

LETTERS

NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia

I. Micu¹, Q. Jiang¹, E. Coderre¹, A. Ridsdale¹, L. Zhang¹, J. Woulfe¹, X. Yin², B. D. Trapp², J. E. McRory³, R. Rehak³, G. W. Zamponi³, W. Wang¹ & P. K. Stys¹

Central nervous system myelin is a specialized structure produced by oligodendrocytes that ensheaths axons, allowing rapid and efficient saltatory conduction of action potentials¹. Many disorders promote damage to and eventual loss of the myelin sheath, which often results in significant neurological morbidity. However, little is known about the fundamental mechanisms that initiate myelin damage, with the assumption being that its fate follows that of the parent oligodendrocyte. Here we show that NMDA (*N*-methyl-*D*-aspartate) glutamate receptors mediate Ca²⁺ accumulation in central myelin in response to chemical ischaemia *in vitro*. Using two-photon microscopy, we imaged fluorescence of the Ca²⁺ indicator X-rhod-1 loaded into oligodendrocytes and the cytoplasmic compartment of the myelin sheath in adult rat optic nerves. The AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)/kainate receptor antagonist NBQX² completely blocked the ischaemic Ca²⁺ increase in oligodendroglial cell bodies, but only modestly reduced the Ca²⁺ increase in myelin. In contrast, the Ca²⁺ increase in myelin was abolished by broad-spectrum NMDA receptor antagonists (MK-801, 7-chlorokynurenic acid, D-AP5^{3,4}), but not by more selective blockers of NR2A and NR2B subunit-containing receptors (NVP-AAM077⁵ and ifenprodil^{2,4}). *In vitro* ischaemia causes ultrastructural damage to both axon cylinders and myelin⁶. NMDA receptor antagonism greatly reduced the damage to myelin. NR1, NR2 and NR3 subunits were detected in myelin by immunohistochemistry and immunoprecipitation, indicating that all necessary subunits are present for the formation of functional NMDA receptors. Our data show that the mature myelin sheath can respond independently to injurious stimuli. Given that axons are known to release glutamate⁷⁻⁹, our finding that the Ca²⁺ increase was mediated in large part by activation of myelinic NMDA receptors suggests a new mechanism of axo-myelinic signalling. Such a mechanism may represent a potentially important therapeutic target in disorders in which demyelination is a prominent feature, such as multiple sclerosis, neurotrauma, infections (for example, HIV encephalomyelopathy) and aspects of ischaemic brain injury.

We measured [Ca²⁺]_i changes from the cytosolic compartment of compact myelin (the space of compact myelin between the cytoplasmic leaflets of the spiralled myelin membranes, the 'major dense line') and oligodendroglial cell bodies in live adult rat optic nerves (Fig. 1). Nerves were rendered chemically ischaemic by using the mitochondrial inhibitor NaN₃ and omitting glucose. Ca²⁺-dependent fluorescence from myelin regions ($F_{Ca,my}$) began to increase within minutes of the onset of ischaemia and rose to 50 ± 22% above baseline after 30 min (mean ± s.d., $n = 94$ myelin regions from individual axons). Ca²⁺-dependent fluorescence from oligodendroglial cytoplasm ($F_{Ca,ol}$) rose with a similar time course,

increasing by 43 ± 27% ($n = 53$) above control at 30 min. Removal of bath Ca²⁺ abolished both the myelinic and oligodendroglial ischaemic Ca²⁺ increase (Fig. 1f), indicating that in both compartments, Ca²⁺ increases are mainly a result of influx from the extracellular space.

