

REVIEW ARTICLE

ELECTRICAL FIELDS, NERVE GROWTH AND NERVE REGENERATION

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INTRODUCTION

This review will examine the proposal that electric fields play a role in controlling aspects of the development and regeneration of the nervous system. Such a proposal was considered seriously in the early part of this century. A revival of these ideas has occurred in the last decade based, in large part, on evidence that endogenous electrical fields exist within developing and regenerating tissues and that neurones respond to electric fields imposed *in vivo* or *in vitro*. For a historical background to the controversy over whether small applied DC fields have any effects on nerve growth, see McCaig (1988). We shall outline only the most salient events of this period.

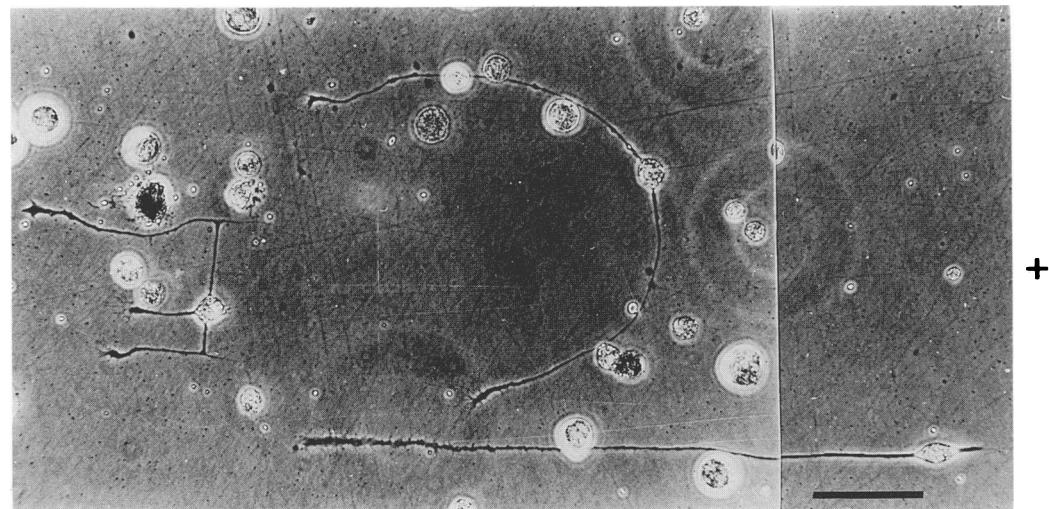


Fig. 1. Cathodotropic responses of *Xenopus* neural tube cells *in vitro*. The electrical field (170 mV/mm) is parallel to the long margins of the photograph, with the cathode to the left and the anode to the right. Three types of responses are evident; sharp, right-angle turns towards the cathode, gradual turns towards the cathode and differential growth, in which cathodal-facing neurites are longer than anodal-facing ones. Scale bar represents 100 μ m (Hinkle, McCaig & Robinson, 1981; Plate 1E).

CULTURED NERVES IN AN APPLIED ELECTRIC FIELD

Effects on nerve orientation

The earliest experiments to test whether an applied electric field could influence nerve growth were made in R. G. Harrison's laboratory shortly after he had invented the tissue culture method. The growth of fibres from explanted chick central nervous tissue was 'along the lines of force in the galvanic field'. Moreover, the cell processes growing towards the anode were morphologically different from those growing towards the cathode (Ingvar, 1920). The veiled inference was that these might represent axons and dendrites. The impact of this work was limited, however, since it only ever appeared as an abstract, without quantification or diagrammatic support. There followed several successes and failures to repeat these observations. The most rigorous of these was work on medullary explants from 7–10 day chick embryos, which showed that processes grew selectively towards the cathode, but were suppressed on the anodal side of the explant (Marsh & Beams, 1946). The threshold levels for these effects were 64 and 53 mV/mm, respectively. Marsh and Beams showed additionally that fibres which emerge at an angle to the current axis displayed a remarkable tropic behaviour, turning in 'more or less continuous curves to grow towards the cathode'. Though this left no doubt that nerves responded tropically in an electric field, it remained possible that nerve guidance was an indirect consequence of field application. The most obvious possibilities which had not been excluded by appropriate controls were: (1) that electrophoresis of charged macromolecules or pH changes at the electrodes might establish a chemical gradient within the culture medium, (2) that the electric field could organize molecular elements of the substrate into physical pathways capable of guiding nerve growth, (3) that a particular pattern of oriented growth could arise from physical translocation of the cell soma (by electrophoresis or by electrically induced fluid flow) rather than field-directed extension of the growth cone. These issues were resolved in more recent experiments, resulting in a revival of interest in galvanotropism.

Neurites from explanted dorsal root ganglia (7–9 day chick embryos) grew about three times faster towards the cathode than the anode. The effect was not seen below a threshold steady field strength of 70 mV/mm (Jaffe & Poo, 1979). Interestingly, no tropic effect of neurites turning to grow to either pole was reported. In general, only very small cathodal displacements of the ganglia relative to the substrate occurred ($\sim 50 \mu\text{m}$), thus eliminating the possibility that field-induced gross movements of the tissue mass might have been responsible for the differential lengths of anodal- and cathodal-facing neurites.

The first studies of single dissociated neurones cultured in a steady applied electric field were of *Xenopus* neural tube cells and indicated striking effects on orientation (Robinson & McCaig, 1980; Hinkle, McCaig & Robinson, 1981). Neurites growing roughly parallel to the electric field grew preferentially towards the cathode (Fig. 1). Since the field did not affect the point of origin of processes from the cell soma, many neurites sprouted perpendicular to the field and subsequently turned to grow towards the cathode (Fig. 1). Both gradual curves and sharp turns were evident at field strengths as low as 7 mV/mm, which corresponds to an external voltage gradient of less than 0.5 mV across a 50 μm diameter growth cone. Differential growth and turning responses were unaltered when culture medium was perfused slowly perpendicular to the electric field vector, such that complete exchange occurred every 10 min (Hinkle *et al.* 1981). This work was confirmed and extended by a contemporaneous study also using dissociated *Xenopus* neural tube cells (Patel & Poo, 1982).

In addition to cathodal tropic behaviour, the applied field increased the number of cells that sprouted processes, and accelerated growth towards the cathode, whilst growth towards the anode was slowed down. An important first step in understanding the mechanisms underlying galvanotropism also was made and will be discussed below (see *Possible mechanisms of orientation*). Patel & Poo (1982) and, more recently Rajnicek, Cork & Robinson (1991) suggest that the electric field may determine the sites on the cell somas from which neurites sprout; more neurites appearing cathodally. This interpretation is complicated by the dynamic behaviour of nerves in an applied field. Complete retraction and loss of neurites occurs within as little as 2–4 h and is 6–8 times more common in neurites facing the anode than in those facing the cathode (McCaig, 1986, 1987). Thus, whilst analyses of cultures after 6–20 h in an electric field certainly show more outgrowths from the cathodal sides of cells, (a phenomenon which could be physiologically significant), this picture probably arises from selective and polarized retraction events, rather than from a polarization of growth initiation sites. Indeed, when the sites of earliest outgrowths were assessed, before much anodal retraction had occurred, an equal number of processes had sprouted from cathodal- and anodal-facing membranes (Hinkle *et al.* 1981).

Whilst developing and regenerating nerves grow in the presence of steady DC electric fields (see below), pulsed and focal electric fields also impinge on growing nerves within a compact, electrically active nervous system. In a uniform pulsed field, the extent of neurite orientation towards the cathode was similar to that produced by a uniform DC field of equivalent time-averaged field intensity, with a threshold of 125 mV/mm (Patel & Poo, 1984). A current-passing micropipette placed close to a growth cone was used to induce a focal electric field. Again, cathodotropic growth occurred, while growth cones turned away from a current source (positive polarity). Threshold DC field values of 0.3–3 mV/mm at the growth cone were sufficient to induce orientation (Fig. 2). However, not all the growth cones responded to focally applied fields. Since the *Xenopus* neural tube preparation contains a heterogeneous population of neurones, this raises the important possibility that different neuronal types might display differing sensitivities in an electric field. We have

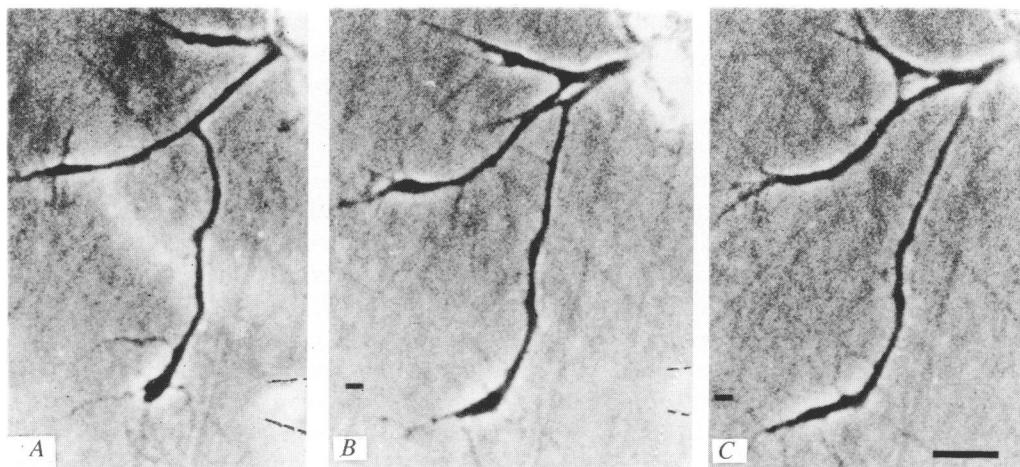


Fig. 2. Cathodal turning induced by a focal DC electrical field. *A*, *Xenopus* neurite growing alongside a microelectrode at right (position of microelectrode shown by dashed lines); 45 min in 900 mV/mm (+3 μ A). *B*, the same neurite after 65 min in 900 mV/mm. The neurite has turned to grow towards the cathode at left. *C*, 75 min in 900 mV/mm. Continued cathodal orientation is evident. Scale bar represents 15 μ m. Reprinted with permission of the Company of Biologists Limited.

pointed out already that nerves from frog neural tube and chick medulla turn tropically towards a cathode, whilst sensory neurones from chick dorsal root ganglia showed no signs of turning behaviour. Perhaps motoneurones respond tropically to an electrical field and sensory neurones do not. Chick motoneurones stain more intensely for the neural cell adhesion molecule (NCAM) than sensory nerves, they elaborate a more complex growth cone in the plexus 'decision' region, during initial growth into chick hindlimb than sensory nerves, and in the absence of motor nerves, sensory nerves fail to project down muscle nerves in chick limb (Tosney & Landmesser, 1985; Tosney, Wanatabe, Landmesser & Rutishauser, 1986; Landmesser & Honig, 1986). Thus, there are precedents for sensory and motor nerves responding differently to the same set of guidance cues *in vivo*.

