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# Compression Injury of Mammalian Spinal Cord In Vitro and the Dynamics of Action Potential Conduction Failure

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## SUMMARY AND CONCLUSIONS

*1.* White matter strips from the ventral spinal cord of adult guinea pigs were isolated in vitro, and their electrophysiological characteristics and response to controlled focal compression injury were examined. A double sucrose gap technique was used for stimulation and recording at opposite ends of a 12.5-mm-diam central well superfused with oxygenated Krebs solution.

*2.* The compound action potential recorded with the sucrose gap was similar in form to single fiber potentials recorded with intra-axonal electrodes, including the presence of a prolonged depolarizing afterpotential.

*3.* Three types of conduction block resulting from compression were identified: an immediate, spontaneously reversible component, which may result from a transient increase in membrane permeability and consequent disturbance of ionic distribution; a second component that was irreversible within 1–2 h of recording, perhaps resulting from complete axolemmal disruption; and a third component, which may have been due to disruption of the myelin sheath, that appeared to be reversible with application of 10–100 $\mu$M of the potassium channel blocker 4-aminopyridine.

*4.* Conduction deficits—decreased amplitude and increased latency of the compound potential—were stable between 5 and 60 min postinjury, and their intensity corellated with the extent of initial compression over a full range of severity.

*5.* Stimulus-response data indicate that mechanical damage to axons in compression was evenly distributed across the caliber spectrum, suggesting that the susceptibility of large caliber axons seen histopathologically after injury in vivo may be based on delayed, secondary processes.

*6.* The model provides the ability to monitor changes in the properties of central myelinated axons after compression injury in the absence of pathological variables related to vascular damage. This initial investigation found no evidence of secondary deterioration of axons in the 1st h after injury, although there was evidence of both transient and lasting mechanical damage to axons and their myelin sheaths.

## INTRODUCTION

Contusion or compression of the mammalian spinal cord results in a characteristic form of tissue injury, marked by hemorrhagic necrosis focused at the center of the cord cross section (Allen 1914; Bresnahan et al. 1976; Dohrmann et al. 1971). Some of the vascular disruption and other cellular damage that marks this condition can be detected more or less immediately (Wagner and Dohrmann 1975) and probably results directly from sudden tissue distortion (Blight and DeCrescito 1986). Additional delayed damage is believed to derive from a variety of interconnected secondary pathological mechanisms such as ischemia, edema, anoxia, energy depletion, excitotoxicity, free-radical damage, and inflammatory responses (e.g., Blight 1985, 1994a; Hall and Braughler 1989; Wrathall et al. 1994; Young 1985, 1993). Many such mechanisms may be common to spinal cord and brain injury (Povlishock and Jenkins 1995). Precise definition of the nature, extent, and timing of secondary damage in the CNS has been impossible, in part because it is obscured by simultaneous degenerative processes that follow primary mechanical damage. This is especially true of injury to myelinated axons in the white matter, because such damage may be scattered and difficult to detect. Disruption of axons in the white matter is the most significant contributor to the devastating clinical deficits that result from spinal cord injury. Better understanding of this pathology should help in the development of therapeutic interventions.

The aim of the present study was to explore the electrophysiological effects of controlled focal compression of spinal cord white matter tracts isolated in vitro, where some of the physiological variables can be simplified or eliminated, particularly those associated with vascular damage, including hemorrhage and ischemia. This model differed from a comparable previous approach (Fehlings and Nashmi 1995) in several ways, including use of physiological temperatures, a wide range of injury intensities, and continuous monitoring of conduction during compression and release. In addition, action potential conduction was measured with the use of a sucrose gap apparatus, which allowed accurate measurement of slower components of the potential and a higher signal-to-noise ratio. The study was carried out with the use of adult guinea pigs, to allow direct comparison with compression injury to the spinal cord in situ, which has been described in detail in this species (Blight 1991a,b, 1993, 1994a; Blight et al. 1995). This in vitro model will form the basis for further experimental studies that address individual components of axonal physiology and their responses to injury. Some of the conclusions of the study have appeared in abstract form (Shi et al. 1995a).

## METHODS

### *Isolation of spinal cord*

The technique for isolation of the cord was similar to that described previously for rat (Blight 1989; Blight and Someya 1985). Guinea pigs were anesthetized deeply with ketamine (80 mg/kg), xylazine (12 mg/kg), and acepromazine (0.8 mg/kg). They were then perfused through the heart with 500 ml oxygenated, cold Krebs solution, to remove the blood and to lower core temperature. The vertebral column was excised rapidly and a complete laminectomy was performed. The spinal cord was removed from the vertebrae and immersed in cold Krebs solution, then immediately subdi-

