Proc. Natl. Acad. Sci. USA
Vol. 77, No. 2, pp. 1209–1213, February 1980
Neurobiology

Large and persistent electrical currents enter the transected lamprey spinal cord

(injury current/axotomy/*p*-aminohippurate/vibrating probe)

RICHARD B. BORGENS, LIONEL F. JAFFE*, AND MELVIN J. COHEN

Department of Biology, Yale University, New Haven, Connecticut 06520

Contributed by Melvin J. Cohen, November 16, 1979

ABSTRACT **The electrical currents at the surface of the proximal portion of an isolated and transected lamprey spinal cord were measured with an extracellular vibrating probe. Soon after transection, currents of about 0.5 mA/cm^2 enter the cut surface of the spinal cord. These currents fall to about a quarter of their initial value within an hour; within the next 2 days they gradually decline from about 100 μA/cm^2 to about 4 μA/cm^2; they then remain constant up to 6 days posttransection, when the measurements were ended. The pattern of current entry included substantial peaks opposite (and presumably into) the cut ends of giant axons. Response to changes in the ionic composition of the medium indicates that about half of the injury current consists of Na^+, and that much of the rest may consist of Ca^{2+}. The measured influx of ions, which adds up to several coulombs per cm^2 in a few days, should radically alter the ionic composition of the terminal few millimeters of neural tissue. Thus it may be important in the degenerative and regenerative responses of neurons to axotomy.**

As early as 1843, Du Bois-Reymond (1) discovered a large potential difference along the surface of a damaged nerve trunk. Relatively sophisticated studies of such demarcation or injury potentials continued until the dawn of the microelectrode age (2, 3). These potentials were understood to result from a flow of current out of the intact regions of the nerve, along the outer surface of the preparation, and into the damaged area. These currents were investigated in an effort to understand the action potential and the resting membrane potential of intact nerves. With the introduction of the microelectrode in 1949, the investigation of injury currents for these purposes became obsolete. However, the involvement of steady currents with a wide range of developmental phenomena (4) has led us to re-examine their possible role in the response of nerve to injury. These previous measurements of injury potentials clearly indicated the direction of the injury currents and gave some measure of their relative change with time. However, nowhere in the literature does one find a determination of the absolute size of the currents nor any indication of the ion species entering the damaged region. In this paper, we provide such data.

MATERIALS AND RESULTS

We have used the excised brain and spinal cord of the larval sea lamprey, *Petromyzon marinus*. This system has three major advantages: First, the central nervous system essentially lacks an intrinsic blood supply, thus permitting the excised brain and spinal cord to be maintained by diffusion in a simple medium for up to 1 wk. Second, it contains giant identifiable reticulospinal neurons whose axons and cell bodies are readily seen in the living preparation (5). Last, in the transected spinal cord, these neurons are known to regenerate their axons (6) and form new synaptic contacts (7).

The ammocoete larvae of sea lampreys were collected in freshwater streams in Maine and held in aerated well water at 13°C in the dark. Animals averaging 8 cm in length were anesthetized in 0.01% Tricaine methanesulfonate (MS-222) and pinned out in sterile saline. A dorsal longitudinal incision was made, and about 4 cm of brain with attached spinal cord was dissected free of the dura and placed in fresh sterile medium. Approximately 1 hr later each preparation was transferred to a coverslip and held down (dorsal side up) by several 8 mm × 1 mm × 50 μm thick Teflon strips whose ends were attached to the glass with petroleum jelly. The spinal cord of each attached preparation was then transected with a single edged razor blade at a level 0.5–1 cm rostral to the point where the cord was dissected free. The time "after transaction" was calculated from this last operation. In some cases electrical measurements were begun immediately after cutting the cord and then made periodically for up to 6.5 hr. In others, the preparations were maintained for up to 6 days in organ culture and current measurements were taken daily. These preparations were cultured in lamprey saline (8) (91 mM NaCl/20 mM $NaHCO_3$/2.6 mM $CaCl_2$/2.1 mM KCl/1.8 mM $MgCl_2$/4 mM *d*-glucose). After aeration the pH was 7.4 and the resistivity was ≈100 ohm cm. Between measurement periods the preparations were kept in sealed chambers filled with 95% O_2/5% CO_2 (clinical grade) at 14–15°C. The saline medium, sometimes containing phenol red, was changed daily. The viability of the cultured preparations was determined at the end of the electrical current measurements by the stimulation and recording of action potentials from the dorsal surface of the cord (see Fig. 7).

Measurements of current densities entering or leaving the cut face of the cord were made with an ultrasensitive vibrating probe (9). The measuring electrode at the tip of the probe (Fig. 1*A*) was a platinum-black ball 20 μm in diameter. The probe registers the minute voltage difference between the extremes of its vibration in the electrical field around the preparation. The local current density in the direction of vibration is calculated from this voltage difference, the amplitude of vibration, and the resistivity of the medium by using an analog of Ohm's law for extended media (10). The probe was periodically calibrated by injecting a known current into the medium from a discrete source. The possibility that the vibrating probe mechanically induced the preparation to produce currents was ruled out as explained below (Fig. 6).