Possible mechanisms of orientation. A phenomenological description of nerve growth in a small electric field has outstripped the emergence of mechanistic explanations, to the extent that even the mechanism underlying cathodal orientation remains unknown. One effect of an applied field is a 50% increase in filopodia on growth cones, together with an induced asymmetry; twice as many filopodia project cathodally as project anodally (McCaig, 1986). A major function of filopodia is the transduction of tension (Bray, 1979) into forward movement of growth cones via actomyosin-mediated filopodial shortening (Heidemann, Lamoureux & Buxbaum, 1990). Therefore, cathodal turning is to be expected once filopodia become concentrated on the cathodal side of growth cones. Such an asymmetry of filopodia is not a prerequisite for field-induced turning, however, because cathodal reorientation persists when growth cones are shorn of filopodia by treatment with the microfilament inhibitor cytochalasin D (McCaig, 1989a). The molecular machinery for turning must therefore exist within the growth cone, irrespective of the elaboration of filopodia.

Other pharmacological treatments also provide clues regarding mechanisms. Galvanotropic orientation is inhibited by the plant lectin, concanavalin A (con A) (Patel & Poo, 1982; McCaig, 1989b), the inorganic calcium channel blockers cobalt and lanthanum (McCaig, 1989b) and the aminoglycoside antibiotic, neomycin (Ahmed, 1990;

C. D. McCaig & T. Ahmed, unpublished observation). The ability of these substances to prevent galvanotropism is consistent with a mechanism of orientation that involves locally altered Ca^{2+} homeostasis and preferential cytoskeletal reorganization on one side of the growth cone. Considering con A first, one major effect of an externally applied electric field is to translocate integral membrane proteins, such that these accumulate in the membrane facing one or the other pole (Jaffe, 1977; Poo & Robinson, 1977). The polarity of the accumulation depends on the charge on the protein and the surface charge on the membrane. Paradoxically, many negatively charged proteins such as con A and acetylcholine (ACh) receptors (a subset of con A receptors) accumulate cathodally. Apparently, this is because the direct electrophoretic influence of the applied field is weaker than the electro-osmotic drive (fluid flow adjacent to the membrane), which physically drags the extracellular elements of transmembrane receptors (McLaughlin & Poo 1981). Field-induced receptor asymmetry and cathodal nerve orientation are inhibited by pre-treatment of neurones with con A, which cross links the receptors, thus inhibiting their lateral mobility.

A recent elegant series of investigations of the ACh receptor has shown that once receptor clustering is initiated by the field, it will continue in the absence of an applied field (Stollberg & Fraser, 1988). This is not simply a function of increasing receptor density, as conditions have been found in which ACh receptors accumulate both anodally and cathodally, but only continue to accumulate cathodally once the field has been switched off (Stollberg & Fraser, 1990a). Two other mechanisms which could trigger this type of receptor clustering have been investigated using alternating or pulsed fields. Slow, linear electromigration of ACh receptors was due to an induced cathodal migration and accumulation of some other non-receptor molecule(s), rather than to a rapid, non-linear, voltage-sensitive mechanism, such as transient activation of voltage-sensitive calcium channels (Stollberg & Fraser, 1990b). The molecules responsible could be one or other of three putative anchoring proteins (a 43 kDa protein, a 58 kDa protein and talin) which co-localize with electric field-induced ACh receptor patches on *Xenopus* muscle cells. These proteins remain localized once the field is removed, indicating that they are stabilized at the ACh receptor patch (Rochlin & Peng, 1989).

Linking these observations with the capacity of migrating embryonic *Xenopus* growth cones to release neurotransmitter (ACh) spontaneously prior to target cell contact (Hume, Role & Fischbach, 1983; Young & Poo, 1983), produces an intriguing, yet speculative scenario. One function of the released neurotransmitter could be transduction and self-guidance on encountering an electric field (endogenous/applied). With an induced accumulation of ACh receptors for example, in the cathodal-facing membrane, self-released ACh would be expected to interact with its own growth cone receptors, activating more ACh receptors cathodally than anodally. Muscarinic ACh receptors on central nervous system (CNS) neurones are coupled to the inositol phosphate second messenger system (Heacock, Fisher & Agranoff, 1987); therefore locally elevated levels of inositol trisphosphate may arise cathodally, causing local release of calcium from intracellular stores. As much as 95% of cellular phosphatidylinositol bisphosphate (PIP_2) in resting cells may be complexed with the actin-binding protein, profilin (see Forscher, 1989). On hydrolysis of PIP_2 , the profilactin complex dissociates, which increases actin monomer levels, whilst the profilin promotes barbed-end assembly at the receptor/cytoskeletal interface. Thus, actin filament assembly could occur predominantly cathodally and this could result in cathodal orientation of the growth cone. Since one of the actions of neomycin, in addition to blocking galvanotropism, is to bind avidly to inositol phospholipids and prevent their break-down by phospholipase C (Lipsky & Leitman,

1982), this would be consistent with the mechanism outlined above. However, the neomycin block of galvanic orientation could be due to an effect independent of the inositol phosphate second messenger system. Neomycin is highly cationic and, by competition, can prevent calcium entry both pre- and postsynaptically. (This may be the basis of its long term side-effect of impairing neuromuscular function (Fiekers, 1983a,b)). However, even with a 16-fold increase of external calcium (to 8 mM), neomycin still totally inhibited cathodal nerve orientation (C. D. McCaig, unpublished observation). Thus, the second messenger block appears the more likely causal action.

An alternative explanation for cathodal orientation could also encompass this action and the observation that inorganic calcium channel blockers also inhibit galvanotropism (McCaig, 1989b). In cells exposed to an applied electric field, cathodal-facing membranes become depolarized and anodal-facing membranes hyperpolarized (Jaffe & Nuccitelli, 1977). It has been suggested that activation of voltage-sensitive calcium channels will increase intracellular $[Ca^{2+}]$ on the cathodal side (e.g. in a growth cone) (Cooper & Schliwa, 1986). A second actin-binding protein, gelsolin, is activated by elevated calcium (more so cathodally?) (Forscher, 1989). Its initial action is to sever actin filaments and thus to promote cytoplasmic transition from a gel to a sol phase (Oster, 1984). Once the initial high calcium influx has been lowered by buffering, actin-gelsolin complex dissociation is promoted locally by PIP_2 . This creates new sites for rapid barbed-end polymerization of actin and hence, site-directed (specific membrane receptors), gelsolin-mediated remodelling of the cytoskeleton (Forscher, 1989). In this way, preferential cathodal entry of calcium could alter both the cytoplasm and the cytoskeleton in ways that would promote growth cathodally.

Delivery of Ca^{2+} or the calcium ionophore A23187 from a micropipette, promotes local cytoplasmic protrusions (veils) from *Aplysia* growth cones (Goldberg, 1988). Locally elevated calcium also induces spreading of neuroblastoma growth cones without neurite advance, whilst calcium hotspots, induced by clustered L-type Ca^{2+} channels are focal points for local expansion of neuroblastoma growth cones (Silver, Lamb & Bolsover, 1989, 1990). The underlying mechanism may involve local activation of gelsolin as outlined above.

Whilst tropic growth in response to an applied field is no longer in doubt, recent work indicates that the direction of tropism may be determined by the nature of the substrate on which growth takes place (Rajnicek *et al.* 1991). *Xenopus* nerves grown on tissue culture plastic or on laminin grew preferentially and turned towards the cathode (as observed previously); however, nerves on polylysine showed preferential growth towards the anode, with some turning to grow anodally (Fig. 3). Varying the surface charge of the culture dish did not alter nerve orientation, therefore anodal as opposed to cathodal growth is unlikely to be due to surface charge-related events (Rajnicek *et al.* 1991). One explanation offered was that different substrates may select for axonal or dendritic growth; with dendrites predominating on laminin and tissue culture plastic, but the polylysine selectively promoting axonal growth. This possibility is being tested currently using the rat hippocampal preparation where axonal and dendritic growth appear in strict temporal sequence (Banker & Cowan, 1979). The ability of a single directional cue to orient axons and dendrites differently, or indeed, to orient some axons differently from others, would increase the scope of influence of electric fields both developmentally and in regeneration, and imply that intriguingly different mechanisms were operative.