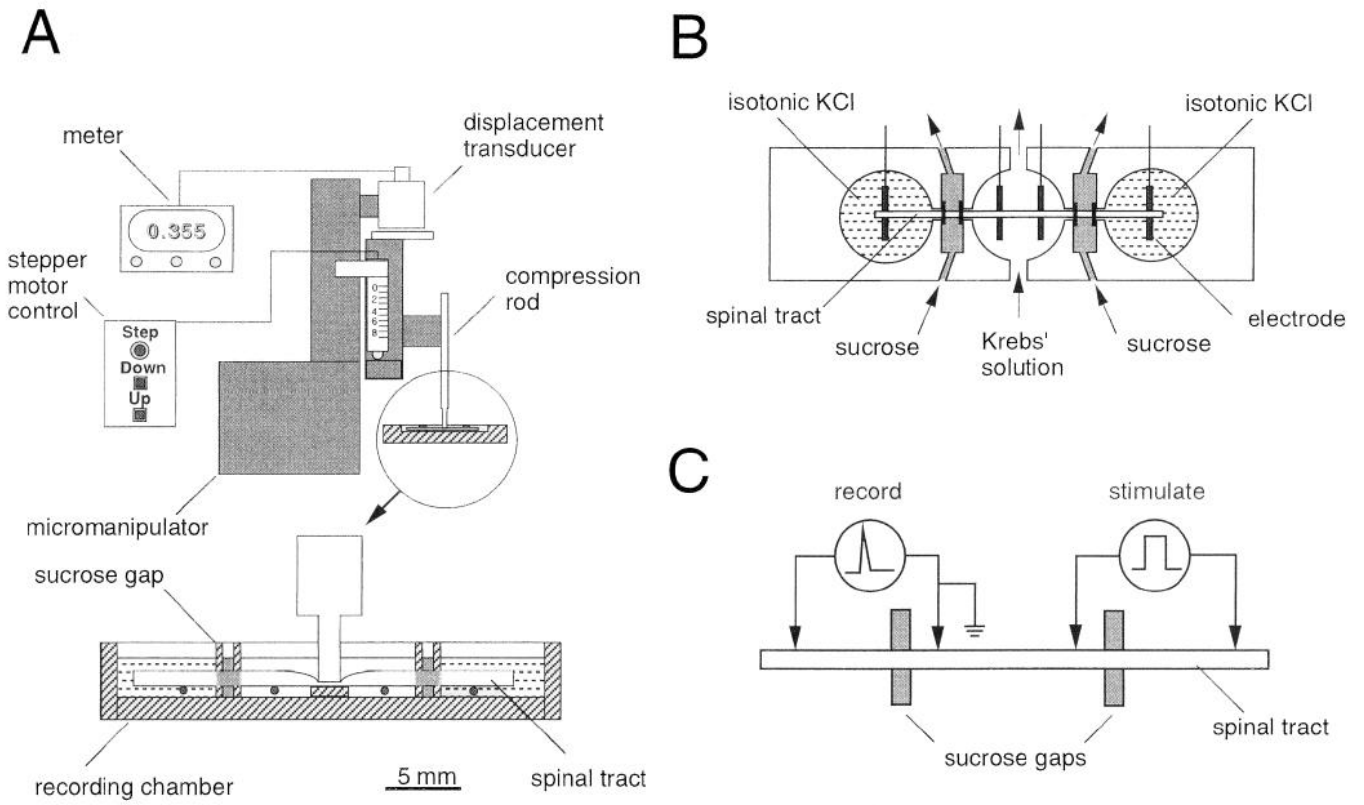


FIG. 1. Diagram showing the injury and recording apparatus. *A*: injuries were produced by compression with a Plexiglas rod advanced in the vertical axis with a micromanipulator. The movements of the rod were monitored with a displacement transducer and activated with a stepper motor control. *B*: recording arrangement. The isolated spinal cord tract is shown mounted in the apparatus, with the injury site placed in the middle of the central well, which is continuously perfused with oxygenated Krebs solution. The 2 ends of the tract were placed in separate wells filled with isotonic KCl, divided from the central well by narrow channels filled with flowing isotonic sucrose solution. *C*: electrical stimulating and recording arrangement, shown in a scaled diagram. The electrodes were formed of silver–silver chloride wires. Action potentials were generated at the right sucrose gap, conducted though the injured part of the spinal cord, and recorded at the left gap with the use of a bridge amplifier.

vided, first along the sagittal midline and then by cutting each half of the cord radially, to produce ventral, lateral, and dorsal strips of white matter that were subsequently incubated in fresh Krebs solution at room temperature, bubbled continuously with 95% oxygen-5% carbon dioxide. The composition of the Krebs solution was as follows (in mM): 124 NaCl, 5 KCl, 1.2 $K_2HPO_4$, 1.3 $MgSO_4$, 2 $CaCl_2$, 20 dextrose, 10 sodium ascorbate, and 26 $NaHCO_3$, equilibrated with 95% $O_2$-5% $CO_2$ to produce a pH of 7.2–7.4.

### *Recording chamber*

The construction of the recording chamber is illustrated in Fig. 1. A strip of isolated spinal cord white matter ~35 mm in length was supported in the three-compartment chamber. The central compartment, 12.5 mm diam, was continuously superfused with oxygenated Krebs solution (~2 ml/min). The ends of the tissue were carried through the sucrose gap channels to side compartments that were later filled with isotonic (120 mM) potassium chloride. The white matter strip was sealed on either side of the sucrose gap channels with the use of shaped fragments of glass coverslip and a small amount of silicone grease to attach the coverslip to the walls of the channel and to block the flow of fluid in the narrow gap between the coverslip and the tissue surface. Isotonic sucrose solution was run continuously through the channel at a rate of 1 ml/min. The temperature of the chamber was maintained with a Peltier unit in the base, thermostatically controlled with a thermistor system (Cambion Instruments), and the temperature was separately monitored across the chamber with additional thermocouple probes. The axons were stimulated and compound action potentials were recorded at opposite ends of the strip of white matter by silver–silver chloride wire electrodes positioned within the side chambers and the central bath. The central bath was similarly connected to instrument ground.

