

# Acute Repair of Crushed Guinea Pig Spinal Cord by Polyethylene Glycol

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**Shi, Riya and Richard B. Borgens.** Acute repair of crushed guinea pig spinal cord by polyethylene glycol. *J. Neurophysiol.* 81: 2406–2414, 1999. We have studied the responses of adult guinea pig spinal cord white matter to a standardized compression within a sucrose gap recording chamber. This injury eliminated compound action potential (CAP) conduction through the lesion, followed by little or no recovery of conduction by 1 h postinjury. We tested the ability of polyethylene glycol (PEG) to repair the injured axons and restore physiological function. Local application of PEG (1,800 MW, 50% by weight in water) for ~2 min restored CAP conduction through the injury as early as 1 min post PEG application. The recovery of the CAP  $\leq$  1 h was significantly greater in treated compared with control spinal cords (controls = 3.6% of the preinjury amplitude; PEG treated = 19%;  $P < 0.0001$ , unpaired Student's *t*-test). Stimulus-response analysis indicated that the susceptibility for recovery was similar for all calibers of axons after PEG application. The enhanced recovery of conduction after PEG treatment was associated with an early alteration in conduction properties relative to control spinal cords. This included increased refractoriness and sensitivity to potassium channel blockade using 4-aminopyridine (4-AP). Normally 4-AP enhanced the amplitude of the recovering CAPs by ~40% in control spinal cords; however this effect was nearly doubled to ~72% in PEG treated spinal cords. Because severe clinical injuries to the spinal cord (and some peripheral nerves) are both resistant to medical treatment and usually produced by compression, we discuss the possible clinical benefits of PEG application.

## INTRODUCTION

The water-soluble polymer polyethylene glycol (PEG) has the unique ability to fuse cell membranes. This class of compounds first was evaluated as a means to couple several individual cells into one as a means to study various aspects of cell biology and to introduce genetic material from one cell into another (Ahkong et al. 1987; Davidson et al. 1976; Nakajima and Ikada 1994; O'Lague and Huttner 1980). Single giant axons of crayfish and earthworm also have been fused using PEG (Bittner et al. 1986; Krause and Bittner 1990; Krause et al. 1991). The ability of these types of polymers to join cell membranes also has been exploited to instantaneously *seal* cell membranes, reversing the permeabilization of the membrane produced by damage or disease. This has obvious medical application where such permeabilization leads to cellular death or tissue atrophy (Lee et al. 1992, 1993).

When the membranes of axons are crushed or compressed, various alterations in their intracellular and extracellular ionic

domains immediately occurs. This is due to a compromised electrical and ionic barrier. These changes also can block the transmission of action potentials. For example, conduction block can occur due to a local collapse of the membrane potential in concert with a higher permeability to  $K^+$  ions. Natural resealing of the compromised membrane eventually may lead to a restoration of ionic equilibrium and conduction (Blight 1993; Shi and Blight 1996). However, if the membrane lesion is severe, the outcome will be a progressive localized dissolution of the membrane and myelin, resulting in permanent conduction block to action potential propagation (Shi and Blight 1996). In the most severe injuries, this process of "secondary injury" will lead to continued permeabilization of the axolemma and its complete collapse, sometimes causing the death of the neuron. This is particularly true if the insult to the nerve process occurs close to the neuron's soma (Lucas et al. 1985; Shi et al. 1989). In surviving neurons, such progressive degeneration eventually will lead to the separation of the axon and Wallerian degeneration of the distal segment (Fawcett and Keynes 1990; Ochs 1980). An artificial means to immediately seal and repair nerve membrane lesions might intervene in these processes, rescuing the distal segment of the axon from eventual dissolution, restoring variable levels of conduction, and to some unknown extent, producing a recovery of lost behaviors.

Here we evaluate the ability of PEG to repair crushed nerve fibers of the CNS. We test the capability for immediate repair of nerve membranes after a standardized and severe compression injury to isolated adult guinea pig white matter.

## METHODS

### *Animal use and care*

Adult female guinea pigs of 350–500 g body wt were used for these studies. The spinal cord was isolated from deeply anesthetized animals (60 mg/kg ketamine hydrochloride, 0.6 mg/kg acepromazine maleate, and 10 mg/kg xylazine, im). After anesthesia, the animal was perfused transcardially with cold (15°C) Krebs solution [which contained (in mM) 124 NaCl, 2 KCl, 1.2  $KH_2PO_4$ , 1.3  $MgSO_4$ , 1.2  $CaCl_2$ , 10 dextrose, 26  $NaHCO_3$ , and 10 sodium ascorbate, equilibrated with 95%  $O_2$ -5%  $CO_2$ ]. The vertebral column was removed rapidly using bone forceps and scissors by previously described techniques (Shi and Blight 1996, 1997). The spinal cord was divided into four longitudinal strips, first by midline sagittal division, then by separating the dorsal and ventral halves with a scalpel blade against a plastic block. Only the ventral white matter was used for this study. These 35- to 38-mm-long strips of spinal cord white matter usually will be referred to below as "cords" or "spinal cords" for ease of description. Spinal

