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REVIEW

Region-Specific Astrocyte Endfeet Disruption as a Driver of Pyramidal Neuron Death after Ischemia-Reperfusion in the Hippocampus

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ABSTRACT: Ischemia-reperfusion (I/R) injury induces region-specific neuronal vulnerability within the hippocampus, with the cornu ammonis 1 (CA1) subfield particularly prone to delayed neuronal death. While intrinsic neuronal factors have been implicated, emerging evidence highlights the decisive contribution of astrocyte endfeet (AEF)—specialized perivascular structures that regulate ion and water homeostasis, glutamate clearance, and blood–brain barrier (BBB) stability. This review synthesizes structural and molecular alterations of AEF across the CA1–CA3 subfields following I/R and their correlation with neuronal fate. In CA1, AEF undergo early-onset swelling and detachment from the vascular basal lamina due to dysfunction of critical proteins such as aquaporin-4 (AQP4) and Kir4.1. These changes impair glutamate uptake, metabolic support, and potassium buffering, contributing to neuronal hyperexcitability and degeneration. In contrast, AEF in CA3 preserves polarity and functional coupling of AQP4 and Kir4.1, conferring regional resilience. At the signaling level, AEF disruption activates mitogen-activated protein kinase (MAPK)/c-Jun N-terminal kinase (JNK) pathways, promotes reactive oxygen species (ROS) accumulation, and induces inducible nitric oxide synthase (iNOS)-mediated inflammation, amplifying neurotoxicity. Furthermore, subfield-specific astrocytic transcriptional profiles modulate inflammatory responses and gliovascular interactions. By reframing AEF not as passive scaffolds but as active regulators of neuronal survival, this review provides novel insight into the astrocyte-dependent mechanisms of hippocampal vulnerability. Therapeutic strategies that preserve AEF structure and function may offer targeted protection against delayed neuronal death in ischemic brain injury.

KEYWORDS: Astrocyte endfeet; hippocampus; ischemia-reperfusion injury; cornu ammonis 1 (CA1) vulnerability; delayed neuronal death; aquaporin-4; neuroinflammation; blood–brain barrier disruption

1 Introduction

Ischemia-reperfusion (I/R) injury in the brain triggers a complex cascade of metabolic, vascular, and cellular disturbances that affect neuronal populations in a regionally selective manner. The hippocampus, a medial temporal lobe structure essential for learning, memory consolidation, and spatial navigation, is among the most vulnerable brain regions to I/R insult [1–3]. Anatomically, the hippocampal formation includes the dentate gyrus, subiculum, entorhinal cortex, and the hippocampus proper, which is subdivided into cornu ammonis (CA) subfields—CA1, CA2, CA3, and CA4 (also referred to as the hilus of the dentate gyrus) [3,4]. Among these, the CA1 region is particularly sensitive to transient global ischemia, exhibiting delayed neuronal death within 2–3 days post-injury despite initial apparent histological preservation [5–7].

Although intrinsic neuronal factors such as high N-methyl-D-aspartate (NMDA) receptor density, elevated oxidative stress susceptibility, and mitochondrial dysfunction have been proposed to explain the selective vulnerability of the CA1 region, emerging evidence suggests that astrocyte-dependent mechanisms, particularly at the level of astrocyte endfeet (AEF), play a decisive role in determining neuronal fate [8–10]. Astrocytes are multifunctional glial cells that maintain brain homeostasis by regulating extracellular ion balance, neurotransmitter clearance, metabolic support, and neurovascular unit (NVU) function [11–13]. AEF, their perivascular terminal expansions of astrocytes, form a critical interface with the vasculature, contributing to blood–brain barrier (BBB) integrity, neurovascular coupling, and fluid exchange via key channel proteins such as aquaporin-4 (AQP4) and Kir4.1 [14].

Recent findings reveal that AEF undergo region-specific and early pathological alterations following I/R injury. In the CA1 region, AEF exhibit rapid-onset swelling, redistribution or mislocalization of AQP4/Kir4.1, and detachment from the vascular basal lamina—events that precede and likely contribute to pyramidal neuron degeneration [15,16]. In contrast, AEF in CA2 and CA3 subfields are relatively preserved, maintaining ion homeostasis and structural integrity, which may underlie the relative resistance of neurons in those regions. This astroglial heterogeneity across hippocampal subfields has spurred new interest in understanding the astrocyte–neuron–vessel triad as a determinant of selective neuronal vulnerability.

While many reviews have addressed astrocyte reactivity or global hippocampal injury, few have specifically focused on AEF-centered mechanisms, nor compared these changes across CA subregions in the context of neuronal death vs. resistance.

