AD-A221 580

Electrically Mediated Trauma Repair

Final Report

Richard B. Borgens
Director/Associate Professor

January 1989

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-86C-6068

Purdue University
West Lafayette, Indiana 47907

DTIC
ELECTE
S MAY 16 1990
E D

DOD DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an
official Department of the Army position unless so designated
by other authorized documents

90 05 15 017

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
Unclassified	

2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT
	Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	

4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
Purdue University		

6c. ADDRESS (City, State, and ZIP Code)	7b. ADDRESS (City, State, and ZIP Code)
West Lafayette, IN 47907	

8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research and Development Command	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
		DAMD17-86-C-6068

8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS			
Fort Detrick Frederick, MD 21701-5012	PROGRAM ELEMENT NO. 62772A	PROJECT NO. 3S1-622772A874	TASK NO. EB	WORK UNIT ACCESSION NO. 139

11. TITLE (Include Security Classification)

Electrically Mediated Trauma Repair

12. PERSONAL AUTHOR(S)

Borgens, Richard B.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 1/12/85 TO 5/15/89	1989 January	26

16. SUPPLEMENTARY NOTATION

17. COSATI CODES

FIELD	GROUP	SUB-GROUP
06	01	
06	05	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Spinal cord regeneration nerve recovery cutaneous trunci muscle reflex (CTM) behavioral RA II

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

> Acute, severe trauma to the musculoskeletal system and nervous system is the unfortunate - but common- consequence of active engagement. These injuries (which sometimes occur even during military training), produce the most intractable medical problems for the military medical command. Of the types of trauma for which there is no known acute treatment applicable during the first hours to days after injury which might offer the promise of significant recovery, injury to the central nervous system is the most serious. Survival means years of chronic rehabilitation with little hope of substantial change in behavioral deficit. Our group has concentrated on the development of the use of applied electrical fields as an acute treatment, relatively simple in application, which may significantly alter the outcome of nervous system trauma.

(continued on following page)

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mrs. Virginia Miller	(301) 663-7325	SGRD-RMI-S

Applied electrical fields are known to be able to strikingly modify the development and elongation of nerve processes in culture conditions and in _in vivo_ studies of nonmammalian vertebrates. In the last contract period we have firmly established that steady DC fields also facilitate regeneration of ascending (sensory) components of the adult guinea pig spinal cord. Moreover, we have demonstrated that growth processes initiated by applied fields _within_ the transected spinal cord of the guinea pig is associated with a _functional recovery_ of a specific, analyzable, behavioral deficit.

The deficit we have chosen to study is the Cutaneous Trunci Muscle Reflex (CTM). Stop frame video analysis, electromyography, and other analytical techniques clearly demonstrate a ca. 25% recovery rate in electrically treated animals with the permanent deficit in the CTM remaining _unchanged_ in sham-treated controls. We have begun development of a model system designed to test the relevance of these applications using acute clinical cases of severe spinal cord injuries in dogs (brought to the Small Animal Clinic, Clinical Sciences Department, School of Veterinary Medicine). These injuries closely, if not perfectly, simulate the symptomology of spinal cord injury in humans and are secondary to gunshot wounds, automobile impact, and intravertebral disc herniation. We are also thoroughly exploring similar applications as possible adjuncts to the conventional treatment of _peripheral nerve injury_. Here we test if applied fields can facilitate the regeneration and behavioral recovery of severed and reanastomosed peripheral nerves in adult mammals. Overall, these experiments offer the promise of a relatively simple surgical technique, applied soon after injury, that may markedly enhance the prognosis for nervous system repair.

Accession For

NTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	

By
Distribution/
Availability Codes

| Dist | Avail and/or Special |

A-1

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

TABLE OF CONTENTS

A) STATEMENT OF PROBLEM

Support of our research by the Department of Defense, Department of the Army, into the electrical control of trauma has allowed us to demonstrate that axonal regeneration within the spinal cord of adult guinea pigs can be facilitated. Ascending sensory axons of the dorsal columns were induced to grow through the fiber's glial scar, to the original plane of transection, and then to deviate around the lesion projecting into the rostral segment of spinal cord (Borgens et al., 1986b). Recently ascending components of the ventrolateral funiculus have been shown to do likewise (Borgens et al., 1989). Using a sensitive indicator of spinal cord integrity, the CTM long tract reflex (Nixon et al., 1984; Thierault et al.1988; and Blight et al., 1990) we have further demonstrated that functional deficits produced by specific subtotal lesions of the cord can be recovered in about 15-25% of experimental animals (Borgens et al., 1987; Borgens et al., 1990). Problem left to be resolved are: a) what is the optimum field strength necessary to produce the most optimum recovery? b) Can neurotrophic substrates or substrates of high affinity be implanted into the lesion to allow a more direct trajectory for regenerating nerves? c) Can a modification of these applications be developed which will result in the enhancement of regeneration (and perhaps functional recovery), in both ascending and descending tracts simultaneously? (These are prerequisites for a clinically relevant procedure.) d) What type of animal or behavioral model provides a credible test of the _clinical_ relevance of these procedures?

In other investigations we have been testing the effectiveness of applied fields on the regeneration of a peripheral nerve (peroneal) and its association with the onset of functional recovery. Presently our findings are negative. Problems to be address are: a) is the imposed field _of sufficient_ magnitude to facilitate regeneration? b) Would better surgical anastomoses lead to conditions that would allow an enhancement effect to be discerned?

We have also pursued the idea that applied fields may increase the rate of fresh fracture healing. (Such applications are now commonplace treatments for clinical cases of chronic non-union in humans (reviewed by McGinnis 1989). In collaboration with David Van Sickle's laboratory (Department of Anatomy, School of Veterinary Medicine), we have used a mechanically induced tibial osteotomy in canine subjects as a laboratory model. Though some promising trends were observed after microradiological analysis, histological analysis, and biomechanical load testing, overall the low numbers of animals and overlap in results of controls and experimental subjects does not allow rigorous conclusions to be drawn.

In summary, we have made substantial progress in demonstrating the efficacy of fields as therapeutic agents for the treatment of CNS injuries and need to refine these applications and rigorously test these notions at the clinical level. In the peripheral nervous system, we have still more experiments to perform before we can accurately address the potential of these application to clinical PNS trauma.

B. BACKGROUND

Applied Fields and Axonal Regeneration and Development

It is now clearly established that an applied electric field can profoundly affect the development and regeneration of nervous tissue. This fact has been established by modern, well-controlled experiments performed on: disaggregated neuroblasts developing in culture (McCaig and Robinson, 1980, Hinkle et al. 1980; Patel and Poo, 1982, 1984; McCaig, 1985a & b); cultured ganglia (Jaffe and Poo 1979); identifiable giant axons regenerating within the severed spinal cord of the larval Lamprey (Borgens et al. 1981; see also Roederer et al. 1983); and on a facilitation of regeneration of dorsal column axons in spinal cord of adult guinea pigs (Borgens et al., 1986b; reviewed by Borgens 1988a and b; and 1989a and b). Such axonal responses to applied fields include: an increase in the rate of growth or regeneration in fibers facing the cathode; a decrease in growth rate in fibers facing the anode; resorption of newly formed neurites by the cell body when such fibers face the anode; an inhibition of axonal degeneration in transected fibers facing the cathode; an increase in retrograde axonal degeneration in transected axons facing the anode; an increase in axonal branching in transected regenerating axons; an overall enhancement in the regenerative response in transected axons; an increase in neurite outgrowth from cultured ganglion; induced - and oriented - growth towards the cathode and away from the anode; an increase in the rate of growth (about 2-3 fold) in fibers that turn toward the cathode; a 2-3 fold increase in periods of fiber quiescence during growth (Argiro, et al., 1984); an increase in growth cone filopodia and cytoplasmic spines in the presence of an applied field. It is also interesting that a weak electrical field (10 - 100 mV/mm) can overcome and redirect neurite projections based on contact guidance cues (McCaig 1986).

