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# Behavioral recovery from spinal cord injury following delayed application of polyethylene glycol

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## Summary

**Topical application of the hydrophilic polymer polyethylene glycol (PEG) to isolated adult guinea pig spinal cord injuries has been shown to lead to the recovery of both the anatomical integrity of the tissue and the conduction of nerve impulses through the lesion. Furthermore, a brief (2 min) application of the fusogen ($M_r$ 1800, 50 % w/v aqueous solution) to the exposed spinal cord injury *in vivo* can also cause rapid recovery of nerve impulse conduction through the lesion in association with functional recovery. Behavioral recovery was demonstrated using a long-tract, spinal-cord-dependent behavior in rodents known as the cutaneus trunci muscle (CTM) reflex. This reflex is observed as a contraction of the skin of the back in response to tactile stimulation. Here, we confirm and extend these preliminary observations. A severe compression/contusion injury to the exposed thoracic spinal cord of the guinea pig was performed between thoracic vertebrae 10 and 11. Approximately 7 h later, a topical application of PEG was made to the injury (dura removed) for 2 min in 15 experimental animals, and levels of recovery were compared with those of 13 vehicle-treated control animals. In PEG-treated animals, 93 % recovered variable levels of CTM functioning and all recovered some level of conduction through the lesion, as measured by evoked potential techniques. The recovered reflex was relatively normal compared with the quantitative characteristics of the reflex prior to injury with respect to the direction, distance and velocity of skin contraction. Only 23 % of the control population showed any spontaneous CTM recovery ($P$=0.0003) and none recovered conduction through the lesion during the 1 month period of observation ($P$=0.0001). These results suggest that repair of nerve membranes by polymeric sealing can provide a novel means for the rapid restoration of function following spinal cord injury.**

Key words: spinal injury, neurotrauma, spinal cord, nerve injury, central nervous system injury, fusogen, polyethylene glycol, guinea pig.

## Introduction

It is both instructive and convenient to consider the catastrophic loss of behavioral function following spinal cord injury (SCI) as two different syndromes: the initial, or acute, phase and the subsequent chronic condition. Theoretical biological approaches to treating each are not mutually exclusive, but must take into account the distinct pathologies characteristic of each phase of the injury.

During the acute phase, a delayed, progressive and self-propagating wave of cell and tissue destruction is initiated, with the injury leading to cell death, the formation of a cicatrix and the irreversible loss of distal segments of axons through Wallerian degeneration (Griffin et al., 1995). This latter dynamic is often referred to as ‘secondary injury’ (Honmou and Young, 1995), and it is the loss of white matter, which cannot be replaced, that frames the more permanent behavioral loss accompanying the long-term, or chronic, phase of the injury.

Techniques aimed at regenerating new and functional connections by promoting nerve regeneration from intact proximal segments show clinical promise in cases of SCI. Inhibition of endogenous inhibitors of central nervous system (CNS) regeneration, application of novel growth factors to the lesioned spinal cord, neurotransplantation of both fetal nervous tissue and activated peripheral nervous system macrophages into the spinal lesion and the application of direct current electrical fields are all thought to facilitate regrowth of spinal cord white matter and/or to facilitate new functional synaptic connections (Schwab et al., 1993; Bregman et al., 1996; Benowitz et al., 1999; Lazarov-Spiegler et al., 1996; Borgens et al., 1999). The last three techniques mentioned have now moved into human clinical study in cases of severe SCI. Another approach, which is aimed at recovering functional deficits irrespective of the time since the original spinal injury, is to restore physiological conduction through intact but non-

functional white matter by $K^+$ channel blockade. This technique is also at the stage of human clinical testing (Shi and Blight, 1997; Hansebout et al., 1993; Hayes et al., 1993).

It is a matter of debate whether greater success in reducing long-term injury has been achieved in recent years by attacking the initial phase of the insult. One such example, adopted as a means of standard management of acute spinal cord injury, has been the administration of large doses of the steroid methylprednisilone within hours of the injury (Bracken et al., 1990). This approach, often referred to as 'neuroprotection', claims to ameliorate behavioral loss by reducing the extent of secondary injury, although the efficacy and safety of this therapy are now under question (Short et al., 2000; Pointillart et al., 1999).

We suggest another approach to treating the acute injury. In this method, the application of a hydrophilic surfactant physically repairs damaged membranes, leading to a rapid (minutes to hours) recovery of cellular structure and function. This treatment is designed to permit immediate recovery of nerve impulse conduction in injured fibers, to reverse the permeabilization of the plasmalemma and immediately to seal breaches in it that would probably progress to dissolution and axotomy.

We have reported that a brief administration of the fusogen polyethylene glycol (PEG) to completely transected, but reapposed, adult guinea pig spinal axons can induce anatomical reconnection of the severed proximal and distal segments and immediate recoveries of compound action potential conduction through fused axons (Shi et al., 1999). We have also shown that a similar application of PEG to severe compression injuries also leads to an immediate recovery of compound action potential conduction through the lesion (Shi and Borgens, 1999). These procedures were repeated *in vivo* in experiments in which an aqueous solution of PEG was applied for 2 min to a standardized compression injury to adult guinea pig thoracic spinal cord. A swift recovery of both spinal cord conduction, measured by somatosensory evoked potentials (SSEPs), and behavioral function, measured by the recovery of the cutaneus trunci muscle (CTM) reflex (Borgens et al., 1987; Blight et al., 1990), occurred in PEG-treated animals (Borgens and Shi, 2000).

Here, for the first time, we evaluate fully the behavioral character of the recovered CTM reflex produced by a delayed application of PEG and confirm our observations of the physiological recovery of conduction in 100 % of these spinally injured animals.

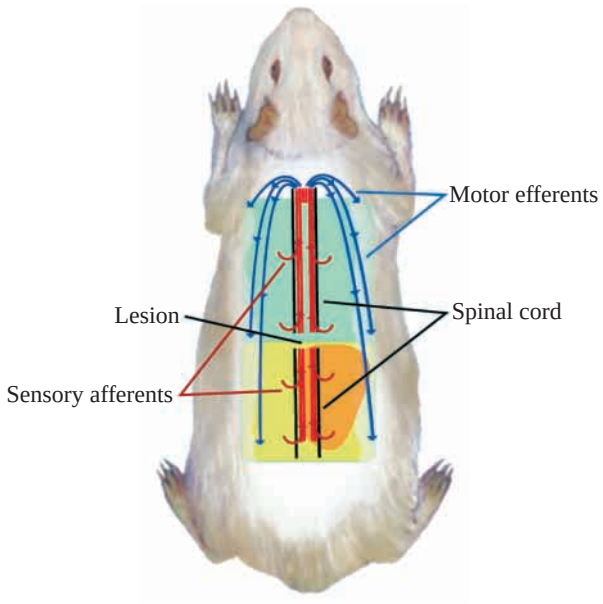


Fig. 1. The circuitry of the cutaneus trunci muscle (CTM) reflex and its interruption by injury. Afferent sensory axons project from nociceptive and mechanosensory receptors in the skin into the spinal cord at the dorsal root of each vertebral segment on both the left and right sides *via* the dorsal cutaneous nerves, as shown in red. These synapse on second- and third-order neurons whose long-tract projections ascend the spinal cord in the ventrolateral funiculus to synapse on CTM motor neurons clustered as bilateral nuclei at the cervical–thoracic junction. There is no anatomical connection between the left- and right-side motor neuron pools. Efferent axons (blue) project out of the spinal cord as a component of the brachial plexus terminating on the cutaneus trunci muscle beneath the skin of the back *via* the lateral thoracic branch of the plexus. In the intact animal, the green and yellow/green area represents the entire normal bilateral receptive fields of the CTM sensory neurons. Tactile stimulation of this region produces skin contractions, but contractions are not elicited outside this region. Note that a lesion to the spinal cord blocks ascending conduction of the afferent sensory tracts of the CTM. This produces a region of areflexia below the level of the lesion in which tactile stimulation no longer produces CTM contractions (highlighted in yellow/green); the rostral fields are unaffected by the injury. The orange-shaded area represents a region of partial recovery within the area of areflexia below the level of the lesion. This drawing is not to scale and, for clarity, does not include every component in the CTM circuit (such as commissural or contralateral projections) (see Blight et al., 1990; Borgens et al., 1990).