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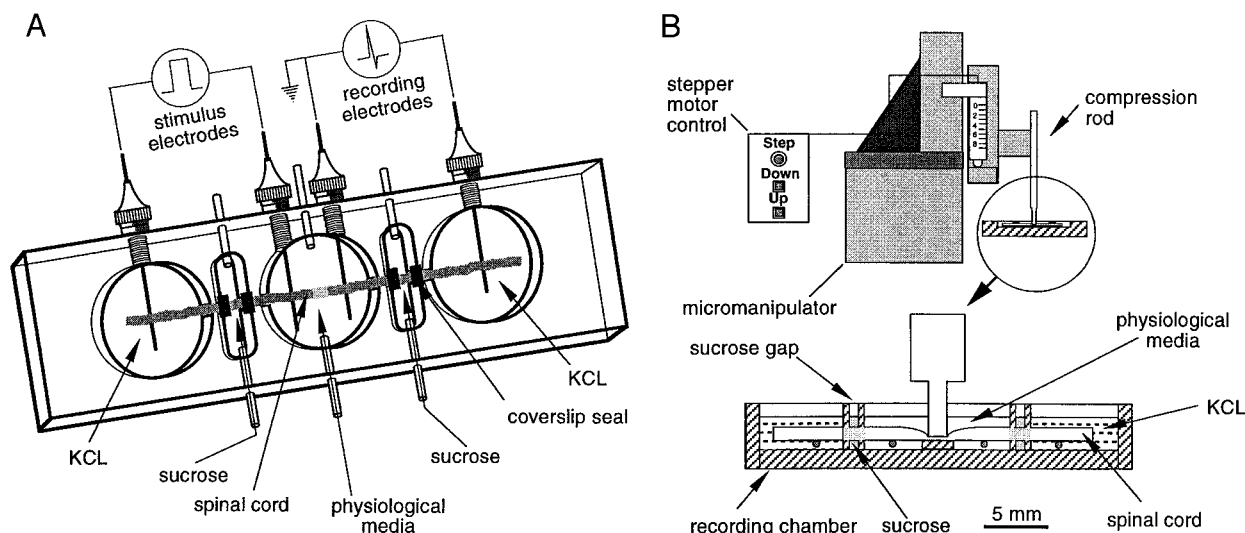


FIG. 1. Double sucrose gap chamber and the experimental injury. *A*: drawing of the double sucrose gap recording chamber. *Left to right*: 1st large compartment contains 120 mM KCl, the central large compartment contains the physiological test solutions, and the 3rd compartment also contains 120 mM KCl. The small chambers on either side of the central compartment contain 230 mM sucrose. Sucrose and the physiological test media are exchanged continuously by capillary pumping into the chambers and by aspiration of the media from the chambers at the same rate through the small tubes as shown. Seals fashioned from coverslips are secured in place with high vacuum silicone grease at the locations shown to inhibit the exchange of the various media from one compartment to the next. AgAgCl electrodes for recording and stimulation are in series with socket connectors at the locations shown. Note that the central chamber is at ground potential for recording. Spinal cord strips (~35 mm long) were placed in the chamber along its long axis and immersed in the various media in all compartments before the placement of the coverslip seals. Action potentials were stimulated at the left side of the spinal cord strip as shown, conducted through the spinal cord in the central compartment (also including the injury site), and recorded at the right side of the spinal cord strip as shown. Additional diagrams and fabrication detail can be found in Shi and Blight (1996, 1997). *B*: apparatus used to produce a standardized crush to the isolated spinal cord at its midpoint within the central compartment is shown. Contact with the spinal cord used a Plexiglas rod with a flattened tip advanced downward to contact the tissue at a standardized rate of 25  $\mu\text{m/s}$ . This end of the rod provided a compression surface of 2.5 mm along the length of the tissue, and a transverse width of 7 mm, such that it was always wider than the spinal cord strip, even under full compression. Compression of the spinal cord involved crushing it between the tip of the compression rod and a raised Plexiglas stage supporting the cord at this site. Positioning of the compression rod was accomplished with a micromanipulator. Standardized injury was controlled with a stepper motor to produce a finely graded crush just sufficient to eliminate all compound action potential (CAP) propagation (which was monitored continuously during the procedure). State of complete CAP failure was maintained for an additional 15 s before the abrupt upward withdrawal of the compression rod by the stepper motor. Position of the spinal cord injury within the central chamber is shown at *bottom*.

cords were maintained in continuously oxygenated Krebs solution for 1 h before mounting them within the recording chamber. This was to ensure their recovery from dissection before experiments were begun.

#### Double sucrose gap recording technique

The double sucrose gap recording chamber and *in vitro* injury model already have been described in previous publications, and we refer the interested reader to these reports (Shi and Blight 1996, 1997). The construction of the recording chamber and the placement of the spinal cord within it is illustrated in Fig. 1, *A* and *B*.

A standardized compression injury was produced with a stepper-motor-controlled rod, which compressed the spinal cord while suspended inside the recording chamber (Shi and Blight 1996) (Fig. 1*B*). The basic recovery profile after such standardized compression in normal Krebs solution has been characterized previously and published (Shi and Blight 1996).

Every electrophysiological test was digitized in real time and captured to the computer for subsequent quantitative evaluation. All records also were recorded on VHS magnetic tape as a means of back up documentation. All solutions used in the PEG repair process were made on the day of their use.

#### PEG repair procedure

First, typical physiological functioning of the isolated white matter strip removed to the recording chamber required ~0.5–1 h of incu-

bation time while immersed in oxygenated Krebs to stabilize. In initial experiments, once the CAP propagation had stabilized, the Krebs solution was replaced with  $\text{Ca}^{2+}$  free Krebs ( $\text{Ca}^{2+}$  replaced with equimolar  $\text{Mg}^{2+}$ ).

Second, the spinal cord strip then was crushed by the techniques described in the preceding section, while simultaneous stimulation and recording continued.