Stimuli in the form of constant-current unipolar pulses 0.1 ms in duration were controlled with a pulse generator connected through a WP Instruments isolation unit. Recordings were made with the use of a Neurodata Instruments bridge amplifier and Neurocorder, for digital data storage on videotape. Subsequent analysis was performed with the use of Labview software (National Instruments) on a Macintosh Power PC computer.

### *Compression*

A flat, raised surface was provided at the center of the recording chamber, against which the isolated white matter strip could be compressed with the use of a rod attached to a motorized micromanipulator (Fig. 1*A*). The end of the rod provided a compression surface of 2.5 mm along the length of the tissue, with a transverse width of 7 mm, such that it was much wider than the tissue, even under compression. The compression rod was positioned perpendicularly to the tissue and was brought to a point of contact with its surface. After baseline measurements of conduction were obtained, the rod was advanced by means of the manipulator motor

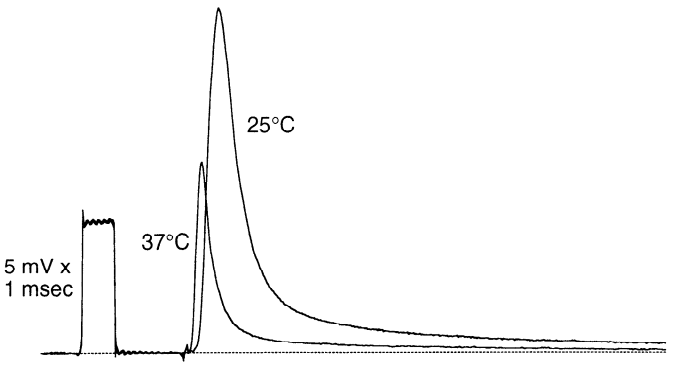


FIG. 2. Superimposed recordings of the maximal compound action potential recorded at 37°C and at 25°C, showing, at higher temperature, the reduction in peak amplitude, depolarizing afterpotential amplitude, and conduction delay. The stimulus artifact is seen in the small biphasic potential before the foot of the action potential.

at a speed of 24 $\mu$m/s. The compound action potential and the displacement of the rod were monitored during the compression and the compression was stopped when the potential reached a set target amplitude. The rod was then removed rapidly upward to relieve pressure on the tissue and the recovery of the compound potential was monitored (see Fig. 3).

### *4-Aminopyridine*

4-Aminopyridine (Sigma) was dissolved in the same Krebs solution used for normal superfusion. The pH of the solution was adjusted where necessary by addition of a small amount of hydrochloric acid.

## RESULTS

### *Sucrose gap recording of action potentials*

The form of the compound action potential recorded from uninjured ventral white matter strips was similar to intracellular recordings of action potentials in individual large axons of rat spinal cord (Blight and Someya 1985). A monophasic action potential, typically several millivolts in amplitude, was followed by a long-lasting depolarizing afterpotential (Fig. 2). The afterpotential could be decomposed into two to three exponential components. As expected, the duration, amplitude, and conduction delay of the recorded action potential decreased with increase in temperature from 25° to 37°C. The amplitude of the depolarizing afterpotential also decreased. In a sample of five spinal cord strips, the compound potential amplitude at 37°C was 5.5 ± 2.5 (SD) mV, or 67.5 ± 2.5% (SD) of that at 25°C. The "gap potential"—a reflection of compound resting membrane potential in the same fiber tract, determined by cutting through the tissue at the outer face of the recording gap—was 16 ± 3 mV, or 338 ± 145% of the compound potential amplitude. With gradually increasing stimulus amplitude, the form of the compound action potential changed in characteristic manner, consistent with recruitment of additional fiber populations, as described below.

### *Response to compression*

The maximal amplitude of the compound potential decreased with compression of the spinal cord strip, and this is illustrated in Fig. 3. As the compression gap decreased below one half the initial thickness of the strip, the amplitude decreased dramatically and the potential was almost completely abolished with ~80% compression. During this loss, there was a simultaneous increase in latency and time course of the potential. With immediate relief of compression, the compound potential recovered rapidly but only partially in amplitude, and with a maintained increase in latency and peak duration (Fig. 3*C*). The compound potential amplitude continued to increase and latency to decrease more slowly, usually attaining stable plateau levels with 5–10 min of recovery (Fig. 4, *A* and *B*). The extent of recovery of amplitude and latency varied with the degree of initial compression and potential loss at the peak of compression (Fig. 5), and the plateau levels showed a similar degree of stability at all levels of injury (Fig. 4*A*).

The decrease in amplitude of the response was not the result of a change in activation probability (threshold), because the relation between stimulus and response amplitude was similar before and after injury (Figs. 6 and 7). The response amplitude increased rapidly to a maximal level over a range of ~500 $\mu$A stimulus current, and then increased little over a further order of magnitude increase in stimulus (Fig. 6). A comparison of the response amplitude before and after injury to set stimulus currents showed no significant change in the relative decrease in amplitude at different stimulus intensities over a wide range. Figure 7 shows data from six different strips that were stimulated at similar current steps before and after injury and the pre- and postinjury response amplitudes compared in absolute terms (Fig. 7*A*) and as a percentage of the maximal amplitude (Fig. 7*B*). The near unity slope of the latter relation indicated that there was no consistent selectivity of loss of conduction in fibers of lower or higher threshold after compression.

