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Calcineurin/NFAT Signaling in the Temporal Integration of Ca^{2+} Stress in Neurodegeneration

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Abstract

The calcineurin (CaN)/nuclear factor of activated T cells (NFAT) signalling axis is a Ca^{2+}-responsive pathway that translates intracellular Ca^{2+} signals into long-term transcriptional programs. Chronic disruption of intracellular Ca^{2+} homeostasis is a convergent feature of neurodegenerative disorders, particularly Alzheimer's disease (AD) and Parkinson's disease (PD). In these conditions, sustained or repetitive Ca^{2+} elevations promote prolonged activation of the CaN/NFAT pathway, thereby linking Ca^{2+} dysregulation to persistent cellular responses. In this review, we summarize the molecular organization and regulation of the Ca^{2+}/CaN/NFAT pathway and discuss its physiological roles in neurons and glial cells, including synaptic plasticity, neurodevelopment, neurogenesis, and neuroinflammatory responses.

We critically examine experimental evidence linking CaN/NFAT signalling to AD and PD, distinguishing direct mechanistic roles from associative and model-dependent findings. Across disease contexts, the CaN/NFAT axis appears to function as a molecular node at which diverse insults, including amyloid-β and tau aggregates, α-synuclein toxicity, mitochondrial dysfunction, and chronic inflammatory cues, converge under conditions of sustained Ca^{2+} dysregulation. We propose that the pathological relevance of CaN/NFAT lies less in pathway activation per se than in its capacity to convert chronic Ca^{2+}-dependent stress signals into persistent transcriptional states affecting synaptic integrity, inflammatory tone, and cellular resilience. We conclude by discussing current therapeutic strategies targeting this pathway, their limitations, and the need for temporally and cell-type-specific modulation.

Facts

● Chronic Ca^{2+} dyshomeostasis is a recurrent feature of neurodegenerative disorders and provides a common condition for sustained activation of the CaN/NFAT signaling axis.

● CaN /NFAT signaling is unlikely to act as a primary initiator of AD or PD; rather, it functions as a stress-responsive node that links prolonged Ca^{2+} disturbances to long-term transcriptional changes.

● In neurons, sustained CaN /NFAT activation is associated with altered synaptic maintenance, dendritic remodeling, and reduced cellular resilience.

● In astrocytes and microglia, NFAT signaling shapes the duration and intensity of neuroinflammatory responses, acting in concert with pathways such as NF-κB.

● Therapeutic targeting of this pathway requires temporal and cell-type-specific precision, because broad CaN inhibition may disrupt essential physiological functions in the CNS.

Open questions

● When does CaN /NFAT activation shift from an adaptive response to a maladaptive transcriptional program in neurodegeneration?

● Which neuronal and glial populations are most vulnerable to maladaptive NFAT activation in AD and PD?

● How do different sources of Ca^{2+} dysregulation, including receptor-mediated entry, mitochondrial dysfunction, membrane disruption, and calpain-dependent calcineurin activation, shape NFAT-dependent transcription?

● Can selective or temporally restricted modulation of CaN/NFAT signaling prevent pathological persistence while preserving physiological Ca^{2+}-dependent functions?

● Which biomarkers could identify patients or disease stages most likely to benefit from CaN/NFAT pathway modulation?

Introduction

Neurodegenerative disorders, particularly Alzheimer's disease (AD) and Parkinson's disease (PD), represent a growing medical and societal challenge worldwide [1] for which effective disease-modifying therapies remain unavailable[2–6]. Substantial progress in elucidating disease-associated mechanisms such as protein misfolding, amyloid and tau pathology, oxidative stress, synaptic dysfunction and neuronal loss has been made in recent years.

Among them, chronic disruption of intracellular calcium (Ca^{2+}) homeostasis has emerged as a recurring feature of neurodegeneration [7,8]. Ca^{2+} is a ubiquitous second messenger that links extracellular stimuli to intracellular signalling pathways and plays a central role in neuronal excitability, synaptic plasticity and gene expression, as well as in glial cell function [9]. Alterations in Ca^{2+} dynamics have been reported in both AD and PD, where sustained elevations of intracellular Ca^{2+} are thought to exacerbate synaptic failure, mitochondrial dysfunction and neuroinflammatory responses [10,11].

A key molecular effector translating Ca^{2+} signals into long-lasting cellular responses is the calcineurin (CaN)/nuclear factor of activated T cells (NFAT) signalling pathway. CaN is a Ca^{2+}/calmodulin-dependent phosphatase that responds to the amplitude and duration of intracellular Ca^{2+} signals and regulates the nuclear translocation and transcriptional activity of NFAT family members. In the central nervous system (CNS), CaN/NFAT signalling has been implicated in the regulation of neuronal survival, synaptic plasticity and glial inflammatory programs [12,13]

While the CaN/NFAT signalling axis is essential for physiological brain function, its sensitivity to Ca^{2+} dynamics also makes it vulnerable to pathological activation [14]. Indeed, sustained alterations of intracellular Ca^{2+} concentration can lead to prolonged CaN activation and persistent NFAT-dependent transcription, potentially linking synaptic dysfunction to maladaptive neuroinflammatory amplification [15]. Therefore, CaN and NFAT are unlikely to be a primary cause of neurodegeneration, but they more realistically represent a central signalling node through which diverse pathological insults, such as amyloid-β and tau-associated stress in AD, α-synuclein toxicity and mitochondrial Ca^{2+} burden in PD, converge to trigger shared transcriptional and cellular responses [13].

In this review, we summarize the molecular organization and regulation of the Ca^{2+}/CaN/NFAT pathway and discuss its physiological roles in neurons and glial cells. We also emphasise recent

advances that refine the causal chain between pathological Ca^{2+} dyshomeostasis and CaN/NFAT-dependent transcription. These include membrane-level mechanisms of Aβ-induced Ca^{2+} entry, and proteolytic mechanisms that may convert transient CaN activation into persistent phosphatase activity. We then evaluate experimental evidence implicating CaN/NFAT signalling in the pathogenesis of AD and PD, highlighting where data support direct mechanistic links versus associative or model-dependent relationships. Finally, we discuss therapeutic strategies aimed at modulating this pathway, from classical CaN inhibitor, including preclinical low dose therapeutic treatments, to NFAT-selective approaches and Ca^{2+}-modulating interventions, with particular attention to their translational potential and limitations in the central nervous system.

Calcineurin: structure, regulation and functional role in the CNS

Calcineurin (CaN), also known as protein phosphatase 2B (PP2B), is a Ca^{2+}/calmodulin-dependent serine/threonine phosphatase originally identified in brain tissue as a major calmodulin-binding protein [16]. Structurally, CaN is a heterodimer composed of a catalytic A subunit (CnA) and a regulatory B subunit (CnB) (Figure 1A). Three CnA isoforms (CnAα, CnAβ and CnAγ) have been described, of which CnAα and CnAβ are predominantly expressed in the brain, whereas CnAγ shows restricted expression in testis and peripheral tissues [17].

The CnA subunit contains the catalytic domain, a calmodulin-binding region and a C-terminal autoinhibitory domain (AID) (Figure 1A). Under basal conditions, the AID occludes the active site, maintaining CaN in an inactive state. Elevation of intracellular Ca^{2+} leads to Ca^{2+} binding to CnB and calmodulin, promoting calmodulin association with CnA and displacement of the AID, thereby activating the phosphatase [18–20] (Figure 1A). This activation mechanism enables CaN to decode both the amplitude and duration of Ca^{2+} signals, distinguishing transient physiological Ca^{2+} fluctuations from sustained pathological elevations.

In the CNS, CaN is widely expressed in neurons, astrocytes and microglia, where it regulates processes including synaptic plasticity, neuronal excitability, and inflammatory responses [12,21]. At synapses, CaN is a critical mediator of activity-dependent signalling, particularly in pathways associated with long-term depression (LTD), where modest and sustained Ca^{2+} increases preferentially activate phosphatase-driven responses over kinase-dominated pathways [22].

CaN substrates bind to discrete non-catalytic docking surfaces on the CnA subunit via conserved PxIxIT and LxVP motifs. These interactions promote substrate recruitment and proper positioning

within the catalytic cleft, thereby conferring specificity and efficiency to Ca^{2+}-dependent dephosphorylation [23,24].

CaN activity is tightly controlled by multiple regulatory mechanisms that are especially refined in neural tissue. Members of the regulator of CaN (RCAN) family, including RCAN1/DSCR1, act as feedback inhibitors of CaN activity and thereby buffer excessive Ca^{2+}-dependent signalling [25]. An additional layer of regulation is provided by proteins such as Cabin1, which interacts with CaN and restricts NFAT activation under specific cellular contexts [26]. CaN function is also sensitive to the cellular redox state, with RCAN1, the best-characterized member of the calcipressin family, providing short-term protection against acute oxidative stress and other Ca^{2+}-mediated stresses [27]. A further mechanism by which Ca^{2+} stress may regulate CaN activity in neurons involves Ca^{2+}-activated calpain proteases. Calpain-1 and calpain-2 are Ca^{2+}-regulated cysteine proteases whose activation results in the removal of autoinhibitory domain leading to constitutive CaN activation (Figure 1B) [28].