## Materials and methods

### *Surgery and anesthesia*

Twenty-nine adult (300 g) guinea pigs were used in this experiment. They were divided into two groups, PEG-treated ($N$=15) and sham-treated ($N$=14). One animal died following surgery in the control group. Guinea pigs were anesthetized with an intramuscular injection of 100 mg $kg^{-1}$ ketamine HCl and 20 mg $kg^{-1}$ xylazine prior to surgical exposure of the cord by dorsal laminectomy and removal of the dura (Borgens et al., 1986, 1990). A standardized injury was produced between thoracic vertebrae 10 and 11 with a constant-displacement 15 s compression of the cord using specially constructed forceps possessing a détente (Blight, 1991) (see also Moriarty et al., 1998). This lesioning procedure had been calibrated to produce a total loss of compound action potential conduction through the spinal cord injury and behavioral functioning of the CTM reflex (Borgens and Shi, 2000). To sedate animals for behavioral and physiological testing, guinea pigs were injected

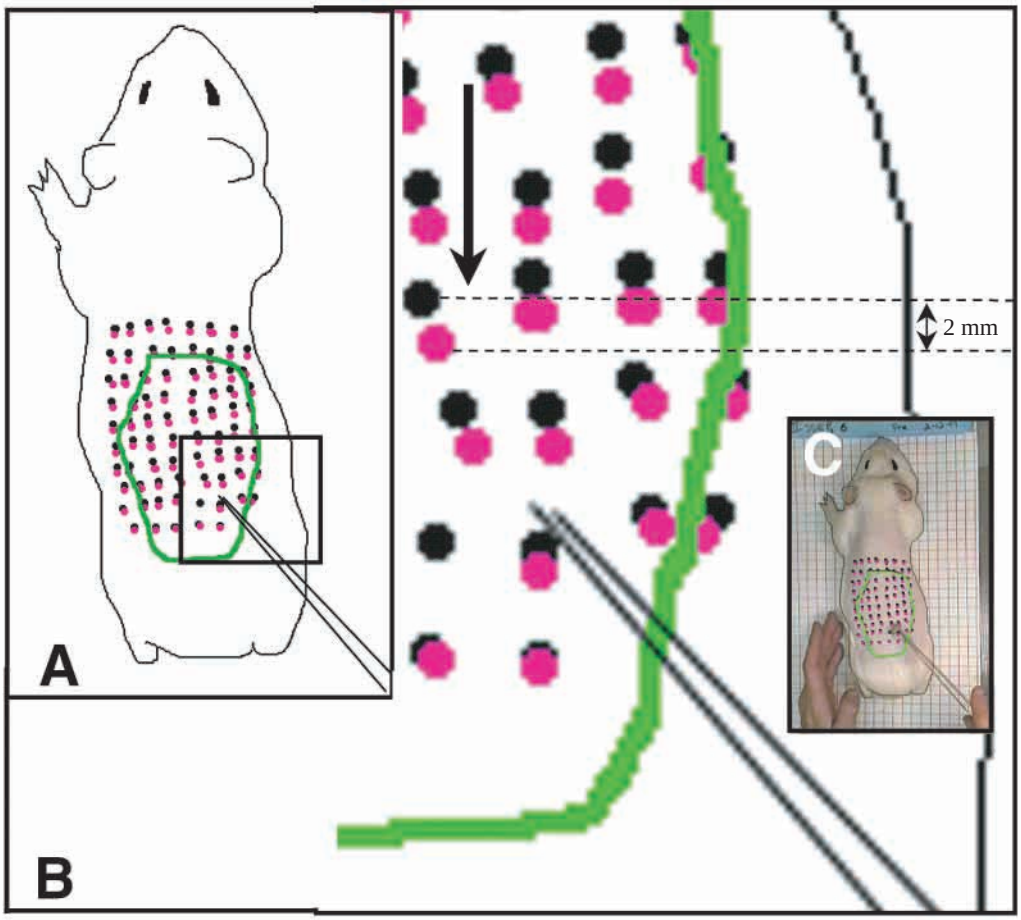


Fig. 2. Dot matrix evaluation of the cutaneus trunci muscle (CTM) reflex. (A) An overlay drawing of superimposed video images of a guinea pig. Black dots are permanent markers tattooed onto the shaved back and pink dots show the positions of the markers at the peak of skin contraction, captured by stop- frame video analysis (see Materials and methods). The exact point of tactile stimulation producing these CTM contractions is shown by the position of the monofilament probe used to stimulate the skin. During the period of testing, a boundary line was drawn on the back of the animal with a marker (highlighted in green) to reveal the total CTM receptive field. Stimulation within the circumscribed area produced skin contractions; stimulation outside the area did not. The actual video image source of the drawing is shown in C. The box in A is magnified in B, showing that the direction of skin contraction is generally towards the probe (arrow); the distance moved by a marker during contraction is also shown (hatched lines). This distance (2 mm) divided by the time required to produce it (0.12 s) gives the velocity of skin contraction (16.7 mm $s^{-1}$).

with 0.1 ml of sodium pentobarbital (50 mg $ml^{-1}$). All surgical procedures and testing were carried out under protocols approved by the Purdue University Animal Care and Use Committee in accordance with Federal, State and University guidelines governing the use of animals in research.

### *Application of PEG*

An aqueous solution of PEG ($M_r$ 1800, 50 % w/v in distilled water) was applied with a Pasteur pipette directly onto the exposed injury (dura removed) for 2 min and then removed by aspiration. This procedure was performed approximately 7 h after the lesioning procedure (see above). The region was immediately washed with isotonic Krebs' solution (NaCl, 124 mmol $l^{-1}$; KCl, 2 mmol $l^{-1}$; $KH_2PO_4$, 1.24 mmol $l^{-1}$; $MgSO_4$, 1.3 mmol $l^{-1}$; $CaCl_2$, 1.2 mmol $l^{-1}$; dextrose, 10 mmol $l^{-1}$; $NaHCO_3$, 26 mmol $l^{-1}$; sodium ascorbate, 10 mmol $l^{-1}$), which was also aspirated to remove excess PEG. In sham-treated animals, the injury site was re-exposed surgically at approximately 7 h post-lesion, a control application of water ('vehicle') was applied for 2 min, followed by a lavage with Krebs' solution subsequently removed by aspiration. The wounds were closed, and the animals were kept warm with heat lamps until awaking. Guinea pigs were housed individually and fed *ad libitum*.

### *Behavioral analysis*

The CTM reflex (Fig. 1) is observed as a rippling of the skin on the back of the animal following light tactile stimulation. These contractions can be measured by tattooing a matrix of dots on the animal's shaved back. When the skin contracts towards the point of tactile stimulation, the dots move in this direction. Practically, a receptive field is determined prior to injury by stimulating the back skin of the sedated animal by lightly touching it with a monofilament probe. When skin outside the receptive field is stimulated, there is no response. By exploring the entire back with the probe, the boundary of the receptive field can be established. This boundary is drawn directly onto the shaved back of the guinea pig using a marker (Fig. 2). Stimulation inside the boundary produces CTM contractions, but stimulation outside it does not. The entire procedure was videotaped by a camera mounted approximately 0.92 m above the examination table. The same procedure was used to determine the region of CTM loss (areflexia) and subsequent recovery, when it occurred. These behavioral tests (and

the physiological test described below) were performed approximately 24 h, 3 days, 2 weeks and 1 month post-treatment.

To quantify the CTM behavior, we evaluated four individual components of it by stop-frame analysis of the videotaped recordings. (i) The area of recovery of receptive fields below the level of the lesion was measured by planimetry from videotape images captured to the computer. These data were normalized by dividing the area of CTM loss by the total area of the receptive field prior to injury and expressing the result as a percentage. Similarly, the area of CTM recovery was determined and then divided by the original area of CTM areflexia to give the percentage recovery of the CTM reflex. (ii) The direction of peak skin movement in normally functioning and recovered receptive fields following injury was determined as a vector. (iii) The distance of skin movement during peak skin contraction was measured. (iv) The velocity of skin contraction following tactile stimulation was computed using these data.