In this review, we aim to bridge this knowledge gap by examining the structural and molecular profiles of AEF, with a focus on region-specific alterations in CA1, CA2, and CA3 following I/R injury. We further evaluate how these changes impact BBB stability, potassium buffering, glutamate clearance, metabolic coupling, and inflammatory signaling—each of which plays a decisive role in determining neuronal survival or degeneration. By integrating anatomical, functional, and mechanistic insights, this review proposes a framework for understanding AEF-mediated mechanisms underlying hippocampal region-specific vulnerability in ischemic brain injury.

2 Selective Vulnerability and Delayed Neuronal Death (DND) in the Hippocampus

I/R injury induces region-specific patterns of neuronal degeneration in the brain, with the hippocampal CA1 subfield demonstrating marked vulnerability relative to adjacent regions such as CA2 and CA3 (Table 1, Fig. 1). A hallmark of this selective vulnerability is DND—a phenomenon in which CA1 pyramidal neurons initially appear morphologically intact post-ischemia but undergo apoptosis-like degeneration within 2–3 days [5,6]. This temporal lag distinguishes DND from necrotic death seen in more acutely damaged brain regions. Notably, CA1 neurons are highly sensitive to brief global ischemia, while CA2 neurons exhibit partial resistance, and CA3 neurons are relatively spared. This gradient of susceptibility has traditionally been ascribed to intrinsic neuronal characteristics such as NMDA receptor density, calcium-buffering capacity, and mitochondrial resilience [7,17]. However, accumulating evidence implicates astrocytic dysfunction—particularly at the level of AEF—as a critical extrinsic factor that exacerbates or mitigates DND. Structural alterations of AEF, mislocalization of ion–water channels (e.g., AQP4 and Kir4.1), and impaired metabolic coupling to vasculature have all been linked to CA1 neuronal demise following I/R insult [16,18,19].

Table 1: Region-specific patterns of delayed neuronal death (DND) across animal models and humans

Species/Model	Ischemia induction method	CA1 DND pattern	CA3/Other regions	Reference
Gerbil	5-min BCCAO (due to incomplete Circle of Willis)	Consistent CA1 neuronal death	CA3 spared	[5,20]
Rat	4-VO or cardiac arrest-resuscitation; 10–15 min	CA1-selective delayed death	Relative CA3 resistance	[21–23]
Mouse	BCCAO ± hypotension; genetic variants used	Reproducible CA1 loss	CA3 relatively preserved	[24,25]
Human	Postmortem after cardiac arrest or hypoxic-ischemic encephalopathy	CA1 most affected	CA3 often preserved	[26,27]

Note: Abbreviation: DND, delayed neuronal death; CA, cornu ammonis; BCCAO, bilateral common carotid artery occlusion; 4-VO, four-vessel occlusion.

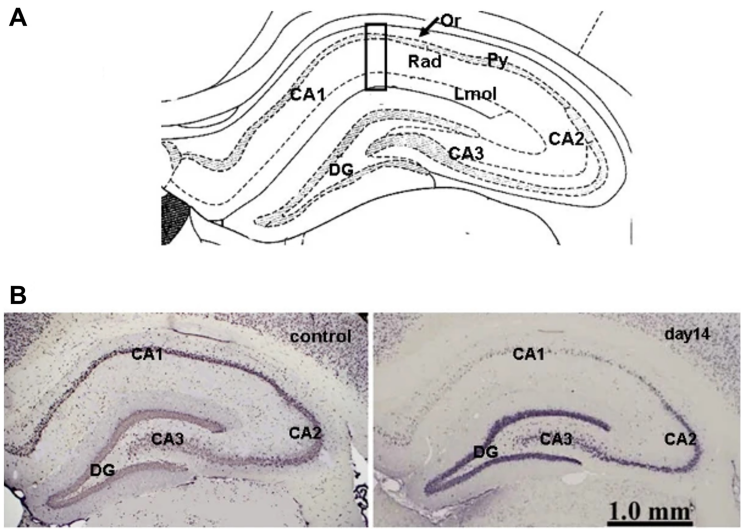

Figure 1: Selective delayed neuronal death in hippocampal subfields after transient global ischemia. (**A**) Schematic of hippocampal subregions. (**B**) Representative NeuN-stained sections show selective neuronal loss in CA1 but preservation of CA2 and CA3 neurons at 14 days post-ischemia. Abbreviations: CA, cornu ammonis; DG, dentate gyrus; Lmol, stratum lacunosum-moleculare; Or, stratum oriens layer; Py, stratum pyramidale; Rad, stratum radiatum. Adapted from [28]. Licensed under CC BY 2.0