Together, these regulatory layers ensure that CaN activity remains closely coupled to physiological Ca^{2+} signalling while preventing chronic overactivation. Disruption of this balance, most commonly through sustained alteration of Ca^{2+} homeostasis, can result in prolonged CaN activation and altered downstream signalling, creating conditions under which CaN-dependent pathways may contribute to synaptic dysfunction and neuroinflammatory responses observed in neurodegenerative diseases [8,29].

Nuclear Factor of Activated T Cells: structure, regulation and functional roles in the CNS

The nuclear factor of activated T cells (NFAT) family comprises a group of transcription factors originally characterized in immune cells as key regulators of Ca^{2+}-dependent gene expression. Subsequent studies demonstrated that NFAT proteins are also expressed in the CNS, where they mediate activity-dependent transcriptional programs in neurons and glial cells [30].

In mammals, the NFAT family consists of five members: NFATc1 (NFAT2), NFATc2 (NFAT1), NFATc3 (NFAT4), NFATc4 (NFAT3), and NFAT5, also known as tonicity-responsive enhancer binding protein (TonEBP). The canonical NFAT proteins (NFATc1-c4) share a conserved regulatory architecture that confers sensitivity to Ca^{2+}/CaN signalling, whereas NFAT5 lacks CaN-binding motifs and is regulated primarily by osmotic stress [31,32].

Canonical NFAT proteins are characterized by two major domains: an N-terminal regulatory region, termed the NFAT homology region (NHR), and a C-terminal Rel homology region (RHR) that mediates DNA binding. The NHR contains multiple serine-rich regions and serine-proline motifs that are targets of kinases that are active in resting cells, notably GSK3, CK1, and DYRK1A, with additional regulation by PKA in response to cAMP signaling., as well as the two conserved CaN-docking motifs (PxIxIT and LxVP) and a nuclear localization signal (NLS) [23,24] (Figure 1C).

Under resting conditions, NFAT proteins are highly phosphorylated and retained in the cytoplasm. Elevation of intracellular Ca^{2+} activates CaN, which dephosphorylates specific serine clusters within the NHR, resulting in exposure of the NLS and rapid nuclear translocation of NFAT [31,33] (Figure 1C). Termination of NFAT signalling occurs when Ca^{2+} levels decline, allowing rephosphorylation by GSK3β, CK1 and DYRK1A, thereby promoting nuclear export and restoring cytoplasmic sequestration [34].This activation-deactivation cycle enables NFAT to function as a decoder of Ca^{2+} signal duration rather than amplitude, preferentially responding to sustained Ca^{2+} elevations.

NFAT proteins bind DNA with relatively low affinity as monomers and typically require cooperative interactions with other transcription factors to achieve stable promoter occupancy. The best-characterized partners are members of the AP-1 family (Fos/Jun), with which NFAT forms composite transcriptional complexes at regulatory elements of target genes [35]. This requirement for transcriptional cooperation provides an important layer of context specificity, integrating Ca^{2+} signals with parallel pathways activated by growth factors, stress responses or inflammatory cues. Importantly, disruption of NFAT-AP-1 coupling can alter transcriptional outcomes, allowing NFAT to engage alternative gene programs. This principle, well established in immune cells, is increasingly recognized as relevant in neural contexts, where NFAT-dependent transcription may diverge depending on cell type, developmental stage and signalling milieu [36].

Within the CNS, canonical NFAT isoforms (NFATc1-c4) are differentially expressed in neuronal and glial populations, although their distribution is partially overlapping and varies across developmental stages and experimental models.

NFATc3 and NFATc4 have been detected in neurons and have been implicated in activity-dependent transcriptional responses. Experimental studies have linked CaN/NFAT signalling in neurons to processes such as neurite extension, synaptic plasticity, and regulation of activity-

responsive genes, including brain-derived neurotrophic factor (BDNF) and growth-associated protein 43 (GAP-43) [37]. However, most evidence derives from *in vitro* systems or defined experimental paradigms, and comprehensive isoform-specific mapping *in vivo* remains incomplete.

NFATc1 and NFATc2 are also expressed in the CNS and have been reported in astrocytes and microglia, where CaN/NFAT signalling has been associated with regulation of inflammatory mediators and glial activation markers [38,39]. In microglia in particular, NFAT-dependent transcription has been involved in cytokine production in response to inflammatory stimuli [40]. Nevertheless, these associations do not imply strict cell-type specificity, as multiple NFAT isoforms can be detected in both neuronal and glial compartments [41–43].

Isoform-specific functions remain ill-defined, because many studies rely on pharmacological CaN inhibition or overexpression, which do not discriminate between NFAT-family members [44]. Moreover, functional redundancy among NFAT isoforms has been documented in other tissues and may also apply within the CNS. Thus, direct comparative analyses across cell types and disease models would be required to clarify these aspects of the NFAT biology [45].

Under physiological conditions, CaN/NFAT signalling contributes to Ca^{2+}-dependent transcriptional programs in both neurons and glial cells. In neurons, NFAT activation has been associated with activity-dependent gene regulation, whereas in glial cells it has been linked to inflammatory and reactive responses. In pathological contexts characterized by sustained Ca^{2+} dysregulation or chronic inflammatory signalling, increased NFAT nuclear localization has been reported in both neuronal and glial populations [12,13]. Although current evidence supports an association between NFAT activation and neurodegenerative conditions, NFAT do not appear to function as initiating drivers, and their contribution depends on cell type, stimulus, disease model and the isoform-specific.

Physiological functions of the Ca^{2+}/NFAT pathway in the CNS

Synaptic Plasticity

Synaptic plasticity encompasses activity-dependent modifications of synaptic strength that underlie learning, memory and circuit refinement. Long-term potentiation (LTP) and long-term depression (LTD) represent two well-characterized forms of plasticity, distinguished by the magnitude and temporal profile of intracellular Ca^{2+} signals triggered by synaptic activity [22].

In canonical hippocampal models, high-frequency stimulation generates rapid, high-amplitude postsynaptic Ca^{2+} transients through NMDA receptors, preferentially activating kinase-dominated pathways such as Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII) and the MAPK/ERK cascade [22,46]. These events lead to AMPA receptor phosphorylation and insertion into the post-synaptic membrane of dendritic spines and engage LTP transcriptional programs that support the maintenance of late-phase LTP [47,48]. In contrast, lower-frequency but more sustained Ca^{2+} increases preferentially activate phosphatase-driven signalling pathways, particularly CaN. Through a CaN-PP1 cascade, AMPA receptors are dephosphorylated, promoting their internalization from the postsynaptic membrane and leading to expression of NMDAR-dependent LTD [21,22].

Therefore, CaN is considered a critical mediator of NMDAR-dependent LTD induction. Its role in synaptic weakening is supported by extensive electrophysiological and pharmacological evidence demonstrating that CaN inhibition disrupts LTD in hippocampal CA1 neurons while leaving LTP largely intact [49,50].

The involvement of NFAT downstream of CaN in synaptic plasticity is less well established than that of CaMKII or CaN itself. NFAT activation depends on sustained Ca^{2+} elevations and CaN activity, and NFAT-dependent transcription has been implicated in the regulation of genes associated with structural remodelling and neuronal excitability, including RCAN1 and, in some contexts, BDNF [51,52]. These observations support a role for NFAT in consolidating longer-term adaptations associated with synaptic activity rather than in the rapid induction of synaptic weight changes.

Importantly, most forms of early-phase LTP and LTD occur independently of transcription and rely on post-translational mechanisms. NFAT-dependent transcription is therefore unlikely to be required for the immediate expression of synaptic plasticity. Instead, available evidence suggests that NFAT may contribute to activity-dependent transcriptional programs engaged during late-phase plasticity or longer-term adaptations to sustained neuronal activity, although direct causal links to synaptic consolidation remain incompletely defined [53–55].

Experimental evidence linking NFAT directly to synaptic plasticity is largely derived from genetic manipulation or chronic pharmacological modulation of CaN/NFAT signalling. In these contexts, altered NFAT activity has been associated with changes in dendritic spine density, activity-dependent synaptic pruning, and structural aspects of synaptic maintenance [52]. However,

pharmacological inhibition of CaN affect multiple downstream pathways beyond NFAT, and even genetic approaches may engage compensatory mechanisms [56]. These factors complicate the attribution of observed phenotypes specifically to NFAT-dependent transcription rather than to broader CaN-mediated effects [57,58].

These limitations are particularly relevant when extrapolating physiological roles to pathological contexts. In neurodegenerative conditions, chronic Ca^{2+} dyshomeostasis can shift CaN activity from transient, activity-coupled signalling toward sustained activation. Under such conditions, prolonged NFAT activity has been linked to transcriptional changes correlated with synaptic weakening, loss of dendritic spines and altered excitability. Although supported by correlative and mechanistic studies, these findings do not establish NFAT activation as an initiating driver of pathology. Rather, maladaptive outcomes are likely to reflect disruption of the temporal constraints that normally restrict CaN/NFAT signalling during physiological synaptic activity [11,13,59] (Figure 2A,B).