One could reasonably suggest that these responses are more profound in scope than the responses of developing neurites to Nerve Growth Factor (NGF). Moreover, responses of nerves to applied fields have been observed in mammalian and non - mammalian CNS and PNS tissue as well as in adult and fetal vertebrate tissue (reviewed by Borgens 1989; and Borgens and McCaig 1989).

It is felt that the biological rationale underlying these responses lies in the electrophysiology of development. Steady, persistent currents are driven through the embryonic neural tube (Robinson and Stump, 1984) and neural folds (Borgens, work in progress). Strong outward currents are found leaving the lateral margins of the neural folds balanced by weak currents entering most of the rest of the embryo.
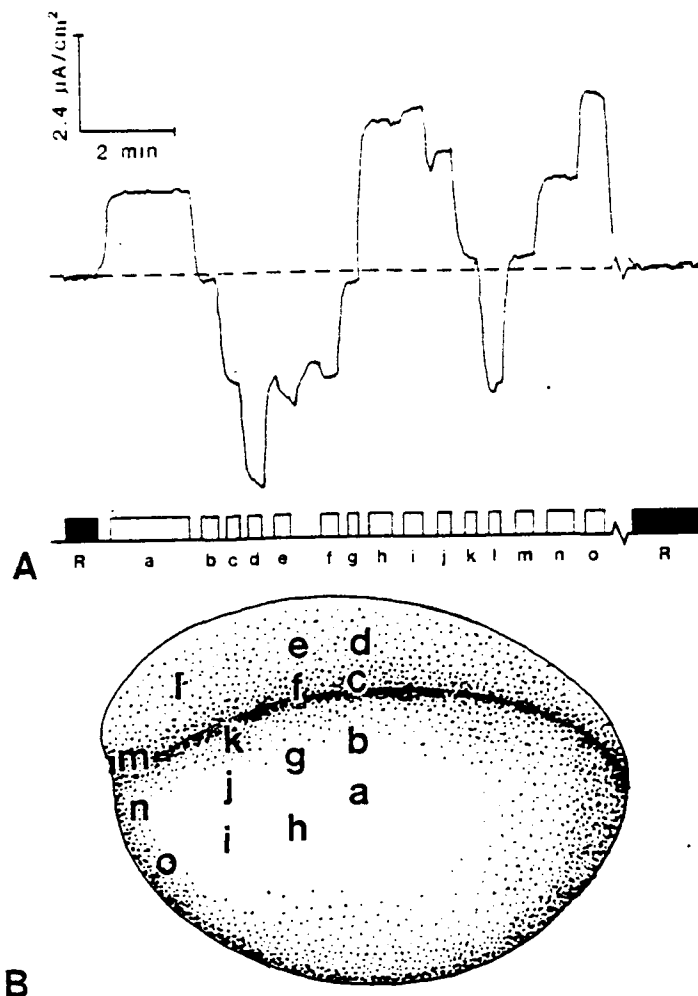
Figure 1.

Endogenous currents leaving the neural folds of the *Xenopus* neurulae as measured with a vibrating electrode for the detection of extracellular current (Jaffe and Nuccitelli, 1974; Borgens et al., 1983) (stage 17-18). A). Chart recording of currents detected entering and leaving the lateral body surface of the embryo at positions a-o, shown graphically below in B. Reference position is where the vibrating electrode is out of the electrical field (ca 2 cm from embryo) deflections above reference indicate current <u>entering</u> the surface; below reference current <u>leaving</u> the embryo's surface. Note that current (ca 2-3 uA/cm^2) leaves the walls of the developing neural fold while entering the rest of the embryo's surface.

These outcurrents, driven through the embryo by the invaginating neuroepithelium of the presumptive neural tube, would be associated with voltage gradients perpendicular to the long axis of the body and negative at the lateral margins with respect to the midline (see Borgens and McCaig 1989). Since neurites elongate parallel to the long axis of an applied field and myoblasts develop their bipolar axis of symmetry perpendicular to the long axis of the field (Hinkle et al., 1981), this arrangement suggests the actual spatial character of developing nerve and muscle at this early stage of

development. Moreover. these investigators found that the presence of a field stimulated neuroblast development in culture over cultures without an imposed field, suggesting that a normal extracellular component important to nervous system development may be the presence of a persistent steady voltage gradient. Steady fields are also claimed to exist at the tips of growth cones - supported by a predominantly inward calcium current at the apex (Freeman et al. 1985). This ion in particular is known to be involved in a variety of mechanisms of growth control in both animals and plants (Jaffe 1979, 1980, 1981; Borgens 1982, Borgens and McCaig 1989; Llinas 1979; Lasek and Hoffman 1976 for reviews).
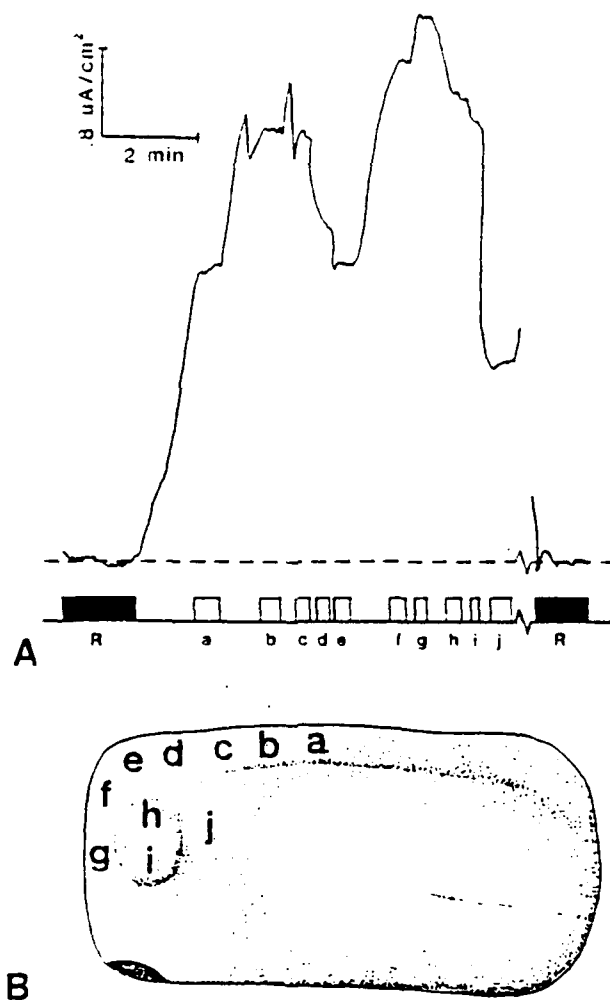

Figure 2.

Endogenous currents entering the well-formed neural tube in a stage 23 *Xenopus* larvae. Reference position and current convention as in fig. 1. Note the change in scale. Also note that after the closure of the neural folds forming the neural tube (and during the development of CNS specializations) outcurrents at the folds disappear completely.

8

Thus, circumstantial evidence suggests endogenous currents and voltages may be components of the natural controls of early nervous system patterning and perhaps in the growth and regenerative process of single neurons. Moreover, it is certain that artificially applied electrical fields can modulate nerve growth (in vivo and in vitro) and regeneration (in vivo) in a very striking way as discussed above.