Immediately after cord transection, we observed intense currents (0.3–0.7 mA/cm^2) entering the cut end of the cord. These extraordinary currents were observed in every one of the 44 animals examined. Fig. 2 shows such a case. The density of current entering the cord declined to approximately one quarter of the initial value within 1 hr. Over the next 2 days the entering

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

* Present address: Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

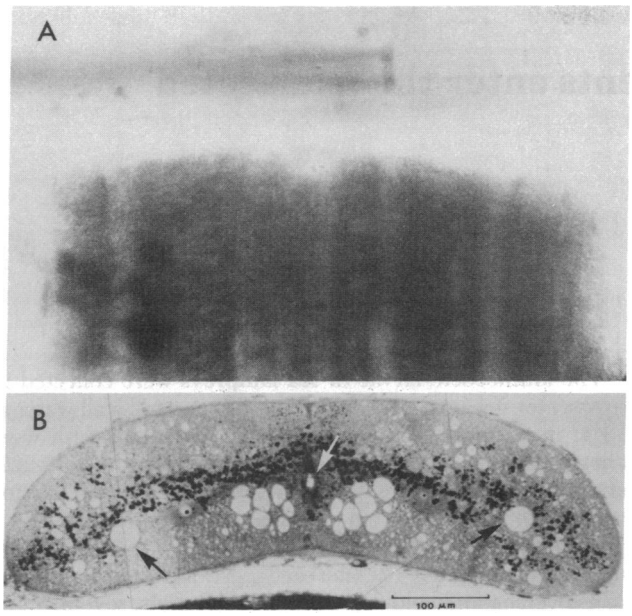

FIG. 1. (*A*) Photograph of the proximal end of a living spinal cord severed 15 min previously, showing the probe vibrating at 400 Hz in a standard measuring position. The 20-μm spherical tip of the electrode is visualized stroboscopically at the two extremes of its 30-μm excursion. Each laterally located Mauthner axon and the more medially spaced groups of Müller axons are seen as light bands running along the axis of the cord. (*B*) Light micrograph montage of transverse 10-μm thick sections showing the distribution of the major giant axons in the lamprey spinal cord approximately 1 cm posterior to the medulla. The pair of laterally located Mauthner axons (black arrows) and the groups of ventro-medially located Müller axons are seen as large clear areas against the background of neuropile. The white arrow indicates the central canal and surrounding ependymal layer. The transverse band of black spheres running the width of the cord is lipid material located within glial cells. Scale is approximately the same in both figures.

current gradually declined from approximately 100 μA/cm^2 to 4 μA/cm^2; it then remained relatively constant for the balance of the 6-day period over which measurements were obtained (Fig. 3).

During the first 20 min after transection, the current appears to enter the entire cut surface of the cord uniformly. Thereafter, a measure of nonuniformity develops, with sharp peaks of current observed opposite the cut ends of individual Mauthner axons and also opposite the midventral group of cut Müller axons. For a Mauthner neuron, the peak is opposite the center of the cut end of the axon. Upon shifting the probe 30–40 μm either horizontally or vertically (about one axon diameter) away from the center of the axon end, the observed current density

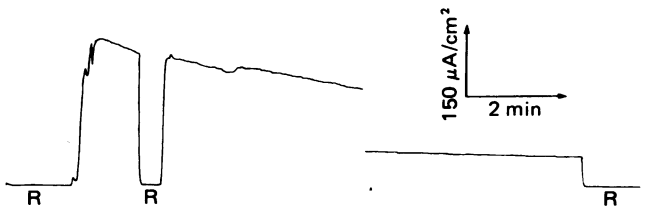

FIG. 2. Portions of recordings taken on August 14, 1979 showing the intense current entering the proximal end of a severed spinal cord cut 4 min previously and measured from 4 min to 44 min posttransection. The reference level (R) was obtained with the probe located more than 1 cm from the cut surface and out of the electric field. Test recordings were taken with the center of vibration 68 μm from the surface. The break in the record represents 30 min not portrayed.