We then explored potential routes of Ca²⁺ entry into both oligodendrocytes and myelin. Kynurenic acid (1 mM), a broad-spectrum antagonist of ionotropic glutamate receptors, completely blocked the increase in $F_{Ca,ol}$ (-8 ± 8% of baseline at 30 min ischaemia, $n = 10$, $P = 1.6 \times 10^{-6}$ versus drug-free ischaemia) and $F_{Ca,my}$ (-1 ± 16%, $n = 29$, $P = 2.0 \times 10^{-6}$). The more selective AMPA/kainate receptor antagonist NBQX (30 μ M) completely blocked the rise in $F_{Ca,ol}$ (-5 ± 11% of control at 30 min, $n = 33$, $P = 1.6 \times 10^{-6}$) but had only a modest effect on myelinic Ca²⁺ rise (40 ± 18%, $n = 57$ in NBQX versus 50 ± 22% in drug-free ischaemia, $P = 0.003$; Fig. 2). This finding has two important implications. First, the fact that an AMPA/kainate receptor antagonist is able to dissociate the ischaemic Ca²⁺ increase in the two compartments indicates that the myelinic Ca²⁺ increase is not merely a passive phenomenon attributable to diffusion of Ca²⁺ from the parent cell body, but is instead mediated by a distinct mechanism. Second, the pharmacological profile suggests an unexpected route of Ca²⁺ influx into myelin. Although AMPA and kainate receptors are known to be present and functional in white matter glia, NMDA receptors are generally thought to be absent¹⁰; yet the above results suggest that NMDA receptors mediate ischaemic Ca²⁺ rise in the myelin sheath.

To explore this possibility more directly, we treated ischaemic optic nerves with selective NMDA receptor antagonists. The non-competitive channel blocker MK-801 (10 μ M), and competitive glutamate (D-AP5, 100 μ M) and glycine (7-chlorokynurenic acid, 200–500 μ M) site antagonists³, completely blocked the increase in myelinic Ca²⁺ (-1 ± 19%, $n = 50$ for MK-801; -16 ± 17%, $n = 103$ for D-AP5; -20 ± 14%, $n = 45$ for 7-chlorokynurenic acid at 30 min, versus 50 ± 22% without drug; $P = 2.1 \times 10^{-6}$ for all groups; Fig. 2c). All three agents also reduced the ischaemic Ca²⁺ increase in oligodendrocyte cell bodies (20 ± 35%, $n = 20$, $P = 0.0002$; -20 ± 20%, $n = 49$, $P = 1.8 \times 10^{-6}$; -22 ± 23%, $n = 23$, $P = 1.8 \times 10^{-6}$, respectively, versus 43 ± 27% without drug), suggesting that NMDA receptors may also directly admit Ca²⁺ into these cells. This is consistent with the observation of NMDA-induced currents in rat spinal cord oligodendrocytes¹¹.

Functional NMDA receptors are heteromers composed of NR1 and NR2 subunits, which express the glycine and glutamate recognition sites, respectively^{2,4}. Discovered more recently, the NR3 subunit may serve a modulatory role¹². A number of splice variants have been identified for each subunit, resulting in a potentially very diverse family of receptors. More specific blockers of NR2A- and

¹Ottawa Health Research Institute, Division of Neuroscience and Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario K1Y 4K9, Canada.

²Department of Neurosciences, Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA. ³Department of Physiology and Biophysics, Hotchkiss Brain Institute, University of Calgary, Alberta T2N 4N1, Canada.

NR2B-containing receptors (NVP-AAM077⁵, tested at 0.4–5 μ M; ifenprodil⁴, 10 μ M) did not significantly reduce the ischaemic increase in $F_{Ca,my}$ ($43 \pm 14\%$, $n = 22$ for 0.4 μ M NVP; $63 \pm 34\%$, $n = 28$ for ifenprodil, versus $50 \pm 22\%$ without antagonist) (Fig. 2c). Taken together, our pharmacological data suggest that NMDA receptors (possibly those not composed of NR2A or NR2B subunits) mediate the bulk of pathological Ca^{2+} accumulation into the myelin sheath of adult optic axons. This is in contrast to oligodendrocytes, in which Ca^{2+} increase and cell death are mediated mainly, but possibly not exclusively (see above), by glutamate receptors of the AMPA/kainate class as previously reported¹³ (but see ref. 14).

We next used immunohistochemistry to localize NMDA receptor subunits (Fig. 3). NR1 subunits were detected just outside axon cylinders. Because virtually all adult rat optic axons are myelinated, regions immediately adjacent to axon cylinders represent myelin. NR2 and NR3 showed a similar distribution. Staining for all three subunits often appeared in discrete high densities in myelin of larger

