Cytoskeletal Disruption Following Contusion Injury to the Rat Spinal Cord

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Abstract. Following experimental spinal cord injury (SCI), there is a delayed loss of neurofilament proteins but relatively little is known regarding the status of other cytoskeletal elements. The purpose of the present study was to compare the extent and time course of the MAP2 loss with that of neurofilament proteins, and to examine tau protein levels and distribution following SCI. Within 1 to 6 hours following SCI, there is rapid loss of MAP2, tau, and nonphosphorylated neurofilament proteins at the injury site. In contrast, the loss of phosphorylated neurofilament proteins was not significant until 1 week postinjury. In addition to the loss of MAP2 protein, there was extensive beading of MAP2-immunoreactive dendrites extending into the white matter. This was most pronounced 1 hour after injury and gradually resolved such that beading was no longer evident 2 weeks after SCI. The time course of beading resolution is similar to that of behavioral recovery following SCI, but the functional significance of the beading remains to be determined. Together, these results demonstrate that there are 2 phases of cytoskeletal disruption following SCI: a rapid loss of MAP2, tau, and nonphosphorylated neurofilament proteins, and a delayed loss of phosphorylated neurofilaments.

Key Words: Dendrites; Microtubule-associated proteins; Neurofilament proteins; Tau proteins.

INTRODUCTION

The pathogenesis of traumatic spinal cord injury involves 2 components: the initial mechanical injury and subsequent secondary events including ischemia, edema, inflammation, and loss of ionic homeostasis, resulting in extensive neuronal degeneration over the next few days (for review see refs. 1–3). The secondary events, particularly ischemia, result in elevated extracellular levels of excitatory amino acids and excessive calcium entry into cells (4–7). A critical step in the evolution of secondary injury is thought to be the activation of calcium-dependent proteases (calpains) and degradation of cytoskeletal proteins (8).

Proteolysis of neurofilament proteins has been examined extensively following spinal cord injury. Loss of both 68 kDa and 200 kDa neurofilament proteins is relatively slow and progressive (8). Much of the neurofilament loss is in white matter axons and is thought to result from elevations in intracellular Ca$^{2+}$, leading to the activation of calpain (calcium-activated neutral protease). There is evidence of calpain activation at the injury site as early as 1 h postinjury consistent with the hypothesis that neurofilament loss is calpain mediated. Dephosphorylated neurofilaments are more sensitive to calpain proteolysis than the phosphorylated proteins (11) and dephosphorylated 200 kDa neurofilament protein is preferentially lost following experimental spinal cord injury (12).

Other cytoskeletal proteins, including the microtubule-associated proteins tau and MAP2, are also sensitive calpain substrates, but their status following acute spinal cord injury has not been examined in detail. Both tau and MAP2 are abundant in neurons although they are also present in non-neuronal cells, including oligodendrocytes. Tau is predominantly located in axons, particularly unmyelinated axons (13). In the adult CNS, MAP2 is localized to the neuronal somatodendritic compartment (14). The prevalence of MAP2 and tau in the gray matter may render these proteins particularly vulnerable to spinal cord injury since the secondary ischemia following spinal cord injury is particularly severe in the gray matter (1) and dorsal gray matter is the site of most severe calcium derangement (5). MAP2 may be especially vulnerable to excitotoxic insult since excitatory amino acid receptors are mainly localized to dendrites and neuronal cell bodies (15–17), and calpains are also enriched in dendrites (18, 19).

Previously, we observed that MAP2 levels declined rapidly following spinal cord injury, accompanied by an increase in 150 kDa spectrin breakdown products and an increase in the ratio of activated calpain I (76 kDa) relative to the precursor (80 kDa) (10). However, the previous study was restricted to 24 hours (h) following injury and did not examine levels of cytoskeletal proteins other than MAP2. The present study compares the relative loss of MAP2, neurofilament proteins, and tau, at time points up to 4 weeks postinjury.

