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DYNAMIC ASPECTS OF AMPHIBIAN NEURITE GROWTH AND THE EFFECTS OF AN APPLIED ELECTRIC FIELD

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SUMMARY

1. The dynamics of growth of earliest spinal neurites from *Xenopus laevis* have been studied *in vitro* in the presence and absence of an applied d.c. electric field.

2. Control and cathode-directed neurites grew at a rate of about 30 μm/h: growth of anodal-facing neurites was 8 times slower.

3. Periods of arrested growth were common in cultured neurones; these lasted 2–3 times longer in an applied electric field.

4. The likelihood and the severity of neurite reabsorption was greatest in neurites directed towards the anode.

5. Many neurites turned to direct their growth towards the cathode. As this happened their rate of growth increased 2–3-fold.

6. The electric field further shaped neurite morphology by increasing the number of filopodia at the growth cone and by increasing the number of cytoplasmic spines along a neurite shaft. The electric field induced an asymmetry in the distribution of these cytoplasmic projections; greater numbers being found on the cathodal-facing than on the anodal-facing side.

7. Implications of these data for nerve growth in development and in regeneration are discussed.

INTRODUCTION

Experimental support for the long-held notion that an electric field can guide axon growth was obtained first by Ingvar (1920) but has been challenged, most influentially by Weiss (1934). Recently several well-controlled studies have established beyond doubt that the neurites of chick sensory ganglia and of amphibian neural tube cells direct their growth preferentially towards an external cathode in a steady d.c. electric field (Jaffe & Poo, 1979; Hinkle, McCaig & Robinson, 1981; Patel & Poo, 1982). Sources of steady endogenous electrical fields in embryos include avian and amphibian epidermis and avian neural tube (Jaffe & Stern, 1979; Robinson & Stump, 1984; Erickson & Nuccitelli, 1984). The magnitude and distribution of the electric fields set up within the confined tissue spaces of the embryo are not known. Pulsed and focal electrical fields have been shown to be similarly effective in guiding neurite

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growth (Patel & Poo, 1984). Chick sensory ganglion neurites grow faster towards the cathode than towards the anode (Jaffe & Poo, 1979). This information was obtained from measurements of a ganglion explant at a single time point. Little else is known about the responses of anodal-facing neurites. I have studied the growth responses of individual anodal and cathodal-facing neurites over time in an electric field. In some cases this allows comparison of growth rates of anodal and cathodal-facing processes from the same cell body.

The use of d.c. electric fields to enhance repair of damaged peripheral nerve (Ziegenbein, Westerman, Silberstein, Krantz, Cassell, Finkelstein & Bettess, 1983) or of spinal cord (Borgens, Roederer & Cohen, 1981; Roederer, Goldberg & Cohen, 1983) has begun to be investigated, in the latter case using larval lampreys, with encouraging positive results. Some suppression of growth and reabsorption of anodal-facing processes may occur in an electric field (e.g. Hinkle *et al.* 1981). It is important therefore to have details on the time course and the rate at which these and other events occur.

The mechanisms responsible for electrical guidance of neurites remain unclear but most likely involve molecular events occurring at the growth cone. I have studied growth cones by time-lapse photomicrography and report that in neurites destined to turn towards the cathode, greater numbers of filopodia are present on the cathodal-facing than on the anodal-facing side of the growth cone tip.