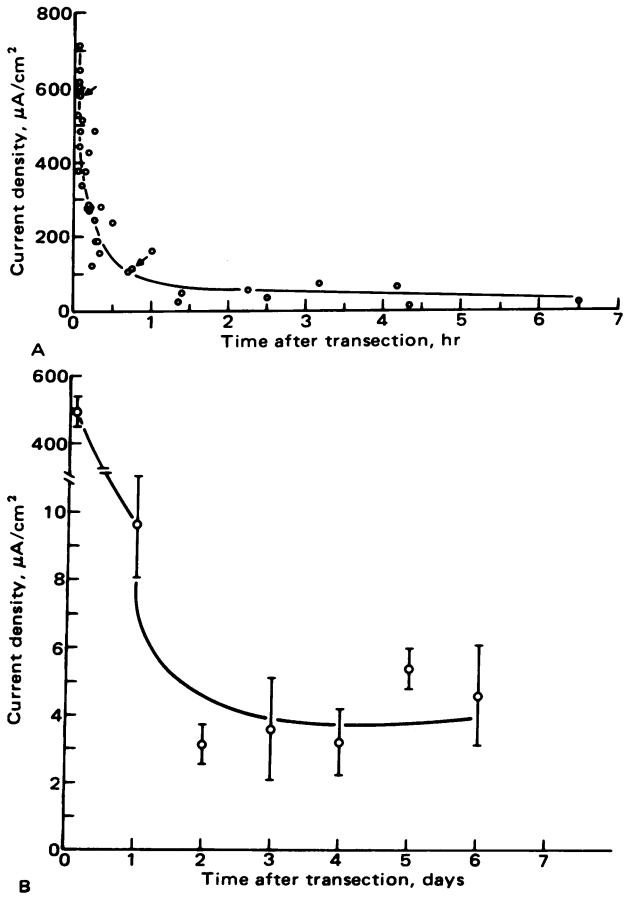

FIG. 3. Decline of current density entering the cut surface of the cord during the first week after transection. (*A*) Rapid decline during the first 7 hr. Each data point represents a single measurement of the peak current density entering the cut end of one spinal cord. Two or three measurements were taken at different times on each of the 12 cords represented in this figure. Arrowed points show the current at the start and end of the record shown in Fig. 2. (*B*) Time course of entry current measured in cords up to 6 days posttransection, beginning on August 1, 1979. Note the relatively stable current density entering the cut surface of the cord after the first day after transection. Data points through 4 days represent averages from the same six animals. Data points at 5 and 6 days were taken from an additional two animals whose cords were cut on August 1, 1979 but not perturbed by intervening measurements. Indicated surface densities in both graphs were obtained by a 2-fold correction of the density measured 68 μm away from the cut surface.

falls by 20–50%. Such abrupt shifts in current density were also seen at what appeared to be the margins of the group of Müller axons. We regularly observed these sharp localizations from 1 to 6 days after transection when the densities had fallen to 100 μA/cm^2 or less; we often saw them as early as a few hours after transection. Between the giant axon regions in the cord, the current density was relatively uniform. We did not observe any change in current density associated with the central canal.

So far, we have discussed only the pattern of current densities entering the cut surface of the transected cord. Systematic exploration was made around the edge of this face and for some distance proximally along the lateral surface of the cord in six preparations. Fig. 4 shows the results of such a scan made 4 hr after transection. Over the edge of the cut face and for about 0.5 mm proximally, current still entered the lateral margins of the cord. For a short distance thereafter, current actually left the lateral margins of the cord. These current densities were 1/5th to 1/10th of those entering the cut face of the cord. Similar patterns of current distribution were seen from 1 to 6 days after

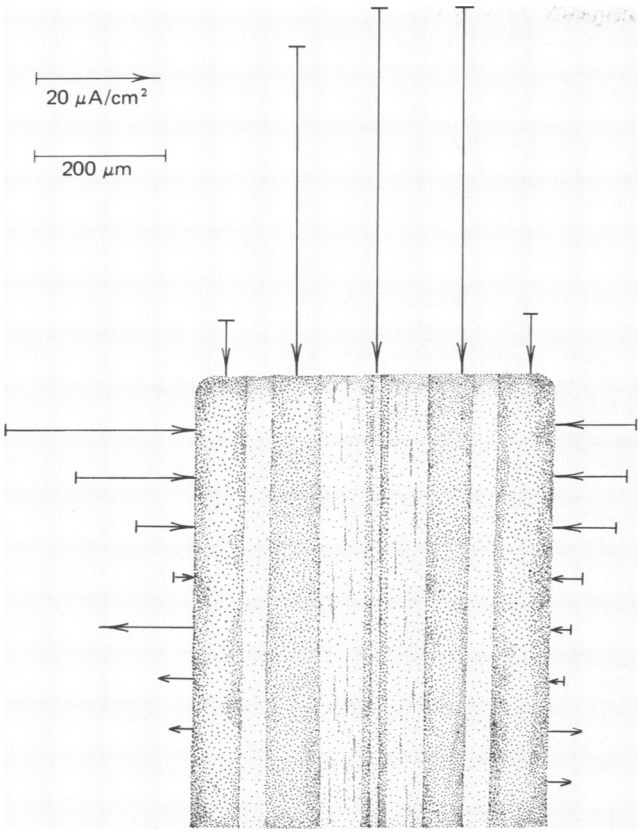

FIG. 4. Scan of perpendicular component of current entering or leaving cut end of a spinal cord 4 hr after transection. The lengths of the arrows are proportional to the density of current entering or leaving the cord. Note that the direction of current flow reverses 300–400 μm proximal to the cut surface. The giant axons are shown as light bands parallel to the long axis of the spinal cord.

transection. We also attempted to explore the pattern of current densities along the entire dorsal and lateral surface of the cord in two preparations. These data proved complex and include the observation that there are alternating (1–2 mm wide) bands of inward and outward current along the length of the cord between the cut end and the brain. The densities of these latter currents are also approximately 1/10th of those entering the face.