In the last two years we have investigated more fundamental mechanisms underlying acute axonal injury to applied electric fields. In collaboration with Alan Strautman and Kenneth Robinson, we have studied the relationship between intracellular Ca^{++} concentration, axonal transection, and applied fields. Large increases in intracellular Ca^{++} is well known to cause the destabiliztion of the cytoskeleton, an interruption in axoplasmic transport, and other catabolic events within a nerve fiber (reviewed by Borgens and McCaig 1989). The first step in our investigation was to identify the time course of Ca^{++} movement into the severed end of axons, determine the spatial extent of a gradient of inwardly and electrogenically driven Ca^{++} (Borgens et al., 1981), and quantitatively determine the actual concentration changes involved. This was accomplished using the fluorescent, intracellular Ca^{++} probe - Fura 2. Fura 2 was injected inside lamprey giant reticulospinal axons and the resting levels of Ca^{++} determined (Ca 60 um) as well as the changes in $[Ca^{++}]$ with time after lesion. A large $[Ca^{++}]$ gradient was determined, extending proximally. These data are described in Strautman et al., (in the appendix) and set the stage for determinations of the way an extracellularly applied field may reduce or otherwise alter the kinetics of Ca^{++} entry into lesions to nerve processes. Though we have hypothesized the character of these changes based upon an evaluation of the cable characteristics of nerve processes (Borgens, 1988a; and Borgens and McCaig, 1989) we are now poised to test these notions.

The CTM Pathway and its Functional Recovery

The CTM reflex is a behavioral function of cervical spinal cord motor units that depends on sensory input from lumbar and thoracic dorsal roots. The underlying circuitry has been described in some detail in the rat by Nixon et al., (1984) and Thierault et al., (1988), and in the guinea pig by Blight et al (enclosed in appendix materials). The CTM behavior is grossly manifested as a phasic rippling of the backskin in response to tactile stimulation. This rippling is mediated by the cutaneous trunci muscle which tightly adheres to most of the skin of the back (fig. 3).

Sensory receptors in the skin are in continuity with the dorsal spinal cord via the Dorsal Cutaneous Nerves (DCN). Ascending components of the reflex are localized in the ventrolateral white matter, projecting to motor nuclei in the thoracocervical junction. The efferent motor projections (out of the cord to the CTM muscle) course through the lateral thoracic branch of the brachial plexus. Section of, or impact to, the ventrolateral white matter creates a chronic unilateral loss of responsiveness to ipsilateral stimulation below the level of the lesion. It is important to point out that unlike most other spinal cord mediated behaviors that are used as indices of spinal cord injury -
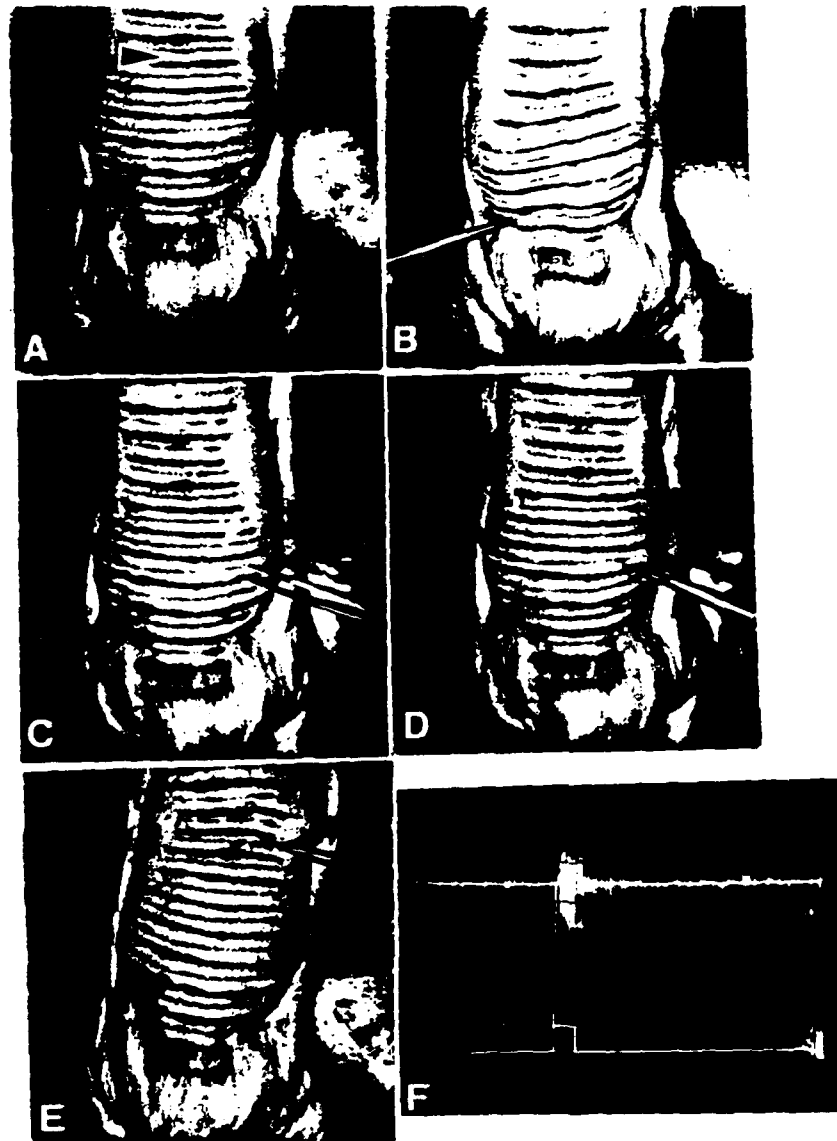
Figure 3.

Photographs illustrating the cutaneous trunci muscle (CTM) reflex in a guinea pig 6 months after thoracic right lateral hemisection of the spinal cord (arrow). A: The back skin of the guinea pig was shaved and marked with ink grid lines. B: Forceps lightly pinching flank skin produce a contraction of the CTM muscle (note drawing together of grid lines rostral to stimulus on the *side of stimulation*). *C*: Forceps poised for stimulation of the right side below the lesion to show the grid lines. D: Stimulation by pinching lightly below the lesion does not produce a CTM reflex. E: Stimulation above the level of the lesion on the right successfully elicits CTM contraction. F: Oscilloscope trace of electromyogram recorded from subcutaneous stainless steel wire electrodes. The lower trace indicates application of tactile stimulation. Scale: sweep duration = 1 second. Full scale = 5 mV.

10

there is no incidence of a spontaneous recovery of this reflex. Deficits in the CTM are truly permanent. Furthermore, though there is a contralateral component (crossed innervation) within the reflex circuitry, there is no capacity for this contralateral projection to contribute to functional restoration of the <u>ipsilateral reflex</u>. The reflex behavior can be photographically documented and quantified by digital analysis of "stop - frame" video tape records and by electromyography (see methods section below and appendix materials). These analytical methods allow complete documentation of changes in the response subsequent to spinal lesions and to treatment paradigms.

The importance of this behavioral model is that it is exquisitely <u>quantifiable</u>, dependent on a <u>known</u> and <u>localized</u> projection of spinal cord neuronal elements, and defects in the behavior resulting from spinal cord injury are <u>permanent</u>.

Functional recovery of the CTM is easily visualized. First, any spontaneous recoveries (i.e. arising from incomplete lesions to the ventrolateral funiculus) appear within one week post hemisection and are eliminated from the study populations. Video reconstruction of the receptive fields and videographic "dot matrix" movements (see following section) convincingly show a persistent and unchanging region of areflexia ipsilateral and below the level of hemisection (usually performed midthoracic). Since the adjacent three quadrants of flank are unaffected by ventrolateral section, the animal can serve as its own control; for example, if depth of anesthesia may reduce CTM functioning, this can easily be detected in the contralateral CTM response.

Presently we have no evidence of any spontaneous recovery of this long tract reflex in over 200 animals, some for periods of observation up to two years. Sham stimulator implanted animals show no change in the CTM deficit as do electrically treated animals where the <u>cathode</u> is <u>located caudal to the hemisection</u> (Borgens et al., 1990). Recoveries occur only in electrically treated animals with cathodes located <u>rostral to the lesion</u>, at about 100 days post-transection. Moreover, the physiological and behavioral character of the <u>recovered</u> reflex has been demonstrated to be nearly <u>identical</u> to the normal CTM (Borgens et al., 1990). Differences from the normal CTM include; a slight depression in the strength of the CTM contraction, a "patchiness" in the receptive field, and a heightened sensitivity to Nembutal anesthesia. (The normal CTM is extremely resistant to Nembutal). Overall, we are clearly dealing with a "recovery of function" that is nearly complete for this one complex sensorimotor long tract reflex. Furthermore, this recovery is now known to be associated with the formation of new connections within the central neuraxis; induced by only one polarity of an applied field (Ca. 300 uV/mm cathode rostral to the lesion); and not dependent on any effects of fields on peripheral innervation or sprouting. These observations offer great promise for clinical testing.