One study may be significant in this regard. Different substrates alter both the ionic conductance properties and the overall morphology of leech Retzius neurones (Ross, Arechiga & Nicholls, 1988). Nerves grown on con A had broad, flat growth cones and

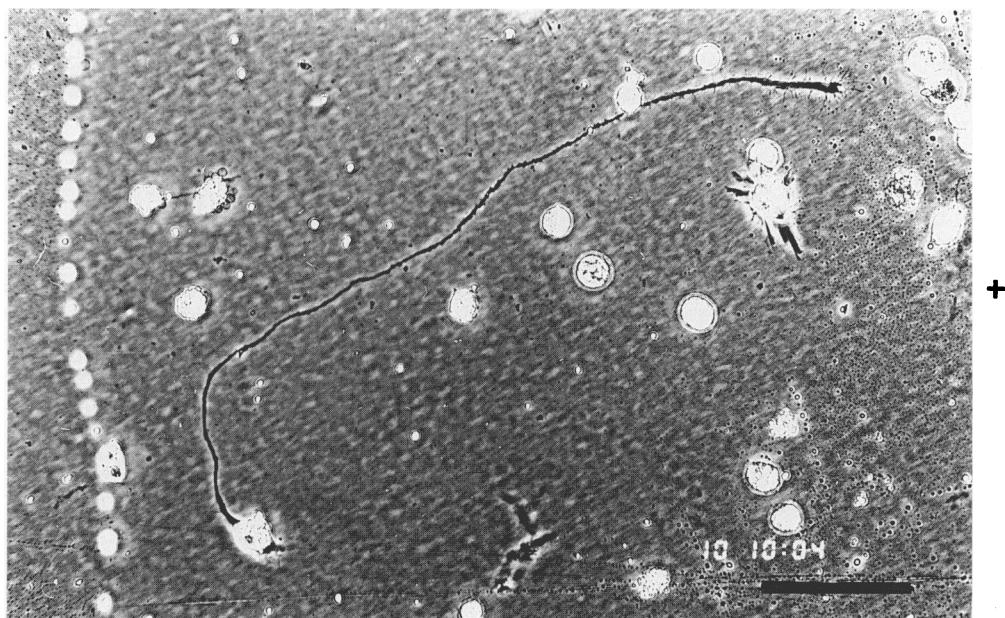


Fig. 3. Anodal orientation of a *Xenopus* neurite growing on tissue culture plastic treated with polylysine and an overlying layer of laminin. The field (113 mV/mm) is parallel to the long edges of the photograph, with the cathode to the left and the anode to the right. Scale bar represents 100 μm .

stout, often fasciculated neurites. The same cells grown on leech laminin had small growth cones and long, fine multiply branching processes. A substantial component of the evoked impulse in Retzius neurones involves Ca^{2+} entry, but only in processes grown on leech laminin. Processes on con A showed no detectable Ca^{2+} component in evoked impulses. Thus, the substrate may affect the number and distribution of functional calcium channels (Ross *et al.* 1988). Some interaction of this sort may underlie the reversed galvanotrophic response seen in *Xenopus* neurites on polylysine.

Profound effects of an electric field are not confined to growth cone orientation. Rates of nerve growth and nerve branching characteristics are also affected.

Effects on nerve growth rates

Anodal-facing nerves grow between 3 and 8 times slower than cathodal-facing nerves *in vitro* (Jaffe & Poo, 1979; Patel & Poo, 1982; McCaig, 1986). This is reversed on switching field polarity; indeed, reabsorption of an individual nerve can be replaced by maximal rates of extension within 10–35 min, whilst a marked slow-down occurs when nerves are switched from facing the cathode to facing the anode (McCaig, 1987). Pharmacological intervention, either with the plant lectin con A (80 $\mu\text{g}/\text{ml}$) or the antibiotic neomycin (0.5–1 mM), eliminated the unequal rates of growth normally evident between cathodal- and anodal-facing nerves (Ahmed, 1990; McCaig, 1990*b*; C. D. McCaig & T. Ahmed, unpublished observation).

Retraction of nerves is also influenced by an applied electric field, being 2.5 times more common in neurites facing an anode than in control cultures, whilst cathodal-facing neurites are spared from retraction (McCaig, 1987). These phenomena have a parallel in growth cone morphology, in that retracting, anodal-facing neurites have collapsed growth cones with few, if any filopodia. Growth cone collapse can be induced on contact with specific cell surface molecules and such avoidance reactions may be important in neuronal

guidance (Cox, Muller & Bonhoeffer, 1990; Raper & Kapfhammer, 1990). It is possible that the electric field may operate by activating the same collapse mechanism.

Pharmacological treatments together with applied electric fields have been studied both to find ways of improving nerve growth and to probe the mechanisms of interaction of electric fields with growing nerves. The adenyl cyclase activator forskolin, when used alone, increased the rate of regeneration of freeze-lesioned frog sciatic nerve (Kilmer & Carlsen, 1984). Forskolin ($50 \mu\text{m}$) had little effect on control rates of growth of cultured *Xenopus* embryonic nerves, but together with an electric field produced a 60% increase in growth rate (McCaig, 1990*b*).

Melanocortins have been used in efforts to improve nerve regeneration (De Koning & Gispens, 1988; Strand, Rose, King, Segarra & Zuccarelli, 1989), and recent work in this laboratory has coupled the use of applied electric fields with exposure to melanocortins. Neither α -melanocyte-stimulating hormone (α -MSH; $2 \mu\text{g/ml}$) nor adrenocorticotropic hormone (ACTH; $0.1 \mu\text{g/ml}$) alone had any effect on control nerve growth in culture; however, when presented together with an electric field, there was a 2- to 3-fold increase in growth rates over the already stimulated levels seen with the electric field alone (Stewart, 1990; R. Stewart & C. D. McCaig, unpublished observation). Indeed, with ACTH, rates of growth of some nerves were so high (up to $322 \mu\text{m/h}$) that augmented rates of cytoskeletal assembly, and an increased rate of delivery of cytoskeletal elements to the growth cone by slow axonal transport may also have been required. The normal rate of microtubule elongation in PC12 cells for example, is about $90 \mu\text{m/h}$ (Okabe & Hirokawa, 1988), which would not sustain the very fast rates of nerve growth seen with ACTH and an electric field. Whether new membrane in the shape of fast axonally transported vesicles also requires increased rates or volumes of delivery is unclear. These experiments raise the issue that one interaction of an electric field with nerve may be to influence axonal transport mechanisms. Given the degenerative effects on anode-facing neurites, such modulation may be either stimulatory or inhibitory.

Effects on nerve branching

Lateral processes resembling growth cone filopodia project from the shaft of both cultured embryonic neurones and pioneer sensory neurones in grasshopper embryos (Bray & Chapman, 1985; Anderson & Tucker, 1988; Dotti, Sullivan & Bunker, 1988). Cathodally projecting nerves possess almost twice as many lateral projections as control nerves, whilst on nerves growing perpendicular to an electric field these processes are distributed asymmetrically, with twice as many projecting towards the cathode than the anode. Some processes are clearly focal points for branch development (McCaig, 1986), suggesting that electric fields may be one of the intrinsic signals influencing nerve branching. An electric field focally applied from a micropipette also induces lateral processes from the shafts of individual nerves. This could occur within minutes, with some processes persisting as growth cone-tipped motile branches, whilst others either remained as single filopodia or withered. Again, this was a polarized event, with processes sprouting from the cathodal sides of neurites (McCaig, 1990*a*). These observations indicate a role for electric fields in modifying and directing cytoskeletal assembly/disassembly. In several neuronal types, disassembly of microtubules (via colchicine or nocadazole) produced fine lateral processes along the neuronal shaft (Bray, Thomas & Shaw, 1978; Matus, Bernhardt, Bodmer & Alaimo, 1986; Joshi, Baas, Chu & Heidemann, 1986). The remaining cytoskeletal polymers became splayed out perpendicular to the nerve shaft, indicating a reduced cross-linking and allowing microfilaments more lateral mobility (Joshi *et al.* 1986). Focally applied electric fields also may induce lateral sprouting by first inducing localized microtubule disassembly.

Microtubules are polarized within axons such that the assembly-favoured '+' ends face the growth cone. Indeed, such an orientation may be essential for growth cone formation (Baas, White & Heidemann, 1987). (No such orientation of microtubules is seen in dendrites (Baas, Deitch, Black & Bunker, 1988)). Cytoskeletal assembly at induced branch points must be polarized by the electric field, since new processes appeared from the cathodal side of nerves. That many of these processes were tipped with growth cones and became fully grown branches, suggests moreover that orientation of microtubules may have occurred, with '+' ends distally, towards the cathode.

Branching was augmented further by combining field application with some pharmacological treatments. For instance, although neither DMSO (dimethyl sulphoxide) nor the ganglioside, GM₁ alone increased branching over control levels, in combination with an applied field, both promoted profuse branching from cathodal facing sides of neurites (McCaig, 1990b). Enhanced branching of nerves in an applied electric field has been observed also *in vivo* in regenerating lamprey spinal cord (Borgens, Roederer & Cohen, 1981), and for intact rat saphenous nerve (Pomeranz, Mullen & Markus, 1984).

ENDOGENOUS ELECTRIC FIELDS

Given these wide-ranging influences on nerve growth, do endogenous electric fields exist either during development or regeneration? Much work using the vibrating microelectrode technique (Jaffe & Nuccitelli, 1974) suggests that they do. A voltage-sensing microelectrode is vibrated rapidly near a cell or tissue in a conductive liquid medium. The difference between the two voltages detected at the extremes of the electrode's excursion can be converted to current flow at the cell/tissue source using Ohm's law. Thus, non-invasive measurements of very small, steady currents can be made for many hours, days or weeks. In *Xenopus*, steady endogenous current of around 10 $\mu\text{A}/\text{cm}^2$ flows in through the skin and exits from the developing blastopore (Robinson & Stump, 1984). This current is carried predominantly by Na⁺, the transport of which sustains the potential difference across the embryonic epithelium (McCaig & Robinson, 1982). In both developing *Xenopus* and axolotl, localized, steady, endogenous current leaves the embryo at a location predictive of the future site of limb bud development, but the ions involved have not been identified (Robinson, 1983; Borgens, Rouleau & DeLaney, 1983).

