosphorylation of BAD. Science 1999, 284 (5412), 3339.

Spence, A. M.; Flippo, K. H.; Lobas, M. A.; Houtman, J. C.; Strack, S. A calcineurin docking motif (LXVP) in dynamin-related protein 1 contributes to mitochondrial fragmentation and ischemic neuronal injury. J. Biol. Chem. 2013, 288 (17), 12353.

Manczak, M.; Reddy, P. H. Abnormal interaction between the mitochondrial fission protein Drp1 and hyperphosphorylated tau in Alzheimer’s disease neurons: implications for mitochondrial dysfunction and neuronal damage. Hum. Mol. Genet. 2012, 21 (11), 2538.

Qi, X.; Qvit, N.; Su, Y.-C.; Mohly-Rosens, D. A novel Drp1 inhibitor diminishes aberrant mitochondrial fission and neurotoxicity. J. Cell Sci. 2013, 126 (3), 789.

Faelber, K.; Held, M.; Gao, S.; Posor, Y.; Haucke, V.; Noé, F.; Daumke, O. Structural insights into dynamin-mediated membrane fission. Structure 2012, 20 (10), 1621.

Fröhlich, C.; Grabiger, S.; Schwefel, D.; Faelber, K.; Rosenbaum, E.; Mears, J.; Rocks, O.; Daumke, O. Structural insights into oligomerization and mitochondrial remodelling of dynamin-1-like protein. EMBO J. 2013, 32 (9), 12880.

Archer, S. L. Mitochondrial dynamics—mitochondrial fission and fusion in human diseases. N. Engl. J. Med. 2013, 369 (23), 2236.

Youle, R. J.; Karbowski, M. Mitochondrial fission in apoptosis. Nat. Rev. Mol. Cell Biol. 2005, 6 (8), 657.

Breckenridge, D. G.; Stojanovic, M.; Marcellus, R. C.; Shore, G. C. Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome c release to the cytosol. J. Cell Biol. 2003, 160 (7), 1115.

Germain, M.; Mathai, J. P.; McBride, H. M.; Shore, G. C. Endoplasmic reticulum BIK initiates DRP1-regulated remodelling of mitochondrial cristae during apoptosis. EMBO J. 2005, 24 (8), 1546.

Martinou, J.-C.; Youle, R. Which came first, the cytochrome c release or the mitochondrial fission? Cell Death Differ. 2006, 13 (13), 1291–1295.

Chen, H.; Detmer, S. A.; Ewald, A. J.; Griffin, E. E.; Fraser, S. E.; Chan, D. C. Mitofusins Mfn1 and Mfn2 coordinate regulation
mitochondrial fusion and are essential for embryonic development. *J. Cell Biol.*, **2003**, *160* (2), 189.

(122) Zamponi, G. W. Targeting voltage-gated calcium channels in neurological and psychiatric diseases. *Nat. Rev. Drug Discovery*, **2016**, *15* (1), 19.

(123) Burté, F.; Carelli, V.; Chinnery, P. F.; Yu-Wai-Man, P. Disturbed mitochondrial dynamics and neurodegenerative disorders. *Nat. Rev. Neurol.*, **2015**, *11* (1), 11.

(124) Kaur, H.; Sarmah, D.; Saraf, J.; Vats, K.; Kalia, K.; Borah, A.; Yavagal, D. R.; Dave, K. R.; Ghosh, Z.; Bhattacharya, P. Noncoding RNAs in ischemic stroke: time to translate. *Ann. N. Y. Acad. Sci.*, **2018**, *1421* (1), 19–36.

(125) Feigin, V. L.; Forouzanfar, M. H.; Krishnamurthi, R.; Mensah, G. A.; Connor, M.; Bennett, D. A.; Moran, A. E.; Sacco, R. L.; Anderson, L.; Truelsen, T. Global and regional burden of stroke during 1990–2010: findings from the Global Burden of Disease Study 2010. *Lancet*, **2014**, *383* (9913), 245.

(126) Sarmah, D.; Agrawal, V.; Rane, P.; Bhute, S.; Watanabe, M.; Kalia, K.; Ghosh, Z.; Dave, K. R.; Yavagal, D. R.; Bhattacharya, P.; Mesenchymal Stem Cell therapy in ischemic stroke: A meta-analysis of preclinical studies. *Clin. Pharmacol. Ther.*, **2018**, *103* (6), 990–998.