C. APPROACH AND METHODOLOGY

General Surgical Procedures

Adult female guinea pigs of the Hartley strain are used in all of our studies (400 - 600 g body weight). They are housed individually after surgery. Animals are anesthetized for surgery by intramuscular injection of a mixture of 60 mg/kg ketamine HC1, 0.6 acepromazine maleate, and 12 mg/kg xylazine. Stimulator units designed for subcutaneous placement and voltage source replacement (refer to above) are located beneath the backskin and stimulating electrodes routed to positions approximately 2 vertebral segments on either side of the compression injury to the spinal cord. Here a partial laminectomy is performed, exposing the spinal cord for a distance of about 3 mm between two vertebra. Stimulating electrodes are located within these partial laminectomies and sutured to the dorsal fascia so that the electrodes are near the exposed cord (1-2 mm) but cannot touch it (fig. 4). Sham and operative stimulator units are identical in appearance and coded by the fabricator, thus the surgeon is blinded to the application at the time of implantation. (All subsequent tests and analytical procedures are performed by individuals blinded to an individual animals experimental status).

Figure 4.

This radiograph of an adult guinea pig displays the location of a current regulated DC stimulator (s) within the peritoneal cavity. The uninsulated platinum iridium electrode coils (e) were sutured in partial laminectomies on either side of the spinal cord hemisection (arrow) and connected to the stimulator via subdermally routed, insulated electrode leads (1).

DC Stimulator - Design and Fabrication

The stimulator that we have designed (Fig. 5) uses two 3-volt lithium dioxide cells (Ray-O-Vac #BR 1225 or equivalent) connected in series. Silver epoxy is used to attach leads to the cells or the cells to each other if stacked. These small crimp-style cells are not capable of withstanding normal soldering temperatures. The stainless steel case of the cells should be roughened to enhance the adhesion of the epoxy. A constant-current source (LM 334, National Semiconductor) and two resistors are soldered together and attached to the battery with silver epoxy. One resistor, R_{set}, is used to set the current level (1) to any value between 1 uA and 10 mA. The proper value for the resistor is given by the equation:

$$R_{set} = \frac{70.4 \text{ mV}}{1} \quad \text{at } 37^{\circ}C$$

This resistor is connected between pins and 3 of the LM 334. One can also place a 1,000-ohm resistor, R_m, in series with the output, allowing the current to be monitored by measuring the voltage drop across the resistor. (One microampere of current will produce one millivolt of potential). Monitoring leads are soldered on either side of R_m. THe monitoring leads, as well as the electrode leads, are made of a highly flexible silicone insulated multistrand wire (AS-155-36, Cooner Electronics, Chatsworth, CA). The monitoring leads can either be left long and exteriorized percutaneously or cut short and left inside the body. When left in the body, the cut ends are capped with medical-grade silicone rubber (Medical Adhesive Silicone Type A, Cat. #891, Dow Corning, Midland, MI). These ends are placed subcutaneously and are later exposed through a skin incision and the ends stripped for voltage measurements. The electrodes consist of coils of platinum-iridium (90%/10%; 0.178 mm in diameter; Engelhard, Carteret, NJ) soldered to the silicone insulated wire with the solder joint covered by silicone rubber. The electrode leads are then either soldered or epoxied to the rest of the circuit. The unit is then suspended by the electrode leads and lowered into melted beeswax. Several dippings produce a 1-2-mm-thick coating of wax that inhibits rusting or corrosion of the electronics. The unit is then dipped in a silicone elastomer. We use Silastic 3110 by Dow Corning which, although not labeled as medical-grade or biocompatible, appears to be well tolerated by guinea pigs. The unit is then gas-sterilized (ethylene oxide) and allowed to degas for 1 week before implantation. The electronics can be fashioned in several shapes to meet the experimental needs. For subcutaneous implants we use a flat arrangement, while for peritoneal implants we use a stacked arrangement. The length of the leads is tailored to the situation.
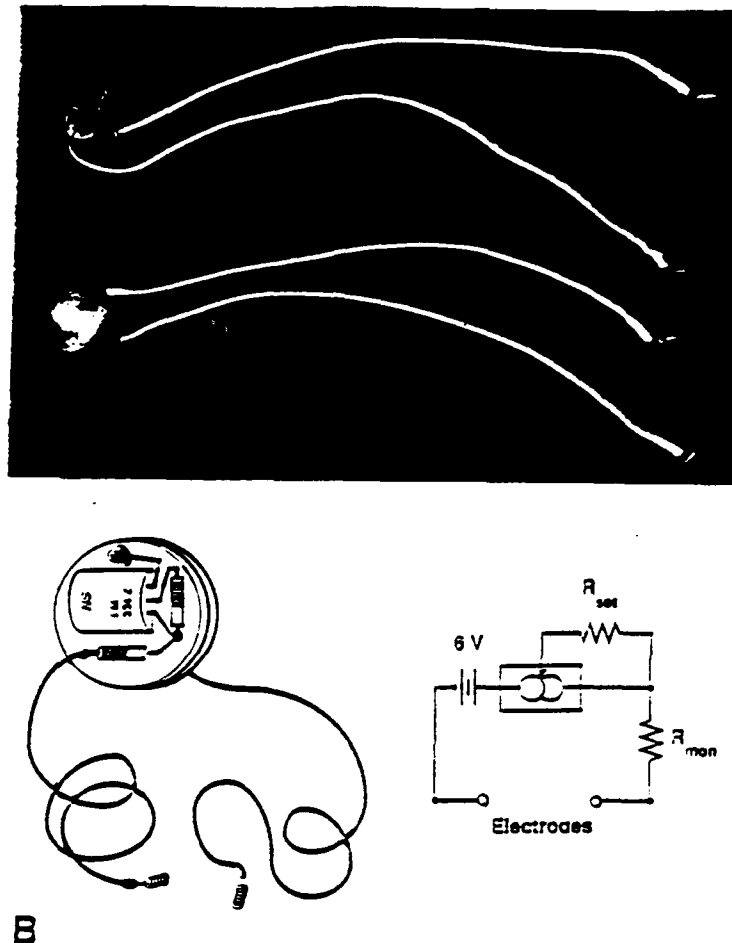
B

Figure. 5. Stimulator

DC constant current stimulator. Two 3 V lithium dioxide cells and a LM 334 constant current source provide regulated current to two Pt. coil electrodes. Current magnitude is determined by the value of R_{set} and the current is monitored by measuring the voltage drop across R_{mon}.

A) A completed stimulator before and after coating with a biocompatible silicone elastomer.
B) Schematic and circuit diagram showing the constant current source (LM 334 Z), the set resistor (R_{set}) and the monitoring resistor (R_{mon}).

Behavioral Analysis of the CTM

To visualize and record skin movements in response to tactile or electrical stimulation, we first shave the back of the animal and mark the skin with a matrix of black dots (using either indelible ink or a more permanent "tattoo"). These dots move during episodes of skin contraction.