There was only a small change in the refractory period of the compound potential. In a sample of nine spinal cord strips where the absolute refractory period was measured at three time points, the absolute refractory period before injury was 0.64 ± 0.05 ms and increased significantly ($P < 0.005$, paired *t*-test) to 0.96 ± 0.10 ms at 30 min postinjury and 0.97 ± 0.10 ms at 1 h. The two postinjury values were not significantly different from each other. The relative refractory period over the same intervals did not change significantly, with values of 6.4 ± 0.9, 7.2 ± 0.8, and 6.6 ± 0.7 (SE) ms.

### *4-Aminopyridine effects on conduction in the plateau recovery phase*

Application of the potassium channel blocker 4-aminopyridine to the superfusion medium produced a marked increase in the amplitude of the compound potential during the plateau phase of recovery. An example is shown in Fig. 8, *A* and *B*, where superfusion with 100 $\mu$M 4-aminopyridine at 1 h postinjury produced a 50% increase in the amplitude of the compound potential with no change in latency or in the overall shape of waveform other than a slight increase in afterpotential. The increase in compound potential amplitude appeared to be complete within 15 min of superfusion, and was reversed with a similar time course on wash with normal Krebs solution. By contrast, application of a similar concen-

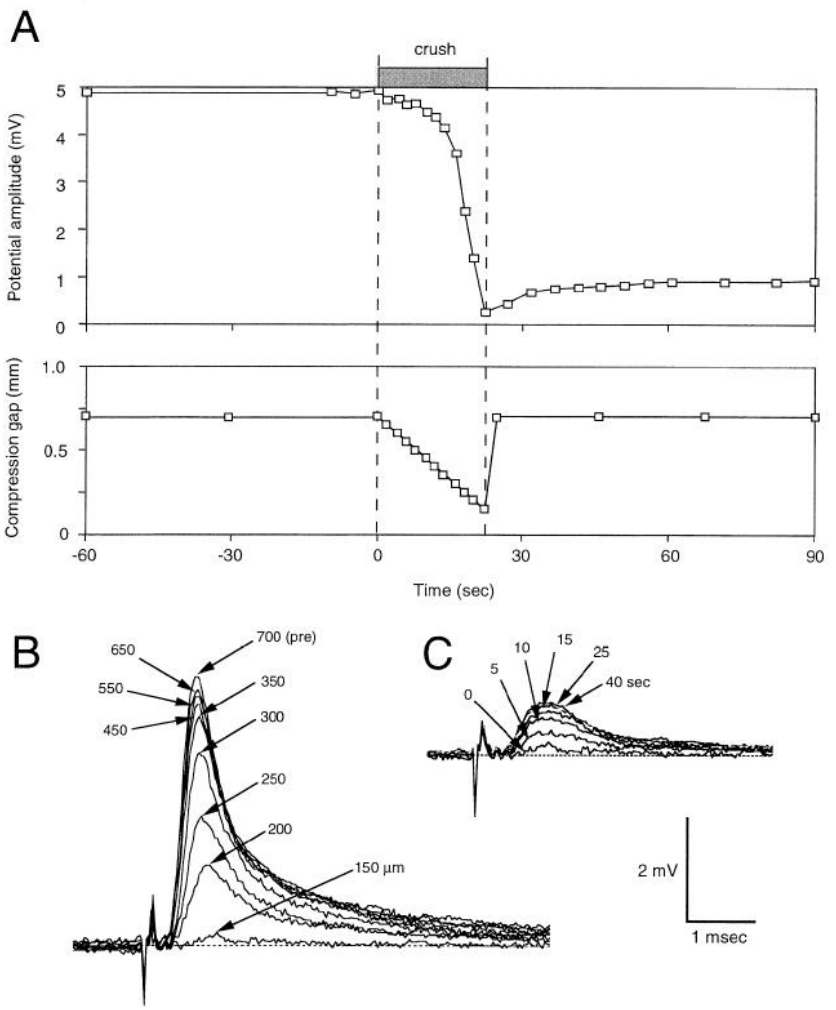


FIG. 3. Example of loss and partial recovery of the compound potential in response to compression. *A*: change in amplitude of the compound potential and the size of the compression gap are plotted against time, showing a nonlinear decrease in conduction. There was a small partial recovery of conduction over the course of 30 s from the relief of compression. *B*: compound action potentials recorded at different compression gap widths (given in $\mu$m) are shown superimposed, and illustrate the loss of amplitude with relatively little change in delay. *C*: compound action potentials recorded at different times after the relief of compression, superimposed to show recovery of amplitude, but with a marked increase in delay of the potential peak that was not apparent during compression (see also Fig. 8*A*).

tration of 4-aminopyridine to uninjured white matter strips produced no significant increase in amplitude of the compound potential, and only a similar small increase in the amplitude of the depolarizing afterpotential (Fig. 8*C*).

## DISCUSSION

### *Different forms of conduction deficit*

The principal finding of the study was that focal compression injury of central white matter produces at least three different forms of conduction deficit, marked by distinct temporal characteristics and degrees of reversibility. There was also a striking stability of conduction in vitro between 5 min and 1 h postinjury, indicating a lack of clear secondary deterioration or spontaneous repair in this period. During the course of compression, a graded decrease in amplitude of the compound potential was accompanied by a small increase in conduction delay (Fig. 3). Some of the amplitude loss remained irreversible within the course of the experiments performed, and the amount of irreversible loss related directly to the extent of compression and initial compound potential loss. A proportion of the conduction loss was spontaneously reversible within 20–30 s from release of compression for all but the most severe injuries. A further slight recovery of amplitude and latency occurred over 3–5 min, and this was followed by a prolonged period of stable amplitude and latency (Figs. 3 and 4). Exposure to the potassium channel blocker 4-aminopyridine during the stable period produced a reversible increase of amplitude of the compound potential without change of peak latency (Fig. 8*B*). This indicates that a component of the amplitude loss over more prolonged periods is based on conduction block in axons that remain in continuity through the area of injury. Studies in chronic injury show that similar deficits are present even several months after compression trauma in vivo (Blight 1983, 1989; Shi et al. 1995b).

