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Review

Reversible vs. Irreversible Neuronal Injury in Brain Ischemia: Role of the Mitochondrial–Synaptic Axis

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Abstract

Ischemia-reperfusion (I/R) injury triggers a rapid and self-amplifying cascade of mitochondrial dysfunction, synaptic instability, and neuroinflammation that ultimately determines neuronal survival and functional recovery. Early bioenergetic collapse—driven by mitochondrial depolarization, Reactive oxygen species (ROS) overproduction, and impaired mitophagy—initiates excitotoxic signaling and calpain-mediated structural degradation at vulnerable synapses. These events converge with microglial and astrocytic activation to exacerbate cytokine release, blood-brain barrier (BBB) disruption, and delayed neuronal injury. Therapeutic strategies targeting this axis show considerable promise. Mitochondria-directed interventions—including mitophagy modulation, Dynamin-related protein 1 (Drp1) inhibition, antioxidant nanocarriers, and emerging mitochondrial transplantation—restore Adenosine triphosphate (ATP) production, stabilize membrane potential, and prevent downstream excitotoxic injury. Synaptic protection via N-methyl-D-aspartate (NMDA)/postsynaptic density protein-95 (PSD-95) uncoupling, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor stabilization, and calpain inhibition preserves dendritic architecture and neurotransmission. Modulation of inflammatory pathways, particularly Interleukin-1β (IL-1β), Tumor necrosis factor-α (TNF-α), and Interleukin-6 (IL-6) signaling, as well as cell-specific microglial and astrocytic reprogramming, further attenuates secondary degeneration. Gene- and RNA-based therapies, nanoparticles engineered for BBB penetration, and extracellular vesicle–mediated delivery systems represent additional advanced platforms moving toward clinical translation. Despite substantial progress, challenges remain, including timing constraints, region-specific vulnerability (e.g., CA1 vs. CA3), limited BBB permeability, and species differences in mitochondrial dynamics and glial responses. Integrative multimodal strategies—combining mitochondrial repair, synaptic stabilization, and immunomodulation—along with advanced imaging, spatial transcriptomics, and patient-derived organoids, may accelerate the development of precision therapies for I/R injury. This review synthesizes mechanistic and translational evidence demonstrating that the mitochondria–synapse–inflammation axis forms a unified pathological framework across experimental and clinical I/R settings, including transient focal ischemia, global ischemia following cardiac arrest (CA), and reperfusion after thrombolysis or thrombectomy.

Keywords: brain ischemia; mitochondria; synapses; inflammation; calpain; drug carriers; genetic therapy

1. Introduction

Despite extensive experimental and clinical research efforts, effective neuroprotective therapies for acute ischemic stroke and ischemia-reperfusion (I/R) brain injury remain elusive. Comprehensive clinical and translational analyses have consistently emphasized that excitotoxicity, oxidative and nitrosative stress, and neuroinflammation act in concert to drive neuronal injury following ischemia and reperfusion, rather than operating as isolated pathological processes [1]. These observations underscore a major limitation of past neuroprotection strategies, which have largely focused on single molecular targets and failed to address the integrated nature of post-ischemic injury cascades.

At the early stage of ischemic injury, excessive glutamatergic signaling and pathological activation of N-methyl-D-aspartate (NMDA) receptors play a central role in neuronal dysfunction. A landmark study demonstrated that selective disruption of NMDA receptor–postsynaptic density protein 95 (PSD-95) interactions confers robust neuroprotection by uncoupling toxic calcium signaling from physiological synaptic transmission, without impairing normal synaptic function [2]. These findings provided critical proof-of-concept that synaptic signaling complexes, rather than neurotransmitter receptors alone, represent key determinants of neuronal fate after ischemic stress.

In parallel with NMDA receptor–dependent excitotoxicity, ischemia and hypoxia profoundly alter α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking and synaptic efficacy. Experimental studies in focal cortical ischemia and hypoxic conditions have shown that prolonged adenosine A1 receptor activation facilitates clathrin-mediated AMPA receptor endocytosis, leading to sustained synaptic inhibition in hippocampal

CA3–CA1 circuits [3]. These synaptic alterations highlight that ischemic injury involves not only neuronal death but also long-lasting disturbances in synaptic transmission and circuit function, which may precede irreversible degeneration.

Downstream of excitotoxic signaling, calcium-dependent proteolytic pathways critically shape the progression from reversible neuronal dysfunction to delayed neuronal death. The calpain–calpastatin–caspase hypothesis proposes that sustained activation of calpains following transient ischemia initiates cytoskeletal breakdown and apoptotic signaling, particularly in vulnerable hippocampal neurons [4]. This framework provides a mechanistic explanation for delayed neuronal death, a hallmark of global and transient cerebral ischemia, and emphasizes the importance of temporal dynamics in post-ischemic injury.

Beyond intrinsic neuronal mechanisms, post-ischemic neuroinflammation has emerged as a major contributor to secondary brain injury. Microglial and astrocytic activation after stroke can amplify excitotoxic and oxidative damage, but may also participate in repair and remodeling, depending on timing and context. Importantly, inflammatory signaling is closely linked to disruption of the blood-brain barrier (BBB), which facilitates leukocyte infiltration, edema formation, [5] and further neuronal stress during reperfusion [6]. These findings reinforce the concept that I/R injury is a multicellular process involving neurons, glia, and the neurovascular unit.

More recently, therapeutic strategies targeting mitochondrial dysfunction and delivery limitations have attracted increasing attention. Mitochondrial transplantation has shown promising neuroprotective effects in experimental I/R models and has begun to advance toward early-stage clinical evaluation, suggesting that restoration of mitochondrial bioenergetics may directly influence neuronal survival and recovery [7,8]. In parallel, the development of polymeric nanocarrier-based delivery systems offers new opportunities to enhance BBB penetration and achieve targeted delivery of neuroprotective agents in ischemic stroke [9].

At the molecular level, regulation of mitochondrial dynamics has emerged as a critical determinant of neuronal vulnerability. Inhibition of dynamin-related protein 1 (Drp1), a key mediator of mitochondrial fission, has been shown to preserve mitochondrial integrity and provide neuroprotection in both *in vitro* and *in vivo* models of ischemic injury [10]. Together, these advances highlight the growing recognition that mitochondrial integrity, synaptic stability, and inflammatory signaling are tightly interconnected in shaping neuronal outcomes after I/R.

In this review, we integrate these lines of evidence by adopting a mitochondria–synapse axis perspective to examine how mitochondrial dysfunction, synaptic destabilization, and neuroinflammatory amplification collectively define the transition from reversible neuronal impairment to irreversible degeneration following ischemia–reperfusion. Detailed mechanistic analyses are presented in the subsequent sections, while the Introduction focuses on establishing the conceptual framework, clinical relevance, and therapeutic challenges that motivate this integrated approach.

Methodology

This narrative review was conducted using a structured, multi-stage literature search designed to capture mechanistic, experimental, and translational evidence related to mitochondrial dysfunction, synaptic pathology, and neuroinflammation in I/R injury. A comprehensive search of PubMed, Scopus, and Web of Science was performed for studies published between 1990 and January 2025, using combinations of the following terms: “I/R”, “mitochondria”, “synapse”, “excitotoxicity”, “calpain”, “microglia”, “astrocyte”, “cytokines”, “nanocarrier”, “mitochondrial transplantation”, “gene therapy”, “microRNA (miRNA)”, “stroke”, “cardiac arrest (CA)”, “global ischemia”, and related phrases. Reference lists of key articles and recent reviews were hand-searched to identify additional relevant studies.

Given the mechanistic focus of this review, inclusion criteria emphasized: (1) original studies describing mitochondrial dynamics, oxidative stress, synaptic remodeling, calpain activation, inflammatory pathways, or cell–cell communication in experimental I/R models (rodent, gerbil, or cell-based), (2) translational or preclinical studies evaluating therapeutics targeting mitochondria, synapses, cytokine networks, or neuroinflammatory cascades, (3) clinical or clinical-translational studies where available, particularly those addressing mitochondrial transplantation, cytokine-targeted therapies, or BBB-penetrant delivery systems, and (4) recent systematic reviews (2020–2025) providing integrated mechanistic or therapeutic insights. Exclusion criteria included studies lacking mechanistic relevance, non-central nervous system (CNS) ischemia, purely computational work without biological validation, or reports with incomplete methodological descriptions. Because the field integrates decades of mechanistic neuroscience with emerging molecular therapeutics, older foundational studies were included when they provided canonical evidence (e.g., NMDA–PSD-95 interaction disruption, calpain–calpastatin axis). All included literature underwent critical appraisal for methodological rigor, model relevance (e.g., Middle cerebral artery occlusion [MCAO], global ischemia, CA/return of spontaneous circulation [ROSC]), and translational significance.

This review synthesizes findings along three mechanistic axes—mitochondrial integrity, synaptic stability, and neuroinflammatory modulation—to build a unified framework for understanding vulnerability and therapeutic opportunity in cerebral I/R injury. While this review is primarily narrative and focuses on the qualitative integration

of mechanistic pathways, it aims to provide a conceptual framework to guide future quantitative meta-analyses as standardized experimental designs in this field continue to evolve.

2. Mitochondrial Dynamics in Neuronal Stress

Having established the integrated mitochondria–synapse–neuroinflammation framework, the following section examines the core mitochondrial processes—bioenergetics, reactive oxygen species (ROS) generation, Calcium ion (Ca^{2+}) buffering, and fission–fusion dynamics—that ultimately dictate whether ischemic neurons recover or proceed toward irreversible degeneration. Mitochondria are central regulators of neuronal viability under ischemic stress due to their dual roles in adenosine triphosphate (ATP) production and cell death signaling. During the early phase of ischemia, neurons often exhibit a reversible decline in $\Delta\Psi m$ associated with transient energy depletion. However, prolonged ischemia or delayed reperfusion induces persistent depolarization, mitochondrial fragmentation, and the release of pro-apoptotic factors such as cytochrome c, which collectively signal a transition from reversible dysfunction to irreversible degeneration [10,11,12,13].

Recent reviews emphasize that mitochondrial dynamics—including fission, fusion, mitophagy, and transport—act as active determinants rather than passive consequences of neuronal injury [14,15]. In particular, the balance between mitochondrial fission and fusion, governed primarily by dynamin-related protein-1 (Drp1) and fusion mediators mitofusin-1/2 (Mfn1/2) and optic atrophy protein-1 (Opa1), is disrupted during ischemic stress. Excessive Drp1-mediated fission has been repeatedly linked to aggravated mitochondrial fragmentation and neuronal loss, whereas promotion of fusion supports mitochondrial network integrity and bioenergetic recovery [14].

Experimental evidence supports these dynamics across multiple ischemic paradigms. In rat global ischemia models (5–8 min bilateral common carotid artery occlusion under normothermia), post-ischemic administration of mitochondrial stabilizers such as cyclosporin A (CsA) significantly restored $\Delta\Psi m$, reduced caspase-3 activation, and preserved hippocampal CA1 pyramidal neurons during the 24–72 h reperfusion period [16,17,18]. These effects are attributed to inhibition of the mitochondrial permeability transition pore (mPTP) and reduction of oxidative stress. Likewise, neuronal *Drp1* gene deletion or pharmacologic inhibition (using Drp1-targeting peptides or small-molecule inhibitors such as mdivi-1) markedly reduced infarct volume and improved motor outcomes in mouse MCAO models [10,12]. In contrast, transient suppression of Drp1 during reperfusion, rather than continuous inhibition, was most effective in maintaining the physiological fission–fusion cycle without impairing mitochondrial turnover [13].

At the ultrastructural level, transmission electron microscopy in gerbil and rat hippocampi has shown that preserved cristae architecture and mitochondrial motility correlate with maintenance of dendritic spines and postsynaptic densities, whereas swollen or immobile mitochondria predict progressive dendritic degeneration [19,20]. These findings underscore that mitochondrial morphology reflects not merely structural integrity but also the metabolic competence essential for synaptic recovery.

Beyond morphology, mitophagy—the selective removal of damaged mitochondria via autophagy—emerges as a critical adaptive process. Studies using oxygen–glucose deprivation (OGD) in primary cortical or hippocampal neurons demonstrate that timely activation of PTEN-induced kinase 1/Parkin (PINK1/Parkin)-dependent mitophagy prevents accumulation of dysfunctional mitochondria, whereas excessive or delayed mitophagy leads to synaptic energy failure and cell death. Inhibition of excessive mitophagy by SIRT3 activation or AMPK modulation has been shown to mitigate neuronal damage following hypoxia-ischemia in neonatal rat and mouse models [21]. These results highlight that mitophagy must remain quantitatively balanced to support neuronal recovery.

Moreover, the spatial distribution and active transport of mitochondria are crucial for neuronal resilience. Live imaging in murine hippocampal neurons reveals that disruption of microtubule-based mitochondrial trafficking, mediated by Miro1 and TRAK-kinesin complexes, limits mitochondrial recruitment to dendritic and axonal terminals, thereby compromising local ATP supply and calcium buffering capacity during reperfusion. Such transport defects accelerate synaptic energy failure and exacerbate neuronal loss [14,19].

Collectively, these data demonstrate that mitochondrial dynamics, bioenergetics, and spatial distribution jointly determine the threshold between reversible and irreversible neuronal injury. Mitochondria thus function as a regulatory hub within the mitochondrial–synaptic axis, integrating metabolic, structural, and signaling cues that dictate neuronal survival or degeneration under ischemic stress. This principle is further reflected in the differential susceptibility of hippocampal subregions, where CA1 neurons exhibit earlier mitochondrial collapse, impaired calcium buffering, and greater ROS sensitivity compared with the more resilient CA3 neurons (Table 1, Ref. [16,17,18,19,20,22,23,24,25,26,27,28,29,30,31,32]).

3. Synaptic Vulnerability and Plasticity After Ischemia

Synapses represent one of the earliest and most sensitive targets of ischemic stress. Because synaptic transmission requires continuous ATP supply and precise Ca^{2+} regulation, transient energy deprivation rapidly disrupts presy-

Table 1. Mitochondrial dynamics as determinants of reversible vs. irreversible neuronal injury after ischemia–reperfusion.

Mitochondrial process	Reversible dysfunction (Early/Adaptive Phase)	Irreversible degeneration (Prolonged/Maladaptive Phase)	Key references
Bioenergetics & ΔΨm stability	Transient ΔΨm depolarization with partial ATP recovery during early reperfusion	Persistent ΔΨm collapse, ATP failure, cytochrome c release	[19,22,23,24]
mPTP regulation	mPTP remains largely closed; mitochondrial swelling is limited	Sustained mPTP opening → mitochondrial swelling, caspase-3 activation, cell death	[16,17,18]
Oxidative stress (ROS)	Moderate ROS signaling supports adaptive responses	Excessive ROS during reperfusion → lipid peroxidation, mitochondrial damage	[25,26,27,28,29,30]
Mitochondrial fission–fusion balance	Physiological Drp1-dependent fission supports mitochondrial quality control	Excessive Drp1-driven fission → mitochondrial fragmentation and neuronal loss	[19,23,24,31,32]
Cristae architecture & ultrastructure	Preserved cristae integrity correlates with synaptic recovery	Cristae disruption and mitochondrial swelling predict dendritic degeneration	[19,20]
Mitophagy (PINK1/Parkin pathway)	Timely mitophagy removes damaged mitochondria and supports recovery	Excessive or delayed mitophagy causes synaptic energy failure	[27]
Mitochondrial transport & spatial distribution	Efficient microtubule-based trafficking maintains local ATP and Ca^{2+} buffering	Impaired transport limits mitochondrial recruitment to synapses	[28,31]
Regional vulnerability (CA1 vs. CA3)	CA3 neurons maintain mitochondrial integrity and adaptive dynamics	CA1 neurons exhibit earlier mitochondrial collapse and degeneration	[28,29,30]

This table provides a comprehensive comparative synthesis of the molecular and structural checkpoints that dictate neuronal fate. By categorizing key mitochondrial processes—ranging from bioenergetic stability and ΔΨm maintenance to cristae ultrastructure and regional vulnerability—the table delineates the precise threshold where early adaptive responses transition into maladaptive, irreversible degeneration. It highlights how the preservation of mitochondrial integrity, particularly at the synaptic interface, serves as a primary marker for salvageable tissue, whereas sustained mPTP opening and transport failure signify a point of no return. This integrated comparison underscores the mitochondrial–synaptic axis as the critical framework for identifying potential therapeutic windows. **Abbreviations**: ΔΨm, mitochondrial membrane potential; mPTP, mitochondrial permeability transition pore; Drp1, dynamin-related protein 1; ATP, adenosine triphosphate; ROS, reactive oxygen species; I/R, ischemia–reperfusion; OGD, oxygen–glucose deprivation; MCAO, middle cerebral artery occlusion; PINK1, PTEN-induced kinase 1; Ca^{2+}, Calcium ion.

naptic vesicle cycling, postsynaptic receptor anchoring, and dendritic spine morphology. Early structural collapse of synapses often precedes overt neuronal death and, importantly, can remain partially reversible during early reperfusion [24,31,32].

3.1 Early Synaptic Disruption and Morphological Changes

Synapses are among the earliest and most vulnerable targets of ischemic injury. Because excitatory glutamatergic transmission depends on a continuous supply of ATP and tightly regulated intracellular Ca^{2+} dynamics, even brief episodes of impaired perfusion or OGD can rapidly disrupt presynaptic vesicle cycling, postsynaptic receptor anchoring, and dendritic spine morphology. The structural collapse of excitatory synapses often precedes neuronal soma loss and, under favorable conditions, may remain partially reversible during the early phase of reperfusion. In organotypic hippocampal slice cultures from rats subjected to OGD (10–20 min), dendritic spine loss becomes evident within 30 minutes but partially recovers when oxygen and glucose are reintroduced for 2–4 hours [24]. *In vivo*, two-photon imaging in Thy1- Green fluorescent protein (GFP) transgenic mice undergoing MCAO (60 min occlusion + 24–48 h reperfusion) reveals rapid spine retraction in cortical layers II–V, followed by gradual regeneration in the peri-infarct cortex [25].

More recently, in C57BL/6J mice equipped with a chronic cranial window over the primary somatosensory cortex (S1), Li et al. (2020) [26] used *in vivo* two-photon imaging combined with electrophysiological recordings to show that transient I/R induces rapid dendritic beading and

spine perturbation, accompanied by cortical hyperexcitability and altered AMPAR trafficking during early reperfusion. These synaptic and receptor changes gradually normalize over the subsequent days, supporting the view that ischemia-induced structural and functional disruption is initially reversible within a limited therapeutic window [26]. Ultrastructural transmission electron microscopy (TEM) studies provide convergent evidence for early yet reversible synaptic injury. In the gerbil hippocampus following 5 min global ischemia, PSD fragmentation and presynaptic vesicle depletion appear at 6 h of reperfusion in the CA1 region, preceding delayed neuronal death at 72 h. In contrast, the CA3 subfield remains largely preserved, highlighting region-specific synaptic resistance [27].

Collectively, these findings indicate that ischemia-induced synaptic degeneration occurs rapidly but not irreversibly, with the potential for recovery dependent on prompt restoration of perfusion and metabolic stability. Synaptic alterations therefore mark a critical early phase of ischemic brain injury—a phase amenable to therapeutic modulation before structural disassembly becomes permanent.

