Chapter 16
Age-Dependent Mechanisms of White Matter Injury After Stroke

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16.1 Introduction

The significance of white matter injury in the clinical manifestations of stroke has been underestimated in experimental animal models. Rodents are the most commonly used animals for the study of stroke but ironically rodent brain constitutes only ~10 % white matter by volume (Fig. 16.1; Zhang and Sejnowski 2000). In addition, the most commonly used stroke model in rodents, the middle cerebral artery occlusion (MCAO) model consistently spares corpus callosum, the white matter tract in rodents (as reviewed in Ginsberg and Busto 1989). This is mainly due to the fact that the middle cerebral is not the main arterial supply to the corpus callosum area. Rodent corpus callosum receives collaterals from deep penetrating pial arteries and striate arteries that arise from the circle of Willis. Conversely, human corpus callosum receives 80 % of its blood supply from the anterior cerebral artery and its branches, the middle cerebral artery being one of them (Wolfram-Gabel et al. 1987; Ture et al. 1996). Predictably, then the injured rodent brain after an ischemic attack is, essentially neuronal injury with minimal or no contribution from white matter. Human brain comprises equal percentages of gray and white matter by volume (Fig. 16.1; Zhang and Sejnowski 2000), which means that injuries sustained after a stroke in humans inevitably involve more white matter than in rodents. In fact, white matter is injured during most strokes, and even small lesions located in a strategic area of white matter can lead to drastic neurological dysfunction and deficits. It is conceivable that all these factors may have contributed to the failure of in clinical trial of drug candidates that selectively conferred protection to neurons in

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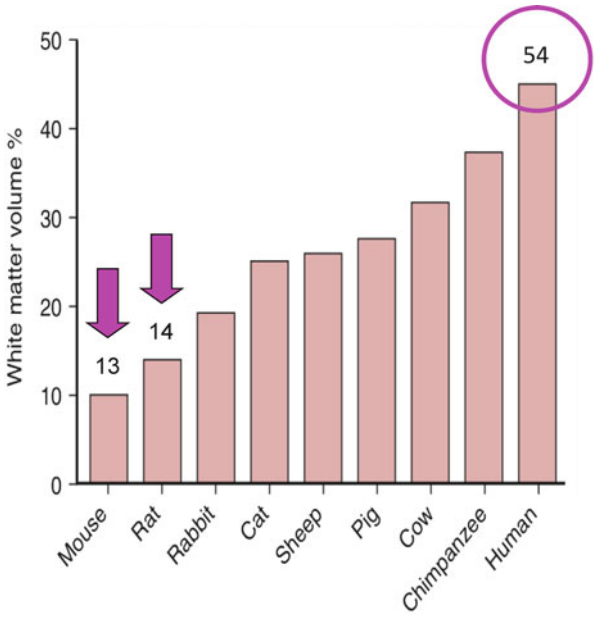

Fig. 16.1 Brain white matter volume expands as brain size enlarges. Histograms show the percentage of cerebral hemisphere volume composed of white matter in several mammals, ranging from mouse to human. (Figure is from Chap. 8 Molecular Pathophysiology of White Matter Anoxic Ischemic Injury by Ransom B, Goldberg MP and Baltan S; Stroke Edited by Mohr, Wolf, Grotta, Moskowitz, Mayberg, von Kummer, Elsevier 2011. Data calculated from Zhang K, Sejnowski TJ: A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Sci U S A 97:5621–5626, 2000)

experimental stroke models (Del Zoppo 1995, 1998; Dirnagl et al. 1999; O'Collins et al. 2006). We suggest that failure to protect white matter is one of the principal reasons contributing to the lack of successful stroke therapy.

The risk of ischemic stroke increases drastically with age. A number of pathological changes occur in aging that preferentially influence brain structures and are candidates for causing age-associated neurological impairment, or increased susceptibility to impairment. The majority of studies of aging brain to date have focused on stereological estimates of total neuron numbers in gray matter. Interestingly, aging has global effects on white matter with relatively little effect on gray matter (Vincent et al. 1989; Peters and Sethares 1993). White matter appears to undergo generalized shrinkage in volume relative to gray matter (Albert 1993), due to the reduction in myelin content (Kemper 1994) and number of axons with age (Yamauchi et al. 2002). Myelin provides important insulating properties to axons allowing for propagation of action potentials over large distances at high velocity. Disruption of the myelin sheath could, therefore, contribute to cognitive impairment, as observed during aging (Peters and Sethares 1993; O'Sullivan et al. 2001; Ferro and Madureira 2002). Imaging studies in elderly people demonstrate

hypodense lesions in white matter, termed leukoaraiosis (Hachinski et al. 1987). The severity of leukoaraiosis may predict high risk for stroke (Yamauchi et al. 2002) and impaired cognitive function (Schmidt et al. 1991). Other imaging techniques (Basser et al. 1994; Pendlebury et al. 1999) show regional variability in white matter signal characteristics, reflecting age-related degenerative effects on myelination and white matter connectivity (Davatzikos and Resnick 2002). However, the cellular basis of these changes cannot be determined from clinical imaging protocols.

White matter is a target of hypoxic-ischemic injury throughout life, in clinical settings ranging from periventricular leukomalacia in neonates, stroke, and cardiac arrest in adults, to dementia in the aging brain (Goldberg and Ransom 2003). Although there is increasing evidence that the degenerative effects of aging on white matter contribute to cognitive impairment, dementia, and increased risk for stroke (Bonita et al. 1994; Kurtzke 1994), structural and functional changes in aging white matter axons and glial cells have been explored only recently (Baltan et al. 2008; Baltan 2009, 2012). With an increase in the elderly population around the world, there is an urgency to view successful brain aging as the goal of scientists in the broad field of neurobiology. Identification of risk factors, awareness of the relative importance of each factor, and knowledge of their interaction should facilitate maintenance of cognitive, emotive, motor, and sensory functions. The focus of this chapter is to summarize the current information on molecular and functional changes in axons and glial cells as a function of age and how these adaptive mechanisms transform the tissue into a state of increased vulnerability in the face of an ischemic attack.

16.2 Intact Optic Nerve and Corpus Callosum Slices as Models to Study White Matter

The optic nerve, a purely myelinated central nervous system (CNS) white matter tract, is sensitive to the aging process (Cavallotti et al. 2002, 2003) and offers several advantages to study the mechanisms of white matter injury: (a) tissue isolation does not require extensive surgical interventions so there is minimal preparation injury, (b) the isolated optic nerve is structurally and functionally stable in vitro for at least 18 h, (c) there are no neurons, therefore no synapses or synaptic machinery to indirectly contribute to white matter injury, (d) axon function can be monitored by recording evoked compound action potentials (CAPs) (Fig. 16.2a), (e) the cellular components of optic nerve can be identified using immunohistochemistry, isoform-specific antibodies and confocal imaging (Fig. 16.2b), (f) amino acid release such as glutamate can be quantitatively monitored (Fig. 16.13), (g) protein levels of interest can be quantified by western blots and (h) intravitreal injections enable a route of delivery to axons.

Rat optic nerve (RON) has been widely used to study white matter injury to determine the effects of anoxia (Stys et al. 1990, 1992), aglycemia (Brown et al. 2001) and ischemia (Garthwaite et al. 1999). Mouse optic nerve (MON), however, has important

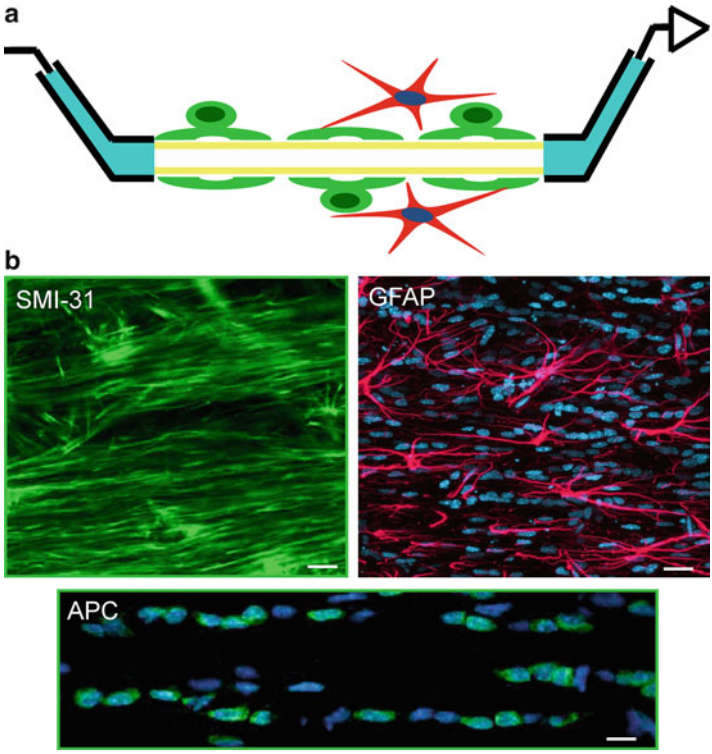

Fig. 16.2 Monitoring white matter function and architecture. (**a**) Use of suction electrodes allows all axons to be stimulated and a compound action potential (CAP) to be recorded. (**b**) Using cell-specific antibodies, white matter axons labeled with SMI-31 for neurofilament (*green*), GFAP for astrocytes (*magenta*) and APC for mature oligodendrocyte cell bodies (*green*). Sytox (+) glial nuclei are in *blue*. Scale bar = 50 μm for SMI-31 and, GFAP, 10 μm for APC. (Reproduced in part from Baltan 2009)

advantages over RON as an in vitro model for studying white matter injury. The diameter of the adult RON is about twice that of the adult MON and metabolism is limited by inadequate glucose diffusion into the RON, especially when glucose utilization is increased as during anoxia (Baltan Tekkök et al. 2002; Tekkok et al. 2003). Taking into account this technical point, and considering the promise of future transgenic mice to analyze specific injury pathways, we use MONs for our experiments.

On the other hand, corpus callosum (CC) slices offer important advantages for the investigation of injury in white matter with neighboring gray matter. Slices preserve the anatomical and structural integrity of the continuity of the axon with the cell body, allowing assessment of axons with or without myelin in the presence of fully differentiated oligodendrocytes. Use of these two in vitro white matter tracts allow exceptional combined function–structure analysis, where glial cells and axons have retained their native relationships to one another within a three-dimensional organization (Fig. 16.2).

An in vivo model that selectively causes ischemic injury to white matter tracts is necessary to test some important parameters of stroke. Several different approaches have been taken to address this issue. For instance, severe applications of the MCAO model cause injury in subcortical white matter in rodents (Pantoni et al. 1996). However, subcortical white matter axons are extensions of cortical neuronal cell bodies (mainly layers 3, 5 and 6). Therefore, subcortical white matter injury in stroke is not a selective white matter injury but is secondary to the injury of cortical neuronal cell bodies. Consequently, neither the injury mechanisms nor the protective approaches can be specified to white matter structures. The modified Rice Vanucci model (carotid artery ligation + recovery under hypoxic conditions) is used to mimic periventricular leukomalacia and/or prenatal hypoxia, and selectively injures immature oligodendrocytes and arrests myelin production in postnatal day 7 rodent brain without any axon damage or motor deficits (Follett et al. 2000; Jensen 2006). Current approaches to corpus callosum injury involve local injections of glutamate analogues (McDonald et al. 1998; Leroux et al. 2010), demyelinating substances (Gadea and Lopez-Colome 2001), or vasoconstrictive substances (Sozmen et al. 2009) to cause glial and/or axon injury. These models do not mimic ischemia and also cause comparable tissue injury due to trauma and volume effects. On the other hand, our earlier efforts to induce ischemic injury in corpus callosum or optic nerve using piglets resulted in inconsistent findings, despite initial promising results (Lee et al. 2006). Primate brains contain 35 % white matter by volume and MCA perfusion territory is closer to human brain, and therefore may be the most appropriate model (Frykholm et al. 2000; Enblad et al. 2001).

16.3 Architectural Organization of White Matter

Structure and function in white matter are integrated: one is a reflection of the other (Fig. 16.2). The challenge, therefore, is to decipher the meaning of molecular architecture of white matter components. Discovery of the structural design of white matter is an evolving process and has so far proved to be more complex than anticipated. Axons and their myelinating oligodendrocytes, together with nurturing astrocytes (Fig. 16.2b, c), defensive microglia (Fig. 16.4) and progenitor cells forming synapses onto axons form an intricate interactive environment. The morphological variability in these white matter components among different white matter tracts further attest to their sophisticated nature to adapt to their regional function. In particular, glutamate homeostasis of adult white matter and intricate distribution of glutamate receptors and glutamate transporters expressed by astrocytes, oligodendrocytes, axons and microglia in a region-specific manner further illustrate the sophistication of white matter architecture (Figs. 16.7 and 16.8). White matter is particularly vulnerable to excitotoxicity during ischemia (Fig. 16.11) and a detailed knowledge of structural elements is key to prevent or restore function after ischemic injury.