Third, a solution of PEG in distilled water (50% by weight) was applied by a pressure injection through a micropipette. A vital dye was added to the PEG solution to monitor its continuous application to the lesion site in a stream ~0.5 mm wide for ~1–2 min. The PEG was applied to one side of the lesion, washed over it, and immediately removed by constant aspiration on the other side using a second pipette.

Fourth, immediately after the PEG application, the bathing media in the central chamber was replaced with a continuous stream of oxygenated normal Krebs solution. The physiological properties of the PEG treated spinal cord were monitored continuously for 1 h. Usually, a weak recovering CAP was evident within 6–15 min of the PEG application.

The preceding technique should be considered as a basic one, from which testing of several variations described in the following text were performed.

For example, we tested the response of “recovering” axons to the additional application of the fast potassium channel blocker, 4-aminopyridine (4-AP). In this trial, five separate cords were treated with

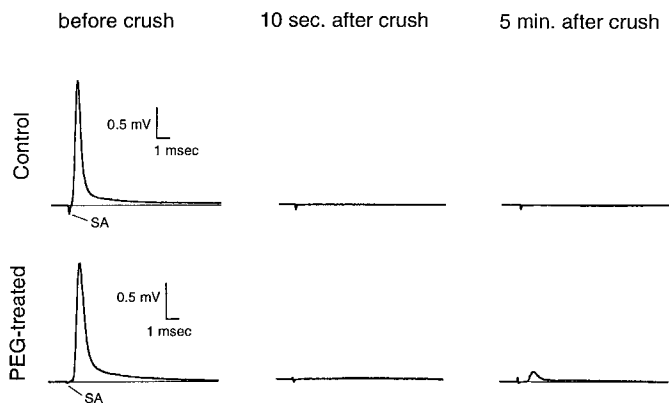


FIG. 2. Acute response of the crushed spinal cord to polyethylene glycol (PEG) treatment. *Top*: electrical records show the CAP before the standardized experimental crush (1st record) and the immediate loss of conduction after the experimental injury. *Bottom*: electrical records show a typical response to acute standardized injury of the isolated spinal cord strip after PEG treatment. Note that at the earliest time point (~5 min postinjury), recovery of a CAP is never observed in the absence of PEG treatment and rarely occurs by 10 min postinjury (data not shown). SA, stimulus artifact.

an application of PEG as described earlier and compared with five control cords. One hour after compression, 100  $\mu$ M 4-AP (in Krebs solution) was applied for 15 min and then washed free with normal Krebs solution as described earlier.

Fifth, in a final series of experiments, the requirement for the injury to be carried out in  $\text{Ca}^{2+}$ -free media was tested. In these experiments, the cord was compressed while it was immersed in normal Krebs' solution.

#### Statistical treatment

Before and after the application of 4-AP, we used Student's *t*-tests to compare recovering action potential amplitude between the control and PEG treated group. Comparisons of action potential amplitude also were made between the two PEG-treated groups.

## RESULTS

### PEG-mediated repair of crushed spinal cord strips

Approximately 0.5 h after the equilibration of the spinal cord strip in the recording chamber, the Krebs solution in the central compartment was replaced with a  $\text{Ca}^{2+}$ -free Krebs and the spinal cord was crushed by previously described techniques. In every spinal cord tested in this group of 20 (10 control and 10 experimental), this procedure resulted in the immediate and total loss of CAP propagation from the point of stimulation to the point of recording. Figure 2 shows an individual record of one typical control experiment and a PEG-treated experimental spinal cord strip. Note the immediate and complete loss of the CAP in both preparations, and the initial recovery of the CAP in the PEG treated spinal cord by 5 min posttreatment (Fig. 2B). The earliest recorded recoveries of a CAP occurred within 1–2 min after PEG treatment. In control preparations, three cords never regained conduction during the 1 h of continuous observation. In contrast, not one PEG-treated spinal cord providing the data summarized in Fig. 3 failed to recover CAP conduction after PEG treatment. In four more control spinal cords, the recovery of the CAP was not observed for ~20 min. Figure 3 provides a summary graph of the 10 control and the 10 experimental spinal cords treated and monitored identically,

save the experimental application of PEG to the lesion site. PEG treatment always provided a striking increase in the amplitude of recorded CAPs, averaging 19% of the original pretransection amplitude, and always facilitated the CAP recovery in 100% of the cases tested. At every time point tested—including the 10-min postinjury period—recovered CAP amplitudes were statistically significantly greater than control preparations (Fig. 3). Figure 3 also shows that the injury need not be carried out in  $\text{Ca}^{2+}$ -free media to produce functional repair as claimed by Bittner for invertebrate axons (Krause and Bittner 1990) (refer to DISCUSSION).

### Electrophysiological properties of the repaired spinal cords

The PEG repaired spinal cords showed typical conduction properties (as observed in recovering untreated cords); however, some differences in their electrophysiological properties were revealed by further evaluation.

Figure 4A shows the effect of injury on the normal recovery of CAP amplitudes. Typically, the recovered CAP was dampened in amplitude across all threshold intensities of excitation. We also evaluated if this reduced magnitude of the CAP occurred across all caliber spectra of injured axons within the spinal cord strip or was manifest in only large or small diameter axons. Figure 4B shows the actual amplitudes of control compound potentials at 1 h postinjury, plotted against the preinjury amplitude at the same stimulus intensity. A least-squares linear regression was not significantly different from 1:1 linearity, suggesting that there was no difference between the susceptibility to damage of axons of different stimulus thresholds.