2.1 Experimental Evidence of Region-Specific DND

Animal models robustly demonstrate region-specific DND patterns (Table 1). In gerbils—characterized by an incomplete circle of Willis—bilateral common carotid artery occlusion (BCCAO) for as little as 5 min reproducibly induces CA1-selective neuronal death without the need for systemic hypotension [5,16]. In contrast, rats require four-vessel occlusion (4-VO) or cardiac arrest-resuscitation paradigms, with ischemic

durations of 10–15 min, to elicit comparable damage [21–23]. Mouse models have evolved to use transient bilateral carotid occlusion with or without hypotension to reliably induce CA1 DND [24,25]. Histological analyses across species consistently reveal profound CA1 neuronal loss with relative CA3 sparing, reinforcing the conserved nature of this spatial vulnerability. In human postmortem studies following cardiac arrest or hypoxic-ischemic encephalopathy, similar patterns of selective CA1 loss—particularly in Sommer's sector—have been documented. These findings correlate with clinical features such as anterograde amnesia or persistent vegetative states, lending translational validity to experimental models [26,27].

2.2 Influence of Experimental Conditions on DND

The emergence and severity of DND are not solely dictated by hippocampal anatomy but are profoundly influenced by experimental and physiological variables. Ischemic duration is a principal factor: in gerbils, 5-min BCCAO induces classic CA1 DND, whereas longer durations (>10 min) expand injury to the CA3 and dentate gyrus, often accompanied by necrotic features [29,30]. Thermal regulation is another critical determinant. Hyperthermia during or after ischemia markedly exacerbates neuronal damage, while post-ischemic hypothermia (32°C–34°C) significantly attenuates both neuronal death and glial activation by reducing metabolic and excitotoxic stress [31–35]. Age-related differences also shape DND outcomes. Young adult rodents exhibit prominent CA1 degeneration, whereas aged animals often show delayed or attenuated neuronal death, potentially due to reduced astrocytic support, altered inflammatory profiles, and compromised mitochondrial function [36–40]. This age-dependent variation bears clinical importance, as elderly stroke patients exhibit distinct recovery patterns and may require age-specific therapeutic approaches.

In summary, DND in the hippocampus reflects a complex interplay between intrinsic neuronal factors and extrinsic modulators, including glial dysfunction and environmental stressors. A multifactorial framework is thus essential to accurately interpret hippocampal vulnerability and guide therapeutic strategies post-I/R injury.

3 AEF: Structure, Composition, and Role in the NVU

AEF are specialized terminal expansions of astrocytic processes that ensheath cerebral microvessels, forming a critical interface between the brain parenchyma and the vascular system [9]. These perivascular structures are integral components of the NVU, which coordinates dynamic interactions among astrocytes, endothelial cells, pericytes, and neurons to maintain cerebral homeostasis and blood flow regulation [41,42]. AEF are enriched with mitochondria and endoplasmic reticulum, allowing for localized protein synthesis, calcium signaling, and metabolic exchange at the gliovascular interface [43,44]. These subcellular organelles support highly compartmentalized calcium dynamics, which are essential for neurovascular coupling—a process often disrupted under pathological conditions such as I/R injury [45,46].

A hallmark of AEF is their AQP4, a water channel primarily localized to the perivascular membrane. AQP4 mediates rapid water transport across the BBB and is critical for brain volume regulation, potassium buffering, and glymphatic clearance [14,47]. Ischemic insults often cause loss of AQP4 polarity, impairing perivascular water clearance and contributing to vasogenic edema [48,49]. Another key AEF component is Kir4.1, an inward-rectifying potassium channel that acts synergistically with AQP4 to maintain extracellular potassium homeostasis during neuronal activity. Kir4.1 dysfunction impairs potassium buffering, leading to extracellular K^+ accumulation and increased neuronal excitability [50,51], particularly exacerbated during I/R injury [52–55]. The synergistic interaction between AQP4 and Kir4.1 at AEF enables coupled water and potassium fluxes, ensuring rapid K^+ clearance and osmotic balance during neuronal activity [56]. I/R injury disrupts this interaction, leading to uncoupled water-ion transport, astrocytic swelling, and neuronal hyperexcitability. Sustained AQP4 mislocalization and Kir4.1 downregulation further compromise

glymphatic clearance and exacerbate secondary injury following I/R insult. AEF also contains connexin 43, forming astrocyte–astrocyte gap junctions that allow for intercellular ion and metabolite exchange [57,58]. During ischemic stroke, connexin 43 undergoes phosphorylation-dependent internalization, leading to the loss of protective astrocyte–astrocyte coupling [57]. Concurrently, aberrant opening of connexin 43 hemichannels promotes the release of ATP and glutamate, amplifying neuroinflammation and neuronal injury [57].

