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## The back and forth of axonal injury and repair after stroke

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### Abstract

**Purpose of review**—The axon plays a central role in both the injury and repair phases after stroke. This review highlights emerging principles in the study of axonal injury in stroke and the role of the axon in neural repair after stroke.

**Recent findings**—Ischemic stroke produces a rapid and significant loss of axons in the acute phase. This early loss of axons results from a primary ischemic injury that triggers a wave of calcium signaling, activating proteolytic mechanisms and downstream signaling cascades. A second progressive phase of axonal injury occurs during the subacute period and damages axons that survive the initial ischemic insult but go on to experience a delayed axonal degeneration driven in part by changes in axoglial contact and axonal energy metabolism. Recovery from stroke is dependent on axonal sprouting and reconnection that occurs during a third degenerative/regenerative phase. Despite this central role played by the axon, comparatively little is understood about the molecular pathways that contribute to early and subacute axonal degeneration after stroke. Recent advances in axonal neurobiology and signaling suggest new targets that hold promise as potential molecular therapeutics including axonal calcium signaling, axoglial energy metabolism and cell adhesion as well as retrograde axonal mitogen-activated protein kinase pathways. These novel pathways must be modeled appropriately as the type and severity of axonal injury vary by stroke subtype.

**Summary**—Stroke-induced injury to axons occurs in three distinct phases each with a unique molecular underpinning. A wealth of new data about the molecular organization and molecular signaling within axons is available but not yet robustly applied to the study of axonal injury after stroke. Identifying the spatiotemporal patterning of molecular pathways within the axon that contribute to injury and repair may offer new therapeutic strategies for the treatment of stroke.

### Keywords

axon; injury; repair; stroke

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### Conflicts of interest

There are no conflicts of interest.

## INTRODUCTION

It has been estimated that every minute an ischemic stroke goes untreated, resulting in the loss of 7 miles of axons [1]. This staggering loss of the vital connections between neurons highlights the pivotal role axons have in the injury response and repair after stroke. Ischemic damage to axons is particularly important during the acute and subacute phases of injury worsening clinical deficits, and yet the axon also plays a crucial role in neural repair mediating recovery after stroke. This review will address recent advances in the study of axonal damage during and after stroke and highlight the importance of axonal neurobiology in repair after stroke.

## NEW INSIGHTS ON THE DYNAMIC FUNCTION OF AXONS

In recent years, the dogma regarding axons as a static unidirectional wire conducting action potentials between neurons has broken down. Cellular machinery for local protein translation resides within the axon [2], not only within the neuronal cell body. Studies of the *Wlds* mutant mouse show that disconnected axons can exist for weeks without the trophic support of the neuronal cell body [3]. Axons have nonneuronal synaptic connections with glial cells along their length [4,5]. Axonal glutamate release at these synapses drives electrical activity in oligodendrocyte precursor cells that may provide a signal for myelination [6,7,8]. Similarly, dorsal column axons have been shown to express both AMPA and kainate receptors [9] that modulate calcium signaling with axons by triggering calcium release from axonal nanocomplexes [10]. Axons are also supported metabolically via the oligodendrocyte monocarboxylate transporter-1 (MCT-1) that functions to transfer lactate from the oligodendrocyte to the axon [11,12], obviating the need to transfer all energy via axonal transport from the cell body. Thus, the axon is a highly dynamic neurobiologic structure that harbors unique molecular pathways that are relevant to stroke-related injury and neural repair after stroke.

In concert with this knowledge of the axon's unique molecular makeup, recent years have advanced our understanding of the molecular organization of myelinated axons. These axons maintain highly specialized regions along their length, termed microdomains, that cluster essential molecules needed for the different roles each segment of the axon mediates [13, 14]. Adjacent to the neuronal cell body, the axon initial segment clusters a long length of voltage-gated sodium channels ( $Na_v$ ) needed to initiate the action potential [15] as well axo-axonic synaptic proteins that help to modulate its fidelity [16]. In a similar way, myelinated axons have regional specializations along their length including the node, with a high concentration of  $Na_v$ , the adjacent paranode, which mediates cell–cell adhesion with oligodendrocytes, and the juxtaparanode with similar but unique trophic and cell–cell adhesion roles (Fig. 1). Thus, the axon is a dynamic neurobiologic structure that exists as a central part of a complex multicellular system termed the axoglial unit (Fig. 2) [17].

This knowledge of the axon and its complex neurobiology is only recently being applied to stroke. A multitude of prior studies have argued for evidence of axonal injury after stroke by demonstrating beaded neurofilament staining or accumulation of amyloid precursor protein (APP), a marker of axonal transport, near the site of injury [18–22]. These

immunohistochemical hallmarks label end-stage degenerating axons. Beaded neurofilament staining represents a failure of the highly regular distribution of axonal neurofilaments and fundamental breakdown of the axonal cytoskeleton. Amyloid precursor protein is hypothesized to be involved in axonal transport [23] and dense, focal increases in APP immunostaining within axons are noted in multiple disorders including traumatic brain injury [24,25], neurodegenerative disease [26–29], and stroke [30–32]. This staining pattern indicates a failure of axonal transport mechanisms. Although these immunohistochemical hallmarks indicate degenerating axons, they likely underestimate the degree of injury to axons after stroke and can no longer serve as sufficient evidence of the extent of axonal injury. Axons without an intact cytoskeleton or normal axonal transport mechanisms are clearly damaged and are likely not amenable to axonal rescue or repair therapies. Extending further away from the stroke core, these fundamental axonal systems may be preserved but  $\text{Na}_V$  lost at the node rendering the axons dysfunctional yet possibly amenable to recovery. To demonstrate axonal rescue, one must demonstrate preservation of the functional molecular organization of axons with normal axonal initial segment, nodal and paranodal regions, intact axonal transport functions, intact axoglial synaptic activity, and/or a functional axoglial relationship. Proving the normal molecular organization is maintained or re-established after injury greatly strengthens the argument that candidate therapeutics will have a lasting effect on functional recovery and increase the likelihood of successful translation to patients. In the following sections, this review will frame axonal injury in the context of typical clinical stroke syndromes and explore the emerging molecular principles that underlie the intriguing back and forth of axonal injury and repair after stroke.