3.2 Molecular Mechanisms of Synaptic Destabilization

Building on recent findings, Li et al. (2020) [26] demonstrated that transient cerebral I/R induces aberrant trafficking of AMPARs in the mouse somatosensory cortex. This receptor redistribution enhances postsynaptic Ca^{2+} influx and contributes to neuronal hyperexcitability during early reperfusion. Sustained Ca^{2+} accumulation perturbs endoplasmic reticulum (ER)–mitochondrial Ca^{2+} exchange, leading to mitochondrial depolarization and generation of ROS. Collectively, these observations delineate a mechanistic continuum linking AMPAR-mediated excitotoxicity to mitochondrial stress, thereby initiating early post-ischemic synaptic destabilization [26]. Ischemic excitotoxicity arises primarily from excessive glutamate release and prolonged activation of N-methyl-D-aspartate receptors (NMDARs) and AMPARs. In rat hippocampal slice cultures subjected to OGD (20 min), sustained Ca^{2+} influx activates calpain I/II, which cleaves spectrin, PSD-95, and other cytoskeletal proteins [28]. This proteolytic cascade destabilizes the PSD scaffold, causing dendritic-spine retraction and impaired synaptic transmission. Pharmacological calpain inhibition with compounds such as MDL-28170 or calpeptin markedly attenuates these alterations, preserving synaptic organization and reducing delayed neuronal degeneration. Further supporting this mechanism, Peng et al. (2011) [29] demonstrated that administration of calpeptin to Sprague–Dawley rats subjected to transient focal cerebral I/R MCAO (90 min ischemia + 24 h reperfusion) significantly reduced neuronal apoptosis in the cortex and hippocampal CA1 region while improving behavioral outcomes. Similarly, Yildiz-Unal et al. (2015) [30] reported that MDL-28170 and calpeptin prevent calpain-mediated cytoskeletal degradation and preserve neuronal morphology in ischemic and traumatic brain-injury models. Together, these data indicate that calpain inhibition stabilizes postsynaptic architecture and limits delayed neuronal loss following ischemic stress. As a downstream consequence of Ca^{2+} overload, mitochondrial dysfunction further exacerbates synaptic injury. In mouse MCAO models (60 min occlusion + 24 h reperfusion), Huan et al. (2023) [13] showed that excessive activation of Drp1 promotes mitochondrial fission, loss of $\Delta\Psi m$, and ROS accumulation in cortical neurons. These changes impair ATP production, disrupt presynaptic vesicle cycling, and aggravate synaptic failure. Conversely, pharmacological Drp1 inhibition using the selective small-molecule inhibitor mdivi-1 (1.2 mg/kg i.p., administered immediately after reperfusion) or genetic deletion of the neuronal Drp1 activator in Drp1-deficient mice and primary cortical neurons exposed to OGD (60 min ischemia + 24 h reoxygenation) mitigated mitochondrial fragmentation, preserved $\Delta\Psi m$ integrity, and reduced neuronal death, thereby maintaining synaptic ultrastructure and transmission fidelity [10]. Oxidative and nitrosative stress further propagate these cascades. Jurcau and Ardelean (2022) [33] reported that I/R in the rat hippocampus elevates ROS and reactive nitrogen species (RNS) within hours of reperfusion, activating nuclear factor-κB (NF-κB) and p53 signaling. These transcriptional events upregulate pro-apoptotic genes (e.g., *Bax*, *PUMA*) and inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). This secondary wave of transcriptional activation exacerbates mitochondrial injury and contributes to further synaptic degeneration and neuronal loss. At the transcriptomic level, ischemia-induced oxidative and metabolic stress remodel synaptic-gene expression. Using primary cortical neurons exposed to OGD and C57BL/6 mouse cortex following transient MCAO (60 min occlusion + 24 h reperfusion), Gupta et al. (2025) [34] found that ROS accumulation drives epigenetic modifications of synaptic genes through altered histone acetylation and DNA methylation, suppressing transcripts involved in vesicle recycling and synaptic plasticity. This oxidative epigenetic reprogramming constrains long-term synaptic recovery even after reperfusion has restored cerebral blood flow.

Collectively, these findings delineate a multistage pathogenic sequence: (1) AMPAR/NMDAR hyperactivation initiates Ca^{2+} influx and receptor-trafficking abnormalities; (2) Ca^{2+}-dependent calpain activation dismantles the PSD architecture; (3) Drp1-mediated mitochondrial fission and depolarization impair energy metabolism; and (4) ROS-driven transcriptional pathways perpetuate synaptic degeneration. Each stage amplifies the next through interconnected feedback loops, linking excitatory receptor signaling at the membrane to mitochondrial collapse and, ultimately, irreversible neuronal injury.

3.3 Cytokine-Mediated Neurotoxicity

Cytokine dysregulation is a critical downstream mediator of ischemia-induced synaptic and neuronal injury. Following reperfusion, excessive production of pro-inflammatory cytokines—IL-1β, TNF-α, and interleukin-6 (IL-6)—acts in concert with excitotoxic and oxidative pathways to impair synaptic signaling, mitochondrial metabolism, and neuronal viability [35]. These cytokines are predominantly released by activated microglia and astrocytes, but also by endothelial cells and infiltrating leukocytes, generating a neuroinflammatory milieu that extends beyond the ischemic core. Elevated cytokine levels suppress glutamate uptake, enhance ROS production, and activate apoptotic signaling cascades, thereby coupling immune activation to synaptic degeneration.

3.3.1 Human Evidence

Clinical investigations and meta-analyses consistently indicate that systemic IL-6 elevation correlates with both ischemic-stroke incidence and unfavorable outcomes. A comprehensive meta-analysis of prospective cohorts demonstrated that higher circulating IL-6 concentrations were associated with increased risk of ischemic stroke, and Mendelian-randomization data supported a causal contribution of IL-6 signaling to specific stroke subtypes [36]. Within the CNS, early lumbar-puncture studies have shown elevated cerebrospinal-fluid (CSF) TNF-α and IL-6 levels during the acute phase of ischemic stroke, correlating with clinical severity [37]. Neuroinflammation imaging using translocator-protein positron-emission tomography (TSPO-PET) with [^{11}C]PK11195 has revealed persistent microglial activation for several weeks after stroke [38]. Collectively, these human data underscore the dual roles of IL-6, TNF-α, and IL-1β as biomarkers and mechanistic drivers of sustained neuronal dysfunction after I/R.

3.3.2 Animal and Cellular Models

Experimental evidence corroborates these clinical observations. In primary rat hippocampal neurons, recombinant IL-1β (3 ng/mL, 24 h) decreases postsynaptic PSD-95-GFP puncta (~23% loss) and presynaptic Synaptophysin-GFP clusters. These changes are prevented by Interleukin-1 receptor antagonist (IL-1Ra, 1 μg/mL), by N-methyl-D-aspartate receptor (NMDAR) blockade (MK-801/AP5), or by cyclo-oxygenase-2 (COX-2) inhibition (NS-398), indicating that IL-1β-induced synaptic disassembly operates via glutamate/NMDAR- and prostaglandin-dependent mechanisms [39]. In rat MCAO (90 min ischemia + 24 h reperfusion), systemic administration of the calpain inhibitor calpeptin significantly reduces infarct volume and apoptotic cell death in cortex and hippocampus, improving neurological scores—supporting a cytokine → Ca^{2+} → calpain → postsynaptic density injury axis *in vivo* [29]. Complementary analyses demonstrate that MDL-28170, a brain-permeable peptide-aldehyde and selective calpain-1/2 inhibitor, together with calpeptin, consistently attenuates calpain-mediated degradation of cytoskeletal and PSD proteins and preserves neuronal architecture in ischemic and traumatic brain-injury models [30,40]. Cytokine signaling also converges on mitochondrial dysfunction. In cultured neurons, TNF-α rapidly induces mitochondrial depolarization, ROS generation, and ATP loss via TNF-receptor-1 (TNFR1); neutralizing TNF-α/TNFR1 interactions rescues mitochondrial ΔΨm and ATP levels [41]. Under mouse MCAO and primary cortical neuron OGD/R conditions, excessive Drp1 activation drives mitochondrial fission and ΔΨm loss [10]. Finally, several *in vivo* studies confirm that suppressing IL-1 signaling alleviates ischemic brain injury. Overexpression or systemic delivery of IL-1Ra reduces infarct size and leukocyte infiltration in mouse/rat MCAO models, validating the IL-1 axis as a translational target [42]. Collectively, evidence from both cellular OGD and rodent MCAO models demonstrates that pro-inflammatory cytokines such as IL-1β and TNF-α directly damage synapses and mitochondria. Specifically, cytokine exposure reduces PSD-95 and Synapsin I expression, while promoting mitochondrial depolarization (ΔΨm↓), ROS accumulation, and Drp1-dependent fission. Conversely, blocking IL-1 signaling, calpain activation, or Drp1-mediated mitochondrial fragmentation markedly preserves synaptic integrity and neuronal survival.

3.3.3 Mechanistic Convergence

At the mechanistic level, IL-1β and TNF-α jointly amplify Ca^{2+} dysregulation and oxidative stress. IL-1β potentiates NMDAR-mediated Ca^{2+} entry via NR2A/GluN2B phosphorylation in hippocampal neurons, increasing excitatory drive [43]. In parallel, TNF-α enhances AMPAR surface expression and promotes GluA2-lacking AMPAR trafficking to synapses, thereby heightening Ca^{2+} permeability and excitotoxic vulnerability [44]. This receptor redistribution further depletes neuronal ATP and intensifies ROS accumulation. Concurrently, IL-6–signal transducer and activator of transcription 3 (STAT3) signaling regulates mitochondrial gene programs critical for neuronal bioenergetics; STAT3 translocates to mitochondria and modulates oxidative-phosphorylation capacity, linking cytokine signaling to delayed energetic imbalance after ischemia [45]. Together, these interconnected processes create a feed-forward loop wherein cytokine-induced Ca^{2+} and oxidative stress sustain synaptic collapse and neuronal death.

In summary of this section, cytokine-mediated neurotoxicity represents a pivotal link between neuroinflammation and neuronal degeneration following I/R. Human and animal studies converge to show that excessive IL-1β, TNF-α, and IL-6 disrupt synaptic structure and mitochondrial homeostasis through receptor- and transcription-dependent signaling. Targeting cytokine receptors or their downstream cascades (e.g., NF-κB, Janus kinase (JAK)/STAT3) may therefore provide a dual benefit—attenuating in-

flammation and preserving synaptic integrity in the post-ischemic brain. This conceptual framework is illustrated in Fig. 1, which shows how mitochondrial status during hypoxia and reoxygenation ultimately determines whether synapses undergo recovery or structural collapse.

4. Mitochondria–Synapse Axis: Converging Pathways

The integrity of the mitochondria–synapse axis is a central determinant of neuronal survival after I/R injury. Synaptic transmission and structural plasticity rely on continuous ATP supply, calcium buffering, and redox regulation provided by mitochondria strategically positioned within presynaptic terminals and dendritic spines. Conversely, synaptic activity shapes mitochondrial trafficking, fission–fusion balance, and metabolic load. During I/R, this bidirectional coupling becomes critically disrupted: mitochondrial depolarization, excessive ROS, impaired calcium handling, and altered dynamics directly destabilize synaptic vesicle cycling, receptor trafficking, and spine architecture. Thus, mitochondrial dysfunction and synaptic failure do not occur as isolated events but as tightly linked processes that amplify each other, accelerating the transition from reversible synaptic impairment to irreversible neuronal degeneration.

4.1 Overview and Conceptual Framework

Neurons rely on a tightly coordinated partnership between mitochondria and synapses to sustain excitatory transmission and structural plasticity. Presynaptic mitochondria generate ATP essential for synaptic vesicle recycling and buffer local Ca^{2+} transients during high-frequency activity, whereas postsynaptic mitochondria support receptor stabilization and cytoskeletal remodeling during activity-dependent spine plasticity [46,47]. This spatial and functional alignment constitutes the mitochondria–synapse axis, a central determinant of neuronal resilience under metabolic or oxidative stress. Following I/R, this axis becomes markedly disrupted. Transient ATP depletion and excessive Ca^{2+} influx overload mitochondrial buffering capacity, triggering mitochondrial depolarization ($\Delta\Psi m$ loss), ROS accumulation, and Drp1–mediated mitochondrial fission. These changes have been demonstrated in mouse MCAO (60 min ischemia followed by 24 h reperfusion) models and in primary cortical neurons exposed to OGD/R [6]. At the synaptic level, impaired mitochondrial transport and docking limit energy delivery to presynaptic boutons, restricting vesicle cycling, while postsynaptic mitochondria undergo fragmentation and detach from the PSD, promoting dendritic spine collapse and synapse loss. Electron microscopy and live-cell mitochondrial imaging in rodent focal ischemia and OGD models support these observations [12]. Further evidence for this bidirectional relationship between mitochondrial distress and synaptic dysfunction comes from *in vivo* imaging studies. In Thy1-mito-CFP transgenic mice subjected to transient MCAO (60 min ischemia followed by 24–72 h reperfusion), mitochondrial fragmentation and reduced motility closely parallel dendritic spine loss in peri-infarct cortical neurons. Pharmacological stabilization of mitochondrial integrity using CsA or the Drp1 inhibitor mdivi-1 preserves mitochondrial continuity and prevents spine elimination [15,48]. Conversely, enhancement of mitochondrial resilience through SIRT3 activation restores synaptic proteins such as PSD-95 and Synapsin I and improves behavioral outcomes in mouse global ischemia models [21].

Collectively, these findings indicate that the mitochondria–synapse axis is not a passive casualty of ischemic injury but an active determinant of neuronal outcome. Failure of this interdependent system produces a reinforcing cycle: mitochondrial energy loss destabilizes synapses, and synaptic hyperexcitability further injures mitochondria through Ca^{2+}-dependent ROS generation. Understanding this cross-talk establishes a foundational framework for the mechanistic and therapeutic convergence explored in subsequent sections.

4.2 Presynaptic Energy and Mitochondrial Coupling

Presynaptic terminals depend on a continuous supply of ATP and localized Ca^{2+} buffering to support synaptic vesicle exocytosis, endocytosis, and vesicle recycling. Mitochondria positioned within or adjacent to presynaptic boutons generate ATP through oxidative phosphorylation and modulate rapid Ca^{2+} transients during high-frequency neuronal activity. Under physiological conditions, this mitochondria–presynaptic coupling sustains vesicle availability, maintains transmission fidelity, and prevents failures in energy-dependent synaptic processes [49,50]. During I/R, this coupling is rapidly compromised. Early ATP depletion and persistent Ca^{2+} influx overwhelm mitochondrial buffering capacity, leading to mitochondrial depolarization (loss of $\Delta\Psi m$), impaired electron transport chain activity, and reduced motility of axonal or bouton-resident mitochondria. Single-organelle imaging studies demonstrate that hypoxia–reoxygenation triggers transient "superoxide flashes", reflecting acute oxidative stress and early mitochondrial instability [51]. Excessive Ca^{2+} and ROS also promote opening of the mPTP, a critical event that precipitates $\Delta\Psi m$ collapse, bioenergetic failure, and heightened neuronal vulnerability during reperfusion [52,53]. These early mitochondrial insults occur before overt structural synaptic degeneration, indicating that presynaptic metabolic destabilization is a key initial driver of functional decline. Presynaptic metabolic failure directly impairs synaptic vesicle recycling. Depletion of mitochondrially derived ATP disrupts vesicle endocytosis and recycling and reduces the capacity of terminals to maintain neurotransmitter release [46]. Altered mitochondrial dynamics—particularly excessive Drp1-mediated mitochondrial fission—further compromise ATP availability at presynaptic boutons, weaken vesicle mobilization from

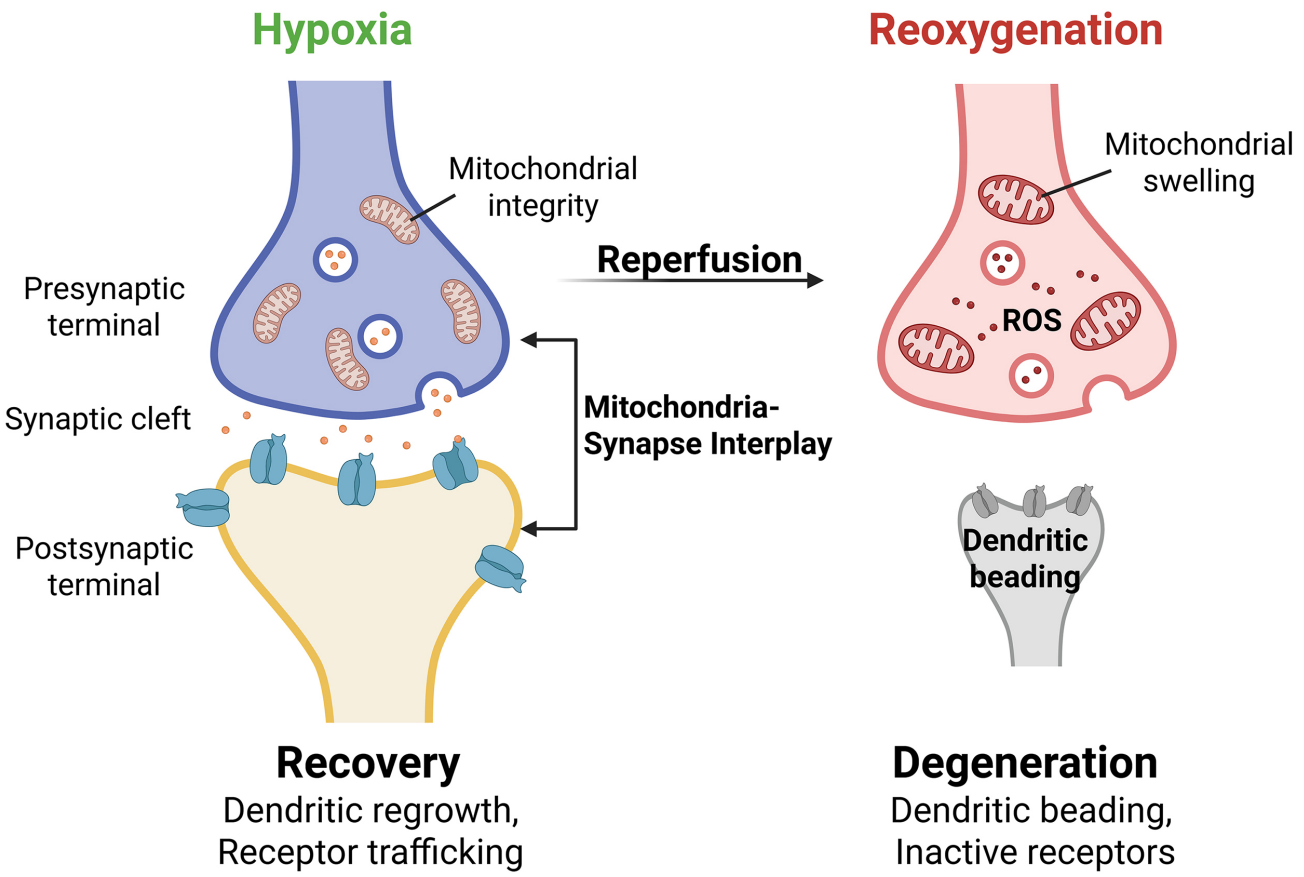

Fig. 1. The mitochondrial-synaptic axis as a metabolic checkpoint during the hypoxia-reoxygenation cycle. This schematic elucidates the divergent fates of synaptic terminals—recovery versus degeneration—as dictated by mitochondrial bioenergetic status. (Left panel: Hypoxia → Recovery) Under moderate hypoxic conditions, synaptic terminals that preserve mitochondrial integrity can sustain essential vesicle cycling. In this neuroprotective trajectory, functional mitochondria provide the necessary ATP for neurotransmitter release, receptor trafficking, and subsequent dendritic regrowth, thereby facilitating synaptic plasticity and structural restoration. (Right panel: Reoxygenation → Degeneration) In contrast, maladaptive reoxygenation triggers a pathological cascade characterized by ROS overproduction and mitochondrial swelling. This failure is marked by a collapsed mitochondrial membrane potential ($\Delta\Psi m$) and ATP depletion, which collectively precipitate dendritic beading, receptor inactivation, and irreversible synaptic collapse. Central concept: Synaptic viability is governed by a bidirectional dependency between mitochondrial health and synaptic architecture; preserved bioenergetics favor functional salvage, whereas mitochondrial exhaustion shifts the balance toward terminal degeneration. Abbreviations: ATP, adenosine triphosphate; I/R, ischemia–reperfusion; ROS, reactive oxygen species. Created in BioRender. Yoo, K. Y. (2026) https://BioRender.com/549oja8.

reserve pools, and destabilize presynaptic release machinery [25,54]. These findings collectively indicate that maintenance of presynaptic vesicle cycling is tightly linked to mitochondrial positioning, integrity, and metabolic output.

Together, presynaptic energy depletion, impaired mitochondrial trafficking, and $\Delta\Psi m$ loss constitute an integrated pathogenic cascade during I/R. Failure of mitochondria to reach or remain anchored at active boutons results in insufficient ATP delivery, reduced Ca^{2+} buffering capacity, and progressive arrest of vesicle recycling. These changes represent one of the earliest—and potentially reversible—components of post-ischemic synaptic dysfunction, underscoring the mitochondria–presynaptic interface as a promising target for neuroprotective intervention.

