15

Effects of Electromagnetic Fields on Nerve Regeneration

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INTRODUCTION

The problem of regeneration in the central and peripheral nervous system has been the topic of numerous investigations and a variety of techniques have been used to effect complete restoration (discussed in a later section) (1,2). In the past 10 years a resurgence of interest has developed in employing electric fields to stimulate regrowth. The purpose of this Chapter is to present results obtained with these fields and to explore future avenues of research.

IN VITRO STUDIES

The pioneering work of Ross Harrison (3) demonstrated that isolated nerve cells are capable of forming nerve processes (neurites) when isolated in a dish containing proper nutrient medium (tissue culture). This tissue-culture technique has been used to study the effects of various electric fields on neuronal growth. Since the tissues are denervated at the time of culture, the regrowth response is termed "neuronal regeneration" and it is assessed by determining outgrowth of neurites from the neuronal cell bodies, and survival of neurons with reestablishment of neurotransmitter synthesis and neurophysiological activity.

The first report of electric field effects on neuronal regeneration *in vitro* was by Ingvar (4), who found that regenerating cell processes oriented along direct current (DC) field lines (0.1–0.2 $\mu A/cm^2$). In 1934 Karssen and Sager (5) confirmed these findings using currents less than 1 $\mu A/cm^2$. In the same year Paul Weiss (6), a noted embryologist, maintained that nerves grow along the electrically-induced stress lines of the plasma clot so that growth was only secondarily affected. In 1946 Marsh and Beams (7) presented definitive proof that the neurons were directly influenced by DC (120 $\mu A/cm^2$). Not only did the newly formed axons grow preferentially to the cathode, but growth was reoriented in response to cathode placement.

Since that time, other studies using DC have demonstrated stimulation of growth and directional response to the cathode (8-14). More recently, noninvasively induced current

via pulsed electromagnetic fields (PEMF) has been applied to cultured neurons resulting in both stimulatory and inhibitory effects (see below).

LEVELS OF APPLIED DIRECT CURRENT

Two schools of thought appear to exist for applying optimum levels of DC to neuronal systems *in vitro*. One concept stems from work with the application of DC current on the order of nanoamperes using in-dwelling metal (platinum or tantalum) electrodes for long time periods (15). A second view comes from the application of DC currents on the order of milliamperes using agar bridge electrodes for relatively short time periods, using a technique introduced by Marsh and Beams (7). Cathodally-oriented neurite growth has been observed in both systems.

The original justification for using fields of 100 µV/cm or less arose from the work of Becker and Murray (16) and Pilla (17), who found that fields of 10^{-8}–10^{-4} V/cm provoked morphological changes in amphibian red blood cells (RBCs) to a "dedifferentiated" state. Pilla observed the greatest change at the electrodes where the calcium ions moved more slowly. Addition of bulk calcium ions to a solution of RBCs produced the same dedifferentiated response in 10% of the cells. Electrochemical tests of different electrodes indicated the superior quality of titanium or tantalum as electrodes; both demonstrated little faradaic current flow over a 2-volt range. In 1975, Sisken and Smith (14) reported on the stimulation of neuronal regeneration in vitro. DC (0.001–11.5 nA/mm^2) applied via point platinum electrodes to trigeminal ganglion cultures enhanced neurite growth that was oriented to the cathode; the rate of growth was 0.1 mm/hr. neuronal survival was also enhanced. These studies have been extended using dorsal root sensory ganglia to include and compare PEMF effects with those produced by applied DC in a nonuniform field (18-21). Our aims have been to assess electric field effects on neuronal regeneration, including studies on the nerve cell body itself. We exposed our neuronal population to minute levels of DC for up to 3 days and observed effects at that time, or after an additional 3 days of culture. The results of these experiments indicated that not only was neurite growth enhanced, but protein content was significantly increased over control cultures (22). These observations were not dependent upon the number of non-neuronal cells (assessed by ^{3}H-thymidine incorporation, unpublished observations). This line of study differs from the studies of those who study electrophoresis-dependent neurite growth and movement of surface receptors.

More recently we tested different levels of DC on the peripheral-ganglia model using agar or metal (platinum, tantalum) electrodes (23), or tantalum electrodes driven by constant current sources (24). Maximal stimulation of regeneration was obtained with a constant current of 10 nA total current (agar electrodes) or 60 nA total current (tantalum electrodes) in chick sensory ganglia. Similar experiments using neuroblastoma cell lines (25) indicated that greater numbers of cells formed processes after application of 10 nA

total current. Increasing the constant current above 10 nA did not increase transformation.

Milliampere levels of DC were employed first by Marsh and Beams (7) and then by Jaffe and Poo (11). The model developed by Jaffe and Poo used agar electrodes to expose dorsal root ganglia (cultured in the presence of nerve growth factor) to a uniform electric field created by applying up to 140 mV/mm across the dish for up to 20 hours. Field strengths of 70–140 mV/mm induced faster outgrowth of neurites oriented toward the cathode. The effective average current density in these experiments was 14.3 mA/cm^2, which was in the same range as that used by Marsh and Beams (Table 1). They postulated that the applied electric fields could cause electrophoresis of nerve growth factor receptors to the growing neurite. Hinkle et al. (10) used a similar system to expose single neurons from *Xenopus* neural tube. Preferential growth to the cathode was obtained with exposure times of 18–20 hours; threshold values for this response were 6–8 mV/mm. Patel and Poo (12) applied steady electric fields of 0.1–10 V/cm to single *Xenopus* neurons and also found stimulated growth of neurites facing the cathode. The number of neurite-containing neurons was increased in these treated cultures as was neurite length. Addition of concanavalin A (ConA) abolished the electrically induced effects while fluorescently labeled ConA receptor accumulated at the cathode. This cathodal accumulation of growth-controlling surface glycoproteins was implicated as the mechanism by which electric fields exerted their effect.

Table 1. Values of Electric Field, E, Current Density, J, and Time, T (in hours) for *In Vitro* Experiments on Neuronal Tissue

	E	J	T (hours)
Ingvar (1920)	—	0.0015 μA/mm^2	—
Marsh and Beams (1946)	65 mV/cm	12 mA/cm^2	29
Sisken and Smith (1975)	80 μV/cm	11.5 nA/mm^2	96
Jaffe and Poo (1979)	1000 mV/cm	14.3 mA/cm^2	4–8 (20 max)
Hinkle et al. (1981)	70–1900 mV/cm	1–27 mA/cm^2	18–20
Patel and Poo (1982)	100–10,000 mV/cm	1.4–143 mA/cm^2	6
Patel and Poo (focal) (1984)	3–30 mV/cm	0.2–2 pA/μm^2	0.25
Sisken (1984)	0.6 μV/cm	9 nA/cm^2	72
Freeman (1985)	70–350 mV/cm	1 mA/cm^2	—