Collectively, these molecular components confer AEF with critical roles in BBB maintenance, neurovascular signaling, ion and water homeostasis, and metabolic regulation [14,59,60]. However, their integrity is highly susceptible to I/R-induced disruption, including protein mislocalization, cytoskeletal breakdown, or detachment from the vascular basal lamina. These alterations destabilize NVU function and contribute to region-specific neuronal injury, particularly in the CA1 hippocampus [15,16]. Thus, the molecular architecture and polarization of AEF are indispensable for maintaining CNS homeostasis under both physiological and ischemic conditions. Fig. 2 provides a schematic overview of the NVU, highlighting AEF components. The next section explores how AEF responds to I/R differ across hippocampal subfields, shedding light on their role in selective neuronal vulnerability.

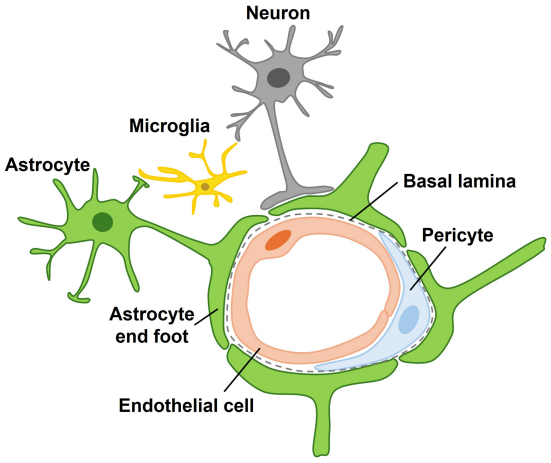

Figure 2: Schematic representation of astrocyte–neuron–vessel interactions. The neurovascular unit shows an astrocyte (green) contacting both a pyramidal neuron (gray) and a blood vessel (red) through its end foot. The end foot maintains blood–brain barrier integrity, regulates ionic and metabolic homeostasis, and modulates vascular tone

4 Region-Specific Alterations of AEF in the Hippocampus after I/R Injury

The hippocampus exhibits regional heterogeneity in its vulnerability to I/R injury, with pyramidal neurons in the CA1 subfield being markedly more susceptible to delayed neuronal death than those in CA2 or CA3 [5,7,35,61]. This differential vulnerability is closely mirrored by AEF pathology, which varies significantly across hippocampal subfields [15,16]. Following I/R insult, AEF in the CA1 region undergoes distinct pathological alterations, including pronounced swelling and detachment from the vascular basal lamina. These changes are strongly associated with BBB disruption and impaired neurovascular coupling [16,48,62]. In contrast, CA3 astrocytes tend to preserve AEF structure and maintain their perivascular association, suggesting greater regional resilience and more effective ionic/osmotic regulation [54,63,64]. These differences are underpinned by region-specific expression and localization of key proteins. In CA1, ischemic insults lead to depolarization and mislocalization of AQP4 and Kir4.1, disrupting water clearance and potassium buffering [49,52,55,65]. In contrast, CA3 astrocytes typically retain AQP4 polarization and Kir4.1 function, facilitating more effective homeostatic regulation [66]. The polarized distribution of AEF proteins, including

AQP4 and Kir4.1, is regulated and stabilized by the dystrophin-associated protein complex (DAPC), especially α-syntrophin and dystrophin, which anchor AQP4 to the perivascular membrane [48,67]. Loss of DAPC integrity—more pronounced in CA1 than in CA3—leads to AQP4 displacement and impaired glymphatic and ionic regulation [62,68–71].

Collectively, these regional disparities in AEF structure and molecular architecture provide a mechanistic basis for the selective vulnerability of CA1 neurons to I/R injury. AEF damage precedes neuronal death and contributes to it through impaired BBB integrity, disrupted neurovascular signaling, and ionic imbalance. Table 2 provides a comparative overview of AEF and neuronal changes across CA1, CA2, and CA3 subfields, integrating experimental data to emphasize the broader heterogeneity of hippocampal responses to I/R stress. The next section explores how these AEF alterations temporally relate to pyramidal neuron degeneration and highlights the astrocyte–neuron interactions that shape regional vulnerability.

