be stable with Hd decaying to it by beta emission.

13. Perhaps neutral heavy stable particles would bind only to nuclei with $Z > 1$ and thus would not have been detected in the experiment of (*3*). Such behavior is known for the Λ^0, which binds to ^{4}He but not ^{1}H or ^{2}H.
14. Technetium has been detected in uranium ore, where it occurs as a product of spontaneous and neutron-induced fission, as well as in the spectra of stars [for example, see G. E. Boyd and Q. V. Larson, *J. Phys. Chem.* **60**, 707 (1956)]. Minute traces of promethium have been found in nature and attributed to neutron irradiation of neodymium [see F. Weigel, *Chem. Ztg.* **102**, 339 (1978)]. These traces are identified by their radioactivity and thus cannot be related to the superheavy isotopes we seek, which would not be radioactive..
15. See M. E. Weeks and H. M. Leicester, *The Discovery of the Elements* (Journal of Chemical Education, Easton, Pa., ed. 7, 1968), pp. 834–883.
16. See, for example, J. M. Blatt and V. F. Weisskopf, *Theoretical Nuclear Physics* (Wiley, New York, 1952), figure 3.1, p. 575.
17. We are indebted to E. K. Hulet and R. Lougheed for this suggestion.
18. See, for example, R. J. Holt *et al.*, *Phys. Rev. Lett.* **36**, 183 (1976), where limits for bismuth are discussed. Their experiment set very stringent limits on the occurrence of abnormal nuclei of a particular kind, but was insensitive to the sort of matter we are discussing.
19. A. G. W. Cameron, in *Explosive Nucleosynthesis*, D. N. Schramm and W. David, Eds. (Univ. of Texas Press, Austin, 1973), p. 3.
20. We thank L. W. Alvarez, F. Asaro, R. Hagstrom, R. Muller, E. Segrè, and R. Wagoner for their assistance and advice. R.N.C. was supported in part by an A. P. Sloan fellowship and expresses his thanks to the Aspen Center for Physics, where a portion of this work was completed. S.L.G. acknowledges the kind hospitality of the Lawrence Berkeley Laboratory, where this work was begun. He also thanks C. K. Jorgensen for several enlightening conversations and communications. This research was supported in part by the High Energy Physics Division of the U.S. Department of Energy under contract W-7405-ENG-48.

Enhanced Spinal Cord Regeneration in Lamprey by Applied Electric Fields

Richard B. Borgens, Ernesto Roederer, Melvin J. Cohen

Summary. After a weak, steady electric current of approximately 10 microamperes was imposed across the completely severed spinal cord of the larval lamprey *Petromyzon marinus*, enhanced regeneration was observed in the severed giant reticulospinal neurons. The current was applied with implanted wick electrodes for 5 to 6 days after transection (cathode distal to lesion). The spinal cords were examined 44 to 63 days after the operation by means of intracellular fluorescent dye injections and electrophysiology. Extracellular stimulation of whole cords showed that action potentials in most of the electrically treated preparations were conducted in both directions across the lesion, but they were not conducted in either direction in most of the sham-treated controls. In most of the electrically treated animals, processes from giant axons with swollen irregular tips, indicating active growth, were seen in or across the lesion. Only a few of the sham-treated controls showed these features. It is possible that these facilitated regenerative responses were mediated by the effects of the artificially applied electric fields on the natural steady current of injury entering the spinal lesion.

The notion that an imposed electrical field can enhance or direct nerve growth began with the work of Sven Ingvar in 1920 (*1*). However, various technical and procedural difficulties rendered the results of many early studies ambiguous (*2–4*). Several experiments have been reported that clearly show that an imposed steady electric field has an effect on nerve growth in culture. Elaborating on an experimental design first used by Marsh and Beams in 1946 (*5*), Jaffe and Poo (*3*) demonstrated that the outgrowth of neurites emerging from explanted chick dorsal root ganglia is increased toward the negative pole (cathode) when an electrical field is imposed across the culture chamber. More recently, Robinson and McCaig (*6*) and Hinkle *et al.* (*4*) reported that single or multiple neurites produced by individual neuroblasts in culture show accelerated growth toward the cathode and will deviate toward it, bending away from the antipode.

Recent studies on the effects of applied electric current on the regeneration of frog forelimbs have indicated that nerve growth in vivo can also be modified by applied electric currents. Borgens *et al.* (*7, 8*) demonstrated that minute, steady electrical fields imposed within the forelimb stumps of adult frogs can initiate limb regeneration. These workers used wick electrodes to deliver current and thus avoided the possible effects of electrode products associated with implanted metal electrodes. The enhanced limb regeneration was correlated with a striking hyperinnervation of the electrically treated limbs in both *Rana* (*7, 8*) and *Xenopus* (*9*). This suggested that the induced limb regeneration might be mediated by a facilitated nerve growth within the terminal portions of the limb stump, in much the same manner that surgical hyperinnervation was shown by Singer (*10*) to initiate limb regeneration in *Rana*. Borgens *et al.* (*7, 8*) established that this induced limb regeneration and increased nerve growth was due directly to the artificially imposed current of 200 nanoamperes with the cathode oriented distally within the limb stump.