MATERIALS AND METHODS

Spinal Cord Injury

Adult, female, Long-Evans rats weighing 200–220 g were used in this study. Spinal cord contusion injuries were performed using the New York University impactor device, which provides an accurate and reproducible method for producing...
Fig. 1. Alterations in MAP2 levels and immunoreactivity following spinal cord injury. In the normal rat spinal cord (A), MAP2 immunoreactivity is largely restricted to gray matter neurons. Within 1 h following contusion injury to the spinal cord (NYU impactor, 25 mm setting), a pronounced loss of MAP2 is evident at the lesion epicenter (B). One week following injury, MAP2 immunoreactivity appears normal in a section just caudal to the lesion (C). In addition to MAP2 immunoreactivity within the gray matter, MAP2 immunoreactive dendrites extend into the white matter in the lateral and ventral funiculus. D–F: The
contusion injury to the rat spinal cord (20) and is utilized by the Multicenter Animal Spinal Cord Injury Study (21). Prior to receiving injury, animals were anesthetized with sodium pentobarbital (50 mg/kg, ip) and a dorsal laminectomy was performed to expose the spinal cord at thoracic (T) level T10 with the dura intact. Clamping at vertebrae T6 and T11 stabilized the vertebral column. The 10-g impact rod was dropped from a height of 25 mm, inflicting a contusion injury of moderate severity (22). Control animals received a dorsal laminectomy. Postinjury care included administration of the analgesic buprenorphine hydrochloride (0.1 mg/kg, im, 2 × daily, for 2–3 days), the antibiotic Polystart (3 mg/kg, sc, once), and 0.9% saline solution (10 ml, sc, daily, for 5–7 days) to prevent dehydration. If blood was detected in the urine, the rats were treated with Polystart until the urine became clear. The animals were weighed once a day until they regained their preoperative weight (usually 10–14 days) and twice weekly thereafter. The rats were euthanized with an overdose of sodium pentobarbital (100 mg/kg, ip) and decapitated. The spinal cords were removed quickly, frozen in powdered dry ice, and stored at −80°C until the tissue was homogenized in 360 μl solution of Tris-saline (50 mM, pH 7.5) containing protease inhibitors (2.5 mg/ml leupeptin, 1 mg/ml pepstatin, and 20 mM 4-(2-Aminoethyl)benzenesulfonylfluoride, HCl). The homogenate was centrifuged at 100,000 g for 30 minutes, the supernatant was collected and stored at −70°C. The pellet was resuspended in homogenization buffer with 2% SDS added. Protein content was determined using the BCA protein assay (Pierce, Rockford, IL). MAP2 and tau were localized almost exclusively in the supernatant, whereas neurofilament proteins were present in both the supernatant and the pellet fractions.

Tissue Processing

For light microscopy and immunocytochemistry, the rats were transcardially perfused with a saline solution followed by phosphate-buffered 4% paraformaldehyde. A 30-mm spinal cord segment containing the impact site was cut into 10 blocks of 3 mm each, the sections were placed in an embedding cassette and immersed in the same fixative overnight. After dehydration with graded alcohol, the tissue blocks were cleared in xylene and embedded in paraffin wax. Eight-μm-thick cross sections of the spinal cord were cut with a microtome and mounted onto Fisher Superfrost Plus microscope slides.

To obtain immunoblots, the animals were given an overdose of pentobarbital (120 mg/kg) and decapitated. The spinal cords were removed quickly, frozen in powdered dry ice, and stored at −70°C. A 10-mm spinal cord segment containing the impact site was homogenized in 360 μl solution of Tris-saline (50 mM, pH 7.5) containing protease inhibitors (2.5 mg/ml leupeptin, 500 mM EDTA 1 mg/ml pepstatin, and 20 mM 4-(2-Aminothyl)benzenesulfonylfluoride, HCl). The homogenate was centrifuged at 100,000 g for 30 minutes (min), the supernatant was collected and stored at −70°C. The pellet was resuspended in homogenization buffer with 2% SDS added. Protein content was determined using the BCA protein assay (Pierce, Rockford IL). MAP2 and tau were localized almost exclusively in the supernatant, whereas neurofilament proteins were present in both the supernatant and the pellet fractions.