### *Injury severity*

The extent to which the initial block recovers depends on the intensity of the compression phase, and at severe compression there is no recovery of conduction. By definition, a sufficiently severe compression (transection) will produce complete disruption of axons running through the site of compression. Experiments in which horseradish peroxidase staining of the compressed cords was used suggest that disruption of some axons occurs as a component of all such injuries, the extent and distribution of disruption being

graded with the intensity of compression (Asano et al. 1995). The stability of deficits appears to be remarkably similar over a wide range of injury intensity (Fig. 4*A*).

*Double pulse responses*

Measurements of refractory period with double pulse stimulation showed only a small increase in refractory period for the compound potential at 30 min and 1 h postinjury. The responses to trains of stimulation were not examined in this study, in the interest of minimizing damage to the strips during prolonged recordings, but these refractory period measurements indicate that repetitive conduction was not markedly compromised by the compression injury.

*Response to 4-aminopyridine*

The increase in amplitude of the compound potential may have been due to an increase in the number of fibers conducting through the injury site or to a change in the properties of the action current. If 4-aminopyridine broadens consituent action potentials near the site of recording, this might increase the amplitude of the recorded compound potential without an accompanying increase in the number of conducting fibers. Even changes in spike duration remote from the gap interface could change the amplitude of the recorded potential, depending on the electrotonic properties of the axons. However, several considerations support an increase in the number of fibers conducting as the most likely explanation of these data. Similar levels of 4-aminopyridine had no effect on the amplitude of the action potential recorded from uninjured spinal cord strips (Fig. 8*C*). The site of compression is removed from the recording gap by >5 mm, which is more than twice the length constant expected of the largest myelinated fibers (Frankenhaeuser and Waltman 1959). Therefore effects of the drug would have to occur at nodes of Ranvier far removed from the site of compression to be noticed in the recording. The absence of any profound effect of the injury on the electrical characteristics (particularly input impedance) of the fibers near the sucrose gaps is also supported by the stability of stimulus-response characteristics at the stimulating gap, shown in Figs. 6 and 7. Most important, there was no change in the peak latency or overall waveform of the compound potential with the increase in amplitude (Fig. 8*B*), and such a change would be

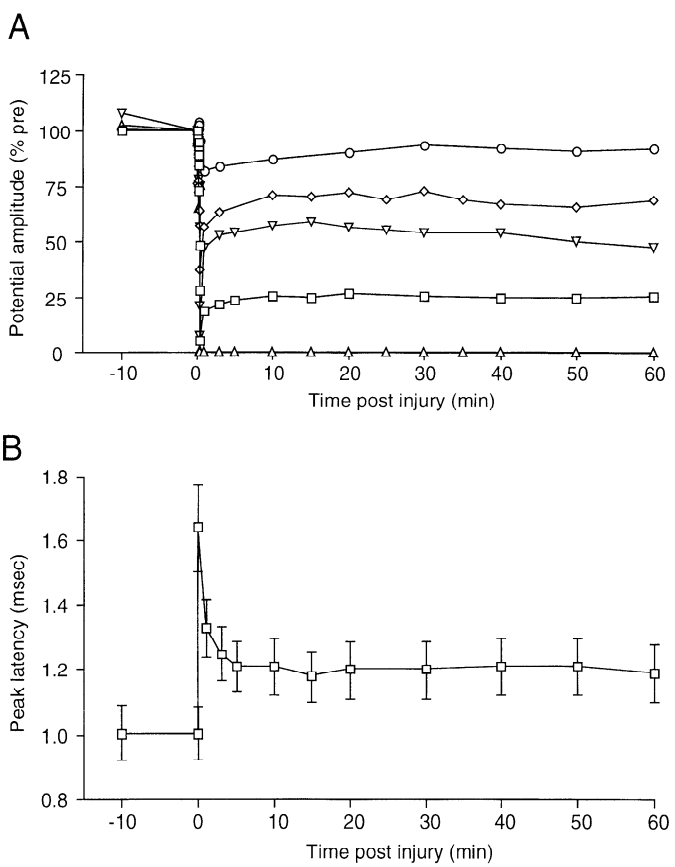


FIG. 4. Graphs showing the change in amplitude (*A*) and peak latency (*B*) of the compound potential after compression injury. *A*: 5 different cases in which the degree of compression was varied over a wide range to produce a full range of recovery of conduction. Open squares: example in Fig. 3. Note the stability of conduction between 5–10 min and 1 h postinjury. *B*: mean ± SD of the latency of the peak of the compound potential, plotted against time, for a set of 27 spinal cord strips. Again, the deficit appears to be stable between 5 and 60 min postinjury.