In optic nerve from rat, mouse, or rabbit oligodendrocytes express AMPA receptors that are composed of GluR1, GluR3, and GluR4, but GluR3 and GluR4 in spinal cord (rat) and corpus callosum (mouse). On the other hand, oligodendrocytes from optic nerve are rich in GluR6, GluR7, KA1, and KA2 subunits of kainate receptors while in spinal cord GluR5, GluR6, KA1 form the subunit composition of kainate receptors. Interestingly corpus callosum oligodendrocytes do not express kainate receptors (Baltan and Goldberg, unpublished data). The AMPA and kainate receptors are mainly located on oligodendrocyte cell bodies and are believed to be involved in axon–myelin signaling.

Oligodendrocytes express NMDA receptors that consist of NR1, NR2A-NR2C, and NR3A subunits. Remarkably, NMDAR are expressed in clusters on oligodendrocyte processes, whereas AMPA and kainate receptors are diffusely located on oligodendrocyte soma. In addition, immunogold electron microscopy revealed that NR1, NR2, and NR3 subunits are present in the myelin sheath. Functional aspects of NMDA receptors on oligodendrocytes were discovered by application of several approaches. NMDA-mediated currents were recorded in mature oligodendrocytes from corpus callosum and cerebellar white matter (Karadottir et al. 2005), influx of Ca was identified in myelin formed by oligodendrocytes in the RON (Micu et al. 2006) and the protein levels of NMDA receptor subunits NR1, NR2A-D, and NR3A were detected in different type of oligodendrocytes (Karadottir et al. 2005; Salter and Fern 2005; Micu et al. 2006).

Glutamate is found in ample amount in white matter (Fig. 16.7) and is released during oxygen and glucose deprivation (OGD) (Fig. 16.13), causing overactivation of AMPA/kainate receptors (Tekkok et al. 2007). Potential sources of glutamate include axons (Li et al. 1999), oligodendrocytes (Fern and Moller 2000), and astrocytes via the reversal of Na^+-dependent glutamate transporters (Li et al. 1999; Tekkok et al. 2007), Ca^{2+} increases (Parpura et al. 1994), or via release from swelling-activated anion channels and hemichannels (Ye et al. 2003). Vesicular glutamate release by vesicular glutamate transporter 1 (VGLUT1), 2 (VGLUT2) and 3 (VGLUT3) is Ca^{2+}-dependent and modulated by stimulation frequency in myelinated (Kukley et al. 2007) and in unmyelinated axons (Ziskin et al. 2007) of the corpus callosum. However Na^+-dependent glutamate transport inhibitors greatly diminish glutamate release in MONs during ischemia (Tekkok et al. 2007). Astrocytes express the greatest density of Na^+-dependent glutamate transporters in white matter; glutamate transporter-1 (GLT-1) and glutamate aspartate transporter (GLAST) (Figs. 16.7 and 16.8). Energy deprivation causes a gradual dissipation of the transmembrane Na^+ gradient in astrocytes setting up conditions for reverse exchange and glutamate release (Longuemare et al. 1999).

16.4 White Matter Components Reorganize with Aging

Across the age groups, there is no change in the proportion of total APC (+) oligodendrocytes, GFAP (+) astrocytes, NG2 (+) precursor cells (Fig. 16.3). Interestingly, microglial processes and the thickness and the length of these

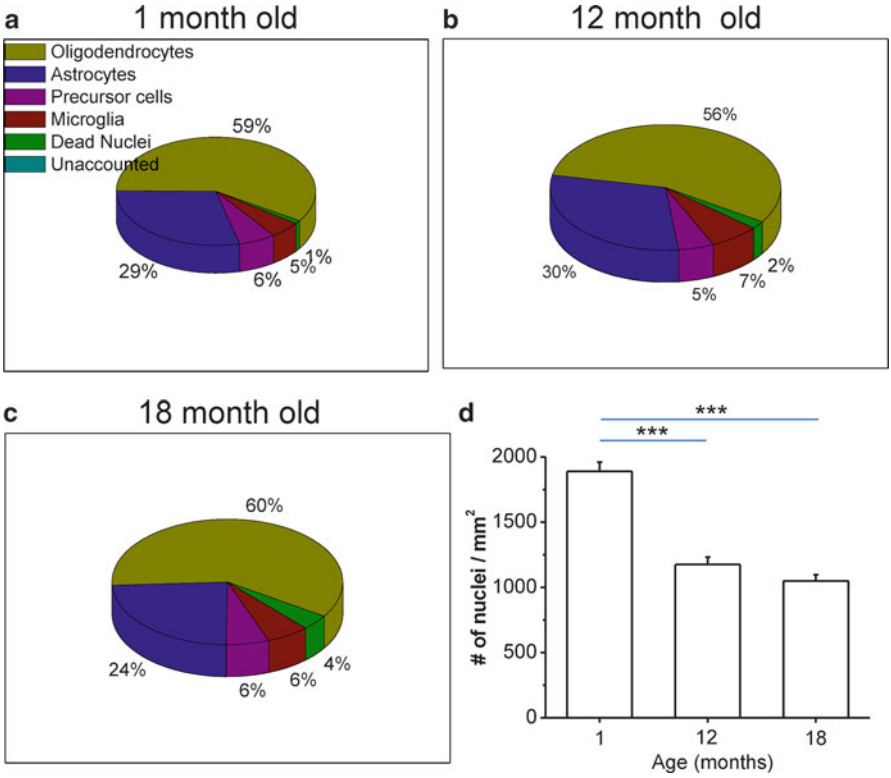

Fig. 16.3 Aging results in loss of cellularity but relative percentage of glial cells is preserved. (**a**–**c**) Pie charts summarize percentile of glial cells (percent of total nuclei) in white matter from 1-, 12-, and 18-month-old optic nerve. (**d**) Histograms demonstrate ~35 % fewer glial cells by 12 months of age. ***$p < 0.001$, one-way ANOVA

processes (ramification) increase three to fivefold in older white matter (Fig. 16.4), in agreement with age-related activation of white matter microglia in monkey (Sloane et al. 1999) and rats (Ogura et al. 1994; Morgan et al. 2004). Additionally, each microglial cell with its processes appears to occupy a distinct domain within the tissue, reminiscent of astrocytic domains (Oberheim et al. 2008) in young white matter. This territorial organization is lost with age as numerous processes reached out towards neighboring domains. Microglial activation is a pathological hallmark of stroke. Curiously, aging seems to unmask this capacity of microglia without any further insult. It is unclear whether the effect of activated microglia on white matter is beneficial, detrimental, or a combination of both.

Astrocytes exhibit thicker processes that change their orientation from transverse to a more longitudinal orientation with aging (Baltan et al. 2008, also see Fig. 16.8). However, the GFAP protein levels or the number of astrocytes do not change in older white matter (Baltan et al. 2008). Because astrocyte processes form the end-feet on the vascular wall and contribute to the neurovascular unit, the change in their direction raises the possibility that they may be adapting to vascular changes with aging.

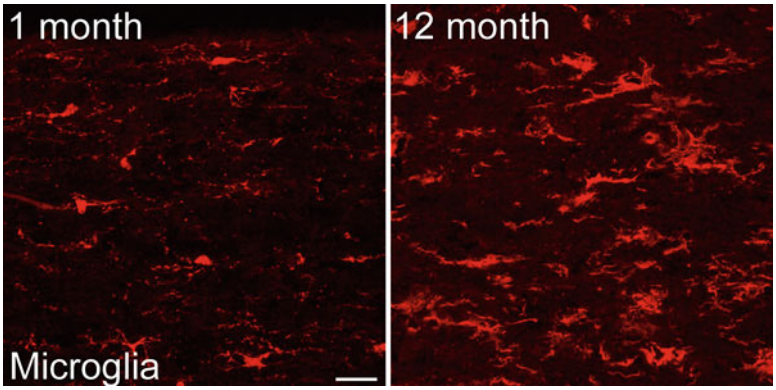

Fig. 16.4 Aging causes microglial activation. A few Iba (+) microglial cells (*red*) with their small cell bodies and few thin processes can be detected in 1-month-old MONs (*left*). Aging causes elaborate changes in their morphology including cell body size, and process ramification (*right*). Calibration bar = 20 μm

In addition, astrocyte processes elongate to the nodes of Ranvier and to sense the metabolic demands of axon function. Age-dependent reorganization of axonal architecture (nodal, paranodal, myelin content-see Figs. 16.5 and 16.6) may need expanded points of surveillance by astrocytes to maintain the metabolic burdens of aging axons.

Myelin provides a unique architecture to allow high-fidelity conduction along axons. Labeling studies for different myelin proteins reveal a significant loss of myelin basic protein (MBP) and myelin associated glycoprotein (MAG) as a function of age. On the other hand, and consistent with preserved oligodendrocyte numbers, CNPase levels show a small drop (Fig. 16.5). Oligodendrocytes and possibly myelin express AMPA/kainate and NMDA receptors (Micu et al. 2006; Baltan et al. 2008) which mediate excitotoxic injury during ischemia. Age-dependent changes in glutamate receptor type and subunit composition is of utmost interest, for they dictate the ensuing injury.

Clustering of Nav1.6, the predominant sodium channel at the nodes of Ranvier (Goldin et al. 2000) is an indicator of proper axo–glial contact dictated by myelin (Boiko et al. 2001) to support saltatory conduction. The contactin-associated protein (CASPR) rich paranodes are flanked by juxtaparanodal Kv1.1 or Kv1.2 potassium channels. Immunohistochemical analysis for CASPR and Nav1.6 indicate that CASPR (Fig. 16.6, green) is longer and thinner, while Nav1.6 clusters (Fig. 16.6, red) become shorter with aging. Although Nav1.6 is also found in unmyelinated axons (Black et al. 1999), it is not detectable in the internodal region of myelinated axons. However, aging causes ectopic regions of Nav1.6 immunoreactivity (Fig. 16.6, white arrows) which might lead to deleterious effects on axons especially if colocalized with the Na^+–Ca^{2+} exchanger (Craner et al. 2004). A majority of axon profiles with diffuse Na^+ channel immunoreactivity is associated with reduced MBP

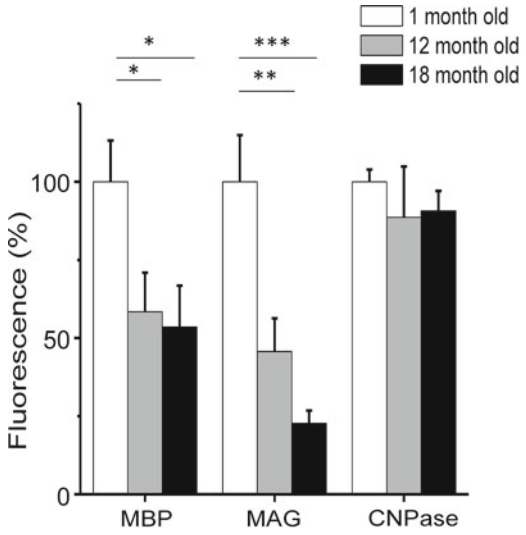

Fig. 16.5 MBP and MAG are down regulated with aging. Quantification of labeling intensity reveals that MBP and MAG decrease with age compared to 1 month old mice. *$p<0.05$, **$p<0.01$, ***$p<0.001$, one-way ANOVA

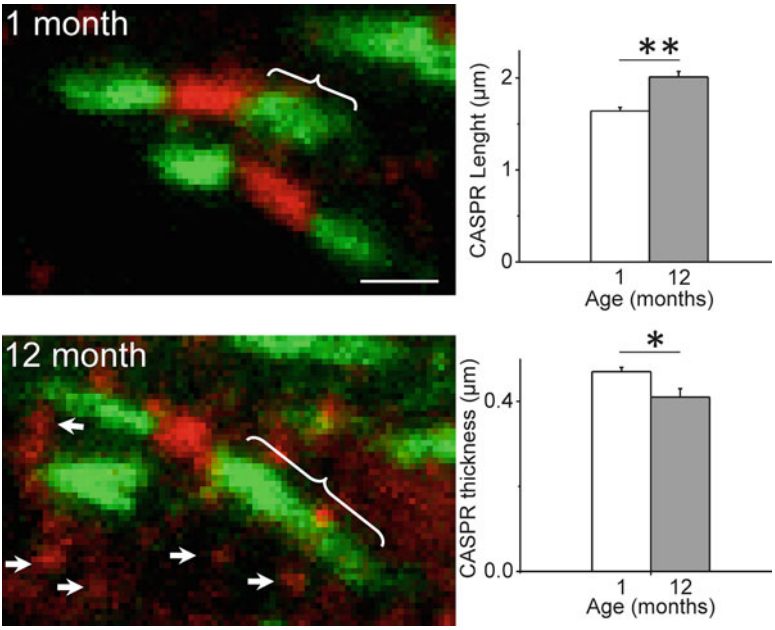

Fig. 16.6 Nodal and paranodal structures reorganize with aging. Immunolabeling of Nav1.6 sodium channels at the nodes of Ranvier show that sodium channel clusters (*red*) become smaller and assume an extranodal localization (*line with arrows*) in aging MONs. Paranodal CASPR (*green*) elongates (*brackets*) and becomes thinner, as summarized in the histograms. *$p=0.0317$, **$p=0.0019$, two-tailed Student's *t*-test. Calibration bar = 2 µM

immunolabeling in spinal cord experimental allergic encephalomyelitis (Craner et al. 2004) which paralleled structural changes in aging axons in optic nerve. In addition, Kv1.2 (+) potassium channels overlap with CASPR immunostaining indicating loss of the characteristic demarcation between paranodal and juxtaparanodal structures (Dupree et al. 1999; Bhat et al. 2001). Displacement or aberrant localization of nodal, paranodal, juxtaparanodal structures suggest that axonal function at the node may be compromised with age. Similar findings in the aging monkey and RON (e.g., Hinman et al. 2006) implicate age-related molecular reorganization at the nodes of Ranvier as intrinsic to white matter which crosses over species.