To analyze the movements we use a commercially available (Magic) video-image digitizer attached to a Macintosh Plus computer. This allows succeeding frames of the video recordings to be digitized in a dot matrix pattern. Each digitized image is then transferred to a graphics program (Superpaint) which

allows superimposition of a succession of images. Finally, the image from the most extreme point of skin contraction can be superimposed on the image preceding the onset of contraction to give a complete vectorial representation of the movement of the skin. To make this image still clearer, we have found it useful to replace the simple digitized image with a pattern of standardized dots obtained by superimposing "graphics tool" patterns over the center of each digitized ink mark before the stage of superimposition of frames. A selection of recordings with this procedure in the normal animal is shown in the following Figures 6 and 7 (see also Borgens 1989b and Borgens et al., 1990). This newly developed system allows us to analyze the movements in great detail and reveals important features of the process of functional recovery in the CTM system, both with regard to spatial distribution and timing.
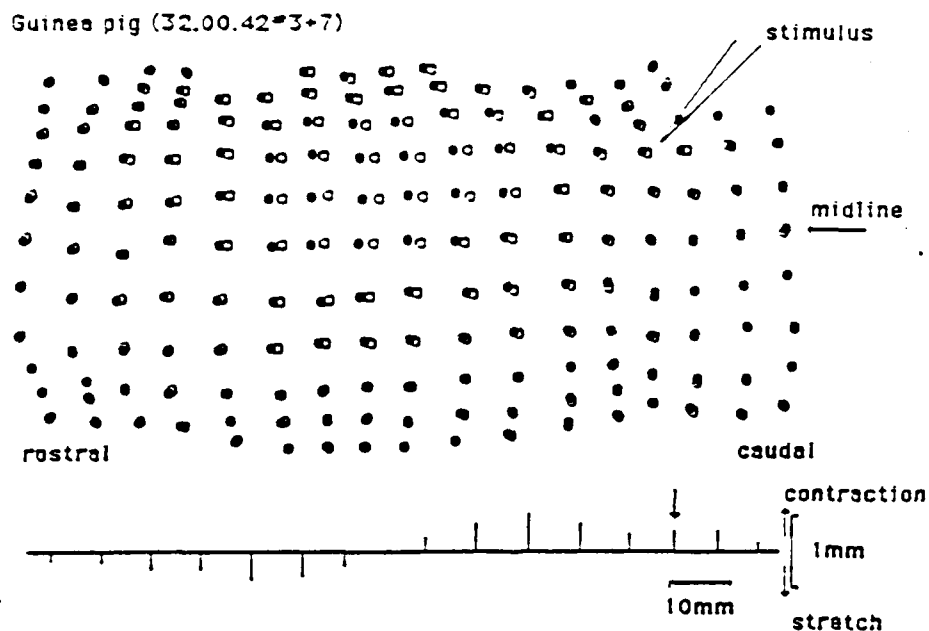

Fig. 6.

The CTM reflex of guinea pig compared by digitized analysis of videotape recordings. Animals were lightly anesthetized with sodium pentobarbital (30 mg/kg), the back shaved and a pattern of india ink dots applied to the skin. The animal was placed on a graduate background, together with a stop-watch, and recorded from above with a video camera. The guinea pig was stimulated with light touch. Individual frames of videotape were digitized with a Magic interface and stored on a Macintosh Plus computer. A graphics program (Superpaint) was used to superimpose uniform dots on the ink marks. Two frames were selected for superimposition, one just before the response (filled symbols) and one at the peak of skin contraction (open symbols), for each animal. A more accessible measure of the pattern of skin contraction was obtained by measuring the distance between pairs of dots along the rostro-caudal axis, in line with the stimulus point. The movement over 3 rows and 3 columns of dot pairs was averaged to give the histogram display shown below the overall representation of the back skin. The site of contraction of the skin relates directly to the stimulus point, though most of the observed *movement* in this instance is rostral to the stimulus.
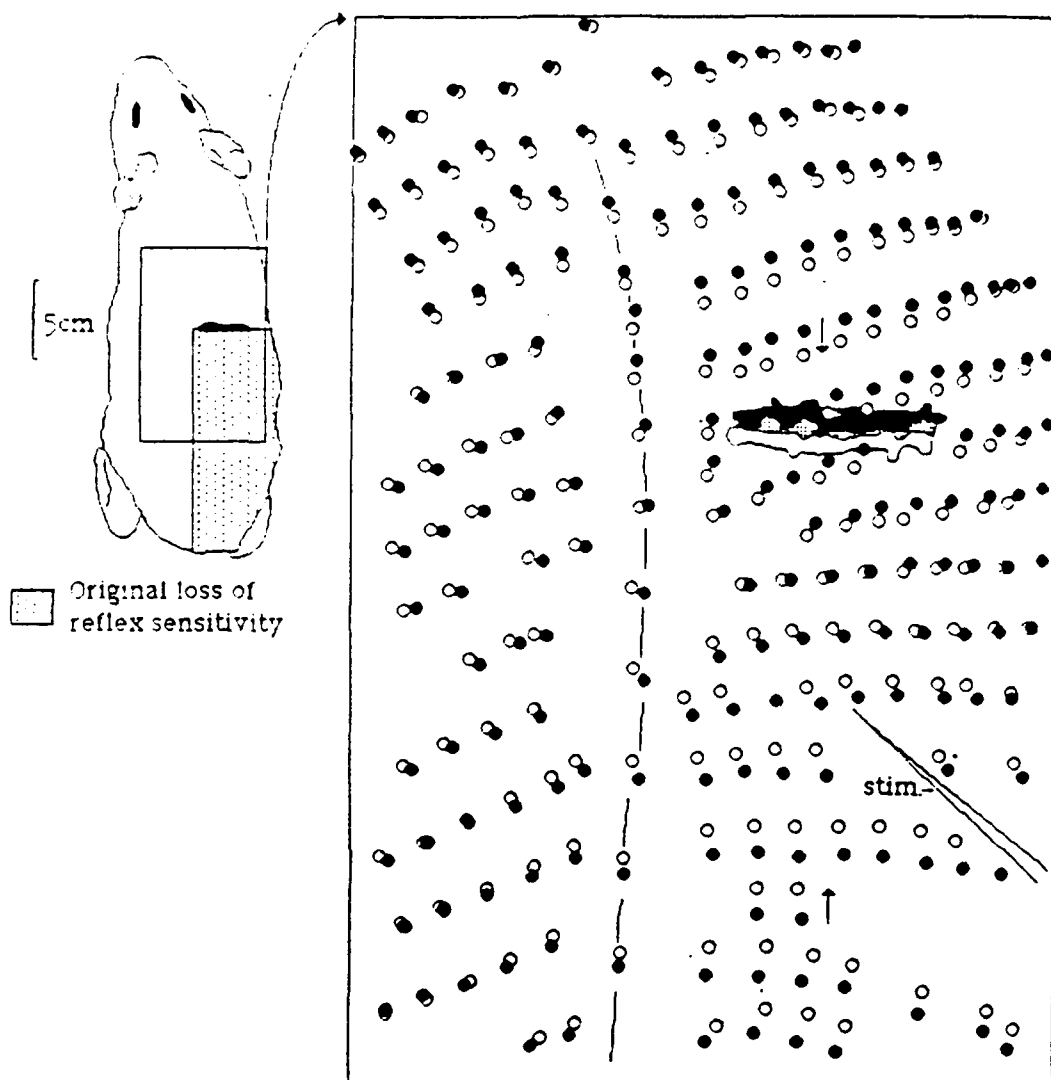
RL3/37   14-1-88   Time: 05:26:90 (●) and 05:27:12 (○)

Figure 7.   Recovery of Function in the CTM Reflex after Electrical Stimulation.

This drawing was made by a computer-graphics integrated system. First the outline of the guinea pig was rendered by a laser printer from a stop-frame video tape image-phased to the graphics analyzer. A dot-matrix pattern was rendered from a videoframe chosen immediately prior to stimulation (not probe) and after stimulation. The closed circle is the position of the dots on the backskin, and the open circle is the position they occupy after CTM skin movement (superimposed by the computer). In this fully recovered animal note that stimulation ipsilateral and below the level of the hemisection once again produces a typical phasic rippling of the skin in response to tactile stimulation. This reflex recovered 121 days after right lateral hemisection in response to a rostrally-negative applied field of about 100 uV/mm.