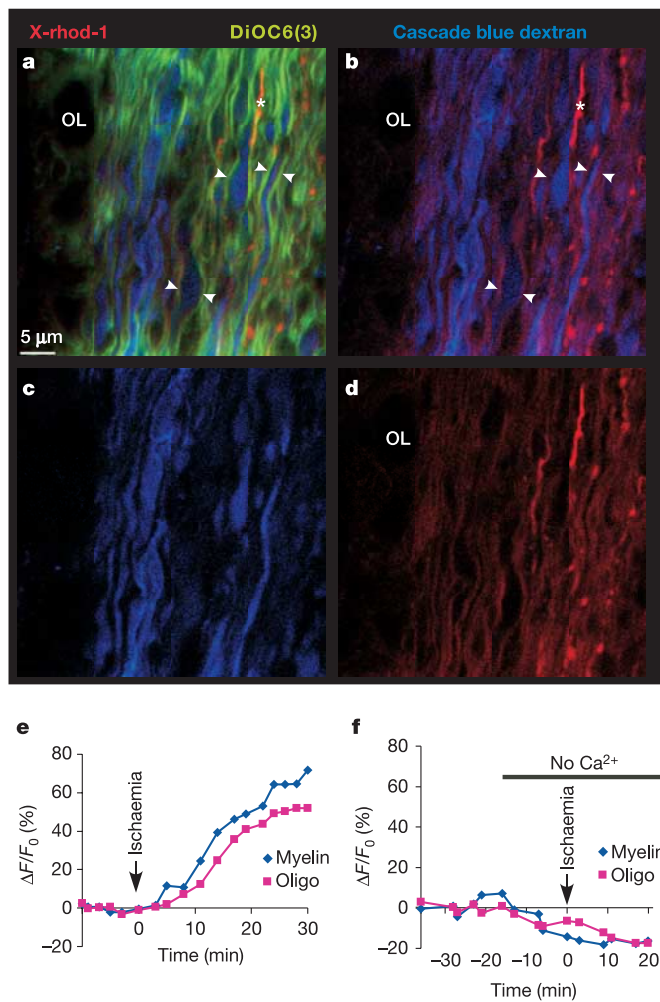


Figure 1 | Ca^{2+} imaging in optic nerve oligodendrocytes and myelin. **a–d**, The same image is shown, but with various colour channels suppressed. Cascade blue dextran outlines axon cylinders, DiOC6(3) strongly stains myelin (arrowheads in **a**), and X-rhod-1 partitions into myelin (arrowheads in **b**) and oligodendrocytes (OL). Glial processes unstained by DiOC6(3) (asterisk in **a** and **b**) were excluded. **e**, Representative time course of X-rhod-1 fluorescence change in oligodendrocytes and myelin. Fluorescence began to increase in both compartments within minutes of ischaemia, rising to ~50–60% above control levels after about 30 min. **f**, This increase was prevented in Ca^{2+} -free aCSF (with 1 mM EGTA added to chelate residual Ca^{2+}), indicating that Ca^{2+} increase in oligodendrocytes and myelin depends on influx from the extracellular space.

axons. Immunoelectron microscopy detected labelling of myelin internodes with antibodies against NR1, NR2 and NR3 (Fig. 3e–g), with gold particles conspicuous in the inner and outer margins of myelin. Furthermore, NR1 was detected on the rough endoplasmic reticulum and Golgi membranes located in the perinuclear cytoplasm of oligodendrocytes (Fig. 3h), supporting the idea of NR1 synthesis by the myelin-forming cell.

We extracted the myelin fraction from whole optic nerve on a sucrose gradient (see Supplementary Methods). NR1, NR2 and NR3

