sections were prepared from OCT-embedded brain tissue, mounted on microscope slides, fixed in 4 percent paraformaldehyde, and stored in 70 percent ethanol at 4°C until hybridized. Slide preparations were then acetylated, treated with 0.1*M* tris-HCl (*p*H 7.0) and 0.1*M* glycine, and hybridized with 10^6 count/min of ^{35}S-labeled RNA transcribed from *p*BH10-R3 [8.9-kb-long Sst I–Sst I viral insert from HTLV-III clone λBH-10 (*9*) subcloned into pSP64]. Hybridization was performed in 50 percent formamide, 2× SSC, and 10 m*M* DTT for 3 hours at 50°C. Slides were rinsed in 50 percent formamide, 2× SSC at 52°C, treated with ribonucleases A and T_1 for 30 minutes, and dehydrated in ethanol. Preparations were autoradiographed with Eastman NTB2 emulsion, exposed at 4°C for 2 days, developed in Dektol developer, and stained with Wright stain. For these in situ hybridization studies, sections were not subjected to DNA strand separation (denaturation), thereby ensuring that hybridization of the HTLV-III probe was to viral RNA and not DNA.

32. M. E. Harper *et al.*, in preparation.

33. We thank L. Rankin and A. B. Minnefor for providing clinical data and postmortem material, L. Marselle for technical assistance, and R. Singer for discussions. G.M.S. was funded through the Intergovernmental Personnel Act of the National Institutes of Health in conjunction with the Ohio State University College of Medicine, and B.H.H. was supported by the German Science Foundation and the Fogarty Center for International Studies. R.W.P. was supported, in part, by New York AIDS institute grant number A0159. L.G.E. is an assistant professor of Neuroscience and Pediatrics at the University of Medicine and Dentistry of New Jersey and is currently a visiting scientist at the NIH.

13 November 1984; accepted 6 December 1984

Targeted Modulation of Acute Inflammation

Abstract. *Localized inflammation of the lungs was induced with the neutrophil chemoattractant,* N*-formylmethionylleucylphenylalanine (FMLP) in combination with magnetically responsive albumin microspheres, a drug carrier that provides efficient, extremely rapid localization in tissue. Intravenous targeting to rat lungs was accomplished by means of an external thoracic magnet. This caused progressive local accumulation of neutrophils, extravascular cell migration, and acute tissue injury. Microscopic findings favored chemotaxis as the principal mechanism of cell accumulation. This system provides a new experimental model for acute alveolar damage, a rapid in vivo assay for drugs that modulate neutrophil chemotaxis, and a new therapeutic approach to focusing inflammation in patients with chemotactic defects.*

Targeted modulation of acute inflammation requires the combination of a potent neutrophil attractant and a drug carrier that provides efficient intravascular targeting, rapid carrier transport across vascular endothelium, and controlled drug release within tissues. The neutrophil chemotactic peptide, *N*-formylmethionylleucylphenylalanine (FMLP) is an ideal local biomodulator because of its high affinity of binding to specific neutrophil receptors and its chemical stability (*1, 2*). However, it is also extremely toxic or lethal when administered in a freely circulating form. This limits the choice of carriers to ones that encapsulate the biomodulator (preventing intravascular availability) and become localized within several minutes of injection. The only carrier that has both of these properties is the magnetically responsive albumin microsphere (*3*). Such spheres localize up to 80 percent of entrapped drug in target tissues when captured with an 8000-gauss magnetic field (*3, 4*).

FMLP spheres were prepared by emulsifying an aqueous mixture of the chemoattractant, human serum albumin, and fluidic magnetite (Fe_3O_4) in cottonseed oil; sonicating to produce particles smaller than 1 μm; and heating at 135°C to obtain controlled FMLP release (*5, 6*). The efficiency of peptide entrapment was 11 percent, and the resulting FMLP and magnetite contents were 0.65 and 20 percent (by weight), respectively. The preparation had a mean particle diameter of 612 nm and a shelf life of more than 1 year. The half-time for release in vitro of [^{3}H]FMLP was 20 minutes (*6, 7*). This was optimal for triggering acute tissue changes. All of the FMLP released between 0 and 24 hours was biologically active, as evaluated by neutrophil chemiluminescence assays (*8, 9*).