The overall pattern of skin movement can be quite complex in response to focal stimulation. Thus, we chose to restrict our quantitative evaluation of back skin movement to the peak contractions in response to stimulation, i.e. to the region of skin where markers were displaced by the greatest distance. When the region of peak skin contraction had been determined, the videotape was reversed to a time just prior to stimulation and skin movement. The videotape was then advanced at intervals of 1/24th of a second so that a time point prior to, and just at the peak of skin contraction, could be captured to the computer. These frames were superimposed over images of the animals, and the distance of peak contraction was divided by the time required to produce it. This provided a measure of the velocity of skin contraction (Fig. 2). The character of skin movement following tactile stimulation was determined at the pre-injury evaluation for all but four animals, and for all animals at 1 day, 3 days, 2 weeks and 1 month post-treatment. When the peak contraction was determined for any one animal, a protractor was used to measure the angle at which the skin was pulled towards the monofilament probe relative to an imaginary line perpendicular to the long axis of the animal at the midline. The peak contraction of the skin was recorded as a positive angle when the skin pulled towards the probe and as a negative angle in the infrequent cases in which the skin pulled away from the probe. We also recorded whether this peak response occurred on the same side of the midline as the point of stimulation or on the other side (a contralateral response).

*Physiological recordings of somatosensory evoked potentials*

Subdermal electrodes stimulated nerve impulses from the tibial nerve of the hindleg (stimuli trains in sets of 200 at 3 Hz; stimulus amplitude ⩽3 mA square wave, 200 μs in duration) (Fig. 3). Evoked potentials, more properly termed somatosensory evoked potentials (SSEPs), were conducted through the spinal cord to the sensory cortex of the brain. To record SSEPs, a pair of subdermal electrodes, located above the level of the contralateral cortex, was used with a reference electrode usually located in the ipsilateral pinna of the ear. The

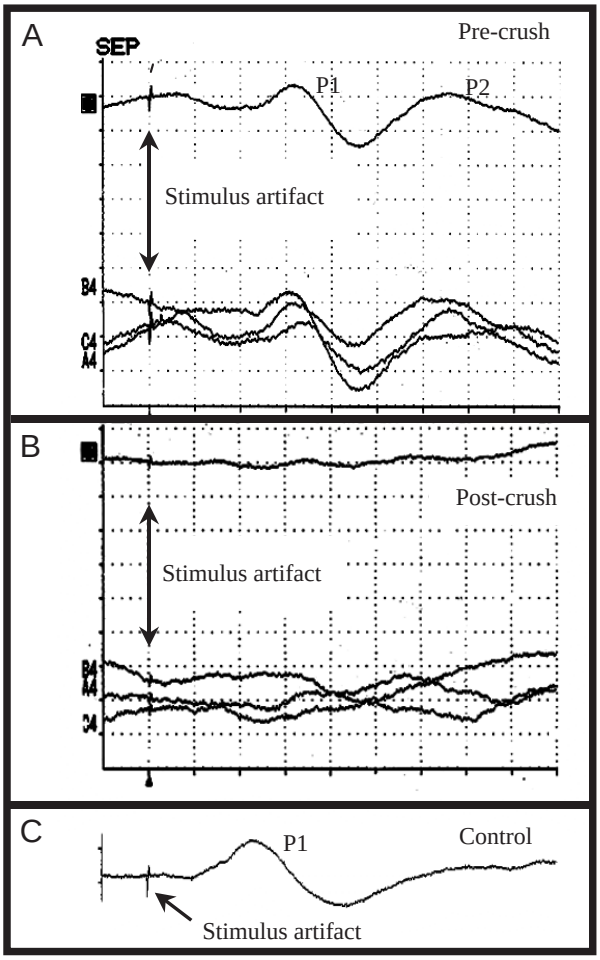


Fig. 3. Somatosensory evoked potentials (SSEPs). (A,B) Complete recordings of SSEP tests. In the pre-injury recording (A), the bottom three overlapping traces were produced by three separate sets of standard stimulations (refer to Materials and methods). These signals were averaged to produce the single top trace revealing two peaks of early-arriving (P1) and late-arriving (P2) evoked potentials at the brain (refer to Materials and methods). The peaks shown are characteristic SSEPs produced by tibial nerve stimulation in adult guinea pigs. The double-headed arrow shows the stimulus artifacts. A similar recording is shown in B, but this was taken within 30 min of a standardized compression to the mid-thoracic spinal cord. Note the complete loss of all ascending SSEPs. In C, a single averaged trace is shown of a median nerve stimulation recorded in this same animal, as a control procedure, within minutes of the traces shown in B.

stimulation and computer management of evoked potential recordings utilized a Nihon Kohden Neuropak 4 stimulator/recorder and PowerMac G3 computer. In all animals, failure to record an SSEP was confirmed to be due to the absence of evoked potential conduction through the lesion by a control test carried out at the same time. The medial nerve of the forelimb was stimulated, initiating evoked potentials in a neural circuit above the level of the crush injury. To perform this test, recording electrodes were left in place while stimulating electrodes were relocated to stimulate the median nerve of the foreleg.

Table 1. *A comparison of behavioral and physiological recovery from spinal cord injury in PEG-treated and control guinea pigs*

| | Cutaneus trunci muscle recovery | | | | | | | Somatosensory-evoked potential recovery | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N$[1] | Post[2] | Day 1[3] | Day 3 | Week 2 | Week 4 | Statistic[4] | Post[2] | Day 1 | Day 3 | Week 2 | Week 4 | Statistic[4] |
| Experimental | 15 | 0 | 11 | 12 | 12 | 14 | $P \leqslant 0.0003$ | 0 | 13 | 14 | 14 | 15 | $P \leqslant 0.0001$ |
| Control | 13 | 0 | 0 | 1 | 2 | 3 | | 0 | 0 | 0 | 0 | 0 | |

[1]$N$ is the total number of animals in the experimental and control groups.

[2]The total number of animals showing CTM responses when measurements were made within 30 minutes of the spinal cord injury, but prior to the PEG or sham treatment. Note that both CTM reflexes and SSEP conduction were eliminated in all animals.

[3]The number of animals showing recovery of either CTM functioning or SSEP conduction at the times specified.

[4]$P$ values are for all comparisons between control and experimental animals at each time point; Fisher's exact test, two-tailed.

Table 2. *Characteristics of cutaneus trunci muscle prior to and 1 month after injury*

| | | Cutaneus trunci muscle performance | | | | |
|---|---|---|---|---|---|---|
| | | $N$[1] | Direction[2] (degrees) | Distance[3] (mm) | Velocity ($mm\,s^{-1}$)[4] | Range ($mm\,s^{-1}$)[5] |
| Experimental | Pre[6] | 11 | 10/11 (88.7±1.2) | 1.2±0.12 | 15.7±1.9 | 8.4–25 |
| | Post | 15 | 13/15 (80.7±6.5) | 1.3±0.17 | 19.9±4.4 | 8.4–50 |
| Statistic[7] | | | 0.2 | 0.62 | 0.4 | – |
| Control | Pre | 3 | 3/3 (90)[8] | 1.0[8] | 25[8] | – |
| | Post | 3 | 3/3 (90) | 1.0 | 20.8±4.2 | – |

| | | CTM angle of contraction[9] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Ipsilateral | | | | Contralateral | | |
| | | $N$ | Angle (degrees)[10] | Range (degrees) | Statistic | Angle (degrees)[10] | Range (degrees) | Statistic |
| | Pre | 11 | 45±18.7 | –90/90 | $P$=0.44 | 49.4±25.9 | –90/90 | $P$=0.43 |
| | Post | 11 | 70±24.2 | –80/90 | | 19.4±25.1 | –90/90 | |

[1]$N$, total number of animals in pre-surgery (Pre) and post-surgery (Post) data sets.