Currents of around 20 and 100 $\mu\text{A}/\text{cm}^2$, respectively, leave the primitive streak region in developing chick and mouse embryos (Jaffe & Stern, 1979; Winkel & Nuccitelli, 1989). In no case is the voltage gradient resulting within the tissues from these steady currents known. Recently, however, developmentally regulated large currents ($112 \pm 10 \mu\text{A}/\text{cm}^2$) were found to leave the posterior intestinal portal (the opening into the hindgut from the yolk sac) in 2.5–4 day chick embryos (Fig. 4; Hotary & Robinson, 1990). This occurred during the period of tail gut reduction, when epithelial cells lining cloacal regions of the hindgut are dying, thus creating a low-resistance pathway for current flow out of the embryo. Currents entered the intact epithelium over other regions of the embryo. Again, at least a part of the current (50%) was carried by Na⁺. Transepithelial potential difference measurements made lateral to the neural tube on the dorsal surface revealed regional variations which were used to calculate an intraembryonic voltage gradient. When the outward current at the posterior intestinal portal was greatest, the caudally negative voltage gradient was as great as 33 mV/mm. This is the first direct measurement of an intraembryonic extracellular voltage gradient. Moreover, the magnitude is certainly sufficient to provide directional information to growing or migrating cells (Robinson, 1985). The hindgut forms the cathode of this natural electric field and many cell types,

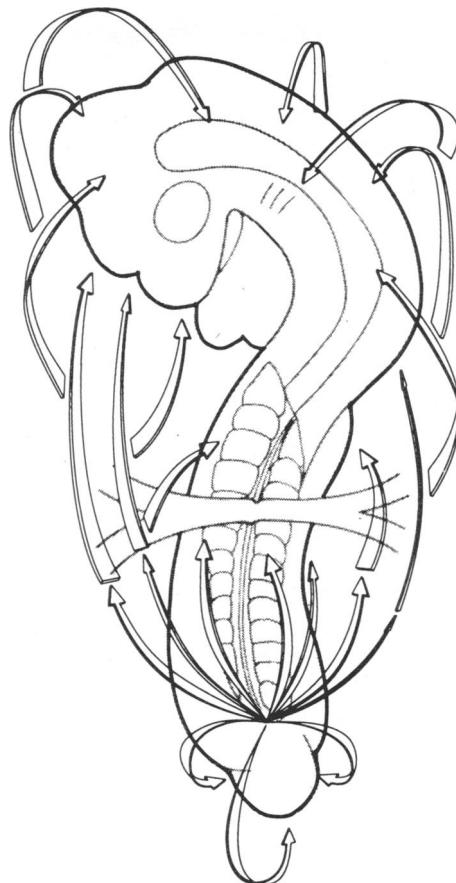


Fig. 4. Presumed three-dimensional pattern of endogenous current flow around a developing chick embryo. This map was derived from current measurements made using a vibrating microelectrode. Current exits the embryo via the posterior intestinal portal and enters diffusely over regions of intact epithelium. The direction of the arrows indicates net flow of positively charged ions. Reproduced with permission of K. B. Hotary.

including neural crest cells, migrate cathodally *in vitro* (Stump & Robinson, 1983). Hotary & Robinson (1990) suggest that both epithelial cells and neural crest cells may respond to these endogenous voltage gradients. The possibility of an electrical influence on neural crest cells, which condense to form Remak's ganglion at a time and place when posterior intestinal portal currents and associated electrical fields are maximal, is being explored.

The same group have measured a voltage gradient across the neurones of the developing *Xenopus* neural tube. Apparently, the voltage within the lumen of the neural tube is 18 mV negative relative to the interstitial spaces. Based on a wall thickness of 40 μm , this represents a huge voltage drop of over 400 mV/mm across the two-cell thick layer of developing neuroepithelium (Hotary & Robinson, 1991). The influence of this internal field on directed axonal outgrowth would be clearer if inhibition of the transluminal potential difference could be correlated with aberrant axonal outgrowth from the developing neural tube.

Endogenous currents are correlated also with episodes of nerve regeneration, both in severed limbs and in transected spinal cords (Borgens, 1989b; Borgens & McCaig, 1989). Large currents (up to 100 $\mu\text{A}/\text{cm}^2$) leave the stumps of transected axolotl limbs; blocking these with the epithelial Na^+ channel blocker amiloride inhibited natural limb regeneration

(Borgens, Venable & Jaffe, 1977a, 1979a). Stump currents in *Xenopus*, in which natural limb regeneration is limited, were much smaller ($5 \mu\text{A}/\text{cm}^2$). However, artificially augmenting the stump current with an implanted battery and a wick electrode drawing current out of the stump for about 2 weeks improved limb regeneration (Borgens, Venable & Jaffe, 1979b). Most notable was the heavy innervation of the regenerate, indicating substantial ingrowth of cathodally directed nerve.

In larval lamprey, transection of the spinal cord leads to natural axonal regeneration (e.g. Mackler & Selzer, 1985). Vibrating microelectrode measurements indicate a large injury current entering the severed cord immediately and persisting at a lower level for several days (Borgens, Jaffe & Cohen, 1980). Artificially reversing the direction of net current flow by drawing current out through the severed cord speeded up the time course of Mauthner axon regeneration, probably by reducing the extent of axonal dieback that occurred during the first few days after transection (Roederer, Goldberg & Cohen, 1983). Recent work indicates that calcium entry into transected axons is reduced in quantity and depth of penetration by an electric field applied rostro-caudally (Strautman, Cork & Robinson, 1990).

These studies were precursors of a number of attempts to use applied electric fields in efforts to promote regeneration in both mammalian peripheral (PNS) and central nervous systems.

EFFECTS OF APPLIED DC FIELDS ON NEURONES *IN VIVO*

Application of electric fields to damaged neurones represents a new frontier in the treatment of nervous system injury. There have been several attempts to enhance mammalian neuronal regeneration with electromagnetic fields induced by Helmholtz coils (e.g. Ito & Bassett, 1983), as well as with AC or DC fields. Direct comparison of these studies is complicated by the use of a wide variety of model systems, different types of stimulators and techniques to assay for recovery. In the interest of clarity, we will limit ourselves to a discussion of the responses of neurones to DC stimulation *in vivo*.

Application of DC fields to peripheral nerve

The first indication that peripheral nerves responded to exogenous electric fields was discovered during attempts to stimulate regeneration of amputated frog limbs (Borgens *et al.* 1977b). When the cathode was positioned at the plane of amputation, as much as 20% of the distal area of the stump was composed of nerve. The response was dependent on current polarity; in anode-distal stumps and those to which no current was applied, only 1% of the volume of the limb terminus consisted of nerve trunks. This result is consistent with the polarity of the field responses of frog neurones *in vitro* (Hinkle *et al.* 1981).

There have been several attempts to influence peripheral nerve growth in mammals using applied DC fields. The first examined field effects on intact rat saphenous nerves as they branched in response to sciatic nerve transection (Pomeranz *et al.* 1984). Saphenous nerves grow slowly as they extend to fill the region normally served by the sciatic nerve, so any field-enhanced branching or increase in growth rate would be detected as an enlargement in the region of tactile sensitivity on the hindpaw.

Stainless-steel electrodes were inserted into the most lateral digit of the hindpaw and into the tail of the rat. Direct current was applied for 30 min each day for 23 days, with the cathode located in the tip of the paw and the anode in the tail (or vice versa). Controls received no current or no electrodes. Functional recovery was assessed by determining the area of the paw over which the animal exhibited flexor withdrawal reflexes. DC stimulation with the cathode in the hindpaw enlarged the zone of responsiveness significantly. This

behavioural response was attributed to increased sprouting of the saphenous nerve since histological examination of silver-stained tissue from that group revealed a 10-fold increase in the number of nerve fibres in the most lateral regions of the receptive field. The lateral regions were not innervated in control animals or those with the anode in the paw.

Whilst this work indicated that DC fields enhanced growth of intact peripheral neurones, most studies have examined field-induced effects on regrowth of damaged rat sciatic nerves. In one of the earliest reports (McDevitt, Fortner & Pomeranz, 1987) sciatic nerves were severed and resutured (cut-and-suture) or crushed. DC stimulation (30 min daily) was provided via stainless-steel electrodes for 18–20 days for crush injured animals or for 30 days for cut-and-suture animals. Motor nerve function was assayed by recording electromyograms (EMGs) from the hindpaw muscles upon electrical stimulation of the sciatic nerve just proximal to the injury site. EMG activity was observed in twelve of eighteen cut-and-suture rats that received cathode distal current, but few rats with anode distal current (two of twelve rats) or no current (two of twelve rats) showed EMG activity. Likewise, only two of twelve rats in each of the crush injury groups displayed EMG activity, regardless of electrical treatment. The cathode distal current did not enhance neuronal maturation or myelinization because conduction velocity measurements were not different between groups. A similar response was observed in a preliminary report for animals treated with a chronic, cathode distal current supplied via wick electrodes (Pomeranz, 1986), so it is unlikely that any field effects were attributable to electrode products.

Animals with their sciatic nerves transected, leaving a gap between the cut ends, also responded favourably to applied DC current (Roman, Strahlendorf, Coates & Rowley, 1987). A cuff electrode, consisting of a Silastic tube with a platinum wire inserted through the wall into the lumen, was positioned around the nerve with the wire midway between the nerve stumps. The electrode in the tube was the cathode and a platinum wire in a nearby muscle was the anode. After three weeks, scanning electron micrographs revealed well-formed neural bridges across the gaps. Central regions of the bridges were twice as large as in sham-treated control nerves. Frozen sections provided evidence for neovascularization and a threefold increase in the total area occupied by myelinated and non-myelinated nerve fibres (relative to controls). Although these results are encouraging, the small number of animals used (three per group) and the absence of an anode distal group minimize their impact. It is not known whether the enhanced morphological regeneration manifested itself behaviourally since no functional assessment was made.