Immunocytochemistry

Following deparaffinization and rehydration, the tissue sections were incubated at 100°C for 15 min in antigen retrieval Citra solution (BioGenex, San Ramon, CA). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide (H2O2) in 50 mM Tris-buffered saline, pH7.5, containing 0.1% Triton X-100 for 30 min (TTBS). The sections were then incubated in TTBS containing 2% normal horse serum for 1 h at room temperature, followed by incubation with primary antibodies in a humid chamber overnight at room temperature. Primary antibodies used in this study included tau-1 (1:1,000, gift from Dr. L.I. Binder), AP-14 (1:2,000, gift from Dr. L.I. Binder), and SMI-31 (1:2,000, Sternberger-Meyer Immunocytochemicals, Baltimore, MD). The antibodies were diluted in TTBS containing 1% normal horse serum. After rinsing 3 times, the sections were incubated in 1:200 biotinylated goat anti-mouse IgG (Vector, Burlingame, CA) for 1 h and then in 1:50 streptavidin peroxidase (Jackson Immunoresearch, Costa Mesa, CA) for 1 h in TTBS containing 1% horse serum. Immunoperoxidase activity was visualized by incubating in a 3,3′-diaminobenzidine solution (DAB, 0.5 mg/ml TTBS) with 0.01% H2O2 for 3–5 min. The sections were rinsed, dehydrated, cleared, and coverslipped. Some sections were stained with hematoxylin and eosin as a counter stain. For negative controls, immunostaining was performed as described above except that the primary antibodies were omitted.

Immunoblots

SDS-PAGE was performed according to the method of Laemmli (23) using a mini-gel apparatus (Bio-Rad, Rockville Center, NY). Protein samples (5 to 20 μg protein) were mixed with sample buffer (2:1), heated to 100°C for 5 min, and loaded onto 6.5% (for MAP2 and NF) or 10% (for tau) Tris-glycine polyacrylamide gels and run at 150 V for approximately 90 min. Two lanes of each gel contained an internal control, consisting of pooled control spinal cord samples, to allow for comparison between gels. The proteins were then transferred to the nitrocellulose membranes (0.45 μm) in tris-glycine/methanol buffer at 100 V for 60–80 min at 4°C. The nitrocellulose membranes were blocked for 60 min in 5% nonfat milk in TTBS at room temperature. Blots were then incubated overnight at room temperature in primary antibodies diluted in TTBS. Primary antibodies included AP-20 (1:5,000, Sigma, St. Louis, MO), tau-1 (1:10,000, gift from Dr. L.I. Binder), 8C11 (1:10,000, gift from Elan Pharmaceuticals), SMI-31 (1:10,000, Sternberger-Meyer Immunocytochemicals), SMI-32 (1:10,000, Sternberger-Meyer Immunocytochemicals). After rinsing 3 times in TTBS, blots were incubated for 1 h at RT with peroxidase-conjugated goat anti-mouse IgG (1:5,000, Jackson). After rinsing 4 × 10 min,
blots were incubated for 1 min in the chemiluminescent substrate (SuperSignal West Pico, Pierce, Rockford, IL) and exposed to an X-ray film (T-Mat, Kodak, Rochester, NY). Some blots were incubated for 90 min at RT with alkaline phosphatase conjugated goat anti-mouse IgG (1:5,000, Sigma) in TTBS (pH 8.0), then developed in nitro-blue tetrazolium (NBT, 0.33 mg/ml) and 5-Brom-4-chlor-3-indolylhydrogenphosphat-p-tolidin (BCIP, 0.16 mg/ml) (Promega, Madison, WI).

Data Analysis

Changes in the immunocytochemical staining pattern for the cytoskeletal proteins were examined using an Olympus AX70 microscope. The rostral and caudal extent of the observed changes was measured relative to the center of the impact site.

Immunoblots were scanned (Umax Astra 1200 S), the image was captured to a Power Macintosh 8500/120 computer and analyzed using gel subroutine of the public domain NIH Image 1.62 (http://rsb.info.nih.gov/nih-image/) to determine the relative density of each individual band. Since film response is not linear, various protein concentrations (0.1–40 μg) of control homogenates were immunoblotted using each of the primary antibodies, and the relative density units plotted against protein concentration to obtain a standard curve. Values from the various blots were compared using the internal controls. Final results are expressed as a percentage of control values (mean ± SEM). Statistical analysis of the immunoblot data was performed using a one-way ANOVA for overall significance, followed by the Fisher Least Significant Difference test when the F ratio was significant. A minimum of 4 animals was examined at each postinjury time point.