White matter glutamate homeostasis and the related machinery also go through a series of age-related restructuring (Figs. 16.7 and 16.8). The dominant glutamate transporter, GLT1, plays an essential role in removing glutamate from the extracellular space and maintaining glutamate below neurotoxic levels under normoxic conditions (Rothstein et al. 1996; Hazell et al. 2001). Although these transporters are predominantly expressed on astrocytes in young white matter, they extend to additional structures with aging (Fig. 16.8), implying that additional white matter constituents may contribute to toxic glutamate accumulation in aging white matter. In addition to GLT-1, the essential members to maintain glutamate homeostasis are GLAST, glutamate and glutamate synthatase (GS). White matter glutamate content increases considerably and in correlation with increased GS levels with age (Fig. 16.8). Together with a twofold increase in GLT-1 levels in older white matter (Baltan et al. 2008), these adjustments may infer an age-related adaptive mechanism in white matter to remove and to convert excessive glutamate to glutamine so as to maintain glutamate homeostasis. As a result glutamate levels under control conditions (Fig. 16.13, Baltan et al. 2008) and axon conduction across aging axons remains impressively stable over time under normoxic conditions. The number of GLT-1 transporters determines the capacity of the tissue to move glutamate between internal and external compartments (Fig. 16.13). However, it is the direction of the pump that acts to save or injure the tissue. During ischemia, these measures act against the tissue due to an accelerated Na^+ overload as a result of decreased tolerance to energy deprivation in aging white matter. Therefore, numerous GLT-1 transporters reverse and lead to early robust release of glutamate causing enhanced excitotoxicity (Fig. 16.13). Moreover, in young white matter glutamate resumes to baseline levels after the end of OGD, which is a sign of efficient astrocyte uptake of glutamate. However, glutamate levels remain elevated in old white matter, suggesting that aging astrocytes cannot take up excess glutamate and thereby expanding the excitotoxicity duration into the recovery period (Fig. 16.13). Despite the possibility that glutamate may be released from multiple sources in aging white matter, astrocytes are expected to remove and store glutamate efficiently. Therefore, these results suggest a prominent change in aging astrocyte capacity to remove glutamate.

On the other hand, GLAST expression in white matter falls with age, raising the possibility that, glutamate transporters can functionally substitute for one another with age (Fig. 16.7). It is well-known that the upregulation of GLT-1 participates in the induction of brain ischemic tolerance in gray matter (Romera et al. 2004; Kawahara et al. 2005; Zhang et al. 2007) and in reactive astrocytes in human periventricular leukomalacia (Desilva et al. 2008). Aging seems to saturate these

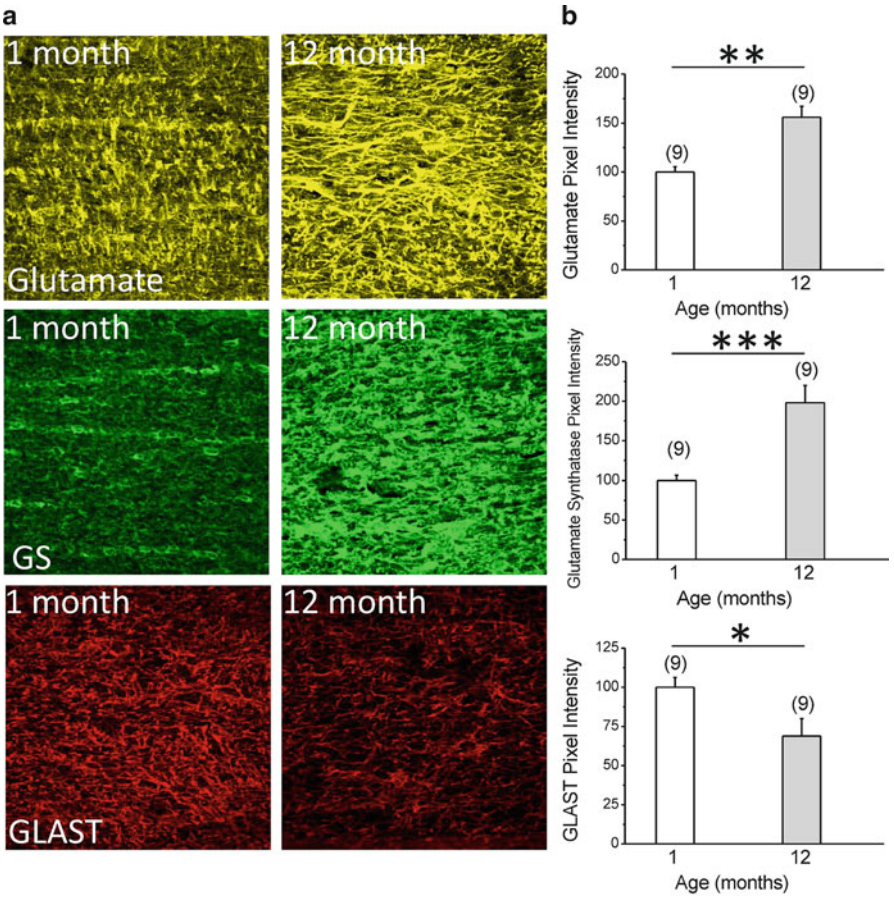

Fig. 16.7 Glutamate and glutamate synthatase (GS) expression increase in 12-month-old MONs. (**a**) Immunolabeling and (**b**) quantification of glutamate, GS and GLAST immunolabeling revealed that glutamate and GS labeling intensity increases by 156 ± 11.1 % and 198 ± 21.9 %, respectively, in 12 month old MONs. Note that GLAST labeling intensity decreased to 68.9 ± 11.2 % in 12 month old MONs. *$p = 0.0278$, **$p = 0.004$, ***$p = 0.0006$, two-tailed Student's *t*-test

mechanisms (Fig. 16.8), and it is intriguing whether preconditioning is impaired in white matter with aging. Unfortunately, these questions will remain unanswered until an in vivo model of adult white matter stroke is available.

16.5 Age-Dependent Mechanisms of Ischemic White Matter Injury

White matter axons are dependent on a constant supply of oxygen and glucose to faithfully transmit signals. We reported that CNS white matter function is exceptionally tolerant to a complete lack of oxygen (Tekkok et al. 2003) while there is

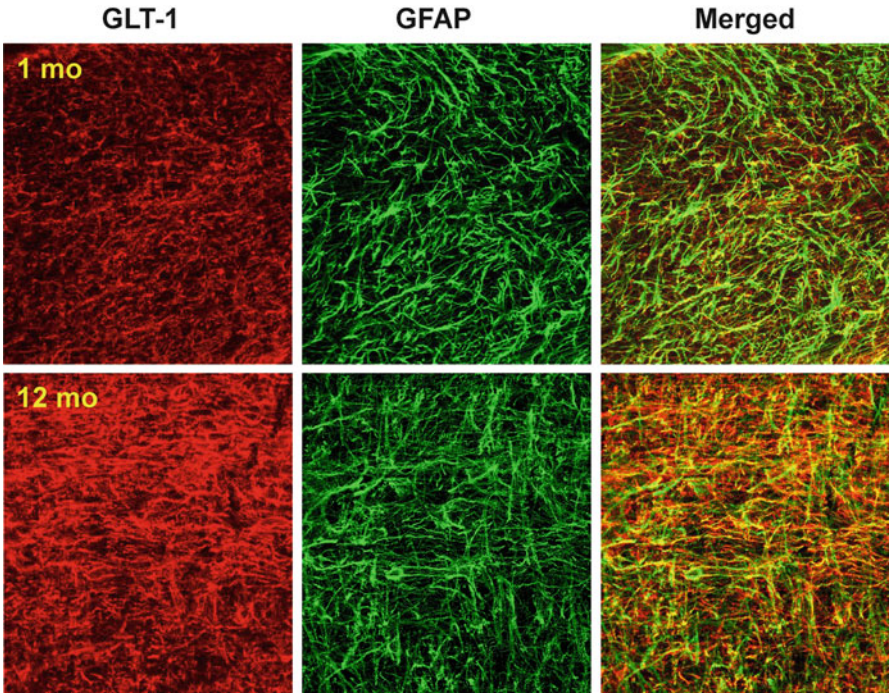

Fig. 16.8 Aging is correlated with upregulation of GLT-1. The overlap of GLT1 (*red*) and GFAP (*green*) labeling indicated that GLT-1 was mainly expressed in astrocytes (*merged*) in 1-month-old MONs. MONs. There was a twofold increase in GLT-1 pixel intensity (188.6 ± 18.5 %) with age. The pattern of GFAP expression in MONs changed with age but without an increase in GFAP pixel intensity. (Reproduced from Baltan et al. 2008)

regional variability in the ability to function and survive anoxia (Baltan 2006). However, young adult white matter is readily susceptible to ischemia induced by combined OGD (Fig. 16.9). Mechanisms underlying ischemic white matter injury prove to be unpredictably complex (Fig. 16.10) (Wrathall et al. 1994; Agrawal and Fehlings 1997; Fern and Ransom 1997; McDonald et al. 1998; Sanchez-Gomez and Matute 1999; Follett et al. 2000; Tekkök and Goldberg 2001; Stys 2004; Tekkok et al. 2007). White matter contains no neuronal soma but instead has myelinated axons, oligodendrocytes, and astrocytes. The cellular elements of white matter are individually under attack during ischemia, while they still remain interactive with each other in intricate mechanisms that are not well understood. Axons are injured (Fig. 16.10) directly by ionic mechanisms, resulting in accumulation of intracellular Na^+ and Ca^{2+} (Stys et al. 1990; Fern et al. 1995; Wolf et al. 2001; Ouardouz et al. 2003; Underhill and Goldberg 2007) while astrocytes, via the reversal of Na^+-dependent glutamate transporters (Li et al. 1999; Tekkok et al. 2007) initiate an excitotoxic path resulting in oligodendrocyte death and myelin damage (Fig. 16.11, Tekkök and Goldberg 2001; Tekkok et al. 2007). Glutamate accumulation concomitantly initiates the oxidative pathway attacking white matter constituents due to