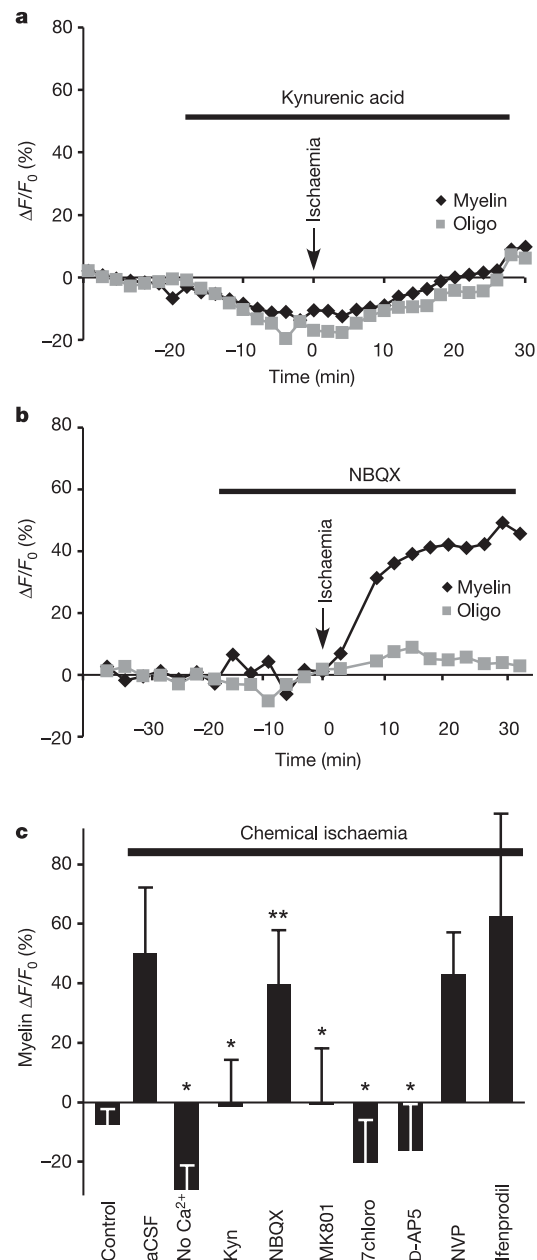


Figure 2 | Effects of glutamate receptor antagonists on ischaemic Ca^{2+} increase. **a**, Kynurenic acid greatly reduced the ischaemic Ca^{2+} increase in both oligodendrocytes and myelin (compare to Fig. 1e). **b**, NBQX blocked the Ca^{2+} increase in oligodendrocytes much more potently than in myelin. **c**, Bar graph summarizing X-rhod-1 fluorescence change (mean \pm s.d.) at 30 min of ischaemia compared to pre-ischaemic/pre-drug baseline. Together, these data indicate that NMDA receptors are largely responsible for myelinic Ca^{2+} accumulation. * $P < 10^{-5}$; ** $P = 0.003$ versus drug-free ischaemia (aCSF) (\pm DMSO as applicable). For each treatment group, 29–104 myelin regions of interest from at least three different experiments were analysed.

subunits could not be detected in the unpurified fraction (not shown). After immunoprecipitation, however, we were able to detect NR1-, NR2- and NR3-positive bands at the expected molecular masses by immunoblotting. Moreover, NR2 and NR3 subunits were detected in lysate precipitated by NR1 antibodies from the myelin fraction (Fig. 3i). We note that the NR2 blot revealed a band of approximately 140 kDa, which, given the known cross-reactivity of the antibody used (see Supplementary Methods), could be consistent with NR2C and/or NR2D subunits. Overall, our biochemical analysis supports the hypothesis that myelin expresses functional NMDA receptor complexes.

The expression of functional NMDA receptors in the mature myelin sheath and their ability to mediate ischaemic Ca^{2+} accumulation suggests that these receptors may have an important role in the degeneration of myelin, and may be an early trigger for eventual demyelination. Examination of ischaemic optic nerve axons by electron microscopy showed marked destruction of axoplasmic constituents, with disappearance of microtubules and neurofilament profiles⁶ (Fig. 4). In addition, the myelin sheath began to split focally (Fig. 4a, arrowheads), with loosening and separation of the lamellae. NMDA receptor inhibition significantly reduced the pathological changes in the myelin sheath; in contrast, there was little evidence that axon cylinders *per se* were protected, indicating that these elements are damaged by another mechanism (Fig. 4b). This finding is consistent with our observation that NMDA receptor block failed to blunt axoplasmic (as opposed to myelinic) Ca^{2+} increase during ischaemia (Supplementary Fig. 3).

Myelin is a vital structure that has been observed histopathologically to degenerate in a broad range of CNS disorders¹⁵. These demyelinated central axons conduct aberrantly or not at all, and account for significant clinical morbidity. During development,

reciprocal axo–glial signalling has a key role in the initiation and completion of myelination¹⁶. In contrast, little is known about the signalling that may take place between axons and the mature myelin sheath. Numerous studies have demonstrated the presence of many enzymes¹⁷ (including Na^+ , K^+ -ATPase¹⁸), connexins¹⁹ and AMPA/kainate receptors^{20,21} in mature myelin, suggesting that this structure may be more dynamic and metabolically active than previously thought, actively participating in signal transduction and translocation of ions and small molecules. Here we show that mature myelin is compromised by ischaemia and that Ca^{2+} ions accumulate in its cytosolic domain in an NMDA receptor-dependent fashion. Given the presence of Ca^{2+} -sensitive enzymes such as calpain-1 and phospholipase C in central myelin^{17,22}, it is possible that such Ca^{2+} accumulation then promotes degradation of a number of key structural myelin components.