METHODS

Embryos of *Xenopus laevis* at stage 19/20 (Nieuwkoop & Faber, 1956) were used. The method for obtaining cultures of spinal neurites was similar to that reported previously (Hinkle *et al.* 1981). Briefly, the dorsal third of an embryo was excised in Steinberg solution (composition in mM: NaCl, 58; KCl, 0·67; $Ca(NO_3)_2$, 0·44; $MgSO_4$, 1·3; Tris, 4·6; pH 7·9) and transferred to a Steinberg solution containing 1 mg/ml of collagenase (Type 1; Sigma Ltd.) for 10–15 min. This facilitated dissection of the clean neural tube, the top two-thirds of which was pipetted into a Ca^{2+}/Mg^{2+}-free Steinberg solution containing 0·4 mM-EDTA for 20–30 min. The dissociated tissue was drawn up through a fine, flame-drawn pipette and dispersed into culture medium in a Falcon plastic tissue culture dish (Type 3003F). Culture medium was Steinberg solution supplemented with 20% v/v Liebowitz L15 solution, 1% v/v Foetal Bovine Serum and 2% v/v penicillin (5000 i.u./ml)/streptomycin (5000 μg/ml) (all from Flow Laboratories, Irvine) and used at pH 7·9. Total ionic strength was 81 mM. This solution lay in a trough formed by two parallel strips of No. 1 cover glass (64 × 10 mm) glued about 1 cm apart. After 20 min attachment time, a roof of No. 1 cover glass was applied and sealed with silicone grease to complete the chamber through which constant current would be passed. Chamber dimensions were 64 × 10 × 0·5 mm.

Cells were allowed to develop for about 4–5 h (first outgrowths appear after 3–4 h) before the electric field was applied to experimental dishes through a pair of agar–salt bridges, long enough (15 cm) to prevent diffusion of electrode products into the culture chambers. Three groups of cells were investigated: (1) controls; (2) those with anodal-facing neurites; (3) those with cathodal-facing neurites. For cells in an electric field control values were established in a 10–20 min period before the field was switched on. A constant field of variable strength (30–233 mV/mm), measured directly at the end of the experiment, was applied across the chambers for the duration of the observation period, 2–4 h, and photographs taken, under phase contrast, of individual neurites as they grew over time. Photographs were printed at a final magnification of 545 times and all measurements of angles and lengths made from these prints. Neurite length was measured from the cell body to the tip of the phase-dark growth cone area, but did not include the length of any filopodia or lamellipodia which projected along the direction of growth. The number of filopodia at the growth cone and the number of spines projecting from the shaft of the neurite were counted by examining

each print under a dissecting microscope (Fig. 1 *A*). Angles of neurite projection were measured as shown in Fig. 1 *B* and *C*.

At stage 19/20 the first motor nerves are emerging from the neural tube and will innervate the myotomes in a rostro-caudaul sequence (Blackshaw & Warner, 1976; Kullberg, Lentz & Cohen, 1977). Sensory nerve processes do not appear until stage 22–24 (Roberts & Taylor, 1982; Taylor & Roberts, 1983), 3–7 h after the motor nerves at 22 °C (Nieuwkoop & Faber, 1956). The present

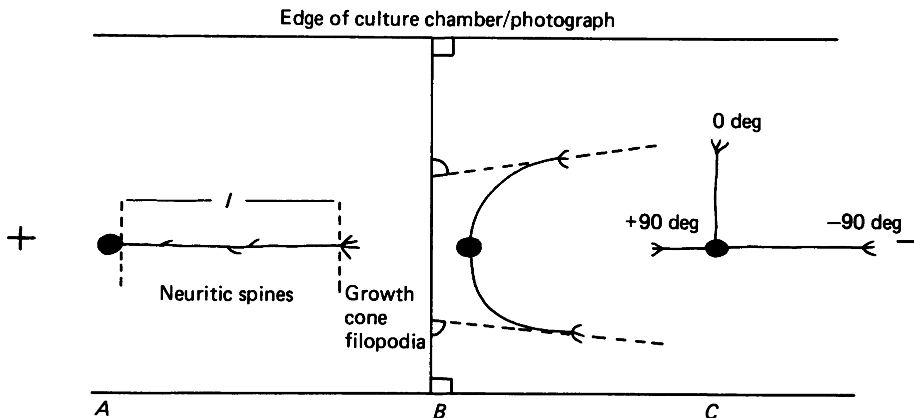

Fig. 1. Schematic representation of method of measuring *A*, neurite length *l*, number of filopodia and number of neuritic spines; *B* and *C*, angle of neurite growth relative to perpendicular: towards anode (positive values), towards cathode (negative values).