## PHASES OF AXONAL DAMAGE IN ISCHEMIC STROKE AND ITS MAJOR SUBTYPES

Ischemic stroke is common but its clinical presentation varies substantially, resulting in wide variation in the degree of axonal injury. Large vessel occlusions often result in significant areas of brain infarction affecting large regions of cortex including their intrahemispheric and interhemispheric axonal fibers. Smaller cortical strokes, such as those caused by distal emboli, largely produce only focal neuronal loss and injure local axonal projections. Lacunar infarcts are often isolated within the subcortical white matter affecting only axons and glia or occur within regions of white matter and deep gray structures, producing a mixed neuronal and axonal injury. The study of axonal injury in stroke should be considered in the context of these clinical variations and likely occurs in three spatially and temporally related phases (Fig. 3).

A large hemispheric stroke affecting primary motor cortex serves as an excellent example to consider these phases. The stroke results in immediate ischemic injury to large areas of cortex and white matter supplied by the occluded vessel. The first phase of axonal injury is a primary ischemic one involving both the lateral intracortical and descending axonal fibers from the affected cortex that experience insufficient oxygen and metabolic support to maintain cellular membranes. The catastrophic loss of energy in the infarct core initiates a molecular cascade that reverses  $\text{Na}^+$  and  $\text{Ca}^+$  exchange across the axolemma, which in turn triggers intra-axonal  $\text{Ca}^+$  waves [33,34\*] that mediate the secondary and tertiary phases

described. Within the surrounding ischemic penumbra, an important second phase of ischemic axonal injury ensues. This progressive phase is marked by partial ischemia that may not result in complete cellular loss and necrosis. In this phase, the axon and its parent neuronal cell body are both affected. This penumbral injury to axons renders them damaged and dysfunctional. As a result, they are vulnerable to progressive axonal degeneration [35,36] as well as rescue with early and successful revascularization [37]. In the third phase of axonal injury after a large hemispheric stroke, important degenerative and regenerative events occur in parallel. The far distal portions of the disconnected axons that were not primarily damaged by ischemia may undergo anterograde Wallerian degeneration [38], while simultaneously peri-infarct cortex experiences axonal sprouting that leads to new functional connections and mediates recovery [39]. These three phases of axonal injury after stroke have different spatiotemporal patterning and unique molecular cascades underlying their progression and contribute differently to the ultimate outcome after stroke.

Now consider a focal lacunar stroke resulting from occlusion of a single penetrating arteriole, producing a limited clinical syndrome and affecting the subcortical white matter. This type of stroke accounts for 25% of all clinical presentations of stroke annually in the United States [40] and occurs silently at a rate that is substantially higher [41]. In this instance, the portion of axons experiencing the primary ischemic phase of axonal injury after stroke is limited. Surrounding this small focal ischemic core is an adjacent region of white matter penumbra experiencing partial ischemia [42,43–45]. This progressive injury phase typically spares the parent neuronal cell body localized elsewhere away from the ischemic injury, making this ischemic injury unique to the axon. The distal portion of the ischemic axons will undergo Wallerian degeneration while important retrograde signals of axonal injury are passed back to the parent neuronal cell body during the third degenerative/regenerative phase.

Thus, the progressive phase of axonal injury after stroke will vary substantially dependent on the clinical stroke subtype. In both types of stroke, the parent neuronal cell body receives retrograde axonal injury signals but in a large hemispheric stroke, the neuron is battling its own ischemic injury and that of its neighbors, whereas in a lacunar stroke, the retrograde axonal injury signals are received by a nonischemic, undamaged parent neuronal cell body. Important molecular distinctions can and should be made between these processes [34] as each may represent a unique therapeutic target after stroke (Table 1). Targeted axonal rescue therapies might have most relevance to lacunar strokes, although both axonal and neuroprotective strategies would be needed for a larger hemispheric stroke [33,46].

## THE BACK: PROGRESSIVE AXONAL DEGENERATION AFTER STROKE

In the minutes to hours after ischemic injury onset, the axon experiences energetic failure at several adenosine triphosphate-dependent ionic channels that triggers massive calcium release from intra-axonal calcium stores [47]. This ionic flux destabilizes the axolemma and activates calcium-dependent proteases, such as calpain, that chew up neurofilament proteins and other structural components [48]. Notably, neurofilament proteins can be detected in the blood within hours after acute stroke [49,50]. This immediate early phase of axonal injury has rarely been studied using animal models of stroke but rather using ex-vivo axonal and

myelinated nerve preps or other models of axonal injury [51–54]. The kinetics of measuring calcium flux and the need for high-resolution imaging make studying these aspects of axonal injury using in-vivo stroke modeling technically challenging. Despite this challenge, future translational studies can target these early molecular events using clinically relevant pre-treatment strategies and continued identification of unique axonal molecular pathways.

Following the acute primary ischemic injury, surrounding axons undergo continued local degeneration over days during an important and understudied second progressive phase of axonal injury. Morphologically, this process involves axonal swelling and the formation of retraction bulbs [55,56]. The molecular pathways that drive this degeneration after stroke are unknown, although the glial metabolic support of axons may be particularly relevant. Monocarboxylate transporters reside in the plasma membrane and transfer lactate and pyruvate across the membrane [57]. Multiple isoforms of this class of transporters exist, but in the brain, MCT-1 is predominantly expressed by oligodendrocytes [12] and is proposed as a central molecule that allows axons to circumvent having to receive all their energy from the neuronal cell body. In the absence of MCT-1, spinal motor neurons undergo a degenerative process that mimics amyotrophic lateral sclerosis [11]. Translating this paradigm to stroke-related axonal injury, as blood flow drops, oligodendrocytes and axons will be increasingly dependent on glycolytic energy metabolism. The ability of oligodendrocytes to efficiently transfer lactate to partially ischemic penumbral axons may be crucial to preventing reversal of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger [33,34], thereby reducing progressive axonal degeneration and selective neuronal loss. The maintenance of cell–cell adhesion and axoglial contact after stroke would be vital to maintaining this energy dependence. These molecular pathways seem deserving of additional study in stroke-related axonal injury.

In-vitro assays of axonal degeneration suggest that death receptor-6 (in the tumor necrosis factor superfamily of molecules) binds with APP to trigger a caspase-6-dependent degeneration [58]. While intriguing given the evidence of APP accumulation in injured axons, whether this system is applicable to ischemic axonal injury remains unclear. Recent work by Akpan *et al.* [59] demonstrated that caspase-9 activation occurs early after transient middle cerebral artery occlusion (tMCAO) triggering activation of the caspase-6-dependent axonal degeneration pathway in the ischemic penumbra. In a novel translational approach, this group used intranasal caspase-9 inhibition to block axonal degeneration after stroke and improve functional recovery. Additional data from lower organisms also support a role for the caspases in axonal degeneration [60], although their relevance to stroke deserves further study.