[2]The direction and angle of orientation of cutaneus trunci muscle (CTM) skin contraction. The number of observations where skin pulled towards the stimulus is given over the total animals evaluated. The angle of skin contraction is expressed relative to an imaginary line perpendicular to the long axis of the animal, and the mean angle of contraction and its standard error for these animals are given in parentheses.

[3]The mean distance of peak contraction and its standard error.

[4]The mean velocity of CTM contraction and its standard error.

[5]The minimum and maximum velocities for each group.

[6]Four PEG-treated animals did not receive a pre-surgery evaluation.

[7]Statistical evaluation comparing the mean value in each column used the Mann–Whitney two-tailed test.

[8]Values for all three control animals were identical in these measurements, so there was no standard error to report.

[9]The angle of skin contraction relative to an imaginary line perpendicular to the long axis of the animal. Contractions pulling towards the probe were assigned positive values, and contractions pulling away from it were assigned negative values. The data are given for animals in which the peak contraction of skin was detected on the same side of the midline of the animal as the stimulus (ipsilateral) and when the peak contraction was detected on the other side of the midline (contralateral). Note that these data are presented for PEG-treated animals only since values for all three control recoveries were identical and all peak responses were ipsilateral to the point of stimulation. Note also that values obtained prior to surgery (Pre) were not statistically different from those obtained 1 month post-surgery (Post).

[10]The mean angle and its standard error.

### *Computer management of behavioral data*

Video images were acquired to an Intel Dual Pentium Pro computer. Superimposition of images, the coloring of receptive field boundaries made on the back of the animal during CTM testing and the general management of video images were performed using Adobe Photoshop software. The final figures were constructed with Microsoft PowerPoint software and printed on an Epson Stylus Color 800 printer. Quantitative planimetry of the area of receptive fields or of the regions of behavioral loss and recovery from these video images was carried out using IP Lab Spectrum software.

### *Statistical analyses*

The Mann–Whitney two-tailed test was used to compare the means of the data derived from experimental and sham-treated groups. To compare the proportions between groups, Fisher's

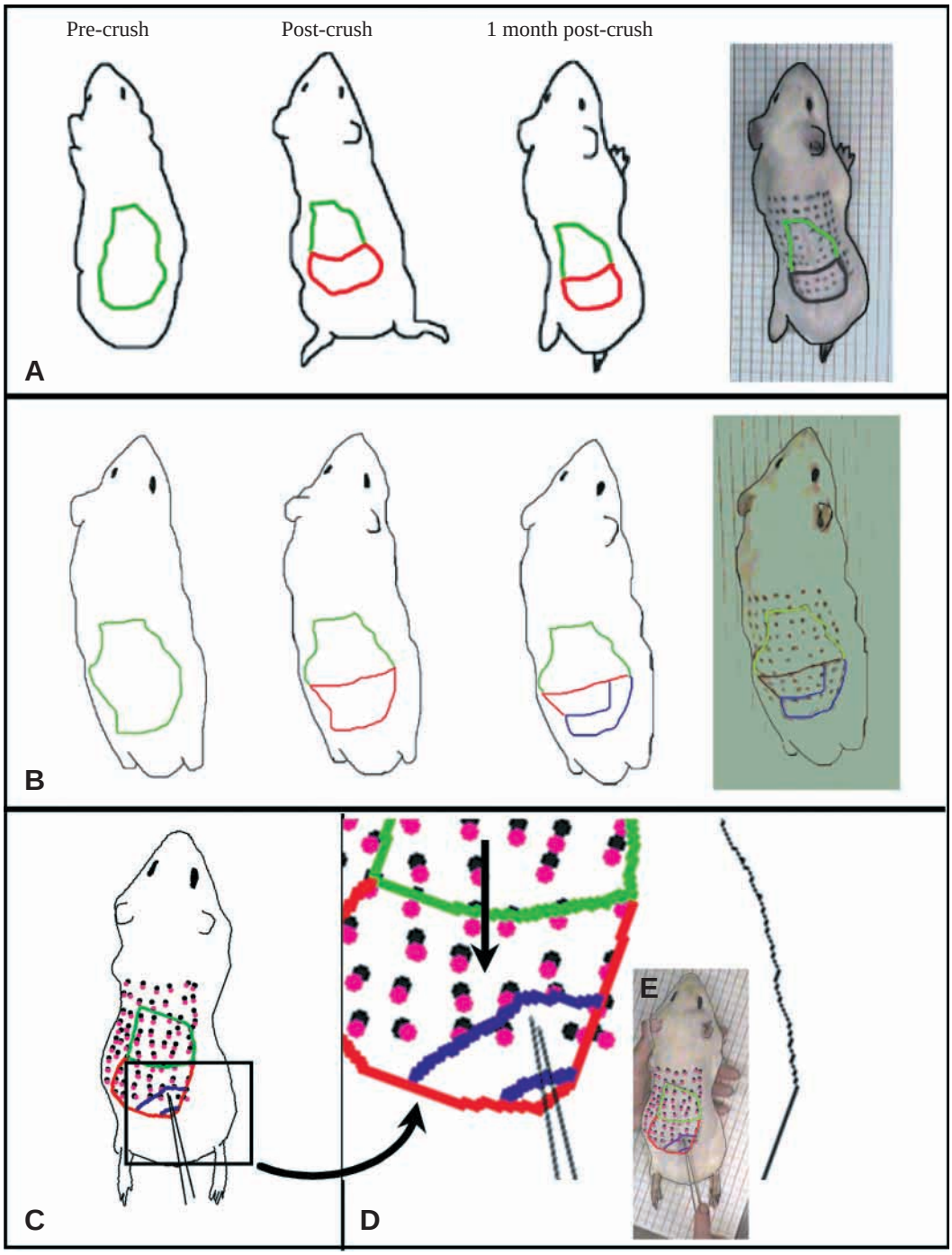


Fig. 4. The loss and recovery of cutaneus trunci muscle (CTM) receptive fields. (A) A normal, complete receptive field is shown highlighted in green on the tracing of a control guinea pig, as shown in Fig. 2 and described in Materials and methods. The region of areflexia is outlined in red on the next image, 24 h post-injury to the spinal cord. Note that approximately half the total CTM receptive field is lost as a result of severe spinal cord compression. One month later, this region remains unchanged. The video image used to produce the last drawing is shown on the far right. (B) From left to right, a similar set of images to those in A. The normal receptive field (green) and the region of areflexia (red) after spinal cord injury are marked. The region of CTM recovery in response to PEG application is outlined in blue in the last drawing and demonstrates a region of recovery of CTM sensitivity comprising approximately 42 % of the original region of areflexia. (C) Note the modest region of PEG-mediated CTM recovery outlined in blue. In the magnified section shown in D, note the position of the probe within the region of recovery and the movement of the skin marker dots towards the probe (arrow). Probing outside the region circumscribed in blue did not produce CTM reflex movements, but probing in the region marked in green (above the level of the injury), did elicit contractions. E shows the actual video images collapsed in layers to produce C and D.

exact test was used. All tests were performed using Instat software.

## Results

Only one animal died (after surgery) during the course of this study. The loss of the receptive fields subsequent to lesioning of the spinal cord resulted in a region of areflexia that was similar in all animals (mean loss of the total receptive field in sham-treated animals, 59.2±5.0 %; in PEG-treated animals, 52.2±2.4 %, means ± S.E.M.; $P$=0.19; Mann–Whitney two-tailed test). Thus, the standardized injury technique produced a mean CTM loss that was similar in both groups.

As will be discussed below, since none of the control animals recovered an SSEP, 13 of the 15 experimental animals with the best electrical recordings were evaluated quantitatively. Similarly, a full evaluation of CTM functioning by stop-frame videographic analysis was carried out on the three controls that recovered the reflex and on 13 of the 15 recovered PEG-treated animals for comparison.