We have asked whether comparable electric fields, when imposed across the severed spinal cord of a primitive vertebrate, will enhance the regeneration of axons in the cord. For our experiments we used the ammocoete larva of the lamprey (*Petromyzon marinus*), for the following reasons: (i) The lamprey central nervous system (CNS) possesses giant reticulospinal neurons that are morphologically identifiable at various locations within the brain and spinal cord (*11*). The cell bodies of these Mauthner and Müller cells lie within the brain and project giant axons (about 40 micrometers in diameter) down the spinal cord in well-characterized tracts. This permits one to compare the reactions of these individual identified neurons to axonal transection in control and experimental animals. (ii) These giant neurons are already known to regenerate across a spinal lesion (*12, 13*). Normally, a few of the axons will regenerate across the lesion and form new synapses (*14*) and eventually, swimming is restored. (iii)

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Since blood vessels are found only at the surface of the cord, vascular exchange occurs primarily by diffusion from capillaries at the surface. The preparation is therefore relatively insensitive to the vascular insults usually associated with spinal transection. (iv) This metabolic exchange by diffusion from the cord surface permits complete removal of the CNS to simple organ culture where it is viable for at least a week [see (*15*)]. Therefore, the spinal cord of this primitive vertebrate presents an excellent model for studying the effects of applied electric fields on the regeneration of axons in the vertebrate CNS. We chose to keep the animals at 16°C and to observe the extent of regeneration at 40 to 60 days after transection, because few giant axons would be expected to have grown across the lesion at this time in untreated animals (*14*). This regime provided the baseline of regenerative growth (relative to the lesion) for measuring the effects of the electrical treatment.

The Imposed Electric Field and General Assay Procedure

Using long flexible saline bridges as wick electrodes, we imposed an electric field across the completely severed spinal cord of lamprey larvae for 5 to 6 days, with the anode rostral and the cathode caudal to the lesion (Fig. 1). In control animals we implanted electrodes spanning the spinal transection, but no current was passed (sham treatment). Of the 15 sham-treated controls and 15 electrically treated experimental animals, 13 and 11 survived, respectively, to provide the data presented here.

For assay purposes, the animals were anesthetized and pinned out in fresh lamprey Ringer solution (*16*). The dorsal surface of the brain and about three-fourths of the spinal cord, including the site of lesion, were exposed by dissection. In half of all the experimental and control animals studied, electrophysiological tests were made on this exposed CNS in situ. In most instances, after electrical records were taken, the entire CNS was dissected free and individual axons were visualized by transillumination, impaled with a microelectrode, and marked by iontophoretic injection of the fluorescent dye Lucifer Yellow (*17*). In the remaining half of all the spinal cords studied, the entire CNS was removed immediately after the animals were anesthetized and placed in lamprey Ringer solution. We studied the electrical responses and axon morphology of these isolated preparations.

The spinal cords of all the animals were tested for the conduction of action potentials across the lesion site in both directions. One pair of silver electrodes (36 gauge) was placed on the dorsal surface of the cord just caudal to the hindbrain (2 to 3 centimeters proximal to the lesion). The other electrode pair was placed at various distances just distal to the site of the lesion (Fig. 2a). After delivering a stimulus pulse of short duration to either electrode pair, we monitored any evoked action potentials that crossed the lesion with the other pair. By switching connections to the electrodes, either pair could be used to stimulate or record without our having to move them from their positions on the cord.

Electrophysiological Results

When the normal intact spinal cord is stimulated by extracellular surface electrodes, a characteristic pattern of action potentials can be easily evoked and recorded with another pair of surface electrodes at some distant point (Fig. 2b). After the complete transection of this cord between the electrodes, this pattern is totally abolished (Fig. 2c). When these extracellular electrode pairs were placed on either side of the spinal transection site (Fig. 2a), striking differences were observed between the animals that were sham-treated and those that were electrically treated. In 8 of 11 (73 percent) of the experimental animals, stimulation of the cord on either side of the lesion evoked action potentials that propagated across the site of transection (Table 1 and Fig. 2, d and e). In controls, we could evoke action potentials that crossed the lesion in both directions in only 3 out of 13 preparations (23 percent) ($P = .021$, Fishers' exact test). In 9 of 13 (69 percent) of the control group, action potentials were not propagated across the lesion in either direction. When no action potentials could be induced to cross the lesion (Fig. 2f), we increased the stimulus intensity as much as four- to sixfold. This was often sufficient to stimulate directly adjacent musculature, and