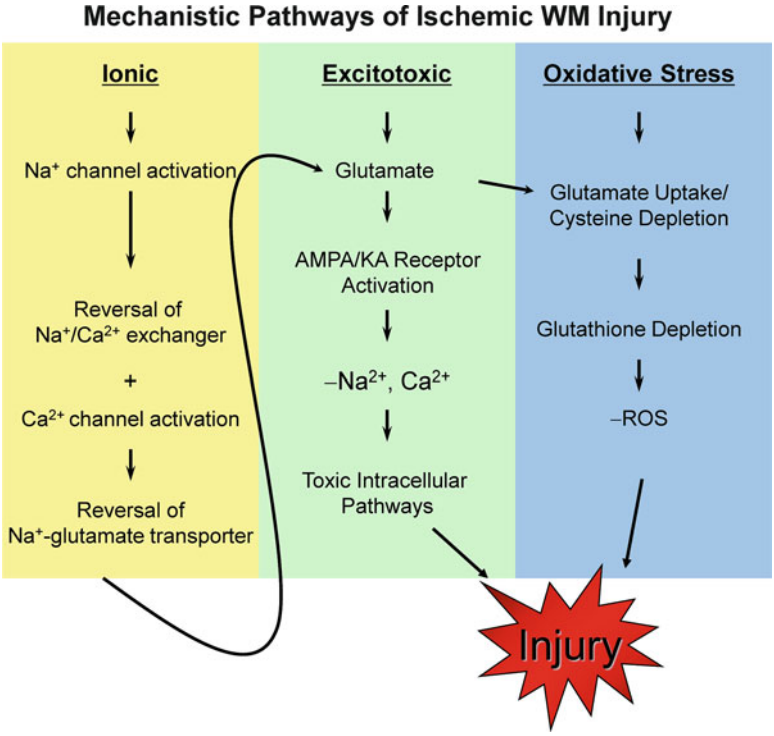

Fig. 16.9 Putative mechanisms of ischemic white matter injury. Ionic, excitotoxic, and oxidative stress converge in sequential order to cause irreversible injury in white matter during ischemia. Note that glutamate release, due to reverse Na^+-dependent transport dictates irreversible nature of the injury. (Reproduced from Baltan 2009)

formation of free radicals mediated by glutamate competing with cysteine at the glutamate-cysteine pump (Oka et al. 1993) and glutamate disrupting mitochondrial function (Chang and Reynolds 2006). Glutamate is necessary but not sufficient to explain ischemic injury in white matter. Consistent with this, exogenous application of glutamate or its agonists fails to initiate injury. Only a short and reversible OGD (15 min) combined with glutamate (or analogues) mimic ischemic injury (Tekkok et al. 2007). In accordance, glutamate levels remain very stable during the initial stage of OGD (25–30 min) and then steadily start to accumulate (Fig. 16.12). These results point to a sequential order of injury pathways converging in order for irreversible injury to ensue, such that an essential first stage of ionic dysfunction primes white matter for glutamate toxicity (Fig. 16.9; Tekkok et al. 2007). The sequential convergence of these pathways also manifests itself as duration-dependent injury (Tekkok et al. 2007; Baltan 2009). Predictably, removal of extracellular Ca^{2+}, blockade of AMPA/kainate receptors (Fig. 16.11), blockade of reverse glutamate transport (Tekkok et al. 2007), or prevention of reactive oxygen species (ROS) generation reduces ischemic white matter injury. On the other hand, although developing

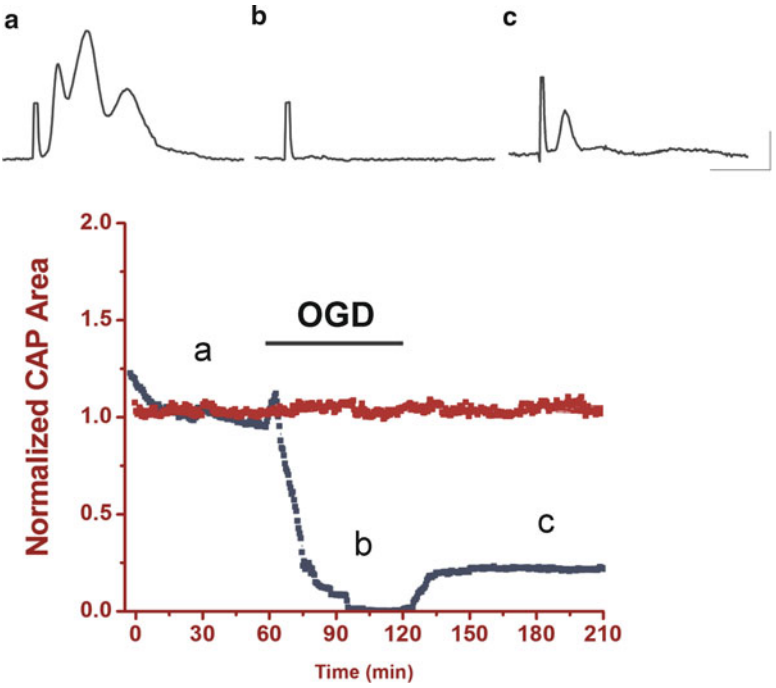

Fig. 16.10 White matter is susceptible to ischemic injury. Axon function is quantified as the area under the CAP, normalized to control, and plotted against time. Under normal conditions CAP area remains stable over time (*brown*). A 60 min period of oxygen glucose deprivation (OGD) depresses the CAP gradually until conduction along the axons is completely lost (*gray*) typically around 30 min. Restoring oxygen and glucose, axon function recovers to ~25 %. Sample traces from control (**a**), OGD (**b**) and recovery (**c**) periods are shown above the plot

oligodendrocyte processes (Salter and Fern 2005), mature oligodendrocyte cell bodies (Karadottir et al. 2005; Baltan et al. 2008), and myelin (Micu et al. 2006) express functional NMDARs, blockade of these receptors does not improve axon function after ischemia in young optic nerve (Tekkok et al. 2007) or corpus callosum (Tekkök and Goldberg 2001). These results do not negate the activation of NMDAR during ischemia but imply that their activation does not contribute to axonal damage and raises a caution for clinical implications of NMDAR antagonism during ischemia, particularly in aging white matter (see below). In fact, oligodendroglial NMDAR activation preserves axon function against ischemia by coupling axonal and glial energy metabolisms in developing and young optic nerves (Saab et al. 2012).

Although valid mechanisms, all of these studies were performed entirely on young animals. Because the risk for stroke increases with age, a thorough understanding of white matter ischemic injury in age-appropriate populations is of central importance to meet the challenge of developing effective stroke therapy. Indeed, CNS white matter becomes intrinsically more vulnerable to OGD in older animals and the mechanisms of white matter injury change as a function of age (Baltan et al. 2008). This increased sensitivity to OGD is due, in part, to increased susceptibility

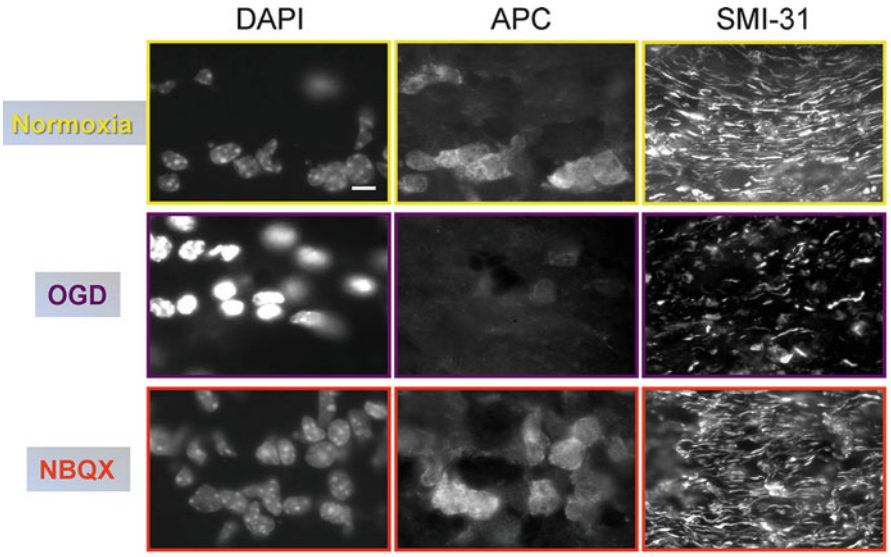

Fig. 16.11 Overactivation of AMPA/kainate receptors cause oligodendrocyte death and axon disruption. Under normoxic condition, DAPI (+) glial nuclei are mostly APC (+) oligodendrocytes among SMI-31 (+) labeled axons. A 30 min period of OGD in corpus callosum slices causes widespread loss of APC (+) oligodendrocytes and SMI-31 (+) axons with pyknotic bright DAPI (+) dead nuclei. Blockade of AMPA/kainate receptors with NBQX prevents oligodendrocyte death and axon disruption. Scale bar = 10 μm. (Reproduced from Tekkök and Goldberg 2001)

of aging axons to a lack of oxygen (Fig. 16.12). Mitochondrial dysfunction and disruption with age presumably underlie the loss of aerobic capacity of aging axons (Fig. 16.13, see mitochondria in insets). On the other hand, whether aging axons are less tolerant to removal of glucose is under investigation.

Accumulation of Ca^{2+} is an important step in the development of ischemic injury in young white matter, while Ca^{2+}, influx may be more vital to aging axons. The lack of protection by removal of extracellular Ca^{2+} or by blockade of Ca^{2+} entry secondary to reverse operation of the Na/Ca exchanger (NCX), denotes that preventing Ca^{2+} influx is not sufficient to preserve older axon function. Ironically, removal of extracellular Ca^{2+} worsens axon function recovery after OGD, a perplexing observation that implies Ca^{2+} entry during ischemia is a protective measure. It is possible that Ca^{2+} release from intracellular Ca^{2+} stores (ICS), and the interplay between intracellular and extracellular Ca^{2+}, is more critical during ischemia in aging axons. Although a role for Ca^{2+} release from endoplasmic reticulum via IP3 and ryanodine receptors is described in young white matter (Thorell et al. 2002), various Ca^{2+}-dependent neurophysiological (Landfield and Pitler 1984; Campbell et al. 1996) and signaling pathways (Verkhratsky et al. 1998) remain unexplored in white matter, particularly as these relate to ischemia and aging.

Because blockade of NMDARs cause a similar worsening of axon function recovery, it points to Ca^{2+} entry through NMDARs as an important element to protect aging axon function against ischemia. Alternatively the role of NMDAR as the axon–glia

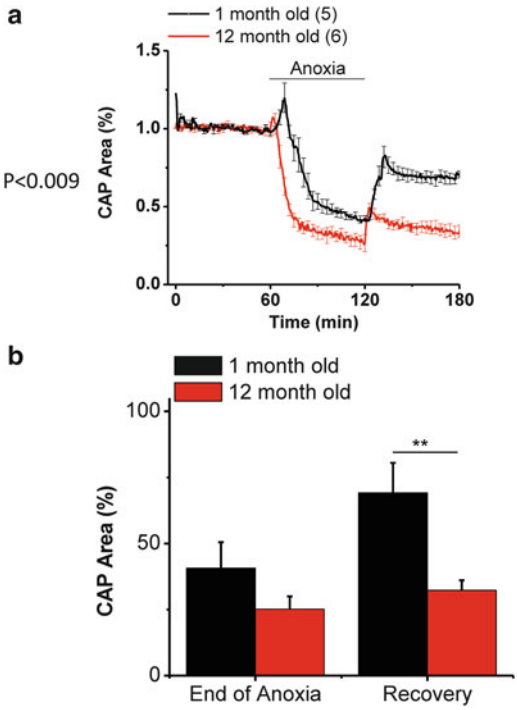

Fig. 16.12 Aging axons are more vulnerable to anoxia. Normalized data show that the CAP area rapidly falls and recovers less after 60 min of anoxia in 12-month-old (*red*) compared to 1-month-old (*black*) MONs. The CAP area and SEM are plotted every 3 min. $^{**}p<0.009$, one-way ANOVA

metabolic couplers becomes more critical to support the increased energy burden of aging axons. It is also plausible that removal of Ca^{2+} may lead to glutamate release via hemichannels from aging astrocytes thus exacerbating excitotoxic injury. These mechanisms remain to be explored.

The Ca^{2+}-independent nature of ischemic injury in aging white matter raises another important question as to how AMPA/kainate receptor activation mediates injury. Because blockade of AMPA/kainate receptors promotes axon function recovery across all age groups, it suggests that Na^+ entry, via AMPA/kainate receptors, rather than Ca^{2+}, mediates injury during ischemia in older white matter. Overload of Na^+ exhibits irreversible toxic swelling, even in the absence of extracellular Ca^{2+} (Rothman and Olney 1995) and a rise in intracellular Na^+ promotes reversal of the Na^+-dependent glutamate transporter, resulting in glutamate accumulation (Szatkowski et al. 1990). Together with the upregulation in GLT-1 expression, increases in intracellular Na^+ may be the leading cause of increased and early release of glutamate, overactivating AMPA/kainate receptors and creating a vicious cycle that underlies the vulnerability of aging white matter to ischemia (Fig. 16.13). Furthermore, an increase in Na^+ concentration interferes with maintenance of the transmembrane ion gradient. This challenges the Na–K ATPase pump, compromises the ability of aging axons to maintain membrane properties and axonal excitability and contributes to an increased vulnerability to ischemia (Fig. 16.17, Scavone et al. 2005).