Taken together, current evidence supports a model in which CaN plays a direct and well-established role in LTD and synaptic weakening, whereas NFAT functions as a downstream transcriptional integrator that may contribute to longer-term synaptic and structural adaptations [57]. The extent to which NFAT-dependent transcription is engaged during physiological synaptic plasticity likely depends on the duration, spatial distribution and cellular context of Ca^{2+} signals, emphasizing the importance of temporal regulation in distinguishing adaptive from maladaptive signalling outcomes [60].

Neurodevelopment and adult neurogenesis

The Ca^{2+}/CaN/NFAT signalling pathway plays well-established roles during embryonic development, where it regulates cell-fate specification, tissue morphogenesis and differentiation across multiple organ systems, including the cardiovascular, immune and nervous systems [61,62]. In the developing CNS, genetic and cellular models have implicated CaN/NFAT signalling in neuronal differentiation, axonal and dendritic growth, and activity-dependent refinement of neural circuits [63].

During neurodevelopment, Ca^{2+} signals arising from growth factor receptors, voltage-gated Ca^{2+} channels and neurotransmitter receptors activate CaN thereby regulating NFAT nuclear translocation and transcriptional activity [58]. Genetic and conditional knockout studies

demonstrate that NFAT family members contribute to neural development in isoform- and context-specific manners [62,64]. However, the functional consequences of NFAT activation are highly dependent on developmental stage and cellular context. In some experimental systems, NFAT activity promotes neuronal differentiation and neurite extension, whereas in others it has been implicated in influencing progenitor cell fate decisions, including glial lineage specification. These findings underscore the importance of developmental stage, cellular identity and signalling environment in shaping NFAT-dependent functional outcomes [65].

In the adult brain, the CaN/NFAT pathway is expressed in restricted neurogenic niches, most prominently in the subgranular zone of the hippocampal dentate gyrus. Adult hippocampal neurogenesis relies on tightly regulated Ca^{2+}-dependent signalling to control neural stem and progenitor cell proliferation, survival and differentiation [52,66]. Experimental evidence indicates that NFAT-dependent transcription can be engaged downstream of neurotrophin in neural progenitor populations, suggesting a potential role for this pathway in aspects of adult neurogenesis [63].

However, the contribution of NFAT to adult neurogenesis should be interpreted cautiously. Changes in NFAT activity correlate with altered proliferation or differentiation of neural progenitors in experimental models, often under conditions of sustained signalling manipulation or genetic perturbation. Direct evidence demonstrating that NFAT acts as a physiological rate-limiting determinant of adult neurogenesis under basal conditions remains limited, and functional redundancy among transcriptional regulators may constrain the impact of NFAT signalling *in vivo* [63,67,68].

Ageing and neurodegenerative conditions are associated with impaired adult hippocampal neurogenesis [69]. Altered Ca^{2+} homeostasis and increased CaN activity have been proposed as contributing factors in this decline [59]. In this context, NFAT activation has been implicated in transcriptional changes accompanying reduced neurogenic capacity. However, available data do not support a simple reactivation of embryonic developmental programs in the adult diseased brain. Sustained Ca^{2+}/CaN/NFAT signalling may influence the balance between proliferation, differentiation and survival within adult neural stem cell niches, particularly when temporal regulation of signalling is disrupted [70].

In conclusion, CaN/NFAT signalling seems to contribute to neurodevelopmental and adult neurogenic processes in a context-dependent manner. NFAT functions as a Ca^{2+}-responsive

transcriptional regulator capable of shaping neural differentiation and plasticity when appropriately constrained. In adult neurogenesis and disease-related states, however, its role appears modulatory rather than deterministic. This distinction is critical for interpreting NFAT activation in pathological settings and for evaluating the therapeutic implications of targeting this pathway.

Ca^{2+}/NFAT signalling and neuroinflammation

Neuroinflammation is a prominent feature of many neurodegenerative diseases and reflects the activation of resident glial cells, primarily microglia and astrocytes. Although these cells do not generate action potentials, they rely extensively on intracellular Ca^{2+} signalling to regulate gene expression, metabolic adaptation and inflammatory responses [41,42]. Dysregulation of Ca^{2+} homeostasis in glial cells has been proposed as an important contributing mechanism to sustained neuroinflammatory states [12].

Astrocytes respond to diverse stimuli, including neurotransmitters, extracellular ATP and inflammatory mediators, through Ca^{2+} elevations that can engage Ca^{2+}-dependent transcriptional pathways. Experimental studies demonstrate that mechanical injury, purinergic signalling and glutamatergic dysregulation can activate the CaN/NFAT pathway in astrocytes [71,72]. NFAT activation in astrocytes has been associated with changes in gene expression that influence neuronal excitability and synaptic function [37] (Figure 2C).

It functions as a modulatory pathway that integrates Ca^{2+} signals generated downstream of neuronal activity or tissue damage. Importantly, astrocytic inflammatory gene expression is strongly regulated by NF-κB and STAT signalling pathways, and current evidence does not support NFAT as a primary or independent driver of astrocyte-mediated neuroinflammation [12,71].

Microglia serve as the innate immune cells of the central nervous system and exhibit highly dynamic activation states in response to tissue perturbation. Canonical microglial activation is initiated through pattern recognition receptors, such as Toll-like receptors (TLRs), which engage NF-κB and MAPK signalling cascades to induce pro-inflammatory gene expression [38,73].

In parallel, sustained increases in intracellular Ca^{2+} can activate CaN and promote NFAT nuclear translocation in microglia. Prolonged Ca^{2+} signalling in immune cells, leading to NFAT activation, is often maintained through store-operated Ca^{2+} entry (SOCE), mediated by STIM and ORAI1

proteins, a mechanism well characterized in peripheral leucocytes and increasingly reported in microglial systems [74,75]. NFAT activation in microglia has been shown to contribute to the expression of selected cytokines and chemokines, including TNF-α, IL-1β and MCP-1, particularly under conditions of sustained inflammatory stimulation [40] (Figure 2C).

As in astrocytes, NFAT signalling in microglia does not operate in isolation. NF-κB remains the principal transcriptional driver of acute inflammatory responses, whereas NFAT appears to modulate the magnitude, persistence and cellular consequences of inflammation. Consistent with this division of labour, pharmacological or genetic inhibition of NFAT attenuates, but does not abolish, microglial inflammatory responses [39,76].

Several Ca^{2+}-permeable receptors expressed by microglia may reinforce NFAT activation during neuroinflammation. Among these, the purinergic P2X7 receptor (P2X7R), activated by high extracellular ATP concentrations released during tissue damage, induces robust Ca^{2+} influx that can support CaN signalling promoting transcriptional programs associated with inflammatory amplification [77]. While NFAT has been implicated in the transcriptional regulation of inflammatory mediators downstream of P2X7R activation, the extent to which NFAT directly regulates P2X7R expression or establishes feed-forward loops that enhances microglial responsiveness under chronic inflammatory conditions remains to be fully defined [78].

Beyond cytokine production, NFAT signalling has also been associated with transcriptional regulation of genes linked to the cell cycle, including c-MYC, in immune cells, and similar mechanisms have been proposed in microglia [43], where this proliferative response may contribute to the expansion of reactive populations in chronic neurodegeneration [79].

In excitotoxic neuronal models, calpain-mediated cleavage of the CnA autoinhibitory domain generates a truncated CaN species with prolonged activity, thereby converting elevated Ca^{2+} exposure into sustained CaN signalling and neuronal injury [80]. This proteolytic mechanism provides an additional link between chronic Ca^{2+} dysregulation and persistent CaN/NFAT activation in neurodegenerative contexts (Figure 2C).

Taken together, Ca^{2+}/NFAT signalling may act as a modulatory component of neuroinflammatory responses rather than as a primary initiating pathway. NFAT integrates sustained Ca^{2+} signals with innate immune signalling to influence the persistence and qualitative features of glial activation [38]. Disruption of the temporal constraints that normally restrict Ca^{2+}/CaN/NFAT signalling in

glia and neurons has been proposed as a mechanism contributing to maladaptive neuroinflammation in neurodegenerative disease [39,81].

CaN/NFAT pathway dysfunction in neurodegenerative diseases

Alzheimer's Disease

Alzheimer's Disease (AD) is characterized by progressive cognitive decline associated with synaptic dysfunction, neuronal loss, extracellular accumulation of amyloid-β (Aβ) peptides, and intracellular aggregation of hyperphosphorylated tau [4]. In addition to these hallmark pathologies, accumulating evidence indicates that disruption of intracellular Ca^{2+} homeostasis is a consistent feature of AD and contributes to both neuronal dysfunction and glial activation [7].

Aβ oligomers perturb neuronal Ca^{2+} handling through multiple mechanisms, including altered NMDA receptor activity, changes in AMPA receptor composition, and impaired intracellular Ca^{2+} buffering. These alterations can result in sustained elevations of intracellular Ca^{2+}, particularly in vulnerable neuronal populations. Under these conditions, increased or sustained CaN activation has been reported [82] (Figure 3A).