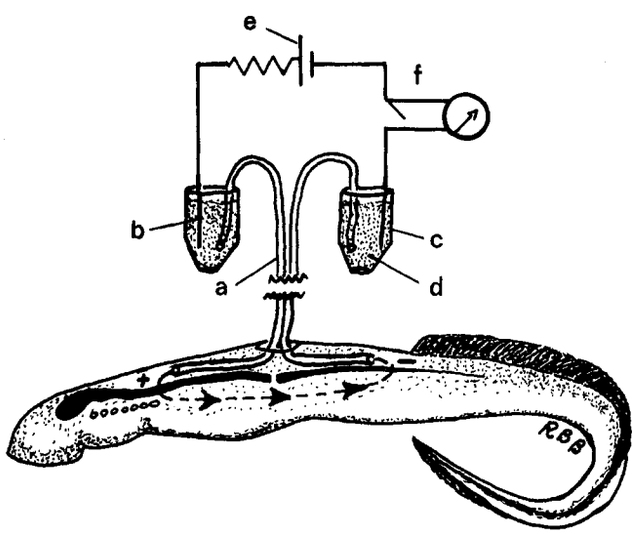


Fig. 1. Surgical and electrical manipulation of lamprey preparation. Larvae (10 to 15 cm in length) were anesthetized in a saturated solution of benzocaine in well water and pinned out in lamprey Ringer solution (*16*). An incision, 5 to 7 mm long, was made in the dorsal midline such that the spinal cord could be exposed and completely severed. This was performed at a point about midway between the head and the end of the tail. Wick electrodes (a) were made by pulling a cotton thread through a 14- to 16-cm-long tube made of medical grade elastomer (0.51 mm inner diameter, 0.94 mm outer diameter) and filling this tube with lamprey Ringer solution. The end of one electrode was routed through the small incision on the back of the animal, beneath the skin, sometimes penetrating the musculature. The uninsulated open tip of this electrode was left in a position about 1.5 to 2.5 cm anterior to the spinal cord transection. Similarly, a wick electrode was placed about the same distance posterior to the transection. The animal was then placed in a plastic container filled with aerated well water. The loose ends of the wick electrodes were immersed in two small plastic reservoirs (c) of lamprey Ringer solution (d), and connected in series with an external voltage source outside the animal's container by way of silver–silver chloride electrodes (b). The voltage source (e) was a 30-volt battery (Eveready No. 413). Since the overall resistance of the individual wick electrodes varied somewhat, an appropriate resistor was chosen (usually on the order of 2 to 4 megohm) so that a total of about 10 μA was driven through this simple circuit. The direction of current flow through the animal is indicated by the arrows. The circuit could be checked by inserting a Keithley model 602 electrometer (f). Sham-treated controls were treated in the same manner except that the ends of the indwelling electrodes were not connected to any external power supply. In most instances, one experimental animal and one control animal were paired in the same plastic container separated by a fiber glass screen. In other instances, animals were individually housed in plastic containers containing aerated well water. The electrodes were implanted at the time of spinal cord transection and removed 5 or 6 days later. All animals were kept in the dark at 15° to 16°C until they were assayed for regeneration.

still no action potentials were seen to cross the lesion. Such a complete absence of activity across the lesion was noted in only 2 of 11 (18 percent) of the electrically treated animals ($P = .001$, Fishers' exact test). One could suggest that this absence of activity across the lesion might be due to retrograde degeneration of axons proximal to the lesion. This was tested by placing both pairs of electrodes on the proximal cord segment (rostral to the lesion). In all instances, a large complement of the action potentials seen in normal spinal cords was evoked with this electrical stimulation (Fig. 2g).

When electrically evoked action potentials crossed the lesion, we could be sure these were produced by spinal cord axons that traversed the lesion by the following tests: (i) A secondary transection of the proximal segment (between the electrode pairs) abolished all such propagated action potentials; (ii) cord action potentials could not be evoked by direct stimulation of the adjacent musculature in animals in which the electrophysiological tests were performed on the exposed spinal cord in situ; and (iii) about half of all tests were carried out on isolated CNS preparations and no differences were observed between these preparations and the records taken in situ. Additionally, the action potentials that crossed the lesion were eventually lost when the distal electrode pair was moved to successively more posterior locations on the cord, away from the lesion. Moreover, when moving the distal electrode pair incrementally, we observed a corresponding shift in the latency of recorded action potentials because of the increased separation between the stimulating and recording electrodes.

To explore further the observed differences between the experimental and control animals, we combined extracellular measurements with intracellular recording to identify individual axons anatomically. This was accomplished by first placing extracellular stimulating and recording electrodes on either side of the lesion as described above. We then impaled individual giant axons within the cord between 5 and 8 millimeters proximal to the lesion with a microelectrode (Fig. 2a). This recording microelectrode was filled with Lucifer Yellow (*17*). After simultaneous extracellular and intracellular records were taken from each axon, we injected it iontophoretically with dye. This enabled us to compare the neurons that propagated action potentials across the lesion with those neurons that did not. Thus, when an axon was impaled, one of two experimental situations arose: either an intracellularly recorded antidromic action potential could be evoked by stimulation distal to the lesion, or it could not. All impaled axons showed intracellularly recorded orthodromic action potentials when stimulated from a point proximal to the recording electrodes.

The records presented in Fig. 3, a to d, were taken from the spinal cord of a sham-treated control. They are typical of axons from either sham-treated or elec-

Table 1. The distribution of extracellularly recorded action potentials (AP's) evoked and recorded across the lesion in electrically treated and sham-treated animals.

Animals tested	Days after transection (mean ± S.E.M.)	Action potentials evoked across lesion		No AP's evoked‡
		In both directions*	In one direction†	
Experimental ($N = 11$)	55.6 ± 1.5	8	1	2
Control ($N = 13$)	53.2 ± 1.4	3	1	9

*The location of stimulating and recording electrodes relative to the lesion made no difference to the incidence of evoked AP's in these animals. Stimulation either proximal or distal to the site of transection caused AP's to propagate across the lesion. †In a few instances, AP's evoked by stimulating proximal to the lesion propagated across the lesion in one direction, but corresponding spikes could not be elicited by stimulating distally and recording proximally to the transection site. ‡Spinal cords in which no AP propagated across the lesion in either direction.