Few groups have demonstrated the effect of ischemia on the molecular organization of axons. Schafer *et al.* [61] showed that the axon initial segment undergoes progressive degeneration and loses its unique molecular markers in the hours after tMCAO. These authors also demonstrated that systemic calpain inhibition before stroke partially reduces the axon initial segment loss after stroke. The loss of the axon initial segment after stroke observed in this study may be a feature of ischemia-induced neuronal apoptosis as the authors focused their analysis on cells in the ischemic core, rather than the surviving penumbra. Mild global reduction in cerebral blood flow by carotid artery coiling produces a

rapid elongation of nodes and paranodes [62]. This elongation is hypothesized to slow axonal conduction velocity by approximately 65% [63]. We have recently shown that axons adjacent to human lacunar infarcts demonstrate profound disorganization of their nodal and paranodal domains (including elongation) despite maintaining their fundamental organization as measured by the presence of myelin and neurofilaments (J.D. Hinman, M.D. Lee, S. Tung, H.V. Vintners and S.T. Carmichael, unpublished observation). Together these studies demonstrate that the molecular organization of the axon at key points of axonal function (the node and paranode) is vulnerable to ischemia and future studies on axonal injury after stroke should include analysis of axonal microdomains and related molecular systems.

## BACK AND FORTH: CANDIDATE AXONAL MOLECULAR PATHWAYS IN STROKE

As axonal injury and repair factors centrally in many neurologic disorders, considerable advances have been made in understanding the molecular events that occur within axons to indicate injury, degrade disconnected segments and initiate axonal repair. Delaying or limiting anterograde axonal degeneration may be beneficial in limiting the degree of injury in a partially ischemic penumbral field after stroke. Anterograde Wallerian degeneration of disconnected axons is now a well described molecular process that appears to be mediated at least in part by nicotinamide mononucleotide adenylyltransferase 1 (NMNAT1). Occurring spontaneously in a breeding colony of C57/Bl6 mice, an 85 kb tandem triplication on chromosome 4 resulted in slow Wallerian degeneration after nerve injury [64]. This mutation results in a gene encoding a fusion protein linking NMNAT1 with the ubiquitination factor e4b (*Ube4b*). Wallerian degeneration is delayed by at least 3 weeks after peripheral nerve injury [3]. This mutated fusion protein generates excess NMNAT1 protein that drives an increase in nicotinamide adenine dinucleotide (NAD<sup>+</sup>) synthesis protecting the axon from energy deprivation once disconnected from the neuronal cell body [65], although recent work indicates that the exact mechanism for delayed axonal degeneration remains unclear [66]. Administration of one of the precursors of NAD<sup>+</sup>, nicotinamide, at the time of or after peripheral nerve injury resulted in a decrease in degenerating axons [65]. To date, no studies have examined the effect of delayed Wallerian degeneration on limiting acute injury or enhancing repair after stroke. This pathway could be considered as a viable molecular target for focal lacunar stroke administered as a rescue therapy or provided prophylactically for patients with cumulative white matter microvascular infarcts and ongoing axonal ischemic injury.

Stroke within the white matter causes a focal axonal injury that needs to be communicated back to the neuronal cell body. Recent insights on the retrograde axonal injury signals come from studies of the retina and axotomy in lower organisms, such as nematodes and *Drosophila*, as well as in mammalian peripheral nerve. A series of studies from peripheral nerve and the optic nerve indicate that the mitogen-activated protein (MAP) kinase system is vital to retrograde signaling of axonal injury that is capable of shifting the neuron back to its developmental programme initiating axonal outgrowth and reconnection [67\*]. Briefly, dual leucine zipper kinase (DLK) acts as a local injury signal that increases within the optic nerve

after crush injury [68<sup>\*\*\*</sup>]. DLK locally phosphorylates STAT3 within the axon and also results in JNK3 activation. Together this complex migrates back to the nucleus wherein these molecules drive c-Jun expression [34<sup>\*</sup>,69]. In the absence of phosphatase and tensin homolog (PTEN) expression in the neuronal cell body, an axonal outgrowth programme is initiated [68<sup>\*\*\*</sup>]. If DLK is blocked, then axotomy does not trigger axonal outgrowth [70–72]. The role of these systems in axonal injury and repair after stroke has not been studied. If DLK activation occurs after ischemic axonal injury in the brain as in the retina and peripheral nerve, then shifting the stroke-injured neuronal cell body towards an axonal outgrowth programme by modulating downstream MAP kinase pathways holds promise from neural repair after certain types of stroke.

## THE FORTH: THE AXON IN NEURAL REPAIR AFTER STROKE

Recovery and neural repair after stroke is ultimately mediated by the development of new axonal connections between disconnected brain regions. Ischemic stroke results in limited axonal sprouting in peri-infarct cortex that can be promoted by the inhibition of myelin-associated growth inhibitors, ephrins, extracellular matrix proteins or an increase in activity-induced plasticity [73<sup>\*</sup>]. Although much of this axonal sprouting occurs in surrounding cortex, some occurs as far away as the contralateral cortex and in the case of motor fibers, also projects to the contralateral spinal cord [74<sup>\*\*\*</sup>]. Most of these studies determined that their progrowth axonal sprouting therapies improved behavioral outcomes after stroke implying functional axons but not demonstrating functional organization of sprouted axons with proper molecular organization of axon initial segments, nodes, paranodes and myelination. To this end, within peri-infarct cortex wherein post-stroke axonal sprouting is robust, the number of axon initial segments increases in upper cortical layers and supernumerary axon initial segments within individual neurons can be demonstrated (Fig. 4) [75<sup>\*\*\*</sup>]. Increased numbers of axon initial segments in peri-infarct cortex suggest that post-stroke sprouted axons eventually undergo maturation as axonal connections are strengthened; an effect that could be promoted by behavioral or molecular therapy. The finding of a secondary axon initial segment arising from a single cell (supernumerary axon initial segment, Fig. 4) indicates that the stroke-injured neuron is capable of fundamentally altering its cell biology and excitability profile to support recovery. These data show that post-stroke axonal sprouting is not a black box and that its molecular correlates can be demonstrated. Combining neuroanatomical tracing techniques with transgenic and lentiviral cellular labeling techniques could be used to demonstrate the degree to which axonal organization and myelination occur in post-stroke sprouted axons. With the knowledge that multiple temporally spaced molecular and behavioral approaches can augment post-stroke axonal sprouting, promoting myelination of sprouted axons after stroke may represent a unique combinatorial translational approach to neural repair after stroke (Table 1).