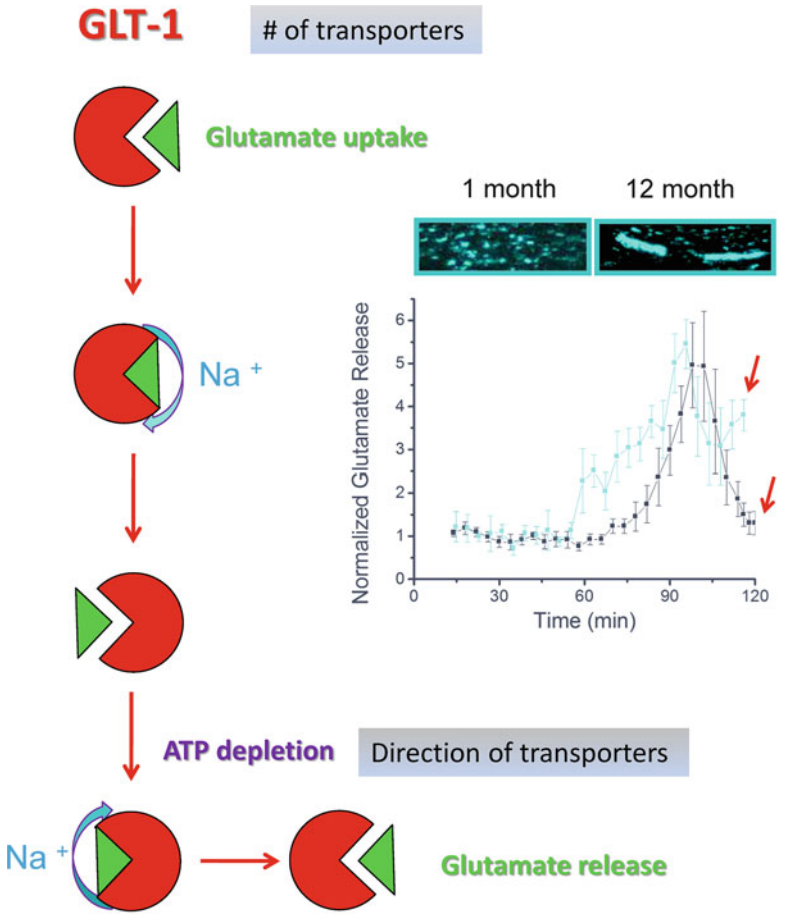

Fig. 16.13 Enhanced excitotoxicity due to impaired mitochondrial function leads to early and robust glutamate release in aging white matter. The principle Na^+-dependent glutamate transporter, GLT-1, typically takes up glutamate with co-transport of Na^+. During ATP depletion, due to increased intracellular Na^+ levels, the transporter reverses and releases glutamate. Therefore the number of transporters determines the capacity of the system for the amount of glutamate that can be transported, but it is the ATP levels that determine the direction of the transporter (to remove or release glutamate). Mitochondria in aging axons become longer and thicker compared to young axons, which may hinder ATP production and drive GLT-1 in reverse mode. Consistent with this, there is an early and robust glutamate release in aging white matter (*blue*) compared to optic nerves from young mice (*gray*). Note, that the glutamate levels return to baseline in young but remain elevated in aging white matter (*red arrows*). (Reproduced in part from Baltan et al. 2008)

The finding that blockade of NMDARs worsens outcome in older white matter (Baltan et al. 2008) has important clinical implications as it explains why NMDAR antagonists, that conferred protection to neuronal cell bodies in experimental stroke settings, failed to provide any benefit in clinical stroke trials. In this aspect, these results challenge the existing convention in stroke research that assumes a common

mechanism of injury in the brain across the life span. They also question the well-established agreement that glutamate receptor over-activation unequivocally causes injury. It is critical to consider that therapeutic options beneficial for white matter tracts may be ineffective for the gray matter and that therapeutic options proving successful in gray matter may be less useful or even harmful for white matter, especially when mechanisms of injury change as a function of age.

16.6 Mitochondrial Dysfunction Underlies Vulnerability of Aging Axons to Ischemia

The bioenergetics of mitochondria in neurons and their role in glutamate excitotoxicity are well described in gray matter (Nicholls et al. 2007). Mitochondrial dysfunction and excitotoxicity share common aspects and are believed to act synergistically by potentiating each other (Albin and Greenamyre 1992; Jacquard et al. 2006; Silva-Adaya et al. 2008). Mitochondria are dynamic organelles that travel along microtubules, using axonal transport to reach peripheral locations (Hollenbeck 2005; Hollenbeck and Saxton 2005) (Fig. 16.13). They constantly undergo fission and fusion events (Karbowski et al. 2004), and the relative rates of mitochondrial fusion and fission have been implicated in the regulation of their number, size and shape (Mozdy and Shaw 2003; Scott et al. 2003; Chen et al. 2007). The balanced delivery of mitochondria to cell body, dendrites and axons helps them serve multiple functions, including energy generation, regulation of Ca^{2+} homeostasis, cell death, synaptic transmission and plasticity (Chang and Reynolds 2006). Expectedly, an exciting link between a variety of neurological diseases, as well as aging, and defects in mitochondrial fusion and distribution is emerging (Karbowski and Youle 2003; Chen et al. 2007). Older neurons become more susceptible to glutamate excitotoxicity due to loss of mitochondrial membrane depolarization and increased ROS generation leading to reduced energy supply (Parihar and Brewer 2007). Mitochondria exhibit a cell-specific morphology; neuronal mitochondria are small and round as opposed to the longer tubular mitochondria in white matter axons (Fig. 16.14). Dynamin-related protein 1 (Drp-1) is known to affect the distribution of mitochondria (Otsuga et al. 1998; Smirnova et al. 1998), and Drp-1 protein levels show cell-specific variation, being expressed more in astrocytes compared to neurons (Uo et al. 2009). Mitochondrial function appears to decline in older animals, presumably causing reduced ATP production. This has been demonstrated in cardiac (Lesnefsky et al. 2001), liver (Selzner et al. 2007), and brain (Toescu 2005). Ion transport accounts for about 50 % of all ATP utilization and Na^+/K^+ ATPase activity alone is responsible for the majority of this consumption (Erecinska and Silver 1994). A loss of ATP reserve, diminishing the activity of this key enzyme with advanced age, is a plausible contributor to heightened white matter injury susceptibility (Scavone et al. 2005). Consistent with this axon function, when transiently challenged with OGD, *was* slower to restore normal ion gradients, permitting pathological processes related to ion derangement to operate for longer periods

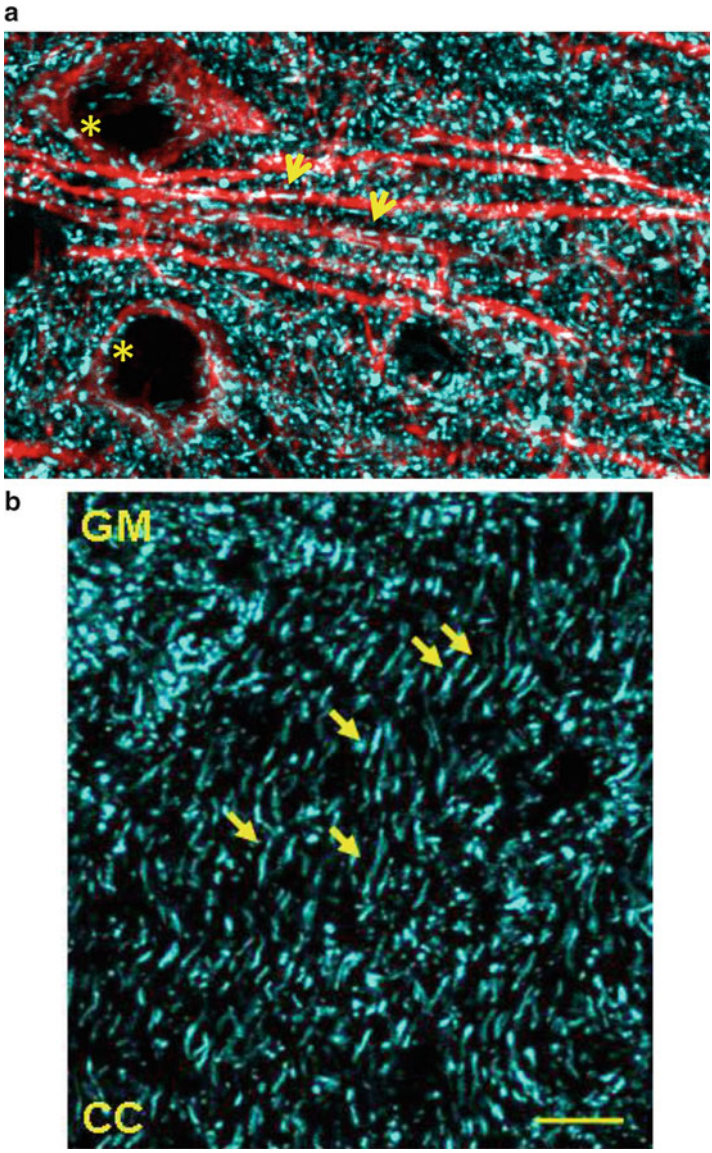

Fig. 16.14 CFP (+) somal and axonal mitochondria exhibit region-specific morphology. (**a**) Neuronal mitochondria are observed as CFP (+) structures in Thy-1 mito mice (Misgeld et al. 2007). CFP (+) mitochondria are small and round in neuronal cells bodies (*yellow asterisk*) labeled with MAP2 (*red*) but more linear and tubular in primary dendrites (*yellow arrows*). (**b**) Numerous long tubular mitochondria (*yellow arrows*) are evident in young corpus callosum. Note, the smaller round mitochondria in GM. Scale bar = 5 μm

(hence reversing Na^+-dependent glutamate transporter(s) earlier), and producing more injury in older MONs (Baltan et al. 2008). The disadvantage of compromised ATP levels in older animals was further verified by better recovery of white matter function in older animals when OGD was imposed at lower temperature (Baltan et al. 2008). Axons with high ATP requirements have many more mitochondria per unit length of process (Bristow et al. 2002); therefore, these axons would be preferentially targeted by low ATP conditions.

Excitotoxicity and elevated Ca^{2+} induce marked changes in mitochondrial morphology, arresting their motion (Rintoul et al. 2003; Barsoum et al. 2006; Chang and Reynolds 2006) and generating ROS in neurons (Nicholls et al. for review). In young white matter, activation of either AMPA or kainate receptors (Baltan et al. 2008) loads mitochondria with Ca^{2+} and fission is enhanced, associated with loss of fluorescence of mitochondria genetically tagged with CFP (Fig. 16.15a; Misgeld et al. 2007). A Ca^{2+} overload activates n-NOS to produce nitric oxide (NO) and ROS, which are proposed as diffusible second messengers linking oligodendrocyte excitotoxicity to axon injury (Matute et al. 2001; Ouardouz et al. 2006). Axon function directly correlates with tissue energy reserves, since Na^+–K^+ ATPase activity is intimately dependent on ATP levels. As a result, OGD causes a significant reduction in ATP levels and CFP (+) mitochondria, which could be prevented by AMPA/kainate receptor blockade (Fig. 16.14).

Aging stimulates mitochondrial fusion (Fig. 16.13) and this may be accompanied by a reduction in Drp-1 levels. The regulated process of mitochondrial fusion and fission controls the spatiotemporal properties of mitochondrial Ca^{2+} responses and the physiological and pathophysiological consequences of Ca^{2+} signals (Szabadkai and Rizzuto 2004; Szabadkai et al. 2004). By enhancing fusion or inhibiting fission, elongated mitochondria possibly absorbs Ca^{2+} efficiently preventing n-NOS activation and subsequent ROS production (Cheung et al. 2007, Fig. 16.17). However, this age-related adaptive reorganization of mitochondria becomes detrimental under ischemic conditions. Ischemia, in aging white matter, further enforces mitochondrial fusion as a result of the age-dependent drop in Drp-1 and age-dependent loss of mitochondrial motility with exposure to glutamate (Chang and Reynolds; Parihar and Brewer). Mitochondria fuse to collectively counteract the already increased excitotoxicity and Ca^{2+} load with aging, and this age-related change in mitochondrial dynamics could hinder ATP production. Because basal ROS generation is already elevated with aging, further increases in ROS accumulation under ischemic conditions result in increased vulnerability to ischemia (Fig. 16.17).

These results were further verified in a series of experiments investigating the protective effects of Class I HDAC inhibitors in young white matter (Baltan et al. 2011a, b). These HDAC inhibitors promoted functional recovery of axons and preserved white matter cellular architecture. This protection correlated with the upregulation of an astrocyte glutamate transporter, delayed and reduced glutamate accumulation during OGD, preservation of axonal mitochondria and oligodendrocytes, and maintained ATP levels in young optic nerves (Fig. 16.16) verifying the proof of principle that excitotoxic injury leads to mitochondrial dysfunction in white matter axons.