The effects of electric fields on central nervous system regeneration *in vitro* have also been investigated. Khan and Gaik (26) placed rat embryo spinal cord explants on carbon fibers (8–10 μm in diameter) in a culture dish and applied 20 nA DC to the fibers. Cathodally oriented growth (length and number of neurites) was significantly enhanced in this system. In our laboratory, spinal-cord explants of 8-day chick embryos or 16-day rat fetus were treated with 10 nA DC or single-pulse 72-Hz PEMF in the presence of

varying concentrations of cytosine arabinoside, ara C (15). The area of the explant, total area of outgrowth, and area occupied by neurites were assessed on cultures stained with Bodian silver or on radioautographs of explants showing incorporation of 3H-proline. Both DC (3-day, continuous) and PEMF (12 hr/day for 3 days) significantly stimulated neurite outgrowth relative to controls.
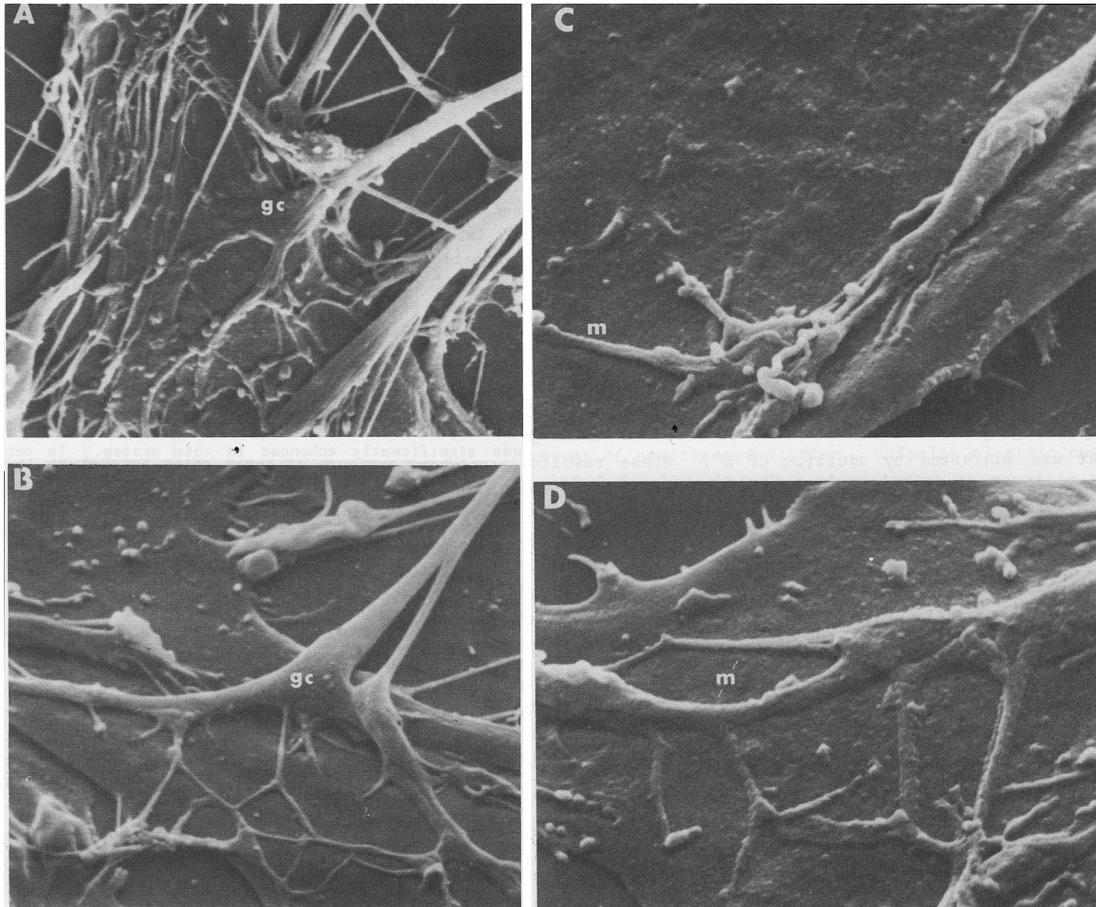

Figure 1. Scanning electron microscopy of neurite growth cones of 8-day chick embryo trigeminal ganglia after 4 days *in vitro*. The growth cones (gc) were found at the edge of the explant lying on top of the fibroblast mat. Note the expanded growth cones of the DC-treated ganglia and increased numbers of micro-spikes (m). A, (control culture) and B (10 nA DC-treated culture) x 2400. C, (control culture) and D (10 nA DC-treated culture) x 9750.

Freeman et al. (8) described the development and use of a circularly vibrating probe on central (retinal) neurons of the goldfish. This probe was capable of discriminating current densities of 5 nA/cm^2. They provided evidence for the existence of currents of 10–100 nA/cm^2 flowing into the filopodia of growth cones of cultured retinal ganglion cells and back out from near the junction of growth cone and filopodia. The currents were produced only during active growth and were believed to be carried primarily by calcium ions. To determine whether such endogenous fields were capable of directing filopodial

growth toward or away from a point source of current, retinal ganglion cells were exposed to point sources. Threshold current for orientation was 40 nA (70 mV/cm). Endogenous currents generated an axial current within filopodia of 4 μA/cm^2, with an extracellular electric field of 0.3 mV/cm. These endogenous fields were 2 orders of magnitude lower than those used exogenously to cause lateral electrophoresis of surface macromolecules, but the authors suggested that they might be involved in polarization and lateral electrophoresis of molecules within the filopodia.

PULSED ELECTROMAGNETIC FIELDS

The first studies reporting the effects of pulsed electromagnetic fields on nerve tissue *in vitro* were those of Bawin et al. (27). Using 147 MHz, 0.8 mW/cm^2, amplitude modulated by slow sinusoidal signals, the release of bound ^{45}Ca^{2+} was increased maximally at a modulation frequency of 16 Hz. Such applied electrical gradients mimic the intrinsic extracellular electric field of the EEG of 50–100 mV/cm. Continuation of these studies (28) demonstrated that this response was unaffected by changing the calcium concentration of the bathing solution, but was inhibited by addition of H$^+$. These results indicated that these weak fields affected calcium bound to extracellular negative binding sites, and that H$^+$ competed for the same sites. There are many other studies describing PEMF effects on isolated pieces of normal tissue such as those by Wachtel (29), Wheeler (30), Bawin et al. (31), and Gundersen and Greenbaum (32).

Regeneration induced by PEMF applied to goldfish retina cultures for 24 hours was reported by Schwartz et al. (33). PEMF (perpendicular orientation of coils) exposure for 0.5 minute at 100 V, 1,000 Hz, 5 μsec and 100 V, 200 Hz, 25 μsec stimulated exaggerated process regrowth. PEMF-induced regrowth resembled that induced by brain-derived growth factors or glial cell conditioned medium. This is the only short exposure time study (0.5 min) found in the literature.