4.3 Postsynaptic Energy Demand and Spine Remodeling

Postsynaptic compartments require substantial ATP to support receptor trafficking, actin cytoskeleton remodeling, and maintenance of dendritic spine structure. Postsynaptic mitochondria positioned beneath the PSD provide ATP for activity-dependent spine enlargement and stabilize local Ca^{2+} dynamics during synaptic potentiation [55,56]. Under physiological conditions, this mitochondria–spine alignment enables efficient AMPAR insertion, actin polymerization, and long-term potentiation (LTP), thereby supporting both structural and functional plasticity. Recent evidence further indicates that postsynaptic mitochondria contribute directly to the structural diversity and stability of dendritic spines. Using *in vivo* two-photon cal-

cium imaging combined with high-resolution serial block-face scanning electron microscopy (SBF-SEM) and correlative light–electron microscopy (CLEM) in adult ferret visual cortex pyramidal neurons, Thomas et al. (2023) [57] demonstrated that the density, morphology, and spatial proximity of postsynaptic mitochondria are strong predictors of spine subtype distribution and their activity-dependent remodeling. These findings indicate that postsynaptic mitochondria act not only as bioenergetic organelles but also as structural regulators that shape synaptic architecture and guide spine-specific plasticity trajectories. These postsynaptic mitochondrial functions during I/R become rapidly impaired. Excessive Ca^{2+} influx through NMDARs and voltage-gated Ca^{2+} channels overwhelms mitochondrial Ca^{2+} buffering, leading to mitochondrial depolarization ($\Delta\Psi m$ loss), increased ROS production, and activation of Drp1–mediated mitochondrial fission [6]. In rodent neuronal models, metabolic stress such as OGD/R induces pronounced postsynaptic mitochondrial fragmentation through excessive activation of Drp1. This mitochondrial fission response reduces the residency of mitochondria at dendritic spine bases, limiting local ATP availability and impairing Ca^{2+} buffering. Using brain-specific Drp1 conditional knockout mice and live two-photon imaging, Itoh et al. (2019) [58] demonstrated that loss of Drp1 function prevented stress-induced mitochondrial fragmentation and preserved dendritic spine density, confirming a causal role for Drp1-mediated mitochondrial dynamics in postsynaptic structural stability. More broadly, as reviewed by Todorova and Blokland (2017) [59], mitochondrial positioning and morphological integrity are essential for sustaining postsynaptic protein trafficking, PSD organization, and spine remodeling, particularly under conditions of metabolic or oxidative challenge. Classical work further demonstrates that dendritic mitochondria are required to support AMPA receptor (AMPAR) stabilization and spine head enlargement during synaptic potentiation; removal of mitochondria from spine bases impairs LTP-associated structural remodeling and promotes spine collapse [55].

Recent work also suggests that restoration of mitochondrial integrity may facilitate recovery of postsynaptic structure after injury. In a systematic review of mitochondrial transplantation approaches, Wang et al. (2024) [60] summarized evidence from rodent spinal cord injury models in which exogenous mitochondria—isolated from healthy donor tissue and delivered via intraparenchymal or intrathecal injection—enhanced neuronal survival, increased synaptic protein expression (e.g., PSD-95, Synapsin I), and promoted structural remodeling of damaged neural circuits. Although these findings were obtained outside the cerebral ischemia field, they reinforce a generalizable principle: successful mitochondrial repair or augmentation is closely linked to postsynaptic reconstruction, a concept directly relevant to I/R injury in the brain.

At the molecular level, Ca^{2+}-dependent activation of calcineurin and cofilin drives actin depolymerization, destabilizing spine cytostructure and limiting AMPAR retention. Concurrent ROS elevation oxidizes actin-regulatory proteins and disrupts PSD scaffolding. These combined events impair the postsynaptic capacity for synaptic strengthening and bias the spine population toward immature or unstable forms.

Collectively, these processes indicate that postsynaptic energy demand and mitochondrial positioning are integral to spine resilience. When I/R disrupts mitochondrial ATP generation, Ca^{2+} handling, or local anchoring at the PSD, dendritic spines lose both metabolic support and structural integrity. This breakdown of postsynaptic bioenergetics is a central determinant of early synaptic failure and limits the brain's capacity for plasticity-driven recovery after ischemic injury. This integrative relationship among mitochondrial dynamics, postsynaptic structure, and inflammation-driven metabolic stress is conceptually summarized in Fig. 2, which highlights how disruptions across the mitochondria–synapse–inflammation axis collectively drive postsynaptic degeneration after I/R injury.

4.4 Bidirectional Feedback Loops Between Mitochondrial Dysfunction and Synaptic Failure

The mitochondria–synapse axis operates as a tightly regulated bidirectional system in which perturbations to one component rapidly propagate to the other. During I/R, this reciprocal relationship shifts from a physiological coupling that supports synaptic plasticity into a pathological series of self-amplifying positive-feedback loops that collectively increase neuronal vulnerability. These feedback cycles accelerate metabolic collapse and synaptic degeneration and help define the molecular thresholds that separate reversible synaptic dysfunction from irreversible structural injury.

4.4.1 Mitochondria → Synapse (Energy Failure and ROS as Primary Drivers of Synaptic Collapse)

Post-ischemic mitochondrial dysfunction generates two major stressors—energy depletion and oxidative damage—that directly impair synaptic structure and function. Loss of $\Delta\Psi m$ reduces ATP availability at both presynaptic and postsynaptic terminals, thereby disrupting vesicle recycling, glutamate-receptor stabilization, and actin-cytoskeletal dynamics. In rodent MCAO models and hippocampal OGD/R assays, early mitochondrial depolarization correlates with dendritic-spine retraction and decreased PSD integrity, demonstrating that mitochondrial bioenergetic failure initiates synaptic dismantling [15,23].

Mitochondria-derived ROS additionally oxidize actin-regulatory proteins, destabilize PSD scaffolds, and impair glutamate-receptor trafficking [61,62]. Excess ROS sensitizes NMDARs and AMPARs, further increasing Ca^{2+} influx and intensifying excitotoxic signaling [26,63]. Together, these events directly couple mitochondrial instabil-

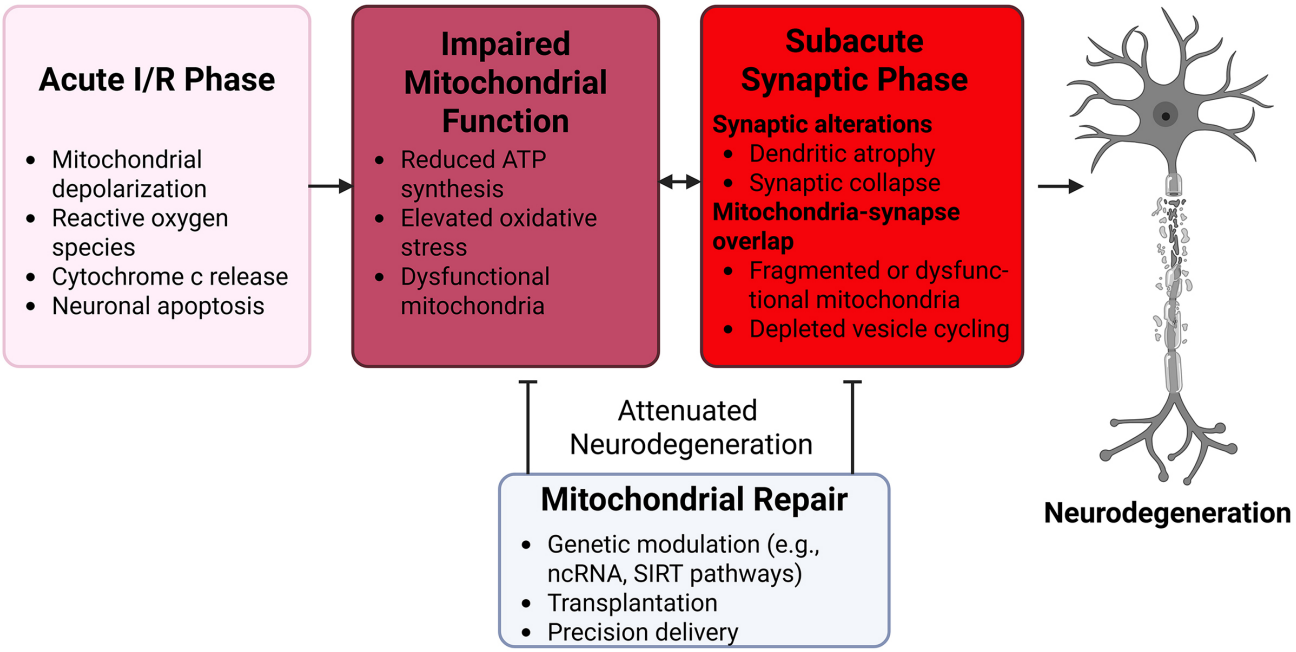

Fig. 2. Spatiotemporal cascade of mitochondrial-synaptic failure and strategic therapeutic intersections. This conceptual flowchart delineates the pathological transition from acute ischemia–reperfusion (I/R) injury to subacute synaptic collapse. The upper panels map the initial metabolic catastrophe, where mitochondrial depolarization and oxidative stress culminate in cytochrome-c release and neuronal apoptosis. These early bioenergetic failures subsequently evolve into persistent mitochondrial dysfunction, characterized by an elevated oxidative burden and a critical deficit in ATP synthesis. As the injury reaches the subacute phase, structural and functional synaptic impairments emerge, including dendritic atrophy and vesicle cycling failure—processes that are further exacerbated by mitochondrial fragmentation at the synaptic interface. Collectively, these events form a self-perpetuating cycle toward neurodegeneration. The lower panels identify multifaceted therapeutic windows aimed at rescuing the mitochondrial-synaptic axis. Potential interventions include genetic modulation (e.g., ncRNA and SIRT signaling pathways), autologous mitochondrial transplantation, and precision nanocarrier-based delivery systems. These combined strategies are designed to restore mitochondrial homeostasis, bolster synaptic resilience, and ultimately attenuate long-term neurological deficits. Abbreviations: ATP, adenosine triphosphate; BBB, blood-brain barrier; I/R, ischemia–reperfusion; ncRNA, non-coding RNA; ROS, reactive oxygen species; SIRT, sirtuins. Created in BioRender. Yoo, K. Y. (2026) https://BioRender.com/549oja8.

ity to synaptic failure, forming a unidirectional degenerative cascade when reparative capacity is exceeded.

4.4.2 Synapse → Mitochondria (Hyperexcitability and Ca^{2+} Overload as Catalysts of Mitochondrial Injury)

Conversely, synaptic hyperactivity during early reperfusion imposes substantial metabolic and ionic stress on mitochondria. Aberrant AMPAR and NMDAR trafficking increases postsynaptic Ca^{2+} influx, overwhelming Ca^{2+}-buffering capacity of dendritic and axonal mitochondria, as shown in mouse somatosensory-cortex I/R models and primary cortical-neuron OGD/R systems [26]. Sustained Ca^{2+} elevation promotes excessive activation of Drp1, driving mitochondrial fission, $\Delta\Psi m$ loss, and ROS accumulation in both primary cortical neurons and mouse MCAO tissue [13]. Hyperexcitability also increases presynaptic glutamate release, elevating neurotransmitter-cycling demands and taxing mitochondrial ATP production [46,50]. Fragmented and immobile mitochondria show reduced trafficking toward active release sites, accelerating presynaptic energy failure [12,49]. Therefore, synaptic excitotoxicity acts as an upstream trigger for mitochondrial destabilization, reinforcing the positive-feedback loop [15].

4.4.3 Self-Reinforcing Cycles (Drp1 Activation, mPTP Opening, and Cytokine Signaling)

A central feature of these bidirectional feedback loops is the presence of molecular events that reinforce themselves in a feed-forward manner. In primary cortical neurons and mouse MCAO models, excessive activation of Drp1 triggers pathological mitochondrial fission, increasing mitochondrial ROS production; this ROS surge further enhances Drp1 phosphorylation, establishing a persistent cycle of fragmentation and bioenergetic decline [10]. Complementary studies in rodent global- and focal-ischemia paradigms show that opening of the mPTP induces abrupt mitochondrial membrane-potential ($\Delta\Psi m$) collapse and impairs ATP synthesis, thereby heightening neuronal sus-

ceptibility to synaptic and excitotoxic stress [52,53]. Inflammatory signaling provides a third reinforcing component of this loop. In rat hippocampal-neuron cultures and mouse MCAO or OGD/R models, IL-1β and TNF-α potentiate NMDAR activation, elevate intracellular Ca^{2+}, and promote mitochondrial depolarization, further integrating cytokine-driven excitotoxicity with mitochondrial dysfunction [39,41]. Together, these Drp1-dependent, mPTP-mediated, and cytokine-driven mechanisms form interconnected positive-feedback loops that progressively erode synaptic and mitochondrial homeostasis, delineating the mechanistic continuum through which neurons shift from reversible functional impairment to irreversible degeneration.

4.4.4 Functional Implication (A Convergent Pathway Toward Irreversibility)

The convergence of these feedback loops establishes a "vicious cycle" framework: synaptic hyperexcitability damages mitochondria, dysfunctional mitochondria exacerbate synaptic collapse, and inflammatory/oxidative signaling reinforces both processes [31,33]. This reciprocal amplification explains how even brief ischemic episodes can trigger progressive synaptic loss and delayed neuronal death, particularly in selectively vulnerable regions such as the hippocampal CA1 subfield [27,64]. Thus, the mitochondria–synapse feedback circuitry represents a mechanistic nexus that determines whether neurons recover, remain in a reversible state, or cross the threshold into irreversible degeneration [15,23].

In summary, I/R initiates a series of mutually reinforcing events in which synaptic hyperexcitability, mitochondrial fragmentation, mPTP opening, and cytokine-driven NMDAR sensitization converge to destabilize both synaptic and mitochondrial homeostasis. These bidirectional loops amplify metabolic stress, Ca^{2+} overload, and ROS generation, progressively reducing the reversibility window of neuronal injury. As these processes accumulate, neurons transition from transient functional impairment to structural degeneration, providing a mechanistic basis for the threshold separating reversible and irreversible injury. This integrated framework also helps explain regional susceptibility, such as the heightened vulnerability of hippocampal CA1 neurons, which is addressed in Section 4.5.

4.5 Region- and Circuit-Specific Determinants of Mitochondrial–Synaptic Vulnerability

I/R injury manifests heterogeneously across hippocampal subfields, with CA1 pyramidal neurons displaying a characteristic pattern of delayed neuronal death, whereas CA3 and dentate gyrus (DG) neurons often exhibit relative resistance to comparable transient ischemic insults. Foundational work in gerbil and rat global ischemia models established this selective vulnerability pattern [27,64]. Current evidence indicates that regional differences in mitochondrial robustness, synaptic excitability, antioxidant defenses, and glial reactivity collectively determine whether each subfield crosses the threshold from reversible dysfunction to irreversible degeneration.

4.5.1 Mitochondrial Quality Control, ROS Handling, and Bioenergetic Reserve

Mitochondrial antioxidant capacity and stress tolerance vary significantly across hippocampal regions. Transcriptomic profiling of rat hippocampus exposed to oxidative stress shows that CA1 neurons express lower levels of antioxidant and mitochondrial maintenance genes than CA3, producing a more vulnerable redox environment [65]. Likewise, comparative ischemia experiments in gerbil and rat models demonstrate that CA1 mitochondria undergo earlier and more pronounced depolarization, swelling, and cristae disruption, whereas CA3 mitochondria better preserve ultrastructure and partially recover $\Delta\Psi m$ during reperfusion [66]. These intrinsic differences indicate that CA1 neurons operate near maximal oxidative capacity under physiological conditions, leaving minimal reserve during ischemic ATP collapse and thereby narrowing the temporal window in which depolarization remains reversible.

4.5.2 Synaptic Load, Hyperexcitability, and the Metabolic Burden

Circuit-level features further shape mitochondrial vulnerability. CA1 pyramidal neurons receive dense Schaffer collateral input (from CA3) and temporoammonic projections (from entorhinal cortex), relying heavily on NMDARmediated synaptic integration. Experimental reperfusion studies (mouse and rat cerebral I/R models) show that I/R induces sustained neuronal hyperexcitability, increased AMPAR trafficking, and elevated intracellular Ca^{2+} influx, collectively imposing substantial metabolic demands on CA1 synaptic mitochondria [26]. In contrast, CA3 recurrent circuits exhibit lower presynaptic release probability and robust short-term facilitation, as demonstrated in rat hippocampal slice cultures and electrophysiological recordings [67,68]. These properties moderate activity-dependent Ca^{2+} loading, thereby reducing excitotoxic drive and supporting comparatively better mitochondrial preservation after I/R.

4.5.3 Glial–Neuronal Interactions and Subfield-Specific Mitochondrial Dynamics

Glial responses to oxidative and ischemic stress also differ by subregion. In a rat oxidative-stress model, PH domain leucine-rich repeat protein phosphatase 1 (PHLPP1) regulated region-specific astroglial mitochondrial fission, with CA1 astrocytes showing exaggerated Drp1 activation, while DG astrocytes maintained more stable mitochondrial morphology through resilient Protein kinase B (AKT) – glycogen synthase kinase-3β (GSK3β) – Drp1 signaling

[69]. Neuroinflammatory cascades further amplify CA1 susceptibility. In primary hippocampal neurons, IL-1β enhances NMDAR-mediated Ca^{2+} influx via Src-family kinase activation [43], thereby increasing mitochondrial Ca^{2+} burden and promoting ΔΨm depolarization. After I/R, CA1 astrocytes and microglia frequently adopt a more pro-inflammatory phenotype, producing higher levels of cytokines such as IL-1β and TNF-α, which reinforce excitotoxicity and accelerate mitochondrial fragmentation preferentially in CA1 circuits [70]. These region-specific glial responses intensify mitochondrial instability and synaptic stress in CA1, creating a permissive environment for feed-forward excitotoxic signaling and lowering the threshold at which reversible dysfunction progresses toward irreversible neuronal injury.

4.5.4 Integrative Model: Why CA1 Crosses the Irreversibility Threshold Faster

The accelerated transition of CA1 neurons toward irreversible injury reflects the convergence of multiple vulnerabilities: (1) reduced antioxidant and mitochondrial maintenance capacity [65]; (2) greater mitochondrial structural fragility [66]; (3) higher synaptic load and reperfusion-induced hyperexcitability [26]; (4) astroglial Drp1 hyperactivation and fission-biased mitochondrial states [69]; (5) cytokine-driven amplification of NMDAR excitotoxicity [43]. Together, these mechanisms create a self-reinforcing mitochondrial–synaptic collapse loop, sharply narrowing the reversibility window in CA1 neurons and predisposing them to delayed neuronal death, whereas CA3 and DG retain more robust compensatory and antioxidant capacity, conferring relative resistance.

4.6 Cellular and Time-Dependent Determinants of Irreversible Neuronal Injury

I/R injury evolves through well-defined temporal phases, during which cellular and molecular checkpoints determine whether neurons recover or progress toward irreversible degeneration. The transition is governed by the interplay between mitochondrial bioenergetics, Ca^{2+} homeostasis, redox balance, and programmed cell-death pathways. Importantly, these processes are constrained by cell-type-specific properties, such that pyramidal neurons, interneurons, astrocytes, and microglia contribute differentially to either narrowing or preserving the reversibility window.

4.6.1 Temporal Phases of the Reversibility Window

4.6.1.1 Early Phase (0–30 minutes). In this phase, energetic depletion and reversible synaptic failure occurs. Immediately after reperfusion, neurons exhibit transient synaptic silencing and mitochondrial depolarization that can remain reversible. ATP depletion impairs synaptic vesicle cycling, and brief, reversible mPTP flickering may occur without structural mitochondrial collapse. In rodent global-ischemia preparations (for example, transient bilateral common carotid artery occlusion in rats or gerbils), early loss of ΔΨm in CA1 pyramidal neurons is recoverable if mitochondrial Ca^{2+} influx and ROS remain below critical thresholds [71,72].

4.6.1.2 Intermediate Phase (30 minutes–6 hours). In this phase, mitochondrial fragmentation, ROS escalation, and Ca^{2+} overload occurs. As reperfusion progresses, neurons enter an intermediate phase characterized by Drp1-mediated mitochondrial fission, rising ROS, and pathological Ca^{2+} overload. Studies using mouse MCAO and hippocampal OGD/R models show that sustained Drp1 activation, prolonged mPTP opening, and progressive mitochondrial swelling mark the transition at which ΔΨm depolarization becomes irreversible [10,73]. During this window, delayed NMDARdependent excitotoxicity further amplifies metabolic deterioration.

4.6.1.3 Late Phase (6–24 Hours). In this phase execution of cell death pathways occurs. Neurons that remain in a state of persistent mitochondrial depolarization progress to intrinsic (mitochondrial) apoptotic signaling. Mitochondrial oxidative stress induces cytochrome-c release, followed by DNA fragmentation, marking an irreversible commitment to cell death in mouse focal-ischemia models [74]. Loss of anti-apoptotic Bcl-2 further accelerates this transition, shifting neurons from reversible bioenergetic failure to caspase-dependent degeneration, as demonstrated in a rodent focal-ischemia model where antisense suppression of endogenous Bcl-2 markedly enhanced ischemic neuronal death [75].

4.6.2 Cell-Type-Specific Determinants of Irreversibility

4.6.2.1 Pyramidal Neurons. They show high metabolic load and limited mitochondrial reserve. CA1 pyramidal neurons possess high synaptic activity and lower antioxidant capacity compared with CA3 neurons. Their mitochondria exhibit earlier ΔΨm collapse, higher ROS sensitivity, and less structural resilience [66]. In mouse global-ischemia models, CA1 mitochondria show pronounced swelling and cristae disruption, whereas CA3 mitochondria partially preserve ultrastructure and recover ΔΨm during reperfusion [66]. These intrinsic bioenergetic and structural constraints shorten the reversibility window in CA1 and predispose this subfield to delayed neuronal death.