In an attempt to determine which ions carry the intense currents entering the cut face soon after transection, we systematically replaced the major ions in the saline medium and observed the responses as shown in Table 1. These data suggest that about half of the inward current consists of Na^+; that much of the remainder may consist of Ca^{2+}; and that the other ions examined are only small components of the current. Note that if an outflow of Cl^- were a substantial part of the inward charge current, then a purely passive response to the replacement of 90% of the Cl^- by an inert but nonpermeating substitute should give a measurable *increase* in this density, rather than the slight decrease actually observed. However, with methane sulfonate substitution we did observe oscillations of inward current of about 10% in amplitude and with an approximate period of 45 sec. This suggests some significant anionic component to the current or some special response to the methane sulfonate medium. Moreover, a remarkable oscillatory response was induced by a medium in which 90% of the Cl^- was replaced by *p*-aminohippurate (Fig. 5).

There remains to discuss specific corrections and controls.

(*i*) One might imagine that the vibration of the probe itself could induce the cord to produce some part of the measured currents. In order to maximize any possible stimulation by the probe, we moved the probe as close as possible to the cord and varied the amplitude of vibration over a wide range (8–68 μm).† If the probe did not stimulate the preparation, then the voltage

Table 1. Effects of ionic changes on spinal cord injury current

Ion replaced	Replacement ion	Concentration change, mM	% change in current density
Na^+	Choline	111–20	−45%; −47%
Ca^{2+}	Mg^{2+}*	2.6–0	−2%
Ca^{2+}	1 mM La^{3+} added	None	−32%
K^+	Na^+	2.1–0	+7%
Mg^{2+}	Na^+	1.8–0	−9%
Cl^-	Isethionate	102–11	−5%
Cl^-	Methanesulfonate	102–11	−15%†
Cl^-	*p*-Aminohippurate	102–11	Strong active response (see Fig. 5)

Measurements were made on six spinal cords 1–4 hr after transection when the injury currents ranged from 20 to 60 μA/cm². For each cord, the current in standard medium was relatively stable during the measurement period. Each ion substitution test was made as follows: A sequence of four or five measurements of peak current density was made in standard medium. The standard medium was then replaced by the test medium and another sequence of current measurements was made within 2–3 min. This was followed by a return to the standard medium and a final sequence of four or five measurements was taken. There was little variation among the four or five individual measurements in any one sequence. Any obvious changes induced by the test media were apparent within minutes and were also reversible upon return to the standard medium. The % change in current densities reported above was computed from these data. For most ions tested the sequence of measurements described above was made on one spinal cord. For the striking responses shown by sodium and *p*-aminohippurate, the sequence of measurements was repeated on a second cord. All media had a pH of 7.3–7.6 and the ion concentrations were adjusted to minimize osmotic pressure changes.

* Mg^{2+} was raised from 1.8 mM to 10.0 mM to stabilize the membrane.

† Indicates fairly regular oscillations of about 10% in amplitude and a 45-sec period.

† At least some components of velocity in the complex flow pattern near such a vibrating probe are known to increase with the 2–2.5 power of the vibration amplitude. Thus these components would increase by 100-fold or more over this range of vibration amplitudes (9).

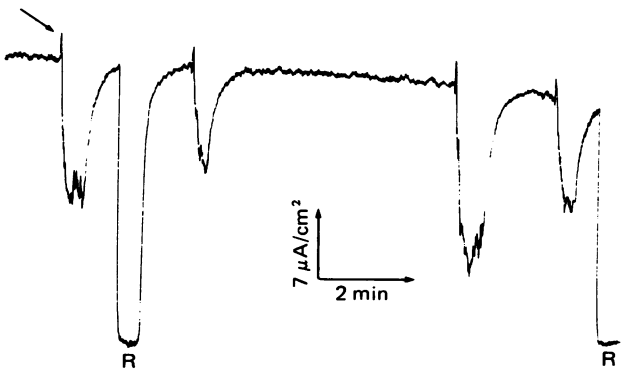

FIG. 5. Response to *p*-aminohippurate. Thirteen minutes before this section of the record, the cord was shifted from standard medium to one in which 90% of the chloride was replaced by *p*-aminohippurate. Note the repeated characteristic oscillatory episodes interrupting the slow decline of inward current. Each episode starts with a 6-sec spike (arrow) followed by a sharp fall and a slow return to the relatively steady level. During the 13-min period prior to the record shown, 4–5 similar episodes occurred. R, reference positions.