The effect of applied current on damaged sciatic nerves with non-cellular scars has been examined by transecting the nerves, freezing the proximal stump and then suturing the two ends together (Politis, Zanakis & Albala, 1988a). Chronic current was supplied to damaged nerves using cuff electrodes (anode or cathode distal), or non-functional nerve cuffs. Anatomical regeneration was assessed by staining sections of nerve distal to the injury with a fluorescently labelled antibody specific for neurofilaments. There was no assessment of functional recovery. The cathode-distal group had a significantly higher number of neurofilament-positive profiles relative to the sham-treated and anode-distal groups 6, 12 and 18 days after surgery. There were fewer positive profiles in the anode-distal group relative to the cathode-distal group at days 12 and 18 post-injury, suggesting a directional effect of the field.

Field-enhanced regeneration of rat sciatic nerves has also been studied following crush injuries coupled with local application of phenol, which produces a neuroma-like 'scar' (Beveridge & Politis, 1988). Silicone cuff electrodes (cathode distal) were put in place and

activated 3 weeks after injury. Regeneration was assessed after 3 weeks of chronic current. Field-treated nerves showed a fourfold increase (relative to controls) in the number of neurofilament-positive profiles distal to the injury site. Behavioural regeneration was assessed by examining various parameters of the rat's stride. Footprint patterns indicated that electrically treated animals were significantly improved relative to controls.

Despite these indications that applied current affects growth of intact peripheral nerves and that a cathode-distal field improves recovery from sciatic nerve injury, the definitive study remains to be done. Such an experiment needs to: (1) use sufficient animals for statistics, (2) describe details of the stimulator unit, (3) measure directly the electrical field produced within the tissues, (4) assess anode- and cathode-distal fields, plus sham-treated control animals, (5) account for possible effects of electrode products, and (6) correlate histological and behavioural evidence for enhanced recovery in the same animals. Point (4) is important since anode-distal current slightly enhanced motor neurone regeneration in one preliminary study (Pomeranz, 1986) and neurites *in vitro* grew predominantly towards the anode on an extracellular matrix extract (Rajnicek *et al.* 1991).

Application of DC fields to the central nervous system

Immediately after spinal cord injury. There has been growing interest in field-enhanced recovery of the CNS within the past decade (see Borgens, 1989a; Zanakis, 1988 for reviews). In the first attempt, wick electrodes delivered current (cathode caudal) to transected spinal cords of larval sea lampreys for 5–6 days (Borgens *et al.* 1981). Recovery of function was assessed by recording electrically evoked action potentials traversing the lesions 44–63 days after transection. This occurred in eight of eleven (73%) field-treated animals, but in only three of thirteen (23%) sham-treated controls. Intracellular injection of Lucifer Yellow from the recording electrode, allowed morphological examination of identified regenerating fibres. Giant axons with swollen, irregular tips were found caudal to the lesion in most field-treated cords, but in few control cords. Branching was also enhanced in field-treated fibres (nineteen of twenty-six fibres; 73%) compared to controls (eight of twenty-seven fibres; 30%). Enhanced branching of cathode-facing neurites has also been reported for intact rat saphenous nerve (Pomeranz *et al.* 1984) and for cultured *Xenopus* spinal neurones (McCaig, 1990a; Rajnicek *et al.* 1991).

How the applied current facilitated regeneration was addressed in a subsequent study of lamprey spinal cords (Roederer *et al.* 1983). Large currents, carried mainly by Ca^{2+} and Na^+ had been measured entering severed lamprey spinal neurones (Borgens *et al.* 1980) and elevated levels of intracellular free calcium were postulated to promote dieback of severed axons. Reduced influx of calcium in an externally applied electric field therefore might alter the extent of retrograde degeneration of the axonal stump, thereby enhancing recovery in a distally negative field.

The polarity of the imposed field had a profound effect on the extent of retrograde degeneration. After 5 days, Lucifer Yellow-filled axons terminated 750 μm (cathode distal), 2820 μm (cathode proximal) or 1750 μm (controls) from the plane of transection. Some component of current flow into the severed axons may cause retrograde degeneration and yet be offset by application of cathode distal electric current. Measurements of intracellular calcium confirmed that a substantial gradient of free calcium exists within the first 1500 μm of several lamprey axons, and that the extent of calcium entry can be manipulated by an applied electric field (Strautman *et al.* 1990). However, a direct relationship between the level of dieback and the extent of elevated intracellular calcium remains to be shown.

The effects of DC fields on anatomical and behavioural recovery of severed spinal

neurones have also been examined in mammals. Anatomical recovery of severed dorsal column axons was induced by weak, chronic DC fields in guinea-pigs (Borgens, Blight, Murphy & Stewart, 1986). This report was the first to demonstrate an effect of electric fields on mammalian CNS regeneration, and the response was evident at very low field strengths. Resistance measurements within the current path *in vivo* indicated that the 10 μ A stimulators (the largest used in the study) produced fields on the order of 0.04 mV/mm, at least 100 times weaker than the minimum fields that influence growth *in vitro* (Hinkle *et al.* 1981).

Dorsal hemisections were performed on adult guinea-pigs and chronic DC current was delivered via wick electrodes (cathode rostral) for approximately 50 days. Horseradish peroxidase (HRP), packed into a shallow second lesion caudal to the injury, was used to label regenerated axons. Axons entering the cord more rostrally were not filled, so any labelled axons at (or rostral to) the level of the lesion must have regenerated. Sham-treated control animals showed little evidence of spontaneous regeneration; ascending axons terminated predominantly caudal to the glial scar, rarely within the scar and never crossed into the rostral cord. In contrast, field-treated axons in most animals grew into the glial scar as far as the plane of transection. In a few cases, axons crossed the level of the lesion by growing around the glial scar (never directly through it) and continuing rostrally for about 1 mm.

Do these regenerating fibres make appropriate connections? Field-induced behavioural recovery of a spinal reflex in guinea-pigs suggests that they do (Borgens, Blight & McGinnis, 1987). Physiological and anatomical aspects of the cutaneous trunci muscle (CTM) reflex system have been examined in detail (Blight, McGinnis & Borgens, 1990). The CTM reflex involves a transient wrinkling of the skin in response to light tactile or electrical stimulation of the back skin. It is eliminated in regions ipsilateral and caudal to a lateral hemisection of the thoracic spinal cord. The deficit is permanent below the lesion but the reflex is unaffected contralaterally. Since the response is dependent on ascending pathways only, the receptive field of the reflex can be exploited as an objective, non-invasive indicator of discrete spinal tract function.

Lateral hemisections were made at the mid- to lower thoracic level of guinea-pigs and wick electrodes supplied 50 μ A of current (cathode rostral to the lesion) for up to 4 weeks. Recovery of the CTM response was assayed visually and electromyographically. Five of the twenty-eight animals receiving electrical stimulation recovered the reflex, the earliest at 56 days after injury. None of the twenty-nine sham-treated control animals recovered the reflex within 140 days post-injury. The possibility that enhanced recovery was due to an influence of the field on collateral sensory innervation, rather than a direct effect upon ascending lateral tract axons was also considered. Three dorsal cutaneous nerves (sensory fibres that drive the reflex) below and ipsilateral to the hemisection were severed in a recovered animal, resulting in complete loss of responsiveness in the skin normally innervated by those nerves.

A more complete investigation of functional recovery in guinea-pig spinal cords has appeared (Borgens *et al.* 1990) with platinum-iridium electrodes sometimes replacing wick electrodes for current delivery. In some animals, the stimulators were monitored throughout the experiment via external leads, thus removing ambiguity about the magnitude and duration of current application. When measured directly, the 35 and 50 μ A stimulators yielded field strengths within the tissues of 0.3–0.4 mV/mm. Another addition to the experimental design was an assay for the vestibulospinal free-fall response (FFR); spreading of the toes upon rapid downward displacement of the guinea-pig. The CTM

reflex depends on ascending spinal tracts and the FFR on descending tracts, so both reflexes were assessed in each animal to determine the influence of field polarity on behavioural recovery.

Only animals that lost CTM function completely 2 weeks after lateral hemisection were included in the study. All sham-treated ($n = 33$) and cathode-caudal ($n = 62$) animals failed to recover CTM function, but nine of the sixty-seven cathode-rostral animals exhibited the reflex between 56 and 139 days after injury. The percentage recovery and the time course for reappearance of the reflex (13%, 56 days) were comparable to the preliminary study (18%, 56 days) (Borgens *et al.* 1987). The FFR was not an effective indicator of the influence of field polarity on functional recovery because 80–90% of the animals in all groups showed spontaneous recovery of the reflex by 3 months.

Taken together, these studies indicate that an exogenous electric field of approximately 0.3–0.4 mV/mm is sufficient to induce functional regeneration of mammalian CNS nerve fibres directed towards the cathode. A differential effect of field polarity on functional recovery of oppositely projecting spinal paths has not been shown, however.

The adult rat spinal cord has also been used to investigate field-induced recovery of the mammalian CNS. Recovery from acute 50 g clip compression injuries has been examined in rats treated with 14 μ A of chronic DC current (cathode distal) (Wallace, Tator & Piper, 1987). This injury models compression of the cord due to a fracture dislocation of the spinal column; the most common form of mammalian spinal injury (Rivlin & Tator, 1977). Recovery was assessed histologically after 15 weeks of treatment and weekly by an inclined plane test. The angle at which the rat could no longer maintain its position was used to assign a behavioural score. The authors claim to find enhanced clinical recovery in the cathode-distal animals but significant increases in inclined plane scores were evident at only three of the fourteen time points.