Studies in our own laboratory (34) on the effects of 72 Hz, single pulse PEMF on chick sensory ganglia indicated that threshold levels of 400 nA/cm^2 of induced current significantly enhanced neurite outgrowth. This stimulation was comparable to that obtained with DC application of 9 nA/cm^2. Using capacitor plates, Yoshioka et al. (35) reported that 27 MHz electric fields had no effect on rat sensory neurons at 1, 20, 50 and 610 mV/cm; significant stimulation was observed at 10 mV/cm. Addition of nerve growth factor to cultures treated with 10 mV/cm did not change the growth response. In 1985, continuing results from Albert's (36) laboratory indicate that 1 mV/cm electric field repeated at 7 Hz inhibited neurite outgrowth of 14–15 day rat ganglia in the presence or absence of nerve growth factor.

Field effects on non-neural cells (Schwann cells) produced by 60-KHz capacitively coupled electric fields using various voltages (2–4 days' exposure) were studied (37). A myelin marker (gal C) was preferentially retained at 250 and 375 V, but no differences

between groups were obtained in ^{3}H-thymidine uptake studies.

The effects of spatially uniform pulsed fields, focally applied DC fields, and focally applied pulsed fields on orienting neurite growth from *Xenopus* neurons was described by Patel and Poo (13). Uniform pulsed fields of an equivalent time-averaged field intensity as uniform DC fields produced the same extent of neurite orientation. Unipolar electric current pulses applied focally through a micropipette to neuritic growth cones modulated the rate of neurite growth. Negative (sink) current increased growth rates while positive (source) currents were inhibitory. The threshold current density to obtain a growth cone response within 15 minutes with focal DC was 0.2–2 pA/μm^2; a similar response was obtained with focally-applied pulsed current of 4 pA/μm^2 at 10 Hz.

IN VIVO STUDIES

Electrical recordings of transected nerve activity were first reported by DuBois-Reymond (38). A more detailed study was performed by Genell and Burr (39) using Ag-AgCl electrodes to measure potential differences on the limb surface of rabbits before and after nerve section, and in man after ulnar nerve injury. In each case a marked positive shift in potential was obtained, suggesting that such recordings could be used clinically to determine the extent of nerve injury.

EFFECTS OF ELECTRIC FEIDLS ON PERIPHERAL NERVE REGENERATION

The application of electric fields to injured nerve tissue has been a recent event. A few early studies (40,41) using DC, or AC, have been followed by a fairly large number of reports. Hoffman (41) stimulated the spinal cord of large nerve trunks (10–60 minutes with 1.5 mA at 50–100 Hz) after transection of the 5th lumbar nerve of rats. Significant acceleration in reinnervation of denervated muscle fibers was found. Bodemer (40) stimulated nerve fibers of the brachial plexus *in situ* in an attempt to increase nerve activity in an amputated stump. The electrical stimulation resulted in partial regeneration of the limbs in adult frogs (42,43) (also see S.D. Smith, this volume). A brief report was presented by Romero-Sierra et al. (44) in 1971 on the application of 27 MHz for 5–30 minutes to non-transected, desheathed sciatic nerves *in situ*. Histological investigation of such exposed nerves revealed various degrees of demyelination, Schwann cell damage and distortion of collagen fiber pattern close to the nerve. In the same conference, Yorde et al. (45) reported on the ultrastructural effects of DC on cortical synapses in biopsies of the monkey brain. DC (2.5 mA) was applied through surface electrodes for 1–2 minutes to the monkey cortex. A biopsy of this cortex was examined and synaptic vesicles counted; the depletion of vesicles after short periods of DC application was correlated with stimulation of synaptic transmission.

In 1976 Wilson (46) used a non-invasive technique to study the effects of PEMF on nerve and spinal cord regeneration. He applied a radiofrequency signal (Diapulse, 5–

120 mW/cm^2) to transected median-ulnar nerves of rats for 15 minutes/day for 30–60 days. By 30 days, PEMF-treated animals showed significant restoration of nerve conduction activity and the histological presence of large-diameter nerve fibers.

The effects of daily stimulation on reinnervation and acceleration of nerve growth after lesioning the sciatic nerve of the rat were reported by Sebille and Bondoux-Jahan (47). Using an intensity 10 times that needed to elicit muscle contraction (30 mV at 50 Hz, pulse duration of 1 msec) for 30 minutes/day via electrodes attached to the leg skin, the tow-spreading reflex was observed and evaluated. Muscle stimulation significantly increased the rate of recovery. The increased muscle contractions resulting from the applied electrical signal probably hastened the functional recovery of the end plate zone when the neurites reach the muscle membrane. A similar paradigm was used by Nix (48) after crushing the common peroneal nerve of the rabbit. Stimulation of the external digitorum longus muscle with 10–12 Hz for 8 hours/day via implanted electrodes into the muscle increased the time course of contraction and relaxation thus preventing denervation-induced slowing of muscle activity seen in controls.

In 1981, Winter et al. (49) reported that intraluminal insertion of Pt/Ag bimetallic electrodes (100 nA) stimulated regeneration of transected sciatic nerves. Regeneration was determined by analyzing the compound action potential obtained; only when the cathode was present and distally located was the DC effective. Maehlen and Nja (50) investigated the effects of electrical stimulation of pre- and postsynaptic cells on sprouting after denervation in the guinea pig superior cervical ganglion. Preganglionic stimulation on the cervical sympathetic trunk for 1 hour only immediately after denervation (100 pulses at 20 Hz every 25 seconsd) increased the number of axons innervating each ganglion cell. This effect was abolished with hemamethonium, which blocked ganglionic transmission. The findings support a mechanism whereby retrograde transsynaptic trophic effects are modulated by impulse activity. Part of the stimulus for sprouting after denervation may be enhanced by a brief period of hyperactivity induced by the electrical stimulation followed by a period of subnormal activity.

In contrast to direct stimulation of nerves via electrodes, Ito and Bassett (51) subjected the entire body of rats to pulsed electromagnetic fields after transection of the sciatic nerve. The PEMF consisted of Helmholtz aiding coils delivering a repetitive single pulse of 380 μsec positive-going, quasi-rectangular waveform repeating at 72 Hz. All rats were treated for 12 hours/day. Motor function was evaluated by plantar-flexion force produced by stimulation of the nerve proximally. Return of motor function occurred within 4 weeks after PEMF in contrast to controls at 8 weeks.

Raji and Bowden (52) tested the effects of PEMF delivered by a Diapulse machine (1 mW/cm^2) on regeneration of the transected common peroneal nerve in rats. PEMF was administered for 15 minutes daily for periods of 3 days to 8 weeks. PEMF caused significant increases in skin, deep-tissue and rectal temperatures which returned to

normal after treatment. The size of the intraneural blood vessels was increased after treatment and the amount of collagenous tissue fibrosis was reduced with PEMF. Most importantly, these studies support those of Wilson (46) on the PEMF-acceleration of regeneration and maturation of myelinated axons.