In Fig. 4C, we plotted two hypothetical lines representing outcomes after PEG treatment. Note that if larger axons of a lowered stimulus threshold were more susceptible to PEG, the data would be shifted as in the gray line (a). In the opposite situation, the hatched line (c) shows a shift in the opposite

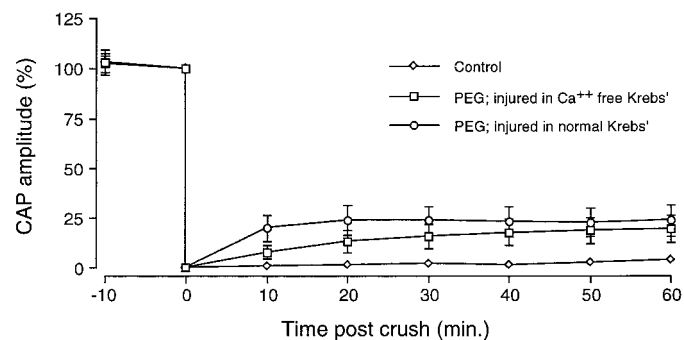


FIG. 3. Recovery of PEG-treated and control spinal cords by 1 h after injury. Abscissa displays the recovery of the CAP as a percentage of the precrush amplitude. Ordinate is time. Average CAPs and their SE are displayed for 10 spinal cord strips for each group. Note that the control group shows a barely detectable CAP (3.6%) even by 1 h postinjury, whereas average recovered CAPs in PEG-treated cords increase ~20%, ranging to as much as 69% of the precrush amplitude. PEG facilitated increase in CAP amplitude was significantly higher at every time point studied ( $P < 0.05$ , Student's *t*-test, two-tailed). CAP recovery was facilitated when the injury was not carried out in  $\text{Ca}^{2+}$ -free Krebs solution. Amplitude of the recovered CAP in normal Krebs at the first time point (10 min postinjury) was elevated statistically over the recovered CAP observed when the injury was performed in  $\text{Ca}^{2+}$ -free media ( $P < 0.05$ ; unpaired Student's *t*-test). Every subsequent time point was still higher in this data set with no reverse trends, but without statistical significance.