In addition to receptor-mediated mechanisms, Aβ assemblies can directly affect membrane permeability. Early work showed that β-amyloid peptides destabilize neuronal Ca^{2+} homeostasis and increase vulnerability of human cortical neurons to excitotoxic stimuli [83]. Subsequent biophysical and electrophysiological studies supported the idea that Aβ1-42 can form irregular cation-permeable channel-like structures in lipid bilayers, with conductance properties influenced by solvent conditions and pH [84]. Consistent with this view, Aβ-induced membrane perturbation in hippocampal neurons has been proposed to create leaky bilayer states and abnormal Ca^{2+} influx, providing an additional route by which extracellular Aβ burden can be translated into chronic intracellular Ca^{2+} stress [85].

Experimental studies in AD models consistently show increased CaN activity in affected brain regions, and pharmacological or genetic reduction of CaN activity attenuates synaptic deficits and neuronal vulnerability [13,86]. These findings support a direct role for CaN as a mediator linking Ca^{2+} dysregulation to synaptic and cellular dysfunction in AD. Consistent with prolonged Ca^{2+}/CaN activation, increased nuclear localization of NFAT has been observed in both neurons and glial cells in AD models [82,87].

In neurons, NFAT-dependent transcription has been associated with changes in genes involved in synaptic stability, dendritic structure and excitability [88–90]. However, direct evidence that NFAT activation alone is sufficient to drive neuronal death or synaptic loss in AD remains limited. Most studies rely on CaN inhibition or broad NFAT suppression, approaches that affect multiple CaN-dependent pathways simultaneously [87].

The amyloidogenic processing of amyloid precursor protein (APP) by β-site APP-cleaving enzyme 1 (BACE1) is a central pathogenic mechanism in AD. NF-κB signalling has been clearly implicated in transcriptional regulation of BACE1 [91,91]. In contrast, the involvement of NFAT in BACE1 regulation is indirect and context-dependent. Although modulation of NFAT activity can influence BACE1 expression in cellular and animal models, direct binding of NFAT to the BACE1 promoter has not been conclusively demonstrated [92]. Collectively, these observations suggest that Ca^{2+}/CaN/NFAT signalling modulates amyloidogenic pathways under pathological conditions, rather than directly initiating Aβ production. Such modulation may contribute to feed-forward interactions between Ca^{2+} dysregulation, synaptic dysfunction and amyloid pathology without implying linear causality.

Neuroinflammation is a prominent feature of AD and is mediated by sustained activation of microglia and astrocytes. In glial cells, NFAT activation occurs downstream of Ca^{2+} signals generated by inflammatory stimuli, extracellular ATP and purinergic receptor activation [12,38].

In microglia, NFAT contributes to the expression of selected cytokines and chemokines, including TNF-α and IL-1β, particularly under conditions of chronic stimulation. However, NF-κB remains the primary driver of acute inflammatory gene expression, whereas NFAT is thought to modulate the persistence and magnitude of inflammatory responses [39,93]. In astrocytes, NFAT activation has been linked to transcriptional programs that influence neuronal excitability and synaptic function, potentially contributing to pathological dysfunction in AD [82].

Tau hyperphosphorylation and aggregation represent a second major pathological axis in AD. Although NFAT does not directly regulate tau expression or phosphorylation, CaN can influence phosphorylation dynamics through multiple mechanisms, including direct dephosphorylation of tau and modulation of kinase activity such as that of GSK3β [94]. In AD mouse models, CaN inhibition reduces both Aβ burden and tau pathology, even though these effects cannot be attributed unequivocally to NFAT inhibition and likely reflect broader modulation of CaN-dependent pathways [86]. Collectively, available evidence suggests that Ca^{2+} dyshomeostasis and

sustained CaN activation represent important contributors to synaptic dysfunction and neuroinflammation in AD. NFAT activation emerges as a downstream transcriptional response that integrates chronic Ca^{2+} signals in neurons and glial cells. Under pathological conditions, prolonged engagement of this pathway may reinforce maladaptive gene expression programs, suggesting a context-dependent modulatory role for NFAT and highlighting the relevance of temporal and spatial control of Ca^{2+}/CaN signalling.

Parkinson's Disease

Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by the selective loss of dopaminergic neurons in the substantia nigra pars compacta and by the accumulation of misfolded α-synuclein in Lewy bodies and Lewy neurites[95–97]. In addition to protein aggregation, PD pathology involves mitochondrial dysfunction, oxidative stress and a prominent neuroinflammatory component, all of which contribute to neuronal vulnerability [6,95]. A defining physiological feature of nigral dopaminergic neurons is their autonomous pacemaker activity, which relies in part on L-type voltage-gated Ca^{2+} channels, particularly Cav1.3. This intrinsic activity results in sustained Ca^{2+} influx and imposes a high metabolic burden, increasing mitochondrial stress and reactive oxygen species (ROS) production [98–100]. In PD, mitochondrial impairment and α-synuclein-mediated toxicity further compromise Ca^{2+} handling, predisposing neurons to elevated cytosolic Ca^{2+} stress (Figure 3B).

In this context, increased or sustained CaN activation has been reported in vulnerable neurons. Experimental models of PD demonstrate increased CaN activity, and pharmacological inhibition of CaN attenuates dopaminergic neuron loss and motor deficits in toxin-based PD models [101].

NFAT nuclear translocation has been reported in both neurons and glial cells exposed to α-synuclein aggregates or inflammatory stimuli[102,103]. In neurons, NFAT-dependent transcription has been associated with alterations in genes regulating synaptic function and neuronal resilience. However, direct evidence that neuronal NFAT activation alone is sufficient to induce dopaminergic neuron death is limited. As in AD, most experimental evidence relies on CaN inhibition or broad NFAT suppression, which affects multiple CaN substrates and complicates attribution of neuronal phenotypes specifically to NFAT.

Neuroinflammation is a key contributor to PD progression. Misfolded or aggregated α-synuclein can activate microglia through TLR2 and TLR4, leading to NF-κB-driven transcription of pro-

inflammatory cytokines [104,105]. In parallel, inflammatory signalling and extracellular ATP release promote sustained Ca^{2+} elevations in microglia, providing conditions permissive for CaN/NFAT activation.

NFAT signalling in microglia has been associated with the expression of selected inflammatory mediators and with proliferative responses under chronic inflammatory conditions [43,76]. However, NF-κB remains the dominant driver of acute inflammatory gene expression, whereas NFAT appears to modulate the persistence and amplification of inflammatory responses. Experimental attenuation of NFAT signalling reduces, but does not abolish, neuroinflammatory activation in PD models, supporting a modulatory role [76]. Sustained neuroinflammation can exacerbate α-synuclein pathology by promoting oxidative stress and impairing proteostatic mechanisms, thereby reinforcing neuronal injury. Conversely, increased α-synuclein burden further activates glial cells, establishing a feed-forward loop that may accelerate disease progression[106,107]. Ca^{2+}/CaN/NFAT signalling likely participates in this loop by integrating prolonged Ca^{2+} signals into transcriptional programs associated with chronic glial activation.

Together the data suggest that altered Ca^{2+} homeostasis may represent a central determinant of neuronal vulnerability in PD, driven by intrinsic pacemaker activity, mitochondrial dysfunction and α-synuclein toxicity. CaN activation constitutes a key molecular consequence of sustained Ca^{2+} stress, while NFAT functions as a downstream transcriptional regulator in both neurons and glial cells, contributing to maladaptive inflammatory and stress-response programs under pathological conditions. Its relevance is therefore best understood as context-dependent and contingent upon chronic disruption of Ca^{2+} signalling control.

Targeting Ca^{2+}/CaN/NFAT signalling in neurodegeneration: opportunities and limitations

The Ca^{2+}/CaN/NFAT axis represents an attractive therapeutic target because it integrates intracellular Ca^{2+} dynamics with transcriptional programs implicated in synaptic dysfunction and neuroinflammation [76,101,108]. However, its central physiological roles in multiple cell types impose substantial constraints on therapeutic modulation, particularly in the CNS (Figure 3C).

Classical CaN inhibitors (CNIs), such as cyclosporin A (CsA) and tacrolimus (FK506), effectively suppress CaN activity and NFAT-dependent transcription (Table1). In experimental models of neurodegeneration, CNIs attenuate synaptic deficits, reduce neuroinflammatory markers and

improve behavioural outcomes [59,101], establishing CaN inhibition as a valid proof-of-principle strategy.

Nevertheless, CNIs lack pathway specificity and broadly interfere with CaN-dependent processes beyond NFAT signalling, including regulation of synaptic vesicle cycling, cytoskeletal dynamics and mitochondrial function [21]. Moreover, chronic CNI administration is associated with well-documented adverse systemic effects such as nephrotoxicity, hypertension and increased infection risk, rendering long-term use in chronic neurodegenerative diseases unlikely to be feasible in their current form [109].