4.6.2.2 Interneurons. Fast-spiking metabolism and vulnerability to oxidative stress. Parvalbumin-positive (PV^{+}) interneurons, characterized by fast-spiking firing patterns and high mitochondrial demand, are particularly susceptible to oxidative and metabolic stress. In mouse models of transient global ischemia (for example, CA and resuscitation with *in vivo* two-photon imaging of somatosensory cortex), optogenetic stimulation and calcium imaging show that

PV^+ interneurons undergo acute dendritic beading and prolonged suppression of stimulation-evoked γ-aminobutyric acid (GABA)ergic network activity despite partial recovery of dendritic structure and intrinsic excitability. This functional dissociation weakens inhibitory control and predisposes cortical circuits to post-ischemic hyperexcitability [76]. Beyond ischemia, redox-imbalance paradigms (including genetic or pharmacologic perturbation of antioxidant systems and disruption of perineuronal nets in rodents) consistently demonstrate that oxidative stress and perineuronal net degradation preferentially impair PV^+ interneurons, underscoring the intrinsic vulnerability of these fast-spiking cells to oxidative imbalance [77,78,79,80].

4.6.2.3 Astrocytes. They regulate mitochondrial stability and maintain redox homeostasis. Astrocytes buffer extracellular glutamate, regulate synaptic activity, and supply metabolic substrates (for example, lactate) to neurons. However, astrocytic mitochondrial dysfunction can exacerbate neuronal injury. In rodent I/R models, reactive astrocytes in the hippocampal CA1 region show altered mitochondrial dynamics, impaired glutamate uptake, and excess ROS production, creating a microenvironment that favors excitotoxicity [81]. Astrocytes also function as central hubs for redox homeostasis by generating and recycling glutathione (GSH), detoxifying peroxides through the glutathione peroxidase (GPx) and catalase systems, and supplying neurons with γ-glutamyl precursors essential for neuronal GSH synthesis [82]. Moreover, astrocytic energy–redox cross-talk ensures that glycolytic and pentose-phosphate pathway flux is dynamically coupled to Nicotinamide adenine dinucleotide phosphate (NADPH) production and *de novo* GSH biosynthesis, enabling rapid buffering of ROS during metabolic stress [83]. During I/R, depletion of astrocytic GSH reserves and impaired NADPH-dependent antioxidant recycling amplify lipid peroxidation and mitochondrial susceptibility to ΔΨm collapse. Recent work in rat hippocampus further indicates that the phosphatase PHLPP1 regulates region-specific astroglial mitochondrial fission; CA1 astrocytes exhibit exaggerated Drp1 activation and fragmented mitochondrial morphology, whereas DG astrocytes maintain more stable mitochondria through resilient AKT–GSK3β–Drp1 signaling [69]. These astroglial alterations compromise neuronal metabolic support, glutamate clearance, and antioxidant defense, thereby lowering the threshold for irreversible neuronal injury.

4.6.2.4 Microglia. They display timing-dependent roles in injury or recovery. Microglia respond rapidly to I/R and can adopt either protective or detrimental phenotypes depending on the activation stage. Early microglial responses may supply neurotrophic factors or anti-inflammatory mediators, but sustained activation leads to excessive release of IL-1β, TNF-α, and ROS/RNS, which amplify excitotoxic Ca^{2+} influx and destabilize neuronal mitochondria. IL-1β enhances NMDAR-mediated Ca^{2+} entry through Src-family kinase signaling in cultured hippocampal neurons, thereby increasing mitochondrial Ca^{2+} loading and lowering the threshold for ΔΨm depolarization [43]. Complementary evidence from primary cortical neurons shows that TNF-α induces rapid mitochondrial depolarization, ATP loss, and ROS generation [41]. These microglia-derived cytokine cascades are consistent with *in vivo* observations that oxidative stress and neuroinflammation synergistically drive mitochondrial collapse and delayed neuronal death during reperfusion after acute ischemic stroke [33]. Blocking IL-1 signaling with IL-1Ra in mouse MCAO models markedly reduces ischemic neuronal damage, underscoring the pathological role of sustained microglial IL-1β during the progression toward irreversible injury [42].

4.6.3 Molecular Checkpoints Governing the Irreversibility Transition

The transition from reversible to irreversible neuronal injury is governed by a set of molecular checkpoints that integrate mitochondrial dynamics, redox status, and excitatory signaling. These checkpoints function as threshold-based switches: once mitochondrial Ca^{2+} load, ROS accumulation, or depolarizing inputs exceed critical limits, neurons enter self-amplifying cascades of mitochondrial collapse, oxidative damage, and Ca^{2+} overload. The following mechanisms represent the principal determinants defining this irreversibility boundary during I/R.

4.6.3.1 mPTP Opening Threshold. The mPTP undergoes brief flickering during early reperfusion, a reversible state that allows partial recovery of ΔΨm. However, once mitochondrial Ca^{2+} and ROS levels cross critical thresholds, mPTP shifts to prolonged opening, resulting in persistent ΔΨm collapse, ATP depletion, matrix swelling, and loss of mitochondrial integrity [73]. This checkpoint marks one of the earliest irreversible commitments to mitochondrial failure.

4.6.3.2 Drp1 Activation Cycle. Pathological mitochondrial fission is another determinant of irreversibility. Sustained phosphorylation of Drp1 at Ser616 drives excessive mitochondrial fragmentation and prevents fusion-mediated repair. Both in cultured neurons exposed to OGD/R and in mouse MCAO models, prolonged Drp1 activation correlates with ΔΨm instability, bioenergetic collapse, and progression toward cell death [10].

4.6.3.3 Redox Imbalance and Antioxidant Saturation. Reperfusion triggers a surge of ROS that challenges the antioxidant buffering capacity of mitochondria and glia. When GSH and thioredoxin systems become saturated, ROS neutralization fails, leading to rapid lipid peroxidation, mitochondrial DNA (mtDNA) damage, and inhibi-

tion of respiratory-chain complexes. This oxidative tipping point accelerates mitochondrial depolarization and amplifies Ca^{2+}-dependent injury cascades [72].

4.6.3.4 Sustained NMDAR Hyperactivation. Persistent activation of NMDARs, driven by glutamate accumulation and pro-inflammatory cytokines such as IL-1β and TNF-α, markedly amplifies Ca^{2+} influx. In hippocampal CA1 neurons—which already operate near their metabolic and oxidative limits—this sustained Ca^{2+} load synergizes with mitochondrial dysfunction to trigger caspase activation and structural degeneration [43]. Thus, excitatory signaling acts as a final convergence point accelerating the transition to irreversible injury.

In summary of Section 4.6, temporal progression (minutes → hours → late phase) and cell-type-specific vulnerabilities converge to determine neuronal fate after I/R. CA1 pyramidal neurons, with their high synaptic and metabolic burden, comparatively weaker antioxidant defenses, and proximity to pro-inflammatory glial responses, cross the irreversibility threshold earlier than CA3 or dentate gyrus neurons. PV^+ interneurons, astrocytes with region-specific mitochondrial fragility, and microglia that shift from transiently protective to chronically pro-inflammatory states all shape the dynamics of the mitochondria–synapse axis. These multilevel determinants highlight the importance of early mitochondrial stabilization, glial modulation, and excitotoxicity control in extending the reversibility window and preventing delayed neuronal death.

4.7 Threshold-Based Integration of Neuronal and Glial Determinants of Irreversibility

The transition to irreversible neuronal injury after I/R does not arise from a single catastrophic event, but instead from the convergence of multiple threshold-dependent processes across neuronal and glial populations. These processes—including mitochondrial metabolic instability, redox exhaustion, inhibitory-circuit breakdown, and glial-mediated inflammatory signaling—interact in a nonlinear and mutually reinforcing manner. Surpassing one threshold lowers the barrier for others, progressively restricting the window in which hippocampal circuits can recover. This framework explains how the initially reversible phase of reperfusion evolves into delayed and irreversible neuronal death, particularly within the metabolically vulnerable CA1 region.

4.7.1 Mitochondrial Vulnerability and Cross-Cellular Propagation

Neurons and glia are coupled through intense metabolic cross-talk, so mitochondrial failure in one cell type can propagate instability throughout the local network. CA1 pyramidal neurons, which operate near their maximal oxidative and synaptic load, exhibit early ΔΨm collapse, impaired oxidative phosphorylation, and heightened susceptibility to ROS in rodent global and focal ischemia models [66]. Once mitochondrial Ca^{2+} buffering capacity is exceeded, prolonged opening of the mPTP leads to irreversible ΔΨm loss, ATP depletion, and cytochrome-c release, committing neurons to apoptotic or necrotic death pathways [73]. In parallel, recent work in the rat hippocampus indicates that phosphatase regulates region-specific astroglial mitochondrial fission; CA1 astrocytes show exaggerated Drp1 activation and fragmented mitochondria, whereas DG maintain more stable mitochondrial networks via resilient AKT–GSK3β–Drp1 signaling [69]. These neuron–glia interactions form a feed-forward loop in which mitochondrial stress in one compartment aggravates dysfunction in the other. Consistent with this, a recent review highlights mitochondrial stress as a central driver of neuroinflammation and injury amplification in experimental stroke, emphasizing the reciprocal reinforcement between mitochondrial damage and inflammatory signaling [84].

4.7.2 Redox Exhaustion as a Common Bottleneck

Redox buffering capacity represents a shared bottleneck for neurons and astrocytes during reperfusion. Astrocytes are the principal regulators of brain GSH metabolism; they synthesize GSH and recycle it via NADPH–dependent pathways, thereby supporting both their own antioxidant defenses and those of neighboring neurons [82]. Contemporary work on astrocyte–neuron metabolic cooperation emphasizes that redox metabolism is tightly integrated with energy supply, such that perturbations in one domain (for example, mitochondrial respiration) rapidly compromise antioxidant capacity [83]. When GSH and thioredoxin systems become saturated during the reperfusion-associated ROS burst, lipid peroxidation, mitochondrial DNA damage, and impairment of respiratory-chain complexes accelerate, further undermining ΔΨm stability and ATP production [72,76]. Loss of astrocyte-driven redox support thus shortens the reversibility window for both neuronal and glial mitochondria.

4.7.3 Circuit-Level Destabilization Driven by Interneuron Failure

At the circuit level, PV^+ interneurons act as high-frequency "brakes" that stabilize pyramidal cell output. In mouse global ischemia models, *in vivo* imaging and optogenetic stimulation reveal that PV^+ interneurons develop acute dendritic beading and prolonged suppression of stimulation-evoked γ-frequency inhibitory activity, even when their dendritic morphology and basic excitability partially recover [76]. These durable functional deficits weaken perisomatic inhibition of pyramidal neurons and promote network hyperexcitability. Independent redox-imbalance paradigms demonstrate that oxidative stress preferentially impairs PV^+ interneurons and disrupts perineuronal nets, suggesting an intrinsic vulnerability of these

fast-spiking cells to oxidative and metabolic insults [79]. Once PV^+ interneuron function falls below a critical threshold, uncontrolled glutamatergic drive and NMDAR activation in pyramidal neurons further increase Ca^{2+} loading and mitochondrial strain, linking local inhibitory failure to downstream bioenergetic collapse.

4.7.4 Inflammatory Acceleration via Microglial Cytokine Signaling

Sustained activation of microglia accelerates the transition to irreversibility through persistent release of IL-1β, TNF-α, ROS, and RNS. In rodent hippocampal neurons and slice preparations, IL-1β enhances NMDAR-mediated Ca^{2+} influx via Src-family kinase signaling, thereby promoting mitochondrial Ca^{2+} overload, $\Delta\Psi m$ depolarization, and synaptic dysfunction [43]. TNF-α induces rapid mitochondrial depolarization, ATP loss, and ROS generation in primary neuronal cultures, further lowering the threshold for irreversible mitochondrial failure [41]. In mouse MCAO models, overexpression or exogenous administration of IL-1Ra significantly reduces infarct volume and delayed neuronal death, underscoring the causal contribution of IL-1 signaling to injury progression [42]. Recent reviews of microglial biology in ischemic stroke further emphasize the time-dependent shift from early, potentially beneficial phenotypes to later, pro-inflammatory states that sustain neurotoxicity, with IL-1β and TNF-α serving as central hubs in these transitions [85]. In parallel, comprehensive analyses of microglia–astrocyte crosstalk following ischemic stroke highlight how microglia-derived cytokines and extracellular vesicles (EVs) drive neurotoxic A1 astrocyte polarization and BBB disruption, amplifying neuronal vulnerability and limiting recovery [86]. Together, these findings position microglial cytokine cascades as late-phase accelerators that couple neuroinflammation to mitochondrial collapse and circuit failure.

In summary of Section 4.7, when mitochondrial instability, redox exhaustion, inhibitory-circuit failure, and chronic microglial inflammation converge, hippocampal networks cross a systems-level point of no return. In this integrated threshold model, CA1 pyramidal neurons become disproportionately vulnerable because they operate at high metabolic and excitatory loads, depend heavily on astrocytic metabolic and antioxidant support, and reside in microenvironments where glial inflammatory responses are particularly intense. This framework explains why interventions that stabilize mitochondria, reinforce astrocytic redox capacity, preserve PV^+ interneuron function, or limit microglial cytokine release are most effective when applied before these processes become mutually amplified. It also provides a mechanistic rationale for targeting neuron–glia–vascular interactions as a functional unit—rather than focusing on isolated pathways—to extend the reversibility window and prevent delayed neuronal death after I/R.

5. Mechanistic Injury Pathways and Therapeutic Relevance

I/R injury activates a coordinated network of mechanistic pathways that converge on mitochondrial failure, synaptic destabilization, and neuroinflammatory amplification. Disrupted bioenergetics, excessive calcium influx, oxidative stress, and cytokine-driven signaling interact to lower the threshold for irreversible neuronal injury, particularly in metabolically vulnerable regions such as hippocampal CA1. These interconnected processes not only explain the rapid transition from early reversible dysfunction to delayed neuronal death but also define mechanistic entry points for therapeutic intervention. Understanding how individual pathways—excitotoxicity, mitochondrial dysregulation, cytoskeletal collapse, and glial-mediated inflammation—shape synaptic and cellular outcomes is essential for designing targeted and multimodal neuroprotective strategies.

5.1 Early Mitochondrial Stabilization Strategies

Mitochondrial failure represents one of the earliest checkpoints that determine whether I/R injury remains reversible or progresses toward irreversible neuronal death. Because mitochondrial permeability, calcium homeostasis, and redox capacity jointly control the metabolic resilience of neurons, early stabilization of these processes has emerged as a central therapeutic strategy. Current experimental and translational evidence highlights three major mitochondrial targets: inhibition of cyclophilin-D (CypD)–dependent mPTP opening, suppression of mitochondrial calcium uniporter (MCU)–mediated Ca^{2+} influx, and restoration of mitochondrial energetic/redox capacity through enhancement of NAD^+ metabolism or delivery of mitochondria-targeted antioxidants.

5.1.1 CypD and mPTP Inhibition

Opening of the mPTP is a decisive molecular event that converts transient mitochondrial stress into irreversible collapse of membrane potential ($\Delta\Psi m$). CypD, a matrix peptidyl-prolyl cis–trans isomerase, sensitizes the pore to Ca^{2+} and ROS during early reperfusion. In mouse models of focal cerebral ischemia, CypD deletion or pharmacologic inhibition (e.g., CsA) reduces mitochondrial swelling, delays cytochrome-c release, and markedly decreases infarct volume [16]. Complementary work in cultured neurons and cardiac mitochondria demonstrates that CypD inhibition preserves ATP production, limits oxidative damage, and stabilizes mitochondrial morphology under oxidative stress [87,88]. A recent comprehensive review consolidates advances in defining the molecular identity and regulatory partners of the mPTP, reinforcing the concept that early pore stabilization remains a high-priority therapeutic target for I/R injury [17]. In rat global ischemia, CsA attenuates necroptotic, autophagic, and apoptotic sig-

naling, demonstrating that mPTP inhibition influences not only mitochondrial integrity but also downstream cell-death programs [18].

5.1.2 Modulation of MCU

The MCU is the primary channel responsible for rapid Ca^{2+} uptake into the mitochondrial matrix. During reperfusion, pathological Ca^{2+} influx through the MCU accelerates $\Delta\Psi m$ dissipation, ROS generation, and activation of mitochondrial permeability pathways. Tamoxifen-inducible knockdown of MCU in adult mice reduces cortical tissue loss, preserves respiratory capacity, and improves neurological outcomes after hypoxic/ischemic injury [89]. Similarly, *in vitro* neuronal studies demonstrate that MCU inhibition suppresses excessive mitophagy, limits mitochondrial fragmentation, and improves cell viability following I/R-like stress [90]. The cell-permeable inhibitor Ru265, which selectively targets MCU, has shown strong protective effects in both OGD models and *in vivo* ischemia, preserving mitochondrial respiration and reducing infarct volume [91]. Recent mechanistic synthesis confirms that MCU-targeting approaches show broad therapeutic relevance not only in stroke but also across neurodegenerative and neurovascular conditions [92].

5.1.3 Restoring NAD^+ and Redox Capacity

Reperfusion induces rapid consumption of NAD^+ through PARP activation and mitochondrial oxidative stress, thereby impairing oxidative phosphorylation and DNA damage repair. In neurodegenerative models, NAD^+ replenishment suppresses cGAS– Stimulator of interferon genes (STING)–mediated inflammatory responses, suggesting additional benefit in chronic I/R-related pathology [93]. A recent 2025 review synthesizes evidence demonstrating that NAD^+-salvage precursor administration reduces Drp1-mediated mitochondrial fragmentation, lowers ROS burden, and enhances neurological recovery after cerebral I/R, supporting NAD^+ restoration as a convergent mechanism for maintaining mitochondrial network stability [94].

5.1.4 Mitochondria-Targeted Antioxidants

Because mitochondria generate the majority of ROS during reperfusion, agents that accumulate in mitochondria offer a uniquely proximal means of preventing oxidative collapse. MitoQ, a ubiquinone conjugated to a triphenylphosphonium (TPP^+) cation, preferentially localizes to the inner mitochondrial membrane and preserves $\Delta\Psi m$, limits mtDNA damage, and suppresses IL-1β/TNF-α signaling in rat I/R models [95]. Broader families of mitochondria-targeted antioxidants—including plastoquinone-based compounds and TPP^+-linked scavengers—have been shown to reduce mitochondrial swelling, attenuate oxidative damage to cardiolipin, and improve behavioral outcomes across multiple I/R paradigms [96]. These mitochondrial redox stabilizers complement mPTP and MCU-targeting strategies by directly preventing ROS-induced positive feedback loops that drive $\Delta\Psi m$ collapse.

In Summary, mPTP inhibition, MCU suppression, NAD^+ restoration, and mitochondria-targeted antioxidants converge on a common principle: maintaining mitochondrial integrity during the early reversible phase of reperfusion. Because mitochondrial permeability, Ca^{2+} loading, and redox balance are tightly interconnected, combination therapies aimed at multiple nodal points are likely to achieve superior protection. Emerging evidence from 2024–2025 highlights that intervening before these mitochondrial events become self-amplifying is essential for translating mechanistic insights into clinical benefit.

5.2 Targeting Excitotoxicity and Synaptic Ca^{2+} Dysregulation

Excitotoxicity represents a central driver of delayed neuronal death after I/R injury. During the early reperfusion period, excessive glutamate release, impaired astrocytic clearance, and pathological activation of NMDARs combine to produce sustained dendritic Ca^{2+} loading, particularly in hippocampal CA1 pyramidal neurons. This Ca^{2+} accumulation promotes mitochondrial depolarization, enhances ROS formation, and activates Ca^{2+}-dependent enzymes that progressively convert reversible synaptic dysfunction into irreversible structural degeneration. Thus, excitotoxicity-targeted interventions complement mitochondrial-protective strategies described in Section 5.1.