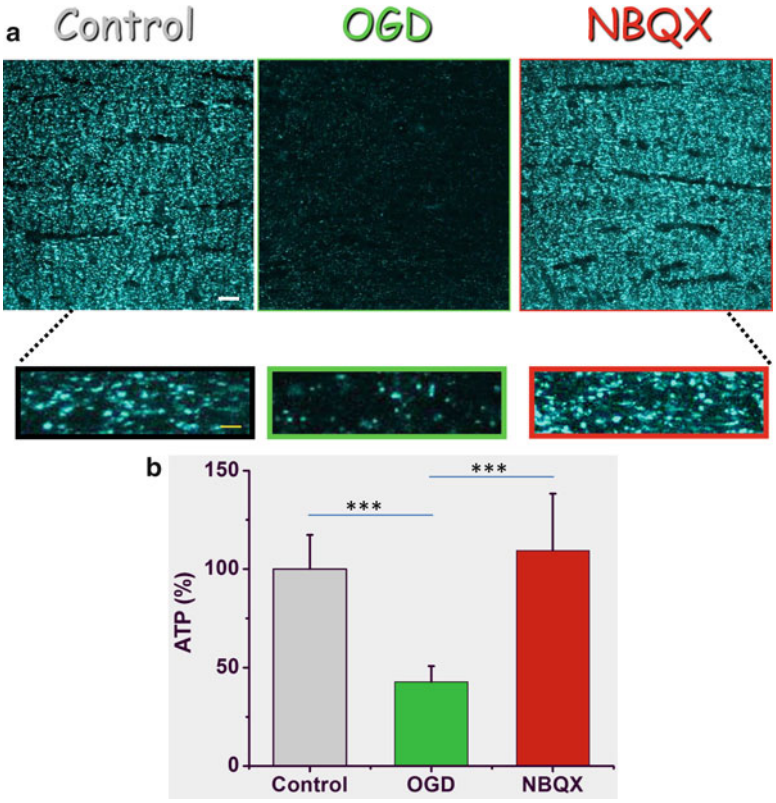

Fig. 16.15 Blockade of excitotoxicity preserves CFP (+) axonal mitochondria and ATP levels in response to OGD. (**a**) OGD drastically reduced CFP fluorescence in MONs from mito CFP (+) mice and pretreatment with NBQX (30 μM) protected against this loss. Note the change in mitochondrial morphology from small and tubular to tiny and punctuated form with OGD. Scale bar = 10 μm (insets = 2 μM) (**b**) Consistent with the preservation of CFP pixel intensity, NBQX pretreatment conserved ATP levels in MONs. ***$p < 0.0001$, one-way ANOVA. (Reproduced in part from Baltan et al. 2011b)

16.7 Region-Specific Mechanisms of Ischemic WM Injury

There is a growing sense that the mechanisms of white matter injury vary from one area of the brain to another (Tekkok et al. 2007). The explanations for regional differences in white matter injury are not yet understood at a cellular level. Regional differences in white matter oligodendrocytes and/or axons are logical possibilities but differences in astrocytes, cells rich in glutamate, should also be considered. Moreover, there may be regional differences in the glutamate receptors that

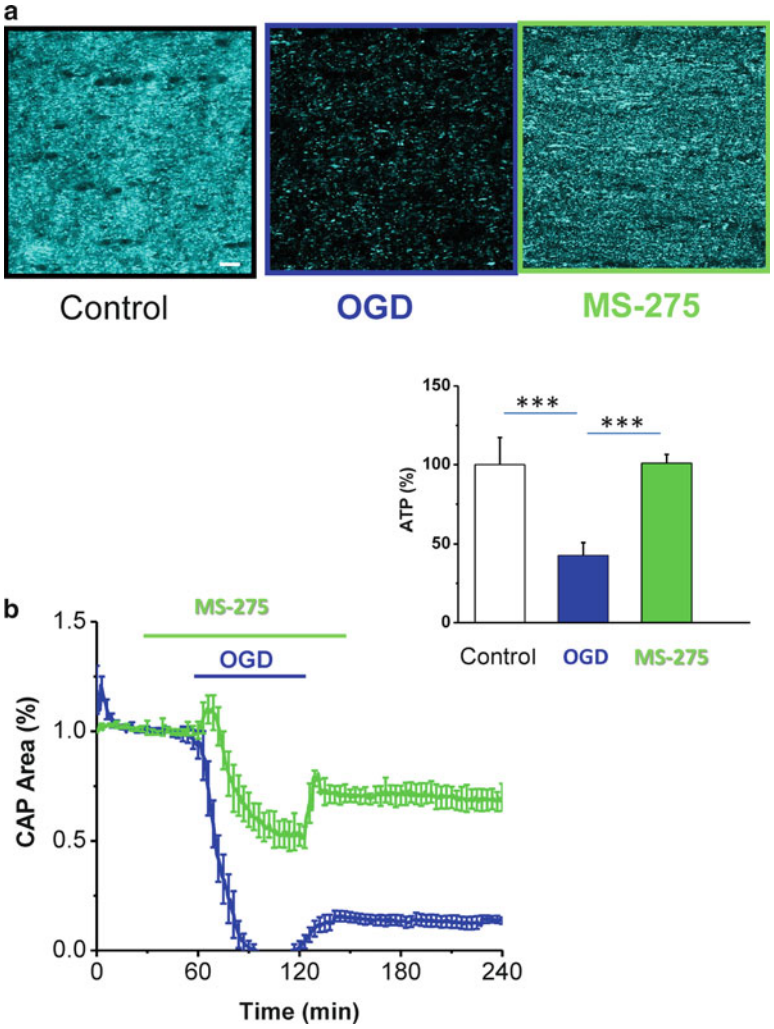

Fig. 16.16 The HDAC inhibitor MS-275 preserves CFP (+) axonal mitochondria and ATP levels in response to OGD. (**a**) OGD (*blue*) drastically reduced CFP fluorescence in MONs from mitoCFP (+) mice and pretreatment with MS-275 (1 μM) protected against this loss. (**b**) Consistent with the preservation of CFP pixel intensity, MS-275 pretreatment promoted axon function recovery (*green*) and conserved ATP levels in MONs. ***$p < 0.0001$, one-way ANOVA. Scale bar = 10 μm. (Reproduced in part from Baltan et al. 2011b)

participate in the injury process (Gallo and Russell 1995; Brand-Schieber and Werner 2003a, b; Tekkok et al. 2007). These differences in glutamate receptor pharmacology of white matter injury may reflect regional differences in the receptors themselves (e.g., degree of expression or subunit composition), regional variability in the amount or onset of glutamate release during ischemia, or the existence of regionally diverse oligodendrocytes. The latter point is particularly noteworthy,

given that the ratio of myelinating to non-myelinating oligodendrocytes vary between areas where all axons are myelinated, like the optic nerve (Foster et al. 1982), compared with areas containing many non-myelinated axons such as the corpus callosum (Olivares et al. 2001). These molecular properties indeed determine the mechanism of injury and the set of receptors that mediate that injury. For instance, activation of either AMPA or kainate receptors in MONs is sufficient to cause injury (Tekkok et al. 2007) while activation of Ca^{2+}-permeable AMPA receptors exclusively mediate the ischemic injury in corpus callosum slices (Tekkök and Goldberg 2001). Moreover, axon function is significantly more resilient to ischemia in optic nerve compared to corpus callosum while corpus callosum axons are more tolerant to anoxia compared to optic nerve. These findings point to a curious divergence between anoxic and ischemic injury mechanisms in white matter. Ischemia causes progressive injury as a function of glutamate accumulation. Anoxia does not cause glutamate release, therefore no region-specific glutamate receptors are involved in the injury process, implying a rather stagnant course for anoxic injury. It is conceivable that axons use energy from glycolysis to prevent Na+/K + pump failure during anoxia to suppress subsequent membrane depolarization and limit the rise in intracellular Ca^{2+} levels. Since more of the smaller diameter unmyelinated axons of corpus callosum survive anoxia, myelin may be the structural element underlying the vulnerability to anoxia, inferring that unmyelinated axons and/or the smallest axons with the thinnest myelin sheath are the resistant group (Baltan Tekkök and Ransom 2004). These results reveal that ischemia and anoxia are not interchangeable forms of injury in white matter and that CNS white matter is remarkably tolerant of anoxia although there is regional variability in their ability to function or survive (Baltan 2006).

16.8 Conclusions

The main goal of this review is to establish the proof of principle that CNS white matter becomes inherently more susceptible to an ischemic attack with age and that the molecular and cellular mechanisms of ischemic injury change as a function of age (Fig. 16.17). Predictably, age-related changes in the molecular architecture of white matter dictate the predominant injury mechanisms and determine the functional outcome. Consequently, protective interventions in young white matter such as removal of extracellular Ca^{2+} (Fig. 16.17a, black arrow), reduce functional recovery in aging axons (Fig. 16.17a, black dotted arrows). Together with the observation that blockade of reverse NCX fails to protect function in older mice (Fig. 16.17b, Baltan et al. 2008), these results propose a diminished role for the ionic pathway with aging. On the other hand, aging causes a prominent increase in the expression pattern of glutamate, GS and GLT-1 levels that extend to additional structures in white matter. These modifications may imply an age-related adaptive mechanism to maintain glutamate signaling and homeostasis. However, during an ischemic episode these adaptive changes act against the tissue and expedite and aggravate glutamate release (Fig. 16.17a, red dotted arrow) and expand the excitotoxic injury

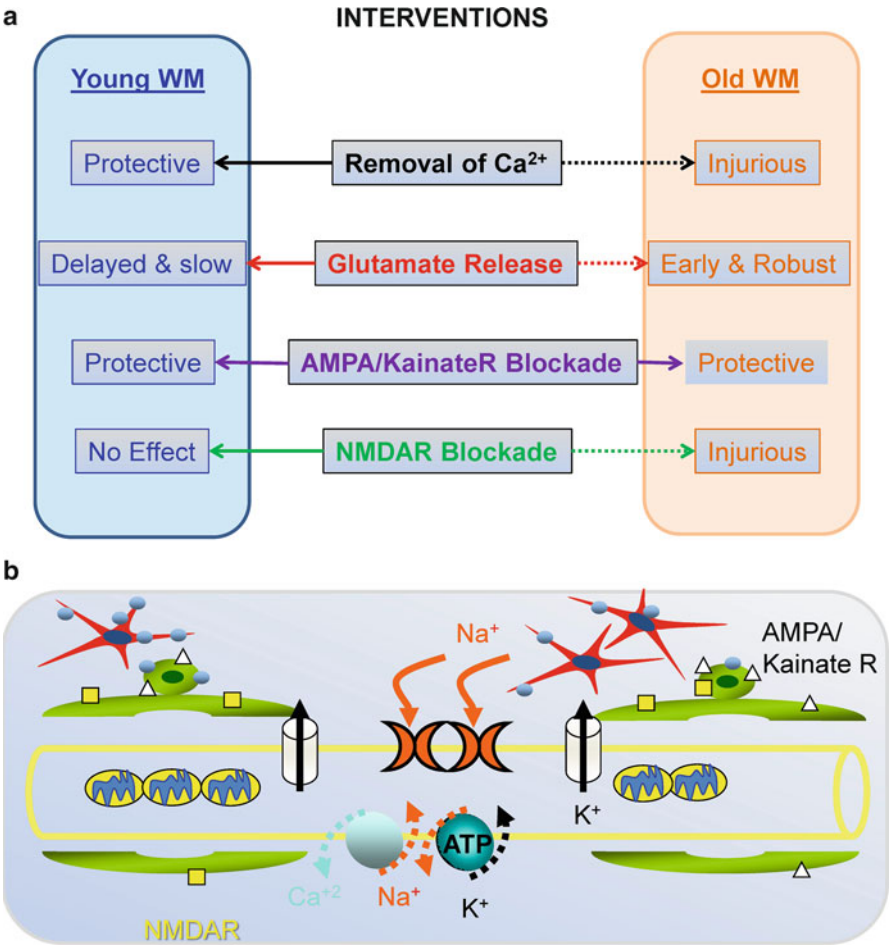

Fig. 16.17 Putative molecular and cellular mechanisms responsible for ischemic white matter injury. (**a**) Protective intervention in young white matter may become injurious or remain the same or become enhanced in aging white matter. (**b**) Age-related cellular reorganization of white matter components determines the injury mechanisms and functional outcome

into recovery period. Interestingly, AMPA/kainate receptors (Fig. 16.17b, white triangles) mediate the ischemic injury across age groups (Fig. 16.17a, purple arrows) indicating that certain steps of injury are preserved irrespective of age. Surprisingly, activation of NMDARs turns into an essential mode of protection for aging axons such that blockade of NMDARs impedes functional recovery after ischemia (Fig. 16.17a, green dotted arrow). NMDARs are expressed in oligodendrocyte cell bodies in young white matter but expand to myelin and to myelin processes with age (Fig. 16.17b, yellow squares; Baltan et al. 2008). Whether this modification of NMDA receptor expression enhances axon–glia metabolic coupling or mediates efficient Ca^{2+} influx to myelin (Micu et al. 2006) to promote aging axon function recovery remains to be explored.