## CONCLUSION

Damage to axons occurs in several important phases after stroke that likely vary dependent on the clinical subtype of stroke: a primary ischemic injury, a secondary progressive injury, and third degenerative/regenerative phase. Each phase offers unique therapeutic opportunities for axonal rescue that can be realized with an improved approach to the study

of the axon during and after stroke. Recent advances in axonal neurobiology indicate an elegant molecular organization of axons that dictates function and involves important intercellular molecular pathways that should be the standard for evaluating axonal injury after stroke. Work in other model systems suggests several important molecular systems that may play important roles in axonal degeneration and regeneration and are deserving of study in the appropriate stroke model.

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## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Saver JL. Time is brain: quantified. *Stroke*. 2006; 37:263–266. [PubMed: 16339467]
  - 2▪. Holt CE, Schuman EM. The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron*. 2013; 80:648–657. This is an insightful review of local translational events in the axon and dendrite and how they impact plasticity. [PubMed: 24183017]
  3. Mack TG, et al. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmнат chimeric gene. *Nat Neurosci*. 2001; 4:1199–1206. [PubMed: 11770485]
  4. Kukley M, Capetillo-Zarate E, Dietrich D. Vesicular glutamate release from axons in white matter. *Nat Neurosci*. 2007; 10:311–320. [PubMed: 17293860]
  5. Ziskin JL, et al. Vesicular release of glutamate from unmyelinated axons in white matter. *Nat Neurosci*. 2007; 10:321–330. [PubMed: 17293857]
  6. Kukley M, Nishiyama A, Dietrich D. The fate of synaptic input to NG2 glial cells: neurons specifically downregulate transmitter release onto differentiating oligodendroglial cells. *J Neurosci*. 2010; 30:8320–8331. [PubMed: 20554883]
  - 7▪. Malone M, et al. Neuronal activity promotes myelination via a cAMP pathway. *Glia*. 2013; 61:843–854. This is a review of the proposed mechanisms of white matter injury in stroke and possible strategies to protect the white matter from injury. [PubMed: 23554117]
  8. Wake H, Lee PR, Fields RD. Control of local protein synthesis and initial events in myelination by action potentials. *Science*. 2011; 333:1647–1651. [PubMed: 21817014]
  9. Ouardouz M, et al. Glutamate receptors on myelinated spinal cord axons: II. AMPA and GluR5 receptors. *Ann Neurol*. 2009; 65:160–166. [PubMed: 19224531]
  10. Stys PK. The axomyelinic synapse. *Trends Neurosci*. 2011; 34:393–400. [PubMed: 21741098]
  11. Lee Y, et al. Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature*. 2012; 487:443–448. [PubMed: 22801498]
  12. Rinholm JE, et al. Regulation of oligodendrocyte development and myelination by glucose and lactate. *J Neurosci*. 2011; 31:538–548. [PubMed: 21228163]
  - 13▪. Yoshimura T, Rasband MN. Axon initial segments: diverse and dynamic neuronal compartments. *Curr Opin Neurobiol*. 2014; 27C:96–102. This is an excellent review of the recent advances in the understanding of the molecular organization and function of the axon initial segment. [PubMed: 24705243]
  14. Rasband MN. Composition, assembly, and maintenance of excitable membrane domains in myelinated axons. *Semin Cell Dev Biol*. 2011; 22:178–184. [PubMed: 20932927]