Table 2: Regional comparison of astrocyte endfeet (AEF) alterations and neuronal vulnerability across hippocampal subfields following I/R injury

Region	AEF response	Neuronal outcome	Experimental model	References
CA1	AEF swelling, polarity loss, mislocalization of AQP4 and Kir4.1, detachment from basal lamina, severe gliosis	Delayed neuronal death, high vulnerability to I/R injury	Gerbil (5-min BCCAO), Mouse (BCCAO ± hypotension), Rat (4-VO, cardiac arrest)	[5,35,49,52]
CA2	Moderate AEF alterations, partial polarity loss, mild gliosis	Relative resistance to neuronal death	Gerbil (hyperthermia + ischemia)	[35,61]
CA3	Preserved AEF structure, stable AQP4/Kir4.1 expression, minimal gliosis	Low vulnerability, high survival after I/R injury	Gerbil, Mouse (transient ischemia models)	[54,63,64]

Note: Abbreviation: AEF, astrocyte endfeet; I/R, ischemia-reperfusion; AQP4, aquaporin-4.

5 Temporal Association between AEF Dysfunction and Pyramidal Neuron Death

The hippocampal CA1 subfield exhibits DND, typically emerging 2–4 days after cerebral I/R injury, in contrast to the relative preservation of CA2 and CA3 neurons [5,7]. This temporal delay strongly implicates post-ischemic secondary mechanisms—rather than immediate necrosis—as central drivers of neuronal demise [6,72]. Among these, astrocyte dysfunction—particularly at the level of AEF—has been increasingly recognized as a pivotal initiator of CA1 vulnerability. Histopathological and ultrastructural analyses consistently demonstrate that AEF swelling and separation from the vascular basal lamina occur within hours after reperfusion—preceding overt neuronal degeneration [16,48,73,74].

These early astroglial alterations compromise the BBB and impair critical homeostatic functions, including water and potassium buffering. Key molecular components, such as AQP4 and inward rectifying potassium channel Kir4.1, become mislocalized or downregulated in CA1 astrocytes following I/R injury, disrupting potassium clearance and astrocytic membrane potential [52,54,55]. This ionic dysregulation increases neuronal excitability and potentiates glutamate-mediated excitotoxicity, particularly under conditions of astrocyte depolarization. Beyond ionic imbalance, AEF detachment also disrupts astrocyte–neuron metabolic coupling. AEF are critical sites for monocarboxylate transporter (MCT)-mediated lactate and

glucose transfer—essential energy substrates during metabolic stress [43,63]. Loss of this coupling deprives neurons of metabolic support, exacerbating mitochondrial failure and energy depletion. Furthermore, astrocytic glutamate transport via excitatory amino acid transporters (EAATs)—especially EAAT2 (glutamate transporter 1, GLT-1)—is often reduced or mislocalized in I/R conditions, impairing extracellular glutamate clearance and further amplifying excitotoxic signaling [75–77]. AEF disruption also initiates astrocyte-derived proinflammatory responses, including upregulation of interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase (iNOS). These mediators activate pro-apoptotic pathways in neighboring neurons, contributing to progressive neuronal degeneration [78,79].

In summary, early AEF dysfunction precedes and predicts delayed pyramidal neuron death in the CA1 region by initiating a cascade of secondary insults—including ionic dysregulation, excitotoxicity, metabolic uncoupling, and neuroinflammation. These processes act synergistically to promote irreversible neuronal injury. Fig. 3 provides a schematic representation of these temporally ordered events, linking AEF pathology to subsequent neuronal loss.