Nix and Hope (53) crushed the motor innervation to the soleus muscle in rabbits and stimulated the nerve proximal to the lesion with stainless steel electrodes sewn into a cuff placed around the nerve. Stimulation was performed for 4 weeks with a Grass S88 stimulator using rectangular pulses of 0.2 msec duration, at 4 Hz. Twitch force, tetanic tension, and muscle action potential amplitude measurements were taken pre- and post-operatively for each animal. In each case, significant differences were obtained as a function of treatment, and reinnevation was enhanced. The authors concluded that either the nerves grew faster or they established functional connections to the muscle sooner than untreated animals. They implicated the electrical stimulatory effect with maintenance of large myelinated fibers; it is well-known that increased motor activity enhances motor nerve regeneration. The slow-frequency pattern used for stimulation was chosen because the soleus muscle is a slow muscle; it could be that fast muscles were reflexively stimulated by this slow pattern.

Preliminary studies were reported on transected rat sciatic nerves treated with a PEMF clinical pulse-burst signal (15 Hz, Electrobiology, Inc.) for five days following transection (54). Assessment of regeneration indicated a faster return of neurophysiologically recorded function and more myelinated axons/mm^2 in the nerve distal to the transection than in untreated control animals.

Singer and Mehler (55) questioned whether increased 2-deoxyglucose uptake in axotomized motor neurons of the hypoglossal was associated with increased electrical activity or protein and RNA synthesis. They recorded spike activity in normal and axotomized hypoglossal nuclei, and observed 2-deoxyglucose localization radiographically in the same nuclei. Increased uptake was noted in the axotomized nucleus but no differences were ob served in numbers of action potentials. The authors conclude that increased uptake associated with axotomy was not the result of increased action potential activity, but rather correlated with synthesis of protein, RNA and lipid during regeneration.

The effects of PEMF on regeneration of the common peroneal nerve of the cat was determined using a multidisciplinary approach (56). Five days after transection, the cats were exposed to PEMF for 10 hours/day, 6 days/week for 12 weeks. Two different signals were tested: a pulse-burst signal used clinically for bone repair (15 Hz, 380 μsec positive, 20 μsec negative), and a single repetitive pulse (200 μsec positive, 6 msec negative, 72 Hz). Electrophysiologic data was collected pre- and postoperatively. Muscle biopsies were taken for fiber typing, the nerves biopsied for fiber counts, and retrograde transport of horseradish peroxidase to the motor neurons in the spinal cord were used for

assessing regenerative events. No significant differences were noted between controls and either PEMF signal in muscle-fiber diameter, numbers of fibers/mm^2, axon-fiber caliber, areas of nerve compound action potential, or muscle compound action potential. However, the numbers of motor neurons retrogradely labeled in spinal cords of cats treated with the pulse burst signal were significantly increased (96.8% more than the unoperated side). This study represents the most in-depth exploration of electrophysiological and morphological/morphometric parameters of peripheral nerve regeneration.

Pomeranz et al. (57) reported on accelerated sprouting of intact saphenous nerves after sciatic nerve transection. Electric current was applied using 1 µA DC or AC (20 Hz, 1000 µA/pulse) delivered through stainless steel electrodes placed in the skin of the digit of the hindpaw. No description of how the signals were generated was given. Only distally placed negative electrodes were effective. Roman et al. (58) applied 10 µA Dc to rat sciatic nerves transected and left with a 5-mm gap. Proximal and distal ends of the nerve were placed inside a Silastic tube; the cathode-stimulator (Pt wire) was inserted into the tube close to the distal stump. Direct current (1 µA/cm^2) was administered using a battery and variable resistor. The contents of the Silastic tube were fixed at 3 weeks and examined histologically. A portion of the DC-treated tubes was filled with blood vessels and axon bundles; the total contents were two times larger than controls. The number of myelinated axons per tube was increased by a factor of three. In another series, the tube contained the proximal stump and the cathode stimulator alone, and no distal stump was present. Only 10 µA stimulated growth of nerve bundles.

EFFECTS OF ELECTRIC FIELDS ON SPINAL CORD REGENERATION

In 1976 Wilson (46) provided preliminary evidence on the effects of PEMF via Diapulse signals (see above) on hemicordectomies of the upper lumbar segment in cats. The experimental protocol was to expose the cats for 30 minutes/day for 30 days using 50 mW/cm^2, 400 Hz. Three months after lesioning, the cords were fixed and sectioned for histology. PEMF-treated cords exhibited decreased scarring and regenerating neurites traversing the lesioned area.

Cohen and collaborators (59) investigated the effects of applied DC (10 µA) on the regeneration rate of lamprey spinal cord neurons. Wick electrodes were placed distally to the lesion and current was delivered for 5 days. Axonal die-back was significantly correlated with the direction of the applied current; die-back of axons was increased with the anode and decreased (promotion of regeneration) with the cathode. The authors proposed that die-back was associated with entry of cations (primarily calcium ions) into the transected stump, and that the DC interacted with endogenous currents so that increasing or decreasing cation flow resulted in enhancement or reduction of axonal die-back, respectively.

A contusion-injured cat model has been used extensively by W. Young and his collaborators in studies on spinal cord injury. Application of a Diapulse signal (65 µsec pulses, 27.12 MHz, average power of 100 mW/cm^2) for 1 hour daily beginning 4 hours post-injury demonstrated beneficial effect on preservation and maintenance of function (60). Short-term analyses of PEMF effects (3 hours post-9injury for 2 hours) on ionic changes showed that it had no effect on sodium, potassium, or water content of the contused area, but that it significantly decreased calcium accumulation in the cord (61,62). These results support the findings of beneficial effects of PEMF on restoration of peripheral nerve function cited above, and they provide another example of electric field action on decreasing calcium entry into injured spinal cord.

Functional electrostimulation (FES) has come of age following the original design of the pacemaker. Different types of FES are now in use or in different stages of development for treating various clinical states. FES has been used to alleviate pain, induce artificial respiration (diaphragm pacing), cause contraction (continence restoration) or evacuation (mictuation reflex) of the bladder, and inhibit muscle atrophy and improve motor function.

THEORIES ON THE MECAHNISM OF ACTION OF ELECTRIC FIELDS ON NERVE REGENERATION

The following discussion on mechanisms of action of imposed electric fields strongly implicates the role of calcium ions, and models originally proposed by Pilla (63), Jaffe et al. (64), and Bawin et al. (27) have addressed this point. In 1974, Pilla proposed a theoretical model to explain electrical effects on tissues and cells based on charges at the interface of the cell membrane and intra- and extracellular fluids. Strong electric fields exist at this site and energies of the order of 1 mW are capable of perturbing the interfacial structure. He suggested that alterations of a few millivolts could result in gross alterations of specifically adsorbed or bound species resulting in electrochemical information transfer. He demonstrated that DC fields of 0.01–100 µV/cm produced morphological changes in RBC, and implicated the slower migrating calcium ions in this phenomenon. This hypothesis was reinforced when addition of calcium alone to these cells induced similar morphological changes. At the same conference, Jaffe et al. (64) proposed an electrical hypothesis for localized growth, suggesting that the plasma membrane of a growth region becomes relatively leaky to cations such as calcium, magnesium, sodium and hydrogen which exist extracellularly at higher concentrations. As the cations enter the growth point of the cell, an electric current and field are generated which pull more negative cytoplasmic substances and vesicles (which form new membrane) toward the leaky area, thus generating more leaky membrane. This "positive feed-back loop" would increase the probability of localized growth and membrane expansions. This hypothesis was tested by measuring electric currents around

developing fucoid eggs, finding growth points of current entry which contain calcium and sodium components. Application of fields of 200 mV/egg to naïve eggs induced blister "fertilization-like" vesicle formation at the positive side of the egg.