An initial bioassay was performed in vivo, without magnetic targeting, by injecting FMLP spheres (20 mg/kg) intravenously into Sprague-Dawley rats. Spheres localized spontaneously in the spleens, and targeting was maximal isotopically in 5 minutes. This was followed by a progressive local accumulation of neutrophils (Table 1). On the basis of these results, a targeted bioassay was designed to test if a lower dose of FMLP spheres (6.6 mg/kg) could be magnetically localized to produce inflammation in a nonreticuloendothelial organ, namely, lung. The dose was selected to produce lung concentrations of total (entrapped plus free) FMLP in the range of $2.9 \times 10^{-6}M$ [which induced a 75 percent maximal chemiluminescence response in vitro (*9*)] and concomitantly to maintain plasma concentrations of free FMLP at levels of $1.9 \times 10^{-8}M$ (which induced <10 percent of maximal chemiluminescence). Spheres were injected intravenously and captured in the right lung with a 5500-gauss gradient (300 gauss per 0.1 cm) magnetic field oriented perpendicular to the right lateral thorax. Lungs were removed 0 to 70 minutes later, and histologic sections were stained by a newly devised method that allows both neutrophil esterase (*10*) and microsphere magnetite iron (*11*) to be visualized in the same tissue section.

Localization of [^{3}H]FMLP spheres occurred during the first circulatory pass and was maximal in the outer 0.5 cm of lung (*12*). The concentration of total FMLP in target tissue reached maximal levels within 5 minutes at $2.9 \times 10^{-6}M$ and remained unchanged over the next 70 minutes. By 70 minutes, 71 percent of this FMLP was estimated (from in vitro kinetics) to be released as free tissue

Table 1. Neutrophil accumulation in rat target tissues after intravenous injection of saline or microspheres.

Treatment	Minutes after injection	Magnet	Neutrophils per ×50 field*
Spleen			
Saline	70	−	46.6 ± 6.0†
Placebo spheres (20 mg/kg)	70	−	49.2 ± 5.2†
FMLP spheres (spheres, 20 mg/kg; FMLP, 47.6 μg/kg)	5	−	49.0 ± 4.0†
	20	−	92.6 ± 14.0‡
	40	−	164.0 ± 17.0‡
	70	−	254.4 ± 18.2‡
Lung			
Saline	70	+	43.4 ± 4.6†
Placebo spheres (6.6 mg/kf)	70	+	40.8 ± 3.8†
FMLP spheres (spheres, 6.6 mg/kg; FMLP, 15.7 μg/kg)	70	−	48.0 ± 4.7†
	5	+	42.4 ± 4.4†
	20	+	74.5 ± 7.7‡
	40	+	122.5 ± 12.9‡
	70	+	163.8 ± 13.5‡

*×50 fields are 800 × 500 μm in area and 5 μm thick; $n = 5$. Data are reported as mean ± 1 standard deviation. †Groups do not differ at $P = 0.05$ (Newman-Keuls multiple comparison test). ‡Each group differs significantly from all others of the respective target tissue at $P < 0.001$ (Newman-Keuls multiple comparison test).

drug. Total plasma FMLP peaked within 2 minutes at $13 \times 10^{-8}M$ and fell rapidly to $6.2 \times 10^{-8}M$ at 5 minutes. Free plasma FMLP remained below $1.9 \times 10^{-8}M$ at all times. At 5 minutes after injection, when sphere localization was maximal and neutrophil localization had not yet begun, the white cell fraction of blood (*13*) contained no detectable FMLP (limit, $9.8 \times 10^{-11}M$). This indicated that FMLP spheres did not adhere to circulating neutrophils. By electron microscopic analysis, 93 and 99 percent of the targeted spheres were extravascular within 5 minutes (Fig. 1E) and 20 minutes (Fig. 1, A and F), respectively, and none of the remaining spheres were adherent to neutrophils. After 20 minutes, significant quantities of sphere material had broken free into the small air spaces (Fig. 1F), and by 40 minutes much of this was phagocytized by alveolar macrophages (Fig. 1G). Small fragments of spheres remained within the tissue at 70 minutes for local chemoattraction of neutrophils (Fig. 1H).