### *The cutaneus trunci muscle reflex*

Application of PEG produced a very rapid recovery of CTM function in 73 % of treated animals within the first 24 h compared with a complete lack of spontaneous recovery in sham-treated animals at this time (Fig. 4). Some spontaneous recovery of the CTM reflex in controls began to appear on day 3, resulting in three recoveries out of a total of 13 animals (23 %) by 1 month (Table 1). In marked contrast, 11 of 15 PEG-treated animals recovered the reflex activity within the region of areflexia during the first day post-treatment (Table 1) (Fig. 4), and another three animals by 1 month (total number of animals showing 93 %; $P \leqslant 0.0003$; Fisher's exact test, two-tailed) (Fig. 4). The area of recovered areflexic back skin was 27.6±8.6 % in the 15 PEG-treated animals and 18.3±3.4 % (means ± S.E.M.) in the three controls. Thus, the total area of PEG-mediated recovery was not statistically different from that occurring spontaneously, although infrequently ($P$=0.28; Mann–Whitney, two-tailed test).

In general, back skin contracts towards the point of stimulus when the CTM reflex is activated. The largest response usually occurs ipsilateral and rostral to the point of stimulation because the reflex occurs bilaterally. Recall that the spinal lesion produces a bilateral injury eliminating the entire CTM receptive field below the level of injury on both sides (Fig. 4). In the normal reflex, there is a minor contralateral contraction in response to ipsilateral stimulation of the skin, so we provide details of the region of peak skin contraction, no matter which side of the midline on which it occurred, with reference to the region of stimulation producing it (Table 2). However, we observed only three occasions where the peak contraction occurred contralateral to the point of stimulation (normal or recovered CTM) and only in PEG-treated animals. In the three control recoveries, the peak contraction was always ipsilateral, as expected. Furthermore, we observed only six examples out of 52 separate comparisons (experimental and control; left and right sides) where the peak contraction was directed away from the focal stimulus in the uninjured animal, emphasizing that this is normally an infrequent occurrence. When the direction of skin contraction, its angle and velocity were compared between the pre-injury and post-injury data in PEG-treated animals, there were no statistical differences; thus, the recovered reflex was faithfully reproduced by PEG treatment (Table 2).

We also evaluated the change in direction of skin contraction on both the left and right sides of the animals by paired comparison of the angle of skin contraction in PEG-treated animals only (as will be emphasized below, control CTM behavior did not change following its spontaneous recovery in just three animals). The mean angle of skin contraction following ipsilateral CTM stimulation was not significantly different after PEG-mediated recovery from the normal CTM in the same animals prior to injury ($P$=0.43; Mann–Whitney, two-tailed paired comparison). This was also true for the contralateral responses ($P$=0.44, same test).

In the three spontaneous recoveries in control animals, the peak distance of contraction (1 mm) and its velocity (25 mm $s^{-1}$) were identical in two of them and the reflex was unchanged after recovery. In the third case, a reduction in the velocity of CTM contraction was measured, while the angle of contraction and the peak distance of contraction remained unchanged (Table 2).

### *Recovery of conduction through the injured spinal cord*

As in previous studies, SSEPs usually segregated into two peaks following tibial stimulation in the uninjured animal: an early-arriving peak (approximately 20–35 ms) and a late-arriving peak (40–50 ms). Table 1 shows the proportion of animals recovering an SSEP in the experimental population, which was 87 % for the first day after PEG treatment and by week 4 had reached 100 %. Not one sham-treated animal recovered conduction over the same period (Table 1). Fig. 5A shows a typical example of an SSEP recorded from a sham-treated animal, as well as a median nerve control procedure. Such control procedures were undertaken for any measurement that failed to demonstrate a repeatable SSEP and, in every case, demonstrated that the absence of evoked potentials was due to a failure to conduct them through the lesion. In Fig. 5B, a typical process of PEG-mediated recovery is shown. Note that the latency of the recovering evoked potentials was greater than normal in the early stages of recovery, but gradually declined with time (depicted for the early-arriving peaks). Fig. 6 shows that normal latency was not reached during the 1 month of observation and also plots the magnitude of the early-arriving evoked potentials, which recover to more than 50 % of their pre-injury values.

## Discussion

This report both confirms and extends an earlier one that PEG treatment can, within hours of its application, reverse the behavioral and conduction losses that follow severe spinal

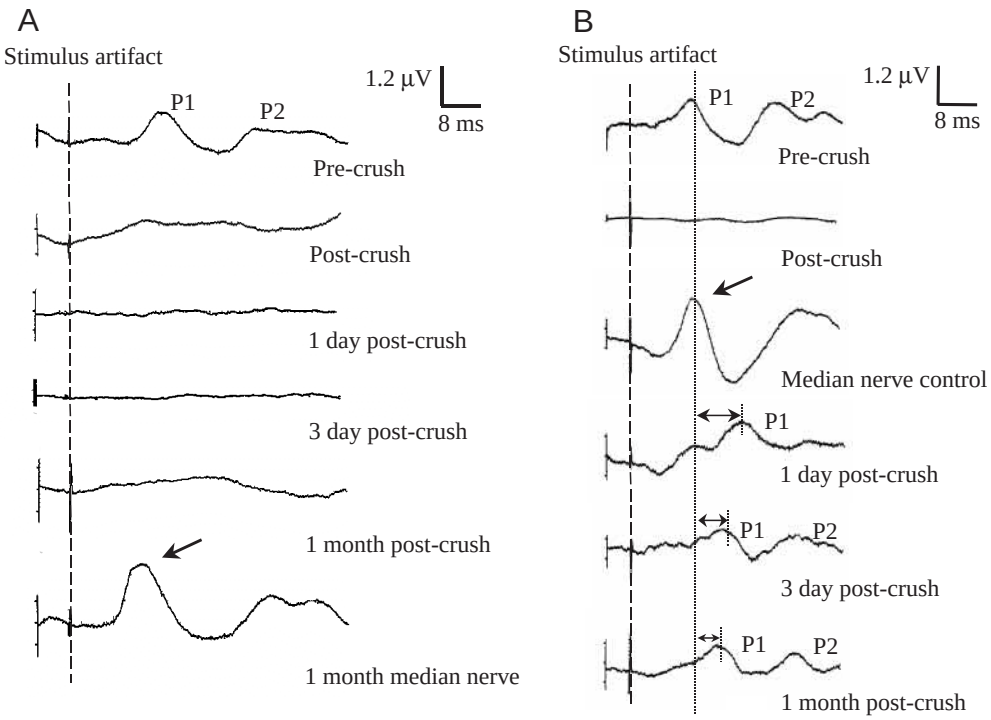


Fig. 5. Evoked potentials. (A; from top to bottom) Averaged traces of evoked potentials are shown, all obtained from the same animal, from the pre-injury electrical recording to those obtained 1 month post-injury. Note the complete absence of somatosensory evoked potentials (SSEPs) following spinal cord injury. This was characteristic in 100 % of the control population at all times tested. A median nerve control stimulation was also carried out at these times, but only the 1 month recording is shown in the bottom trace, the arrow pointing to a strong early-arriving evoked potential (for an explanation of P1 and P2, refer to Fig. 3). (B) Recovery of SSEP conduction in a PEG-treated animal. The characteristic double SSEP peaks are present in the uninjured animal. Note the complete loss of these peaks following injury and the positive median nerve control procedure carried out at this time point. One day post-injury to 1 month post-injury recordings show the recovery of SSEP conduction. The dotted line marks the approximate peak magnitude of the early-arriving SSEP. Note that the latency to peak contraction is reduced over time (refer to Fig. 6). Such recovering SSEPs were characteristic of 100 % of the PEG-treated animals and are in contrast to the complete absence of such conduction in all control animals.

cord injury. Here, we have focused exclusively on a 7 h delayed administration of PEG to a standardized spinal cord injury.