5.2.1 Differential Roles of NMDAR Subunits in Excitotoxic Ca^{2+} Loading

NMDAR-mediated Ca^{2+} influx depends strongly on receptor subunit composition and subcellular localization. Liu et al. (2007) [97] demonstrated that, using primary cortical neurons and *in vivo* rodent models, Glutamate ionotropic receptor NMDA type subunit 2B (GluN2B)-containing NMDARs generate greater Ca^{2+} currents and stronger excitotoxic coupling than GluN2A-containing receptors, consistent with their enrichment at extrasynaptic sites linked to pro-death signaling. Using focal ischemia models and biochemical assays, Tymianski (2011) [31] established that GluN2B C-terminal interactors, such as PSD-95, criticaly exacerbate ischemia-induced Ca^{2+} influx. Conversely, McQueen et al. (2017) [98] leveraged genetic and electrophysiological approaches to reveal a more nuanced paradigm. They demonstrated that GluN2B-driven toxicity can also propagate through multiple convergent downstream pathways independent of specific scaffold interactions, underscoring a highly complex regulatory network. Recent analysis by Yu et al. (2023) [99] further highlights extrasynaptic GluN2B activation as a major determinant of acute I/R-induced excitotoxicity and chronic neu-

rodegeneration, emphasizing the translational advantage of GluN2B-selective modulation over global NMDAR antagonism, which disrupts physiological synaptic function. Together, these findings support precision-targeted GluN2B modulation as the most feasible strategy for limiting pathological Ca^{2+} entry while preserving normal synaptic transmission.

5.2.2 Astrocytic Glutamate Clearance and Excitatory Amino Acid Transporter 2 (EAAT2) (GLT-1) Preservation

Astrocytic excitatory amino acid transporter-2 (EAAT2/GLT-1) removes the majority of extracellular glutamate in forebrain regions and is essential for preventing prolonged NMDAR activation. During I/R, oxidative and inflammatory stress reduce EAAT2 expression and membrane localization, enhancing glutamate spillover. Rothstein et al. (2005) [63] demonstrated that β-lactam antibiotics (e.g., ceftriaxone) markedly increase EAAT2 expression and protect neurons from glutamate-mediated toxicity. Recent findings extend this concept. Yu et al. (2023) [99] reported that EAAT2 internalization in reactive astrocytes promotes extrasynaptic NMDAR activation, amplifying GluN2B-mediated Ca^{2+} influx. Collectively, the evidence indicates that preserving or restoring EAAT2 expression—via transcriptional modulation, pharmacological upregulation, or stabilization of plasma-membrane localization—represents a high-impact upstream strategy to reduce excitotoxic Ca^{2+} burden.

5.2.3 Preservation of Inhibitory Interneuron Function

PV^+ interneurons impose fast, precise inhibition that restrains pyramidal neuron excitability during reperfusion. Xie et al. (2014) [76] showed that although PV^+ interneurons regain morphological integrity after global ischemia, their evoked network activity remains impaired, leading to persistent disinhibition of CA1 pyramidal neurons and increased dendritic Ca^{2+} spiking. A recent synthesis by Perovic et al. (2025) [100] underscores broader post-stroke GABAergic instability, including altered interneuron–pyramidal interactions. These findings suggest that maintaining inhibitory tone—through GABA_A receptor enhancement, neurosteroid-based modulation, or stabilization of interneuron metabolic function—may indirectly reduce excitotoxic Ca^{2+} accumulation. Although direct PV^+ interneuron-targeted interventions remain limited, the evidence supports their inclusion in multitarget neuroprotective strategies.

5.2.4 Downstream Ca^{2+}-Activated Injury Pathways

After excessive Ca^{2+} entry, several Ca^{2+}-dependent enzymes mediate structural and metabolic injury. Calpains degrade cytoskeletal components such as spectrin, contributing to dendritic fragmentation. Bartus et al. (1994) [101] showed, in a rat focal ischemia model, that calpain inhibitor AK295 reduced infarct volume even when given post-occlusion, highlighting ROSC calpain activation as a key mediator of delayed degeneration. Neuronal nitric oxide synthase (nNOS) is another major effector. nNOS-mediated nitric oxide formation facilitates peroxynitrite generation, mitochondrial DNA oxidation, and loss of $\Delta\Psi m$. Huang et al. (1993) [102] demonstrated significantly reduced infarct volumes in nNOS-knockout mice, establishing nNOS as a crucial downstream mediator of Ca^{2+}-driven neurotoxicity. More recent findings refine these mechanisms. Chukai et al. (2024) [103] identified mitochondrial calpain-5 proteolysis as a contributor to mitochondrial swelling and cytochrome-c release after I/R, while Maccallini & Amoroso (2023) [104] reviewed structural and post-translational regulation of nNOS as a promising therapeutic target in experimental stroke models. Together, these studies underscore the importance of pairing upstream Ca^{2+}-entry control (e.g., GluN2B inhibition, EAAT2 preservation) with downstream enzyme inhibition (e.g., calpain, nNOS) to mitigate irreversible mitochondrial injury.

In summary, across molecular, synaptic, and network levels, excitotoxicity forms a coherent mechanistic bridge between early glutamate dysregulation and irreversible neuronal degeneration. Evidence supports four major therapeutic pillars: (1) GluN2B-selective/extrasynaptic NMDAR modulation to limit toxic Ca^{2+} influx. (2) EAAT2 preservation or enhancement to prevent glutamate spillover. (3) Maintenance of inhibitory interneuron function to restore excitatory–inhibitory balance. (4) Downstream enzyme inhibition (calpains, nNOS) to prevent structural failure and mitochondrial collapse. Because excitotoxic Ca^{2+} influx drives key mitochondrial transitions—including depolarization, ROS amplification, and mPTP opening—combined approaches targeting both synaptic Ca^{2+} entry and mitochondrial stability are expected to provide the greatest extension of the reversibility window after I/R injury.

5.3 Mitochondria–Synapse Coupling and the Transition From Reversible to Irreversible Injury

Mitochondria positioned within dendrites and presynaptic terminals form a critical metabolic hub that determines whether neurons remain in a reversible energetic state or transition into irreversible degeneration after I/R injury. These synaptic mitochondria regulate Ca^{2+} buffering, ATP supply, and redox balance; when these functions collapse, excitotoxic signaling rapidly converts to structural disassembly and delayed neuronal death. Thus, the mitochondria–synapse interface acts as a decisive checkpoint where metabolic stress becomes irreversible neurodegeneration.

5.3.1 Early Reversible Mitochondrial Dysfunction at Synapses

Early reperfusion evokes mitochondrial disturbances that are potentially reversible. Using primary neuronal cul-

tures, Nicholls (2004) [71] showed that glutamate-induced mitochondrial depolarization and ROS elevation can recover fully when intracellular Ca^{2+} entry and oxidative burden do not exceed defined thresholds, indicating that early ΔΨm instability represents a reversible "mitochondrial crisis". Complementary I/R metabolic analysis by Chouchani et al. (2014) [72] in mouse cardiac and brain I/R models revealed that ischemic succinate accumulation produces a transient, complex I–derived ROS burst at reperfusion. Importantly, this ROS surge is initially reversible but becomes destructive only when it precipitates sustained ΔΨm collapse and irreversible mPTP opening. More recent synaptic-level insights refine this window. Zaninello et al. (2024) [105] demonstrated that synaptic mitochondria undergo rapid, activity-dependent shifts in fission–fusion balance, supported by local translation of mitochondrial proteins. When these adaptive dynamics remain intact, synaptic mitochondria compensate for short-lived depolarization ("flickering") and transient ROS elevations. Conversely, failure of these regulatory systems predisposes synaptic mitochondria to mPTP opening and structural degeneration. Together, these data indicate that synaptic mitochondrial dysfunction after I/R is not inherently irreversible; a biologically meaningful therapeutic window exists wherein controlling Ca^{2+} influx, complex I–derived ROS, and mPTP opening can restore synaptic bioenergetics.

5.3.2 Synaptic Ca^{2+} Overload and the Collapse of Mitochondrial Homeostasis

Persistent Ca2+ influx through NMDARs acts as the proximal driver of mitochondrial collapse during I/R injury. In a mouse model of focal ischemia, Liu et al. (2007) [97] demonstrated that GluN2B-containing receptors are tightly coupled to dendritic mitochondrial depolarization; disrupting this signaling pathway effectively curtails Ca^{2+} overload and preserves organellar integrity. Expanding this paradigm, McQueen et al. (2017) [98] utilized targeted genetic deletions of the GluN2B C-terminal tail. Their findings indicate that receptor-dependent mitochondrial dysfunction propagates through multiple, convergent downstream cascades. This suggests that a versatile array of GluN2B-specific signaling complexes, rather than a single linear pathway, coordinates the bioenergetic collapse. These mechanistic findings align with structural observations that synaptic mitochondria depolarize earlier and more severely than somatic mitochondria due to their smaller volume and faster Ca^{2+} turnover. Yu et al. (2023) [99], integrating *in vitro*, slice ischemia, and *in vivo* global ischemia evidence, identified extrasynaptic GluN2B activation as the earliest step linking excitotoxic Ca^{2+} influx to mitochondrial collapse. Thus, synaptic Ca^{2+} overload—particularly via extrasynaptic GluN2B receptors—serves as the mechanistic trigger that converts reversible mitochondrial depolarization into irreversible mPTP opening and metabolic failure.

5.3.3 Structural Synaptic Degeneration (Spine Loss and Cytoskeletal Breakdown)

Structural degradation at dendritic spines is a hallmark of irreversible injury. In hippocampal CA1 neurons, spine shrinkage, PSD disassembly, and cytoskeletal breakdown emerge within hours after reperfusion and correlate closely with mitochondrial swelling and ROS increase.

Foundational studies established the enzymatic drivers of this degeneration. In a rat focal ischemia model, Bartus et al. (1994) [101] showed that calpain activation leads to spectrin cleavage and dendritic fragmentation, even with post-occlusion administration of the calpain inhibitor AK295. Similarly, Huang et al. (1993) [102] demonstrated that genetic deletion of nNOS attenuates oxidative/nitrosative injury, thereby preserving mitochondrial membranes and cytoskeletal stability. More recent findings refine this enzymatic framework. Chukai et al. (2024) [103] identified calpain-5, a mitochondria-enriched isoform, as a rapid mediator of mitochondrial swelling and spine loss in mouse I/R. Its inhibition significantly reduced ROS accumulation and prevented early dendritic degeneration. Additional mechanistic insights from Maccallini & Amoroso (2023) [104] showed that post-translational modification of nNOS enhances synaptic oxidative stress, further destabilizing cytoskeletal structures and linking mitochondrial redox failure to synaptic disassembly. Collectively, these data establish that structural synaptic collapse arises from the convergence of Ca^{2+}-dependent protease activation (calpains), mitochondrial ROS amplification, and nNOS-mediated nitrosative stress. Protecting synaptic mitochondria early in reperfusion is therefore essential to prevent irreversible dendritic spine loss and maintain network function.

In summary, a synthesis of classical and recent evidence indicates that preserving mitochondria–synapse coupling requires coordinated, multi-level intervention: (1) limiting excitotoxic Ca^{2+} influx via selective GluN2B modulation (Section 5.2), (2) sustaining astrocytic glutamate clearance through EAAT2 preservation, (3) maintaining inhibitory network stability to prevent high-frequency Ca^{2+} spiking, and (4) direct mitochondrial support, including suppression of calpain-5 activity, mitigation of complex I ROS burst, and prevention of mPTP opening. Protecting synaptic mitochondria during the early reversible phase of reperfusion may extend the neuronal salvage window and delay the transition to irreversible neuronal death.

5.4 Mitochondrial Dynamics (Fission–Fusion) and Synaptic Survival During Reperfusion

Mitochondrial fission–fusion dynamics are essential for sustaining synaptic viability under physiological and pathological conditions. Synaptic mitochondria continuously remodel their morphology to maintain ATP production, regulate Ca^{2+} buffering, and support local proteostasis, functions that become particularly critical during

I/R. Early reperfusion induces rapid mitochondrial remodeling, but the balance between adaptive and pathological fission determines whether synapses remain within a reversible metabolic window or progress toward irreversible degeneration. Disruption of this balance—most commonly through excessive Drp1–mediated fission—promotes ΔΨm collapse, mtROS accumulation, and synaptic structural loss.

5.4.1 Early Activation of Fission–Fusion Dynamics: Protective or Detrimental?

Early reperfusion triggers a modest increase in mitochondrial fission, which may serve a protective role. In primary neuronal cultures, Youle and van der Bliek (2012) [106] demonstrated that Drp1-dependent fission is required for segregating dysfunctional mitochondrial segments, thereby enabling selective mitophagy and preserving overall mitochondrial quality. This suggests that initial fission is not inherently harmful and may support short-term recovery during the reversible phase of reperfusion. Recent work has expanded understanding of synapse-specific mitochondrial behavior. Zaninello et al. (2024) [105] reviewed mitochondrial dynamics in hippocampal synapses and showed that synaptic mitochondria undergo rapid, activity-dependent remodeling—including fluctuations in ΔΨm and local translation of fission/fusion regulators—allowing flexible metabolic responses. These observations reinforce that early fission–fusion remodeling at synapses is dynamic, reversible, and potentially adaptive as long as regulatory mechanisms remain intact.

5.4.2 Pathological Drp1 Hyperactivation and Mitochondrial Fragmentation After I/R

Persistent ischemic stress drives pathological Drp1 hyperactivation, which shifts fission–fusion dynamics toward uncontrolled fragmentation. Evidence across *in vitro* and *in vivo* I/R models supports this transition. Using mouse glutamate-toxicity and OGD paradigms, along with transient MCAO models, Grohm et al. (2012) [10] demonstrated that pharmacological Drp1 inhibition (mdivi-1) or DRP1 knockdown preserves ΔΨm, reduces cytochrome-c release, and decreases neuronal death, providing foundational evidence that excessive Drp1 activity causally contributes to ischemic injury. More recently, Zhang et al. (2024) [107] employed murine hypoxia/ischemia and neuronal culture models to show that ischemia induces CDK5–AMPK–GCN5L1–mediated acetylation of DRP1, enhancing its mitochondrial recruitment and promoting fragmentation, mtROS accumulation, and neuronal apoptosis. Inhibiting Drp1 acetylation preserved mitochondrial morphology and improved neuronal survival, identifying a post-translational mechanism driving pathological fission. A comprehensive synthesis by Liu et al. (2025) [108] further confirmed across rodent MCAO, global ischemia, and OGD models that Drp1 hyperactivation is consistently associated with early ΔΨm loss, mitochondrial swelling, oxidative stress, and irreversible synaptic failure, establishing excessive fission as a conserved pathological event in I/R injury. Together, these data indicate that once Drp1 activity exceeds physiological levels, mitochondrial fission becomes maladaptive, amplifying bioenergetic deficits and promoting structural degeneration at synapses.

In summary, a central insight from experimental and translational studies is that the therapeutic effect of Drp1 modulation depends critically on timing. During early reperfusion, moderate Drp1 activity plays an adaptive role, helping segregate damaged mitochondrial segments for mitophagy and supporting short-term metabolic stabilization as long as fission–fusion balance is preserved. However, in mid-to-late reperfusion, Drp1 becomes pathologically overactivated, driving uncontrolled mitochondrial fragmentation, ΔΨm collapse, mtROS amplification, and ultimately irreversible synaptic dysfunction. Rodent I/R models show that inhibiting Drp1 during this later phase preserves mitochondrial integrity and improves neuronal survival. Thus, Drp1-targeted therapy requires temporal precision—avoiding complete fission blockade early on, while suppressing pathological Drp1 hyperactivation as reperfusion progresses. This principle is well supported by mechanistic work [10] and recent translational reviews [108].

6. Therapeutic Strategies and Emerging Interventions

I/R injury produces a complex interplay of excitotoxicity, mitochondrial dysfunction, oxidative stress, and synaptic destabilization. The integrated mitochondrial–synaptic axis described in earlier sections provides a mechanistic foundation for targeted therapeutic development. Treatments aimed at restoring mitochondrial integrity, reducing oxidative/RNS burden, stabilizing synaptic structures, and modulating neuroinflammation have shown promise in both preclinical and early translational settings. This section synthesizes current therapeutic strategies and evaluates their mechanistic rationale, efficacy across models, and challenges for clinical implementation. To provide an integrated overview of how individual interventions converge on mitochondrial, synaptic, and inflammatory targets, a therapy-by-target matrix is summarized in Table 2.

6.1 Mitochondrial Stabilization and Dynamics Modulation

6.1.1 Targeting mPTP

Early reperfusion rapidly induces opening of mPTP, resulting in abrupt loss of ΔΨm, inhibition of oxidative phosphorylation, and release of apoptogenic proteins. Classical work using mitochondrial-targeted peptides and CsA in rodent ischemia and oxidative-injury models demonstrated that pharmacological desensitization of the pore preserves ΔΨm, limits mitochondrial swelling and ROS production, and attenuates cell death, thereby establishing

Table 2. The integrated therapy-by-target matrix for cerebral ischemia–reperfusion injury.

Therapeutic strategy	Primary target (mitochondria/synapse/inflammation)	Mechanism of action	Representative compound/technique	Key preclinical evidence (summary)	Translational status	Section(s)
mPTP inhibition and cardiolipin protection	Mitochondria	Limit pathological mPTP opening, preserve ΔΨm, stabilize cristae and electron transport	Cyclosporin A (CsA), SS-31 (Elamipretide)	Reduces mitochondrial swelling, ROS generation, and cell death in rodent I/R and oxidative stress models	Early clinical exploration (cardiac); conceptually relevant but unproven in large stroke trials	6.1.1
Modulation of mitochondrial fission–fusion dynamics	Mitochondria	Inhibit excessive Drp1-dependent fission, maintain mitochondrial network integrity and spine-associated mitochondria	Drp1 inhibitor (mdivi-1), Drp1 activator knockout	Limits mitochondrial fragmentation, preserves ΔΨm and dendritic spine density, reduces infarct size in rodent ischemia models	Preclinical stage; target considered promising but safety/specificity unresolved	6.1.2, 4.3
Mitophagy and mitochondrial quality-control tuning	Mitochondria	Enhance selective removal of dysfunctional mitochondria while avoiding depletion of overall mitochondrial mass	PINK1/Parkin pathway modulators, ligustilide, mitophagy-regulating compounds	Balanced mitophagy reduces oxidative stress, neuronal death, and cognitive decline in rodent stroke models	Early preclinical; optimal timing and degree of activation remain to be defined	6.1.3
Direct ROS scavengers and redox buffers	Mitochondria/synapse	Directly neutralize ROS/RNS and stabilize redox state in mitochondria and synapses	Melatonin, vitamins, flavonoids, small-molecule antioxidants	Decrease lipid peroxidation, improve mitochondrial function, and reduce infarct volume in multiple I/R models	Repeated but often inconclusive clinical trials; more promising in multi-target or combinatorial regimens	6.2.1
Enzymatic antioxidant augmentation	Mitochondria/synapse	Increase SOD, GPx, catalase activity and upstream redox-regulatory pathways	Gene transfer, pharmacologic inducers (e.g., Nrf2 activators)	Overexpression or pharmacologic induction reduces oxidative damage, neuronal loss, and vascular dysfunction in rodents	Preclinical to early translational; network-level redox effects require cautious modulation	6.2.2, 6.1
NMDA/AMPA receptor modulation and PSD-95 decoy strategies	Synapse	Reduce excitotoxic Ca^{2+} influx while preserving physiological synaptic transmission and plasticity	PSD-95 decoy peptides (e.g., Tat-NR2B9c), NMDAR antagonists, AMPAR trafficking modulators	Decrease infarct size, limit NO-mediated injury, and stabilize synaptic transmission in rodent focal ischemia	Advanced experimental; limited clinical translation due to safety and timing constraints	6.3.1
Calpain inhibition and preservation of synaptic structure	Synapse/mitochondria	Block Ca^{2+}-activated calpain-mediated cleavage of cytoskeletal, receptor, and mitochondrial proteins	AK295, MDL-28170, calpain inhibitor-I, calpastatin-based approaches	Reduce delayed neuronal death, protect dendritic architecture, and attenuate mitochondrial dysfunction in global and focal I/R models	Multiple preclinical studies; systemic side effects and specificity remain key hurdles	6.3.2
Synaptic reconstruction and plasticity enhancement	Synapse	Promote dendritic spine regrowth, receptor stabilization, and activity-dependent network remodeling	BDNF-related interventions, pro-plasticity natural compounds, enriched environment/rehabilitation paradigms	Improve synaptic density, plasticity markers, and functional recovery in rodent stroke models	Conceptually strong; partly implemented via rehabilitation and neuromodulatory strategies in patients	6.3.3, 7
Cytokine-axis targeting (IL-1β, TNF-α, IL-6/STAT3)	Inflammation/mitochondria/synapse	Block cytokine receptors or downstream signaling that link inflammation to mitochondrial dysfunction and synaptic loss	IL-1 receptor antagonist (IL-1Ra), TNF-α inhibitors, JAK/STAT3 modulators	Reduce infarct size, BBB disruption, neuroinflammation, and synaptic degeneration in experimental stroke	IL-1Ra and related agents tested clinically; timing, dosing, and infection risk limit broad use	6.4.1, 3.3

Table 2. Continued.