NMDA receptors must bind both glutamate and glycine as obligatory co-agonists, and activation additionally requires depolarization to remove Mg^{2+} -dependent block^{2,23}. Both neurotransmitters are taken back up into cells by electrogenic, Na^+ -coupled transporters, which can operate in the reverse, transmitter efflux mode under conditions of increased intracellular $[\text{Na}^+]$ and membrane depolarization^{24,25}. Injured axons accumulate Na^+ , lose K^+ and depolarize (see ref. 26 for a review), thus promoting the release of glutamate via this mechanism⁸. CNS myelinated axons have been shown to express glycine transporters as well²⁷; therefore they could release this amino acid under pathological conditions in a manner analogous to glutamate. Together with ischaemic depolarization of cellular membranes, this would complete the requirements for sustained and deleterious activation of myelinic NMDA receptors.

NMDA receptors have fundamental roles in the normal physiology of the CNS²⁸ and in many diseases^{4,29}. These receptors have not been

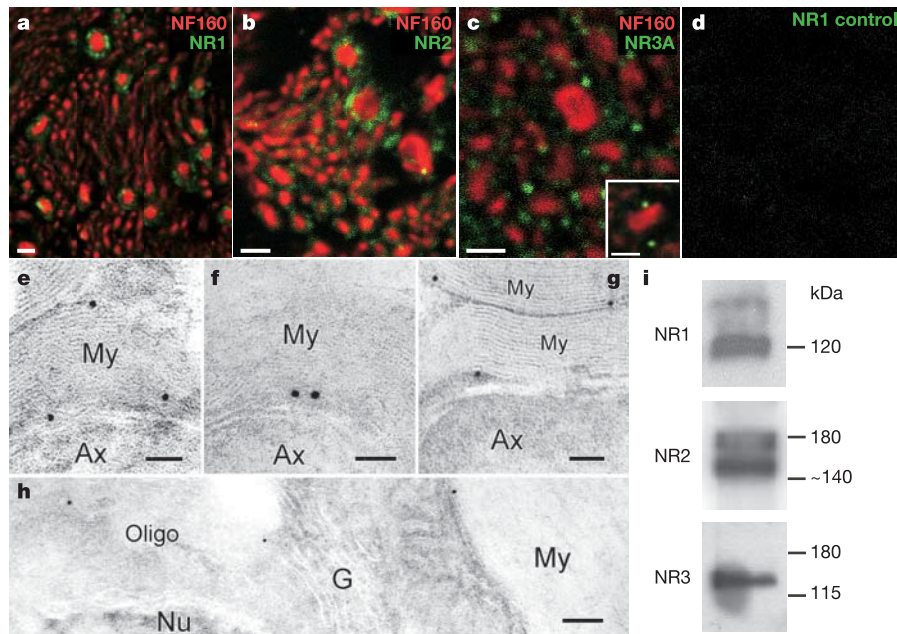


Figure 3 | Immunodetection of NMDA receptor subunits in myelin.

a, Nerves were immunostained for neurofilament (NF)160 and NR1 subunits, and imaged by confocal microscopy. Most axons in adult nerves are myelinated, and therefore regions immediately outside axon cylinders represent myelin sheaths. Faint, homogeneous NR1 signal was seen just outside NF160-positive axon cylinders, and was most apparent in larger axons. Myelin of larger-diameter axons showed occasional punctate regions of more intense NR1 label. **b**, **c**, NR2 and NR3 subunits showed a similar distribution. Inset in **c** shows an example of punctate NR3A staining at the inner and outer myelin rim of a larger axon. **d**, Control with primary antibodies omitted. Controls for NR2 and NR3 were similar.