In rats receiving FMLP spheres, neutrophils accumulated selectively and progressively in the magnetically exposed fields, beginning shortly after the completion of sphere targeting (Table 1 and Fig. 1A). Initially, the cells localized within small vessels next to interstitial spheres (Fig. 1F). These neutrophils were elongated and smooth, contained no detectable sphere material, and did not invade the endothelium. However, by 70 minutes the neutrophils that accumulated in large numbers in similar locations (Fig. 1B) were enlarged and had prominent cytoplasmic processes that extended directly into the tissue around spheres (Fig. 1H). This is strongly suggestive of extravascular chemotaxis. Fragments of spheres were newly present within these late cells (Fig. 1H), and they had also undergone marked degranulation (Fig. 1B). Acute lung injury developed at 70 minutes as indicated by electron microscopic changes (Fig. 1H) and perivascular edema, which formed around capillaries and small arterioles (Fig. 1C) in a fashion similar to that reported for ischemic pulmonary vascular injury (*14*). The edema comprised 5.1 ± 0.6 percent of lung cross-sectional areas in comparison with 1.8 ± 0.5 percent for control rats receiving FMLP spheres without magnetic targeting ($P < 0.001$; Student's *t*-test). Increasing the dose of FMLP spheres to 20 mg/kg accentuated the edema (14.3 ± 2.6 percent of lung areas compared with 2.0 ± 0.5 percent for controls; $P < 0.001$). Targeted placebo spheres (without FMLP) produced no neutrophil accumulation (Table 1) or tissue injury (Fig. 1D), and FMLP spheres injected without magnetic targeting produced no pulmonary histologic changes.

These results indicate that FMLP microspheres can be magnetically localized in a selected tissue site and caused to induce graded local neutrophil accumulation. Morphologic findings suggest that the 6.6 mg/kg dose used in this study also induced a transition of neutrophil responses from chemotaxis to O_2^- and lysosomal enzyme release at the site. The differential levels of plasma and lung FMLP achieved by magnetic targeting were in the appropriate ranges for such a transition to occur in Sprague-Dawley rats (*1, 2, 8, 9, 12*). Selection of a microsphere FMLP dose that maintained the free plasma concentrations of FMLP at liminal levels for chemiluminescence in vitro preserved the local migrational and phagocytic responses of neutrophils in vivo. Smaller doses would be expected