observations were carried out at room temperature 18–20 °C on the first nerves appearing in tissue culture, that is about 3–4 h after seeding. Observation continued for 2–4 h. It is likely, since development of these cells *in vitro* follows a normal or slightly delayed time course over *in vivo* development (Jones & Elsdale, 1963), that most neurites examined would be motor outgrowths, with some of the most mature sensory outgrowths also being included.

RESULTS

In these cultures, 50–80 % of cells with neurites either projected these directly towards the cathode, or the neurites turned to grow towards the cathode. To a limited extent this response of the population of cells was a function of field strength (Hinkle *et al.* 1981).

Mean rate of neurite growth

In control cultures neurites extended at a mean rate of about 30 μm/h (n = 85 neurites). In an applied electric field, growth towards the cathode took place at much the same rate, 35 ± 4 μm/h (n = 44 neurites). The population of neurites growing towards the anode, however, progressed much more slowly, at only 4 ± 3 μm/h (n = 50 neurites; Fig. 2). When expressed as a function of electric field strength, no obvious trend emerged (Table 1). The rate of growth of anodal-facing neurites was suppressed substantially (50 %) within 10 min of field application. Thereafter a reduction in over-all rate occurred gradually over the next 2 h (Fig. 3).

This response shown by the total population of neurites was seen again when the rate of growth of anodal- and cathodal-projecting neurites from the same cell body

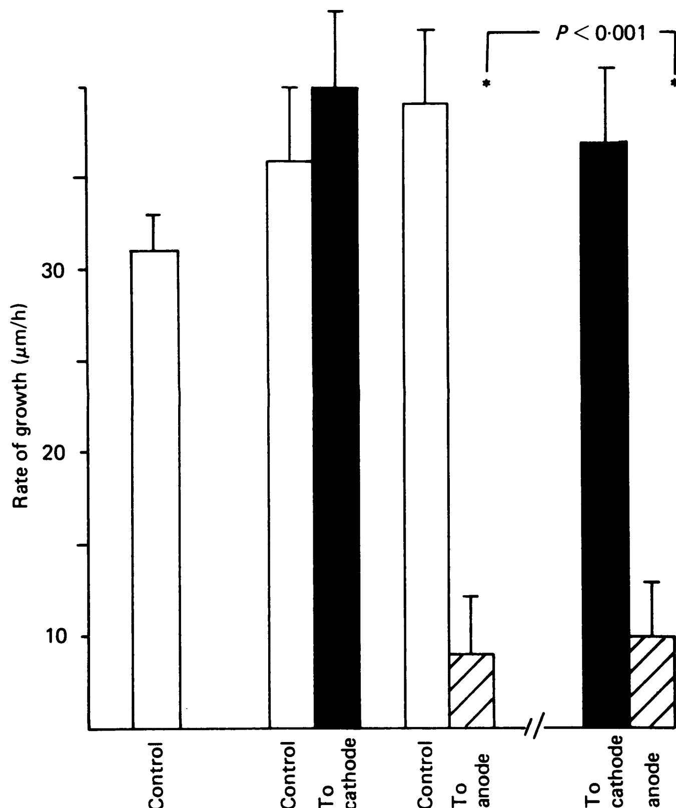

Fig. 2. Rate of growth of neurites *in vitro*. Control ($n = 85$ neurites: 43 neurones). To cathode + pre-field control ($n = 44$ neurites: 38 neurones). To anode + pre-field control ($n = 50$ neurites: 44 neurones). To cathode/to anode, same cell body ($n = 35$ neurones).

was compared. Growth towards the cathode occurred at 32 ± 4 μm/h and towards the anode at 5 ± 3 μm/h ($n = 35$ neurones; Fig. 2).