e–g, Immunogold staining of optic nerve for NR1 (**e**), NR2 (**f**) and NR3 (**g**) showed specific myelin labelling (My), with enrichment of gold particles at the inner and outer margins of the sheath (Ax, axon). **h**, NR1 was also detected on the rough endoplasmic reticulum and Golgi membranes (G) in oligodendrocyte perinuclear cytoplasm (Nu, nucleus). **i**, A pan-NR1 monoclonal was used to immunoprecipitate receptor complexes from myelin fractions in all three blots, which were then probed with anti-NR1, anti-NR2 or anti-NR3 antisera. All three subunits were detected, and both NR2 and NR3 are complexed with NR1. Scale bars, 2 μm (**a–c**), 50 nm (**e–g**), 100 nm (**h**).

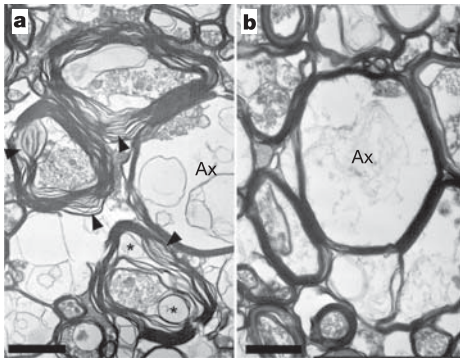


Figure 4 | NMDA receptor inhibition protects myelin against ischaemic injury. **a**, Vehicle-treated nerves showed marked damage to myelin after 60 min of *in vitro* chemical ischaemia, with separation of the compact lamellae (arrowheads), swellings and vacuolations (asterisks). Axon cylinders (Ax) were also severely damaged and swollen, with the disappearance of neurofilament and microtubule profiles. **b**, The NMDA receptor antagonist 7-chlorokynurenic acid (500 μ M) protected myelin. Occasional focal loosening of the compact sheath was observed, but was much less pronounced compared to controls (2.0 ± 1.0 lamellar separations per $65 \mu\text{m}^2$ in 7-chlorokynurenic acid versus 4.0 ± 1.4 in vehicle alone, $P = 0.0016$). In contrast, damage to axon cylinders (Ax) remained severe. Scale bars, 1 μm .

considered important in the physiology or pathobiology of white matter, but it is likely that their presence in myelin suggests a normal physiological role. Action potential traffic releases glutamate from axons^{7,9}, and glycine could also be released in an activity-dependent manner. If myelinic NMDA receptors are composed of NR2C and/or NR2D subunits, as our data suggest, they would be more sensitive to glutamate and glycine^{2,4}. This would assist signalling in a system (myelinated axons) that is, in contrast to synapses, presumably less able to release large local amounts of transmitter. The activity-dependent release of axonal glutamate, in quantities reflecting an integral of ongoing electrical traffic in the sourcing fibre, might activate myelinic NMDA receptors to support axo–glial communication. This would represent an elegant solution for allowing axons, which may be conducting different volumes of impulse traffic, to selectively signal their myelin sheaths about their levels of activity. Such a signal could in turn be transduced to the parent oligodendrocyte for purposes of myelin production commensurate with the degree of electrical activity of individual fibres. Under pathophysiological conditions, excessive stimulation of myelinic NMDA receptors may cause direct Ca^{2+} -dependent damage to this structure. For instance, the NMDA receptor antagonist memantine²⁹ was reported to reduce neurological disability in a rat model of experimental autoimmune encephalomyelitis³⁰. Although the mechanisms are not clear, it is possible that this agent reduced myelin damage by antagonizing the effects of glutamate released in the inflammatory lesions. A more in-depth understanding of such an NMDA-receptor-dependent ‘axo–myelinic’ communication could guide the design of more effective treatments for disorders in which demyelination of central white matter tracts is a prominent and clinically devastating phenomenon.

METHODS

Please refer to Supplementary Information for additional details.