### *The spinal injury*

The means of injury we chose was a constant-displacement injury in which each spinal cord received a severe lateral compression for approximately the same distance and for the same duration. This is a different approach from previous studies of impact injuries (usually dropping a weight, usually 20 g from 20 cm, onto the dorsal aspect of the exposed spinal cord). These different approaches have been discussed and described elsewhere (Blight, 1988, 1991; Collins and Kauer, 1979). We looked for a method that would produce a repeatable injury among animals, at least with respect to the anatomical consequences of the technique. In our hands, the constant-displacement injury method produces lesions that are not statistically different among animals, as determined by three-dimensional computer reconstruction of the vertebral segment containing the injury (Moriarty et al., 1998; Duerstock et al., 2000). The resulting hemorrhagic lesion is fairly typical of clinical injuries in that it spreads in severity from the center of the spinal cord (destroying most gray matter) to the margins, leaving a subpial rim of spared white matter over a longitudinal distance of approximately one vertebral segment (Moriarty et al., 1998) (see also Duerstock and Borgens, 2002). Moreover, the technique does not require special laboratory equipment,

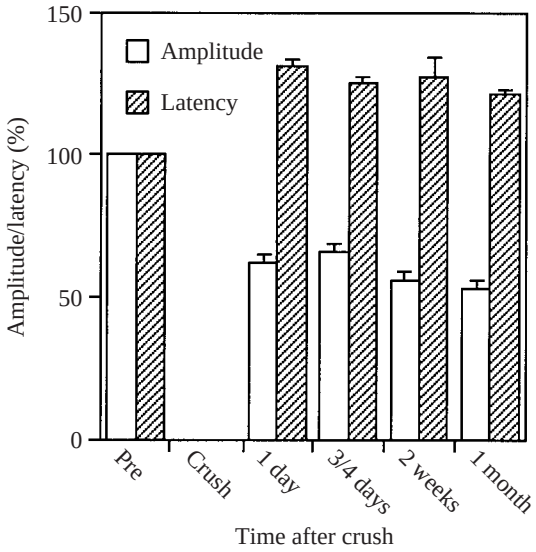


Fig. 6. Amplitude and latency of recovered somatosensory evoked potentials (SSEPs) in PEG-treated animals. The peak normalized amplitude of the early-arriving SSEP is plotted for all time points together with the mean latency (100 % represents the pre-injury value). Note that the mean magnitude of the SSEP is approximately 50 % of its pre-injury level, while the latency declines incrementally. The latency at 1 month was statistically significantly reduced compared with day 1 measurements. Values are means + S.E.M. Measurements from 13 animals are shown for all points except at 2 weeks, where nine animals were used for recordings.

such as stereotaxic frames and impactors, and is simple to carry out. Nevertheless, all attempts at standardizing an injury to the spinal cord are still associated with some variation in functional recovery among animals. This is believed to be due to biomechanical differences between the cords, to the variable level of endogenous sealing of mechanically damaged axons (Krause et al., 1994; Shi et al., 2000) and to other factors that cannot be controlled (Blight, 1991). This is evident in this report from the modest number of control animals showing CTM recovery. A more precise anatomical and behavioral outcome can be obtained by specific transection techniques; however, severing tracts, or the spinal cord itself, is generally not a clinically meaningful injury model (Borgens, 1992).

Other conventional experimental methods have also been vitiated, or found to be unnecessary, by the extremely rapid recovery produced by PEG in prior published experiments and unpublished pilot trials. Thus, blinding of evaluators to the treatment used was not performed in this study since, within a matter of a hours, the experimentally treated animals could be identified with greater than 90 % accuracy in CTM studies or with 100 % accuracy when evaluating SSEP recovery. The most important precaution in these series of experiments was the determination that the measures of damage are statistically similar between animals after injury and before PEG treatment.

### *The CTM reflex as a functional indicator of spinal cord behavioral recovery*

The skin movement is dependent on sensory afferents projecting as a long tract of axons in each ventral funiculus of the spinal cord (just lateral to the spinothalamic tract) to nuclei of CTM motor neurons located at the cervical/thoracic junction left and right of the midline (Blight et al., 1990; Thierault and Diamond, 1998) (Fig. 1). There are some contralateral afferent projections within the cord, but these appear to play only a very marginal role in the normal expression of the reflex. This mainly local response to stimulation is further represented by the fact that there are no contralateral projections between the nuclei of CTM motor neurons (Blight et al., 1990). The reflex is bilaterally organized into segmentally arranged receptive fields, displays little supraspinal control and is lost following spinal injury, producing a region of areflexia below the level of the lesion (Borgens et al., 1990; Blight et al., 1990; Thierault and Diamond, 1988) (Fig. 1). Following transection, recovery of the CTM reflex within this region of areflexia is not usually observed for the rest of the life of the animal and is infrequent ($\leqslant$20 %) following severe crush lesions to guinea pig spinal cord (Blight et al., 1990; Borgens and Shi, 2000; Borgens and Bohnert, 2001).

Although these injuries to the spinal cord also produce locomotor deficits in the animals, we have ignored these deficits and their apparent changes over time. This is because standing, stepping and overall locomotion in small animal models of SCI are locally controlled and generated within the spinal cord and so have little dependence on supraspinal control and organization (for a review, see Borgens, 1992). We also note that the recovery of the CTM and the recovery of SSEP conduction should be considered as independent outcomes. The long tracts associated with the CTM are bilateral and located in the ventrolateral funiculus, while SSEP conduction (initiated by stimulation of the tibial nerve) is more a measure of dorsal column activity (see below).

### *Behavioral recovery*

We have not previously evaluated the character of the CTM behavioral recovery by comparing its characteristics with those of the pre-injury reflex. Here, we have shown that the recovered reflex activity is statistically similar in terms of the direction, distance and velocity of CTM contractions when compared with the normal reflex. The entire receptive field lost after injury was not restored, however. The largest area of back showing recovery after PEG treatment approached 50 % of the original area of areflexia. Given that we evaluated only one specific sensorimotor reflex, which is dependent on an identified white matter tract, this amount of appropriately organized recovery was surprising. The recovery of the receptive fields on the back was also variable in location and was sometimes revealed as islands of CTM-receptive flank that stood out within a still-areflexic region of back skin. This variability in the way in which recovery was re-established is believed to result from the variable re-recruitment of afferent axons into conduction through the lesion (see also Blight et al., 1990; Borgens et al., 1987, 1990). In summary, direct application of this hydrophilic polymer to the site of a spinal cord injury can rapidly reverse behavioral loss, restoring an appropriately organized behavioral response as well as nerve impulse conduction through the lesion within a clinically useful time frame.

### *Physiological and anatomical recovery*

The most striking effect of PEG application in all SCI studies in this series, *in vitro* and *in vivo*, has been the uniform recovery of conduction through the lesion in response both to transection (and reattachment) and to compression injury (Shi and Borgens, 1999; Shi et al., 1999; Borgens and Shi, 2000; Shi and Borgens, 2000; Borgens and Bohnert, 2001). This recovery of conduction, documented by the recovery of either compound action potential *in vitro* or of SSEP conduction *in vivo*, occurred in 100 % of the spinal cords treated with PEG. It is very clear that the fusogenic action of PEG (Nakajima and Ikada, 1994; Davidson et al., 1976) alters the dynamics of axolemma injury to permit a very rapid re-establishment of excitability in the region of conduction block. The magnitude of recovered compound action potentials in spinal cord strips in isolation can be nearly doubled by the addition of the fast $K^+$ channel blocker 4-aminopyridine, suggesting that the 'repaired' region of membrane is still somewhat leaky to $K^+$ and not yet a perfect seal (Shi and Borgens, 1999).

We have also shown that the PEG-restored membrane is repaired sufficiently to exclude the uptake of a large-molecular-mass intracellular tracer, horseradish peroxidase (HRP) (Shi and Borgens, 2000). This label is normally taken up into breaches of the nerve membrane, a common method of

loading neurons or their processes with this intracellular 'dye' (Borgens et al., 1986; Malmgren and Olsson, 1977). Compression-injured spinal cords showed a dramatic uptake of HRP into white matter axons at the epicenter of the injury when evaluated only 15 min post-injury. This uptake was greatly reduced by PEG treatment, and the effect was independent of axon diameter. Thus, the dye-exclusion test (Asano et al., 1995) proved that the polymeric 'seal' is indeed just that and is sufficient to interfere with the ability of even large-molecular-mass compounds to cross the membrane after damage (Shi and Borgens, 2001). Physical reconnection of axons within completely severed strips of guinea pig spinal cord ventral white matter also demonstrated the capability of a brief PEG treatment to reunite membranes sufficiently to seal in small-molecular-mass fluorescent intracellular markers (rhodamine- and fluorescein-decorated dextrans; $M_r$ 8000) (Shi et al., 1999).