RESULTS

Microtubule-associated Protein 2 (MAP2)

Immunocytochemistry: The AP-14 antibody used for immunocytochemical detection of MAP2 was a generous gift from Dr. L. I. Binder and recognizes the high molecular weight MAP2 isoforms, similar to AP-20 (24). In the normal rat spinal cord, MAP2 immunoreactivity was localized predominantly in dendrites within the gray matter and the white matter of the spinal cord (Fig. 1A, D). Most neuronal perikarya exhibited weak immunoreactivity, but staining was stronger in motor neurons in the ventral horn. MAP2 immunoreactivity was not observed in astroglia or oligodendrocytes.

As early as 1 h after injury and at all subsequent time points examined, there was a dramatic decrease in dendritic MAP2 immunoreactivity (Fig. 1B) within 3–4 mm of the lesion epicenter in both the rostral and caudal directions. Remaining dendrites had a beaded appearance. Adjacent to the lesion site, some neurons exhibited much darker MAP2 immunostaining in the perikaryon as described previously (10). In more distal sections, MAP2 immunoreactivity appeared normal in the gray matter (Fig. 1C), but there was a pronounced beading of dendrites extending into the white matter (Fig. 1E). One h and 6 h following SCI, the beading was evident throughout the entire rostral-caudal extent of the spinal cord. The extent of beading was most severe adjacent to the impact site with severity gradually diminishing towards rostral and caudal extremities. In spinal cord cross sections, the most affected region was the lateral funiculus. Beading could also be observed in the ventral funiculus and dorsal funiculus, which contains the corticospinal tract. By 48 h, the beading was diminished in intensity and largely restricted to the thoracic and lumbar regions. One week following SCI, dendritic beading was more restricted, being most pronounced closest to the lesion epicenter. Two weeks following SCI, dendritic beading was no longer detected (Fig. 1F).

ImmunobLOTS: The epitope recognized by the AP-20 antibody (Sigma) used for MAP2 detection on immunoblots is similar to that of the AP-14 antibody used for immunocytochemistry. Both antibodies recognize the same high molecular weight (280 kDa) forms of MAP2 (2a and 2b), but not the embryonic MAP2c isoform (24). MAP2 levels were decreased by 80% 1 h after injury, by 90% at 6 h, and remained at similar levels throughout the 4 week postinjury time course examined (Fig. 1G).

Tau Protein

Immunocytochemistry: Tau protein is predominantly localized to the axons of neurons, but is also present in...
Fig. 3. Alterations in high molecular weight neurofilament (NF200) levels and immunoreactivity following spinal cord injury. In the normal rat spinal cord (A), SMI-31 immunoreactivity is largely restricted to white matter axons. SMI-31 recognizes a phosphorylated NF200 epitope. At higher magnification (B), the white matter staining is relatively even and diffuse. A similar pattern of immunoreactivity is evident in the white matter 1 h following injury (not shown). Six h postinjury (C), the first signs of axonal degeneration are evident within white matter axons, illustrated by accumulation of SMI-31 immunoreactivity in some axon bundles with the surrounding area becoming devoid of staining. Progression of the axonal degeneration is evident at 48 h (D) and 2 weeks (E) after injury. In the supernatant fraction, the decline in levels of SMI-31 immunoreactive protein (F) was not significant up to 48 h postinjury, but was significant at the 1 week and 2 week postinjury time points ($p < 0.05$). In contrast,
the perikarya, dendrites, and in non-neuronal cells, including oligodendrocytes. The tau-1 antibody used for immunocytochemistry in the present study recognizes a nonphosphorylated epitope (Ser 199–202) (13, 25). Tau-1 immunoreactivity was evident in both the gray and white matter, and was prominent in the corticospinal tract (Fig. 2A).

One h after contusion injury, tau-1 immunoreactivity was markedly decreased in the injury epicenter, but not in the spared white matter (Fig. 2B). Tau-1 immunostaining in the corticospinal tract was maintained rostral to the injury site at all postinjury time points (Fig. 2C). Caudal to the injury site, the corticospinal tract was no longer stained with tau-1 one week postinjury and at later time points, indicating degeneration of the tract below the level of injury site (Fig. 2D). Postinjury alterations in tau-1 immunoreactivity in the white matter were similar to those observed for phosphorylated neurofilaments (SMI-31 antibody).