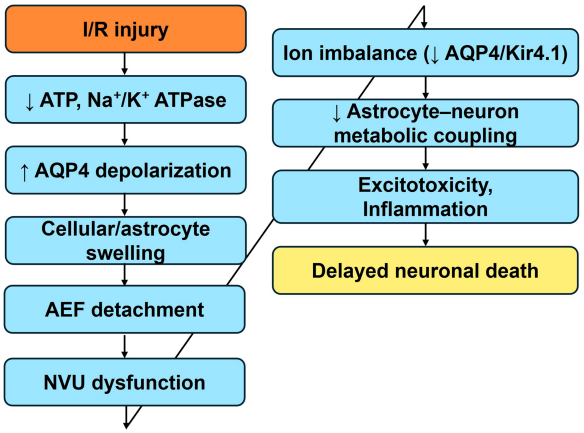

Figure 3: Sequential astrocyte endfeet (AEF) mechanisms contributing to delayed neuronal death in CA1 after I/R injury. AEF dysfunction following I/R injury leads to a cascade of interrelated pathological events that culminate in CA1 pyramidal neuron death. Early events include depletion of ATP and inhibition of Na^+/K^+-ATPase activity, resulting in AQP4 depolarization and astrocytic swelling. Swollen AEF detach from the vascular basal lamina, contributing to NVU disruption. Concurrently, mislocalization or downregulation of AQP4 and Kir4.1 channels impairs potassium buffering and water homeostasis, promoting neuronal hyperexcitability. In parallel, AEF detachment disrupts astrocyte-neuron metabolic coupling via monocarboxylate transporters, reducing the availability of lactate and glucose to neurons. Downregulation of glutamate transporters exacerbates excitotoxicity. Additionally, upregulation of inflammatory mediators amplifies neuronal damage through oxidative stress and apoptotic signaling. Collectively, these mechanisms temporally and functionally link AEF disruption to the selective vulnerability of CA1 neurons. Abbreviations: I/R, ischemia-reperfusion; ATP, adenosine triphosphate; AQP4, aquaporin-4; AEF, astrocyte endfeet; NVU, neurovascular unit

6 Molecular Pathways Linking AEF Dysfunction to Neuronal Vulnerability

AEF dysfunction in the hippocampal CA1 region following I/R injury initiates a cascade of interrelated molecular events that amplify neuronal vulnerability and degeneration. Among the most prominent mechanisms are the activation of mitogen-activated protein kinases (MAPKs), including c-Jun N-terminal kinase (JNK) and p38, which are rapidly upregulated in reactive astrocytes and injured neurons. These kinases mediate inflammatory gene transcription, apoptotic signaling, and cytoskeletal destabilization, thereby exacerbating CA1 neuronal loss [19,80].

In addition to MAPK cascades, the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway also plays a central role in astrocytic and neuronal responses following I/R injury [81,82]. Activation of the PI3K/AKT axis in reactive astrocytes regulates cell survival, redox homeostasis, and inflammatory signaling by activating downstream effectors such as mechanistic target of rapamycin (mTOR), glycogen synthase kinase-3β (GSK-3 β), and nuclear factor erythroid 2-related factor 2 (Nrf2) [83]. Conversely, excessive or insufficient activation of PI3K/AKT signaling exacerbates oxidative stress, mitochondrial dysfunction, and cytoskeletal instability within AEF, thereby amplifying the cascade that culminates in CA1 neuronal degeneration [83].

Another key pathological mediator is oxidative stress, which intensifies during reperfusion. Reactive oxygen species (ROS), primarily generated through mitochondrial dysfunction and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, accumulate within astrocytes and adjacent neurons. ROS induce lipid peroxidation, protein oxidation, and DNA fragmentation, leading to activation of nuclear factor-kappa B (NF-κB) and p53-dependent death pathways [75,84,85]. Furthermore, oxidative stress down-regulates and mislocalizes the EAAT2 (GLT-1), thereby intensifying extracellular glutamate accumulation and glutamate-induced excitotoxicity [75,77].

Nitric oxide (NO), synthesized by iNOS in astrocytes and microglia, contributes to CA1 damage through the formation of peroxynitrite, a potent oxidant formed by the reaction of NO with superoxide. Peroxynitrite disrupts mitochondrial integrity, promotes protein nitration, and triggers apoptotic cascades [86]. Notably, pharmacological inhibition of iNOS mitigates neuronal death in the CA1 region, highlighting a functional link between astrocytic iNOS upregulation and pyramidal neuron injury [87,88].

Inflammatory signaling further amplifies the impact of AEF dysfunction. Disruption of the gliovascular unit promotes astrocytic release of IL-1β, TNF-α, and monocyte chemoattractant protein-1 (MCP-1). These cytokines not only activate resident microglia but also facilitate the recruitment of peripheral immune cells such as monocytes, neutrophils, and T lymphocytes [79,89]. This astrocyte–neuroimmune crosstalk exacerbates BBB leakage and initiates cycles of neuroinflammation and apoptosis, particularly in CA1 neurons [90].

Collectively, these pathways illustrate how AEF dysfunction acts as a central trigger of oxidative imbalance, neurotransmitter dysregulation, mitochondrial impairment, and neuroinflammatory amplification. These intertwined molecular events converge to drive the selective vulnerability of CA1 pyramidal neurons following I/R injury. The representative molecular mechanisms and their functional consequences are summarized in Table 3.