(127) Chamorro, A.; Dinsdagl, U.; Urra, X.; Planas, A. M. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *Lancet Neurol.*, **2016**, *15* (8), 869.

(128) Bhattacharya, P.; Pandey, A. K.; Paul, S.; Patnaik, R. Melatonin renders neuroprotection by protein kinase C mediated aquaporin-4 inhibition in animal model of focal cerebral ischemia. *Life Sci.*, **2014**, *100* (2), 97.

(129) Sarmah, D.; Kaur, H.; Saraf, J.; Pravalka, K.; Goswami, A.; Kalia, K.; Borah, A.; Wang, X.; Dave, K. R.; Yavagal, D. R. Getting Closer to an Effective Intervention of Ischemic Stroke: The Big Promise of Stem Cell. *Transl. Stroke Res.*, **2017**, DOI: 10.1007/s12975-017-0580-0.

(130) Bhosale, G.; Sharpe, J. A.; Sundier, S. Y.; Duchen, M. R. Calcium signaling as a mediator of cell energy demand and a trigger to cell death. *Ann. N. Y. Acad. Sci.*, **2015**, *1350* (1), 107.

(131) Manzanero, S.; Santro, T.; Arumugam, T. V. Neuronal oxidative stress in acute ischemic stroke: sources and contribution to cell injury. *Neurochem. Int.*, **2013**, *62* (5), 712.

(132) Niatetskaya, Z. V.; Susonov, S. A.; Matsuuevich, D.; Uktsina-Susonova, I. V.; Ratner, V. I.; Starkov, A. A.; Ten, V. S. The oxygen free radicals originating from mitochondrial complex I contribute to oxidative brain injury following hypoxia–ischemia in neonatal mice. *J. Neurosci.*, **2012**, *32* (9), 3235.

(133) Qiu, J.; Tan, Y.-W.; Hagenston, A. M.; Martel, M.-A.; Currey, H. N.; Taylor, L. M.; Wheeler, J. M.; Oblak, A. L.; Ghetti, B.; Montine, T. J. The phosphatase calcineurin regulates pathological diseases. *Proc. Natl. Acad. Sci. U. S. A.*, **2014**, *111* (34), E3544.

(134) Pineda, J. R.; Pardo, R.; Zala, D.; Yu, H.; Humbert, S.; Saudou, F. Genetic and pharmacological inhibition of calcineurin corrects the BDNF transport defect in Huntington’s disease. *Mol. Brain*, **2009**, *2* (1), 33.

(135) Grateau, M.; Noël, A.; Julien, C.; Cisbani, G.; Milot-Rousseau, P.; Morin, F.; Dickler, M.; Goupil, C.; Bezeau, F.; Poitras, I. Tau hyperphosphorylation and deregulation of calcineurin in mouse models of Huntington’s disease. *Hum. Mol. Genet.*, **2015**, *24* (1), 86.

(136) Arthur, K. C.; Calvo, A.; Price, T. R.; Geiger, J. T.; Chio, A.; Traynor, B. J. Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. *Nat. Commun.*, **2017**, *8*, 12408.

(137) Renton, A. E.; Majounie, E.; Waite, A.; Simón-Sánchez, J.; Rollinson, S.; Gibbs, J. R.; Schymick, J. C.; Läkkösvirta, H.; Van Swieten, J. C.; Myllykangas, L. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron*, **2011**, *72* (2), 257.

(138) Robberecht, W.; Philips, T. The changing scene of amyotrophic lateral sclerosis. *Nat. Rev. Neurosci.*, **2013**, *14* (4), 248.

(139) Turner, M. R.; Hardiman, O.; Benatar, M.; Brooks, B. R.; Chio, A.; De Carvalho, M.; Ince, P. G.; Lin, C.; Miller, R. G.; Montine, T. J. The phosphatase calcineurin regulates pathological TDP-43 phosphorylation. *Acta Neuropathol.*, **2016**, *132* (4), 545.

(140) Kim, J. M.; Billington, E.; Reyes, A.; Notarrianni, T.; Sage, J.; Agbas, E.; Taylor, M.; Monast, J.; Stanford, J. A.; Agbas, A. Impaired Cu–Zn Superoxide Dismutase (SOD1) and Calcineurin (Cn) Interaction in ALS: A Presumed Consequence for TDP-43 and Zinc Aggregation in Tg SOD1 G93A Rodent Spinal Cord Tissue. *Neurochem. Res.*, **2018**, DOI: 10.1007/s11064-017-2461-z.
Macrez, R.; Stys, P. K.; Vivien, D.; Lipton, S. A.; Docagne, F. Mechanisms of glutamate toxicity in multiple sclerosis: biomarker and therapeutic opportunities. *Lancet Neurol.* 2016, 15 (10), 1089.