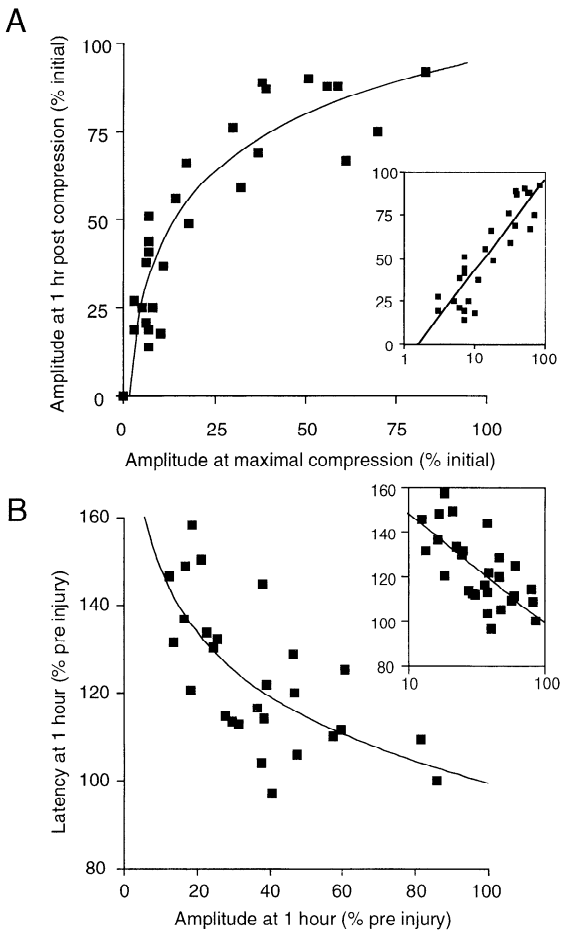


FIG. 5. *A*: graph showing the relation between the initial loss of compound potential amplitude at the peak of compression and the degree of recovery of amplitude in the plateau phase, measured at 1 h. There was a clear exponential relation between the 2 measures (*inset*). *B*: graph showing the relation between the latency and amplitude at 1 h postinjury expressed as percentages of their preinjury values, in a range of different injury intensities. As expected, deficits of conduction speed generally increase with overall injury severity.

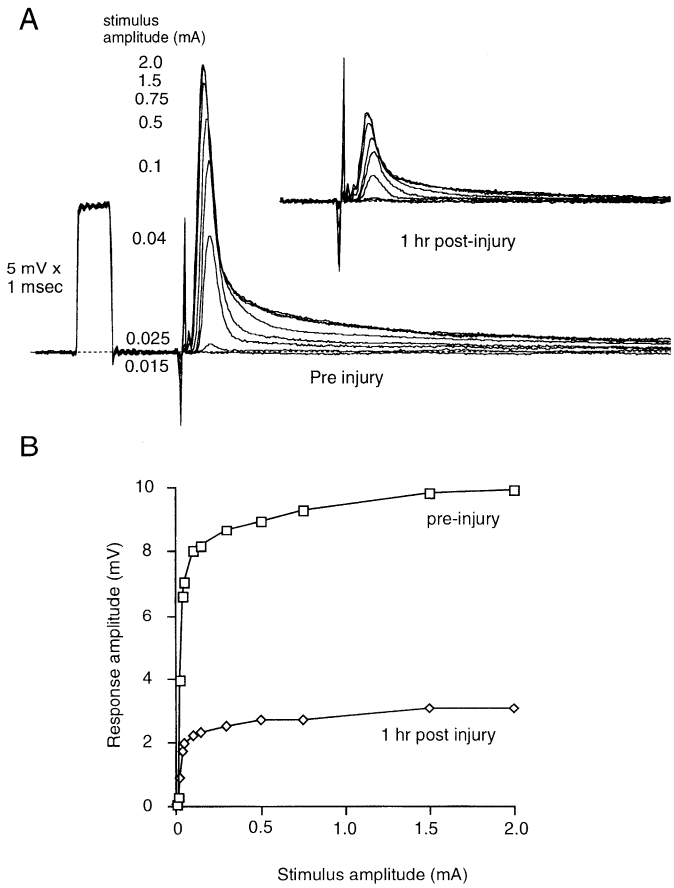


FIG. 6. Examination of the relation between stimulus and response amplitude before and after injury, shown (*A*) in the form of superimposed recordings and (*B*) as a graphic plot. The compound potential amplitude increased rapidly to a maximum within a narrow range of stimulus intensity between 20 and 200 $\mu$A. Above that intensity there was only a slight further increase in amplitude, which was accompanied by decreased conduction delay and increased synchrony of activation. This combination of changes at higher stimulus strengths is consistent with increased current spread, not increased recruitment of nerve fibers. The decrease in amplitude of the compound potential occurred consistently at all stimulus intensities.

expected if the increased amplitude were due to repetitive firing of action potentials or to a local increase in the duration of the action current. Finally, 4-aminopyridine has been shown to promote recovery of conduction in chronically injured spinal cord axons with the use of intracellular microelectrode recording from single fibers (Blight 1989) as well as the double sucrose gap technique (Shi et al. 1995b).

Recovery of conduction is also consistent with earlier studies showing reversal of conduction block with 4-aminopyridine in demyelinated peripheral nerve (Bostock et al. 1981; Targ and Kocsis 1985). Such effects on conduction can be explained as a direct result of the blockade of axonal potassium channels, leading to a reduced shunting of the action current, although the mechanism of effect may include other factors, including alteration of the resting membrane potential.