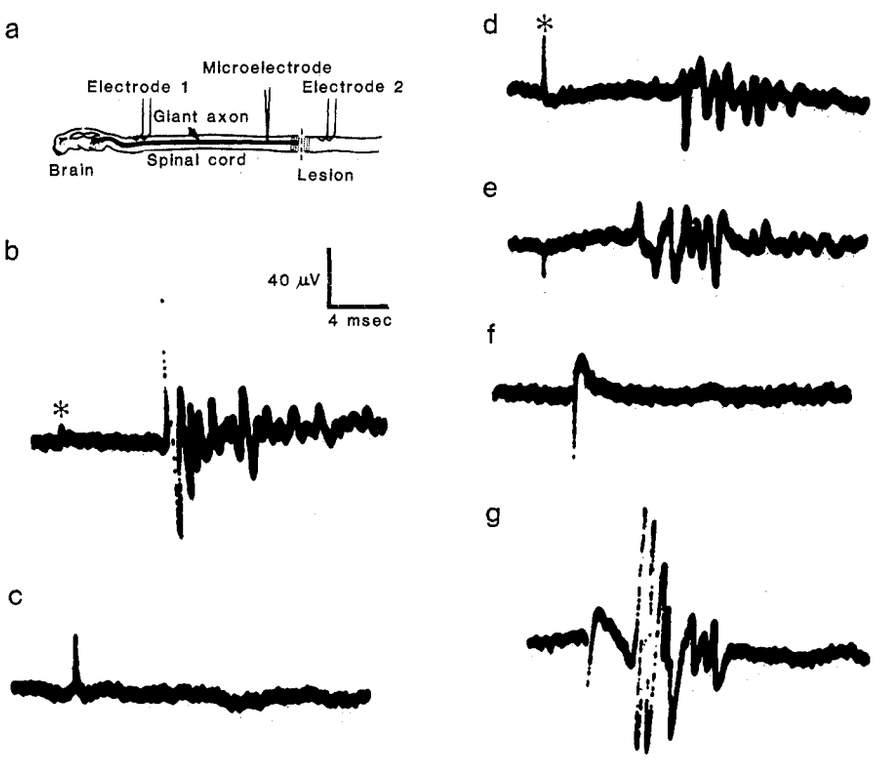


Fig. 2. Extracellular records. (a) Electrode positions. Electrode pairs 1 and 2 are extracellular electrodes on the surface of the spinal cord. Pair 1 was placed on the dorsal surface of the exposed spinal cord, directly caudal to the hindbrain; pair 2 was placed at various distances caudal to the lesion. These electrode pairs were usually about 2.5 to 3 cm apart. Giant axons were impaled with a microelectrode 4 to 8 mm rostral to the lesion within the proximal segment of the spinal cord (these records are presented in Fig. 3). (b) The pattern of evoked orthodromic action potentials (AP's) from an intact spinal cord. The stimulating and recording electrodes were separated by about 2.5 cm. Asterisks mark the stimulus artifacts. (c) The effect of complete transection of this spinal cord 5 mm proximal to the recording electrodes [pair 2 as shown in (a)]. Note the complete loss of recorded action potentials. (d) The pattern of evoked AP's recorded across the lesion in an experimental animal, 51 days after transection. Electrode pair 2 recorded these spikes from a position 1.5 mm caudal to the lesion. (e) Same as (d), except the positions of the stimulating and recording electrodes were reversed. Electrode pair 2 stimulated the cord caudal to the lesion, and electrode pair 1 recorded these spikes just caudal to the brain. The extracellular records in (d) and (e) show propagation of AP's across the lesion in both directions (see Table 1). (f) Extracellular record obtained from a control animal 56 days after transection. Electrode pair 2 was recording, and was placed 1 mm caudal to the lesion. Note the complete absence of AP's distal to the lesion. (g) The same animal as in (f). Both extracellular electrodes were placed on the proximal segment of the spinal cord, with recording electrode pair 2 about 3 mm rostral to the lesion. Note the similarity of the pattern of evoked AP's to that recorded in the intact spinal cord in (b).

trically treated preparations that regenerated across the spinal lesion. Figure 3a shows an intracellularly recorded orthodromic action potential evoked by stimulation of the whole cord proximal to the microelectrode. This intracellular action potential also coincided with the presence of a large spike in the extracellular record obtained caudal to the lesion. Both of these spikes exhibited the same stimulus threshold (compare Fig. 3, a and b). Figure 3c shows that this cell can also propagate a spike antidromically across the lesion when the cord is stimulated distal to the site of transection. Once again the intracellular spike correlates with the large spike in the extracellular record. Subsequent iontophoresis of dye through the intracellular recording electrode showed this to be a Mauthner axon which indeed had crossed the lesion.

Figure 3e shows a record from another sham-treated spinal cord in which an orthodromic action potential could be elicited by stimulation of the cord. In the simultaneously recorded extracellular record, no action potentials were observed to traverse the lesion. When the direction of stimulation and recording was reversed (Fig. 3f), no intracellular action potential could be evoked from this axon by stimulation of the cord 1 mm caudal to the lesion. Three small spikes propagated across the lesion toward the brain; however, none was associated with the impaled fiber. By subsequent intracellular dye injection we identified this fiber as a Mauthner cell axon which ended in small multiple branches about 4 to 5 mm rostral to the lesion.