Ca^{2+} imaging. Rat optic nerves from adult Long Evans rats were loaded with the Ca^{2+} indicator X-rhod-1 AM to report the free $[\text{Ca}^{2+}]$ in myelin and oligodendrocytes, and with DiOC6(3) to outline myelin. Dextran-conjugated Ca^{2+} -insensitive cascade blue was selectively loaded into axons. Two-photon excited fluorescence images were collected every 2 min using a custom-modified Nikon D-Eclipse C1 laser scanning microscope. Emitted fluorescence was collected using appropriate filters. Image data were imported into ImageTrak software (written by P.K.S.; <http://www.ohri.ca/stys/imagetrak>) for analysis. All

quantitative fluorescence changes are reported after a standard 30-min exposure to ischaemia. In-place immunohistochemistry of live nerves was performed to ascertain the localization of X-rhod-1. Fluorescence-lifetime measurements confirmed that fluorescence intensity of X-rhod-1 reported $[\text{Ca}^{2+}]$ changes, and Mn^{2+} quench studies showed that emission originated from the cytosolic compartment of myelin (see Supplementary Methods).

Immunohistochemistry and immunoelectron microscopy. For light microscopy, deeply anaesthetized adult rats were perfused with saline then 4% paraformaldehyde in 0.1 M phosphate buffer. Optic nerves were post-fixed, placed in 20% sucrose overnight, then sectioned at a thickness of 30 μm in a cryostat. All washes were done in TBS/1% Triton X-100, and sections were blocked with 10% normal donkey serum, 3% dry milk for 1 h at 20 °C. Samples were incubated overnight in primary antibodies, then secondary antibodies were applied. Sections were imaged on a Nikon D-Eclipse C1 confocal microscope. Immunoelectron microscopy was performed using standard techniques as described in Supplementary Methods.

Immunoblots and immunoprecipitation. Myelin fractions were incubated on ice for 1 h, centrifuged for 15 min (4 °C) at 15,000g, and the supernatant was collected for overnight dialysis against the binding solution (300 mM NaCl, 50 mM Tris pH 7.5 and 0.1% Triton X-100). Total protein (250 μg in 50 μl) was immunoprecipitated with a pan-NR1 monoclonal antibody. The complex was centrifuged, resuspended and loaded onto a 6% acrylamide SDS–PAGE gel. Samples were transferred to a PVDF membrane and western blot analysis was performed using a different NR1 polyclonal antibody, or NR2 or NR3 antisera. Appropriate negative controls were performed by omitting the primary (precipitating) antibody or probing with secondary antibody only (data not shown).

Received 20 August; accepted 29 November 2005.

Published online 21 December 2005.

- Waxman, S. G., Kocsis, J. D. & Stys, P. K. *The Axon: Structure, Function and Pathophysiology* (Oxford Univ. Press, Oxford, 1995).
- Dingledine, R., Borges, K., Bowie, D. & Traynelis, S. F. The glutamate receptor ion channels. *Pharmacol. Rev.* **51**, 7–61 (1999).
- Waxman, E. A. & Lynch, D. R. *N*-methyl-D-aspartate receptor subtypes: multiple roles in excitotoxicity and neurological disease. *Neuroscientist* **11**, 37–49 (2005).
- Parsons, C. G., Danysh, W. & Quack, G. Glutamate in CNS disorders as a target for drug development: an update. *Drug News Perspect.* **11**, 523–569 (1998).
- Auberson, Y. P. *et al.* 5-phosphonomethylquinolinediones as competitive NMDA receptor antagonists with a preference for the human 1A/2A, rather than 1A/2B receptor composition. *Bioorg. Med. Chem. Lett.* **12**, 1099–1102 (2002).
- Waxman, S. G., Black, J. A., Stys, P. K. & Ransom, B. R. Ultrastructural concomitants of anoxic injury and early post-anoxic recovery in rat optic nerve. *Brain Res.* **574**, 105–119 (1992).
- Lin, S. C. *et al.* Climbing fiber innervation of NG2-expressing glia in the mammalian cerebellum. *Neuron* **46**, 773–785 (2005).
- Li, S., Mealing, G. A., Morley, P. & Stys, P. K. Novel injury mechanism in anoxia and trauma of spinal cord white matter: glutamate release via reverse Na^{+} -dependent glutamate transport. *J. Neurosci.* **19**, RC16 (1999).
- Chiu, S. Y. & Kriegler, S. Neurotransmitter-mediated signalling between axons and glial cells. *Glia* **11**, 191–200 (1994).
- Matute, C., Alberdi, E., Ibarretxe, G. & Sanchez-Gomez, M. V. Excitotoxicity in glial cells. *Eur. J. Pharmacol.* **447**, 239–246 (2002).
- Ziak, D., Chvatal, A. & Sykova, E. Glutamate-, kainate- and NMDA-evoked membrane currents in identified glial cells in rat spinal cord slice. *Physiol. Res.* **47**, 365–375 (1998).
- Das, S. *et al.* Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature* **393**, 377–381 (1998).
- McDonald, J. W., Althomsons, S. P., Hyrc, K. L., Choi, D. W. & Goldberg, M. P. Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. *Nature Med.* **4**, 291–297 (1998).
- Rosenberg, P. A. *et al.* Mature myelin basic protein-expressing oligodendrocytes are insensitive to kainate toxicity. *J. Neurosci.* **Res. **71**, 237–245 (2003).**
- Lazzarini, R. A. *et al.* *Myelin Biology and Disorders* (Elsevier, Amsterdam, 2004).
- Michailov, G. V. *et al.* Axonal neuregulin-1 regulates myelin sheath thickness. *Science* **304**, 700–703 (2004).
- Quarles, R. H., Macklin, W. B. & Morell, P. In *Basic Neurochemistry* (eds Siegel, G. J., Albers, R. W., Brady, S. T. & Price, D. L.) 51–72 (Elsevier, San Diego, in the press).
- Mrsulja, B. J., Zalewski, A. A. & Coping, G. Ultracytochemical localization of ouabain-sensitive K^{+} -dependent, p-nitrophenyl phosphatase in myelin. *Brain Res.* **343**, 154–158 (1985).
- Kamasawa, N. *et al.* Connexin-47 and connexin-32 in gap junctions of oligodendrocyte somata, myelin sheaths, paranodal loops and