Recent preclinical studies have begun to refine this view by testing whether low-dose or subclinical CNI regimens can normalize excessive CaN activity without producing full systemic immunosuppression. In AD model mice, long-term low-dose FK506 normalized CNS CaN activity, reduced neuroinflammatory and AD-associated phenotypes, and did so without blocking peripheral T-cell responses [85]. Similarly, in an aging canine model of AD, tacrolimus and related CaN/NFAT inhibitors preserved cognitive performance and brain health measures, supporting the concept that partial pathway normalization may have preventive or disease-modifying potential [110]. These approaches partially attenuate peripheral toxicity and may improve long-term safety.

To overcome the non-specificity of CaN inhibition, efforts have focused on selectively disrupting CaN-NFAT interactions. Peptide inhibitors derived from NFAT docking motifs, such as VIVIT, selectively block NFAT dephosphorylation without globally suppressing CaN activity [23] (Table 1). In cellular and animal models, NFAT-selective inhibition reduces inflammatory gene expression and attenuates pathological phenotypes while preserving other CaN-dependent functions [76,108,110]. Despite these advantages, NFAT-selective strategies face significant translational barriers. Peptide inhibitors exhibit poor pharmacokinetic properties, limited blood-brain barrier penetration, suboptimal pharmacokinetics, and potential immunogenicity. Furthermore, NFAT family members display partial functional redundancy and context-dependent roles, raising concerns that incomplete or isoform-biased inhibition may yield variable outcomes. At present, NFAT-selective approaches remain primarily experimental tools rather than clinically actionable therapies [19].

An alternative strategy involves indirect modulation of CaN/NFAT signalling by targeting upstream Ca^{2+} sources (Table 1). In PD, blockade of L-type Ca^{2+} channels using dihydropyridines has been proposed to reduce Ca^{2+} burden in dopaminergic neurons and thereby limit CaN

activation [111,112]. Similarly, modulation of NMDA receptor activity and intracellular Ca^{2+} buffering has been explored in AD models [113,114]. Although these approaches may attenuate pathological Ca^{2+} signalling, clinical trials targeting Ca^{2+} channels or glutamatergic receptors have yielded limited or inconclusive results, underscoring the difficulty of selectively dampening pathological Ca^{2+} elevations without disrupting physiological signalling essential for neuronal function [115,116]. These outcomes highlight the limited predictive value of Ca^{2+}-centric interventions when applied without precise temporal and cellular specificity.

A recurring challenge in targeting the Ca^{2+}/CaN/NFAT pathway is its divergent role across neurons, astrocytes and microglia. While NFAT activation in glial cells may contribute to sustained neuroinflammation, NFAT-dependent transcription in neurons can support adaptive responses under physiological conditions. Global pathway inhibition therefore risks suppressing protective as well as maladaptive signalling.

Emerging strategies aimed at cell-type-specific modulation, such as viral vectors, nanoparticle-based delivery systems and gene regulatory approaches, offer conceptual solutions but remain technically immature for widespread therapeutic application in neurodegenerative disease [117,118].

In summary, modulation of the Ca^{2+}/CaN/NFAT pathway holds clear mechanistic relevance for neurodegenerative disease but presents substantial translational challenges. Broad CaN inhibition demonstrates efficacy in experimental models but is incompatible with long-term clinical use. NFAT-selective strategies provide improved specificity yet remain constrained by delivery and redundancy issues. Targeting upstream Ca^{2+} dysregulation may indirectly modulate CaN/NFAT signalling, but thus far has shown limited clinical success.

Conclusions

The Ca^{2+}/CaN/NFAT signalling axis functions in the CNS as a mechanism that translates the temporal structure of intracellular Ca^{2+} signals into sustained transcriptional responses. Although many Ca^{2+}-dependent processes regulate rapid synaptic events, CaN/NFAT signalling is preferentially engaged during sustained or repetitive Ca^{2+} elevations, thereby linking prolonged cellular activity to long-term gene expression programs.

In neurodegenerative disorders, persistent Ca^{2+} dysregulation reshapes this signalling module. Chronic activation of CaN alters the nuclear residence and transcriptional activity of NFAT in both

neurons and glial cells. Under these conditions, NFAT may contribute to the stabilization of transcriptional programs that influence synaptic structure, inflammatory tone and cellular resilience. Its pathological relevance therefore appears to reside not in initiating Ca^{2+} stress, but in consolidating prolonged disturbances in Ca^{2+} homeostasis into durable molecular states.

Across AD and PD, converging evidence suggests that CaN/NFAT signalling participates in the shift from adaptive stress responses to maladaptive persistence. In neurons, prolonged activation is associated with altered synaptic maintenance and increased vulnerability. In glial populations, NFAT contributes to the persistence and qualitative shaping of inflammatory responses. Although these effects are highly context-dependent, they reflect the shared principle that NFAT integrates the duration and intensity of Ca^{2+} signals into transcriptional outputs with extended temporal impact.

Neurodegeneration may therefore be interpreted not solely as a disorder of protein aggregation or inflammatory activation, but as a progressive failure in the temporal regulation of Ca^{2+}-dependent signalling. Within this framework, CaN/NFAT does not function as a classical upstream trigger of pathology. Rather, it occupies a strategic position at the interface between transient cellular stress and long-term transcriptional stabilization. When physiological termination mechanisms become progressively impaired, NFAT-dependent transcription may convert episodic Ca^{2+} disturbances into persistent molecular states that reinforce synaptic fragility and inflammatory tone. This perspective shifts the emphasis from pathway activation *per se* to the loss of signal resolution. The critical pathological event may not be excessive NFAT activity alone, but the inability of neural cells to disengage Ca^{2+}-dependent transcription once stress becomes chronic. In this sense, CaN/NFAT signalling may act less as an initiator of degeneration and more as a molecular memory system that consolidates unresolved cellular stress into durable gene expression programs.

Therapeutically, this implies that effective modulation of the Ca^{2+}/CaN/NFAT axis will require temporal and cellular precision. Broad inhibition of CaN activity risk disrupting essential physiological signalling, whereas strategies aiming at restoring appropriate signal duration, threshold sensitivity or cell-type-specific engagement may hold great promise. Future research should therefore focus not only on pathway inhibition, but on defining how Ca^{2+} signal dynamics are encoded and stabilized by NFAT under physiological and pathological conditions.

We advocate that the field should move beyond asking whether CaN/NFAT is activated in neurodegeneration and instead define how, where and for how long the pathway remains engaged.

Particularly important will be studies that separate receptor-mediated Ca^{2+} entry from membrane-disruptive Aβ actions, distinguish reversible CaN activation from calpain-dependent proteolytic activation, and test whether low-intensity pharmacological normalization can preserve protective CaN functions while preventing maladaptive transcriptional persistence.

Understanding how temporal gating of Ca^{2+} signals becomes destabilized in ageing and disease may therefore be more informative than further cataloguing downstream transcriptional targets. Such an approach reframes therapeutic intervention, rather than suppressing the pathway globally, restoring physiological signal termination and cell-type-specific control may represent a more viable strategy for preventing the transition from adaptive stress responses to irreversible neurodegenerative states.

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Competing interest
The authors declare no competing interests.

Author contributions
BDSO wrote the original draft of the manuscript. MI contributed to writing, and critically revised, and editing the manuscript. FG conceptualized, contributed to writing and critically revised the review. All authors reviewed and approved the final manuscript.

Figures

Figure 1. Structural organization and regulation of the calcineurin/NFAT signaling axis.
(A) Calcineurin (CaN) is a Ca^{2+}/calmodulin-dependent serine/threonine phosphatase composed of a catalytic A subunit (CnA) and a regulatory B subunit (CnB). In the inactive state, the autoinhibitory domain (AID) of CnA blocks the catalytic site. Upon elevation of intracellular Ca^{2+}, Ca^{2+} binding to CnB and calmodulin promotes calmodulin association with CnA, displacement of the AID, and exposure of the catalytic site, resulting in CaN activation.
(B) Canonical NFAT proteins contain an N-terminal regulatory region/NFAT homology region (NHR), serine-rich regions and SP motifs, CaN-docking motifs (PxIxIT and LxVP), a nuclear localization sequence (NLS), and a Rel homology region (RHR) responsible for DNA binding. In resting cells, phosphorylated NFAT is retained in the cytoplasm. Ca^{2+}/CaN-dependent dephosphorylation exposes the NLS and promotes NFAT nuclear translocation, DNA binding, and transcriptional activation. Signal termination occurs through rephosphorylation by kinases including GSK3, CK1, and DYRK1A, which drives NFAT nuclear export.
(C) Under pathological conditions, chronic Ca^{2+} elevation can activate calpain proteases. Calpain-mediated cleavage of the calcineurin AID may generate truncated, persistently active CaN species, thereby prolonging NFAT nuclear residence and reinforcing CaN/NFAT-dependent transcriptional programs. This mechanism provides a disease-relevant route by which sustained Ca^{2+} stress may uncouple CaN activity from physiological temporal control. Created with Biorender.

Figure 2. Ca^{2+}/CaN/NFAT signaling in health and disease. (A) Physiological Ca^{2+} signaling is characterised by transient oscillations that drive brief CaN activation, transient NFAT nuclear translocation, and reversible transcriptional programs that support adaptation. (B) Pathological Ca^{2+} stress produces sustained CaN activation and prolonged NFAT nuclear residence, leading to stable, maladaptive transcriptional states. (C) Persistent CaN/NFAT activity induces distinct, cell-type-specific programs that collectively promote dysfunction across the CNS. Created with Biorender.