Based on these findings, we propose that age-related upregulation of glutamate, GS and GLT-1 (Fig. 16.17b, blue circles) is not limited to astrocytes but extends to other white matter components (Baltan et al. 2008). Na^+-dependent and Ca^{2+}-dependent mechanisms involving astrocytes, oligodendrocytes expressing EAAC1 (Arranz et al. 2008), microglia expressing GLT-1 or axons with VGLUTs (Kukley et al. 2007; Ziskin et al. 2007) become additional sites of glutamate release with aging and contribute to increased excitotoxicity. In young white matter, activation of either AMPA or kainate receptors loads mitochondria with Ca^{2+} and fission is enhanced due to abundant Drp-1 levels. Ca^{2+} overload activates n-NOS to produce NO and ROS which are proposed as diffusible second messengers to link oligodendrocyte excitotoxicity to axon injury (Matute et al. 2001; Ouardouz et al. 2003, 2006). Aging leads to mitochondrial fusion (Fig. 16.17b, elongated mitochondria) due to a reduction in Drp-1 levels. The regulated process of mitochondrial fusion and fission controls the spatiotemporal properties of mitochondrial Ca^{2+} responses and the physiological and pathophysiological consequences of Ca^{2+} signals (Szabadkai and Rizzuto 2004; Szabadkai et al. 2004). By enhancing fusion or inhibiting fission, elongated mitochondria efficiently absorbs Ca^{2+} preventing n-NOS activation and subsequent ROS production (Cheung et al. 2007). However this age-related adaptive reorganization of mitochondria becomes detrimental under ischemic conditions. Ischemia, in aging white matter, further enforces mitochondrial fusion as a result of an age-dependent drop in Drp-1 and an age-dependent loss of mitochondrial motility with exposure to glutamate (Chang and Reynolds; Parihar and Brewer). Mitochondria fuse to collectively counteract the already increased excitotoxicity and Ca^{2+} load with aging, and this age-related change in mitochondrial dynamics could hinder ATP production. This challenges the Na^+/K^+ ATP pump to maintain axon excitability and the associated rise in Na^+ levels challenges the GLT-1 to function in forward a direction to take up glutamate.

An age-specific understanding of the mechanisms of injury processes in white matter is essential to design dynamic therapeutic approaches for stroke victims.

Therefore an age-dependent reduction in mitochondrial bioenergetics may underlie the increased vulnerability of aging axons to ischemia.

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References

Agrawal SK, Fehlings MG (1997) Role of NMDA and non-NMDA ionotropic glutamate receptors in traumatic spinal cord axonal injury. J Neurosci 17:1055–1063

Albert M (1993) Neuropsychological and neurophysiological changes in healthy adult humans across the age range. Neurobiol Aging 14:623–625

Albin RL, Greenamyre JT (1992) Alternative excitotoxic hypotheses. Neurology 42:733–738

Arranz AM, Hussein A, Alix JJ, Perez-Cerda F, Allcock N, Matute C, Fern R (2008) Functional glutamate transport in rodent optic nerve axons and glia. Glia 56:1353–1367

Baltan S (2006) Surviving anoxia: a tale of two white matter tracts. Crit Rev Neurobiol 18:95–103

Baltan S (2009) Ischemic injury to white matter: an age-dependent process. Neuroscientist 15: 126–133
Baltan S (2012) Histone deacetylase inhibitors preserve function in aging axons. J Neurochem 123(suppl 2):108–115
Baltan Tekkök SB, Ransom BR (2004) Anoxia effects on CNS function and survival: regional differences. Neurochem Res 29:2163–2169
Baltan Tekkök S, Brown AM, Ransom BR (2002) Persistence of axonal function during anoxia in mouse optic nerve. Glia S66
Baltan S, Besancon EF, Mbow B, Ye Z, Hamner MA, Ransom BR (2008) White matter vulnerability to ischemic injury increases with age because of enhanced excitotoxicity. J Neurosci 28: 1479–1489
Baltan S, Bachleda A, Morrison RS, Murphy SP (2011a) Expression of histone deacetylases in cellular compartments of the mouse brain and the effects of ischemia. Transl Stroke Res 2: 411–423
Baltan S, Murphy SP, Danilov CA, Bachleda A, Morrison RS (2011b) Histone deacetylase inhibitors preserve white matter structure and function during ischemia by conserving ATP and reducing excitotoxicity. J Neurosci 31:3990–3999
Barsoum MJ, Yuan H, Gerencser AA, Liot G, Kushnareva Y, Graber S, Kovacs I, Lee WD, Waggoner J, Cui J, White AD, Bossy B, Martinou JC, Youle RJ, Lipton SA, Ellisman MH, Perkins GA, Bossy-Wetzel E (2006) Nitric oxide-induced mitochondrial fission is regulated by dynamin-related GTPases in neurons. EMBO J 25:3900–3911
Basser PJ, Mattiello J, LeBihan D (1994) MR diffusion tensor spectroscopy and imaging. Biophys J 66:259–267
Bhat MA, Rios JC, Lu Y, Garcia-Fresco GP, Ching W, St Martin M, Li J, Einheber S, Chesler M, Rosenbluth J, Salzer JL, Bellen HJ (2001) Axon-glia interactions and the domain organization of myelinated axons requires neurexin IV/Caspr/Paranodin. Neuron 30:369–383
Black JA, Cummins TR, Plumpton C, Chen YH, Hormuzdiar W, Clare JJ, Waxman SG (1999) Upregulation of a silent sodium channel after peripheral, but not central, nerve injury in DRG neurons. J Neurophysiol 82:2776–2785
Boiko T, Rasband MN, Levinson SR, Caldwell JH, Mandel G, Trimmer JS, Matthews G (2001) Compact myelin dictates the differential targeting of two sodium channel isoforms in the same axon. Neuron 30:91–104
Bonita R, Beaglehole R, Asplund K (1994) The worldwide problem of stroke. Curr Opin Neurol 7:5–10
Brand-Schieber E, Werner P (2003a) AMPA/kainate receptors in mouse spinal cord cell-specific display of receptor subunits by oligodendrocytes and astrocytes and at the nodes of Ranvier. Glia 42:12–24
Brand-Schieber E, Werner P (2003b) (+/−)-Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainate receptor subunit expression in mouse versus rat spinal cord white matter: similarities in astrocytes but differences in oligodendrocytes. Neurosci Lett 345:126–130
Bristow EA, Griffiths PG, Andrews RM, Johnson MA, Turnbull DM (2002) The distribution of mitochondrial activity in relation to optic nerve structure. Arch Ophthalmol 120:791–796
Brown AM, Wender R, Ransom BR (2001) Metabolic substrates other than glucose support axon function in central white matter. J Neurosci Res 66:839–843
Campbell LW, Hao SY, Thibault O, Blalock EM, Landfield PW (1996) Aging changes in voltage-gated calcium currents in hippocampal CA1 neurons. J Neurosci 16:6286–6295
Cavallotti C, Pacella E, Pescosolido N, Tranquilli-Leali FM, Feher J (2002) Age-related changes in the human optic nerve. Can J Ophthalmol 37:389–394
Cavallotti C, Cavallotti D, Pescosolido N, Pacella E (2003) Age-related changes in rat optic nerve: morphological studies. Anat Histol Embryol 32:12–16
Chang DT, Reynolds IJ (2006) Differences in mitochondrial movement and morphology in young and mature primary cortical neurons in culture. Neuroscience 141:727–736
Chen H, McCaffery JM, Chan DC (2007) Mitochondrial fusion protects against neurodegeneration in the cerebellum. Cell 130:548–562

Cheung EC, McBride HM, Slack RS (2007) Mitochondrial dynamics in the regulation of neuronal cell death. Apoptosis 12:979–992
Craner MJ, Hains BC, Lo AC, Black JA, Waxman SG (2004) Co-localization of sodium channel Nav1.6 and the sodium-calcium exchanger at sites of axonal injury in the spinal cord in EAE. Brain 127:294–303
Davatzikos C, Resnick SM (2002) Degenerative age changes in white matter connectivity visualized in vivo using magnetic resonance imaging. Cereb Cortex 12:767–771
Del Zoppo GJ (1995) Why do all drugs work in animals but none in stroke patients? 1. Drugs promoting cerebral blood flow. J Intern Med 237:79–88
Del Zoppo GJ (1998) Clinical trials in acute stroke: why have they not been successful? Neurology 51:S59–S61
Desilva TM, Billiards SS, Borenstein NS, Trachtenberg FL, Volpe JJ, Kinney HC, Rosenberg PA (2008) Glutamate transporter EAAT2 expression is up-regulated in reactive astrocytes in human periventricular leukomalacia. J Comp Neurol 508:238–248
Dirnagl U, Iadecola C, Moskowitz MA (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 22:391–397
Dupree JL, Girault JA, Popko B (1999) Axo-glial interactions regulate the localization of axonal paranodal proteins. J Cell Biol 147:1145–1152
Enblad P, Frykholm P, Valtysson J, Silander HC, Andersson J, Fasth KJ, Watanabe Y, Langstrom B, Hillered L, Persson L (2001) Middle cerebral artery occlusion and reperfusion in primates monitored by microdialysis and sequential positron emission tomography. Stroke 32: 1574–1580
Erecinska M, Silver IA (1994) Ions and energy in mammalian brain. Prog Neurobiol 43:37–71
Fern R, Moller T (2000) Rapid ischemic cell death in immature oligodendrocytes: a fatal glutamate release feedback loop. J Neurosci 20:34–42
Fern R, Ransom BR (1997) Ischemic injury of optic nerve axons: the nuts and bolts. Clin Neurosci 4:246–250
Fern RJ, Hahm MS, Lu HK, Liu LP, Gorelick FS, Barrett PQ (1995) Ca2+/calmodulin-dependent protein kinase II activation and regulation of adrenal glomerulosa Ca2+ signaling. Am J Physiol 269:F751–F760
Ferro JM, Madureira S (2002) Age-related white matter changes and cognitive impairment. J Neurol Sci 203–204:221–225
Follett PL, Rosenberg PA, Volpe JJ, Jensen FE (2000) NBQX attenuates excitotoxic injury in developing white matter. J Neurosci 20:9235–9241
Foster RE, Connors BW, Waxman SG (1982) Rat optic nerve: electrophysiological, pharmacological and anatomical studies during development. Dev Brain Res 3:371–386
Frykholm P, Andersson JL, Valtysson J, Silander HC, Hillered L, Persson L, Olsson Y, Yu WR, Westerberg G, Watanabe Y, Langstrom B, Enblad P (2000) A metabolic threshold of irreversible ischemia demonstrated by PET in a middle cerebral artery occlusion-reperfusion primate model. Acta Neurol Scand 102:18–26
Gadea A, Lopez-Colome AM (2001) Glial transporters for glutamate, glycine and GABA I. Glutamate transporters. J Neurosci Res 63:453–460
Gallo V, Russell JT (1995) Excitatory amino acid receptors in glia: different subtypes for distinct functions? J Neurosci Res 42:1–8
Garthwaite G, Brown G, Batchelor AM, Goodwin DA, Garthwaite J (1999) Mechanisms of ischaemic damage to central white matter axons: a quantitative histological analysis using rat optic nerve. Neuroscience 94:1219–1230
Ginsberg MD, Busto R (1989) Rodent models of cerebral ischemia. Stroke 20:1627–1642
Goldberg MP, Ransom BR (2003) New light on white matter. Stroke 34:330–332
Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB, Noda M, Tamkun MM, Waxman SG, Wood JN, Catterall WA (2000) Nomenclature of voltage-gated sodium channels. Neuron 28:365–368
Hachinski VC, Potter P, Merskey H (1987) Leuko-araiosis. Arch Neurol 44:21–23
Hazell AS, Rao KV, Danbolt NC, Pow DV, Butterworth RF (2001) Selective down-regulation of the astrocyte glutamate transporters GLT-1 and GLAST within the medial thalamus in experimental Wernicke's encephalopathy. J Neurochem 78:560–568