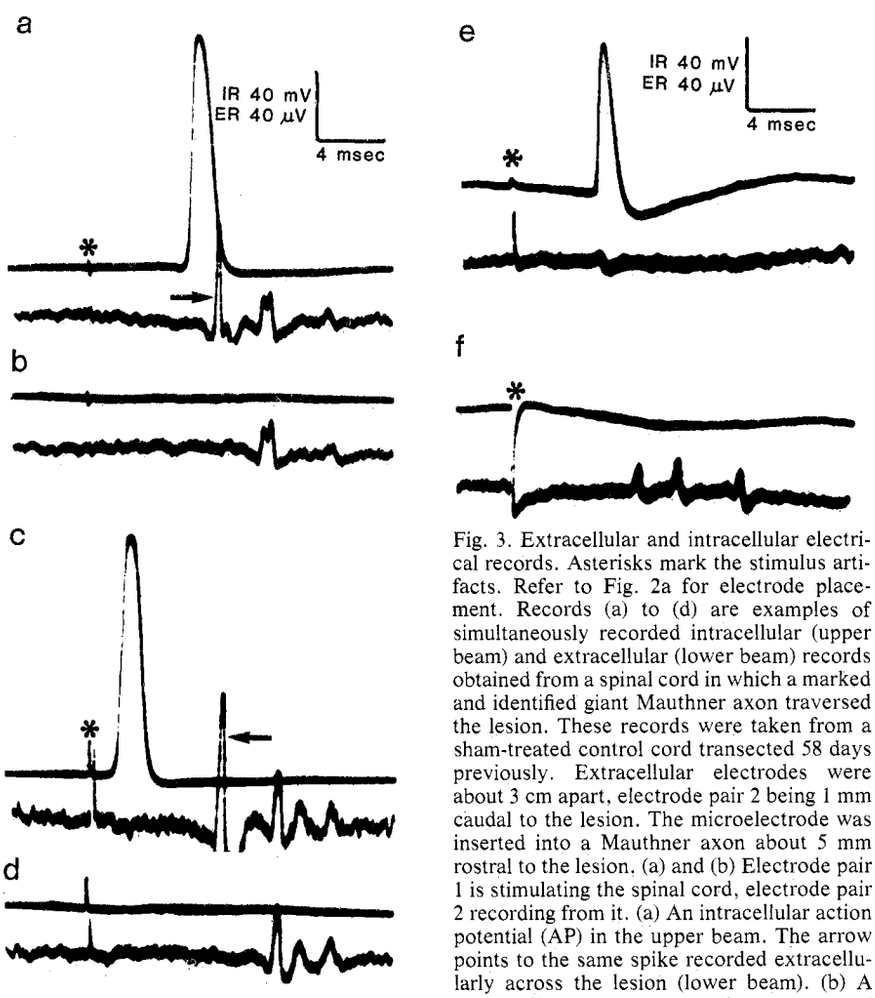


Fig. 3. Extracellular and intracellular electrical records. Asterisks mark the stimulus artifacts. Refer to Fig. 2a for electrode placement. Records (a) to (d) are examples of simultaneously recorded intracellular (upper beam) and extracellular (lower beam) records obtained from a spinal cord in which a marked and identified giant Mauthner axon traversed the lesion. These records were taken from a sham-treated control cord transected 58 days previously. Extracellular electrodes were about 3 cm apart, electrode pair 2 being 1 mm caudal to the lesion. The microelectrode was inserted into a Mauthner axon about 5 mm rostral to the lesion. (a) and (b) Electrode pair 1 is stimulating the spinal cord, electrode pair 2 recording from it. (a) An intracellular action potential (AP) in the upper beam. The arrow points to the same spike recorded extracellularly across the lesion (lower beam). (b) A record obtained during subthreshold stimulation of this fiber. Simultaneous disappearance of the large spike from both records further demonstrates that it represents an AP recorded from the same axon at two separate locations. (c) and (d) The positions of the stimulating and recording electrodes were reversed by switching their connections while leaving them in place on the cord. (c) An antidromic intracellular AP from the same Mauthner axon as in (a) and (b) evoked by stimulating distal to the lesion and recording proximal to it. The arrow indicates the same spike in the extracellular record. (d) Subthreshold stimulation for this neuron. (e) and (f) Examples of simultaneous intracellular (*IR*) and extracellular recordings (*ER*) from a spinal cord in which a marked and identified giant axon did not traverse the lesion but ended about 3 mm proximal to it. These records were obtained from a control cord, 49 days after it was transected. Extracellular electrodes 1 and 2 are 2.3 cm apart, with electrode pair 2 placed 1 mm caudal to the lesion. The microelectrode was inserted into a Mauthner axon about 6 mm rostral to the lesion. The record in (e) shows that this impaled fiber could conduct an orthodromic AP proximal to the lesion when stimulated via electrode pair 1. Record (f) shows that no intracellular response could be obtained from this large fiber when the cord was stimulated distal to the lesion via electrode pair 2. However, three other small AP's were observed in the extracellular record (lower beam), indicating that some smaller axons had crossed the lesion.

Location of Axon Terminals Relative to the Lesion

In all, 62 transected axons were intracellularly injected with dye in the sham-treated controls and the electrically treated experimental animals. These axons were divided into one of two categories: (i) fibers terminating proximally to the lesion, (ii) fibers that ended either within the area of the lesion or actually traversed it (see Fig. 4). We further subdivided the total population of experimental and control axons into two blocks of time after transection—days 44 to 53 and days 54 to 63. By day 53 after transection, a total of 14 fibers was filled with dye in experimentally treated spinal cords; 36 percent (five fibers) terminated proximal to the lesion, 64 percent (nine fibers) were found within the lesion or crossing it. Of the 17 dye-filled fibers in the sham-treated cords during this same time period, 53 percent (nine fibers) ended proximal to the lesion, and 47 percent (eight fibers) were found within or traversing it. These differences are not statistically significant ($P = .28$, Fishers' exact test). However, significant differences were seen within the later time period of 54 to 63 days after transection. In the 16 filled axons from the electrically treated experimental preparations, 94 percent (15 fibers) entered or crossed the lesion. In the 15 filled axons observed in sham-treated controls only 47 percent (seven fibers) entered or crossed the lesion whereas 53 percent (eight fibers) terminated proximal to the lesion ($P = .005$, Fishers' exact test).