16

Electromyography of the CTM Reflex

Electromyographic recordings of skin contractions are performed on animals lightly anesthetized with sodium pentobarbital. EMGs are recorded from subdermal wire electrodes located at the brachial region near the midline of the back in a region of most visible skin contraction. EMGs are amplified with a Grass P 15 D preamplifier and displayed on either a Tektronix 5113 Oscilloscope or a digital oscilloscope (Nicolet #310). Permanent records are polaroid photographs of the sweep (made with a Tektronix oscilloscope camera) of the former. In the latter, records are saved to a floppy disk built into the oscilloscope. Permanent records could be obtained form the disk by plotting on a Hewlett Packard Pro Plotter. In some cases, stimulation of the skin is electrical, using a bipolar stimulation electrode and a Grass S 44 stimulator. All electromyographic recordings are accompanied by a visual scoring of the presence or absence of the CTM reflex. Such observations are made by stimulating the four quadrants of back skin with watchmakers forceps. An example of such physiological recordings of CTM behavior are included here and in the appendix materials.
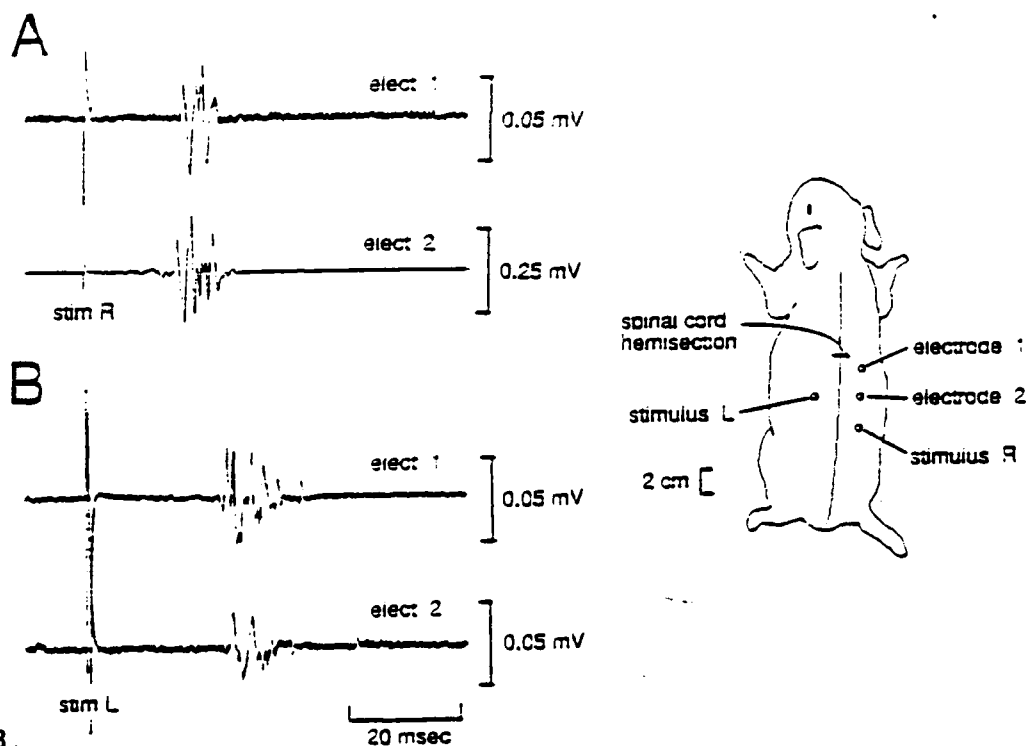

Figure 8.

Electromyograms of the CTM reflex in response to electrical stimulation of the skin on either side of the midline in the recovered animal illustrated in Fig. 7. The characteristics of the ipsilateral response were apparently normal, with well synchronized bursts of activity, larger in amplitude near the stimulus and at a latency of approximately 17 msec, shorter than the latency of the response to contralateral stimulation (B).

Anatomical Analysis of the Lesion and of Nerve Fibers

The extent of the original injury is determined in histological sections of the lesion site after perfusion fixation. Most cords are examined with transverse 0.5-1.0 um plastic section stained with toluidine blue. Others are sometimes sectioned horizontally with the freezing microtome at 30-40 um.

Axonal projections and identification of single processes and sometimes their growth cones are accomplished by using anterograde filling of Horseradish peroxidase. One day prior to the sacrifice of an animal, the guinea pig is anesthetized and a second laminectomy performed - about 1-2 vertebral segments caudal to the level of the original hemisection. At this site, a fresh section of the cord is perfused and crystals of HRP (type II, Sigma) are introduced into this lesion. Gelfoam is layered above this region (to limit diffusion of HRP from the site), the lesion closed, and the animal returned to its quarters for recovery. Twelve to sixteen hours subsequent, the guinea pig is sacrificed by perfusion fixation, the spinal cord dissected free, and the tissues processed for HRP visualization (Borgens et al, 1986a and b). An example of regenerated nerve processes, visualized by this method are provided in fig. 9.
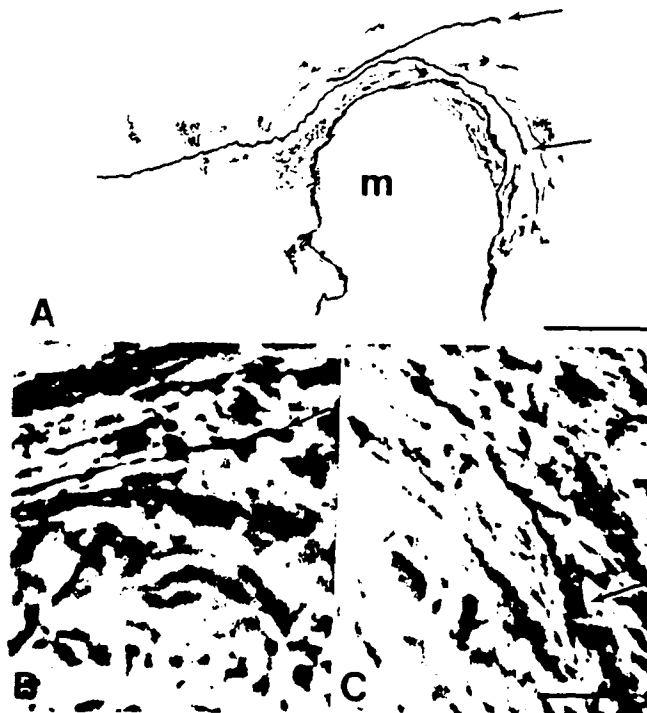

Figure 9.

Camera lucida reconstruction of two regenerating lateral tract axons in guinea pig spinal cord, 59 days post hemisection. Lengths of axons shown here were traced within one 60 um horizontal section. These HRP filled axons ended in growth cones (arrows) after projecting into the rostral segment of spinal cord by growing around the lesion and marker device. m = hole in tissue left by marker. Caudal is to the left. B and C are photomicrographs of the growth cones drawn in A. Scale bar for A = 40 um; scale bar for B and C = 10 um.

Acute Spinal Cord Injury in the Dog: A Clinical Model for Human Spinal Cord Injury

We have developed a clinical model for acute spinal cord damage utilizing naturally occurring injuries in dogs. The most common cause of paralysis in dogs is intervertebral disk degeneration and subsequent disk rupture at or near the thoracolumbar junction in chondrodystrophic breeds. This is generally a non-traumatic, but acute, event that occurs during normal daily activity. Complete herniation of the nucleus pulposis in these dogs causes severe spinal cord compression which leads to complete loss of both sensory and motor function to the pelvic limbs (paraplegia) which is often unresponsive to standard medical and surgical therapy. These dogs and dogs with trauma-induced complete paraplegic (spinal fracture/dislocations, and trauma-induced disk ruptures) comprise the population of interest for our study.