Therapeutic strategy	Primary target (mitochondria/synapse/inflammation)	Mechanism of action	Representative compound/technique	Key preclinical evidence (summary)	Translational status	Section(s)
Microglia polarization and inflammatory pathway modulation	Inflammation/synapse	Shift microglia from M1-like to M2-like states; inhibit pro-inflammatory pathways (e.g., NF-κB, cGAS–STING)	Minocycline, CSF1R inhibitors, HDAC3 inhibitors, quercetin, miRNA-based modulators	Decrease cytokine production, preserve spines, and improve behavioral outcomes in rodent MCAO	Several drugs repurposed/under evaluation; cell-type- specificity and chronic effects under investigation	6.4.2
Astrocyte-targeted interventions	Inflammation/synapse/mitochondria	Enhance glutamate clearance, gap-junction communication, and pro-repair phenotypes; harness astrocyte-to-neuron conversion	EAAT2 upregulators, dexmedetomidine (Cx43), Danshensu liposomes, NeuroD1 AAV, TRPA1-LIF axis	Improve glutamate homeostasis, reduce edema and oxidative stress, and promote functional circuit repair in I/R models	Preclinical; gene and AAV-based strategies at early translational stage	6.4.2, 4.3
Mitochondrial transplantation and mitochondria-containing EVs	Mitochondria/synapse	Supply functional mitochondria or mitochondria-rich EVs to restore ATP production, ΔΨm, and synaptic protein expression	Direct mitochondrial injection, mitochondria-containing EVs from endothelial or donor cells	Reduce infarct size, enhance remyelination, and improve neurological recovery in rodent cerebral and global I/R; Phase 1 trial in acute stroke	First-in-human feasibility shown; dosing, sourcing, and delivery route require optimization	6.5.1
Gene therapy targeting pro-repair pathways	Mitochondria/synapse/inflammation	Deliver genes that promote angiogenesis, white matter repair, trophic support, or immune modulation	AAV-based CXCL12 expression, neurotrophic factor genes	Attenuate white-matter damage, enhance oligodendrocyte survival, and improve functional outcomes in mouse MCAO	Preclinical with emerging delivery platforms (intranasal/targeted AAV); regulatory and safety issues remain	6.5.2
ncRNA/miRNA/circRNA modulation	Inflammation/mitochondria/synapse	Reprogram post-ischemic gene networks controlling microglia activation, endothelial stability, and neuronal survival	miR-126, miR-149-5p, circ-FoxO3, ncRNA-targeted mimics/inhibitors	Reduce cytokine levels, protect BBB and mitochondria, and decrease neuronal apoptosis in rodent I/R models	Early preclinical; requires precise delivery and off-target risk control	6.5.2, 3.2–3.3
Nanocarrier-based precision delivery	Mitochondria/synapse/inflammation	Enhance BBB penetration, cell-/organelle-specific targeting, and controlled drug release	Liposomes, polymeric nanocarriers, ceria nanoenzymes, BBB-targeted LNPs, EV-based carriers	Improve drug accumulation in peri-infarct tissue, reduce infarct size, and support structural/functional recovery in multiple rodent stroke models	Rapidly evolving; some platforms entering early-phase clinical translation	6.5.3, 6.1–6.4

This table provides a multidimensional overview of emerging therapeutic interventions, mapping specific molecular mechanisms to their primary targets within the mitochondrial–synaptic–neuroinflammation axis. By categorizing strategies—ranging from organelle-specific modulation (e.g., mPTP inhibition and fission dynamics) to advanced regenerative platforms (e.g., mitochondrial transplantation and nanocarrier-based delivery)—the matrix offers a translational roadmap from preclinical evidence to current clinical status. Crucially, the table includes section-specific mapping to facilitate a deeper exploration of the combinatorial approaches proposed in this review, emphasizing the transition from benchtop discovery to potential clinical implementation. **Abbreviations**: AAV, adeno-associated virus; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ATP, adenosine triphosphate; BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; CsA, cyclosporin A; Cx43, connexin 43; Drp1, dynamin-related protein 1; EAAT2, excitatory amino acid transporter 2; EV, extracellular vesicle; I/R, ischemia–reperfusion; IL-1Ra, interleukin-1 receptor antagonist; JAK, Janus kinase; LIF, leukemia inhibitory factor; LNP, lipid nanoparticle; LTP, long-term potentiation; MCAO, middle cerebral artery occlusion; mPTP, mitochondrial permeability transition pore; ncRNA, non-coding RNA; NMDAR, N-methyl-D-aspartate receptor; PSD, postsynaptic density; ROS, reactive oxygen species; sEV, small extracellular vesicle; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α.

mPTP as a key early mediator of reperfusion-associated mitochondrial failure [48,109]. Subsequent mechanistic studies have clarified that mPTP opening is not a simple all-or-none event. Rather, it functions as a regulated high-conductance channel whose activation depends on calcium load, redox state, and inner-membrane lipid microenvironment. Bernardi and Di Lisa (2015) [110] summarized evidence that transient, low-probability pore flickering may occur under physiological or mildly stressful conditions, whereas sustained high-conductance opening is associated with irreversible swelling and loss of mitochondrial integrity during I/R. At the structural level, single-channel recordings identified a large mitochondrial megachannel associated with monomeric F_1F_0-ATP synthase, providing a plausible molecular correlate for permeability-transition events [111]. Mitochondria-targeted peptides such as SS-31 (Elamipretide) provide an indirect but complementary approach to mPTP modulation by stabilizing cardiolipin, a phospholipid essential for cristae architecture and electron-transport-chain organization. In ischemic mitochondria, SS-31 binds cardiolipin, improves ATP-generating efficiency, and reduces swelling and depolarization, effectively preserving organellar bioenergetics under reperfusion-related stress [112]. Additional pharmacological studies showed that cardiolipin-protective compounds can restore mitochondrial function across disease contexts in which permeability-transition susceptibility is increased [20]. Supporting these observations, recent studies of cerebral I/R emphasize the broader interdependence between mPTP regulation, cristae stability, and mitochondrial dynamics, reinforcing mitochondria-centered interventions as a rational neuroprotective strategy [15]. Taken together, the classical CsA and peptide studies, combined with contemporary insights into pore regulation, ATP-synthase–associated megachannels, and cardiolipin stabilization, converge on a consistent mechanistic principle: limiting pathological mPTP opening during early reperfusion is essential for preserving mitochondrial structural integrity and mitigating downstream neuronal degeneration.

6.1.2 Regulating Mitochondrial Fission/Fusion

Mitochondrial dynamics are rapidly disrupted during I/R, with excessive fragmentation emerging as a prominent structural correlate of early mitochondrial dysfunction. A major driver of this response is Drp1, which undergoes stress-induced activation and translocation to the outer mitochondrial membrane. Experimental studies in neuronal cultures and rodent ischemia models demonstrate that Drp1 overactivation promotes mitochondrial shortening, cristae deformation, increased ROS generation, and heightened susceptibility to permeability-transition–mediated collapse [10]. Inhibition of Drp1 using mdivi-1, or genetic deletion of upstream Drp1 activators, significantly limits fragmentation, preserves $\Delta\Psi m$, and reduces neuronal degeneration following focal cerebral ischemia, supporting a direct causal relationship between pathological fission and reperfusion-induced mitochondrial injury [12]. Physiological mitochondrial fission is required for quality control, enabling segregation and removal of damaged mitochondrial segments. However, I/R induces a rapid pathological shift in this balance, producing excessive Drp1-dependent fragmentation. Foundational work in neurodegeneration research demonstrates that disruption of the fission–fusion equilibrium leads to network destabilization, ROS amplification, and reduced metabolic resilience [113,114]. Consistent with these findings, Drp1 overactivation during ischemic stress causes abrupt mitochondrial shortening, $\Delta\Psi m$ instability, and increased susceptibility to oxidative and apoptotic injury, whereas targeted Drp1 inhibition preserves mitochondrial structure, reduces ROS accumulation, and limits neuronal death [10]. Genetic deletion of a neuronal Drp1 activator further protects against ischemia by stabilizing mitochondrial dynamics and attenuating tissue injury [12]. Collectively, these results highlight early restriction of pathological Drp1-mediated fission as an important component of mitochondrial preservation during reperfusion.

6.1.3 Enhancing Mitophagy and Mitochondrial Quality Control

Mitophagy plays a critical role in maintaining mitochondrial integrity after I/R, as dysfunctional mitochondria accumulate rapidly and promote ROS generation and metabolic instability. Multiple experimental and translational studies indicate that cerebral ischemia activates PINK1/Parkin-dependent mitophagy, facilitating selective removal of damaged mitochondria and contributing to early neuronal protection [115,116]. However, the timing and magnitude of mitophagy activation determine its functional consequences: adequately regulated mitophagy supports organelle turnover and metabolic recovery, whereas excessive or prolonged activation may deplete mitochondrial mass and exacerbate energy failure, particularly in highly vulnerable neuronal populations [117,118]. Importantly, several experimental interventions have been shown to modulate mitophagy in a manner that improves ischemic outcomes. Natural compounds such as ligustilide, as well as endogenous stress-response modulators, enhance PINK1/Parkin-mediated clearance of dysfunctional mitochondria and reduce oxidative injury in preclinical stroke models [119]. These observations underscore mitophagy as a dynamic and tightly regulated process whose therapeutic potential depends on achieving an optimal balance between clearance of damaged mitochondria and preservation of mitochondrial mass.

6.2 Antioxidant and Redox-Modulating Therapies

Oxidative stress is a central downstream mediator of I/R injury, arising from mitochondrial respiratory chain dysfunction, excitotoxic calcium influx, activation of ox-

idases, and impairment of endogenous antioxidant systems. Reactive oxygen and nitrogen species (ROS/RNS) trigger lipid peroxidation, protein oxidation, DNA damage, and BBB disruption, thereby amplifying neuronal death and neuroinflammation after acute ischemic stroke [33,120,121]. Given this mechanistic profile, antioxidant and redox-modulating therapies have been explored as potential neuroprotective approaches. These strategies are commonly grouped into (i) direct ROS scavengers or redox buffers and (ii) interventions that enhance endogenous enzymatic antioxidant defenses. Although many of these approaches have shown robust effects in preclinical models, their translation to clinical settings has been limited, prompting recent proposals for multi-target or combination antioxidant regimens tailored to the temporal evolution of IR-induced oxidative stress [121,122].

6.2.1 Direct ROS Scavengers and Redox Buffers

Direct ROS scavengers aim to neutralize free radicals generated during early reperfusion and to restore redox homeostasis. Classical small-molecule antioxidants such as vitamin C, vitamin E, and flavonoids showed promising reductions in oxidative biomarkers and infarct size in experimental stroke, but clinical trials have yielded inconsistent results, partly due to suboptimal timing, dosing, and limited brain penetration [120,121]. More recent attention has focused on pleiotropic antioxidants with improved pharmacokinetic and mechanistic profiles. Melatonin is a prototypical example: it is amphiphilic, crosses the BBB, accumulates in mitochondria, and directly scavenges multiple ROS/RNS species while upregulating endogenous antioxidant defenses [123,124]. In rodent models of focal and global cerebral ischemia, melatonin administration reduces markers of lipid peroxidation, preserves $\Delta\Psi m$, attenuates cytochrome-c release, and suppresses inflammatory responses, leading to smaller infarcts and improved functional recovery [123].

Building on these observations, recent reviews argue that direct scavengers are most effective when integrated into multi-antioxidant or multi-target regimens rather than used as stand-alone therapies. Briones-Valdivieso et al. (2024) [122] propose combining radical scavengers, metal chelators, and redox-active compounds to simultaneously target upstream ROS generation, mitochondrial redox signaling, and downstream inflammatory cascades. Jurcau and Ardelean (2022) [33] similarly emphasize that the temporal evolution of oxidative stress after stroke—early mitochondrial ROS bursts followed by prolonged neuroinflammatory ROS/RNS production—likely requires stage-specific antioxidant profiles. Overall, direct ROS scavengers and redox buffers remain biologically plausible and mechanistically supported, but their therapeutic efficacy appears highly dependent on optimized timing, brain delivery, and rational combination with complementary neuroprotective modalities.

6.2.2 Enzymatic Antioxidant Augmentation

Endogenous antioxidant enzymes such as superoxide dismutases (SODs), catalase, and GPx form a tightly regulated defense network that buffers ROS at mitochondrial and cytosolic levels. Experimental studies summarized by Rodrigo et al. (2013) [120] and Li and Yang (2016) [121] indicate that upregulation or overexpression of these enzymes in rodent stroke models can reduce oxidative damage, limit neuronal death, and preserve cerebrovascular function. For example, increased SOD activity enhances dismutation of superoxide to hydrogen peroxide, while catalase and GPxs further detoxify hydrogen peroxide and lipid peroxides, collectively mitigating mitochondrial dysfunction and apoptotic signaling. Conversely, deficiency or inactivation of these enzymes is associated with larger infarcts, worse neurological outcomes, and heightened susceptibility to excitotoxic and inflammatory injury [120,121]. Translational approaches to enzymatic augmentation have included gene-transfer strategies, pharmacological inducers of endogenous antioxidant pathways, and adjunctive use of drugs that secondarily enhance SOD, catalase, or GPx activity. However, clinical implementation remains in an early or experimental stage. Li and Yang (2016) [121] critically note that antioxidant enzymes are embedded in a complex redox network, and that non-specific elevation of enzymatic activity may disrupt physiological redox signaling or inadvertently shift ROS metabolism toward other reactive species. Recent conceptual frameworks therefore advocate “upstream and downstream” targeting: modulating sources of ROS generation (e.g., mitochondrial complex dysfunction, NADPH oxidase activity) while selectively reinforcing antioxidant capacity in high-risk compartments such as mitochondria and the neurovascular unit [33,122]. Within this paradigm, enzymatic antioxidant augmentation is best understood as a complementary component of a broader redox-modulating strategy, rather than a stand-alone therapeutic solution, and may achieve its greatest efficacy when integrated with mitochondrial stabilization and anti-inflammatory approaches.

6.3 Synaptic Stabilization and Anti-Excitotoxic Strategies

Excitotoxicity remains one of the earliest and most destructive mechanisms triggered during I/R, driven by excessive glutamate release, NMDA/AMPA receptor overactivation, calcium influx, and downstream activation of proteases, mitochondrial dysfunction, and structural synapse failure. These events not only produce acute synaptic collapse but also impair long-term synaptic plasticity, ultimately contributing to persistent cognitive and functional deficits after stroke. Accordingly, synapse-centered strategies—ranging from receptor modulation to protease inhibition and plasticity enhancement—represent an essential complement to mitochondrial- and antioxidant-targeted approaches.

6.3.1 NMDA/AMPA Receptor Modulation

Glutamate-mediated excitotoxicity is a central mechanism of acute neuronal injury during I/R. Excessive activation of ionotropic glutamate receptors—particularly NMDA and AMPA receptors—induces calcium overload, free radical generation, and downstream activation of proteases and apoptotic cascades. Early foundational work by Meldrum (2000) [125] reviewed how glutamate, under physiological conditions, supports synaptic transmission and plasticity, but becomes a potent neurotoxin when clearance and receptor regulation fail, such as during ischemic states. In line with this, Chamorro et al. (2016) [1] emphasized that excitotoxicity, together with oxidative and nitrosative stress and inflammation, forms a tightly interlinked triad that drives neuronal death in acute stroke, and thus remains a primary target of neuroprotective strategies. Within this framework, NMDA receptor signaling has been dissected in considerable mechanistic detail. One influential approach targets the interaction between NMDA receptors and the scaffolding protein PSD-95. Aarts et al. (2002) [2] demonstrated that a cell-penetrating peptide that disrupts GluN2B –PSD-95 binding attenuates coupling to nNOS, thereby reducing nitric oxide–mediated injury in rodent models of focal ischemia. This intervention reduced infarct volume and improved functional outcome without globally blocking NMDA receptor function, establishing the concept of selectively interrupting pathological NMDA signaling while preserving physiological synaptic transmission. Earlier pharmacological studies using broad NMDA receptor antagonists such as CGS 19755 also showed that receptor blockade could prevent ischemia-induced reductions in NMDA, PCP, and adenosine A1 receptor binding in gerbil brain, suggesting preservation of receptor integrity and synaptic signaling capacity [126]. However, the limited therapeutic window and adverse effects of non-selective NMDA antagonists in clinical settings have shifted the focus toward more refined modulation strategies.

Beyond simple receptor blockade, post-ischemic changes in NMDA receptor localization and signaling quality have emerged as critical determinants of neuronal fate. Besshoh et al. (2005) [127] reported that transient global ischemia in rats increases phosphorylation of NMDA receptor subunits and redistributes receptors between synaptic lipid rafts and postsynaptic densities, indicating that ischemia reshapes receptor microdomain organization and downstream signaling. Complementary work by Zhang et al. (2011) [128] identified a synaptic NMDA receptor–driven signaling cascade—nuclear calcium–CREB–ATF3—that activates a gene repression program protective against cell death induced by extrasynaptic NMDA receptors and ischemic injury. Together, these studies support a model in which synaptic NMDA receptor activity can engage neuroprotective transcriptional pathways, whereas excessive extrasynaptic activation promotes neuronal death. Thus, therapeutic strategies that preserve or enhance synaptic NMDA receptor signaling while restraining extrasynaptic overactivation may confer more nuanced protection than global receptor inhibition.

AMPA receptors also undergo profound remodeling in response to ischemic insults, with important consequences for synaptic stability and calcium homeostasis. Liu et al. (2006) [129] demonstrated that ischemic injuries direct GluA2-lacking, calcium-permeable AMPA receptors to synaptic sites, thereby increasing Ca^{2+} influx through AMPA channels and amplifying excitotoxic damage. In a complementary hippocampal model, Chen et al. (2014) [3] showed that prolonged activation of adenosine A1 receptors during hypoxia and focal cortical ischemia facilitates clathrin-mediated AMPA receptor endocytosis at CA3–CA1 synapses, leading to long-lasting synaptic inhibition. They further demonstrated that GluA2 and GluA1 subunits are differentially regulated by p38 MAPK and JNK pathways, underscoring the complexity of AMPA receptor trafficking and subunit-specific modulation after ischemia. These findings highlight that both insertion of GluA2-lacking receptors and pathological internalization of AMPA receptors can destabilize synaptic function, and that targeted control of AMPA receptor trafficking may help preserve excitatory–inhibitory balance during reperfusion.

Finally, pharmacological agents that indirectly modulate NMDA and AMPA receptor activity, such as certain antiepileptic drugs, have been proposed as potential adjunctive neuroprotectants. Reviewing preclinical and clinical data, Calabresi et al. (2003) [130] argued that several antiepileptic drugs—by reducing glutamate release, enhancing GABAergic inhibition, or altering ion channel function—may limit excitotoxicity and confer modest protection in brain ischemia. Although their direct impact on ischemic outcome remains incompletely defined, these agents exemplify a broader concept: rather than completely silencing glutamatergic transmission, fine-tuning receptor activity and downstream signaling may achieve a more favorable balance between preserving physiological synaptic function and preventing excitotoxic damage.

Taken together, work on NMDA/PSD-95 interactions, receptor microdomain dynamics, synaptic versus extrasynaptic signaling, and AMPA receptor trafficking converges on the idea that synapse-focused modulation of glutamate receptors is a critical component of synaptic stabilization strategies in the setting of I/R.

6.3.2 Calpain Inhibition and Preservation of Synaptic Structure

Calcium overload during I/R rapidly activates calpain, a Ca^{2+}-dependent cysteine protease that cleaves cytoskeletal proteins, synaptic scaffolds, ion channels, and signaling receptors. Early work by Rami (2003) [4] introduced the calpain–calpastatin–caspase hypothesis, proposing that an imbalance between calpain and its endogenous inhibitor calpastatin facilitates structural protein degrada-

tion and caspase-driven delayed neuronal death in vulnerable hippocampal neurons. This concept places calpain at a central mechanistic intersection between excitotoxic calcium influx, cytoskeletal destabilization, and apoptosis.

Pharmacological studies provided direct evidence that calpain inhibition confers neuroprotection *in vivo*. In a rat focal ischemia model, Bartus et al. (1994) [101] reported that post-occlusion intra-arterial infusion of the calpain inhibitor AK295 reduced infarct volume and neuronal degeneration, demonstrating that calpain blockade is effective even when initiated more than 1 hour after vessel occlusion. Similarly, in a gerbil global ischemia paradigm, Li et al. (1998) [131] showed that postischemic administration of MDL 28170 reduced cortical injury and partially attenuated CA1 neuronal loss, supporting the existence of a therapeutic window for calpain inhibition during reperfusion. Beyond structural targets, calpain also regulates trophic receptor signaling. Lu et al. (2016) [132] demonstrated that brain ischemia triggers calpain-dependent cleavage of ErbB4, disrupting neuregulin-1–mediated pro-survival pathways and exacerbating neuronal death. Recent mechanistic reviews, such as that by Zhao et al. (2024) [133], further highlight isoform-specific roles, calpastatin dynamics, and subcellular localization as key determinants of calpain's pathological actions in ischemic and hypoxic brain injury. Contemporary work extends these findings to combined toxic–ischemic conditions. In a rat model of copper oxide nanoparticle–aggravated cerebral I/R, Karimkhani et al. (2024) [134] observed that calpain inhibitor-I (ALLN) reduced oxidative stress, apoptotic signaling, and histopathological damage. Together, these data indicate that calpain is a convergent driver of synaptic destabilization, mitochondrial impairment, and neuronal death. Timely and targeted calpain inhibition—whether pharmacological or through modulation of endogenous calpastatin—represents a mechanistically grounded approach for preserving synaptic integrity during reperfusion.