- Schmidt-Lanterman incisures: implications for ionic homeostasis and potassium siphoning. *Neuroscience* **136**, 65–86 (2005).
20. Brand-Schieber, E. & Werner, P. 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 (2003).
 21. Li, S. & Stys, P. K. Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. *J. Neurosci.* **20**, 1190–1198 (2000).
 22. Hinman, J. D., Duce, J. A., Siman, R. A., Hollander, W. & Abraham, C. R. Activation of calpain-1 in myelin and microglia in the white matter of the aged rhesus monkey. *J. Neurochem.* **89**, 430–441 (2004).
 23. Mayer, M. L., Westbrook, G. L. & Guthrie, P. B. Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* **309**, 261–263 (1984).
 24. Aragon, C. & Lopez-Corcuera, B. Structure, function and regulation of glycine neurotransmitters. *Eur. J. Pharmacol.* **479**, 249–262 (2003).
 25. Danbolt, N. C. Glutamate uptake. *Prog. Neurobiol.* **65**, 1–105 (2001).
 26. Stys, P. K. White matter injury mechanisms. *Curr. Mol. Med.* **4**, 113–130 (2004).
 27. Spike, R. C., Watt, C., Zafra, F. & Todd, A. J. An ultrastructural study of the glycine transporter GLYT2 and its association with glycine in the superficial laminae of the rat spinal dorsal horn. *Neuroscience* **77**, 543–551 (1997).
 28. Bear, M. F. & Malenka, R. C. Synaptic plasticity: LTP and LTD. *Curr. Opin. Neurobiol.* **4**, 389–399 (1994).
 29. Lipton, S. A. & Rosenberg, P. A. Excitatory amino acids as a final common pathway for neurologic disorders. *N. Engl. J. Med.* **330**, 613–622 (1994).
 30. Wallstrom, E. *et al.* Memantine abrogates neurological deficits, but not CNS inflammation, in Lewis rat experimental autoimmune encephalomyelitis. *J. Neurol. Sci.* **137**, 89–96 (1996).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements This work was supported by the NINDS, CIHR, Heart and Stroke Foundation of Ontario Center for Stroke Recovery, an HSFO Career Investigator Award, the Canadian Institute for Photonic Innovations, and the generosity of private donors to P.K.S. G.W.Z. is a Canada Research Chair and an AHFMR Senior Scholar. This work was additionally supported by the CIHR (G.W.Z., J.E.M.) and NINDS (B.D.T.). We thank Y. Auberson for the gift of NVP-AAM077; J. A. Wang and C. E. Morris for providing HEK293 cells for fluorescence-lifetime measurements; and M. Nikolaeva for assistance with fluo-4 Ca^{2+} imaging.

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to P.K.S. (pstys@ohri.ca).