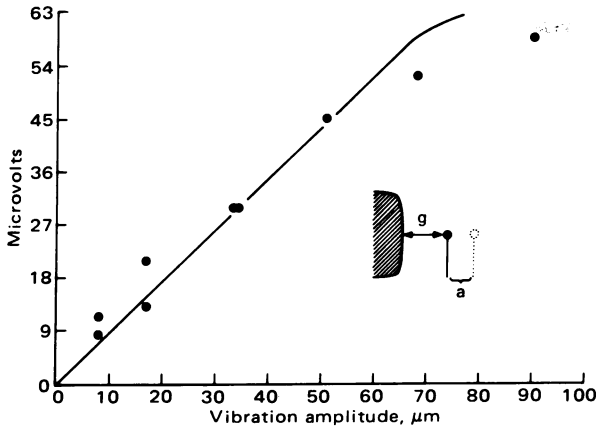

FIG. 6. Voltage differences between the extreme positions of a probe vibrating (at 400 Hz) near a cord as a function of the amplitude of vibration. The gap (g) between the cord and the nearest position of the probe was held constant at 17 μm. The voltage is proportional to amplitude (a), indicating that the vibration of the probe itself is not mechanically inducing these currents.

differences registered in a homogeneous field should have been proportional to this amplitude; but if it did substantially stimulate the preparation, then these differences should have increased much more rapidly. Fig. 6 shows the results of this test. There is no indication of any direct stimulation of the cord by the probe.

(*ii*) The necessary correction for the current density at the surface of the preparation, as opposed to the point of actual measurement, was obtained by measuring the decline of current density with distance from the cut surface, starting as close as possible to the surface. One determination made opposite the center of a cut Mauthner axon yielded the following values: at 34 μm from the surface, a current density of 210.2 μA/cm^2 was measured; at 51 μm, 124.1 μA/cm^2; and at 68 μm, 92.0 μA/cm^2. The ratio of densities measured at the closest accessible point (34 μm) to that at the standard measuring position (68 μm) was 2.3. Two other determinations of this ratio made over a more homogeneous region (toward the center of a group of Müller cells) gave values of 1.8 and 1.6. We have no reliable basis for extrapolating to the cut surface itself from the closest accessible point. So we simply used a value of 2 based on the above measurements as a summary correction factor for estimating the average intensity of current at the cut surface of the preparation.

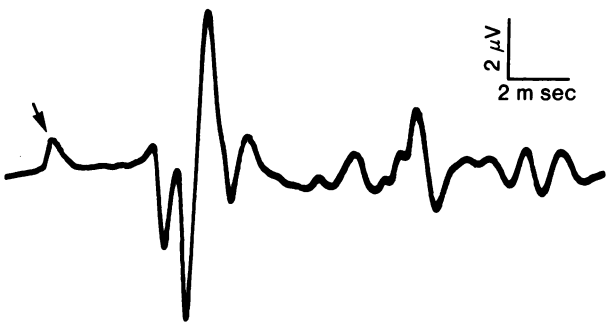

FIG. 7. Action potentials recorded from the surface of an excised spinal cord removed from the animal and maintained in organ culture 4 days prior to recording. The recording electrodes were on the dorsal surface, with the distal member of the pair about 5 mm from the cut end. The stimulating electrodes were placed 1.5 cm proximally. The overall induced pattern of action potentials, including the large spikes indicative of giant fiber activity, is similar to that seen in normal cords *in situ*. This cord was one of those used to provide data for Fig. 3. Arrow indicates stimulus artifact.

(*iii*) In order to determine the viability of the preparations, action potentials were evoked and recorded from the dorsal surface of the cord in every preparation at the end of the last measurement. Fig. 7 is such a record from a cord 4 days after transection showing a pattern of action potentials similar to those seen in the normal cord *in situ*. All eight preparations that provided the data for Fig. 3*B* showed comparable normal action potentials at the end of the measuring period.

DISCUSSION

When the living spinal cord of the lamprey is severed, intense electrical currents begin to enter the cut face immediately. The current density falls rapidly over the first few hours after transection, then declines more slowly to reach a still substantial level. This level is then maintained from the second day to the longest period of measurement, which was 6 days after transection. Moreover, sharp peaks of current enter the cord opposite the cut ends of the various giant axons. An initial analysis suggests that about one-half of this entry current consists of Na^+, and that much of the remainder may consist of Ca^{2+}.

Before discussing the implications of our findings, it is appropriate to consider the classic observations of Lorente de Nó (2, 3) on frog peripheral nerve. Soon after transection, he measured demarcation potentials of about 20 mV, a figure typical of this literature. In a representative plot, this potential fell to a quarter of its initial value in 3 hr, and then to $^1/_{10}$th by one day after injury [ref. 2 (figure 3, p. 9)]. Furthermore, he had evidence that 8 days after nerve injury, demarcation potentials of 1–2 mV could still be measured [ref. 3 (p. 458)]. It is interesting to note that the course of this voltage decline in peripheral nerve is similar to the decline in current density that we have measured after transecting the spinal cord. Moreover, he considered it "thinkable that the permanent flow of demarcation current plays a role in the process of regeneration . . . [perhaps] the continued flow of the demarcation current into the last few millimeters of regenerating nerve is a mechanism by means of which energy is transferred to the regenerating end from points at some distance from it" [ref. 3 (p. 459)].