**Immunoblots:** In normal spinal cord, the monoclonal antibody tau-1 recognized both the normal tau 48–67 kDa tau isoforms present throughout the CNS, as well as the 110 kDa ‘big tau’ isoform present in the spinal cord and PNS. The 110 kDa band is present at lower levels than other tau isoforms. There was a rapid loss of the 48–67 kDa tau isoforms to 35% of control levels within 1 h of SCI, after which tau-1 levels remain relatively constant throughout the 4 week postinjury time course examined (Fig. 2E). In addition, a 25 kDa tau breakdown product became evident. There was also a downward shift in tau migration on SDS-PAGE gels, indicating tau dephosphorylation. Tau levels were also examined using the phosphorylation-independent 8C11 antibody (Fig. 2F). The results obtained were similar to those observed with tau-1. The extent of 110 kDa tau loss was similar to that of the 48–67 kDa isoforms (Fig. 2G, H).

**Neurofilament Protein**

**Immunocytochemistry:** The monoclonal antibody SMI-31 antibody recognizes a phosphorylated epitope on the 200 kDa neurofilament protein (26). Alterations in SMI-31 immunoreactivity were similar to those described previously for neurofilament proteins. In the normal spinal cord, SMI-31 immunoreactivity was present mainly in white matter where staining is relatively diffuse (Fig. 3A, B). A similar pattern of SMI-31 immunoreactivity is observed in spared white matter within 1 h after injury. At 6 h postinjury, SMI-31 immunoreactivity is increased in some axon bundles, but decreased in the surrounding area (Fig. 3C). This reflects the early stages of axonal degeneration and the alterations are restricted to the immediate vicinity of the injury site. Progression of the axonal degeneration is evident at 48 h (Fig. 3D) and 2 weeks (Fig. 2E) after injury. At these later time points, the alterations in SMI-31 immunoreactivity are evident throughout the cord both rostral and caudal to the injury.

**Immunoblots:** In the supernatant fraction, levels of SMI-31 immunoreactive protein decreased progressively following SCI. The decline was not significant up to 48 h postinjury, but was significant at 1 week and 2 weeks after SCI (Fig. 3F). In contrast, SMI-31 immunoreactive protein levels in the 100,000 × g pellet did not exhibit a significant decline until 4 weeks after SCI (Fig. 3G).

High molecular weight neurofilament protein levels were also assessed with the SMI-32 antibody, which recognizes a nonphosphorylated epitope. Levels of SMI-32 immunoreactive protein in the supernatant fraction decreased rapidly following injury to approximately 40% of control levels at 6 h, then remained low at the 48 h and 2 week postinjury time points examined (Fig. 3H).

In contrast, levels of SMI-32 immunoreactive protein in the pellet were not decreased until more than 48 h after injury (Fig. 3I).

**DISCUSSION**

Previous studies of cytoskeletal disruption following spinal cord injury have largely focused on axonal neurofilament proteins (8, 12, 27–30). Proteolytic degradation of neurofilament proteins is thought to result from elevated intracellular calcium levels, due to calcium influx through NMDA receptors and other mechanisms, resulting in the activation of the neutral protease calpain (10, 31–33). The results of the present study are in agreement with previous studies demonstrating a slow or delayed loss of neurofilament proteins following spinal cord injury (8, 30), and the preferential loss of nonphosphorylated neurofilaments (12). The results further indicate a preferential loss of phosphorylated high molecular weight neurofilaments in the soluble versus insoluble fraction. Insoluble neurofilament subunits are associated with the stable neurofilament triplet polymers, whereas soluble subunits are not tightly associated with the NF triplet and

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SMI-31 immunoreactive protein levels in the 100,000 × g pellet were not significantly different from control levels until 4 weeks after SCI (G). NF200 levels were also assessed with the SMI-32 antibody, which recognizes a nonphosphorylated epitope. Levels of SMI-32 immunoreactive protein in the supernatant fraction decreased following injury to 40% of control levels at 6 h. Similar values were observed at later postinjury time points. In the insoluble fraction (pellet), SMI-32 immunoreactive protein was significantly lower than control 2 weeks after SCI, but not at the earlier 6 h and 48 h time points (I). *Significantly different from control levels (p < 0.05).
are typically more heavily phosphorylated than neurofilaments in the insoluble fraction (34, 35). The results suggest that stable neurofilament triplet polymers may be more resistant to proteolytic degradation than the soluble neurofilament subunits.