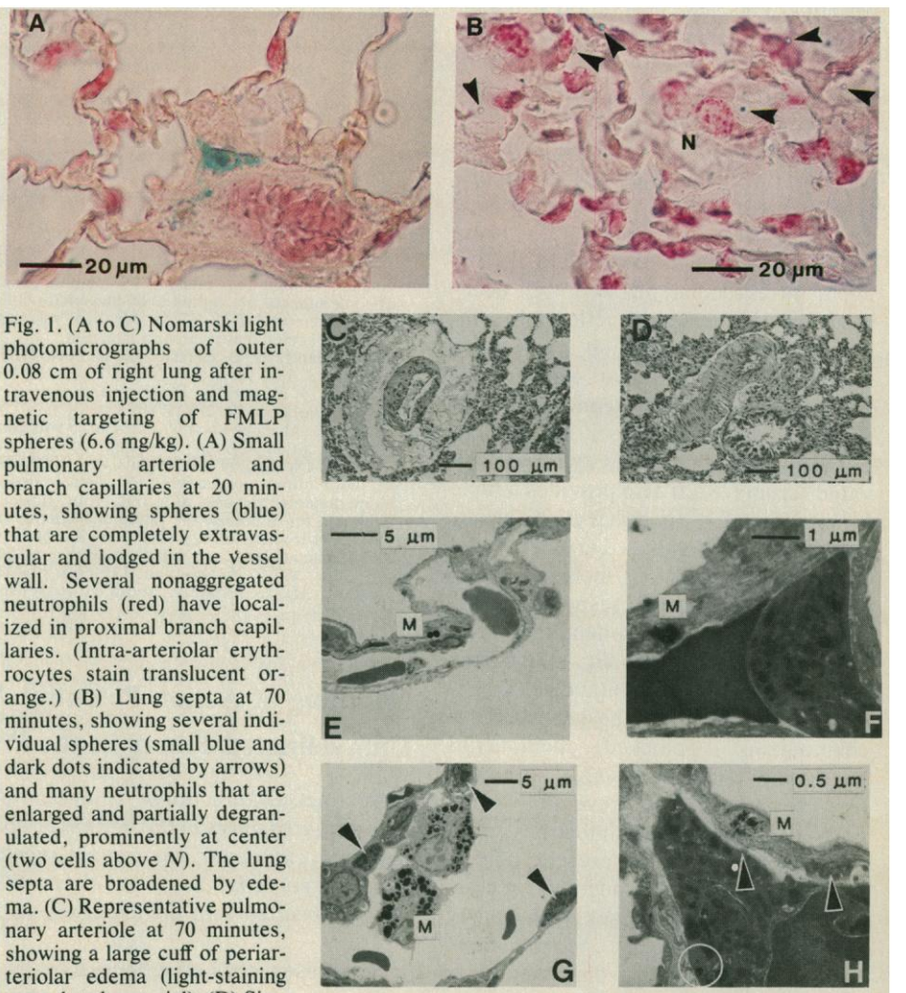

Fig. 1. (A to C) Nomarski light photomicrographs of outer 0.08 cm of right lung after intravenous injection and magnetic targeting of FMLP spheres (6.6 mg/kg). (A) Small pulmonary arteriole and branch capillaries at 20 minutes, showing spheres (blue) that are completely extravascular and lodged in the vessel wall. Several nonaggregated neutrophils (red) have localized in proximal branch capillaries. (Intra-arteriolar erythrocytes stain translucent orange.) (B) Lung septa at 70 minutes, showing several individual spheres (small blue and dark dots indicated by arrows) and many neutrophils that are enlarged and partially degranulated, prominently at center (two cells above *N*). The lung septa are broadened by edema. (C) Representative pulmonary arteriole at 70 minutes, showing a large cuff of periarteriolar edema (light-staining vacuolated material). (D) Similar arteriole from control group 70 minutes after magnetic targeting of non-FMLP spheres. Periarteriolar edema is absent. (E to H) Electron micrographs of microspheres and neutrophils in target lung at increasing times after intravenous injection and magnetic targeting of FMLP spheres (6.6 mg/kg). (E) Magnification (×1200) of small arteriole (center) and efferent capillary (left) at 5 minutes, showing representative extravascular spheres (two dark dots *M* at center). Neutrophils are not yet present. (F) Magnification (×5400) of capillary at 20 minutes showing an intravascular neutrophil (right) adjacent to deformed interstitial sphere material (*M*). A fragment of material is breaking free into the air space (above interstitial deposit). Another fragment is already free in the air space (upper left). Neutrophil borders are smooth, and there are no cell-associated spheres. (Intracellular lysosomal granules stain more lightly than spheres.) Endothelium is smooth, and the vascular basement membrane is preserved. (G) Magnification (×820) of lung septa at 40 minutes showing two alveolar macrophages (center) containing extensive sphere material (*M*). Individual neutrophils are present in capillary loops (arrows). Two extravasated red blood cells are free in the air space, reflecting the onset of tissue injury. (H) Magnification (×820) of representative capillary at 70 minutes, showing an enlarged, partly intravascular neutrophil with irregular cytoplasmic processes (arrows), which extend through the endothelium into the interstitium next to extravascular sphere material (*M*). The neutrophil contains a fragment of phagocytized sphere (circle, lower left). Morphologic indicators of tissue injury are disruption of the basement membrane (right arrow), vesiculation of endothelium (left), and extravasation of plasma proteins (dark smudgy material in left vessel wall).