We are inclined to similar speculation about the role of steady currents through injured nerve. Consider first the possible pathways of ionic current through the cord. It seems likely that the bulk of the current entering the cut surface of the cord enters the cut ends of the axons rather than the glia. For one thing, most of the surface consists of cut axons rather than glia; for another, sharp peaks of current entry were observed over the center of the individual cut Mauthner axons. The current that enters the cut end of the cord from the medium must return to the medium from some other region of the preparation; however in the areas of the preparation thus far explored, we have been unable to account for most of the necessary exit current. (The brain and ventral surface of the spinal cord present certain difficulties to exploration with the vibrating probe and were not examined in this initial study.)

Whatever pathway the current may take through most of the preparation, it seems reasonable to consider the terminal portion of the cord as a region in which ions are flowing longitudinally along the main axis. By integrating Fig. 3, one can calculate the number of charges entering the cut face during various periods after transection. In order to do this we will assume (as our data indicate) that practically all of this current flow consists of positive charges. Straightforward integration yields the following values: from 0 to 1 hr, about 1 coulomb (C) enters each square centimeter of surface; from 1 to 6 hr, another 1 C; from 6 hr to 2 days, 1.4 C; and from 2 to 6 days, another 1.6 C. Therefore in the course of 6 days a total of about 5 C of charge enters each square centimeter of cut surface. We may now ask

to what depth this massive influx of charge displaces the pre-existing ions toward the brain. No precise answer can be given to this question because we do not know the pathways of ion flow within the tissue. Nevertheless, a calculation based on a simple model is instructive. Let us suppose that all of the pre-existing ions are displaced by the influx so that a sharp planar boundary between "new" and "old" ions is established. Let us further suppose (as an adequate approximation) that all the ions concerned are univalent. The total concentration of all positive ions in the tissue can be taken to be about 0.1 M. On the basis of this simple model, one can then calculate that an influx of 1 C/cm^2 will displace the pre-existing ions for a distance of 1 mm. Thus the plane of ion replacement should penetrate the tissue by about 1 mm in the first hour; 2 mm after 6 hr; 3.4 mm by 2 days; and 5 mm by day 6. Because the entry current consists largely of Na^+ and possibly Ca^{2+}, while the pre-existing cations in the tissue should consist largely of K^+, it is evident that the injury current should cause massive changes in the ionic composition in the terminal region of the preparation. In particular there should be a large increase in cytoplasmic Ca^{2+} in this region, not only because of the influx of Ca^{2+} itself, but because the large increase of Na^+ would be expected to release Ca^{2+} from the mitochondria (11). It is also interesting to compare these deductions with a recent abstract of Meiri *et al.* (12). They studied the membrane properties of severed and regenerating giant axons in the cockroach by conventional electrophysiological techniques. A number of interesting changes occurred in the 0.2- to 0.5-mm terminal region. These include two pertinent results: First, the membrane depolarized from −80 to −10 mV within 1 hr after axotomy. Second, 4–24 hr after axotomy, the conductances of both Na^+ and Ca^{2+} greatly increased from close to zero to levels of 0.55 and 0.38 × 10^{-9} Siemens, respectively, whereas the Cl^- conductance remained constant. Furthermore, there was a remarkable shift from sodium-dependent to calcium-dependent action potentials during the same period. These data and our own are complementary in two ways: both suggest a radical change in ionic composition within the terminal millimeter of the cut axons. Furthermore, our data indicate that about 60% of the entry current consists of Na^+ while most of the rest may be Ca^{2+}. This composition of the influx current is about that to be expected from the conductance data of Meiri *et al.* (12).

There remains to consider the possible relationship between these ionic changes and the morphological responses to injury. In various nerves, axotomy is soon followed by a considerable deterioration of the axoplasm for a short distance from the cut surface (13). In isolated axonal segments, the dissolution of axoplasmic microtubules and neurofilaments apparently requires calcium entry (14, 15). It is likely that the Na^+ and Ca^{2+} components of the injury current entering the transected proximal stump results in a similar but local dissolution. The subsequent regeneration of severed axons in the lamprey spinal cord may also be induced by ionic changes produced by steady current flow. In particular we would point out that actin assembly, associated with elongation in various cells, is well known to be under ionic control (16).

In addition to affecting the tissue by ionic changes and gradients, current densities of the order of 30–300 μA/cm^2 within the cut axons should generate voltage gradients of at least 1–10 mV/mm. These in turn should be strong enough to drive some negatively charged macromolecules and particles towards the cut surface. In particular, we wonder if self-electrophoresis plays some direct or indirect role in forming the gross accumulations of various organelles that soon appear on both sides of an axonal lesion; for there is evidence that the accumulation of mitochondria (and perhaps of other components) results from movement towards the lesion rather than synthesis near it (13, 17).

Altogether, we think it likely that the flow of current into cut nerves helps govern their response to injury. Finally, it should be pointed out that these currents through cut lamprey spinal cords add to the emerging picture that large steady currents are present in a wide variety of regenerating and developing systems (4, 18).

We thank A. Breslin for skilled technical assistance and M. R. Wood for providing the photograph in Fig. 1*B*. This work was supported by funds to M.J.C. from National Institutes of Health Spinal Trauma Center Grant 2P50 NS10174-07 and by National Institutes of Health Research Grant NS11545 (L.F.J.). R.B.B. was a Fellow of the National Spinal Cord Injury Foundation.