Table 3: Key molecular pathways linking AEF dysfunction to CA1 pyramidal neuron death

Pathological feature	Astrocytic mechanism	Neuronal consequence	Experimental model	Reference
AQP4 mislocalization	Impaired water clearance and perivascular polarity	Interstitial fluid accumulation, vasogenic edema	Mouse (α-syntrophin KO)	[48]
Kir4.1 downregulation	Impaired K^+ buffering, astrocyte depolarization	Neuronal hyperexcitability	Mouse (Kir4.1 conditional KO)	[52]
EAAT2 reduction	↓ Glutamate uptake via astrocytes	Glutamate excitotoxicity	Mouse (GLT-1 KO)	[76]

(Continued)

Table 3 (continued)

Pathological feature	Astrocytic mechanism	Neuronal consequence	Experimental model	Reference
MCT disconnection (AEF-capillary)	↓ Lactate/glucose delivery to neurons	Energetic failure and metabolic distress	Mouse (wild-type; astrocyte studies)	[63]
Cytokine release (IL-1β, TNF-α)	Inflammatory astrocyte–neuron signaling	Apoptotic and necroinflammatory pathways	Mouse (I/R injury model)	[79]

Note: Abbreviation: KO, knockout; EAAT2, excitatory amino acid transporters; GLT-1, glutamate transporter 1; MCT, monocarboxylate transporter; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-alpha.

7 Therapeutic Implications and Future Directions

Understanding the pivotal role of AEF dysfunction in hippocampal CA1 vulnerability provides a strong rationale for targeting astrocyte-based mechanisms in I/R injury. As summarized in Fig. 4, therapeutic strategies that directly modulate astrocytic pathways—rather than focusing solely on neuronal survival—may yield more robust and sustained neuroprotection.

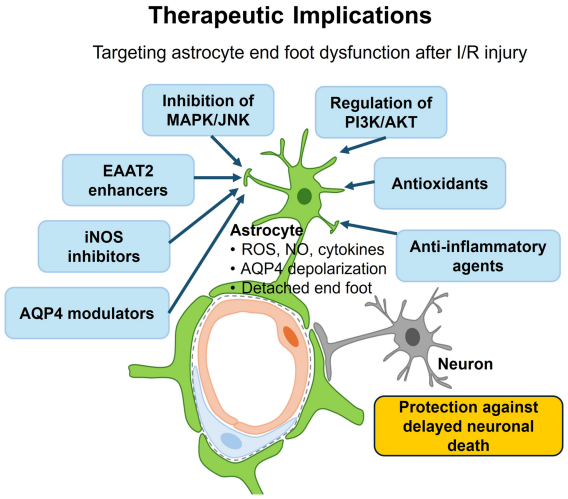

Figure 4: Therapeutic implications of targeting astrocyte end foot dysfunction after ischemia-reperfusion (I/R) injury. This schematic illustrates potential therapeutic strategies aimed at mitigating astrocyte-mediated pathological cascades that contribute to delayed neuronal death in the hippocampal CA1 region following I/R injury. Detachment of perivascular astrocyte endfeet impairs ion and water homeostasis, leading to excessive production of ROS, NO, and proinflammatory cytokines. These processes ultimately promote neuroinflammation and excitotoxicity. Interventions include: inhibition of MAPKs, particularly JNK; regulation of PI3K/AKT; antioxidants to suppress oxidative stress; iNOS inhibitors to limit NO toxicity; EAAT2 enhancers to restore glutamate clearance; anti-inflammatory agents targeting IL-1β, TNF-α, or MCP-1 signaling; and AQP4 modulators to restore AQP4 polarity. Collectively, these approaches aim to protect against astrocyte-driven injury mechanisms and prevent delayed neuronal death. Abbreviations: ROS, reactive oxygen species; NO, nitric oxide; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B; iNOS, inducible nitric oxide synthase; EAAT2, excitatory amino acid transporter 2; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1

Pharmacological inhibition of MAPKs, particularly JNK and p38, has demonstrated efficacy in reducing astrocyte reactivity and delaying neuronal apoptosis in various experimental I/R models [91,92]. Notably, D-JNKI1, a peptide JNK inhibitor, exerts prolonged neuroprotective effects by dampening pro-apoptotic signaling in both neurons and reactive astrocytes. Pharmacological activation of the PI3K/AKT pathway in astrocytes confers neuroprotection by enhancing connexin 43 phosphorylation, maintaining BBB integrity, and suppressing oxidative stress and apoptosis after I/R injury. Vinpocetine activates PI3K/AKT signaling to protect astrocytes and reduce infarct volume [93], while tetramethylpyrazine (TMP) targets the endothelin-1/AKT pathway to stabilize the astrocyte–endothelial interface and limit BBB disruption [94]. Antioxidants that scavenge astrocyte-derived ROS, such as edaravone and astaxanthin, have been shown to attenuate oxidative stress, limit secondary BBB damage, and reduce infarct volume [95–97]. Restoration of astrocytic glutamate transport function—particularly through upregulation or stabilization of EAAT2 (GLT-1)—is another promising strategy. Pharmacological agents like ceftriaxone and gene therapy approaches targeting EAAT2 have successfully reduced glutamate-induced excitotoxicity and improved neuronal viability in animal models [75,77,98]. Similarly, selective inhibition of iNOS, for example, with aminoguanidine or other small-molecule inhibitors, reduces peroxynitrite formation, preserves mitochondrial integrity, and confers indirect protection to neighboring neurons [87,99]. In addition, astrocyte-targeted anti-inflammatory interventions—such as neutralizing antibodies against IL-1β or TNF-α, or inhibition of MCP-1/C-C chemokine receptor type 2(CCR2) signaling—have shown efficacy in attenuating glial reactivity and preventing leukocyte infiltration [79,89,100]. Recent studies propose the restoration of AQP4 polarity via DAPC stabilization or pharmacological blockade using AQP4 modulators such as TGN-020, which reduces water accumulation and vasogenic edema in I/R injury [70,101,102].