6.3.3 Synaptic Reconstruction and Plasticity Enhancement

I/R injury disrupts synaptic integrity at multiple levels, including neurotransmission, dendritic spine structure, and the molecular programs that govern long-term circuit remodeling. A central mechanism underlying synaptic repair failure is the dysregulation of key plasticity-related pathways. Numerous studies have demonstrated that I/R impairs LTP, reduces synaptic density, and downregulates neuroplasticity-associated signaling cascades, ultimately contributing to post-stroke cognitive impairment [135,136]. These deficits involve alterations in excitatory receptor composition, impaired synaptic protein turnover, and reduced activity-dependent structural plasticity. Neuroimmune interactions play a crucial role in shaping the trajectory of synaptic recovery after ischemia. Activated microglia release cytokines, reactive species, and complement proteins that can exacerbate synaptic loss or, under specific conditions, support remodeling and rewiring. Evidence from experimental stroke models shows that microglia actively participate in synaptic reorganization, influencing dendritic spine turnover and neurotransmission in peri-infarct regions [137,138]. Contemporary reviews further highlight that microglial phenotype transitions—particularly shifts from pro-inflammatory to reparative states—modulate neuroplasticity, partly by regulating neurotrophic signaling, synaptic pruning, and astrocytic support functions [139]. This immune–synapse interface is now recognized as a major determinant of whether synaptic circuits degenerate or begin restoring activity patterns after injury. In addition to inflammatory modulation, multilayered mechanisms contribute to synaptic reconstruction. Molecular analyses indicate that ischemia alters key regulators of cytoskeletal remodeling, neurotransmitter receptor trafficking, and dendritic spine stability [135,136]. Microglia-mediated synaptic stripping and reinnervation further define the transition from acute synaptic collapse to structural reorganization [137]. Recent insights into neuroimmunological synapses demonstrate that ischemia reshapes cell–cell signaling domains among neurons, microglia, and astrocytes, affecting calcium signaling, spine dynamics, and circuit responsiveness [138]. Together, these findings indicate that synaptic reconstruction following I/R is not a single-pathway event, but the result of coordinated interactions among neuronal plasticity machinery, immune-derived signals, and glial structural support. Enhancing these pathways—by promoting neurotrophic signaling, modulating microglial phenotypes, and stabilizing synaptic scaffolding—represents a promising strategy to improve long-term functional recovery.

6.4 Anti-Inflammatory and Cytokine-Targeted Therapies

6.4.1 IL-1, TNF-α, and IL-6 Axis Modulation

Pro-inflammatory cytokines released during I/R play a central role in amplifying mitochondrial dysfunction, synaptic failure, and secondary neuronal injury. Among these, IL-1β, TNF-α, and IL-6 constitute a tightly interconnected inflammatory triad that drives excitotoxicity, oxidative stress, BBB breakdown, and leukocyte infiltration [6,140,141]. IL-1β rises within minutes after reperfusion, driven by microglial activation and NLRP3 inflammasome signaling. IL-1β enhances NMDA receptor activity, promotes calcium overload, and destabilizes $\Delta\Psi m$, thereby accelerating neuronal vulnerability. Preclinical stroke studies summarized by Lambertsen et al. (2019) [5] show that pharmacological blockades using IL-1Ra in rodent models of focal IR reduces leukocyte infiltration, attenuates BBB permeability, and lowers infarct volume, establishing IL-1 signaling as a validated therapeutic axis. TNF-α exerts dual receptor-mediated effects, but excessive soluble TNF-α promotes caspase activation, mitochondrial depolarization, and synaptic loss. Experimental and clinical-review data indicate that selective interference with TNF-α signal-

ing can preserve dendritic spine density, limit redox imbalance, and reduce tissue damage in experimental ischemia [141]. IL-6, particularly through the JAK/STAT3 trans-signaling pathway, contributes to delayed neuronal injury, inducing astrocytic metabolic stress, microglial cytokine release, and mitochondrial energy deficits [6]. Anti-IL-6 or STAT3 inhibition reduces metabolic collapse and late-phase oxidative injury in IR models, highlighting IL-6 as a mechanistic link between early inflammatory signaling and longer-term neurodegenerative processes after stroke. Across these studies, a consistent principle emerges: early modulation of the IL-1β/TNF-α/IL-6 network can redirect the inflammatory trajectory, limiting mitochondrial damage, stabilizing synapses, and reducing long-term neurological impairment. However, because these cytokines also participate in debris clearance and tissue remodeling, excessive suppression may interfere with reparative immune signaling, suggesting that future cytokine-targeted therapies should employ temporal- and cell-selective approaches rather than broad anti-inflammatory blockade.

6.4.2 Microglial and Astrocyte Modulation

Microglia and astrocytes rapidly respond to I/R by undergoing metabolic and phenotypic changes that amplify neuroinflammation, oxidative stress, and synaptic instability. Microglial M1-type polarization drives early production of IL-1β, TNF-α, and ROS, promoting BBB leakage and dendritic spine loss, whereas M2-type polarization supports debris clearance and tissue remodeling [141,142,143]. Review and experimental data indicate that shifting microglia/macrophages toward an M2-skewed state is associated with smaller infarcts, reduced inflammatory damage, and improved functional outcome in rodent I/R models [142,143,144]. Pharmacological strategies that suppress pro-inflammatory microglial activation—such as PI3K/Akt/NF-κB inhibition or cGAS–STING suppression—have demonstrated the ability to reduce infarct size and stabilize synapses in rodent MCAO and global I/R models [144,145]. More recent approaches employ nanoparticles and exosome-like vesicles to reprogram microglial responses: Panax notoginseng–derived exosome-like nanoparticles and targeted mRNA nanocarriers promote M2 polarization, preserve BBB integrity, and attenuate I/R-mediated neuroinflammation in experimental stroke models [146,147].

Astrocytes also undergo profound metabolic reprogramming after I/R, shifting from glutamate uptake and trophic support toward inflammation, impaired energy metabolism, and reactive gliosis [148]. Enhancing astrocytic glutamate clearance (EAAT2 upregulation) or modulating astrocyte-intrinsic stress pathways attenuates excitotoxicity and supports synaptic resilience. In rodent ischemic models, gene- and cell-based interventions that convert astrocytes to functional neurons via NeuroD1 overexpression improve long-term circuit repair and behavioral recovery [149]. In parallel, targeted delivery systems—such as astrocyte- and microglia-targeted Danshensu liposomes or agents that upregulate astrocytic connexin 43—reduce astrocytic swelling, protect mitochondrial function, and promote functional recovery after cerebral I/R [150,151]. Notably, astrocytic TRPA1 signaling has emerged as an intrinsic protective system, where TRPA1 activation increases leukemia inhibitory factor (LIF) production and enhances neurovascular resilience in models of vascular cognitive impairment, suggesting that astrocytes can mount endogenous neuroprotective responses that may be harnessed therapeutically [152].

Together, these findings demonstrate that precise modulation of microglial polarization and astrocyte metabolic–structural responses represents a convergent strategy to reduce I/R-induced neuroinflammation, stabilize synapses, and promote long-term recovery.

6.5 Emerging Therapies: Mitochondrial Transplantation, Gene Therapy, and Nanocarriers

6.5.1 Mitochondrial Transplantation

Mitochondrial transplantation has recently emerged as a transformative strategy for I/R injury, based on the premise that exogenous functional mitochondria can restore cellular bioenergetics, stabilize synaptic and structural integrity, and restrain neuroinflammatory cascades. Across rodent and early human studies, transplanted mitochondria have been shown to enhance ATP production, recover ΔΨm, reduce oxidative stress, and support neuronal survival, placing mitochondrial transfer within a broader class of mitochondrial repair–oriented neuroprotective strategies [7,153]. In cerebral I/R models, direct transplantation of isolated mitochondria into peri-infarct tissue reduces infarct size and improves neurological outcomes. In a focal cerebral ischemia model, Xie et al. (2021) [154] demonstrated that locally delivered mitochondria preserved ΔΨm, decreased cytochrome-c release, and attenuated apoptosis, contributing to reduced tissue loss. Their data also suggest that partial disassembly and reassembly of mitochondrial components may facilitate functional integration into host neurons. Extending these results, Chen et al. (2022) [155] showed that mitochondrial transplantation after focal ischemia not only improved early neuronal survival but also promoted remyelination and enhanced long-term locomotor recovery, indicating that exogenous mitochondria can support both axonal integrity and circuit-level plasticity. Global ischemia models following CA/ROSC further reinforce the therapeutic potential of this approach. Xu et al. (2024) [156] reported that autologous mitochondrial injection in a rat CA model significantly restored ATP levels, reduced delayed neuronal death in vulnerable hippocampal and cortical regions, and improved neurological scores, suggesting applicability to severe whole-brain I/R where metabolic collapse is widespread. These mechanistic and preclinical insights are strongly aligned with

stroke pathophysiology, in which early mitochondrial depolarization triggers synaptic failure and inflammatory amplification. Importantly, first-in-human clinical translation has begun. In a Phase 1 trial of acute cerebral ischemia, Walker et al. (2026) [8] demonstrated that intra-arterial autologous mitochondrial transplantation was feasible, well tolerated, and associated with early neurological improvement. Although preliminary, this study represents the first evidence that mitochondrial transplantation can be clinically deployed in the hyperacute stroke setting. Safety and feasibility data are further supported by cardiac and skeletal muscle I/R studies in which mitochondrial transplantation improved tissue recovery with minimal adverse effects [7]. Finally, emerging bioengineering approaches, such as mitochondria-containing EV, offer a complementary or alternative delivery platform. EV-mediated mitochondrial transfer—from brain endothelial cells or engineered donor cells—has been shown to improve cellular respiration, enhance BBB integrity, and reduce neuroinflammation in rodent stroke models [157]. These nanoparticle-like carriers may overcome current limitations in mitochondrial viability, targeting precision, and delivery efficiency.

Collectively, mitochondrial transplantation represents one of the most promising frontier therapies for ischemic stroke, offering multipronged benefits across mitochondrial energetics, synaptic preservation, and inflammatory modulation. Nonetheless, optimization of mitochondrial source (autologous vs. allogeneic), dose, timing, and route of administration—as well as standardized functional quality control of donor mitochondria—will be essential for successful Phase 2/3 trials. Future clinical studies may also integrate mitochondrial transplantation with redox-modulating agents, anti-inflammatory therapies, or synaptic stabilizers to achieve synergistic neuroprotection. Representative preclinical and early clinical studies of mitochondrial transplantation and mitochondria-containing vesicles in I/R injury are summarized in Table 3 (Ref. [7,8,153,154,155,156,157]).

6.5.2 Gene and RNA-Based Therapies

Gene- and RNA-based interventions offer upstream control over the molecular programs that govern inflammation, mitochondrial dysfunction, and synaptic deterioration after I/R injury. Because transcriptional and post-transcriptional disruptions occur within minutes after ischemia and persist through delayed degeneration, these approaches enable coordinated modulation of both acute and chronic injury cascades.

Gene delivery strategies were first explored using viral vectors expressing neuroprotective or trophic genes. Early adeno-associated virus (AAV)–based approaches demonstrated reductions in neuronal apoptosis and improved functional outcomes in rodent stroke models [158,159]. More targeted constructs have since been developed. For example, CXCL12 gene therapy attenuates ischemia-induced white-matter damage and promotes oligodendrocyte survival in mouse MCAO, highlighting chemokine augmentation as a mechanism for circuit-level repair [160]. Contemporary reviews similarly emphasize that gene therapies targeting angiogenesis, glial modulation, or metabolic support hold promise for restoring tissue microenvironments after I/R [161]. RNA interference (RNAi) enables selective suppression of deleterious pathways. Brain-targeted, intranasally delivered protein-based gene constructs carrying RNAi cargo reduce pro-inflammatory signaling, limit infarct size, and improve sensorimotor outcomes in rodent focal ischemia [162]. Earlier nanoparticle-assisted small interfering RNA (siRNA) delivery studies also demonstrated BBB-permeable suppression of inflammatory mediators with substantial neuroprotection in ischemic rats [163]. Together, these findings indicate that RNAi can effectively target upstream regulators of cytokine release, mitochondrial injury, and programmed cell death. ncRNAs—including miRNAs and circular RNAs (circRNAs)—represent another powerful regulatory layer. Systematic reviews highlight that miRNAs shape microglial activation, endothelial function, oxidative stress, and apoptotic signaling after stroke [164,165]. Specific miRNAs such as miR-126 and miR-149-5p confer robust protection in rodent I/R models by reducing inflammatory cytokines, improving vascular integrity, and preventing neuronal apoptosis [166]. The circ-FoxO3 axis mitigates BBB disruption by enhancing autophagy and reducing oxidative injury during I/R [167]. Furthermore, transcriptomic profiling during remote ischemic postconditioning reveals coordinated circRNA/miRNA expression signatures linked to reduced infarction and improved functional recovery, indicating that ncRNAs participate in endogenous ischemic tolerance mechanisms [168]. Gene-level regulators of neuroinflammation provide additional therapeutic rationale. Recent work shows that gain-of-function PPM1D mutations modulate microglial inflammatory output and reduce ischemic injury in mouse models, underscoring immune phosphatases as genetic determinants of stroke susceptibility and repair potential [169]. Meanwhile, single-cell RNA sequencing demonstrates that aging imposes a transcriptional bias toward pro-inflammatory glial states and impaired reparative programs after stroke, suggesting that gene/RNA therapies may be particularly impactful in older patients [170].

Collectively, these studies indicate that gene- and RNA-based strategies can modify multiple pathological nodes—neuroinflammation, mitochondrial failure, endothelial dysfunction, and synaptic destabilization. Most approaches remain at the preclinical or early translational stage, but ongoing advances in cell-type-specific promoters, intranasal and nanoparticle-based delivery, and ncRNA network mapping are progressively clarifying the safest and most effective intervention points for human stroke therapy. Representative gene- and RNA-based strategies targeting

Table 3. Representative evidence for mitochondrial transplantation and mitochondria-containing vesicles in I/R injury.

Species/model	Sample size (*n*/group)	I/R paradigm	Mitochondrial source & delivery	Main mechanistic findings	Functional/structural outcomes	Reference
Rat, focal cerebral ischemia	6	Transient MCAO, cerebral I/R	Isolated mitochondria from healthy tissue; local intracerebral delivery into peri-infarct cortex during early reperfusion	Preserved mitochondrial membrane potential (ΔΨm); reduced cytochrome-c release and apoptosis; attenuation of mitochondrial swelling and ROS accumulation	Decreased infarct volume; improved histological neuronal survival in peri-infarct area	[154]
Mouse, focal cerebral ischemia	10	Transient MCAO, long-term follow-up	Donor mitochondria injected into ischemic region after reperfusion	Enhanced oligodendrocyte survival and remyelination; stabilization of axonal mitochondrial function	Improved locomotor recovery; increased myelin integrity and synaptic protein expression (e.g., PSD-95, synapsin I)	[155]
Rat, global cerebral ischemia after cardiac arrest	6	CA/ROSC model; whole-brain I/R	Autologous mitochondria isolated from peripheral tissue; systemic or targeted injection after ROSC	Restored brain ATP content; reduced delayed neuronal death in vulnerable regions; improved mitochondrial respiratory capacity	Better neurological scores and survival; attenuation of global I/R-induced neurodegeneration	[156]
Mouse, *in vitro* and *in vivo* stroke models (overview)	22	Various focal and global I/R paradigms	Isolated mitochondria; multiple routes (intraparenchymal, intra-arterial, intravenous)	Cross-study evidence for ΔΨm recovery, ROS reduction, and support of endogenous mitochondrial networks	Consistent reduction of infarct size and organ dysfunction across brain, heart, and other tissues	[7]
Mouse, experimental stroke – mitochondrial EVs	10	Focal cerebral I/R	Mitochondria-containing extracellular vesicles from brain endothelial cells; systemic administration	Enhanced mitochondrial respiration in recipient cells; improved BBB stability; reduction of neuroinflammatory signaling	Reduced infarct volume; improved neurobehavioral outcomes; preservation of neurovascular unit integrity	[157]
Human, acute cerebral ischemia (Phase 1 trial)	4	Acute ischemic stroke; endovascularly treatable lesions	Autologous mitochondria isolated from patient tissue; intra-arterial mitochondrial transplantation during acute treatment window	Feasibility and safety demonstrated; no major procedure-related adverse events; suggestion of mitochondrial integration into ischemic territory	Early neurological improvement in several patients; provides first-in-human proof of concept for stroke	[8]
Multiple animal models and organs (heart, brain, skeletal muscle)	N/A (Review)	Focal and global I/R	Isolated mitochondria via various routes (local injection, vascular delivery)	Convergent evidence that exogenous mitochondria restore bioenergetics, limit oxidative injury, and modulate cell death pathways	Broad protection against I/R injury across organs; framework for translating mitochondrial repair to CNS ischemia	[153]

This table summarizes the translational landscape of mitochondrial-based interventions, bridging the gap between mechanistic rodent studies and early clinical proof-of-concept. By delineating the species, ischemia–reperfusion (I/R) paradigms, and delivery routes, the table illustrates how exogenous mitochondria—either as isolated organelles or via extracellular vesicles—restore bioenergetic homeostasis and promote functional recovery. The inclusion of the first-in-human trial highlights the emerging feasibility of these strategies in acute clinical settings, providing a multifaceted overview of this innovative therapeutic frontier. **Abbreviations**: ATP, Adenosine triphosphate; BBB, blood-brain barrier; CA, cardiac arrest; CNS, central nervous system; ΔΨm, mitochondrial membrane potential; EV, extracellular vesicle; I/R, ischemia–reperfusion; MCAO, middle cerebral artery occlusion; PSD-95, postsynaptic density protein-95; ROS, reactive oxygen species; ROSC, return of spontaneous circulation.

Table 4. Representative gene- and RNA-based therapeutic strategies in ischemia–reperfusion (I/R) injury.