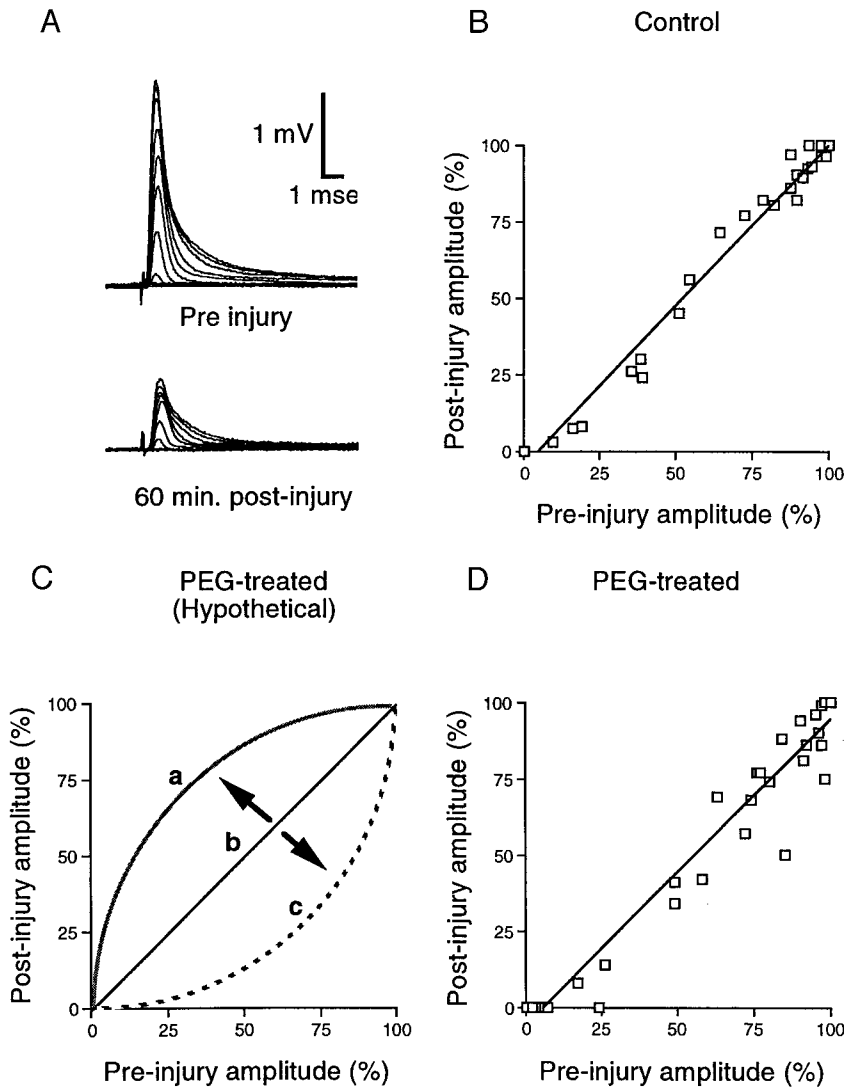


FIG. 4. Evaluation of the CAP amplitude with increasing strength of stimulus: PEG-treated vs. controls before and after injury. *A*: series of 10 superimposed CAPs are shown in response to 10 separate increasing stimulus intensities (0.015–2.0 mA, 100- $\mu$ s duration squarewave stimuli) before the experimental crush and 1 h after it in a control preparation. Note the dampened amplitude of the recovered CAP at all stimulus intensities after injury. *B*: this graph shows the preinjury amplitude vs. postinjury amplitudes for 4 spinal cord strips in a modestly injured control group. Less severe injury was required in these spinal cords to allow an adequate range of recovered CAP amplitudes for this graded evaluation. In the severely injured cords, the maximal recovered CAPs were insufficient to adequately make these comparisons. These data points are shown relative to the maximum amplitude achieved before and after injury. Note that the slope of the least squares regression line is close to unity. *C*: hypothetical skewing of data are shown where (a) more large caliber fibers (with a lower stimulus threshold) are responsible for the CAP or (c) more small caliber fibers are recruited to produce the recovered CAP after injury relative to unity (b). *D*: actual distribution of these data points are shown in the PEG-treated group. Note that the least-square linear regression line is not significantly different from 1:1 linearity, which is again not different from that shown in *B*. In this test, the typical and severe standardized injury was used because PEG-repaired cords showed substantial CAPs sufficient for a graded plot of their amplitudes.

direction should small caliber axons with a higher stimulus threshold be repaired. In Fig. 4D, the actual data taken from the PEG-treated population is plotted in the same manner as in Fig. 4B. The near unity slope of the relation of amplitude response before and after injury indicated no consistent selectivity of PEG-mediated improvement of conduction in fibers of lower or higher threshold.

Although PEG appeared to be able to repair axons of a wide range of calibers similar to the natural recovery process observed in control cords, the electrophysiological properties of PEG-mediated recoveries was not the same as controls. Figure 5A shows the classical relationship between the timing of paired stimuli and the amplitude of the two elicited CAPs. Paired stimuli in which the interstimulus interval was between 0.6 to 15.0 ms demonstrated typical dampening of the CAP amplitude soon after the absolute refractory period. When the interval between the paired stimuli was longer than this, a plateau was reached where the first and second CAPs were of an identical magnitude-marking the extent of the relative refractory period.

Figure 5B shows control data derived from four separate

experiments. The abscissa shows the magnitude of the second CAP of the pair as a percent of the magnitude of the first elicited CAP. The ordinate shows the log of the interstimulus interval ranging from 0.6 to 15 ms. This sigmoidal plot is typical, beginning with stimuli that do not elicit a second AP during the absolute refractory period and ending at the termination of the relative refractory period.

Furthermore Fig. 5B shows that this relationship was not disturbed by the injury, as pre- and postinjury data points were not significantly different along this sigmoidal curve. This did not hold true, however, for PEG-treated spinal cords. The early and robust recovery of CAPs produced by PEG demonstrated a typical period of absolute refractory as before the injury and experimental treatment. Moreover the relative refractory period also appeared to terminate when a similar stimulus interval to control preparations was achieved. During the refractory period of PEG-treated cords, the amplitude of the second CAP was slightly reduced when compared with that before the crush and PEG treatment (Fig. 5C). However, this latter relationship was not statistically significant.