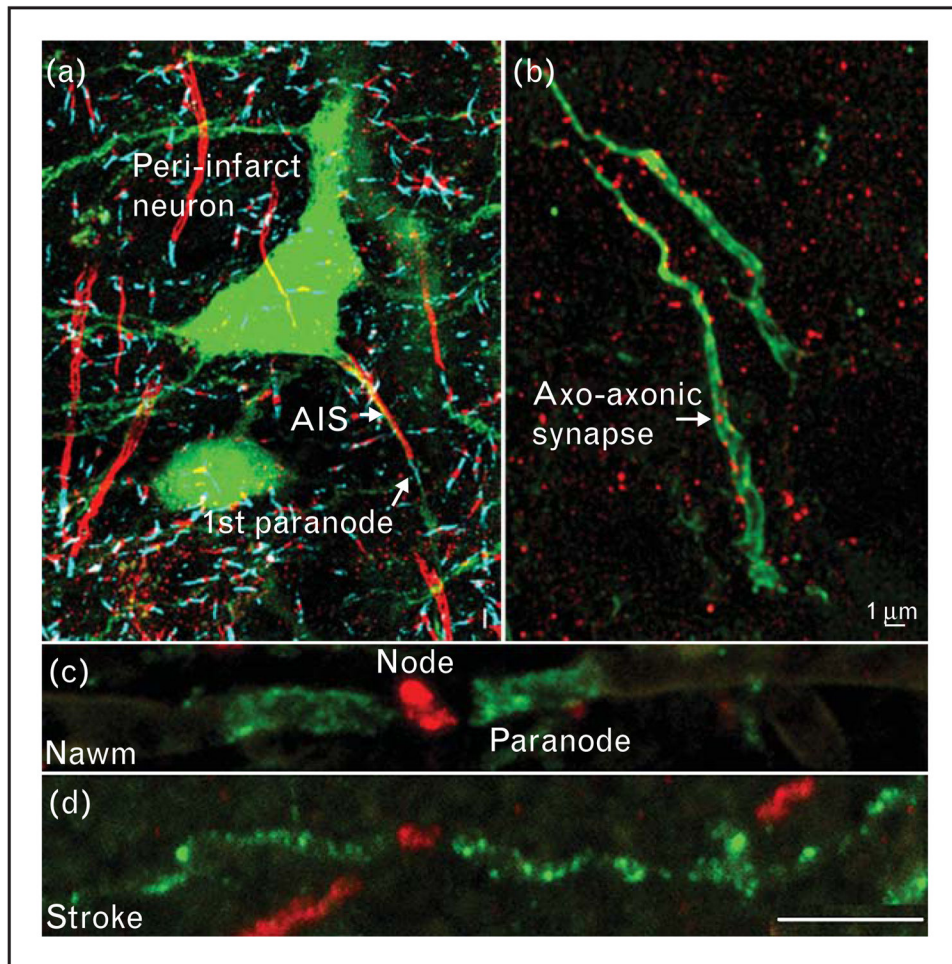
15. Rasband MN. The axon initial segment and the maintenance of neuronal polarity. *Nat Rev Neurosci.* 2010; 11:552–562. [PubMed: 20631711]
16. Howard A, Tamas G, Soltesz I. Lighting the chandelier: new vistas for axo-axonic cells. *Trends Neurosci.* 2005; 28:310–316. [PubMed: 15927687]
17. Hinman, JD.; Carmichael, ST. Acute axonal injury in white matter stroke. In: Baltan, S., et al., editors. *White Matter Injury in Stroke and CNS Disease*. New York: Springer; 2013. p. 521-535.
18. Schroeder E, et al. Neurofilament expression in the rat brain after cerebral infarction: effect of age. *Neurobiol Aging.* 2003; 24:135–145. [PubMed: 12493559]
19. Gresle MM, et al. Injury to axons and oligodendrocytes following endothelin-1-induced middle cerebral artery occlusion in conscious rats. *Brain Res.* 2006; 1110:13–22. [PubMed: 16905121]
20. Kurumatani T, et al. White matter changes in the gerbil brain under chronic cerebral hypoperfusion. *Stroke.* 1998; 29:1058–1062. [PubMed: 9596257]
21. Chu M, et al. Focal cerebral ischemia activates neurovascular restorative dynamics in mouse brain. *Front Biosci (Elite Ed).* 2012; 4:1926–1936. [PubMed: 22202008]
22. Irving EA, Bentley DL, Parsons AA. Assessment of white matter injury following prolonged focal cerebral ischaemia in the rat. *Acta Neuropathol.* 2001; 102:627–635. [PubMed: 11761724]
23. Cottrell BA, et al. A pilot proteomic study of amyloid precursor interactors in Alzheimer's disease. *Ann Neurol.* 2005; 58:277–289. [PubMed: 16049941]
24. Lewen A, et al. Traumatic brain injury in rat produces changes of beta-amyloid precursor protein immunoreactivity. *Neuroreport.* 1995; 6:357–360. [PubMed: 7756628]
25. Sherriff FE, Bridges LR, Sivaloganathan S. Early detection of axonal injury after human head trauma using immunocytochemistry for beta-amyloid precursor protein. *Acta Neuropathol.* 1994; 87:55–62. [PubMed: 8140894]
26. Ohgami T, Kitamoto T, Tateishi J. Alzheimer's amyloid precursor protein accumulates within axonal swellings in human brain lesions. *Neurosci Lett.* 1992; 136:75–78. [PubMed: 1635670]
27. Ichihara N, et al. Axonal degeneration promotes abnormal accumulation of amyloid beta-protein in ascending gracile tract of gracile axonal dystrophy (GAD) mouse. *Brain Res.* 1995; 695:173–178. [PubMed: 8556328]
28. Wirths O, et al. Axonopathy in an APP/PS1 transgenic mouse model of Alzheimer's disease. *Acta Neuropathol.* 2006; 111:312–319. [PubMed: 16520967]
29. Sasaki S, Iwata M. Immunoreactivity of beta-amyloid precursor protein in amyotrophic lateral sclerosis. *Acta Neuropathol.* 1999; 97:463–468. [PubMed: 10334483]
30. Stephenson DT, Rash K, Clemens JA. Amyloid precursor protein accumulates in regions of neurodegeneration following focal cerebral ischemia in the rat. *Brain Res.* 1992; 593:128–135. [PubMed: 1458315]
31. Nukina N, et al. Accumulation of amyloid precursor protein and beta-protein immunoreactivities in axons injured by cerebral infarct. *Gerontology.* 1992; 38 (Suppl 1):10–14. [PubMed: 1459467]
32. Akguchi I, et al. Alterations in glia and axons in the brains of Binswanger's disease patients. *Stroke.* 1997; 28:1423–1429. [PubMed: 9227695]
33. Matute C, et al. Protecting white matter from stroke injury. *Stroke.* 2013; 44:1204–1211. [PubMed: 23212168]
34. Rishal I, Fainzilber M. Axon-soma communication in neuronal injury. *Nat Rev Neurosci.* 2014; 15:32–42. This is an excellent review of axonal mechanisms for sensing injury and signaling back to the neuron. [PubMed: 24326686]
35. Angelo MF, et al. p75 NTR expression is induced in isolated neurons of the penumbra after ischemia by cortical devascularization. *J Neurosci Res.* 2009; 87:1892–1903. [PubMed: 19156869]
36. Baron JC, et al. Selective neuronal loss in ischemic stroke and cerebrovascular disease. *J Cereb Blood Flow Metab.* 2014; 34:2–18. This is an insightful review of the data on selective neuronal loss in the ischemic penumbra and the vulnerability of neurons to delayed degeneration after stroke. [PubMed: 24192635]
37. Tisserand M, et al. Is white matter more prone to diffusion lesion reversal after thrombolysis? *Stroke.* 2014; 45:1167–1169. This is a neuroimaging study demonstrating the ability of the white