It is unclear whether improvement in these animals was due to the imposed field. Only one of the 'active' stimulators was functioning at the end of the experiment; therefore the duration of field exposure for the other animals is uncertain. Although *in vivo* measurements (Fehlings, Tator & Linden, 1988) have shown that stimulators of the same design produced fields sufficient to affect neuronal growth (70 mV/mm), the actual field strength within the tissues was not measured. The basis for a regenerative effect at the tissue level is also unclear because the levels of new myelin formation, and Schwann cell and ependymal cell proliferation in treated animals were not different from controls. Another study, using the same type of injury and stimulator design (Fehlings, Wong, Tator & Tymianski, 1989), suggested that the effect was due to field-enhanced survival or regrowth of axons because the number and calibre of myelinated axons was increased at the injury site relative to controls. The response was observed only in animals that received 53 g injuries (not in those with 17 g injuries). Again, the existence of non-functional 'active' units was not excluded.

In another report, only animals subjected to 53 g injuries (not those with 17 g injuries) showed behavioural and anatomical improvement over sham-treated control rats (Fehlings *et al.* 1988). There was some qualitative improvement in hindlimb function for field-treated rats in the 53 g injury group and inclined plane scores were significantly improved over the other groups by 6 weeks. Improvement continued until the end of the 8 week treatment. In addition, the amplitude of motor-evoked potentials and the number of HRP-labelled cells in the red nuclei, raphe nuclei and vestibular nuclei were higher than in control rats. The presence of HRP label in the brain stem represents axons that crossed the injury site and were capable of retrograde transport. They may therefore be indicative of neuronal regeneration, but some labelled cells may represent survivors of the injury.

Delayed treatment of spinal cord injury. Histological and behavioural studies of regeneration in compression injuries to rat spinal cord have also used delayed field application. Stimulators delivered 3 μ A of current (cathode or anode distal) for 21 days beginning at injury (Politis & Zanakis, 1988a) or 10 days post-injury (Politis & Zanakis, 1989). Animals receiving cathode-distal fields at injury performed statistically better on the inclined plane test than anode-distal or control counterparts after 14 and 21 days. Hindlimb function also improved in field-treated animals. Behavioural improvement correlated well with histological regeneration after 21 days, in that the cathode-rostral group had significantly more neurofilament positive profiles in the dorsal funiculus (rostral to the lesion) than either the anode-rostral or the sham-treated group. Animals that received delayed fields had improved hindlimb function and performed better in behavioural tests than sham-treated control animals by 7 days after stimulator implantation. Surprisingly, the anode-distal animals performed better than the other groups. Twice as many neurofilament positive profiles were present in the dorsal funiculus of cathode-distal cords compared to the other two groups, so the reason for behavioural superiority in anode-distal animals is not apparent. A thorough investigation of the role of field polarity in regeneration of mammalian spinal neurones is needed. It should include the use of a marker to distinguish axons that survive the injury from those that regenerate beyond the lesion and longer term assessment of field-induced recovery.

Damaged optic nerve and hippocampus. Field-enhanced recovery in the mammalian CNS is not limited to the spinal cord. Cathode-distal fields applied via nerve cuff electrodes also increased the number of neurofilament-positive profiles in rat optic nerves following crush injuries (Politis, Zanakis & Albala, 1988b). No positive profiles were found in control or anode-distal animals. Imposed fields also improve regeneration in rat hippocampi (Politis & Zanakis, 1988b). Current (cathode or anode distal) was applied for 4 weeks via platinum-iridium wire electrodes following unilateral fimbrial lesions. Two protocols were used to assess the rat's hippocampal function: (1) the number of alternating choices made while in a three-forked maze and (2) the ability to learn the location of a submerged platform in a water tank. At the end of field exposure, unoperated controls and cathode-distal animals performed similarly in the maze but anode-distal and sham-treated control rats performed significantly worse. Similarly cathode-distal and unoperated controls learned the location of the platform after three daily trials, whereas the anode-distal and sham-treated animals did not. Acetylcholinesterase (AChE) activity in the hippocampi was significantly higher in cathode-distal and unoperated controls relative to the level in anode-distal and sham-treated controls. Increased AChE activity may indicate a greater number of cholinergic neurones in the hippocampi of cathode-distal rats, but no histological evidence for regeneration was presented.

The latter study presents a unique opportunity to correlate field responses of mammalian CNS neurones *in vivo* with those *in vitro*. Preliminary evidence indicates that primary cultures of rat hippocampal neurones (Banker & Cowan, 1977) respond directionally to applied DC electric fields (A. M. Rajnicek, N. A. R. Gow & C. D. McCaig, unpublished observations). These experiments will provide valuable information regarding the optimum magnitude, direction and duration of fields to use in future clinical studies of mammalian CNS regeneration.

Interaction of fields with other regenerative techniques

Other approaches and techniques have made substantial progress towards inducing CNS regeneration. Most notably, the PNS bridging methods, which use lengths of peripheral nerve as a non-hostile environment for promoting and routing regeneration. In adult rats, regenerated long retinal axons leave these conduits and make functional synaptic contacts in the midbrain (Aguayo, Vidal-Sanz, Villegas-Perez & Bray, 1987). Oligodendrocytes possess surface proteins that inhibit nerve growth. In rats, antibodies specific for these molecules mask this inhibition and allow regeneration of long axons in the spinal cord to occur over as much as 11 mm (Schnell & Schwab, 1990). Additionally, the observation that growth of freeze-lesioned sciatic nerves is enhanced in the presence of forskolin (Kilmer & Carlsen, 1984) coupled with the observation that certain pharmacological agents (including forskolin) boost the responses of neurites to electrical fields (McCaig, 1990*b*) suggests that some combination of these techniques could prove effective in a clinical context.

How do electric fields exert their effects in vivo?

The mechanism by which electric fields enhance neuronal regeneration remains unclear but several possibilities exist. (1) A distally negative imposed field reduces inward flow of Ca^{2+} at the cut end of neurones, thus reducing the level of retrograde degeneration following transection. There is evidence that an applied field (cathode distal) virtually eliminates Ca^{2+} influx in lamprey spinal axons (Strautman *et al.* 1990) and that applied fields augment regeneration of these same axons (Borgens *et al.* 1981). Whether these effects are reproduced within mammalian systems is not known. (2) The field may induce branch formation, as has been shown for intact rat peripheral neurones (Pomeranz *et al.* 1984) and cultured frog spinal neurones (McCaig, 1990*a*; Rajnicek *et al.* 1991). Increased branch formation in damaged cells would allow numerous paths to be sampled, thereby increasing the chances of finding a favourable route for regeneration. Alternatively, uninjured neighbouring cells may be stimulated to branch, causing formation of deviant, yet potentially functional synapses. (3) Non-neuronal elements, such as glial cells, fibroblasts or Schwann cells may respond to the fields, thus affecting the physical composition of the scar at the lesion site. For example, fibroblasts, which migrate towards the cathode of an applied field (Erickson & Nuccitelli, 1984), may be arranged more diffusely. The resulting scar would be less dense, possibly permitting penetration by advancing growth cones. Alternatively, exogenous fields, which increase capillary permeability (Nannmark, Buch & Albrektsson, 1985), may affect the inflammatory response following injury. (4) More neuroblasts differentiate in the presence of an applied field *in vitro* (Hinkle *et al.* 1981), so the field may act as a trophic factor, increasing cell survival and differentiation.

SUMMARY

The presence of voltage gradients within developing and damaged tissues led to the notion that the resultant electrical fields provide instructional cues to cells. Field effects on avian and amphibian neurones *in vitro* include increased differentiation, turning of neurites towards the cathode, increased rate of growth towards the cathode, resorption of anode-facing neurites, increased branching and increased filopodial activity. Electric fields enhance regeneration of damaged PNS and CNS neurones in animals as diverse as lampreys, frogs, rats and guinea-pigs, but the mechanisms by which fields produce their

effects are not understood. Further examination of the interaction of fields with intracellular elements, such as the cytoskeleton and second messenger systems, may offer some insight.