There is a common misconception that the dog disk herniation model has little relevance to acute spinal cord trauma in humans. This misconception arises from the assumption that clinical consequences of disk herniations in dogs are similar to those in man. Although the disk herniation event is similar in both species, important anatomic differences between species produce drastic differences in the degree of dysfunction which occurs because of this event. The spinal cord terminates in dogs at the level of L 6, whereas in man it terminates at the level of L 1. Disk herniations in dogs most commonly occur at the thoracolumbar junction, but in man the most common site is L 4/5. Therefore, disk herniations in man lead to nerve root compression and the associated clinical signs of pain and sometimes paresis, whereas in the dog they lead to mechanical distortion of the grey and white matter of the spinal cord and clinical signs ranging from pain and paresis in mild cases to complete paraplegia in severe ones. The clinical signs associated with acute rupture of a large volume of disk material in the dog produce a clinical syndrome very similar to that observed in humans with fracture/dislocations of the thoracic spine: absence of voluntary movement below the level of the injury, incontinence, complete loss of action potential propagation across the lesion [as determined by electrodiagnostic tests including somatosensory evoked potentials (SSEP), sensory evoked potentials (SEP), and motor evoked potentials (MEP), as well as losses of superficial and deep pain appreciation below the level of the lesion]. Therefore the clinical manifestations of acute disc herniation in the dog are directly comparable to acute spinal trauma in humans (and do not resemble human disc herniations in biology or clinical picture). Furthermore, the long term persistence (many years) of such deficits in the dog support the fact that these are indeed spinal cord injuries directly relevant to human SCI. Such animals display hard and soft tissue deterioration, abnormal reflex patterns, spasticity, chronic renal problems (associated with chronic incontinence) in addition to the behavioral defects observed at the time of acute injury.

Preliminary Findings in Dogs with Acute Complete Paraplegia

Twelve dogs with acute (1-16 days duration) complete paraplegia have had exploratory laminectomies performed and have been implanted with either active or sham oscillating field stimulator units. These trials are "double-blind" in construction. Some of these dogs have completed the mandatory six month follow-up period, at which time the code (which reveals the type of implant) is broken. Others listed below have been done more recently and are still in progress. Neurologic improvement has been variable but has included improved urinary bladder control, return of the pelvic limb sensory function, improved pelvic limb motor strength, and in some cases a return to voluntary ambulation without assistance. For dogs in which walking function was restored, the time after surgery at which this change occurred ranged from six weeks to eight months. Preliminary results for the 12 acute paraplegic dogs are tabulated below.

DOGS WITH COMPLETE PARAPLEGIA SECONDARY TO INTERVERTEBRAL DISK HERNIATION OR SPINAL FRACTURE/DISLOCATION

IMPLANT TYPE	# DOGS	NO CHANGE	IMPROVED BLADDER	IMPROVED SENSORY	VOLUNTARY WALKING
Active	5	0	4	4	3
Sham	3	2	0	1[*]	1[*]
Unknown	4	1	1	3	1
	—	—	—	—	—
	12	3	5	8	5

[*] This dog was re-implanted with an active unit when the code was broken six months after surgery. During the period that the dog has worn the active unit improved bladder function and improved hindlimb sensation have been noted.
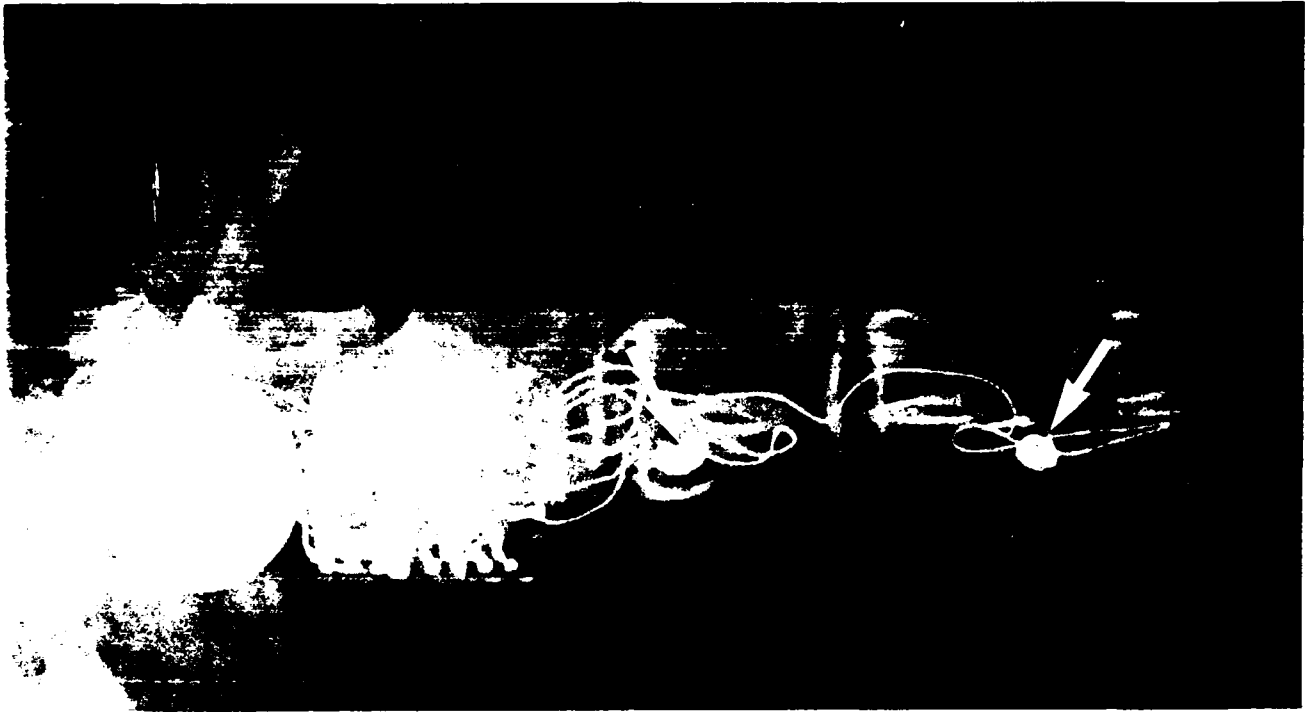
Figure 10.

This radiograph (dorsal view) displays an oscillating field stimulator implanted adjacent the vertebral column of a paraplegic dog. The round voltage source is clearly seen to the left of the oscillator (printed circuit board and electronic components). Coiled platinum/iridium electrodes (arrows) are located on either side of the spinal cord lesion.

D. CONCLUSIONS

1.   Weak (300 uV/mm) electrical fields are able to facilitate the regeneration of afferent spinal cord axons after partial transection of the cord in situ.

2.   Regenerating fibers grow <u>around</u> the plane of transection (and the fibrous component of the scar) and not through it.

3.   A similar magnitude and polarity of field is associated with a 25% recovery of function in a permanent behavioral deficit in the laboratory guinea pig.

4.   Quantitative analysis of the recovered behavior (occurring usually 100 days post transection) by stop-frame video graphics suggest that grossly, the CTM recovered behavior is nearly identical to the behavior lost after spinal cord hemisection.   The electromyography suggests certain differences from the normal CTM reflex (together with a Nembutal sensitivity) that point to the formation of novel pathways between the afferent input to the cord and the motor nuclei that are found on the cervical-thoracic junctions.

5.   Using comparable applied fields we see no comparable effect on peripheral nerve regeneration.

6.   In summary, applied fields appear to have real therapeutic potential for facilitating CNS regeneration, reconnection and recovery of function.   We are testing these notions in clinical cases of naturally occurring acute spinal cord injuries in dogs.   More investigation of field effects on peripheral nerve regeneration will be necessary to exclude applied currents as a potential therapeutic aid.

22

LITERATURE CITED

Argiro, V., M.B. Bunge, and M.I. Johnson (1984) Correlation between growth cone form and movement and their dependence on neuronal age. J. Neurosci. 4:3051-3089.