Bawin et al. (27) implicated a specific class of calcium sites at the extracellular neuronal membrane that were responsive to low-frequency electric fields, and postulated that they play a role in regulating cell excitability. They found that RF fields modulated at brain wave-like frequencies increased calcium efflux from Ca^{45}-loaded cerebral cortex, and that efflux was enhanced in the presence of additional H^+ ions.

Other interpretations of bioelectric effects of external electric fields have been reported, and are reviewed in the following section.

DC LEVELS OF 100µV/cm AND BELOW

As a consequence of the work of Becker and Murray (16) and Pilla (63) who described morphological changes in red blood cells using nanoampere currents, we began our studies on assessing the effects of the se minute fields on cultured neurons (14). The stimulation of neuritic growth, which was cathodally oriented, was postulated to be correlated with changes of calcium ions at the boundary of the cell membrane. Studies on the growth cones of cultures subjected to DC (65) indicated that thy were enlarged. Application of DC (~ 10 nA/cm^2) to trigeminal neurons significantly increased calcium efflux of preloaded ganglia relative to control or nerve growth factor-treated ganglia. Our hypothesis was that the DC acted by decreasing calcium entry into cells by enhancing calcium binding to the external membranous pool.

We tested the influence of calcium ions on growth processes by blocking calcium entry with lanthanum chloride or the drug Verapamil, or increasing calcium entry with added calcium chloride or the calcium ionophore A23187 which opens calcium channels allowing intracellular calcium concentration to increase. These studies indicated that long-term application (6 days) of compounds which inhibited calcium influx (lanthanum, Verapamil) increased neurite formation, while those that stimulated calcium influx (added calcium or the ionophore A23187) inhibited neurite formation (24). These results are consistent with those of Bray et al. (66), who found that low extracellular calcium induced the formation of growth cones all along the length of cultured sensory neurons. It appears very probable that long exposures to minute levels of DC mimic the drug-induced reduction of calcium entry into the neurons; both result in stimulation of nerve process growth.

The role of cations in neuronal growth and differentiation is slowly becoming clear. The regulation of sodium and potassium levels, and of sodium and potassium pump activities by nerve growth factor (NGF) in early stages of neuronal regeneration in culture has been characterized (67). Micromolar levels of calcium are required for growth-cone formation (the growth end of neurites), and mobility (actin network), but

they are not required for neurite formation and (microtubule) stability.

An increase in the number of calcium channels has been correlated with active neurite outgrowth as neurons differentiate (8,68). This calcium requirement is reduced as a function of time in culture. Although the precise role of calcium in neurite formation is not known, Hammerschlag (69) postulated that amino acid uptake and protein transport are dependent upon calcium entry through the cell membrane, and Fukada and Kameyama (68) suggested that increased calcium is needed for protein synthesis for neurite membranous growth. In an elegant study, Anglister et al. (70) detected calcium action potentials in neuroblastoma cells, and found that voltage-activated calcium channels were less abundant in neurite processes, and more abundant in growth cones. Upon depolarization with excess potassium or electrical stimulation, the area of growth cones increased by 20–120% associated with increased neurite outgrowth. This expansion in growth cone area was inhibited with cadmium (calcium blocker) or in low-calcium media. The authors suggested that calcium ion entry functions as a trigger for neurite elongation.

Freeman et al. (8) employed a novel circularly vibrating probe (capable of measuring current densities of 5 nA/cm^2) to measure the direction and magnitude of endogenous current at the growth cone. Steady or slowly varying (not pulsatile) currents in the range of 10–100 nA/cm^2 entered the filopodia tip of the growth cone at the end of a neurite, and flowed down it and back out toward the base. The current appeared to be carried primarily by calcium ions, and was not derived from growth-cone motion or flow of medium over the surface of the plasma membrane. Average applied fields of 70 mV/cm were necessary to orient the growth cones to the cathode; the magnitude of the applied fields was 2 orders larger than those found endogenously. The authors concluded that he endogenous currents were too small to cause lateral electrophoresis of surface molecules, but may be the result of localization of clusters of voltage-sensitive calcium channels at the tips of growth cones. These calcium channel proteins are brought to the growing nerve tips by active transport and are incorporated into newly formed, expanding membrane, and sodium channels arrive later due to a "slower rate of lateral diffusion in the plasmalemma." Expanded growth of the plasma membrane that occurs by fusion of intracytoplasmic vesicles to the membrane requires calcium, some of which is current-generated. Other functions assigned to calcium ions include alignment of actin and myosin in the growth cone, and subsequent calcium-dependent contractile movements. Small changes in the membrane potential resulting from external electric field application affect calcium-ion entry. Depolarization on the side of the cell facing the cathode would increase calcium entry, while that facing the node would inhibit entry. Excess calcium entering the growth cone not used for membrane expansion would be extruded as exocytosis of intracytoplasmic vesicles occurs, thereby maintaining calcium homeostasis.

The paradigm presented by Freeman et al. (8) is provocative. The sequence of

processes suggested to occur at the level of the neuronal growth cone could be applied to other tissues and cells, and should be considered by other workers in this area. In this context, a hypothesis to explain stimulation of regeneration as a function of minute levels of direct current ($\sim$10 nA/cm^2) as studied in our own laboratory incorporates some of this thinking. The fields applied to neuronal (sensory/motor) explants are so small that membrane depolarization is perhaps in the order of 1 mV or less. Attempts to measure small changes have yielded equivocal results (Sisken and Ringham, unpublished results). However, the levels of DC used in our system ($\sim$10 nA/cm^2) is equivalent to that measured by freeman et al. (71) (10–100 nA/cm^2) flowing into the tips of growth cones of retinal neurons. Adding any additional current to that present endogenously would increase calcium entry in growth cones facing the cathode; the cascade of events postulated by Jaffe (64) and Freeman et al. (8) would ensue, resulting in growth cone expansion. Such expansions with microspikes have been observed (65) and are demonstrated in Figure 1. Orientation of the newly formed growth cones to the cathodes by motile (calcium-dependent actomyosin system) growth cones would continue with ensuing lengthening of the neurites; such activity is normally suppressed as the neurons mature (72). Calcium entry would also be instrumental in stimulating amino-acid uptake and protein synthesis (43,65) needed for membrane proteins and microtubule formation. Exposure to DC over hours would be manifest by increased neurite outgrowth. As the neurons differentiate in culture, calcium channels are replaced by sodium channels (68,70) thereby reducing calcium entry. Continued application of minute levels of DC may act to increase the adhesion of non-entering calcium to the external membrane (18). Reduction of excess calcium entry also serves to stabilize the microtubules in the neurites. The foregoing activities require that the parent neuronal cell body be synthesizing relatively large amounts of protein to enable the processes to form and elongate; neurites of 100–150 nm have been observed in our DC-treated cultures. We have found that the protein content in such explants are significantly larger than control explants, and that they approach levels of NGF-treated sister cultures (unpublished observations).