Finally, the companion paper (Duerstock and Borgens, 2002) demonstrates that this anatomical sealing of single cells can also be represented at the level of the whole tissue. The histopathological character of the *in vivo* spinal lesion is also reduced by PEG treatment.

### *The mechanisms of PEG-mediated repair*

This report is one of a series that has explored the ability of a cell fusogen, PEG, to reconnect severed mammalian spinal cord axons and to seal the axolemma of severely compressed/crushed spinal axons. We have previously discussed what are believed to be the mechanisms of action of PEG, in particular, as well as the mechanisms that may be shared with non-ionic triblock polymers such as the poloxamines and poloxamers discussed below (for a review, see Borgens, 2001) (see also Borgens and Shi, 2000; Lee and Lentz, 1997; Lentz, 1994; Marks et al., 2001). Briefly, sealing of membrane breaches by high-molecular-mass molecules such as PEG may involve a dehydration of the plasmalemma where closely apposed regions of the bilayer resolve into each other, i.e. the structural components of the plasmalemma are no longer partitioned by the polar forces associated with the aqueous phase. The lipid core of the membrane, exposed across a breach in it, would be expected to flow together in the absence of an aqueous 'barrier' (following dehydration of the membrane). Subsequent to the removal of the polymer and rehydration, the now-continuous phase undergoes spontaneous reassembly of its structural elements, including protein components and complex lipid components. This reorganization of cellular water is believed to result from the strongly hydrophilic structure of PEG.

Poloxamers are complex polymers of a PEG–propylene glycol–PEG structure. They too can seal membrane breaches (see below), but perhaps through a slightly different mechanism. It is proposed the hydrophobic head group inserts itself into the membrane breach, sealing it by 'plugging' it (Marks et al., 2001). We have preliminary evidence that Poloxamer 188 can also induce axolemma sealing and induce recovery from adult guinea pig SCI in a manner similar to PEG (D. Bohnert and R. B. Borgens, unpublished observations). It is also possible that these non-ionic detergents, like PEG, may seal membrane breaches initially as a surfactant film, although this does not explain the permanent nature of polymer-mediated seals, even after the polymers have been removed.

In all studies, we have used an application of an aqueous solution of PEG (50 % w/v in distilled water) for 2 min. We have detected no difference in response using PEG solutions prepared with polymers with a relative molecular mass of 400 to approximately 3000 (D. Bohnert and R. B. Borgens, unpublished observations), but believe that the viscosity of the solution may be more important to PEG-mediated repair than the molecular mass of the polymer. Using fluorescently decorated PEG, we have traced the distribution of PEG after various routes of administration in guinea spinal cord injury. PEG clearly targets the hemorragic lesion and is only faintly detected in uninjured spinal cord following a direct application to the injury site (Borgens and Bohnert, 2001). More importantly, subcutaneous or intravenous application labels the lesion just as well. This may be more important to an eventual clinical use where intravascular injection of PEG may be beneficial during emergency care and later applied directly to the lesion during surgical management of the injury. At present, we are testing the application of hydrophilic polymers administered both topically and through the blood supply in veterinary cases of severe, acute paraplegia in dogs secondary to disc herniation and fracture dislocation (see also Blight et al., 1991; Borgens et al., 1999).

### *Membrane repair in other types of injury*

As mentioned above, non-ionic detergents, so-called triblock polymers, are mainly composed of PEG and may share mechanisms of action in reversing cell permeabilization. Their structure usually incorporates a high-molecular-mass central hydrophobic core with hydrophilic PEG side chains. Poloxamer 188 (P188) has been shown to reverse muscle cell death subsequent to high-voltage insult (Lee et al., 1992). Isolated rat skeletal muscle cells were labeled with an intracellular fluorescent dye that leaked out of the cells after high-voltage trauma. This insult was sufficient to disrupt muscle membranes, allowing the leakage of the marker in 100 % of the control preparations. Treatment of skeletal muscle cells *in vitro* with P188 reduced or even eliminated leakage of dye following the injury. Further *in vivo* tests extended these results: an intravenous injection of P188 produced physiological and anatomical recovery of rat muscle following electric shock (Lee et al., 1992). This approach has also been tested for its ability to reverse cell death in a testicular reperfusion injury model in rats (Palmer et al., 1998). P188 can also seal heat-shocked muscle cells *in vitro*, as shown by an inhibition of calcein dye leakage from cells induced by elevated temperature (Padanlam et al., 1994). P188 rescues fibroblasts from lethal heat shock (Merchant et al., 1998). Another biocompatible detergent (Poloxamer 1107; administered intravenously) was used in an *in vivo* testicular ischemia/reperfusion injury model in rats as well as for inhibiting the leakage of hemoglobin from irradiated

erythrocytes (Palmer et al., 1998; Hannig et al., 1999). These studies demonstrate that non-ionic biocompatible detergents and large hydrophilic molecules can reverse the permeabilization of cell membranes and also that they can be administered through the vascular system to reach damaged target cells.

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## References

**Asano, T., Shi, R. and Blight, A. R.** (1995). Horseradish peroxidase used to examine the distribution of axonal damage in spinal cord compression injury *in vitro*. *J. Neurotrauma* **12**, 993.

**Benowitz, L. J., Goldberg, D. E., Madsen, J. R., Soni, D. and Irwin, N.** (1999). Inosine stimulates extensive axon collateral growth in the rat corticospinal tract after injury. *Proc. Natl. Acad. Sci. USA* **96**, 13486–13490.

**Blight, A. R.** (1988). Mechanical factors in experimental spinal cord injury. *J. Am. Paraplegia Soc.* **11**, 26–34.

**Blight, A. R.** (1991). Morphometric analysis of a model of spinal cord injury in guinea pigs, with behavioral evidence of delayed secondary pathology. *J. Neurol. Sci.* **103**, 156–171.

**Blight, A. R., Toombs, J. P., Bauer, M. S. and Widmer, W. R.** (1991). The effects of 4-aminopyridine on neurological deficits in chronic cases of traumatic spinal cord injury in dogs: a phase I clinical trial. *J. Neurotrauma* **8**, 103–119.

**Blight, A. R., McGinnis, M. E. and Borgens, R. B.** (1990). Cutaneus trunci muscle reflex of the guinea pig. *J. Comp. Neurol.* **296**, 614–633.

**Borgens, R. B.** (1992). Applied voltages in spinal cord reconstruction: History, strategies and behavioral models. In *Spinal Cord Dysfunction*, vol. 3 (ed. L. S. Illis), pp. 110–145. Oxford: Oxford Medical Publications.

**Borgens, R. B.** (2001). Cellular engineering: Molecular repair of membranes to rescue cells of the damaged nervous system. *J. Neurosurg.* (in press).

**Borgens, R. B., Blight, A. R. and McGinnis, M. E.** (1990). Functional recovery after spinal cord hemisection in guinea pigs: The effects of applied electric fields. *J. Comp. Neurol.* **296**, 634–653.

**Borgens, R. B., Blight, A. R. and Murphey, D. J.** (1986). Axonal regeneration in spinal cord injury: A perspective and new technique. *J. Comp. Neurol.* **250**, 168–180.

**Borgens, R. B. and Bohnert, D.** (2001). Rapid recovery from spinal cord injury following subcutaneously administered polyethylene glycol. *J. Neurosci. Res.* (in press).

**Borgens, R. B., Blight, A. R. and McGinnis, M. E.** (1987). Behavioral recovery induced by applied electric fields after spinal cord hemisection in guinea pig. *Science* **238**, 366–369.