- matter to undergo ischemic reversal with thrombolysis and revascularization. [PubMed: 24519405]
38. Thomalla G, et al. Diffusion tensor imaging detects early Wallerian degeneration of the pyramidal tract after ischemic stroke. *Neuroimage*. 2004; 22:1767–1774. [PubMed: 15275932]
  39. Carmichael ST. Cellular and molecular mechanisms of neural repair after stroke: making waves. *Ann Neurol*. 2006; 59:735–742. [PubMed: 16634041]
  40. Go AS, et al. Heart disease and stroke statistics: 2014 update—a report from the American Heart Association. *Circulation*. 2014; 129:e28–e292. This is an annual update publication by the American Heart Association and American Stroke Association on the incidence and prevalence of stroke. [PubMed: 24352519]
  41. Leary MC, Saver JL. Annual incidence of first silent stroke in the United States: a preliminary estimate. *Cerebrovasc Dis*. 2003; 16:280–285. [PubMed: 12865617]
  42. Maillard P, et al. White matter hyperintensities and their penumbra lie along a continuum of injury in the aging brain. *Stroke*. 2014; 45:1721–1726. This is a neuroimaging study that categorizes white matter hyperintensities on MRI and demonstrates the presence of a white matter penumbra in adjacent regions that progresses over time with longitudinal imaging. [PubMed: 24781079]
  43. Rosso C, Samson Y. The ischemic penumbra: the location rather than the volume of recovery determines outcome. *Curr Opin Neurol*. 2014; 27:35–41. [PubMed: 24275722]
  44. Maillard P, et al. White matter hyperintensity penumbra. *Stroke*. 2011; 42:1917–1922. [PubMed: 21636811]
  45. Murphy BD, et al. White matter thresholds for ischemic penumbra and infarct core in patients with acute stroke: CT perfusion study. *Radiology*. 2008; 247:818–825. [PubMed: 18424687]
  46. Barone FC. Ischemic stroke intervention requires mixed cellular protection of the penumbra. *Curr Opin Investig Drugs*. 2009; 10:220–223.
  47. Stys PK, Steffensen I.  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchange in anoxic/ischemic injury of CNS myelinated axons. *Ann N Y Acad Sci*. 1996; 779:366–378. [PubMed: 8659849]
  48. Stys PK, Jiang Q. Calpain-dependent neurofilament breakdown in anoxic and ischemic rat central axons. *Neurosci Lett*. 2002; 328:150–154. [PubMed: 12133577]
  49. Singh P, et al. Levels of phosphorylated axonal neurofilament subunit H (pNfH) are increased in acute ischemic stroke. *J Neurol Sci*. 2011; 304:117–121. [PubMed: 21349546]
  50. Sellner J, et al. Hyperacute detection of neurofilament heavy chain in serum following stroke: a transient sign. *Neurochem Res*. 2011; 36:2287–2291. [PubMed: 21792676]
  51. Stirling DP, et al. Axoplasmic reticulum  $\text{Ca}^{2+}$  release causes secondary degeneration of spinal axons. *Ann Neurol*. 2014; 75:220–229. [PubMed: 24395428]
  52. Micu I, et al. Real-time measurement of free  $\text{Ca}^{2+}$  changes in CNS myelin by two-photon microscopy. *Nat Med*. 2007; 13:874–879. [PubMed: 17603496]
  53. Petrescu N, et al. Sources of axonal calcium loading during in vitro ischemia of rat dorsal roots. *Muscle Nerve*. 2007; 35:451–457. [PubMed: 17206661]
  54. Nikolaeva MA, Mukherjee B, Stys PK.  $\text{Na}^+$ -dependent sources of intra-axonal  $\text{Ca}^{2+}$  release in rat optic nerve during in vitro chemical ischemia. *J Neurosci*. 2005; 25:9960–9967. [PubMed: 16251444]
  55. Taguohi J, et al. Ischemic axonal injury and its recovery after focal cerebral ischemia. *No To Shinkei*. 1989; 41:813–818. [PubMed: 2679826]
  56. Zhang Q, et al. Transient focal cerebral ischemia/reperfusion induces early and chronic axonal changes in rats: its importance for the risk of Alzheimer's disease. *PLoS One*. 2012; 7:e33722. [PubMed: 22457786]
  57. Halestrap AP, Meredith D. The SLC16 gene family—from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflugers Arch*. 2004; 447:619–628. [PubMed: 12739169]
  58. Nikolaev A, et al. APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. *Nature*. 2009; 457:981–989. [PubMed: 19225519]

59. Akpan N, et al. Intranasal delivery of caspase-9 inhibitor reduces caspase-6-dependent axon/neuron loss and improves neurological function after stroke. *J Neurosci.* 2011; 31:8894–8904. [PubMed: 21677173]
60. Schoenmann Z, et al. Axonal degeneration is regulated by the apoptotic machinery or a NAD<sup>+</sup>-sensitive pathway in insects and mammals. *J Neurosci.* 2010; 30:6375–6386. [PubMed: 20445064]
61. Schafer DP, et al. Disruption of the axon initial segment cytoskeleton is a new mechanism for neuronal injury. *J Neurosci.* 2009; 29:13242–13254. [PubMed: 19846712]
62. Reimer MM, et al. Rapid disruption of axon-glia integrity in response to mild cerebral hypoperfusion. *J Neurosci.* 2011; 31:18185–18194. [PubMed: 22159130]
63. Babbs CF, Shi R. Subtle paranodal injury slows impulse conduction in a mathematical model of myelinated axons. *PLoS One.* 2013; 8:e67767. [PubMed: 23844090]
64. Lyon MF, et al. A gene affecting Wallerian nerve degeneration maps distally on mouse chromosome 4. *Proc Natl Acad Sci U S A.* 1993; 90:9717–9720. [PubMed: 8415768]
65. Wang J, et al. A local mechanism mediates NAD-dependent protection of axon degeneration. *J Cell Biol.* 2005; 170:349–355. [PubMed: 16043516]
66. Conforti L, et al. NAD<sup>+</sup> and axon degeneration revisited: Nmnat1 cannot substitute for Wld<sup>S</sup> to delay Wallerian degeneration. *Cell Death Differ.* 2007; 14:116–127. [PubMed: 16645633]
67. Lu Y, Belin S, He Z. Signaling regulations of neuronal regenerative ability. *Curr Opin Neurobiol.* 2014; 27C:135–142. This is a concise review on recent advances in molecular axonal pathways relevant to axonal regeneration. [PubMed: 24727245]
68. Watkins TA, et al. DLK initiates a transcriptional program that couples apoptotic and regenerative responses to axonal injury. *Proc Natl Acad Sci U S A.* 2013; 110:4039–4044. This study demonstrates that DLK is essential in retrograde signaling after optic nerve crush injury activating proapoptotic and regeneration associated genes. Deletion of DLK prevents retinal ganglion cell degeneration. [PubMed: 23431164]
69. Shin JE, et al. Dual leucine zipper kinase is required for retrograde injury signaling and axonal regeneration. *Neuron.* 2012; 74:1015–1022. [PubMed: 22726832]
70. Hammarlund M, et al. Axon regeneration requires a conserved MAP kinase pathway. *Science.* 2009; 323:802–806. [PubMed: 19164707]
71. Xiong X, et al. Protein turnover of the Wallenda/DLK kinase regulates a retrograde response to axonal injury. *J Cell Biol.* 2010; 191:211–223. [PubMed: 20921142]
72. Yan D, et al. The DLK-1 kinase promotes mRNA stability and local translation in *C. elegans* synapses and axon regeneration. *Cell.* 2009; 138:1005–1018. [PubMed: 19737525]
73. Overman JJ, Carmichael ST. Plasticity in the injured brain: more than molecules matter. *Neuroscientist.* 2014; 20:15–28. This is a review of recent advances in post-stroke plasticity including the role of activity, experience, and injury-dependent plasticity in the context of neural repair after stroke. [PubMed: 23757300]
74. Wahl AS, et al. Neuronal repair. Asynchronous therapy restores motor control by rewiring of the rat corticospinal tract after stroke. *Science.* 2014; 344:1250–1255. This is an important recent study highlighting the spatiotemporal patterning of molecular and activity-dependent plasticity after stroke. Early growth promoting therapy with anti-Nogo followed by intensive behavioral training resulted in improved outcomes after stroke compared with early high-intensity training. [PubMed: 24926013]
75. Hinman JD, Rasband MN, Carmichael ST. Remodeling of the axon initial segment after focal cortical and white matter stroke. *Stroke.* 2013; 44:182–189. This is a study of axon initial segment remodeling after focal cortical stroke and white matter stroke demonstrating a decrease in axon initial segment length and loss of axo-axonic synapses along the axon initial segment in surviving peri-infarct neurons as well as neurons with axons damaged by white matter stroke. [PubMed: 23233385]