Hinman JD, Peters A, Cabral H, Rosene DL, Hollander W, Rasband MN, Abraham CR (2006) Age-related molecular reorganization at the node of Ranvier. J Comp Neurol 495:351–362
Hollenbeck PJ (2005) Mitochondria and neurotransmission: evacuating the synapse. Neuron 47: 331–333
Hollenbeck PJ, Saxton WM (2005) The axonal transport of mitochondria. J Cell Sci 118: 5411–5419
Jacquard C, Trioulier Y, Cosker F, Escartin C, Bizat N, Hantraye P, Cancela JM, Bonvento G, Brouillet E (2006) Brain mitochondrial defects amplify intracellular [Ca2+] rise and neurodegeneration but not Ca2+ entry during NMDA receptor activation. FASEB J 20:1021–1023
Jensen FE (2006) Developmental factors regulating susceptibility to perinatal brain injury and seizures. Curr Opin Pediatr 18:628–633
Karadottir R, Cavelier P, Bergersen LH, Attwell D (2005) NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438:1162–1166
Karbowski M, Youle RJ (2003) Dynamics of mitochondrial morphology in healthy cells and during apoptosis. Cell Death Differ 10:870–880
Karbowski M, Arnoult D, Chen H, Chan DC, Smith CL, Youle RJ (2004) Quantitation of mitochondrial dynamics by photolabeling of individual organelles shows that mitochondrial fusion is blocked during the Bax activation phase of apoptosis. J Cell Biol 164:493–499
Kawahara K, Kosugi T, Tanaka M, Nakajima T, Yamada T (2005) Reversed operation of glutamate transporter GLT-1 is crucial to the development of preconditioning-induced ischemic tolerance of neurons in neuron/astrocyte co-cultures. Glia 49:349–359
Kemper TL (1994) Neuroanatomical and neuropathological changes during aging and dementia. Oxford University Press, New York
Kukley M, Capetillo-Zarate E, Dietrich D (2007) Vesicular glutamate release from axons in white matter. Nat Neurosci 10:311–320
Kurtzke JF (1994) Epidemiology of stroke: methods and trends. Health Rep 6:13–21
Landfield PW, Pitler TA (1984) Prolonged Ca2+–dependent after hyperpolarizations in hippocampal neurons of aged rats. Science 226:1089–1092
Lee L, Baltan Tekkok S, Deem S, Moulding J, Lam AM (2006) A novel model of in vivo pig posterior optic nerve ischemia. American Society of Anesthesiologists, Chicago, 14–17 Oct
Leroux P, Hennebert C, Catteau J, Legros H, Hennebert O, Laudenbach V, Marret S (2010) Neuroprotective effects vary across nonsteroidal antiinflammatory drugs in a mouse model of developing excitotoxic brain injury. Neuroscience 167:716–723
Lesnefsky EJ, Moghaddas S, Tandler B, Kerner J, Hoppel CL (2001) Mitochondrial dysfunction in cardiac disease: ischemia–reperfusion, aging, and heart failure. J Mol Cell Cardiol 33: 1065–1089
Li S, Mealing GA, Morley P, Stys PK (1999) Novel injury mechanism in anoxia and trauma of spinal cord white matter: glutamate release via reverse Na^+-dependent glutamate transport. J Neurosci 19:RC1–RC9
Longuemare MC, Rose CR, Farrell K, Ransom BR, Waxman SG, Swanson RA (1999) K(+)-induced reversal of astrocyte glutamate uptake is limited by compensatory changes in intracellular Na+. Neuroscience 93:285–292
Matute C, Alberdi E, Domercq M, Perez-Cerda F, Perez-Samartin A, Sanchez-Gomez MV (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 24:224–230
McDonald JW, Althomsons SP, Hyrc KL, Choi DW, Goldberg MP (1998) Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. Nat Med 4:291–297
Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, Yin X, Trapp BD, McRory JE, Rehak R, Zamponi GW, Wang W, Stys PK (2006) NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439:988–992
Misgeld T, Kerschensteiner M, Bareyre FM, Burgess RW, Lichtman JW (2007) Imaging axonal transport of mitochondria in vivo. Nat Methods 4:559–561

Morgan SC, Taylor DL, Pocock JM (2004) Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. J Neurochem 90:89–101

Mozdy AD, Shaw JM (2003) A fuzzy mitochondrial fusion apparatus comes into focus. Nat Rev Mol Cell Biol 4:468–478

Nicholls DG, Johnson-Cadwell L, Vesce S, Jekabsons M, Yadava N (2007) Bioenergetics of mitochondria in cultured neurons and their role in glutamate excitotoxicity. J Neurosci Res 85:3206–3212

O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW (2006) 1,026 experimental treatments in acute stroke. Ann Neurol 59:467–477

O'Sullivan M, Jones DK, Summers PE, Morris RG, Williams SC, Markus HS (2001) Evidence for cortical "disconnection" as a mechanism of age-related cognitive decline. Neurology 57: 632–638

Oberheim NA, Tian GF, Han X, Peng W, Takano T, Ransom B, Nedergaard M (2008) Loss of astrocytic domain organization in the epileptic brain. J Neurosci 28:3264–3276

Ogura K, Ogawa M, Yoshida M (1994) Effects of ageing on microglia in the normal rat brain: immunohistochemical observations. Neuroreport 5:1224–1226

Oka A, Belliveau MJ, Rosenberg PA, Volpe JJ (1993) Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms, and prevention. J Neurosci 13:1441–1453

Olivares R, Montiel J, Aboitiz F (2001) Species differences and similarities in the fine structure of the mammalian corpus callosum. Brain Behav Evol 57:98–105

Otsuga D, Keegan BR, Brisch E, Thatcher JW, Hermann GJ, Bleazard W, Shaw JM (1998) The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast. J Cell Biol 143:333–349

Ouardouz M, Nikolaeva MA, Coderre E, Zamponi GW, McRory JE, Trapp BD, Yin X, Wang W, Woulfe J, Stys PK (2003) Depolarization-induced Ca2+ release in ischemic spinal cord white matter involves L-type Ca2+ channel activation of ryanodine receptors. Neuron 40:53–63

Ouardouz M, Malek S, Coderre E, Stys PK (2006) Complex interplay between glutamate receptors and intracellular Ca2+ stores during ischaemia in rat spinal cord white matter. J Physiol 577:191–204

Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. Stroke 27:1641–1646; discussion 1647

Parihar MS, Brewer GJ (2007) Simultaneous age-related depolarization of mitochondrial membrane potential and increased mitochondrial reactive oxygen species production correlate with age-related glutamate excitotoxicity in rat hippocampal neurons. J Neurosci Res 85: 1018–1032

Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG (1994) Glutamate-mediated astrocyte-neuron signalling. Nature 369:744–747

Pendlebury ST, Blamire AM, Lee MA, Styles P, Matthews PM (1999) Axonal injury in the internal capsule correlates with motor impairment after stroke. Stroke 30:956–962

Peters A, Sethares C (1993) Aging and the Meynert cells in rhesus monkey primary visual cortex. Anat Rec 236:721–729

Rintoul GL, Filiano AJ, Brocard JB, Kress GJ, Reynolds IJ (2003) Glutamate decreases mitochondrial size and movement in primary forebrain neurons. J Neurosci 23:7881–7888

Romera C, Hurtado O, Botella SH, Lizasoain I, Cardenas A, Fernandez-Tome P, Leza JC, Lorenzo P, Moro MA (2004) In vitro ischemic tolerance involves upregulation of glutamate transport partly mediated by the TACE/ADAM17-tumor necrosis factor-alpha pathway. J Neurosci 24: 1350–1357

Rothman SM, Olney JW (1995) Excitotoxicity and the NMDA receptor–still lethal after eight years. Trends Neurosci 18:57–58

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16:675–686

Saab A, Baltan S, Möbius I, Tzvetavona J, Hirrlinger B, Goetze HM, Jahn W, Huang A, Pérez-Samartín F, Pérez-Cerdá C, Matute S, Löwel K-A, Nave F, Kirchhoff (2012) Oligodendroglial NMDA receptor signalling in coupling axonal and glial energy metabolism. Society for Neuroscience Abstracts, New Orleans, LA

Salter MG, Fern R (2005) NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438:1167–1171

Sanchez-Gomez MV, Matute C (1999) AMPA and kainate receptors each mediate excitotoxicity in oligodendroglial cultures. Neurobiol Dis 6:475–485

Scavone C, Munhoz CD, Kawamoto EM, Glezer I, de Sa LL, Marcourakis T, Markus RP (2005) Age-related changes in cyclic GMP and PKG-stimulated cerebellar Na, K-ATPase activity. Neurobiol Aging 26:907–916

Schmidt R, Fazekas F, Offenbacher H, Lytwyn H, Blematl B, Niederkorn K, Horner S, Payer F, Freidl W (1991) Magnetic resonance imaging white matter lesions and cognitive impairment in hypertensive individuals. Arch Neurol 48:417–420

Scott SV, Cassidy-Stone A, Meeusen SL, Nunnari J (2003) Staying in aerobic shape: how the structural integrity of mitochondria and mitochondrial DNA is maintained. Curr Opin Cell Biol 15:482–488

Selzner M, Selzner N, Jochum W, Graf R, Clavien PA (2007) Increased ischemic injury in old mouse liver: an ATP-dependent mechanism. Liver Transpl 13:382–390

Silva-Adaya D, Perez-De La Cruz V, Herrera-Mundo MN, Mendoza-Macedo K, Villeda-Hernandez J, Binienda Z, Ali SF, Santamaria A (2008) Excitotoxic damage, disrupted energy metabolism, and oxidative stress in the rat brain: antioxidant and neuroprotective effects of L-carnitine. J Neurochem 105:677–689

Sloane JA, Hollander W, Moss MB, Rosene DL, Abraham CR (1999) Increased microglial activation and protein nitration in white matter of the aging monkey. Neurobiol Aging 20:395–405

Smirnova E, Shurland DL, Ryazantsev SN, van der Bliek AM (1998) A human dynamin-related protein controls the distribution of mitochondria. J Cell Biol 143:351–358

Sozmen EG, Kolekar A, Havton LA, Carmichael ST (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180: 261–272

Stys PK (2004) White matter injury mechanisms. Curr Mol Med 4:113–130

Stys PK, Ransom BR, Waxman SG, Davis PK (1990) Role of extracellular calcium in anoxic injury of mammalian central white matter. Proc Natl Acad Sci U S A 87:4212–4216

Stys PK, Waxman SG, Ransom BR (1992) Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na^+ channels and Na^+-Ca^{2+} exchanger. J Neurosci 12:430–439

Szabadkai G, Rizzuto R (2004) Participation of endoplasmic reticulum and mitochondrial calcium handling in apoptosis: more than just neighborhood? FEBS Lett 567:111–115

Szabadkai G, Simoni AM, Chami M, Wieckowski MR, Youle RJ, Rizzuto R (2004) Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca2+ waves and protects against Ca2+–mediated apoptosis. Mol Cell 16:59–68

Szatkowski M, Barbour B, Attwell D (1990) Non-vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake. Nature 348:443–446

Tekkök SB, Goldberg MP (2001) Ampa/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. J Neurosci 21:4237–4248

Tekkok SB, Brown AM, Ransom BR (2003) Axon function persists during anoxia in mammalian white matter. J Cereb Blood Flow Metab 23:1340–1347

Tekkok SB, Ye Z, Ransom BR (2007) Excitotoxic mechanisms of ischemic injury in myelinated white matter. J Cereb Blood Flow Metab 27:1540–1552

Thorell WE, Leibrock LG, Agrawal SK (2002) Role of RyRs and IP3 receptors after traumatic injury to spinal cord white matter. J Neurotrauma 19:335–342

Toescu EC (2005) Normal brain ageing: models and mechanisms. Philos Trans R Soc Lond B Biol Sci 360:2347–2354

Ture D, Yasargil MG, Krist AF (1996) The arteries of the corpus callosum: a microsurgical anatomic study. Neurosurgery 39:1075–1085

Underhill SM, Goldberg MP (2007) Hypoxic injury of isolated axons is independent of ionotropic glutamate receptors. Neurobiol Dis 25:284–290

Uo T, Dworzak J, Kinoshita C, Inman DM, Kinoshita Y, Horner PJ, Morrison RS (2009) Drp1 levels constitutively regulate mitochondrial dynamics and cell survival in cortical neurons. Exp Neurol 218:274–285

Verkhratsky A, Orkand RK, Kettenmann H (1998) Glial calcium: homeostasis and signaling function. Physiol Rev 78:99–141

Vincent SL, Peters A, Tigges J (1989) Effects of aging on the neurons within area 17 of rhesus monkey cerebral cortex. Anat Rec 223:329–341

Wolf JA, Stys PK, Lusardi T, Meaney D, Smith DH (2001) Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. J Neurosci 21: 1923–1930

Wolfram-Gabel R, Maillot C, Koritke JG, Laude M (1987) [The vascularization of the human tela choroidea of the lateral ventricle]. Acta Anat (Basel) 128:301–321

Wrathall JR, Choiniere D, Teng YD (1994) Dose-dependent reduction of tissue loss and functional impairment after spinal cord trauma with the AMPA/kainate antagonist NBQX. J Neurosci 14:6598–6607

Yamauchi H, Fukuda H, Oyanagi C (2002) Significance of white matter high intensity lesions as a predictor of stroke from arteriolosclerosis. J Neurol Neurosurg Psychiatry 72:576–582

Ye ZC, Wyeth MS, Baltan-Tekkok S, Ransom BR (2003) Functional hemichannels in astrocytes: a novel mechanism of glutamate release. J Neurosci 23:3588–3596

Zhang K, Sejnowski TJ (2000) A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci U S A 97:5621–5626

Zhang M, Li WB, Geng JX, Li QJ, Sun XC, Xian XH, Qi J, Li SQ (2007) The upregulation of glial glutamate transporter-1 participates in the induction of brain ischemic tolerance in rats. J Cereb Blood Flow Metab 27:1352–1368

Ziskin JL, Nishiyama A, Rubio M, Fukaya M, Bergles DE (2007) Vesicular release of glutamate from unmyelinated axons in white matter. Nat Neurosci 10:321–330