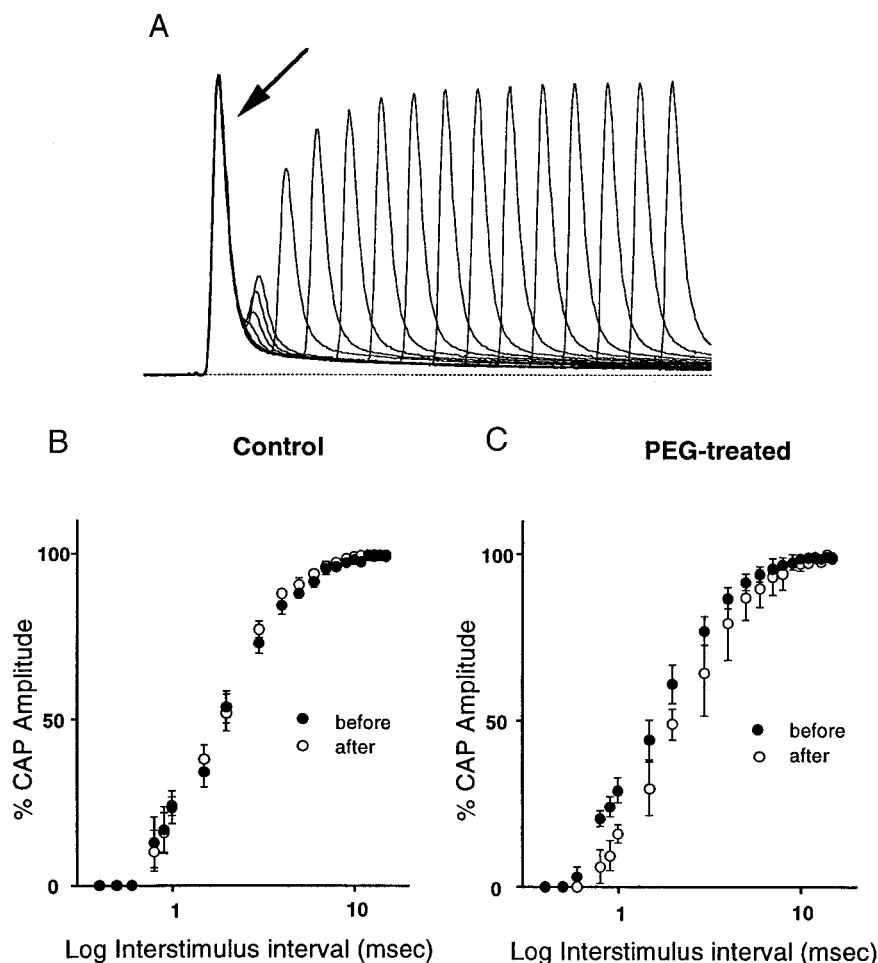


FIG. 5. Refractory period changes after double pulse stimuli. A: 20 individual records of CAP responses to twin pulse stimuli are superimposed. First of these 20 stimuli produced the single large CAP ( $\leftarrow$ ). Because this 1st CAP always is produced by a stimulus of the same intensity, each of these superimposed individual electrical records was identical. Left to right: CAP produced by the 2nd stimulus is shown. Note the typical dampened amplitude of the 2nd CAP when triggered during the relative refractory period followed by the typical plateau in amplitude produced when the second stimulus is applied subsequent to the relative refractory period. B: here, the response to the secondary stimulus (as a percentage of the 1st CAP amplitude) vs. the interstimulus interval is plotted for 4 untreated spinal cord strips. ●, data points before the standardized crush injury; ○, data points obtained 1 h after the injury. Note that in both cases, a typical sigmoidal period of relative refractory is apparent, and there is no significant difference in the average response to the 2nd stimulus before and after the injury. C: same type of data are displayed for the PEG-treated group of 4 spinal cords. Note that the amplitude of the response to the 2nd stimuli of the pair is reduced.

#### Potassium channel blockade as an adjunct to PEG-mediated recovery of conduction

It is a common feature of injured cells to lose intracellular potassium to the extracellular milieu through compromised membrane. In axons, this may be sufficient to suppress action potential conduction. Thus we attempted to determine if blockage of fast potassium channels with 4-AP would affect the properties of conduction immediately after PEG repair.

Figure 6A shows the enhancement of the CAP in crushed (but untreated with PEG) spinal cord by 4-AP. In this individual record, the initial recovered CAP at 1 h postinjury is shown, and the enhanced CAP after 100  $\mu$ M 4-AP treatment is superimposed on it. After documentation of the 4-AP enhanced CAP, the blocker was washed out, and the media in the central compartment was replaced with normal Krebs solution. The CAP fell to pretreatment levels by 15 min and was indistinguishable from the original record. This final waveform is superimposed on the other two CAPs in Fig. 6A but cannot be discriminated from the pretreatment electrical record. In this single test, 4-AP reversibly enhanced the recovered CAP by  $\sim$ 40%.

Figure 6B shows an identical test performed on a PEG-treated spinal cord, in which 4-AP was administered at 1 h post-PEG application. In this individual test, the second CAP was enhanced reversibly by  $\sim$ 70%. After the near doubling of the CAP, 4-AP was washed out as described, and the CAP fell

to pretreatment levels as in controls (Fig. 6A). Figure 6C shows the group data, five spinal cords in each group. The percent enhancement of the PEG-mediated recovery for the group data mirrors that discussed above for the individual experiments ( $\sim$ 70% enhancement in the experimental group;  $\sim$ 40% in the control group). This experimental enhancement was statistically significantly greater than that observed in the controls. ( $P < 0.05$ , unpaired Student's *t*-test)

#### DISCUSSION

After severe compression/contusion of the guinea pig spinal cord, a complete conduction block occurs in response. For reasons that are understood, such as the initiation of endogenous repair mechanisms (Shi and Blight 1996; Xia and Barrett 1991; Yawo and Kuno 1985), and for reasons that are not, a small population of axons seal their cellular lesions and are capable of propagating action potentials through the damaged area once again. In our standardized injury model, compound action potential propagation through the damaged area never recovered before 10 min postinjury, whereas some cords never recovered at all during the hour of measurement and evaluation. Furthermore, this natural recovery process never resulted in CAPs greater than  $\sim$ 7% of the pretransection amplitude.

Within a few minutes after the application of the water-soluble polymer PEG, an immediate recovery of CAP propagation through the lesion occurred. The recovered CAP ampli-

tude slowly increased with time to a peak of ~20% of the initial CAP amplitude. Moreover, this level of recovery was always statistically significantly higher than control amplitudes, observed at every time point tested, and occurred in 100% of the experimentally treated spinal cords. It is clear that a topical application of PEG can immediately repair severe compression injury to the mammalian spinal cord leading to significant increases in functional recovery as defined by the enhanced capacity to propagate nerve impulses through the lesion. This report is the first to demonstrate PEG-mediated repair of crushed mammalian nervous tissue.

We have employed a concentration of PEG (50% by weight in water) and MW (1,800 Da) that commonly has been used by many to fuse both nonneuronal cells and neuron-like cells (PC 12) *in vitro* (Davidson et al. 1976; O'Lague and Huttner 1980). Bittner has used similar PEG MWs and concentrations to fuse or repair single axons in crayfish and earthworms, while suggesting that additional use of a  $\text{Ca}^{2+}$ -free hypotonic bathing medium was required for successful treatment (Bittner et al. 1986; Krause and Bittner 1990; Krause et al. 1991). We have shown that a physiological, balanced media and the aforementioned PEG solution is all that is required to produce functionally significant repair in mammalian spinal cords (see following text). Moreover, in other experiments where completely transected guinea pig spinal cords were fused with PEG, we have learned there was no specific PEG MW critical to the process—having tested PEG solutions using 400, 1,400, 1,800, 2,000, and 3,700 Da (unpublished observations).