1. DuBois-Reymond, E. (1843) *Ann. Phys. U. Chem.* **58,** 1–30.
2. Lorente de Nó, R. (1947) *Institute for Medical Research* (The Rockefeller Institute for Medical Research, New York), Vol 131.
3. Lorente de Nó, R. (1947) *Institute for Medical Research* (The Rockefeller Institute for Medical Research, New York), Vol. 132.
4. Jaffe, L. F. (1979) in *Society of General Physiology Series: Membrane Transduction Mechanisms,* eds. Cone, R. A. & Dowling, J. E. (Raven, New York), Vol. 33, pp. 199–231.
5. Rovainen, C. M. (1967) *J. Neurophysiol.* **30,** 1000–1023.
6. Rovainen, C. M. (1974) *J. Comp. Neurol.* **168,** 545–554.
7. Wood, M. R. & Cohen, M. J. (1979) *Science* **206,** 344–347.
8. Wickelgren, W. O. (1977) *J. Physiol. (London)* **270,** 89–114.
9. Jaffe, L. F. & Nuccitelli, R. (1974) *J. Cell Biol.* **63,** 614–628.
10. Jaffe, L. F. & Nuccitelli, R. (1977) *Annu. Rev. Biophys. Bioeng.* **6,** 445–476.
11. Carafoli, E. & Crompton, M. (1976) in *Symposia of the Society for Experimental Biology: Calcium in Biological Systems,* ed. Duncan, C. J. (Cambridge Univ. Press, Cambridge, England), Vol. 30, pp. 89–115.
12. Meiri, H., Spira, M. E. & Parnus, I. (1979) *Soc. Neurosci. Symp.* **5,** 680 (abstr.).
13. Zelena, J., Lubinska, J. & Gutman, E. (1968) *Z. Zellforsch.* **91,** 200–219.
14. Schlaepfer, W. W. & Hasler, M. B. (1979) *Brain Res.* **168,** 299–309.
15. Schlaepfer, W. W. & Bunge, R. P. (1973) *J. Cell Biol.* **59,** 456–470.
16. Tilney, L. G. (1979) in *Society of General Physiology Series: Membrane Transduction Mechanisms,* eds. Cone, R. A. & Dowling, J. E. (Raven, New York), Vol. 33, pp. 163–186.
17. Zelena, J. (1969) in *Symposia of the International Society for Cell Biology: Cellular Dynamics of the Neuron,* ed. Barondes, S. (Academic, New York), Vol. 8, pp. 73–94.
18. Borgens, R. B., Vanable, J. W., Jr. & Jaffe, L. F. (1979) *Bioscience* **29,** 468–474.

Correction. In the article "Cloning of the active thymidine kinase gene of herpes simplex virus type I in *Escherichia coli* K-12" by Florence Colbere-Garapin, Suzanne Chousterman, Florian Horodniceanu, Philippe Kourilsky, and Axel-Claude Garapin, which appeared in the August 1979 issue of *Proc. Natl. Acad. Sci. USA* (**76**, 3755–3759), the authors request that the following correction be noted. The reference cited in lines 2–4 of the Introduction should be: Kit, S. & Dubbs, D. R. (1963) *Biochem. Biophys. Research Commun.* **11**, 55–59.

Correction. In the article "Inheritance of acquired immunological tolerance to foreign histocompatibility antigens in mice" by R. M. Gorczynski and E. J. Steele, which appeared in the May 1980 issue of *Proc. Natl. Acad. Sci. USA* (**77**, 2871–2875), there was an error in Table 1. Under "Outcross" the entry should be: "Normal CBA♀ × δ_1 (1st gen.$^{tn.}$)."

Correction. In the article "Large and persistent electrical currents enter the transected lamprey spinal cord" by Richard B. Borgens, Lionel F. Jaffe, and Melvin J. Cohen, which appeared in the February 1980 issue of *Proc. Natl. Acad. Sci. USA* (**77**, 1209–1213), there was an error in the affiliation line. The work actually was done in the Biology Department at Purdue University. Two of the authors (R.B.B. and M.J.C.) were in the Biology Department at Yale University and came to Purdue to carry out this study in L.F.J.'s laboratory.

Correction. In the article "Electrical excitability: A spectrum of properties in the progeny of a single embryonic neuroblast" by Corey S. Goodman, Keir G. Pearson, and Nicholas C. Spitzer, which appeared in the March 1980 issue of *Proc. Natl. Acad. Sci. USA* (**77**, 1676–1680), details of some of the figures were not printed well or were retouched incorrectly by the printer. Corrected Figs. 1, 2, and 4 are printed here.