to induce primarily chemotaxis and to avoid significant cellular activation and tissue injury. Intravascular aggregation and passive sequestration of neutrophils were excluded by (i) the very rapid plasma clearance of microsphere [^{3}H]FMLP compared to the gradual accumulation of neutrophils, (ii) different microscopic locations of microspheres and neutrophils, and (iii) absence of pulmonary neutrophil accumulation (and edema) when FMLP spheres were injected without magnetic targeting. This last observation also indicates that lung injury should not occur as a side effect of targeting to nonpulmonary sites. The extravascular migration of neutrophils toward microsphere fragments indicates that chemotaxis is the principal mechanism of cell accumulation. Because FMLP can also produce smooth muscle contraction (*15*), vasospasm might have contributed to the initial intravascular phase of cell accumulation. Magnetic targeting of sufficient chemotactic peptide provides a model for acute alveolar damage (*16*) that minimizes the systemic effects of freely circulating initiators, such as cobra venom factor (*17*) and zymosan-activated serum (*18*). It also provides a rapid in vivo test for pharmaceutical agents that modulate chemotaxis (*19*). Potential therapeutic applications include restoration of local neutrophil responses in patients with specific inflammatory, infectious, neoplastic, metabolic, toxicologic, thermal (*20*), and traumatic disease processes (*21*), in which the generation of chemotaxins is blocked, chemotaxins are oxidatively degraded (*22*), or neutrophil responses are reversibly suppressed. In most local infections, sufficient chemoattractant is present; however, in life-threatening infections, such as intra-abdominal abscesses with sepsis, either receptor-specific (complement-based) or nonspecific deactivation of chemotaxis can occur (*23*). In some cases, the very high local gradients of FMLP produced by targeting may succeed in refocusing the chemotactic and microbicidal activities of the patient's neutrophils if they retain a sufficient fraction of their normal response to FMLP (*23*). However, more commonly, host cells will be almost completely unresponsive, and restoration of local inflammation may require the concomitant transfusion of fresh donor granulocytes.

DAVID F. RANNEY

Laboratory of Targeted Diagnosis and Therapy, Department of Pathology, University of Texas Health Science Center, Dallas 75235

References and Notes

1. E. Schiffmann, B. A. Corcoran, S. M. Wahl, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1059 (1975); L. T. Williams, R. Snyderman, M. C. Pike, R. J. Lefkowitz, *ibid.* **74**, 1204 (1977).
2. H. J. Showell *et al.*, *J. Exp. Med.* **143**, 1154 (1976).
3. K. J. Widder, A. E. Senyei, S. D. Reich, D. F. Ranney, *Proc. Am. Assoc. Cancer. Res.* **19**, 17 (1978); K. J. Widder, A. E. Senyei, D. G. Scarpelli, *Proc. Soc. Exp. Biol. Med.* **158**, 141 (1978); K. J. Widder, A. E. Senyei, D. F. Ranney, *Advances in Pharmacology and Chemotherapy*, R. J. Schnitzer, S. Garattini, A. Goldin, G. Hawking, I. Kopin, Eds. (Academic Press, New York, 1979), vol. 16, pp. 213–271.
4. A. J. Quattrone and D. F. Ranney, *J. Anal. Toxicol.* **4**, 12 (1980); *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **158**, 141 (1980).
5. Ten milligrams of FMLP (Sigma) was dissolved in 0.25 ml of dimethyl sulfoxide and added to an aqueous slurry of 136 mg of ferrofluidic magnetite [25 percent Fe_3O_4 (weight to volume); SE-10, Ferrofluidics Corp.] containing 125 mg globulin-free human serum albumin (Sigma). This mixture was emulsified in 30 ml cottonseed oil (Sargent-Welch) and sonicated (*6*). Heat stabilization and extraction of oil were performed as reported (*6*).
6. K. J. Widder, A. E. Senyei, D. F. Ranney, *Cancer Res.* **40**, 3512 (1980).
7. Spheres were washed to remove rapidly releasable surface peptide (63.3 percent of the entrapped FMLP). Controlled release of the remaining FMLP (36.7 percent) was carried out at 10 mg spheres per milliliter of phosphate-buffered saline (*p*H 7.35, 37°C). Identical washing was performed before in vivo injection.
8. G. E. Hatch, D. E. Gardner, D. B. Menzel, *J. Exp. Med.* **147**, 182 (1978); W. D. Welch, C. W. Graham, J. Zaccari, L. D. Thrupp, *J. Reticuloendothelial Soc.* **28**, 275 (1980).
9. Luminol-enhanced chemiluminescence was performed as in (*8*), by exposing human and Sprague-Dawley rat neutrophils to 1:1000 and 1:100 dilutions, respectively, of control-release supernatants [prepared as in (*7*)]. After backcalculation for dilutions, the ratios of bioactive to total ^{3}H-labeled supernatant FMLP obtained with human and rat target cells were 1.1 ($8 \times 10^{-5}M/7 \times 10^{-5}M$) and 1.0 ($7 \times 10^{-5}M/7 \times 10^{-5}M$), respectively. Midrange responses of human and rat cells to soluble FMLP standards are $3 \times 10^{-8}M$ and $1 \times 10^{-7}M$, respectively. Chemiluminescence was used in preference to chemotaxis because of rapidity and improved quantification of small concentration differences.
10. W. C. Maloney, K. McPherson, L. Fliegelman, *J. Histochem. Cytochem.* **8**, 200 (1960).
11. R. D. Lillie, Ed., *Histopathologic Technic and Practical Histochemistry* (McGraw-Hill, New York, 1965), p. 406.
12. Thirty percent of the total microsphere FMLP injected was captured in thoracic viscera (predominantly right lung). The remainder localized principally in liver (49 percent) and spleen (8 percent). This intermediate targeting efficiency resulted from only partial inclusion of left thoracic structures within the magnetic field.
13. Prepared by hypotonic ammonium chloride lysis of blood (*8*).
14. B. C. Lee, H. van der Zee, A. B. Malik, *J. Appl. Physiol. Respirat. Environ. Exercise Physiol.* **47**, 556 (1979); R. L. Johnson, Jr., S. S. Cassidy, M. Haynes, R. L. Reynolds, W. Schulz, *ibid.* **51**, 845 (1981).
15. W. A. Marasco, J. C. Fantone, P. A. Ward, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7470 (1982).
16. T. Sachs, C. F. Moldow, P. R. Craddock, J. K. Bowers, H. S. Jacob, *J. Clin. Invest.* **61**, 1161 (1978).
17. G. O. Till, K. Johnson, R. Kunkel, P. A. Ward, *ibid.* **69**, 1126 (1982).
18. A. C. Helfin and K. L. Brigham, *ibid.* **68**, 1253 (1981).
19. N. Ackerman, S. Martinez, T. Thieme, A. Mirkovich, *J. Pharmacol. Exp. Ther.* **221**, 701 (1982).
20. G. O. Till *et al.*, *J. Trauma* **23**, 269 (1983).
21. J. I. Gallin, *Rev. Infect. Dis.* **3**, 1196 (1981); J. C. Fantone and P. A. Ward, *Am. J. Pathol.* **107**, 397 (1982).
22. S. J. Weiss, M. B. Lampert, S. J. Test, *Science* **222**, 625 (1983).
23. J. S. Solomkin, M. K. Jenkins, R. D. Nelson, D. Chenoweth, R. L. Simmons, *Surgery* **90**, 319 (1981).
24. I thank R. Mack, B. Beck, M. Glass, and D. Rodriguez for technical assistance and Wilson Engraving, Dallas, Texas, for computer enhancement of the cover photograph. Supported in part by NIH CA15673 and Eli Lilly.