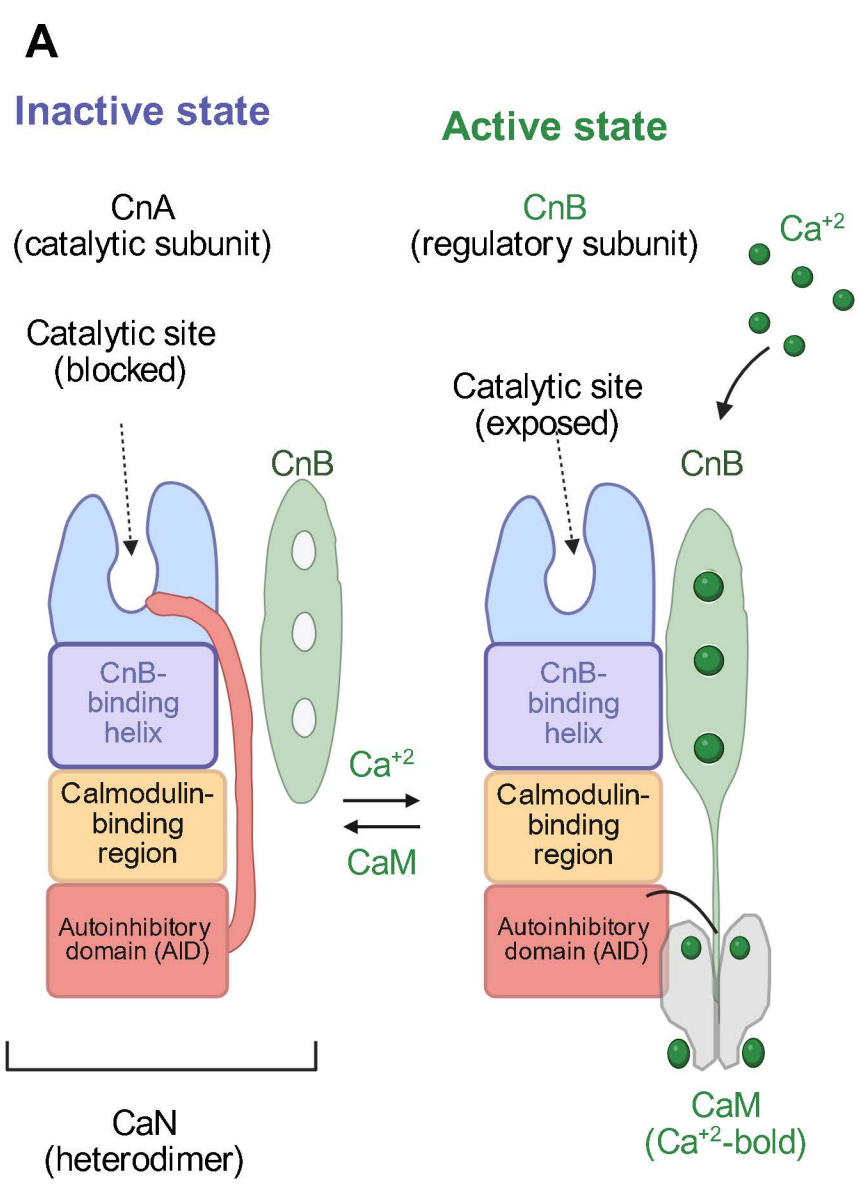
Figure 3. Disease-specific routes converging on Ca^{2+}/CaN/NFAT signaling in neurodegeneration. (A) Alzheimer's disease: distinct sources of Ca^{2+} dysregulation drive sustained Ca^{2+} elevation, CaN activation, and NFAT translocation, leading to synaptic dysfunction and neuroinflammation. (B) Parkinson's disease: multiple mechanisms create a chronic Ca^{2+} burden that activates CaN and NFAT-dependent transcription, promoting dopaminergic neuron vulnerability and persistent glial activation. (C) Therapeutic opportunities and limitations for targeting the Ca^{2+}/CaN/NFAT axis across neurodegenerative diseases. Created with Biorender.