Looking forward, combinatorial therapies that concurrently target multiple astrocytic dysfunctions—including oxidative stress, impaired neurotransmitter clearance, and cytokine-mediated neurotoxicity—represent a compelling next step. Novel approaches such as epigenetic modulation of astrocyte resilience or transplantation of genetically engineered glia could further enhance regional resistance to I/R injury in the hippocampus. By advancing our understanding of AEF–neuron interactions and leveraging astrocyte-specific therapeutic windows, future interventions may prevent or mitigate delayed neuronal death in ischemic conditions.

8 Conclusion

AEF plays a critical and dynamic role in maintaining neurovascular homeostasis, particularly within the hippocampal CA1 region, which is highly susceptible to I/R injury. Accumulating evidence indicates that early structural and molecular alterations of AEF—including swelling, polarity loss, mislocalization of AQP4 and Kir4.1, and detachment from the vasculature—precede and exacerbate delayed neuronal death in CA1 pyramidal neurons. These pathological changes disrupt water and ion homeostasis, compromise glutamate clearance, impair astrocyte-mediated metabolic coupling, and activate inflammatory cascades. Importantly, the selective vulnerability of CA1 pyramidal neurons appears to result not only from intrinsic neuronal properties but also from their critical reliance on tightly regulated astrocyte–neuron–vascular interactions, which are destabilized following I/R injury. Mechanistic insights discussed in this review—such as MAPK/JNK activation, PI3K/AKT dysregulation, oxidative and nitrosative stress, glutamate excitotoxicity, and cytokine-mediated neuroinflammation—highlight the multifactorial nature of AEF-mediated neurodegeneration. From a therapeutic standpoint, targeting AEF-specific dysfunction presents a promising and underexplored strategy for neuroprotection. Interventions that restore astrocytic polarity, EAAT2 expression, inhibit pro-inflammatory signaling, and mitigate ROS–driven damage may delay or prevent CA1 neuronal death. In particular, regionally tailored therapies that account for the distinct vulnerability of hippocampal astrocytes

could enhance clinical translatability. Future research should focus on integrating region-specific astrocyte biology with combinatorial therapeutic approaches and preclinical models of global ischemia, thereby advancing astrocyte-targeted interventions for ischemic brain injury. By redefining AEF not as passive structural elements but as active and modulable regulators of neuronal viability, this review emphasizes their central role in hippocampal pathology and positions them as pivotal therapeutic targets in preventing delayed neuronal death following I/R insults.

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Availability of Data and Materials: All data generated or analyzed during this study are included in this published article.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

Abbreviations

AQP4	Aquaporin-4
AEF	Astrocyte endfeet
BCCAO	Bilateral common carotid artery occlusion
BBB	Blood–brain barrier
JNK	C-Jun N-terminal kinase
CA	Cornu ammonis
CCR2	C-C chemokine receptor type 2
DND	Delayed neuronal death
DAPC	Dystrophin-associated protein complex
EAATs	Excitatory amino acid transporters
GLT-1	Glutamate transporter 1
GSK-3 β	Glycogen synthase kinase-3β
iNOS	Inducible nitric oxide synthase
IL-1β	Interleukin-1β
I/R	Ischemia-reperfusion
MAPK	Mitogen-activated protein kinase
MCT	Monocarboxylate transporter
MCP-1	Monocyte chemoattractant protein-1
mTOR	Mechanistic target of rapamycin
NVU	Neurovascular unit
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
NMDA	N-methyl-D-aspartate
NF-κB	Nuclear factor-kappa B
Nrf2	Nuclear factor erythroid 2-related factor 2
PI3K/AKT	Phosphoinositide 3-kinase/protein kinase B

ROS	Reactive oxygen species
TMP	Tetramethylpyrazine
TNF-α	Tumor necrosis factor-alpha
4-VO	Four-vessel occlusion

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