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REFERENCES

- AGUAYO, A. J., VIDAL-SANZ, M., VILLEGRAS-PEREZ, M. P. & BRAY, G. M. (1987). Growth and connectivity of axotomized retinal neurons in adult rats with optic nerves substituted by PNS grafts linking the eye and the midbrain. *Annals of the New York Academy of Sciences* **495**, 1–9.
- AHMED, T. (1990). Nerve growth; pharmacological treatments and an applied electric field. M.Sc. Thesis, University of Aberdeen.
- ANDERSON, H. & TUCKER, R. P. (1988). Pioneer neurones use basal lamina as a substratum for outgrowth in the embryonic grasshopper limb. *Development* **104**, 601–608.
- BAAS, P. W., DEITCH, J. S., BLACK, M. M. & BANKER, G. A. (1988). Polarity orientation of microtubules in hippocampal neurones: Uniformity in the axon and nonuniformity in the dendrite. *Proceedings of the National Academy of Sciences of the USA* **85**, 8335–8339.
- BAAS, P. W., WHITE, L. A. & HEIDEMANN, S. R. (1987). Microtubule polarity reversal accompanies regrowth of amputated neurites. *Proceedings of the National Academy of Sciences of the USA* **84**, 5272–5276.
- BANKER, G. A. & COWAN, W. M. (1977). Rat hippocampal neurones in dispersed cell culture. *Brain Research* **126**, 397–425.
- BANKER, G. A. & COWAN, W. M. (1979). Further observations on hippocampal neurones in dispersed cell culture. *Journal of Comparative Neurology* **187**, 469–494.
- BEVERIDGE, J. A. & POLITIS, M. J. (1988). Use of exogenous electric current in the treatment of delayed lesions in peripheral nerves. *Plastic and Reconstructive Surgery* **82**, 573–577.
- BLIGHT, A. R., MCGINNIS, M. E. & BORGENS, R. B. (1990). Cutaneous trunci muscle reflex of the guinea pig. *Journal of Comparative Neurology* **296**, 614–633.
- BORGENS, R. B. (1989a). Artificially controlling axonal regeneration and development by applied electric fields. In *Electric Fields in Vertebrate Repair*, pp. 117–170. Alan R. Liss, Inc., New York.
- BORGENS, R. B. (1989b). Natural and applied currents in limb regeneration and development. In *Electric Fields in Vertebrate Repair*, pp. 27–74. Alan R. Liss, Inc., New York.
- BORGENS, R. B., BLIGHT, A. R. & MCGINNIS, E. M. (1987). Behavioural recovery induced by applied electric fields after spinal cord hemisection in guinea pig. *Science* **238**, 366–369.
- BORGENS, R. B., BLIGHT, A. R. & MCGINNIS, M. E. (1990). Functional recovery after spinal cord hemisection in guinea pigs: The effects of applied electric fields. *Journal of Comparative Neurology* **296**, 634–653.
- BORGENS, R. B., BLIGHT, A. R., MURPHY, D. J. & STEWART, L. (1986). Transected dorsal column axons within the guinea pig spinal cord regenerate in the presence of an applied electric field. *Journal of Comparative Neurology* **250**, 168–180.
- BORGENS, R. B., JAFFE, L. F. & COHEN, M. J. (1980). Large and persistent electrical currents enter the transected lamprey spinal cord. *Proceedings of the National Academy of Sciences of the USA* **77**, 1209–1213.
- BORGENS, R. B. & MCCAIG, C. D. (1989). Endogenous currents in nerve repair, regeneration and development. In *Electric Fields in Vertebrate Repair*, pp. 77–116. Alan R. Liss, Inc., New York.
- BORGENS, R. B., ROEDERER, E. & COHEN, M. J. (1981). Enhanced spinal cord regeneration in lamprey by applied electric fields. *Science* **213**, 611–617.
- BORGENS, R. B., ROULEAU, M. F. & DELANNEY, L. E. (1983). A steady efflux of ionic current predicts hind limb development in the axolotl. *Journal of Experimental Zoology* **228**, 491–503.
- BORGENS, R. B., VANABLE, J. W. JR & JAFFE, L. F. (1977a). Bioelectricity and regeneration: Large currents leave the stumps of regenerating newt limbs. *Proceedings of the National Academy of Sciences of the USA* **74**, 4528–4532.
- BORGENS, R. B., VANABLE, J. W. JR & JAFFE, L. F. (1977b). Bioelectricity and regeneration: Initiation of frog limb regeneration by minute currents. *Journal of Experimental Zoology* **200**, 403–416.
- BORGENS, R. B., VANABLE, J. W. JR & JAFFE, L. F. (1979a). Reduction of sodium dependent stump currents disturbs urodele limb regeneration. *Journal of Experimental Zoology* **209**, 337–386.

- BORGENS, R. B., VANABLE, J. W. JR & JAFFE, L. F. (1979b). Small artificial currents enhance *Xenopus* limb regeneration. *Journal of Experimental Zoology* **207**, 217–225.
- BRAY, D. (1979). Mechanical tension produced by nerve cells in tissue culture. *Journal of Cell Science* **37**, 391–410.
- BRAY, D. & CHAPMAN, K. (1985). Analysis of microspike movement on the neuronal growth cone. *Journal of Neuroscience* **5**, 3204–3213.
- BRAY, D., THOMAS, C. & SHAW, G. (1978). Growth cone formation in cultures of sensory neurones. *Proceedings of the National Academy of Sciences of the USA* **75**, 5226–5229.
- COOPER, M. S. & SCHLIWA, M. (1986). Motility of cultured fish epidermal cells in the presence and absence of direct current electric fields. *Journal of Cell Biology* **102**, 1384–1399.
- COX, E. C., MULLER, B. & BONHOEFFER, F. (1990). Axonal guidance in the chick visual system: Posterior tectal membranes induce collapse of growth cones from the temporal retina. *Neuron* **2**, 31–37.
- DE KONING, P. & GISPEN, W. H. (1988). A rationale for the use of melanocortins in neural injury. In *Pharmacological Approaches to the Treatment of Brain and Spinal Cord Injury*, ed. STEIN, D. G. & SABEL, B. A., pp. 233–258. Plenum, New York.
- DOTTI, C. G., SULLIVAN, S. A. & BANKER, G. A. (1988). The establishment of polarity by hippocampal neurones in culture. *Journal of Neuroscience* **8**, 1454–1468.
- ERICKSON, C. A. & NUCCITELLI, R. (1984). Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. *Journal of Cell Biology* **98**, 296–307.
- FEHLINGS, M. G., TATOR, C. H. & LINDEN, R. D. (1988). The effect of direct-current field on recovery from experimental spinal cord injury. *Journal of Neurosurgery* **68**, 781–792.
- FEHLINGS, M. G., WONG, T. H., TATOR, C. H. & TYMIANSKI, M. (1989). Effect of a direct current field on axons after experimental spinal cord injury. *Canadian Journal of Surgery* **32**, 188–191.
- FIEKERS, J. F. (1983a). Effects of the aminoglycoside antibiotics, streptomycin and neomycin, on neuromuscular transmission. I. Presynaptic considerations. *Journal of Pharmacology and Experimental Therapeutics* **225**, 487–495.
- FIEKERS, J. F. (1983b). Effects of the aminoglycoside antibiotics, streptomycin and neomycin, on neuromuscular transmission. II. Postsynaptic considerations. *Journal of Pharmacology and Experimental Therapeutics* **5**, 496–502.
- FORSCHER, P. (1989). Calcium and phosphoinositide control of cytoskeletal dynamics. *Trends in Neurosciences* **12**, 468–474.
- GOLDBERG, D. J. (1988). Local role of Ca^{++} in formation of veils in growth cones. *Journal of Neuroscience* **8**, 2596–2605.
- HEACOCK, A. M., FISCHER, S. K. & AGRANOFF, B. W. (1987). Enhanced coupling of neonatal muscarinic receptors in rat brain to phosphoinositide turnover. *Journal of Neurochemistry* **48**, 1904–1911.
- HEIDEMANN, S. R., LAMOUREUX, P. & BUXBAUM, R. E. (1990). Growth cone behavior and production of traction force. *Journal of Cell Biology* **111**, 1949–1957.
- HINKLE, L., MCCAG, C. D. & ROBINSON, K. R. (1981). The direction of growth of differentiating neurones and myoblasts from frog embryos in an applied electric field. *Journal of Physiology* **314**, 121–135.
- HOTARY, K. B. & ROBINSON, K. R. (1990). Endogenous electrical currents and the resultant voltage gradients in the chick embryo. *Developmental Biology* **140**, 149–160.
- HOTARY, K. B. & ROBINSON, K. R. (1991). The neural tube of the *Xenopus* embryo maintains a potential difference across itself. *Developmental Brain Research* (in the Press).
- HUME, R. I., ROLE, L. W. & FISCHBACH, G. D. (1983). Acetylcholine release from growth cones detected with patches of acetylcholine receptor-rich membranes. *Nature* **305**, 632–634.
- INGVAR, S. (1920). Reactions of cells to the galvanic current in tissue culture. *Proceedings of the Society for Experimental Biology and Medicine* **17**, 198–199.
- ITO, H. & BASSETT, C. A. L. (1983). Effect of weak, pulsing electromagnetic fields on neural regeneration in the rat. *Clinical Orthopaedics and Related Research* **181**, 283–290.
- JAFFE, L. F. (1977). Electrophoresis along cell membranes. *Nature* **265**, 600–602.
- JAFFE, L. F. & NUCCITELLI, R. (1974). An ultrasensitive vibrating probe for measuring steady extracellular currents. *Journal of Cell Biology* **63**, 614–628.
- JAFFE, L. F. & NUCCITELLI, R. (1977). Electrical controls of development. *Annual Review of Biophysics and Bioengineering* **6**, 445–476.