The paradoxical role of DC may be related to the presence of calcium channels (8,71). DC increases calcium intracellularly by adding to preexisting endogenous (calcium) currents. When sodium channels replace calcium channels, calcium entry is regulated precisely by transport processes and depolarization phenomena. Addition of nanoampere levels of DC to relatively more mature neurons does not increase calcium entry, rather it fosters binding of the calcium to the negative sites on the external membrane.

DC LEVELS OF 10 mV/cm TO 1.5 V/cm

The agar wedge system employed by Marsh and Beams (7) was modified by Jaffe and Poo (11) to apply fields of $\sim$100 mV/mm. this model has been used by a number of

investigators (see earlier section) who have examined the role of fields up to 1.5 V/cm on neurite growth and orientation. Jaffe and Poo (11) suggested that such fields caused lateral electrophoresis of nerve growth factor receptors along the plasma membrane. Hinkle et al. (10), however, favored the hypothesis that the imposed electric fields acted on the interior of the growth cone since the voltage drop across the microspikes of the growth cone was of the same magnitude (~0.7 mV/cell) as that found to orient neurites. The mechanism of action of these fields on neurite growth orientation most likely does not involve field-induced mechanical effects or chemical gradients (12), since constant perfusion with fresh medium did not change the observed effects. They proposed three mechanisms to account for oriented neurite growth: (1) an electric field-induced potential change causing redistribution of cytoplasmic material; (2) alteration of membrane potential asymmetry inducing preferential growth; and (3) an electrophoretic redistribution of charged surface molecules in the plasma membrane. Data obtained from their experiments favored the third hypothesis since field strengths used for neurite growth caused fluorescently bound ConA receptors to accumulate on the cathodal side of the neuron, and inhibition of receptor migration abolished the orientation effect.

The electromigration model (73) proposes receptor (acethylcholine, Ach) migration to the synaptic region. Transient fields of 1 V/cm are generated normally by synaptic currents; such fields applied exogenously cause electrophoretic migration of Ach receptors to synaptic contact areas. The proposal offered by these investigators states that "transient electric fields associated with neuronal activities serve to develop, maintain and modulate the topography of the membrane components responsible for these activities."

Finally, the role played by calcium ions in altering locomotor activity after imposition of 0.5–15 V/cm electric fields action was addressed by Cooper and Schliwa (72). Migration of keratocytes to the cathode was observed in these fields, and it was inhibited with calcium channel antagonists. Their hypothesis for cathodal migration requires asymmetric calcium entry into the cell through specific calcium channels to trigger contractile activity and direct orientated cell movement. In addition, they discuss the contradictory role played by calcium, specifically in neuronal systems. Anodal neurite retraction, observed by Patel and Poo (12) in field strengths of 5 V/cm hyperpolarized the neuron resulting in calcium entry and its subsequent axoplasmic filament degradation. However, depolarization at the cathode also increased calcium entry, with consequent increased growth-cone formation and neuronal survival. They did not resolve this discrepancy; it may finally rest on the absolute concentration of intracellular calcium levels which is as dependent upon calcium extrusion as it is on calcium entry.

PULSED ELECTROMAGNETIC FIELDS

The action of various pulsed fields on nerve tissue has been explored and the

association of these effects with calcium changes has been implicated. Efflux studies of calcium after application of low frequency extracellular fields (1–300 Hz) (28,74,75) demonstrated a correlation of enhanced efflux with specific intensity ranges. The amplitude range and frequency of these fields are comparable to those of extracellular brain waves. The hypothesis offered by Bawin et al. (28) proposed that specific negative sites on the plasma membrane were occupied by calcium, and were affected by applied ELF.

Comparative studies of DC and PEMF on promotion of neurite growth (19) correlated stimulatory effects with current density ($0.7\ \mu A/cm^2$) and with orientation of the Helmholtz coils. Only coils oriented so that the magnetic field was parallel to bottom of the dish produced a significant response. Effective levels of DC and PEMF were equivalent (10^{-3} coulombs) on a time exposure basis. Mechanisms associated with the PEMF fields may also involve calcium ion changes (as discussed previously for DC effects) since the effective dosage of both systems is in the same range.

Thresholds of 3–30 mV/cm, DC, or $4\ pA/\mu m2$, pulsed fields, applied focally to influence the direction of neurite growth were found to be similar to those associated with action potentials, and synaptic activity. Patel and Poo (13) suggest that the action of these fields is not to generally guide nerve growth in developing systems, but to modulate localized neurite orientation to specific areas of intense activity. In another study, square-wave pulses comparable to local extracellular AC fields produced by nerve action potentials (100 Hz) (76) redistributed ConA receptors to the cathodal area of cultured myoblasts. These findings add support to the electromigration model proposed by Fraser and Poo (73) that electrical events associated with nerve activity can modulate neuronal topography.

FUTURE STUDIES

In view of the rapid technological advances and the increasing numbers of workers in the area of bioeffects of externally-applied electrical fields, it is anticipated that more clinically-useful procedures will be developed to address nerve regeneration problems. Hopefully these methods will encompass stimulation of regeneration in the spinal cord as well.

Basic studies to determine the mechanism of action of these fields and how such imposed fields relate to normal activity and to activity following degeneration and regeneration should be conducted in parallel with the clinical investigations. It is hoped that advances made in the area of soft tissue regeneration in general, and nerve tissue specifically, will duplicate or exceed that found in the electrical treatment of bone repair.

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REFERENCES

1.   Kao, C., Bunge, R. and Reier, P., Eds. Spinal Cord Reconstruction. New York: Raven Press, 1983.

2.   Seil, F. Nerve, Organ and Tissue Regeneration: Research Perspectives. New York: Academic Press, 1983.

3.   Harrison, R.G. Observations of the living developing nerve fiber. Anat. Rec. **1**:116-128, 1907.

4.   Ingvar, S. Reaction of cells to the galvanic current in tissue cultures. Proc. Soc. Exp. Biol. Med. **17**:198-199, 1920.

5.   Karssen, A. and Sager, B. Sur l'influence de courant electrique sur la croissance des neuroblasts *in vitro*. Arch. Exp. Zellorsch **16**:255, 1934.

6.   Weiss, P. *In vitro* experiments on the factors determining the course of the outgrowing nerve fiber. J. Exp. Zool. **68**:393-448, 1934.

7.   Marsh, G. and Beams, H.W. In vitro control of growing chick nerve fibers by applied electric currents. J. Cell Physiol. **27**:139-157, 1946.