Correlations Between Intracellular Recordings and Anatomy

If we consider all of the dye-filled axons from which we obtained intracellular recordings, we can determine if the anatomical and electrophysiological evidence for regeneration is correlated. We discuss here the pooled results of these tests (for 42 individual fibers) without considering whether the respective cords were electrically treated or sham-treated.

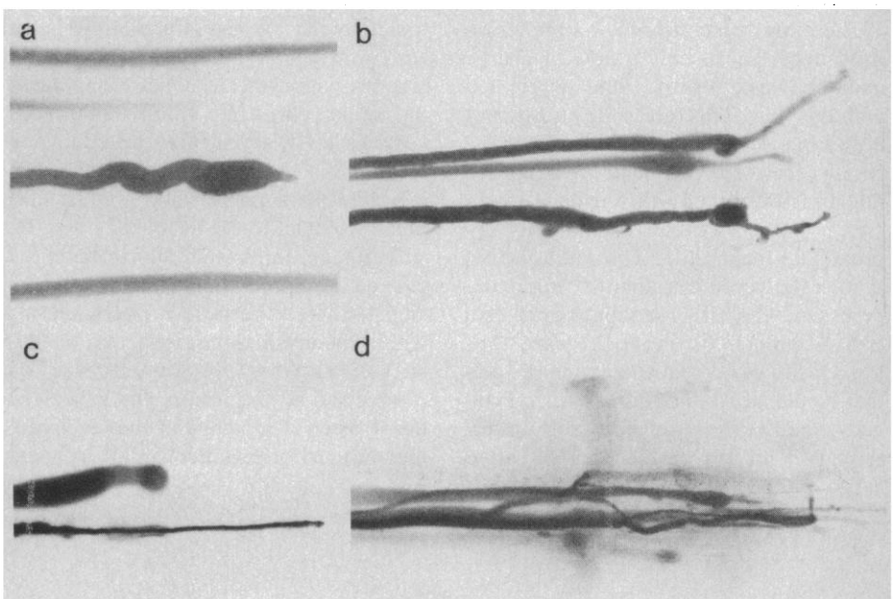
In the three instances in which a filled fiber traversed the lesion and from which intracellular electrical records were obtained, antidromic action potentials propagated back across the lesion when the cord was stimulated distal to the site of transection. Of the 24 fibers terminating in the lesion site but not traversing it, only four could be stimulated with electrodes placed just distal to the lesion. None of the 15 axons ending at a point proximal to the lesion could be fired by stimulating the cord just distal to the lesion. Therefore, these tests demonstrate that the increased occurrence of action potentials propagating across the lesion in electrically treated cords (Table 1) can be ascribed to the increased number of axons ending in or beyond the lesion area.

The Morphology of Cut Axonal Ends

The endings of sectioned giant axons labeled with Lucifer Yellow were examined in spinal cord wholemounts by means of transmission fluorescence optics. We distinguished three general categories of fiber endings: (i) large swollen tips (sometimes in excess of 50 μm) with no protrusions of any kind; (ii) swollen and more blunt endings that exhibited fimbriated edges, other protrusions, or single and multiple long neurites; (iii) blunt or tapered endings with no processes or obvious surface irregularities. About twice as many large swollen tips and tips with neurites were found in the experimentally treated spinal cords as in those that were sham-treated; whereas twice as many fibers with undifferentiated tapered or blunt ends were found in the sham-treated controls compared with the experimental cords (Fig. 4, a to c). There was also a pronounced difference between axons from experimental and control animals with respect to the amount of branching. Such branching was common in the experimental cords and was particularly profuse within the area of the lesion (Fig. 4d), but was rarely seen in the controls. These characteristic morphological differences between electrically treated preparations and the sham-treated controls are illustrated in Fig. 5.

For the following reasons, we believe that all the axon endings were completely filled with dye: (i) Lucifer Yellow is known (*17*) and was observed by us to fill the tiniest processes and neurites visible with the light microscope. (ii) The overall extent of fill was usually about 2 cm or more of axon, and the point of injection was usually only a few millimeters from the ending itself. This ensured a high concentration of dye near the terminus. (iii) The dye diffuses quickly throughout cells, and a period of hours usually elapsed between the end of a filling period and the time the cord was fixed.

Our studies demonstrate that in most of the animals treated with electric current, action potentials elicited by extracellular stimulation of the whole spinal cord were propagated in both directions across the lesion. In most of the sham-treated controls, action potentials did not propagate across the lesion in either direction. When intracellular recordings and fluorescent dye labeling of individual cells were used to characterize the axons responsible for propagating action potentials across the lesion, axons that conducted spikes antidromically across the lesion site were found to traverse it or, in a few cases, terminate within it. Few axons terminating within the lesion, and no axon ending proximal to the lesion, could be fired by stimulation distal to the lesion. The greatest proportion of fluorescently labeled axons in experimentally treated cords was found within or through the lesion by about 58 days after transection. Most of a comparable population of axons in the sham-treated controls ended proximal to the lesion. The morphology of most of the terminal ends of identifiable axons found within experimentally treated cords was indicative of actively growing regenerating fibers. Most of the ends of control fibers were relatively undifferentiated morphologically with the appearance of axons that were in a less active growth state.



e

Individual nerve endings

	Swollen tips	Swollen tips and neurites	Blunt or pointed tips	Fiber branching: Proximal to lesion	Fiber branching: Within the lesion
Experimentals	3	14	9	2	17
Controls	5	4	18	4	4