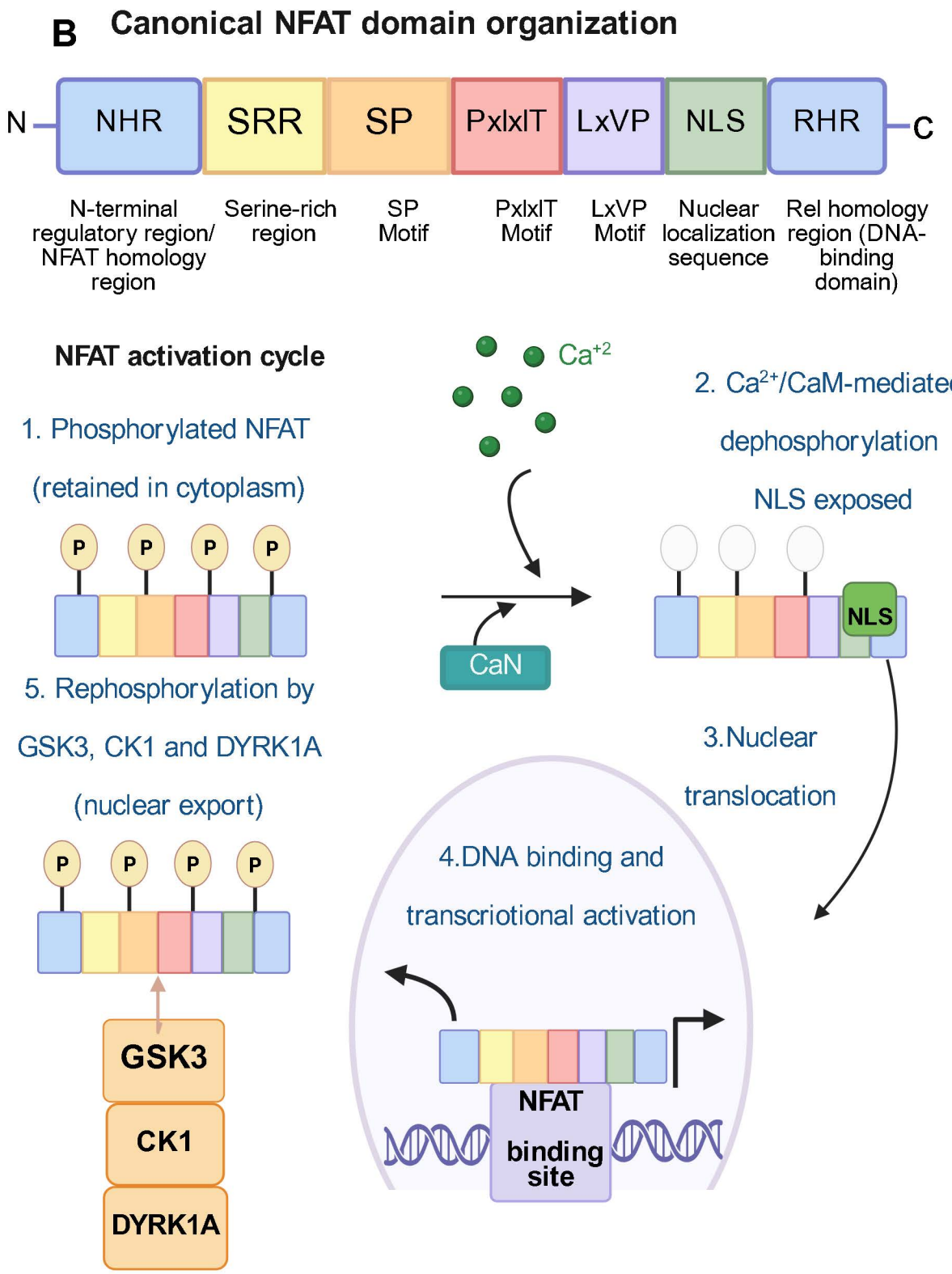
Tables

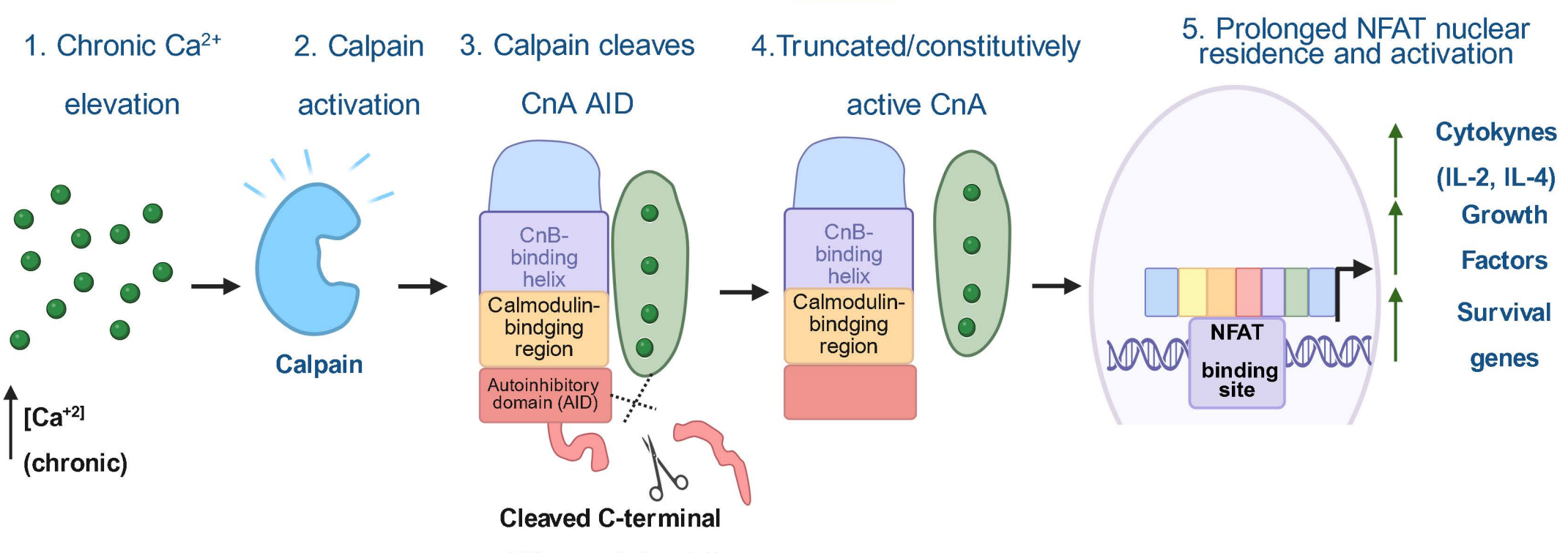
Strategy	Representative Agents	Molecular Target	Key Advantages	Current Limitations & Challenges	Reference
Classical CNIs	Cyclosporine A (CsA), Tacrolimus (FK506)	Calcineurin (CaN) Catalytic Site	Well-establishe clinical use; potent immunosuppression	High toxicity (nephro/neuro); blocks all CaN functions (off-target)	(Kocanci et al. 2025)
Selective Peptide	VIVIT, 11R-VIVIT	CaN-NFAT Docking Sites (PxIxIT/LxVP)	High selectivity; preserves non-NFAT CaN functions	Poor Blood-Brain Barrier (BBB) permeability; requires delivery vectors	(Gal et al. 2014)
Upstream Ca^{2+} Modulation	L-type CCBs (Isradipine), NMDAR antagonists	Intracellular Ca^{2+} influx	Indirectly reduces pathway hyperactivation; existing clinical safety data	May lack specificity for the NFAT pathway; systemic cardiovascular effects	(Little 2021)
Endogenous Modulators	RCAN1-derived peptides, CAIN	Physiological CaN/NFAT interaction	Mimics natural regulatory mechanisms; potentially lower toxicity	Complex dose-response relationship; difficult to deliver exogenously	(Wong et al. 2022)
Genetic Approaches	siRNA, Viral vectors (AAV)	NFAT mRNA / Isoform expression	High specificity for isoforms (e.g., NFATc2); long-term modulation	Delivery challenges; potential for permanent genomic/transcriptional changes	(Si et al. 2026)
Emerging Delivery	Nanoparticles, Exosomes	BBB transport systems	Enhanced CNS penetration; targeted delivery to glia or neurons	Still in early preclinical stages; high production complexity and cost	(Jin et al. 2026)

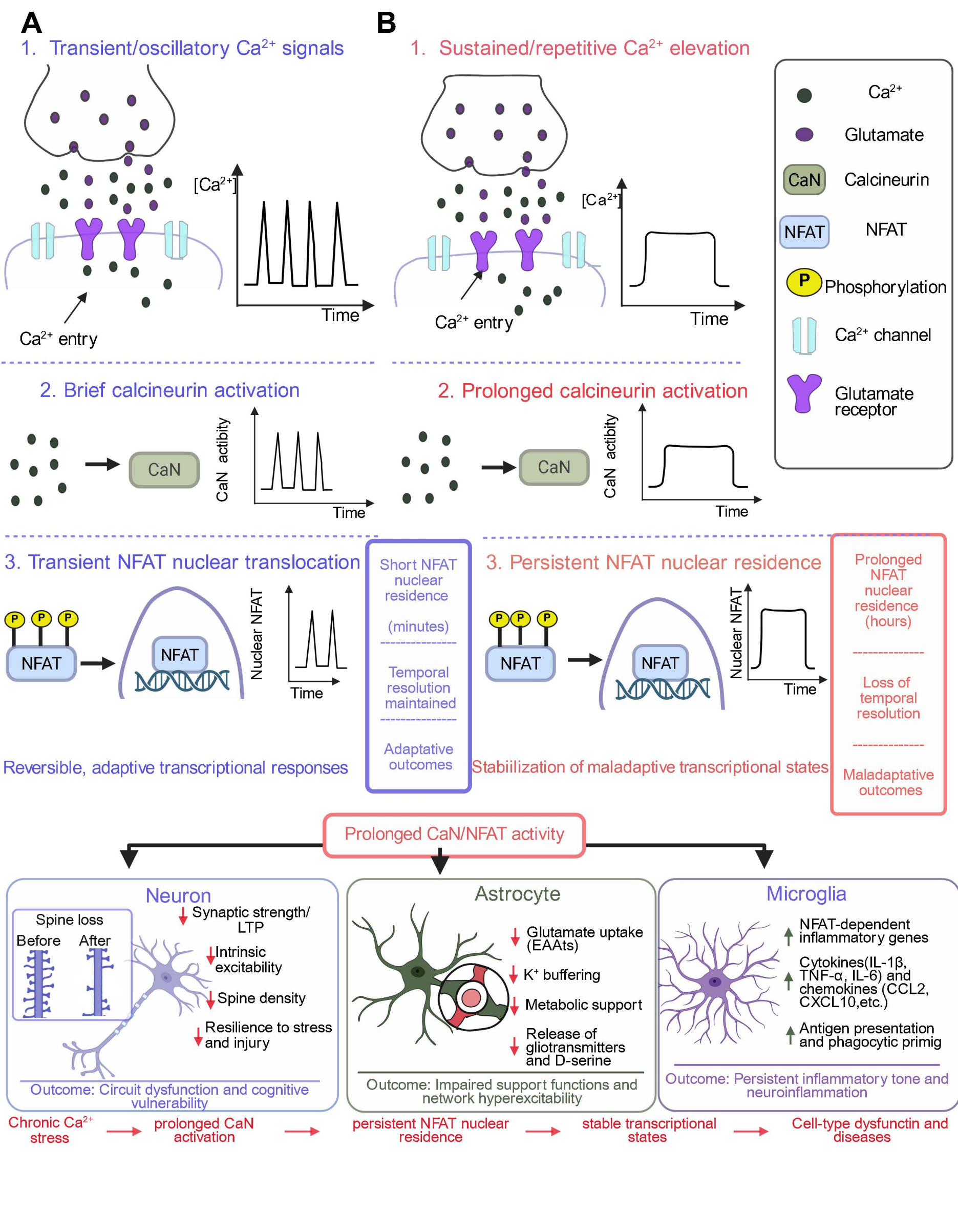
Table 1. Strategies to Modulate the Calcineurin-NFAT Pathway