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

9.   Freeman, J.A., Weiss, J.M., Snipes, G.J., Mayes, B. and Norden, J.J. Growth cones of goldfish retinal neurites generate DC currents and orient in an electric field. Soc. Neurosci. Abstr. **7**:550, 1981.

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

11.   Jaffe, L. and Poo, M.M. Neurites grow faster toward the cathode than the anode in a steady field. J. Exp. Zool. **209**:115-128, 1979.

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

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

14.   Sisken, B. and Smith, S.D. The effects of minute direct electrical currents on cultured chick embryo trigeminal ganglia. J. Embryol. Exp. Morphol. **33**:29-41, 1975.

15.   Sisken, B.F. *In vitro* stimulation of central nervous system regeneration by direct current and PEMF. Annual Meeting of the Bioelectrical Repair and Growth Society. Boston, MA, 1985.

16.   Becker, R.O. and Murray, D.G. The electrical control system regulating fracture healing in amphibians. Clin. Orthop. **73**:169-198, 1970.

17.   Pilla, A.A. Electrochemical information transfer at living cell membranes. Ann. N.Y. Acad. Sci. **238**:149-169, 1974.

18.   Sisken, B.F., Lafferty, J.F. and Acree, D. The effects of direct and inductively coupled current and nerve growth factor on nerve regeneration *in vitro*. in Becker, R., Ed., Mechanisms of Growth Control and their Clinical Applications. Springfield: Charles Thomas, 1981.

19.   Sisken, B.F., McLeod, B. and Pilla, A.A. PEMF, direct current and neuronal regeneration: effect of field geometry and current density. J. Bioelectricity **3**:81-102, 1984.

20.   Sisken, B.F., Roberts, E. and Goetz, I. Triethanolamine, tris, hepes, and cytosine arabinoside show neuritogenic activity in cultured chick embryo ganglia. Exp. Neurol. **88**:27-43, 1985.

21.   Sisken, B.F., Sechaud, P. and Barr, E. Correlation of current delivered versus potential differences imposed on trigeminal ganglia *in vitro*. J. Electrochem. Soc. *129*:579, 1982.

22.   Sisken, B.F., Estes, R.S. and Kryscio, R. The effects of cytosine arabinoside on nerve regeneration, protein content and protein synthesis in NGF or electricity-treated sensory ganglia *in vitro*. Bioelectrical Repair and Growth Society, **3**: 17, 1983.

23.   Sisken, B.F., Barr, E. and Estes, R.S. Electrical effects on nerve ganglia *in vitro*. in Hunt, T.K., Heppenstall, R.B., Pines, E. and Rovey, D., Eds., Soft and Hard Tissue Repair: Biological and Clinical Aspects. New York: Praeger Scientific, 1984.

24.   Sisken, B.F. and Sisken, J.E. Ionic and direct current effects on neurite differentiation in primary cultures of chick sensory ganglia *in vitro*. Bioelectrical Repair and Growth Society, **4**: 22, 1984.

25.   Sisken, B.F. and Estes, R.S. The effects of direct current on neurite growth in N18 neuroblastoma cells treated with Ara C. Trans. Bioelectrical Repair and Growth Society **4**:12, 1984.

26.   Khan, T. and Gaik, G. Preliminary studies of spinal cord explants cultured on carbon filaments and stimulated with direct current. Bioelectrical Repair and Growth Society, **4**: 23, 1984.

27.   Bawin, S.M., Kaczmarek, L.K. and Adey, W.R. Effects of modulated VHF fields on the central nervous system. Ann. N.Y. Acad. Sci. **247**:74-81, 1975.

28.   Bawin, S.M., Adey, W.R. and Sabbot, I.M. Ionic factors in release of $^{45}Ca^{2+}$ from chicken cerebral tissue by electromagnetic fields. Proc. Natl. Acad. Sci. USA **75**:6314-6318, 1978.

29.   Wachtel, H. Firing-pattern changes and transmembrane currents produced by extremely low frequency fields in pacemaker neurons. in Phillips, R., Gullis, M., Kaune, W. and Mahlum, D., Eds., Biological Effects of Extremely Low Frequency Electromagnetic Fields. U.S. Dept. of Energy, 1979, pp. 123-146.

30.   Wheeler, D.D. Effects of polarizing currents and repetitive stimulation on the uptake of amino acids by peripheral nerve. J. Neurosci. Res. **4**:123-131, 1979.

31.   Bawin, S.M., Sheppard, A.R. and Mahoney, M.D. Effect of electric field orientation and strenght on neuronal excitatability in the rat hippocampal slice. 6th Annual Meeting of the Bioelectromagnetics Society, 1984.

32.   Gundersen, R. and Greenbaum, B. The effects of ELF magnetic fields on synaptic function. 7th Annual Meeting of the Bioelectromagnetics Society, 1985.

33.   Schwartz, M., Korenstein, R., Mizarchi, Y., Newman, D. and Kimhi, Y. Neuritic outgrowth from regenerating goldfish retina can be modulated by external factors. Birth Defects **19**:451-456, 1983.

34.   Sisken, B.F., McLeod, B. and Pilla, A.A. PEMF and neuronal regeneration: effect of field geometry. in Pilla, A.A. and Boynton, A.L., Eds., Electrochemistry, Membranes, Cells and the Electrochemical Modulation of Cell and Tissue Function. New York: Springer Verlag, 1984.

35.   Yoshioka, A., Cao, H. and Albert, E. Effects of 27 MHz electric fields on rat DRG neurite growth *in vitro*. 6th Annual Meeting of the Bioelectromagnetics Society, 1984.

36.   Albert, E.N., Yoshioka, A., Cao, H. and Imam, F. Modulation of fetal rat DRG neurite growth by pulsing electromagnetic fields (PEMFs). 7th Annual Meeting of the Bioelectromagnetics Society, 1985.

37.   Culp, R., Osterman, A., Brighton, C., Bora, F., Kreider, B. and Pleasure, D. *In vitro* response of the Schwann cell to various capacitively coupled electrical fields. Bioelectrical Repair and Growth Society **4**:24, 1984.

38.   Dubois-Reymond, E. Vorlaufiger abrifs einer untersuchung uber den sogenannten froschstrom und uber die electromotorischen fische. Ann. Phys. U. Chem. **58**:1, 1843.

39.   Grenell, R.G. and Burr, H.S. Surface potentials and peripheral nerve injury: a clinical test. Yale J. Biol. Med. **18**:517-525, 1946.

40.   Bodemer, C.W. Evocation of regrowth phenomena in anuran limbs by electrical stimulation of the nerve supply. Anat. Rec. **148**:441-457, 1964.

41.   Hoffman, H. Acceleration and retardation of the process of axon-sprouting in partially denervated muscles. J. Biol. Med. Sci. **30**:541-566, 1952.

42.   Borgens, R., Vanable, J. and Jaffe, L. Bioelectricity and regeneration. I. Initiation of frog limb regeneration by minute currents. J. Exp. Zool. **200**:403-416, 1977.