#### *Mammalian spinal cord in isolation*

The ability to resolve even small increases in a recovering CAP was permitted by the use of the double sucrose gap chamber of the design described here and elsewhere (Shi and Blight 1996, 1997). Briefly, the ends of the isolated strip of spinal cord white matter are bathed in isotonic KCL and are approximately at intracellular potential during the recording session, while the central compartment contains physiological media at extracellular potential. The compartments are separated by a viscous sucrose gap, which can be thought of as analogous to a limiting membrane. Although this electrical seal is not perfect, this recording arrangement can produce electrical records of superior resolution—approaching the signal-to-noise ratio of intracellular recordings. In our other studies where PEG has been used to fuse completely transected strips of spinal cord white matter in isolation, CAPs of only 1–2% of the original magnitude could be revealed clearly against noise (to appear elsewhere). In this study, the demand for high-resolution discrimination between preinjury and recovered CAP propagation was less a factor given the immediate recurrence of PEG-mediated CAPs at a time when control spinal cords could not conduct action potentials at all, and the substantial amplitudes of the recovered CAPs at times >10 min postinjury relative to their preinjury levels. We have outlined other advantages when studying the physiology of spinal cord injury using this technique and direct the interested reader to these discussions (Shi and Blight 1996, 1997).

#### *Fusogens and membrane repair*

PEG has been used for >30 yr as a means to fuse cells. This allows the production of giant cells from many small ones

facilitating electrophysiological study or manipulation of membranes. The fusion of cells also facilitated the exchange of genetic material between cells and the formation of hybridomas during the production of monoclonal antibodies, as well as serving as a model for the vesicular fusions that normally occur during the biology of cells (Lee and Lentz 1997; Lentz 1994). Even so, the actual molecular mechanisms of action permitting membrane fusion by PEG is still under investigation (Lee and Lentz 1997). It is clear that cellular fusion occurs in a stepwise manner when adjacent membranes touch in the presence of PEG, membrane fusion occurring before the fusion of the cells and the mixing of cytoplasm (Ahkong et al. 1987). At the level of the membrane, acute dehydration of the fusing plasmalemmas permits the intermingling of glyco/protein/lipidic structures, which resolve into each other first at the outer membrane leaflet and, subsequently, the inner membrane leaflet (Lee and Lentz 1997). Rehydration apparently leads to a spontaneous form of structural self-assembly within the aqueous plane of the membrane.

#### *PEG-mediated axonal recovery*

Severe and local compression to spinal cord white matter produces a ‘lesion’ in axons that in the least may be characterized by a compromised electrical seal and a collapse in the local resting potential of the axon at this site. This usually leads to three possible outcomes: 1) the breach in the membrane at the site of damage seals itself, which may preserve the axon’s anatomic integrity and capacity to propagate APs. 2) In some cases, this natural recovery process may preserve the anatomic integrity of the axon—but may not restore its ability to conduct action potentials in some types of axons. This is especially true if axons within the local area of injury becomes denuded of myelin. And 3) failure in the natural process of axonal sealing may lead to physical separation of the axon and the death of the distal segment in mammals—or of the neuron itself if the injury to the nerve process is close to the soma (Blight 1993; Blight and Decrescito 1986; Lucas et al. 1985; Shi et al. 1989).

We believe the chemical properties of PEG that allow membranes to resolve into each other permitting the complete fusion of two or more closely opposed cells provides a very rapid mechanism of repair of the acute membrane breach. We are using a horseradish peroxidase (HRP) dye exclusion test (Asano et al. 1995), together with a new method of computer-managed morphometry (Moriarty et al. 1998) to anatomically evaluate the difference in axonal membrane repair by PEG relative to control spinal cords. This test was based on the hypothesis that a PEG-induced ‘seal’ or membrane ‘repair’ will exclude the intracellular uptake of HRP by damaged cells after injury. Our preliminary evidence is that PEG-treated spinal cord strips are indeed better sealed than control cords. These studies will be reported elsewhere.

In this physiological study, we have determined similarities and differences between the natural mechanisms of axonal repair and those mediated by PEG. First, a least-squares linear regression analysis of pre- and postinjury CAP amplitudes suggests that PEG-mediated repair can occur across all levels of stimulus thresholds, reflecting axon diameters, as does the natural recovery process in untreated spinal cord strips. In other words, all spinal axons regardless of their caliber are equally susceptible to PEG-mediated repair (see Shi and Blight



FIG. 6. Response of recovered CAPs to the application of 4-aminopyridine (4-AP). *A*: expected increase in recovered CAP amplitude at 1 h postinjury produced by application of 100  $\mu$ M 4-AP to an untreated spinal cord strip. This was expected because fast potassium channel blockade by 4-AP is an effective means to reduce partial to complete conduction block. *B*: similar experiment on an individual spinal cord strip is shown 1 h after PEG treatment. Note the more robust increase in the recovered CAP produced by 4-AP in the PEG-treated cord compared with the control shown in *A*. *C*: group data are shown for 5 control and 5 PEG-treated spinal cords. Increased effect of 4-AP on CAP recovery is statistically significant ( $P < 0.05$ , unpaired Student's *t*-test).

1996 for a similar analysis of axonal recovery from compression injury). The differences between natural repair and that produced by PEG application are more striking. First, this injury is very severe; 30% of control spinal cords never recovered any capacity to conduct CAPs during the 1-h period of evaluation after injury. On the other hand, there was no instance where PEG did not initiate a measurable physiological recovery. On a more subtle level, there appears to be a slightly reduced CAP amplitude during the period of relative refractory in only PEG-mediated CAPs relative to control cords. One explanation for this observation may be that in control cords a severely compromised and dysfunctional population of axons may become completely nonfunctional, revealing more normal conduction properties in that population that survive the injury. PEG may rescue a portion of such severely compromised

axons, recruiting them into the CAP and perhaps accounting for its slightly different conduction properties.