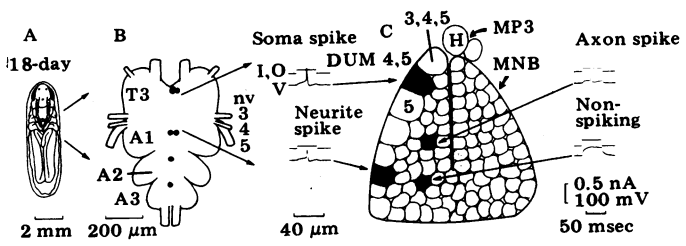

FIG. 1. Grasshopper embryo at day 18; diagrams and camera lucida drawings of living specimens (hatching occurs on day 20 at 35°C). (*A*) Embryo at day 18 viewed within the egg case. (*B*) Dorsal outline of metathoracic ganglion (T3) fused with first three abdominal ganglia (A1–A3), showing the location of the packets of progeny produced by the DUM neuroblast (or median neuroblast, MNB) in each segment (stippled areas) and the one or two remaining progeny of the single cell division of midline precursor 3 (MP3) in each segment (black dots). Lateral nerves (nv) 3–5 are indicated. (*C*) Camera lucida drawing of the packet of ≈100 progeny of the DUM (MNB) neuroblast and two progeny of MP3. Four of the large DUM neurons are individually identified in the drawing (see text). The DUM neuron somata in a packet appear under Nomarski optics to be encased in a glial sheath. The glial sheath has a median boundary that divides the packet into left and right portions. The progeny of the DUM neuroblast show the complete spectrum of electrical excitability, from soma spikes, to neurite spikes, to axon spikes, to nonspiking, I,0 indicate injected current and zero reference voltage; V, the voltage of the intracellular microelectrode. This range of electrical properties is recorded in cells that appear by other criteria to have reached their mature phenotypes.

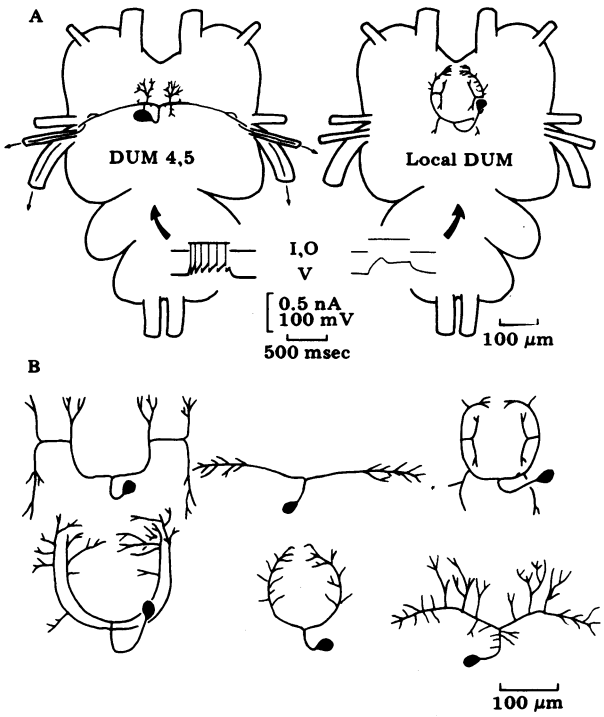

FIG. 2. (*A*) Comparison of the morphology and physiology of two progeny of the DUM neuroblast in the grasshopper: DUM 4,5 and a local DUM neuron. DUM 4,5 has a large-diameter soma and peripheral axons in lateral nerves 4 and 5, and generates overshooting soma spikes. The local DUM neuron has a small-diameter soma, is intraganglionic, and is incapable of generating action potentials in normal saline (it shows delayed rectification). Drawings are based on injections of the dye Lucifer yellow in an 18-day embryo. (*B*) Six other examples of the intraganglionic morphology of small nonspiking DUM neurons in an 18-day embryo.

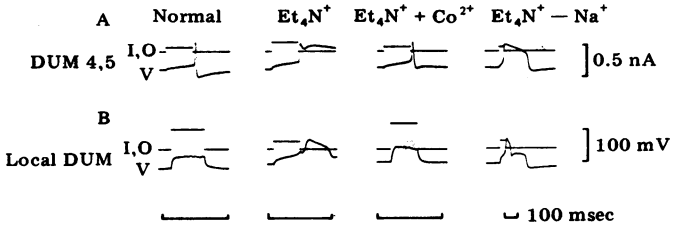

FIG. 4. Comparison of electrical excitability of two progeny of the DUM neuroblast in the grasshopper: DUM 4,5 and a local DUM neuron in an 18-day embryo. (*A*) The soma of DUM 4,5 generates action potentials that depend on Na^+ and Ca^{2+} for their inward current (1, 10, 11). Addition of 30 mM Et_4N^+ converts the brief action potential into a long and more complex response. The long plateau is abolished by the addition of 10 mM Co^{2+}, whereas the initial spike is nearly completely eliminated by removal of Na^+. (*B*) The local DUM neuron does not produce action potentials in normal saline. Addition of 30 mM Et_4N^+ induces the cell to produce long-duration Ca^{2+} action potentials that are blocked by the addition of 10 mM Co^{2+} and are unaffected by the removal of Na^+. See text for discussion of different plateau amplitudes.