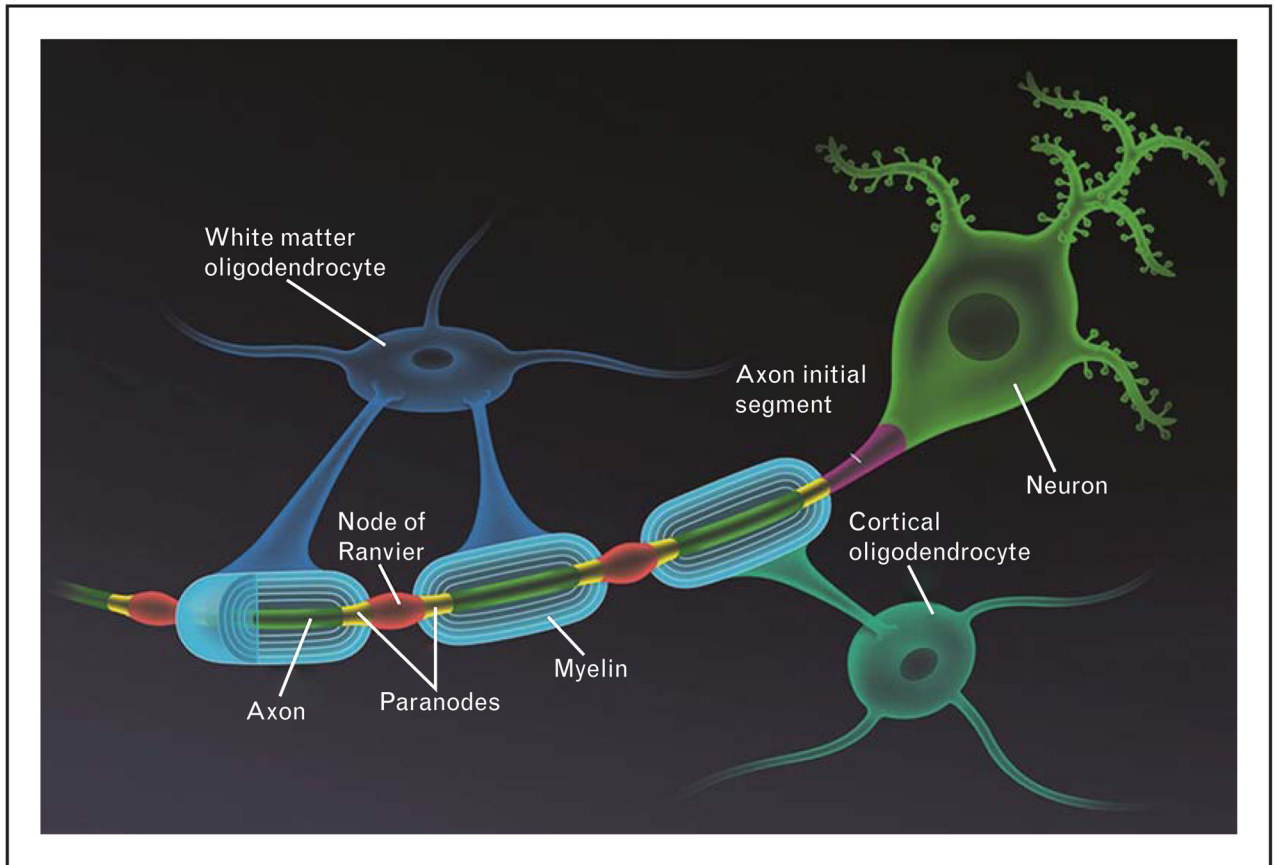
**KEY POINTS**

- Recent years have greatly advanced our understanding of axonal neurobiology and this necessitates advancing the study of axonal injury and repair in stroke beyond the current level to incorporate the molecular organization of axons, the axoglia relationship and axo–glia signaling mechanisms.
- Axonal injury after stroke occurs in distinct phases that vary in relevance depending on the type of clinical stroke.
- Initially, a rapid primary phase involving calcium signaling and energy deprivation leads to local proteolysis of key axonal cytoskeletal elements, followed by a secondary phase of progressive axonal degeneration occurring over hours to days that potentially worsens clinical deficits, with a third phase involving a complex interplay of anterograde Wallerian degeneration, retrograde signaling of axonal injury that triggers axonal sprouting and regeneration.
- Progressive axonal degeneration occurs during and after stroke to worsen the initial injury; emerging molecular pathways relevant to this progressive injury should be studied in stroke.
- Axonal regeneration is fundamental to stroke recovery and can be promoted by behavioral and molecular therapies, although the effect of these treatments on the molecular organization of axons remains poorly understood with the potential to augment neural repair.