### *Previous in vitro studies*

Isolated tracts of chronically injured spinal cord have been used previously to examine conduction deficits in myelinated axons and the effects of 4-aminopyridine by means of intracellular microelectrode recordings (Blight 1983, 1989). More recent studies have used extracellular microelectrode recording of field potentials to examine axonal pharmacology and to determine the response to clip compression injury (Agrawal and Fehlings 1996; Fehlings and Nashmi 1995; Honmou et al. 1993; Sakatani et al. 1991a,b, 1993; Saruhashi et al. 1994). The present study used sucrose gap recording, which has been used previously in a slightly different form to examine 4-aminopyridine sensitivity of action potential generation in normal spinal cord white matter (Kocsis 1985). This technique presents a useful compromise between the detailed but unstable measurements provided by intracellular recording and the more stable but small and less readily interpreted measurements provided by extracellular microelectrodes.

Previous studies of compression injury in vitro (Agrawal and Fehlings 1996; Fehlings and Nashmi 1995) were carried out at room temperature, under which conditions action po-

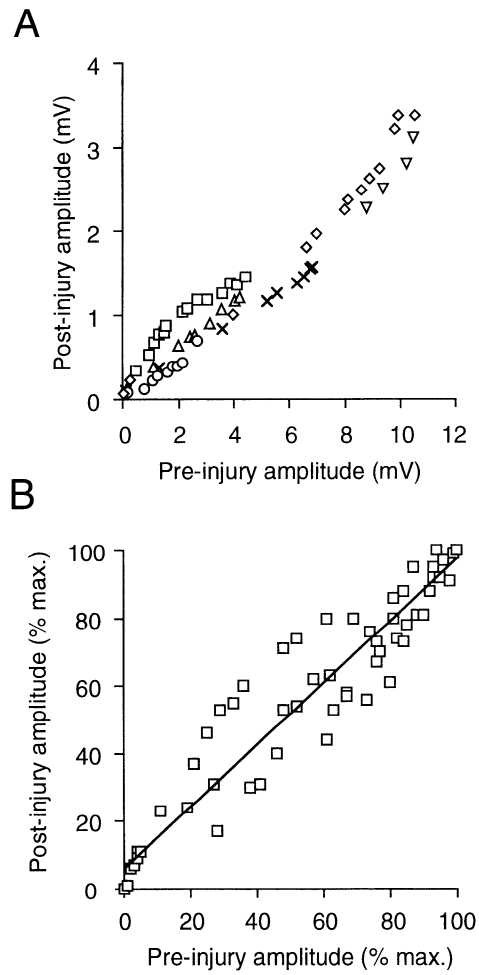


FIG. 7. *A*: amplitude of the compound potential at 1 h postinjury, plotted against the preinjury amplitude at the same stimulus intensity for 6 different spinal cord strips. Some variation in the relation between these measures is apparent between individual strips, but the overall trend appears to be linear. *B*: plotting all the points relative to the maximum amplitude before and after injury, the linear relation is more apparent, and the least-squares linear regression line is not significantly different from 1:1 linearity, indicating that there is little difference in the susceptibility of axons with different stimulus thresholds to damage from focal compression injury.

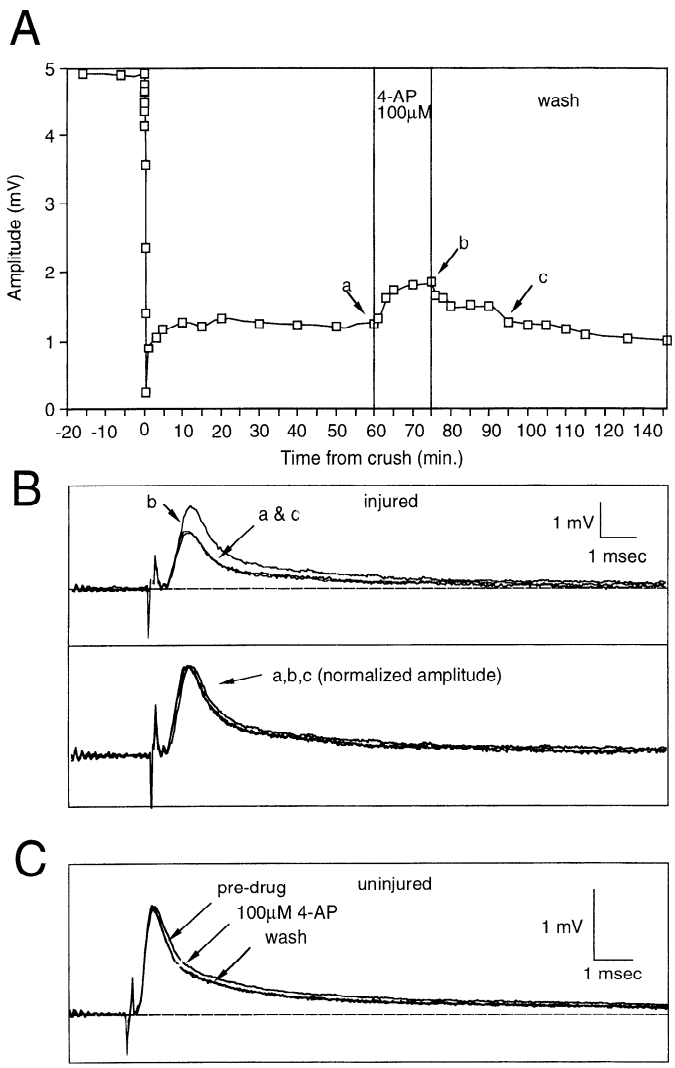


FIG. 8. *A*: plot of compound potential amplitude change with time post-injury, showing the initial loss and recovery, and also the effect of superfusion with 100 $\mu$M 4-aminopyridine (4-AP) during the plateau phase. The amplitude increased within 15 min to a new plateau level almost 50% larger than before application. This increase was reversed by wash with normal Krebs solution. *B*: superimposed traces in the *top panel* show the increase in compound potential amplitude with 4-AP and its complete reversal with 20 min of washing. In the *bottom panel* the same traces are plotted, normalized for peak amplitude, to show the absence of any significant change in waveform accompanying the increase in amplitude with 4-AP. *C*: comparable experiment with uninjured spinal cord, the superimposed traces (with a single vertical scale) showing the lack of effect of 4-AP on compound potential amplitude at this concentration, despite a small change in the amplitude of the depolarizing afterpotential.