Strategy type	Target/cargo	Species/model	Delivery method/vector	Main mechanistic effects	Functional/conceptual outcomes	Reference
Gene therapy – chemokine support	CXCL12 overexpression	Mouse; transient MCAO with white matter injury	AAV-based CXCL12 gene delivery to peri-infarct region	Enhances oligodendrocyte survival; promotes angiogenesis and remyelination; stabilizes neurovascular microenvironment	Reduced ischemia-induced white matter loss; improved sensorimotor recovery	[160]
Viral gene therapy – early proof-of-concept	Neuroprotective/reporter genes	Rodent ischemia models	Defective recombinant AAV vectors injected into brain	Stable transgene expression in ischemic tissue; reduction of delayed neuronal loss in selected paradigms	First demonstration that AAV vectors can be used for ischemia-targeted CNS gene delivery	[158,159]
Protein-based gene therapy (intranasal)	Brain-targeted therapeutic protein/gene construct	Mouse; focal cerebral ischemia	Intranasal, brain-targeting protein-based gene therapy	Suppression of pro-inflammatory cascades; protection of neurons in peri-infarct cortex	Reduced infarct size; improved neurological scores; proof-of-concept for non-invasive CNS gene delivery	[162]
RNA interference (RNAi) nanotherapy	siRNA against pro-death/pro-inflammatory mediators	Rat; focal cerebral I/R (MCAO)	Brain-targeting nanoparticles carrying siRNA (systemic administration)	Knockdown of apoptotic and inflammatory genes; attenuation of oxidative stress and BBB injury	Decreased infarct volume; improved functional recovery; validation of RNAi-based neuroprotection	[163]
miRNA-based therapy – vascular protection	miR-126 modulation	Rodent cerebral I/R models (reviewed)	Viral vectors, miRNA mimics or inhibitors (various platforms)	Regulation of endothelial survival, angiogenesis, and inflammatory signaling; improved vascular integrity	Experimental and conceptual support for miR-126 as a candidate to limit vascular and BBB injury after stroke	[166]
circRNA–miRNA axis and BBB stabilization	circ-FoxO3; circRNA–miRNA networks	Mouse ischemia–reperfusion models	Viral vectors or engineered constructs; transcriptomic profiling	circ-FoxO3 enhances autophagy, limits oxidative damage, and preserves BBB integrity; remote ischemic postconditioning shifts circRNA–miRNA profiles toward neuroprotection	Reduced BBB leakage and infarct size; identification of circRNA–miRNA modules as endogenous ischemic tolerance pathways	[167,168]
ncRNA programs in neuroinflammation (systematic overview)	Broad ncRNA networks (miRNAs, circRNAs)	Human and animal data; multiple stroke models	Narrative/systematic review	ncRNAs regulate microglial activation, cytokine production, oxidative stress, and cell death signaling	Provides mechanistic framework and therapeutic targets for ncRNA-based interventions in I/R injury	[164,165]
Genetic regulators of microglial response	PPM1D gain-of-function mutations	Mouse MCAO; genetic models	Germline or engineered gain-of-function PPM1D	Modulation of microglial inflammatory output; attenuation of post-ischemic neuroinflammatory cascades	Reduced infarct volume and neurological deficits; highlights genetic determinants of stroke susceptibility and repair	[169]
Single-cell transcriptomics – target discovery	Aging-biased transcriptional programs	Mouse stroke + aging models	Single-cell RNA sequencing (scRNA-seq)	Identification of age-dependent microglial and astrocytic states; impaired reparative programs after stroke	Defines cell-type-specific gene/RNA targets for future gene or ncRNA therapies, particularly in aged brain	[170]
Gene therapy & stroke – conceptual framework	Multiple gene targets (angiogenesis, neurotrophins, metabolic support)	Various experimental stroke models	Review of viral and non-viral gene delivery approaches	Summarizes advantages and limitations of gene therapy (timing, cell specificity, safety)	Guides rational design of next-generation gene strategies for ischemic stroke	[161]

This table highlights the rapidly evolving landscape of molecular interventions, from AAV-mediated chemokine support to sophisticated circRNA–miRNA networks. By summarizing diverse modalities—including RNA interference (RNAi) nanotherapy, intranasal protein-based delivery, and single-cell transcriptomic-driven target discovery—the table delineates how genetic reprogramming can stabilize the neurovascular unit and protect the mitochondrial–synaptic axis. These strategies emphasize a shift toward precision neuroprotection, where the modulation of age-dependent or cell-type-specific transcriptional programs offers a promising avenue to mitigate the transition from reversible to irreversible neuronal injury. **Abbreviations**: AAV, adeno-associated virus; BBB, blood-brain barrier; circRNA, circular RNA; CNS, central nervous system; I/R, ischemia–reperfusion; MCAO, middle cerebral artery occlusion; miRNA, microRNA; ncRNA, non-coding RNA; PPM1D, protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1D; RNAi, RNA interference; scRNA-seq, single-cell RNA sequencing; siRNA, small interfering RNA.

Table 5. Nanocarrier-based therapeutic strategies in ischemia–reperfusion (I/R) injury.

Nanocarrier type	Therapeutic cargo/target	Species & model	Delivery method	Main mechanistic effects	Functional outcomes	Reference
Liposomal nanocarriers (general platforms)	Antioxidants, anti-inflammatory and neuroprotective small molecules	Review of rodent focal and global ischemia models	Intravenous or ligand-modified liposomes	Enhanced BBB penetration; prolonged brain retention; reduced oxidative stress and inflammation	Overall reduction in infarct size and improvement in neurological outcomes across models	[171]
Dual-ligand immune-cell–targeted liposomes	Puerarin	Rat MCAO	Intravenous; neutrophil/monocyte-targeting liposomes	Selective adhesion to ischemia-recruited immune cells; suppression of localized inflammatory responses	Reduced infarct volume and improved neurological scores	[172]
Polymeric nanocarriers	Antioxidants, anti-excitotoxic compounds	Rodent MCAO models	Intravenous polymeric nanoparticles	Controlled drug release; enhanced neuronal uptake; attenuation of apoptosis and oxidative injury	Improved histological preservation and behavioral recovery	[9]
Intelligent (stimuli-responsive) nanocarriers	ROS- or pH-responsive neuroprotective agents	Rodent I/R models (review and experimental data)	Targeted nanocarriers responsive to ischemic microenvironment	Protection of neurovascular unit; reduced BBB disruption under ischemic conditions	Improved neurovascular integrity and functional outcomes	[173]
Mitochondria-targeted nanoenzymes	Ceria (CeO_2) nanoenzymes	Mouse MCAO	Intravenous; mitochondrial-localizing nanoenzymes	Scavenging of mitochondrial ROS; stabilization of $\Delta\Psi m$; limitation of neuronal death	Markedly reduced infarct volume and improved functional recovery	[176]
Small extracellular vesicles (sEVs)	AMPKα2	Mouse MCAO	Tail-vein injection of targeted sEVs	Restoration of cellular energy metabolism; suppression of neuroinflammation; BBB protection	Improved motor and sensory outcomes	[174]
EV-based modulation of ncRNA pathways	ncRNAs regulating microglial activation and BBB repair	Human and animal data (review)	Endogenous or engineered EV delivery	Regulation of post-ischemic neuroinflammation and vascular repair via miRNA/circRNA transfer	Conceptual and experimental support for EV-based ncRNA therapy	[175]
Lipid nanoparticles (LNPs)	Gene- or RNA-based therapeutic constructs	Mouse MCAO	Intravenous BBB-targeting LNPs	Efficient BBB crossing; modulation of microglial activation and inflammatory gene expression	Reduced infarct size and stabilization of inflammatory responses	[177]
Theranostic albumin-based nanocarriers	Nerve growth factor (NGF)	Rat MCAO	Intravenous albumin-based theranostic nanocarriers	Enhanced axonal regeneration; combined therapeutic delivery and imaging capability	Improved behavioral recovery and structural repair	[178]

This table provides a comprehensive overview of next-generation delivery systems designed to surmount the physiological constraints of the blood-brain barrier (BBB). By categorizing diverse nanoplatforms—including stimuli-responsive polymeric carriers, mitochondria-targeted nanoenzymes, and engineered extracellular vesicles (EVs)—the matrix delineates how precision nanomedicine can enhance the pharmacokinetic profile and site-specific accumulation of neuroprotective agents. These strategies highlight a shift toward "intelligent" drug delivery, where the ischemic microenvironment itself triggers localized therapeutic release, thereby maximizing efficacy while minimizing systemic off-target effects within the mitochondrial–synaptic–neuroinflammation axis. **Abbreviations**: AMPKα2, AMP-activated protein kinase alpha-2; BBB, blood-brain barrier; EV, extracellular vesicle; I/R, ischemia–reperfusion; IV, intravenous; LNP, lipid nanoparticle; MCAO, middle cerebral artery occlusion; ncRNA, non-coding RNA; NGF, nerve growth factor; ROS, reactive oxygen species; sEV, small extracellular vesicle; $\Delta\Psi m$, mitochondrial membrane potential.

inflammation, vascular stability, and mitochondrial–synaptic integrity in I/R injury are summarized in Table 4 (Ref. [158,159,160,161,162,163,164,165,166,167,168,169,170]).

6.5.3 Nanocarriers for Precision Delivery

Nanocarrier platforms have emerged as a critical component of next-generation stroke therapeutics by enabling BBB-penetrant, cell-selective, and mitochondria-targeted drug delivery. These systems help overcome major translational barriers in I/R injury, including poor drug penetration, rapid systemic clearance, and off-target toxicity.

Liposomal systems constitute one of the most established approaches. Conventional and ligand-modified liposomes improve stability and facilitate targeted delivery of antioxidants, anti-inflammatory agents, and small neuroprotective molecules. Review analyses show that liposomes enhance brain accumulation and prolong therapeutic exposure, making them suitable for early-phase I/R therapy [171]. More recent dual-ligand formulations—such as neutrophil/monocyte-targeted liposomes delivering puerarin—achieve selective adhesion to ischemia-recruited immune cells, thereby reducing localized inflammation and infarct volume in MCAO rats [172]. Polymeric and "intelligent" nanocarriers expand these capabilities further. Polymeric nanoparticles allow modular tuning of surface ligands, drug release kinetics, and mitochondrial targeting. For example, polymeric nanocarriers developed for ischemic stroke enable controlled release of antioxidants and anti-excitotoxic compounds, significantly reducing neuronal apoptosis and improving neurological recovery in rodent MCAO [9]. Intelligent nanocarriers that respond to ischemic microenvironmental cues—such as acidosis, elevated ROS, or endothelial activation—have also shown improved neurovascular-unit protection and reduced BBB disruption [173]. EV–based delivery, particularly small EVs (sEVs), represents a biologically compatible alternative. Targeted sEVs engineered to deliver AMPKα2 demonstrate potent neuroprotection in murine MCAO by restoring energy metabolism, reducing inflammation, and improving functional outcomes [174]. In parallel, review studies highlight that EV-associated ncRNAs regulate post-ischemic neuroinflammation and BBB repair, suggesting that EVs can serve both as endogenous modulators and therapeutic vectors [175]. Mitochondria-directed nanocarriers are an emerging frontier. Ceria-based nanoenzymes that localize to neuronal mitochondria effectively reduce ROS surges, stabilize $\Delta\Psi m$, and limit neuronal death in rodent IR models, establishing mitochondria as a viable nanotherapeutic target [176]. Other platforms—including lipid nanoparticles (LNPs) engineered for BBB targeting—enable delivery of gene- or RNA-based therapies and have shown marked infarct reduction and microglial stabilization after experimental stroke [177]. Finally, specialized theranostic nanocarriers integrating imaging and therapeutic functions have demonstrated enhanced axonal regeneration and synaptic repair after stroke. Albumin-based NGF–loaded nanocarriers, for example, show improved behavioral recovery in rodent MCAO while simultaneously enabling monitoring of biodistribution and target engagement [178]. Overall, nanocarrier technologies provide a versatile and rapidly evolving toolkit that enhances precision delivery to neural, glial, and vascular compartments affected by I/R injury. Their modular design and compatibility with mitochondrial- and gene-targeted agents position them as a foundational platform for future combinatorial stroke therapies rather than stand-alone solutions.

Taken together, nanocarrier platforms provide versatile, BBB-penetrant delivery systems capable of targeting mitochondrial dysfunction, synaptic instability, and post-ischemic inflammation. A comparative summary of major nanocarrier classes—including liposomes, polymeric systems, intelligent stimuli-responsive carriers, EVs, and mitochondria-directed nanoenzymes—is provided in Table 5 (Ref. [9,171,172,173,174,175,176,177,178]). This overview highlights how each platform differentially modulates oxidative stress, cytokine signaling, or energy metabolism across established rodent ischemia models.

6.5.4 Emerging Role of Cell-Based Interventions in Synaptic Recovery

Beyond pharmacological neuroprotection, the potential of cell-based therapies—particularly mesenchymal stem cells (MSCs)—offers a novel paradigm for restoring the mitochondrial-synaptic axis after ischemic insult. Recent evidence suggests that MSCs do not solely function through cell replacement; instead, they act as "bioreactors" that enhance the metabolic resilience of the ischemic penumbra. A key mechanism involves the direct horizontal transfer of healthy mitochondria from MSCs to injured neurons via tunneling nanotubes (TNTs) or EVs [179,180]. This mitochondrial donation is particularly vital for the synaptic compartment, where localized bioenergetic failure often precedes irreversible somatic death. Moreover, the secretome of transplanted stem cells, rich in neurotrophic factors and specialized microRNAs, facilitates synaptic remodeling and stabilizes the presynaptic membrane [181]. These paracrine effects modulate the neuroinflammatory microenvironment, preventing the excessive fission and degradation of synaptic mitochondria. By integrating cell therapy with acute reperfusion strategies, it may be possible to broaden the therapeutic window and promote functional plasticity in neurons that would otherwise progress to irreversible injury [182].

6.6 Translational Challenges and Future Directions

Despite the substantial neuroprotective efficacy demonstrated across mitochondrial-targeted, synaptic-stabilizing, and neuroimmune-modulating interventions in preclinical models, clinical translation remains lim-

ited. Several mechanistic and methodological challenges underlie this gap. First, the therapeutic time window for most I/R interventions is narrow. Mitochondrial permeability transition, excitotoxic calcium influx, and microglial priming occur within minutes after reperfusion, making delayed pharmacological delivery far less effective. Second, the etiological heterogeneity of human I/R conditions—ranging from CA and large-vessel occlusion stroke to perioperative hypoxia—introduces substantial variability in metabolic rate, inflammatory profiles, and recovery trajectories, complicating generalization from controlled animal models.

Third, BBB permeability remains a major bottleneck. Many mitochondria-directed peptides, antioxidants, and RNA-based therapeutics exhibit limited penetration or require high systemic doses, increasing off-target effects. Fourth, species differences in mitochondrial dynamics, dendritic spine recovery, and glial responses complicate translation. Rodent CA1 neurons, for instance, exhibit sharper thresholds for mitochondrial collapse and delayed neuronal death compared with primate or human tissue, raising questions about dose scaling and timing. Another structural challenge involves the regional vulnerability gradient—particularly the differential thresholds for injury in CA1 vs. CA3, cortex vs. striatum, and white vs. gray matter. Region-specific differences in astrocyte–endfeet organization, microglial priming, mitochondrial cristae density, and synaptic firing energetics imply that a single therapeutic agent is unlikely to address all compartments of I/R pathology.

Looking forward, multimodal approaches that integrate mitochondrial repair (e.g., mPTP modulation, Drp1 regulation, mitochondrial transplantation), synaptic stabilization (e.g., PSD-95 disruption, AMPA trafficking control), and targeted immunomodulation (e.g., microglial reprogramming, cytokine axis modulation) are likely to yield the most durable neuroprotection. Emerging platforms—including BBB-targeted nanocarriers, EV therapeutics, and organelle-specific delivery systems—may overcome prior pharmacokinetic limitations. Additionally, the incorporation of advanced *in vivo* imaging (two-photon microscopy, mito-GFP reporters, calcium and ROS biosensors), spatial transcriptomics, and patient-derived organoids will enhance the mechanistic fidelity of preclinical research and help identify human-relevant therapeutic windows. These tools, combined with machine-learning–assisted profiling of mitochondrial and synaptic states, hold promise for bridging the translational divide and establishing personalized treatment strategies for cerebral I/R injury

7. Conclusions and Future Perspectives

I/R injury triggers a tightly interconnected cascade in which mitochondrial destabilization, synaptic structural failure, and neuroinflammatory amplification reinforce one another to determine whether neurons recover or progress toward irreversible degeneration. Across the evidence summarized in this review, a central theme emerges: the mitochondrial–synaptic axis functions as the decisive biological threshold that separates reversible dysfunction from permanent structural loss. Early mitochondrial depolarization, impaired oxidative phosphorylation, and dysregulated fission–fusion dynamics initiate synaptic silencing and dendritic spine retraction, while parallel activation of microglia and astrocytes amplifies excitotoxic, inflammatory, and metabolic stress. Region-specific patterns—particularly the pronounced vulnerability of hippocampal CA1 neurons compared with CA3—reflect differential resilience within this axis, shaped by intrinsic metabolic load, mitochondrial reserve, and glia–synapse coupling. The therapeutic strategies reviewed in Section 6—from antioxidant support and excitotoxicity modulation to glial regulation, mitochondrial transplantation, and gene/RNA-based interventions—collectively underscore that single-target therapies are insufficient for a process driven by multiple, temporally layered injury mechanisms. Instead, convergent modulation of mitochondrial stability, synaptic integrity, and neuroimmune activity offers a unified therapeutic framework. Advanced approaches, such as exogenous mitochondrial transfer, nanoengineered delivery systems, and precision gene/RNA therapeutics, highlight growing recognition that metabolic restoration and structural stabilization must proceed in parallel to achieve durable neuroprotection. Despite promising progress, several translational challenges persist. Human I/R conditions are heterogeneous—ranging from CA and global hypoperfusion to focal large-vessel occlusion—and therapeutic time windows differ substantially across these etiologies. Many candidates with strong preclinical efficacy still face limitations related to BBB penetration, off-target effects, and species-dependent differences in mitochondrial dynamics, microglial responsiveness, and synaptic recovery. Furthermore, aging and comorbidities impose a pro-inflammatory transcriptional bias that may blunt therapeutic responsiveness, emphasizing the need for patient-specific or stratified treatment designs.

Future research should integrate modern high-resolution and multimodal platforms to refine our understanding of neuronal reversibility thresholds. Spatial transcriptomics and single-cell profiling can map region- and cell-type-specific responses to I/R, while *in vivo* mitochondrial imaging and synaptic reporters will allow real-time monitoring of structural and metabolic recovery. Patient-derived organoids and humanized vascular–glial co-culture systems offer promising translational tools to test therapeutic combinations and evaluate BBB-targeted interventions. Ultimately, combination therapies—linking mitochondrial repair, synaptic stabilization, and targeted inflammatory modulation—are likely to yield the most clinically meaningful outcomes. As emerging technologies converge with a deeper mechanistic understanding of the mitochondrial–synaptic axis, translational prospects for

I/R therapy continue to expand, bringing precision and multi-level neuroprotection closer to clinical reality.

In conclusion, the transition from experimental neuroprotection to clinical success likely hinges on multifaceted strategies that address the mitochondrial–synaptic axis through combinatorial vectors. Among the most promising avenues are: (1) the synergy of autologous mitochondrial transplantation with precision nanocarrier platforms, which ensures site-specific organelle delivery while bypassing the BBB, and (2) the integration of ncRNA-based genetic reprogramming with standard mechanical thrombectomy, aiming to extend the therapeutic window by suppressing secondary neuroinflammatory cascades. However, for these strategies to reach bedside implementation, several key questions remain unanswered. Most notably, the precise temporal dynamics of mitochondrial-synaptic cross-talk must be mapped to identify the optimal timing for intervention. Furthermore, establishing standardized protocols for the sourcing and quality control of donor organelles or extracellular vesicles remains a critical hurdle. Addressing these fundamental gaps will be essential to translate the conceptual frameworks presented in this review into tangible clinical outcomes for acute cerebral ischemia patients.

Abbreviations

AAV, Adeno-associated virus; AKT, Protein kinase B; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ATP, Adenosine triphosphate; BBB, Blood–brain barrier; BDNF, Brain-derived neurotrophic factor; Ca^{2+}, Calcium ion; CA, Cardiac arrest; CNS, Central nervous system; CsA, Cyclosporin A; CypD, Cyclophilin D; DAPK1, Death-associated protein kinase-1; DG, Dentate gyrus; Drp1, Dynamin-related protein 1; ΔΨm, Mitochondrial membrane potential; EAAT2, Excitatory amino acid transporter 2; EV, Extracellular vesicle; GABA, γ-aminobutyric acid; GFP, Green fluorescent protein; GluA2, Glutamate ionotropic receptor AMPA type subunit 2; GluN2B, Glutamate ionotropic receptor NMDA type subunit 2B; GPx, Glutathione peroxidase; GSH, Glutathione; GSK3β, glycogen synthase kinase-3β; IL-1β, Interleukin-1β; IL-1Ra, Interleukin-1 receptor antagonist; IL-6, Interleukin-6; I/R, Ischemia–reperfusion; JAK, Janus kinase; LIF, Leukemia inhibitory factor; LNP, Lipid nanoparticle; LTP, Long-term potentiation; MCAO, Middle cerebral artery occlusion; MCU, Mitochondrial calcium uniporter; miRNA, microRNA; mPTP, Mitochondrial permeability transition pore; NADPH, Nicotinamide adenine dinucleotide phosphate; ncRNA, Non-coding RNA; NF-κB, Nuclear factor-κB; NGF, Nerve growth factor; NMDAR, N-methyl-D-aspartate receptor; nNOS, Neuronal nitric oxide synthase; OGD, Oxygen–glucose deprivation; OGD/R, Oxygen–glucose deprivation/reoxygenation; PINK1, PTEN-induced kinase 1; PSD, Postsynaptic density; PSD-95, Postsynaptic density protein-95; PV^{+}, Parvalbumin positive; RNAi, RNA interference; RNS, Reactive nitrogen species; ROS, Reactive oxygen species; sEV, Small extracellular vesicle; siRNA, Small interfering RNA; SOD, Superoxide dismutase; STAT3, Signal transducer and activator of transcription 3; STING, Stimulator of interferon genes; TNF-α, Tumor necrosis factor-α.

Author Contributions

KYY and MHW orchestrated the conceptual framework and designed the research strategy for this review. KYY took the lead in synthesizing the literature, performing the formal analysis, and drafting the initial manuscript, including the preparation of all illustrative figures and tables. BHJ and JHA contributed significantly to the data curation and scrutinized the clinical and mechanistic evidence presented in the tables. MHW provided high-level supervision, secured the necessary resources, and performed a critical appraisal of the intellectual content. All authors participated in the iterative revision process, offered substantial refinements to the text, and have formally approved the final version for publication. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

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