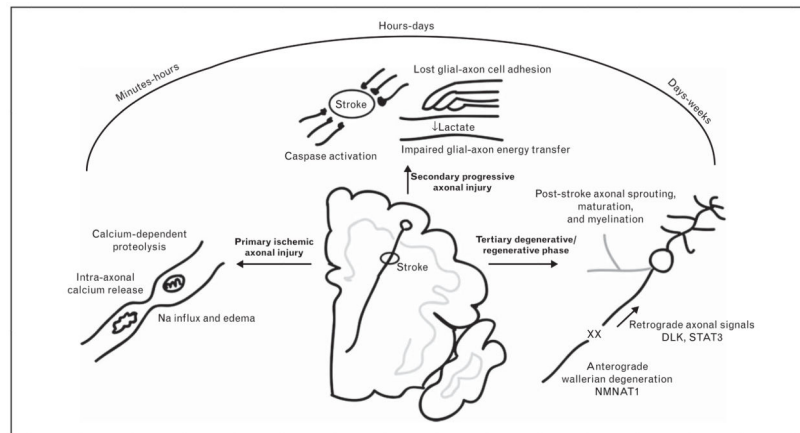


**FIGURE 1.**

Examples of molecular organization of axons and their role in axonal injury after stroke. Surviving layer 5 peri-infarct neuron (green) after photothrombotic stroke with labeling of the axon initial segment (AIS) (red) and the first myelinated segment (1st paranode, cyan) (a). Axo-axonic GABAR- $\alpha$ 2 subunit-positive synapses (red) along the axon initial segment (green) (b) are lost after stroke. Immunofluorescent labeling of nodal (red) and paranodal (green) regions in normal appearing white matter (Nawm) (c). Axons adjacent to human lacunar infarcts show paranodal elongation (d). Scale bars: a = 5  $\mu$ m; b = 1  $\mu$ m; c and d = 5  $\mu$ m.

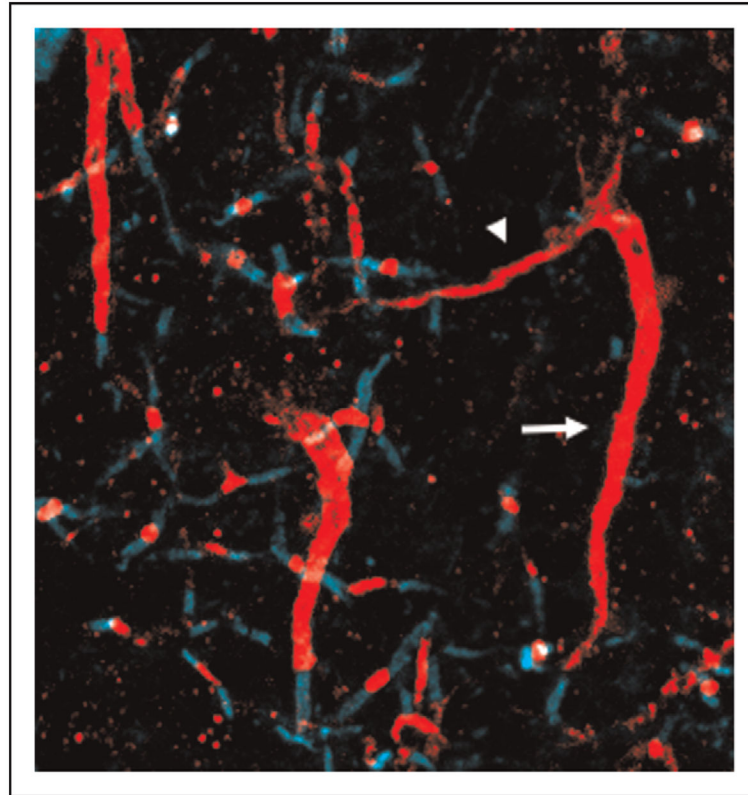
**FIGURE 2.**

Schematic of the axoglial unit showing the unique microdomain organization of axons into the axon initial segment (purple), the node of Ranvier (red), the paranode (yellow) and the intramyelinic axon (green). Ischemic injury to the axon involves multicellular signaling that impairs neurotransmission at the node, cell adhesion at the paranode, as well as anterograde and retrograde molecular signals that modulate neural repair after stroke. (Reproduced with permission from [17].)



**FIGURE 3.**

Schematic figure illustrating the three distinct phases of axonal injury and repair after stroke. As an example, focal lacunar stroke within the centrum semiovale is represented. The primary ischemic phase results from catastrophic failure of adenosine triphosphate availability, leading to  $\text{Na}^+$  retention, axonal swelling, and intra-axonal  $\text{Ca}^{2+}$  release from axoplasmic reticulum. These events trigger calcium-dependent proteolysis and potentially activate the secondary progressive phase of injury. Multiple molecular targets participate in this phase including caspase activation, loss of axoglial contact and/or failure of glial-to-axon energy transfer. A third phase begins in the days to weeks after stroke and involves a combination of anterograde Wallerian degeneration, retrograde axonal injury signaling and activation of axonal sprouting or degenerative pathways.

**FIGURE 4.**

Identification of supranumerary axon initial segments in peri-infarct cortex.

Immunofluorescent labeling of the axon initial segment (beta-IV spectrin, red) allows identification of a typical axon initial segment projecting towards white matter (arrow). Arising laterally and projecting intracortically is a thin, shorter axonal initial segment (arrowhead). Labeling for contactin-associated protein (caspr, teal) shows the distal end of the original axon initial segment is myelinated while the newly sprouted axonal initial segment remains unmyelinated at its distal end.



**Table 1**

Axonal molecular systems with therapeutic potential in stroke

| <b>Molecular system</b>                                    | <b>Phase of axonal injury</b> | <b>Relevant stroke type</b>    | <b>Therapeutic timing after stroke onset</b> |
|------------------------------------------------------------|-------------------------------|--------------------------------|----------------------------------------------|
| Axonal Ca <sup>2+</sup> reversal                           | Phase 1                       | Lacunar and hemispheric stroke | Hours                                        |
| Ca <sup>2+</sup> -dependent proteolysis                    | Phases 1 and 2                | Lacunar and hemispheric stroke | Hours to days                                |
| Axonal caspase activation                                  | Phase 2                       | Lacunar stroke                 | Hours to days                                |
| Glia–axon energy transfer via MCT-1                        | Phase 2                       | Lacunar stroke                 | Days                                         |
| Glia–axon cell adhesion and trophic support                | Phase 2                       | Lacunar and hemispheric stroke | Days                                         |
| Delayed Wallerian degeneration via NMNAT1                  | Phase 2 and 3                 | Lacunar and hemispheric stroke | Days to weeks                                |
| Retrograde signaling via MAPK system                       | Phase 3                       | Lacunar stroke                 | Days to weeks                                |
| Maturation and myelination of post-stroke axonal sprouting | Phase 3                       | Lacunar and hemispheric stroke | Weeks                                        |

MAPK, mitogen-activated protein kinase; MCT-1, monocarboxylate transporter-1; NMNAT1, nicotinamide mononucleotide adenylyltransferase 1.