Evonuk, K. S.; Baker, B. J.; Doyle, R. E.; Moseley, C. E.; Sester, C. M.; Johnston, B. P.; De Sarno, P.; Tang, A.; Gembitsky, I.; Hewett, S. J. Inhibition of system \( \text{xc}^- \) transporter attenuates autoimmune inflammatory demyelination. *J. Immunol.* 2015, 195 (2), 450.

Jung, S.; Yang, H.; Kim, B. S.; Chu, K.; Lee, S. K.; Jeon, D. The immunosuppressant cyclosporin A inhibits recurrent seizures in an experimental model of temporal lobe epilepsy. *Neurosci. Lett.* 2012, 529 (2), 133.

Furman, J. L.; Sompol, P.; Kraner, S. D.; Pleiss, M. M.; Putman, E. J.; Dunkerson, J.; Abdul, H. M.; Roberts, K. N.; Schell, S. W.; Norris, C. M. Blockade of astrocytic calcineurin/NFAT signaling helps to normalize hippocampal synaptic function and plasticity in a rat model of traumatic brain injury. *J. Neurosci.* 2016, 36 (5), 1502.

Gross, C.; Yao, X.; Engel, T.; Tiwari, D.; Xing, L.; Rowley, S.; Danielson, S. W.; Thomas, K. T.; Jimenez-Mateos, E. M.; Schroeder, L. M. MicroRNA-mediated downregulation of the potassium channel Kv4. 2 contributes to seizure onset. *Cell Rep.* 2016, 17 (1), 37.

Azzi, J. R.; Sayegh, M. H.; Mallat, S. G. Calcineurin inhibitors: 40 years later, can't live without… *J. Immunol.* 2013, 191 (12), 5785.

Ishikura, K.; Ikeda, M.; Hamasaki, Y.; Hataya, H.; Shishido, S.; Asanuma, H.; Nishimura, G.; Hiramoto, R.; Honda, M. Posterior reversible encephalopathy syndrome in children: its high prevalence and more extensive imaging findings. *Am. J. Kidney Dis.* 2006, 48 (2), 231.

Pflugrad, H.; Schrader, A. K.; Tryc, A. B.; Ding, X.; Lanfermann, H.; Jäckel, E.; Schrem, H.; Beneke, J.; Barg-Hock, H.; Klempnauer, J. Longterm calcineurin inhibitor therapy and brain function in patients after liver transplantation. *Liver Transplantation* 2018, 24 (1), 56.

Bindu, P. S.; Sinha, S.; Taly, A. B.; Christopher, R.; Kovoor, J. M. Cranial MRI in acute hyperammonemic encephalopathy. *Pediatric neurology* 2009, 41 (2), 139.

Pruitt, A. A.; Graus, F.; Rosenfeld, M. R. Neurological complications of solid organ transplantation. *Neurohospitalist* 2013, 3 (3), 152.

Kumar, D.; LeCorchick, S.; Gupta, G. Belatacept as an alternative to calcineurin inhibitors in patients with solid organ transplants. *Front. Med.* 2017, 4, 60.

Teperman, L.; Moonka, D.; Sebastian, A.; Sher, L.; Marotta, P.; Marsh, C.; Koneru, B.; Goss, J.; Preston, D.; Roberts, J. P. Calcineurin Inhibitor–Free Mycophenolate Mofetil/Sirolimus Maintenance in Liver Transplantation: The Randomized Spare-the-Nephron Trial. *Liver transplantation* 2013, 19 (7), 675.

Webber, A. B.; Vincenti, F. An Update on Calcineurin Inhibitor–Free Regimens: The Need Persists, but the Landscape has Changed. *Transplantation* 2016, 100 (4), 836.

Goede, L.; Pflugrad, H.; Schmitz, B.; Tryc, A. B.; Barg-Hock, H.; Klempnauer, J.; Weissenborn, K.; Lanfermann, H.; Ding, X.-Q. Neurotoxic Side Effects of Calcineurin Inhibitors in Patients After Liver Transplantation: Preliminary Results of a Quantitative MRI Study of the Brain. *J. Clin. Exp. Hepatol.* 2017, 7, S31.

DOI: 10.1021/acscentsci.8b00230