Blight, A.R. (1990) The cutaneous trunci muscle reflex of the guinea pig. J. Comp. Neurol., 296:614-633.

Borgens, R.B. (1982) What is the role of natural electric current in vertebrate regeneration and healing? Int. Rev. Cytol. 76:245-298.

Borgens, R.B. (1988a) Voltage gradients and ionic currents in injured and regenerating axons. In: Advances in Neurology: Functional Recovery in Neurological Disease (S.G. Waxman, ed). New York: Raven. pp. 51-66.

Borgens, R.B. (1988b) Stimulation of neuronal regeneration and development by steady electrical fields. In: Advances in Neurology: Functional Recovery in Neurological Disease. (S.G. Waxman, ed.). New York: Raven Press. pp. 547-564.

Borgens, R.B. (1989a). Artificially controlling axonal regeneration and development by applied electric fields. In: Electric Fields in Vertebrate Repair. New York: Liss. Chapter 4, pp. 117-170.

Borgens, R.B. (1989b) Natural and applied currents in limb regeneration and development. In: Electric Fields in Vertebrate Repair. New York: Liss. Chapter 2, pp. 27-75.

Borgens, R.B., E. Roederer, and M.J. Cohen. (1981) Enhanced spinal cord regeneration in lamprey by applied electric fields. Science 213:611-617.

Borgens, R.B., A.R. Blight, and D.J. Murphy (1986a) Axonal regeneration in spinal cord injury: A perspective and new technique. J. Comp. Neurol. 250:157-167.

Borgens, R.B., A.R. Blight, D.J. Murphy, and L. Stewart (1986b). Transected dorsal column axons within the guinea pig spinal cord regenerate in the presence of an applied electric field. J. Comp. Neurol. 250:168-180.

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

Borgens, R.B. and C.D. McCaig (1989) Endogenous currents in nerve repair, regeneration, and development. In: Electric Fields in Vertebrate Repair. New York: Liss. Chapter 3, pp. 77-116.

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

Freeman, J.A., P.B. Manis, G.I. Snipes, G.N. Mayes, P.C. Samson, J.P. Wikswo, Jr., and D.P. Freeman (1985) Steady growth cone currents revealed by a novel circularly vibrating probe: A possible mechanism underlying neurite growth. J. Neurosci. Res. *13*:257-283.

Hinkle, L., C.D. McCaig, and K.R. Robinson (1981) The direction of growth of differentiating neurones and myoblasts from frog embryos in an applied electric field. J. Physiol. (London) *314*:121-135.

Jaffe, L.F. (1979) Control of development by ionic currents. In: *Membrane Transduction Mechanisms*, (R.A. Cone and J.E. Dowling, eds.). New York: Raven Press, pp. 199-231.

Jaffe, L.F. (1980a) Calcium explosions as triggers of development. Ann. N.Y. Acad. Sci. *339*:86-101.

Jaffe, L.F. (1980b) Control of plant development by steady ionic currents. In:*Plant Membrane Transport: Current Conceptual Issues*, (R.M. Spanswick, W.J. Lucas, and J. Dainty, eds). Amsterdam: Elsevier/North Holland/Biomedical Press, pp. 381-388.

Jaffe, L.F. (1981) The role of ionic currents in establishing developmental pattern. Philos. Trans. R. Soc. Lond. [Biol.] *B295*:553-566.

Jaffe, L.F. and M.-M. Poo (1979) Neurites grow faster towards the cathode anode in a steady field. J. Exp. Zool. *209*:115-128.

Lasek, R.J. and P.N. Hoffman (1976) The neuronal cytoskeleton, axonal transport, and axonal growth. In: *Cell Motility* (R. Goldman, T. Pollard, and J. Rosenbaum, eds.) Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, pp. 1021-1049.

Llinas, R. (1979) The role of calcium in neuronal function. In: *The Neurosciences: Fourth Study Programs* (F.O. Schmitt and F.G. Worden, eds.). Cambridge, Massachusetts: MIT Press, pp. 555-571.

McCaig, C.D. (1985a) Electric fields, contact guidance and the direction of nerve growth. J. Embryol. Exp. Morph. *94*:245-255.

McCaig, C.D. (1985b) Dynamic aspects of amphibian neurite growth and the effects of an applied electric field. J. Physiol. *375*:55-69.

McGinnis, M.E. (1989) The nature and effects of electricity in bone. In: *Electric Fields in Vertebrate Repair*. New York: Liss. (Chapter 6, pp. 225-284.

Nixon, B.J., R. Doucette, P.C. Jackson, and J. Diamond (1984) Impulse activity evokes precocious sprouting of nociceptive nerves into denervated skin. Somatosensory Res. 2:97-126.

Patel, N. and M.-M. Poo (1982) Orientation of neurite growth by extracellular fields. J. Neurosci. 2:483-496.

Patel, N. And M.-M. Poo (1984) Perturbation of the direction of neurite growth by pulsed and focal electric fields. J. Neurosci 4:2939-2947.

Robinson, K.R. and C.D. McCaig (1980) Electrical fields, calcium gradients and cell growth. Ann. N.Y. Acad. Sci. 339:132-138.

Robinson, K.R. and R.F. Stump. (1984) Self-generated electrical currents through *Xenopus* neurulae. J. Physiol. *352*:339-352.

Roederer, E., N.H. Goldberg, and M.J. Cohen (1983) Modification of retrograde degeneration in transected spinal axons of the lamprey by applied DC current. J. Neurosci. *3*:153-160.

Strautman, A.F., R.B. Borgens, and K.R. Robinson ( ) The distribution of calcium in transected spinal axons. (submitted for publication).

Theriault, E., and J. Diamond (1988) Nociceptive cutaneous stimuli evoke localized contractions in a skeletal muscle. J. Neurophys. *60*:446-477.

GLOSSARY

<u>CTM</u> Cutaneous trunci muscle reflex. A sensorimotor long tract reflex that is grossly manifested in the guinea pig as a phasic rippling of the backskin in response to tactile stimulation.

<u>Dorsal Columns</u> These large spinal cord tracts are bundles of neurons that project into the spinal cord from segmental ganglia lying just outside the cord itself. Sensory information (largely) is carried to the brain by these tracts that ascend the cord.

<u>Laminectomy</u> Surgical exposure of the spinal cord within the vertebral column.

<u>Neurite</u> A general and non-specific term for a neuronal process.

<u>Orthodromic and Antidromic stimulation and recording</u>. Experimentally evoked Action Potentials whose conduction pathway is in the same direction as natural conduction are <u>orthodromically</u> stimulated. For example: orthodromic stimulation of a motor neuron would involve stimulating near the soma (or ganglion) and recording at the periphery. Antidromic stimulation and recording would be the reverse of this regimen.

<u>Wick electrode</u> An aqueous "wire". Stimulating electrodes fashioned from a silastic tube, filled with mammalian ringers and a cotton string (the "wick"). Thus, current is carried to the tissues by a conductive solution similar to body fluids and not by metallic wires (which contaminate the tissues with electrolysis products).

DISTRIBUTION LIST

4 copies   Commander
              Letterman Army Institute of Research
              (LAIR), Bldg. 1110
              ATTN: SGRD-ULZ-RC
              Presidio of San Francisco, CA   94129-6815

1 copy   Commander
              US Army Medical Research and Development Command
              ATTN: SGRD-RMI-S
              Fort Detrick, Frederick, Maryland   21701-5012

2 copies   Defense Technical Information Center (DTIC)
              ATTN: DTIC-FDAC
              Cameron Station
              Alexandria, VA   22304-6145

1 copy   Dean
              School of Medicine
              Uniformed Services University of the Health Sciences
              4301 Jones Bridge Road
              Bethesda, MD   20814-4799

1 copy   Commandant
              Academy of Health Sciences, U.S. Army
              ATTN: AHS-CDM
              Fort Sam Houston, TX   78234-6100