#### *Ionic controls of axonal injury and membrane repair*

In other work, we have evaluated the way that ionic changes produced by injury impact the response of axons to insult (Borgens 1988; Borgens et al. 1980; Shi and Blight 1997). It is known that injury to cell membranes leads to an increase in  $K^+$  permeability that contributes to AP conduction block (Blight 1989; Shi and Blight 1996, 1997). In myelinated axons, even acute damage to the myelin sheath leads to  $K^+$  current shunting and depressed or inhibited conduction—both events can be reversed variably by  $K^+$  channel blockade using 4-AP (Blight 1989; Pratt et al. 1995; Shi et al. 1997). This fast potassium channel blocker both enhances the safety factor for conduction as well as extending the length constant for AP propagation across regions of demyelination (Blight 1989; Shi and Blight 1996, 1997). In the studies reported here, we expected an enhanced amplitude of the CAP in even control cords based on

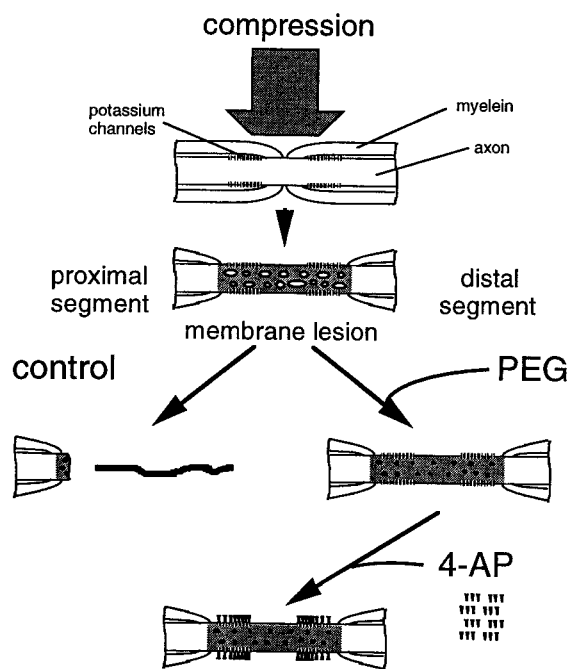


FIG. 7. Proposed mechanism of the synergistic effect of PEG and potassium channel blockade by 4-AP. *Top*: severe mechanical compression of a myelinated axon. Note that the myelin sheath envelops high densities of fast  $K^+$  channels clustered at the paranodal region. Severe crush leads to an immediate exposure of the potassium channels of the paranodal region by a withdrawal or collapse of the myelin lamella at this site (Shi et al. 1997). Exposure of the voltage-gated potassium channels after injury would elevate  $K^+$  conductance, further impeding conduction across this damaged portion of the membrane (gray region showing "holes" in the compromised membrane). In control preparations, partial to complete conduction block results from this localized disturbance of the axolemma, which may progress to complete separation of the axon and loss of the distal axonal segment by Wallerian degeneration (*left*). In PEG-treated axons (*right*), the membrane repair leads to preservation of injured axons as well as improvements in their conduction capabilities (gray regions; membrane holes now sealed). However, elevated  $K^+$  conductance through  $K^+$  channels that still are exposed at the site of repair in PEG-treated nerve fibers still might suppress conduction to some extent. Blockade of these channels with 4-AP (small arrow heads; *bottom right*) would be expected to reduce any outward  $K^+$  conductance and thus enhance conduction.

our prior studies and these mechanisms of action. We were surprised, however, by the nearly doubling of CAP amplitudes in PEG-treated cords, suggesting that PEG repaired membrane itself is particularly leaky to  $K^+$ . We propose a mechanism of synergistic action of 4-AP with PEG repair in Fig. 7. In any event, 4-AP that has been used to restore conduction and behavioral recovery in clinical spinal cord injury should be viewed as a possible adjunct to the development of any clinical use of PEG in nerve repair (see next section).

### Clinical implications

It is likely that this procedure can provide a novel means of treating severe, acute neurotrauma. In addition to immediate improvements in conduction, repair of crushed axons in peripheral nerves leading to a rescue of their distal segments would provide the added benefit of reducing atrophy or degeneration of target cells or so called "end organs." Many target tissues require innervation for sustenance and/or survival. Currently trauma to peripheral nerves close to the trunk of the body are still problematic in that slowly regenerating axons (~1 mm/d) may not reach downstream targets before their irreversible atrophy or degeneration. This is in spite of the fact that fascicular alignment and grafting can provide the enhanced possibility for functional reconnection (Fawcett and Keynes 1990; Ketchum 1982; Thomas 1988). It is also possible that PEG-mediated fusion of even transected axons could become a component of microsurgical grafting techniques because the conventional resection of peripheral nerve trunks before fascicular grafting exposes the severed tips of proximal and distal axonal segments—making them available for fusion.

In the spinal cord, transection of white matter is rarely a clinical occurrence—clinical spinal cord injury usually involves severe compression/contusion of the spinal cord followed by centrally occurring hemorrhagic necrosis of gray and white matter. As discussed earlier, "secondary injury" leads to progressive loss of white matter due to a local deterioration of cell membranes at the site of the lesion. In such a clinical scenario, PEG-mediated repair of crushed white matter—as shown to be possible in this report—could take on real clinical significance. With continued development of these techniques for the clinic, one might reasonably expect more immediate recovery of conduction (and variable function) as well as the rescue of variable amounts of injured white matter. To these ends, we already have moved this treatment approach to experimental tests in vivo using both spinal cord and peripheral nerve injury models in adult guinea pigs.

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