**Borgens, R. B. and Shi, R.** (2000). Immediate recovery from spinal cord injury through molecular repair of nerve membranes with polyethylene glycol. *FASEB J.* **14**, 27–35.

**Borgens, R. B., Toombs, J. P., Breur, G., Widmer, W. R., Water, D., Harbath, A. M., March, P. and Adams, L. G.** (1999). An imposed oscillating electrical field improves the recovery of function in neurologically complete paraplegic dogs. *J. Neurotrauma* **16**, 639–657.

**Bracken, M. B., Shepard, M. J., Collins, W. F., Holford, T. R., Young, W., Baskin, D. S., Eisenberg, H. M., Flamm, E., Leo-Summers, L. and Maroon, J.** (1990). A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. *New Engl. J. Med.* **322**, 1405–1461.

**Bregman, B. S., Dai, H. N., Lin, T., James, C. V., Schwab, M. E. and Schnell, L.** (1996). Transplants, neurotrophic factors and myelin-associated neurite growth inhibitors: effects on recovery of locomotor function after spinal cord injury in rats. *Soc. Neurosci. Abstr.* **22**, 764.

**Collins, W. F. and Kauer, J. S.** (1979). The past and future of animals models used for spinal cord trauma. In *Neural Trauma* (ed. J. J. Popp, R. S. Bourke, L. R. Nelson and H. K. Kimelberg), pp. 273–279. New York: Raven Press.

**Davidson, R. L., O'Malley, K. and Wheeler, T. B.** (1976). Induction of mammalian somatic cell hybridization by polyethylene glycol. *Somat. Cell Genet*. **2**, 271–280.

**Duerstock, B. S., Bajaj, C. L., Pascucci, V., Schikore, D., Lin, K.-N. and Borgens, R. B.** (2000). Advances in three-dimensional reconstructions of the experimental spinal cord injury. *Comput. Med. Im. Grap.* **24**, 389–406.

**Duerstock, B. S. and Borgens, R. B.** (2002). Three-dimensional morphometry of spinal cord injury following polyethylene glycol treatment. *J. Exp. Biol.* **205**, 13–24.

**Griffin, J. W., George, E. B., Hsieh, S. T. T. and Glass, J. D.** (1995). Axonal degeneration and disorders of the axonal cytoskeleton. In *The Axon* (ed. S. G. Waxman, J. Kocsis and P. Stys), pp. 375–390. New York: Oxford University Press.

**Hannig, J., Yu, J., Beckett, M., Weichselbaum, R. and Lee, R. C.** (1999). Poloxamine 1107 sealing of radiopermeabilized erythrocyte membranes. *Int. J. Radiat. Biol*. **75**, 379–385.

**Hansebout, R. R., Blight, A. R., Fawcett, S. and Reddy, K.** (1993). 4-Aminopyridine in chronic spinal cord injury: A controlled, double-blind, crossover study in eight patients. *J. Neurotrauma* **10**, 1–18.

**Hayes, K. C., Blight, A. R. and Potter, P. J.** (1993). Preclinical trial of 4-aminopyridine in patients with chronic spinal cord injury. *Paraplegia* **31**, 216–224.

**Honmou, O. and Young, W.** (1995). Traumatic injury to the spinal axons. In *The Axon* (ed. S. G. Waxman, J. D. Kocsis and P. K. Stys), pp. 480–503. New York: Oxford University Press.

**Krause, T. L., Fishman, H. M., Ballinger, M. L. and Bittner, G. D.** (1994). Extent and mechanism of sealing in transected giant axons of squid and earthworms. *J. Neurosci.* **14**, 6638–6651.

**Lazarov-Spiegler, O., Solomon, A. S., Zeev-Brann, A. B., Hirschberg, D. L., Lavie, W. and Schwartz, M.** (1996). Transplantation of activated macrophages overcomes central nervous system regrowth failure. *FASEB J.* **10**, 1296–1302.

**Lee, J. and Lentz, B. R.** (1997). Evolution of lipid structures during model membrane fusion and the relation of this process to cell membrane fusion. *Biochemistry* **36**, 6251–6259.

**Lee, R., River, L. P., Pan, F. S. and Wollmann, L.** (1992). Surfactant-induced sealing of electropermeabilized skeletal muscle membranes *in vivo*. *Proc. Natl. Acad. Sci. USA* **89**, 4524–4528.

**Lentz, B. R.** (1994). Induced membrane fusion; potential mechanism and relation to cell fusion events. *Chem. Physiol. Lipids* **73**, 91–106.

**Malmgren, L. and Olsson, L.** (1977). A sensitive histochemical method for light and electron microscopic demonstration of horseradish peroxidase. *J. Histochem. Cytochem.* **25**, 1280–1283.

**Marks, J. M., Pan, C.-Y., Bushell, T., Cromie, W. and Lee, R. C.** (2001). Amphiphilic, tri-block copolymers provide potent, membrane-targeted neuroprotection. *FASEB J.* (in press).

**Merchant, F. A., Holmes, H. A., Capelli-Schellpfeffer, M., Lee, R. C. and Toner, M.** (1988). Poloxamer 188 enhances functional recovery of lethally heat-shocked fibroblasts. *J. Surg. Res.* **74**, 131–140.

**Moriarty, L. J., Duerstock, B. S., Bajaj, C. L., Lin, K. and Borgens, R. B.** (1998). Two- and three-dimensional computer graphic evaluation of the subacute spinal cord injury. *J. Neurol. Sci.* **155**, 121–137.

**Nakajima, N. and Ikada, Y.** (1994). Fusogenic activity of various water-soluble polymers. *J. Biomater. Sci. Polym. Ed*. **6**, 751–759.

**Padanlam, J. T., Bischof, J. C., Cravalho, E. G., Tompkins, R. G., Yarmush, M. L. and Toner, M.** (1994). Effectiveness of poloxamer 188 in arresting calcein leakage from thermally damaged isolated skeletal muscle cells. *Ann. N.Y. Acad. Sci*. **92**, 111–123.

**Palmer, J. S., Cromie, W. L. and Lee, R. C.** (1998). Surfactant administration reduces testicular ischemia–reperfusion injury. *J. Urol.* **159**, 2136–2139.

**Pointillart, V., Petitjean, M. E., Wiart, L., Vital, J. M., Lassie, P., Thicoipe, M. and Dabadie, P.** (2000). Pharmacological therapy of spinal cord injury during the acute phase. *Spinal Cord* **38**, 71–76.

**Schwab, M. E., Kapfhammer, J. P. and Bandtlow, C. E.** (1993). Inhibitors of neurite growth. *Annu. Rev. Neurosci.* **16**, 565–595.

**Shi, R., Asano, T., Wining, N. C. and Blight, A. R.** (2000). Control of membrane sealing in injured mammalian spinal cord axons. *J. Neurophysiol.* **84**, 1763–1769.

**Shi, R. and Blight, A. R.** (1997). The differential effects of low and high

concentrations of 4-aminopyridine on axonal conduction in normal and injured spinal cords. *Neuroscience* **77**, 553–562.

**Shi, R. and Borgens, R. B.** (1999). Acute repair of crushed guinea pig spinal cord by polyethylene glycol. *J. Neurophysiol.* **81**, 2406–2414.

**Shi, R. and Borgens, R. B.** (2000). Anatomic repair of nerve membrane in crushed mammalian spinal cord with polyethylene glycol. *J. Neurocytol.* **29**, 633–644.

**Shi, R., Borgens, R. B. and Blight, A. R.** (1999). Functional reconnection of severed mammalian spinal cord axons with polyethylene glycol. *J. Neurotrauma* **16**, 727–738.

**Short, D. J., El Marsy, W. S. and Hones, P. W.** (2000). High dose methylprednisolone in the management of acute spinal cord injury – a systematic review from a clinical perspective. *Spinal Cord* **38**, 273–286.

**Thierault, E. and Diamond, J.** (1988). Noccieptive cutaneous stimuli evoke localized contractions in a skeletal muscle. *J. Neurophysiol*. **60**, 446–447.