- JAFFE, L. F. & POO, M.-M. (1979). Neurites grow faster towards the cathode than the anode in a steady field. *Journal of Experimental Zoology* **209**, 115–128.
- JAFFE, L. F. & STERN, C. D. (1979). Strong electrical currents leave the primitive streak of chick embryos. *Science* **206**, 569–571.
- JOSHI, H. C., BAAS, P., CHU, D. T. & HEIDEMANN, S. R. (1986). The cytoskeleton of neurites after microtubule disruption. *Experimental Cell Research* **163**, 233–245.
- KILMER, S. L. & CARLSEN, R. C. (1984). Forskolin activation of adenylate cyclase *in vivo* stimulates nerve regeneration. *Nature* **307**, 455–457.
- LANDMESSER, L. & HONIG, M. (1986). Altered sensory projections in the chick hindlimb following early removal of motoneurons. *Developmental Biology* **118**, 511–531.
- LIPSKY, J. J. & LEITMAN, P. A. (1982). Aminoglycoside inhibition of a renal phosphatidylinositol phospholipase C. *Journal of Pharmacology and Experimental Therapeutics* **220**, 287–292.
- MCCAIG, C. D. (1986). Dynamic aspects of amphibian neurite growth and the effects of an applied electric field. *Journal of Physiology* **375**, 55–69.
- MCCAIG, C. D. (1987). Spinal neurite reabsorption and regrowth *in vitro* depend on the polarity of an applied electric field. *Development* **100**, 31–41.
- MCCAIG, C. D. (1988). Nerve guidance: A role for bioelectric fields? *Progress in Neurobiology* **30**, 449–468.
- MCCAIG, C. D. (1989a). Nerve growth in the absence of growth cone filopodia and the effects of a small applied electric field. *Journal of Cell Science* **93**, 715–721.
- MCCAIG, C. D. (1989b). Studies on the mechanism of embryonic frog nerve orientation in a small applied electric field. *Journal of Cell Science* **93**, 722–730.
- MCCAIG, C. D. (1990a). Nerve branching is induced and oriented by a small applied electric field. *Journal of Cell Science* **95**, 605–615.
- MCCAIG, C. D. (1990b). Nerve growth in a small applied electric field and the effects of pharmacological agents on rate and orientation. *Journal of Cell Science* **95**, 617–622.
- MCCAIG, C. D. & ROBINSON, K. R. (1982). The ontogeny of the transepidermal potential difference in frog embryos. *Developmental Biology* **90**, 335–339.
- McDEVITT, L., FORTNER, P. & POMERANZ, B. (1987). Application of weak electric field to the hindpaw enhances sciatic motor nerve regeneration in the adult rat. *Brain Research* **416**, 308–314.
- MACKLER, S. A. & SELZER, M. E. (1985). Regeneration of functional synapses between individual recognizable neurones in the lamprey spinal cord. *Science* **229**, 774–776.
- MCLAUGHLIN, S. & POO, M.-M. (1981). The role of electroosmosis in the electric field-induced movement of charged macromolecules on the surfaces of cells. *Biophysical Journal* **34**, 85–93.
- MARSH, G. & BEAMS, H. W. (1946). *In vitro* control of growing chick nerve fibres by applied electric currents. *Journal of Cellular and Comparative Physiology* **27**, 137–157.
- MATUS, A., BERNHARDT, R., BODMER, R. & ALAIMO, D. (1986). Microtubule associated protein 2 and tubulin are differently distributed in the dendrites of developing neurons. *Neuroscience* **17**, 371–389.
- NANNMARK, U., BUCH, F. & ALBREKTSSON, T. (1985). Vascular reactions during electrical stimulation. *Acta Orthopaedica Scandinavica* **56**, 52–62.
- OKABE, S. & HIROKAWA, N. (1988). Microtubule dynamics in nerve cells; analysis using microinjection of biotinylated tubulin into PC 12 cells. *Journal of Cell Biology* **107**, 651–664.
- OSTER, G. F. (1984). On the crawling of cells, *Journal of Embryology and Experimental Morphology* **83**, suppl., 329–364.
- PATEL, N. B. & POO, M.-M. (1982). Orientation of neurite growth by extracellular electric fields. *Journal of Neuroscience* **2**, 483–496.
- PATEL, N. B. & POO, M.-M. (1984). Perturbation of neurite growth by pulsed and focal electric fields. *Journal of Neuroscience* **4**, 2939–2947.
- POLITIS, M. J. & ZANAKIS, M. F. (1988a). Short term efficacy of applied electric fields in the repair of the damaged rat spinal cord: Behavioral and morphological results. *Neurosurgery* **23**, 582–588.
- POLITIS, M. J. & ZANAKIS, M. F. (1988b). Treatment of the damaged rat hippocampus with a locally applied electric field. *Experimental Brain Research* **71**, 223–226.
- POLITIS, M. J. & ZANAKIS, M. F. (1989). The short-term effects of delayed application of electric fields in the damaged rat spinal cord. *Neurosurgery* **25**, 71–75.
- POLITIS, M. J. & ZANAKIS, M. F. ALBALA, B. J. (1988a). Facilitated regeneration in the rat peripheral nervous system using applied electric fields. *Journal of Trauma* **28**, 1375–1381.
- POLITIS, M. J., ZANAKIS, M. F. & ALBALA, B. J. (1988b). Mammalian optic nerve regeneration following the application of electric fields. *Journal of Trauma* **28**, 1548–1552.

- POMERANZ, B. (1986). Effects of applied fields on sensory nerve sprouting and motor nerve regeneration in adult rats. In *Ionic Currents in Development*, ed. NUCCITELLI, R., pp. 251–260. Alan R. Liss, Inc., New York.
- POMERANZ, B., MULLEN, M. & MARKUS, H. (1984). Effect of applied electrical fields on sprouting of intact saphenous nerve in adult rat. *Brain Research* **303**, 331–336.
- POO, M.-M. & ROBINSON, K. R. (1977). Electrophoresis of concanavalin A receptors in the embryonic muscle cell membrane. *Nature* **265**, 602–605.
- RAJNICEK, A. M., CORK, R. J. & ROBINSON, K. R. (1991). Substrate-dependent effects of electrical fields on developing *Xenopus* neurones *in vitro*. *Journal of Cell Science* (in the Press).
- RAPER, J. A. & KAPFHAMMER, J. P. (1990). The enrichment of a neuronal growth cone collapsing activity from embryonic chick brain. *Neuron* **2**, 21–29.
- RIVLIN, A. S. & TATOR, C. H. (1977). Objective clinical assessment of motor function after experimental spinal cord injury in the rat. *Neurosurgery* **47**, 577–581.
- ROBINSON, K. R. (1983). Endogenous electric current leaves the limb and pre-limb regions of the *Xenopus* embryo. *Developmental Biology* **97**, 203–211.
- ROBINSON, K. R. (1985). The responses of cells to electrical fields: A review. *Journal of Cell Biology* **101**, 2023–2027.
- ROBINSON, K. R. & McCAG, C. D. (1980). Electrical fields, calcium gradients and cell growth. *Annals of the New York Academy of Sciences* **339**, 132–138.
- ROBINSON, K. R. & STUMP, R. F. (1984). Self-generated electrical currents through *Xenopus* neurulae. *Journal of Physiology* **352**, 339–352.
- ROCHLIN, M. W. & PENG, H. B. (1989). Localization of intracellular proteins at acetylcholine receptor clusters induced by electric fields in *Xenopus* muscle cells. *Journal of Cell Science* **94**, 73–83.
- ROMAN, G. C., STRAHLENDORF, H. K., COATES, P. W. & ROWLEY, B. A. (1987). Stimulation of sciatic nerve regeneration in the adult rat by low-intensity electric current. *Experimental Neurology* **98**, 222–232.
- ROEDERER, E., GOLDBERG, N. H. & COHEN, M. J. (1983). Modification of retrograde degeneration in transected spinal axons of the lamprey by applied DC current. *Journal of Neuroscience* **3**, 153–160.
- ROSS, W. N., ARECHIGA, H. & NICHOLLS, J. G. (1988). Influence of substrate on the distribution of calcium channels in identified leech neurones in culture. *Proceedings of the National Academy of Sciences of the USA* **85**, 4075–4078.
- SCHNELL, L. & SCHWAB, M. E. (1990). Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. *Nature* **343**, 269–272.
- SILVER, R. A., LAMB, A. G. & BOLSOVER, S. R. (1989). Elevated cytosolic calcium in the growth cone inhibits neurite elongation in neuroblastoma cells: Correlation of behavioural states with cytosolic calcium concentration. *Journal of Neuroscience* **9**, 4007–4020.
- SILVER, R. A., LAMB, A. G. & BOLSOVER, S. R. (1990). Calcium hotspots caused by L-channel clustering promote morphological changes in neuronal growth cones. *Nature* **343**, 751–754.
- STEWART, R. (1990). Melanocortins, electrical fields and neuronal growth. B.Sc. Thesis, University of Aberdeen.
- STOLLBERG, J. & FRASER, S. (1988). Acetylcholine receptors and concanavalin A-binding sites on cultured *Xenopus* muscle cells: Electrophoresis, diffusion and aggregation. *Journal of Cell Biology* **106**, 1723–1734.
- STOLLBERG, J. & FRASER, S. (1990a). Local accumulation of acetylcholine receptors is neither necessary nor sufficient to induce cluster formation. *Journal of Neuroscience* **10**, 247–255.
- STOLLBERG, J. & FRASER, S. (1990b). Acetylcholine receptor clustering is triggered by a change in the density of a nonreceptor molecule. *Journal of Cell Biology* **111**, 2029–2039.
- STRAND, F. L., ROSE, K. J., KING, J. A., SEGARRA, A. C. & ZUCCARELLI, L. A. (1989). ACTH modulation of nerve development and regeneration. *Progress in Neurobiology* **33**, 45–85.
- STRAUTMAN, A. F., CORK, R. J. & ROBINSON, K. R. (1990). The distribution of free calcium in transected spinal axons and its modulation by applied electrical fields. *Journal of Neuroscience* **10**, 3564–3575.
- STUMP, R. F. & ROBINSON, K. R. (1983). *Xenopus* neural crest cell migration in an applied electric field. *Journal of Cell Biology* **197**, 1226–1233.
- TOSNEY, K. W. & LANDMESSER, L. T. (1985). Growth cone morphology and trajectory in the lumbrosacral region of the chick embryo. *Journal of Neuroscience* **5**, 2345–2358.
- TOSNEY, K. W., WANATABE, M., LANDMESSER, L. T. & RUTISHAUSER, U. (1986). The distribution of NCAM in the chick hindlimb during axonal outgrowth and synaptogenesis. *Developmental Biology* **114**, 437–452.

- WALLACE, M. C., TATOR, C. H. & PIPER, I. (1987). Recovery of spinal cord function induced by direct current stimulation of the injured rat spinal cord. *Neurosurgery* **20**, 878-884.
- WINKEL, G. K. & NUCCITELLI, R. (1989). Large ionic currents leave the primitive streak of the 7.5 day mouse embryo. *Biological Bulletin* **176** (S), 110-117.
- YOUNG, S. H. & POO, M.-M. (1983). Spontaneous release of transmitter from growth cones of embryonic neurones. *Nature* **305**, 634-637.
- ZANAKIS, M. F. (1988). Regeneration in the mammalian nervous system using applied electric fields: A literature review. *Acupuncture and Electro-Therapeutics Research, International Journal* **13**, 47-57.