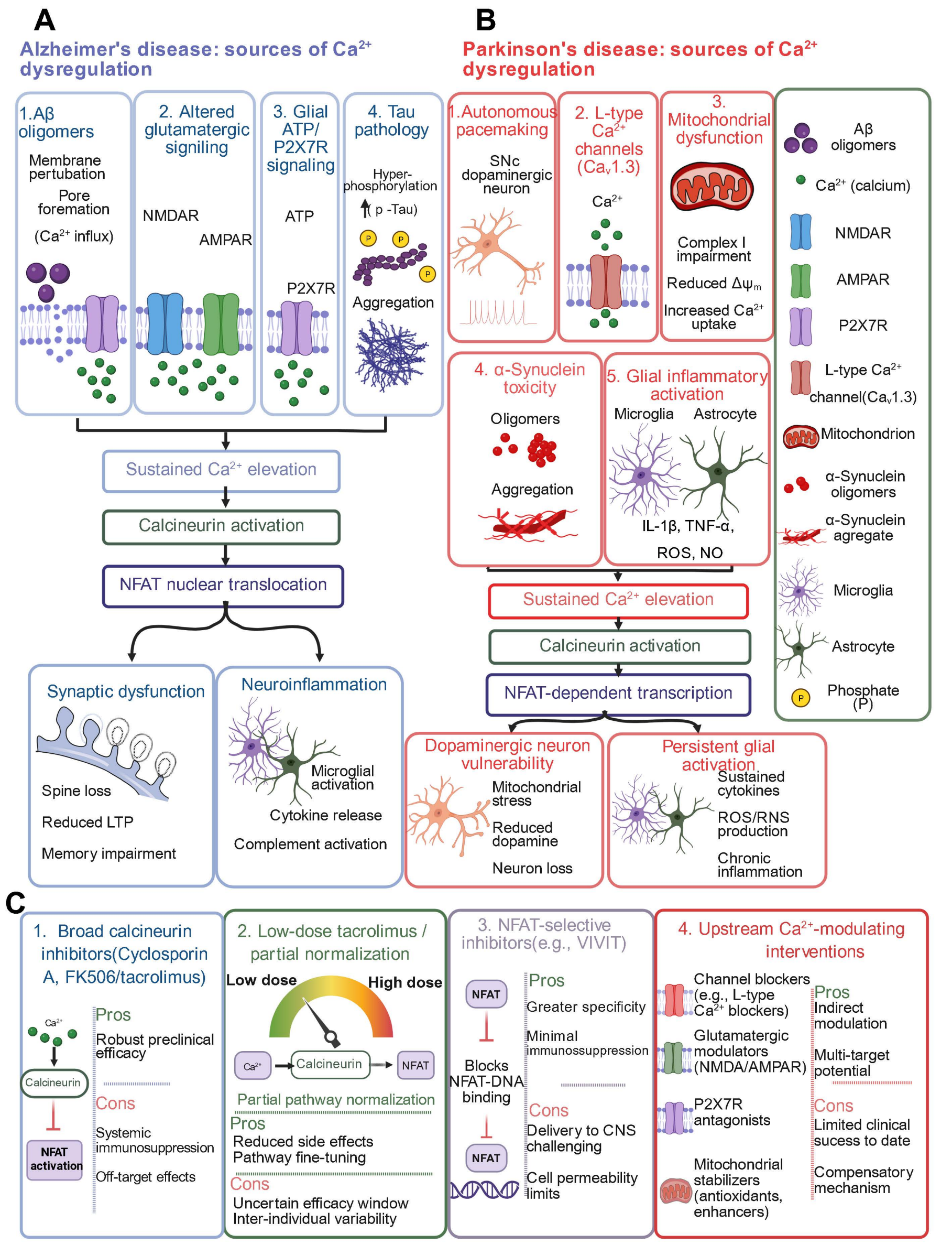
This table summarizes therapeutic approaches targeting the CaN-NFAT signalling axis, detailing representative agents, molecular targets, principal advantages, and major limitations. The strategies include classical calcineurin inhibitors (CNIs), selective peptide-based modulators, Ca^{2+} modulating interventions, endogenous regulatory mechanisms, and genetic approaches. Emerging delivery platforms, such as nanoparticles and exosomes, are noted for their ability to improve central nervous system (CNS) penetration and enable cell-type-specific targeting. However, these technologies are in early preclinical development and meet considerable manufacturing and cost challenges. The table offers a comparative overview of continuing efforts to develop safer and more effective modulators of CaN-NFAT activity in CNS-related disorders. References supporting each strategy are provided.


A
Inactive state
Active state
CnA
(catalytic subunit)
CnB
(regulatory subunit)
Ca+2
Catalytic site
(blocked)
Catalytic site
(exposed)
CnB
CnB
CnB-
binding
helix
Calmodulin-
binding
region
Autoinhibitory
domain (AID)
Ca+2
CaM
CaN
(heterodimer)
CaM
(Ca+2-bold)


B
Canonical NFAT domain organization
N
NHR
SRR
SP
PxIxIT
LxVP
NLS
RHR
C
N-terminal regulatory region/ NFAT homology region
Serine-rich region
SP Motif
PxIxIT Motif
LxVP Motif
Nuclear localization sequence
Rel homology region (DNA-binding domain)
NFAT activation cycle
1. Phosphorylated NFAT
(retained in cytoplasm)
P
5. Rephosphorylation by
GSK3, CK1 and DYRK1A
(nuclear export)
GSK3
CK1
DYRK1A
Ca+2
CaN
2. Ca2+/CaM-mediated
dephosphorylation
NLS exposed
NLS
3.Nuclear
translocation
4.DNA binding and
transcriotional activation
NFAT
binding
site


C
1. Chronic Ca2+
elevation
2. Calpain
activation
3. Calpain cleaves
CnA AID
4.Truncated/constitutively
active CnA
5. Prolonged NFAT nuclear
residence and activation
[Ca+2]
(chronic)
Calpain
CnB-
binding
helix
Calmodulin-
bindging
region
Autoinhibitory
domain (AID)
Cleaved C-terminal
AID-containing tail
NFAT
binding
site
Cytokynes
(IL-2, IL-4)
Growth
Factors
Survival
genes


A
1. Transient/oscillatory Ca2+ signals
[Ca2+]
Time
Ca2+ entry
B
1. Sustained/repetitive Ca2+ elevation
[Ca2+]
Time
Ca2+ entry
Ca2+
Glutamate
CaN
Calcineurin
NFAT
NFAT
P
Phosphorylation
Ca2+ channel
Glutamate receptor
2. Brief calcineurin activation
CaN
CaN actibity
Time
2. Prolonged calcineurin activation
CaN
CaN actibity
Time
3. Transient NFAT nuclear translocation
NFAT
NFAT
Nuclear NFAT
Time
Short NFAT nuclear residence
(minutes)
Temporal resolution maintained
Adaptative outcomes
Reversible, adaptive transcriptional responses
3. Persistent NFAT nuclear residence
NFAT
NFAT
Nuclear NFAT
Time
Prolonged NFAT nuclear residence (hours)
Loss of temporal resolution
Maladaptative outcomes
Stabiilization of maladaptive transcriptional states
Prolonged CaN/NFAT activity
Neuron
Spine loss
Before
After
Synaptic strength/ LTP
Intrinsic excitability
Spine density
Resilience to stress and injury
Outcome: Circuit dysfunction and cognitive vulnerability
Astrocyte
Glutamate uptake (EAAts)
K+ buffering
Metabolic support
Release of gliotransmitters and D-serine
Outcome: Impaired support functions and network hyperexcitability
Microglia
NFAT-dependent inflammatory genes
Cytokines(IL-1β, TNF-α, IL-6) and chemokines (CCL2, CXCL10,etc.)
Antigen presentation and phagocytic primig
Outcome: Persistent inflammatory tone and neuroinflammation
Chronic Ca2+ stress
prolonged CaN activation
persistent NFAT nuclear residence
stable transcriptional states
Cell-type dysfunctin and diseases


A
Alzheimer's disease: sources of Ca2+ dysregulation
1.Aβ oligomers
Membrane pertubation
Pore foremation
(Ca2+ influx)
2. Altered glutamatergic signiling
NMDAR
AMPAR
3. Glial ATP/ P2X7R signaling
ATP
P2X7R
4. Tau pathology
Hyper-phosphorylation
(p -Tau)
Aggregation
Sustained Ca2+ elevation
Calcineurin activation
NFAT nuclear translocation
Synaptic dysfunction
Spine loss
Reduced LTP
Memory impairment
Neuroinflammation
Microglial activation
Cytokine release
Complement activation
B
Parkinson's disease: sources of Ca2+ dysregulation
1.Autonomous pacemaking
SNc dopaminergic neuron
2. L-type Ca2+ channels (Cav1.3)
Ca2+
3. Mitochondrial dysfunction
Complex I impairment
Reduced Δψm
Increased Ca2+ uptake
4. α-Synuclein toxicity
Oligomers
Aggregation
5. Glial inflammatory activation
Microglia
Astrocyte
IL-1β, TNF-α,
ROS, NO
Sustained Ca2+ elevation
Calcineurin activation
NFAT-dependent transcription
Dopaminergic neuron vulnerability
Mitochondrial stress
Reduced dopamine
Neuron loss
Persistent glial activation
Sustained cytokines
ROS/RNS production
Chronic inflammation
Aβ oligomers
Ca2+ (calcium)
NMDAR
AMPAR
P2X7R
L-type Ca2+ channel(Cav1.3)
Mitochondrion
α-Synuclein oligomers
α-Synuclein agregate
Microglia
Astrocyte
Phosphate (P)
C
1. Broad calcineurin inhibitors(Cyclosporin A, FK506/tacrolimus)
Ca2+
Calcineurin
NFAT activation
Pros
Robust preclinical efficacy
Cons
Systemic immunosuppression
Off-target effects
2. Low-dose tacrolimus / partial normalization
Low dose
High dose
Ca2+
Calcineurin
NFAT
Partial pathway normalization
Pros
Reduced side effects
Pathway fine-tuning
Cons
Uncertain efficacy window
Inter-individual variability
3. NFAT-selective inhibitors(e.g., VIVIT)
NFAT
Blocks NFAT-DNA binding
NFAT
Pros
Greater specificity
Minimal immunossuppression
Cons
Delivery to CNS challenging
Cell permeability limits
4. Upstream Ca2+-modulating interventions
Channel blockers (e.g., L-type Ca2+ blockers)
Glutamatergic modulators (NMDA/AMPAR)
P2X7R antagonists
Mitochondrial stabilizers (antioxidants, enhancers)
Pros
Indirect modulation
Multi-target potential
Cons
Limited clinical sucess to date
Compensatory mechanism