tential conduction is more secure because of the increased duration of the action current (Fig. 2) and membrane fluidity is decreased. Those studies examined apparently moderate and mild degrees of injury, respectively, allowing ~50% and 75% recovery of field potential amplitude, and did not allow monitoring of conduction during the phase of compression or immediately after release. The data derived from these clip compression studies, to the degree to which they are comparable, appear partly compatible with the present findings. An apparent discrepancy between the models was the suppressive effects on extracellular field potentials seen with a very high (5 mM) concentration of 4-aminopyridine (Fehlings and Nashmi 1995), compared with the enhancement of conduction seen here with 100 $\mu$M concentration. The paradoxical effects of high concentrations of 4-aminopyridine are discussed at length elsewhere (unpublished data).

### *Comparison with injury in vivo*

Spinal cord contusion injury in situ leads to a rapid loss of conduction in spinal pathways, as measured by evoked potential techniques. Unless the contusion is very severe, there is usually a partial recovery of conduction, which may progress for several hours (e.g., Blight 1994b; Blight and Young 1990; D'Angelo et al. 1973) or require several weeks to become apparent (e.g., Blight and Young 1989). Slow compression of mammalian spinal cord in vivo produces a gradual loss and slowing of conduction, which is followed by a similarly slow rate of incomplete recovery of conduction over the course of several hours after decompression (Sakatani et al. 1987, 1989, 1991c). A similar pattern of slow recovery is seen in the guinea pig spinal cord subjected to more rapid compression in vivo (unpublished observations). The time course of recovery in vivo is therefore quite different from that seen here in vitro. This slow recovery in the intact cord is likely to be at least partly a reflection of the slow recovery of normal extracellular potassium activity (Young and Sakatani 1990). Spinal cord injury is accompanied by a rapid disruption of the selective ionic permeability of cell membranes, allowing a rapid elevation of extracellular potassium activity, accompanied by a balancing loss of extracellular sodium and calcium ions (Chesler et al. 1994; Moriya et al. 1994; Young 1992; Young and Koreh 1986; Young et al. 1982a,b). The initial elevation of potassium may be sufficient in itself to block conduction in axons. In the intact animal, recovery of normal extracellular potassium levels may take up to 1 h (Chesler et al. 1991, 1994). In the isolated tracts, in the absence of significant gray matter, and completely surrounded by the superfusing medium, the rise in extracellular potassium activity may be small or the restoration to normal levels may be much more rapid. Some of the initial rapid recovery of conduction may reflect normalization of extracellular ion levels, but it may also reflect recovery of normal selective permeability of axonal membranes that have been subjected to mechanical distortion.

The increase in latency, which is partly independent of the amplitude changes (being maintained as amplitude recovers—Fig. 3, *B* and *C*) may be explained in a number of ways, perhaps most reasonably by damage to myelin sheaths, resulting in increased internodal capacitance and action potential transit time. There may also be a maintained increase in axonal membrane ionic permeability, or a sustained decrease in excitability through alteration of nodal sodium channel behavior. An alternative possibility, that there has been a change in the distribution of surviving fiber sizes, seems unlikely because there is no evidence of a selectivity of survival of smaller myelinated fibers on the basis of the threshold for activation before and after injury (Fig. 7). Although the relation between axon caliber and electrical stimulation threshold has not be established in this particular preparation, it would be expected to exist on the basis of previous studies of spinal cord white matter (e.g., BeMent

and Ranck 1969; West and Wolstencroft 1983). The lack of selectivity of the initial injury for large axons is also supported by preliminary anatomic evidence of no early selective loss of larger-caliber fibers (Asano et al. 1995). These data also suggest that the selective loss of larger axons seen in vivo (Blight and DeCrescito 1986; Blight 1991a) may result from true secondary pathological mechanisms and perhaps a selective metabolic susceptibility of large fibers.

The lack of evidence of secondary deterioration in vitro, and the evidence of recovery of conduction in vivo during the first few hours after moderate injury, seem paradoxical in the face of histological and ultrastructural evidence of a gradual increase in the extent of axonal deterioration in spinal cord contusion models (Dohrmann et al. 1972; Wagner and Dohrmann 1975). This physiological robustness is also at variance with early signs of progressive axonal pathology in models of diffuse axonal injury in head trauma (Maxwell et al. 1993; Pettus et al. 1994; Povlishock 1992; Povlishock and Jenkins 1995). The paradox may be explained if axons that appear to be intact ultrastructurally, yet proceed to show gradual axoplasmic deterioration and eventual widespread axolemmal breakdown, have actually suffered sufficient initial axolemmal damage to make them incapable of action potential conduction. Slow deterioration of disrupted fibers may be a feature of injuries in vivo because of the very low extracellular calcium activities that may be present within the injured area for some hours (Moriya et al. 1994; Stokes et al. 1983; Young et al. 1982b).

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