1 December 1983; accepted 11 July 1984

Coupling of Action Potential Activity Between Unmyelinated Fibers in the Peripheral Nerve of Monkey

Abstract. *Bidirectional coupling of action potential activity occurs between unmyelinated fibers in the normal peripheral nerve of monkey. The site of coupling is near the cutaneous nociceptive receptor associated with one of the fibers. This coupling could be due to an electrical synapse and could provide the basis for the flare associated with the axon reflex.*

Somatosensory nerve fibers have generally been assumed to have no chemical or electrical synapses with one another outside the central nervous system. However, cross talk between nerve fibers, presumably due to electrical coupling, has been observed in various forms of nerve injury (*1*). This prompted us to examine whether similar interactions between fibers might occur in normal peripheral nerves.

Monkeys (*Macaca fasicularis*, 3.5 to 6.5 kg) were anesthetized with an intravenous injection of pentobarbital (3 to 6 mg kg^{-1} $hour^{-1}$) or by inhalation of a mixture of halothane and N_2O (0.8 percent and 67 percent, respectively). The animals were paralyzed with pancuronium to eliminate artifacts caused by muscle twitching and were mechanically ventilated to maintain the end-tidal pressure of CO_2 at 32 to 40 torr. Recordings from single fibers in the peripheral nerve (superficial radial, ulnar, median, and sural) were obtained from finely teased nerve strands by the use of standard techniques (*2*). The strands were cut proximally so that only centripetally directed action potentials were recorded (Fig. 1a). Tripolar stimulating electrodes were placed on the parent nerve proximal and distal to the recording site and were insulated from surrounding tissue with plastic (*3*). The preparation was bathed in mineral oil. Action potentials recorded at the recording electrode in response to stimulation from the proximal electrode indicated coupling of ac-