Other cytoskeletal proteins including tau and MAP2 are also sensitive calpain substrates (36, 37) and the somatodendritic localization of MAP2 would be expected to enhance its vulnerability to spinal cord injury and excitotoxic mechanisms. The results of the present study suggest that tau protein is an excellent marker of neuronal damage following spinal cord injury. Tau is present in both the gray and white matter, and although predominantly localized to axons, may also be present in dendrites (13). In the spinal cord, tau-1 immunoreactivity is greater in dorsal corticospinal tract than other ascending or descending axonal fibers such as cuneate fiber tract. Within 1 week of injury, tau-1 immunoreactivity is lost in the corticospinal tract caudal to the injury site, but is maintained on the rostral side. This is consistent with previous studies indicating that neurons of the rat corticospinal tract survive axotomy and remain functional even 1 year after transection at the cervical level (38, 39). This contrasts with the much slower loss of neurofilament protein in the same pathway (29). On immunoblots, loss of both normal tau (48–67 kDa) and 'big tau' (110 kDa) was evident following SCI. This was accompanied by the appearance of a 25 kDa breakdown product, consistent with calpain-mediated tau proteolysis (36).

Previously, we observed a rapid decline in MAP2 levels following SCI, accompanied by calpain activation (10). The present study extends the time course of the previous study and provides a direct comparison of MAP2 and neurofilament changes following SCI. The rapid and pronounced loss of MAP2 contrasts with the slow, progressive decline in phosphorylated NF200. These results confirm the sensitivity of MAP2 to SCI, and suggest that MAP2 levels may serve as an excellent marker of injury severity. In a separate study, we demonstrated that MAP2 levels correlate with injury severity and that interventions that protect against MAP2 loss (NMDA receptor antagonists, AMPA receptor antagonists, and calpain inhibitors) also result in improved locomotor behavior following SCI (manuscript in preparation). In the adult CNS, MAP2 is exclusively localized to the gray matter, suggesting that the extent of MAP2 loss reflects the extent of gray matter damage. Following traumatic brain injury, MAP2 loss is evident (40, 41) and is highly correlated with neuron loss (42). MAP2 loss has also been used as a quantifiable marker of neuronal damage following focal cerebral ischemia, preceding and predicting neuron loss and lesion volume (43). MAP2 loss and dendritic breakdown is also an early indicator of neuron loss in transient cerebral ischemia (44).

The MAP2 loss observed following ischemia and traumatic brain injury is thought to be functionally significant and to reflect the neuron loss contributing to the behavioral deficits. Following experimental SCI at T10, it is unlikely that the gray matter loss contributes to the behavioral deficits. The neurons at this level innervate abdominal regions, but contribute little to locomotor behavior. Kainate-induced destruction of gray matter at T10 does not impair open field locomotor performance (45). Thus, following experimental spinal cord injury in the thoracic region, MAP2 loss is an excellent marker of injury severity and gray matter damage, but the functional deficits are due to the loss of descending axonal projections.

In addition to MAP2 loss in the lesion epicenter and cavity, we observed dendritic beading over the full extent of the spinal cord. This was most pronounced at the 1 h postinjury time point and gradually resolved over 2 weeks. This time course of the disappearance of MAP2 beading approximately parallels behavioral recovery following SCI (22). In vitro, dendritic beading is reversible, resolving within 2 h after removal of a mild excitotoxic insult (46). Although dendritic beading is often thought to represent an early sign of neuronal degeneration, beading is also evident following mild insults that afford neuroprotection, such as sublethal ischemia (46). Contrasting electrophysiologic studies suggest that beaded dendrites correspond with impaired excitability (47) or hyperexcitability (48). The functional significance of the dendritic beading observed following SCI remains to be determined.

In summary, the results illustrate 2 phases of cytoskeletal disruption following spinal cord injury. Cytoskeletal proteins in the gray matter, including MAP2, tau, and nonphosphorylated high molecular weight neurofilament subunits are decreased within the first few hours after SCI. In contrast, loss of phosphorylated neurofilament proteins in white matter axons is delayed. These results are consistent with the rapid calpain activation following SCI, largely due to Ca\(^{2+}\) influx through NMDA receptors, and the degradation of sensitive calpain substrates. The delayed loss of phosphorylated neurofilament proteins in the white matter also results from calpain activation (8, 49), suggesting 2 phases of calpain activation following spinal cord injury (8). In addition to the rapid loss of cytoskeletal proteins at the injury site, widespread MAP2 immunoreactive dendritic beading may represent mild neuronal insult throughout the spinal cord following SCI.

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