Fig. 4. Types of axonal endings observed in dye-filled severed axons. From a total of 62 dye-injected axons, 53 provided terminal endings suitable for a detailed morphological analysis. Three general categories were seen. (a) Giant swollen tips with relatively smooth surface. This example measured about 65 μm at its widest diameter. (b) Swollen tips that gave rise to processes (neurites), fimbria, or filopodial extensions. We also included in this category two blunt endings that gave rise to very long single or multiple neurites. (c) Blunt or tapered endings that neither gave rise to any neurites nor showed any marked surface irregularity. Branching occurred in some of the individual fibers listed above, and also in many of the nine remaining dye-injected axons in which we were unable to examine closely the axonal terminus. An example of such branching is shown in (d). In (e) we summarize the occurrence of these morphological categories in the experimental and control populations. Note that 73 percent (19 of 26) of the axons from experimental animals show branching compared to 30 percent (8 of 27) from the controls. Photographs (a) through (d) are reverse positive prints made from color photomicrographs of the fluorescently labeled cells photographed in dark field.

Nature of the Stimulus

Are the responses described here in fact due to the imposed electric current? The data we have presented were obtained from experiments where the imposed current was delivered to the tissues through 14- to 16-cm-long wick electrodes. Moreover, electrical contact with the battery was established by immersing a silver–silver chloride electrode and each wick electrode in a reservoir of lamprey Ringer solution. It is unlikely that any metallic ions traversed the reservior and the wick before removal of the wick from the animal 5 to 6 days later (*7*). In addition, the exposed end of each electrode was placed about 2 cm either side of the lesion, thus separating the transection site from possible chemical reactions occurring near the tips of the implanted electrodes.

Therefore, we conclude that the lesion itself experienced only a flow of charge and the weak electric field associated with this flow. This raises the question of how large an electric field might have resulted between the electrodes. We did not measure directly this voltage gradient; however, we can estimate its approximate magnitude. The total current that we delivered was about 10 microamperes and the cross-sectional area of a typical lamprey is about 0.1 cm^2, thus the density of current traversing the animal would be about 100 $\mu A/cm^2$. Typically, the resistivity of most soft animal tissues is of the order of 1000 ohm-centimeter (*18*), hence, the voltage drop between the electrodes would be on the order of 10 millivolts per millimeter. Voltage gradients in the tens of millivolts per millimeter are roughly comparable to those known to influence neurite outgrowth in culture (*3–5*) and in electrically treated amphibian limbs (*7–9*). These reported effects, including the responses reported here, are all mediated by distally negative imposed fields.

Nature of the Response

Are there alternative possibilities for explaining the propagation of action potentials in axons regenerating across the cord transection?

First, one possible cause for the propagation of an antidromic spike evoked by stimulation distal to the lesion is electrotonic spread of the stimulating pulse across the lesion to excite the large axon endings within the proximal segment of the spinal cord. We think this occurs very little, if at all, for the following reasons: (i) No electrically and morphologically characterized fiber ending at a point proximal to the lesion was responsible for an antidromic action potential evoked by stimulating distal to the lesion. In fact, very few fibers ending within the lesion gave rise to extracellular activity when the stimulus was applied distal to the lesion. (ii) When the distal electrode pair was moved incrementally, a corresponding shift in latency of the response was observed. This would not be the case for passive current spread. We are thus confident that the antidromic action potentials in the proximal cord evoked by stimulating distal to the lesion are a good index of axon growth into or across the lesion.

Second, one might suggest that these action potentials may be synaptically mediated and may not represent a whole fiber or its process, which indeed crosses the lesion. Synapses in regenerating central nervous tissue have been observed proximal to the lesion in teleosts (*19*), lampreys (*14*), and leeches (*20*); however, again we think we can safely exclude this possibility: (i) We marked individual axons and their branches and observed their endings within or traversing the lesion. (ii) The action potentials that we are addressing here as pertinent indices of regeneration propagate in either direction across the lesion. This excludes most chemical synapses, which are generally polarized; and these regenerating neurons do not form electrical junctions (*14*). (iii) Finally, we found that these large spikes did not fatigue after several seconds of stimulation at frequencies of over 100 hertz.

With respect to the morphology of the axons injected with dye, a greater number and variety of axon endings that could be classified as regenerating appeared in the experimentally treated animals. Swollen tips, especially those with fimbriated edges, filopodial extensions, neurites, or very long processes have all