43.   Sisken, B.F., Estes, S., Barr, E. and Kryscio, R. Morphological and biochemical effects of NGF or applied electric fields in the presence or absence of non-neuronal cells of chick dorsal root ganglia. Soc. Neurosci. **9**:5, 1983.

44.   Romero-Sierra, C., Halter, S. and Tanner, J.A. Effect of an electromagnetic field on the sciatic nerve of the rat. in Wulfsohn, N.L. and Sances Jr., A., Eds., The Nervous System and Electric Currents. New York: Plenum Press, 1971, pp. 81-84.

45.   Yorde, D.E., Siegesmund, K.A., Sances, A. and Larson, S.J. The effects of DC current on synaptic endings. in Wulfson, N.L. and Sances, A., Eds., The Nervous System and Electric Currents. New York: Plenum Press, 1971.

46.   Wilson, D.H. and Jagadeesh, P. Experimental regeneration in peripheral nerves and the spinal cord in laboratory animals exposed to a pulsed electromagnetic field. Paraplegia **14**:12-20, 1976.

47. Sebille, A. and Boundoux-Jahan, M. Effects of electric stimulation and previous nerve injury on motor function recovery in rats. Brain Res. **193**:562-565, 1980.

48. Nix, W.A. The effect of low-frequency electrical stimulation on the denervated extensor digitorum longus muscle of the rabbit. Acta Neurol. Scand. **66**:521-528, 1982.

49. Winter, W., Schnutt, R., Siskenm, B.F. and Smith, S.D. Effects of low levels of direct current on peripheral nerve regeneration. 27th Annual Orthopaedic Research Society Meeting. Las Vegas, 1981.

50. Maehlan, J. and Nja, A. The effects of electrical stimulation on sprouting after partial denervation of guinea-pig sympathetic ganglion cells. J. Physiol. **322**:151-166, 1982.

51. Ito, H. and Bassett, C.A.L. Effect of weak, pulsing electromagnetic fields on neural regeneration in rat. Clin. Orthop. **181**:283-290, 1983.

52. Raji, A.R.M. and Bowden, R.E.M. Effects of high-peak pulsed electromagnetic field on the degeneration and regeneration of the common perineal nerve in rats. J. Bone Joint Surg. **65**:478-492, 1983.

53. Nix, W.A. and Hope, H.C. Electrical stimulation of regenerating nerve and its effect on motor recovery. Brain Res. **272**:21-25, 1983.

54. Parker, B., Bryant, C., Apesos, J., Sisken, B.F. and Nickell, T. The effects of pulsed electromagnetic fields (PEMF) on rat sciatic nerve regeneration. Bioelectrical Repair and Growth Society **3**:19, 1983.

55. Singer, P. and Mehler, S. Role of electrical activity in axotomy-induced increased glucose use. Exp. Neurol. **80**:601-612, 1983.

56. Orgel, M.G., O'Brien, W.J. and Murray, H.M. Pulsing electromagnetic field therapy in nerve regeneration: an experimental study in the cat. Plast. Reconstr. Surg. **73**:173-183, 1984.

57. Pomeranz, B., Mullen, M. and Markus, H. Effect of applied electrical fields on sprouting of intact saphenous nerve in adult rat. Brain Res. **303**:331-336, 1984.

58. Roman, G.C., Rowley, B.A., Strahlendorf, H.K. and Coates, P.W. Electric low-intensity direct currents stimulate sciatic nerve regeneration. Neurology **36**:80, 1986.

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

60. Gruner, J.A. and Young, W. An objective test of descending motor function to study the efficacy of pulsed electromagnetic fields and ganglioside treatments of spinal injury. American Association of Neurological Surgeons. Atlanta, GA, 1985.

61. Young, W. Pulsed electromagnetic fields alter calcium in spinal cord injury. Central Nervous System Trauma **1**:100, 1984.

62. Young, W., DeCrescito, V., Flamm, E.S. and Ransohoff, J. Pulsed electromagnetic fields (PEMF) and methylprednisolone (MP) alter calcium and functional recovery in spinal cord injury. Annual Meeting of the American Association of Neurological Surgeons: 127-128, 1985.

63. Pilla, A.A. Mechanisms of electrochemical phenomenon in tissue repair and growth. Bioelectrochem. Bioenerg. **1**:227-243, 1974.

64.   Jaffe, L.F., Robinson, K.R. and Nuccitelli, R. Local cation entry and self-electrophoresis as an intracellular localization mechanism. Ann. N.Y. Acad. Sci. **238**:372-389, 1974.

65.   Sisken, B.F. and Lafferty, J.F. Comparison of the effects of minute levels of direct current and nerve growth factor on embryonic sensory ganglia *in vitro*. Bioelectrochem. Bioenerg. **5**:459-472, 1978.

66.   Bray, D., Thomas, C. and Shaw, G. Growth cone formation in cultures of sensory neurons. Proc. Natl. Acad. Sci. USA **75**:5226-5229, 1978.

67.   Skaper, S.D. and Varon, S. Nerve growth factor influences potassium movements in chick embryo dorsal root ganglionic cells. Exp. Cell Res. **131**:353-361, 1981.

68.   Fukada, J. and Kameyama, M. Enhancement of Ca spikes in nerve cells of adult mammals during neurite growth in tissue culture. Nature **279**:546-548, 1979.

69.   Hammerschlag, R., Bakhit, C. and Chu, A. Role of calcium in the initiation of fast axonal transport of protein: effects of divalent cations. J. Neurobiol. **8**:439-451, 1977.

70.   Anglister, L., Farber, I.C., Sharar, A. and Grinvald, A. Localization of voltage-sensitive calcium channels along developing neurites: their possible role in regulating neurite elongation. Develop. Biol. **94**:351-365, 1982.

71.   Freeman, J.A., Mayes, B., Snipes, G.J. and Wikswo Jr., J.P. Real-time measurements of minute neuronal currents with a circularly vibrating microprobe. Soc. Neurosci. Abstr. **12**:302, 1982.

72.   Cooper, M.S. and Schliwa, M. Electrical and ionic controls of tissue cell locomotion in DC electric fields. J. Neurosci. **13**:223-244, 1985.

73.   Fraser, S.E. and Poo, M. Development, maintenance, and modulation of patterned membrane topography: models based on the acetylcholine receptor. Curr. Top. Dev. Biol. **17**:77-100, 1982.

74.   Bawin, S.M. and Adey, W.R. Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency. Proc. Natl. Acad. Sci. USA **73**:1999-2003, 1976.

75.   Blackman, C.F., Benane, S.G., Kinney, L.S., Joines, W.T. and House, D.E. Effects of ELF fields on calcium ion efflux from brain tissue *in vitro*. Brain Res. **92**:510-520, 1982.

76.   Lin-Liu, S. and Adey, W.R. Migration of cell surface concanavalin A receptors in pulsed electric fields. Biophys. J. **45**:1211-1218, 1984.