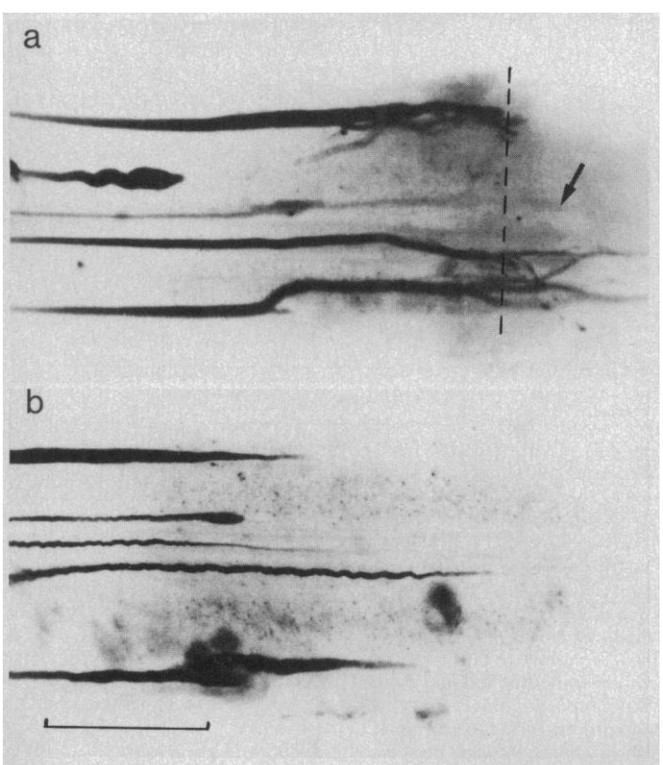


Fig. 5. Photomicrographs of spinal cord wholemounts taken from experimentally treated and sham-treated preparations. (Reverse positive prints made from dark-field photomicrographs of Lucifer Yellow-injected preparations.) In some of these photomicrographs, the lateral margins of the spinal cord are not visible. The width of most cords ranged from 0.8 to 1 mm. (a) An example of axon regeneration in an electrically treated spinal cord 59 days after transection. The Mauthner cell axon (at the lower margin) gave rise to a large bore process which, like the Müller cell axon medial to it, ramified throughout the area of the lesion and traversed it. The contralateral Mauthner cell axon (upper margin) had many large and small diameter processes within the area of the lesion, but none of these was observed to cross it. One giant axon ended in a giant swollen tip proximal to the lesion, and one very small unidentified fiber (centrally located and faintly filled) also traversed the lesion. Arrow points to the swollen central canal. (b) A typical sham-treated spinal cord 50 days after transection. The lesion is not within the photographic field and was about 3 mm further distal (to the right-hand margin of this figure). Of the three centrally located Müller cell axons, two taper to fine sharp points, the other ends in a swollen tip. One Mauthner axon at the upper margin ends in a tapered point; the contralateral Mauthner axon ends in a swollen tip that gives rise to a tapered extension.

been associated with axons that are actively growing in other giant fiber systems (*21*) as well as in the lamprey (*12–14*). This type of ending was most common in the electrically treated preparations. Smooth blunt or tapered terminal endings are not usually associated with actively growing fibers. A greater proportion of these was observed in the sham-treated control cords. Degenerating fibers sometimes display swellings near or at the end of the fiber; however, this is usually accompanied by various swollen areas along the length of the axon. We think it implausible that the swollen endings described here could be associated with degenerating fibers; they occurred within the area of the lesion itself, where most fibers in the experimental cords were found, and at a time when regenerated synapses are in the process of formation (*12–14*).

The procedure for impaling and dye injecting these giant axons is in itself free of bias. The experimenter simply visualizes the vague boundaries of the giant axons within the cord through a stereo microscope. The endings themselves, deep in the neuropile or lesion, are invisible at this magnification in the living unstained preparation.

One may suggest that the relatively high proportion of growing endings in experimental animals represent spinal cords that were simply more viable than the controls. However, our data do not support such a conclusion. When both stimulating and recording extracellular electrodes were placed on the proximal segment of the spinal cord, the character of the evoked action potentials was equivalent in experimentals and controls.

Thus we think that these results indicate an enhanced regeneration of giant axons in the severed lamprey spinal cord in response to an applied electric field.

Possible Mechanisms

Jaffe and co-workers (*3, 22*), Hinkle *et al.* (*4*), and Borgens *et al.* (*7*) have discussed the possible mechanisms by which steady fields can affect the individual cell, specifically the neuron. We will not repeat these discussions here, except to suggest that a likely means by which such imposed fields can influence an individual neuron is to act on various charged membrane surface components. Moreover, the "lateral electrophoresis" of a variety of membrane macromolecules has been demonstrated experimentally (*23*) in response to weak steady fields.

However, there is an additional fact that merits consideration here. Immediately after it is transected, the severed spinal cord of the lamprey drives intense currents (0.5 milliampere per square centimeter) into its cut face; and much of this current is driven into the injured ends of individual neurons (*15*). The magnitude of this inward current drops precipitously over the next 36 hours to reach steady levels of about 4 to 5 $\mu A/cm^2$. This steady current continues to enter the injured end of the spinal cord for at least the next 4 days. The steady component of this injury-induced current may indeed last longer, but we have not made measurements beyond this point. Moreover, there is reason to believe that this endogenous current may be involved with the overall responses of neurons to injury: this flow of charge may account for the radical alteration in ionic character of the axoplasm occurring at the terminal ends of severed nerves (*15, 21*), the alterations in the ionic basis of conductance at their ends (*21*), the initial and severe dissolution of cytoskeletal elements that occurs in a localized "pellet" at the end of a truncated nerve (*24*), and possibly the accumulation of organelles that occurs at their cut ends (*24*). Since this natural, injury-induced current enters the cut face of the proximal cord segment, the extracellular voltage gradient produced along its length by this current flow would be distally negative, and would correspond in direction to the artificially imposed fields described here. Furthermore, if the imposed current penetrates the nerves themselves [and there is sound theoretical reason to suppose it might—see (*22*)], its direction (proximal–distal) within the axon would be opposite to the natural current entering the end of the severed nerve. Perhaps this produces a rectification effect, reducing the early (and probably destructive) effects of the current of injury. We think it is possible (and testable) that our imposed field may act by modulating physiological processes associated with this endogenous current of injury.

It would be interesting to determine the effects of applied electrical fields that are positive distal to the lesion in severed spinal cords of the lamprey. This polarity of applied current has been associated with a general degeneration of tissue, both in amphibian limbs (*7*) and in bone (*7, 25*). The present results in this primitive vertebrate encourage further testing of the hypothesis that applied electrical fields may also enhance the regrowth of injured CNS elements in higher vertebrates.

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