The values for growth rates of individual neurites at different times showed considerable variation, indicating that neurites could speed up or slow down quite dramatically during the course of normal growth *in vitro*. As much as a 9-fold difference in rate of growth was seen in one control case; the neurite growing initially at 8 μm/h, then at 72 μm/h 30 min later. Variation, however, was usually about 4-fold. This variation was unrelated to time of observation and thus to the length of the neurite. However, high rates of growth often were associated with changes in the direction of neurite growth (see (*c*) below).

Variability of rate of extension

(*a*) *Periods of arrested growth*. Roughly one in four cultured neurones showed periods in which no growth took place. This was true of control neurites and of neurites projecting to either the anode or to the cathode. Table 2 shows, however, that periods of arrested growth were 2–3 times longer when neurones were exposed to an electric field than in controls.

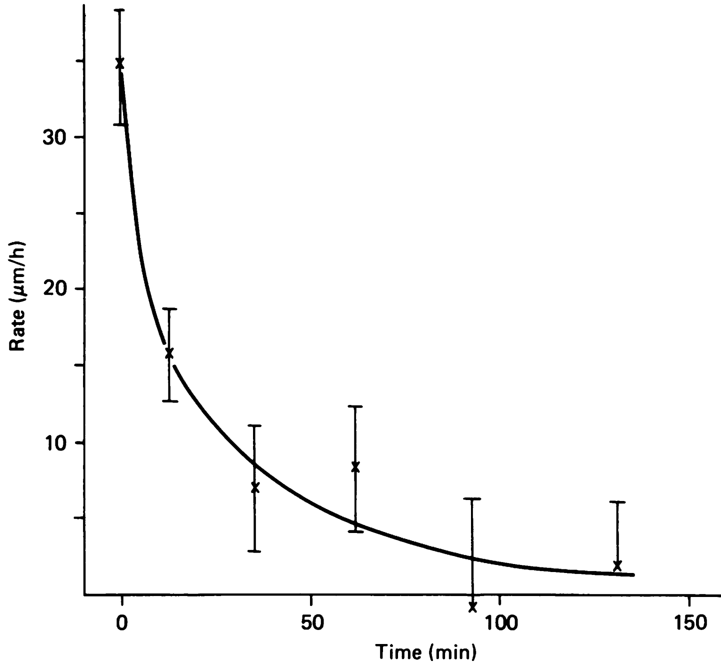

Fig. 3. Time course of suppression of growth rate towards the anode. Mean neurite growth rate is plotted at different times after the onset of the electric field. Number of neurites included is between 53 at 0 min and 33 at 131 min.

TABLE 1. Rate of growth of cathodal-directed and anodal-directed neurites at various strengths of electric field. Field applied for 2–3 h

Field strength (mV/mm)	Number of neurites	Mean angle relative to perpendicular (deg)	Length (μm) Pre-experiment	Length (μm) Post-experiment	Rate (μm/h) Pre-experiment	Rate (μm/h) During experiment
		Cathodal directed				
233	11	−7→−54	59±6	155±15	30±4	41±6
120–153	19	−38→−66	69±6	156±27	36±6	22±7
30–90	16	−44→−67	56±6	167±18	29±7	43±5
		Anodal directed				
233	4	+84→+80	66±9	84±11	37±5	4±4
120–153	26	+70→+65	74±5	82±11	34±4	−1±6
30–90	16	+70→+62	51±5	89± 9	37±8	10±3

(*b*) *Incidence of reabsorption.* In previous work some anodal-directed neurites were reabsorbed although it was not determined whether this was caused by the electric field (Hinkle *et al.* 1981). Fig. 4 shows that 39 % of neurites in control cultures (33/85) showed some degree of reabsorption within a 2–4 h period and lost on average 14 ± 2 μm of neurite length. A significantly greater incidence of reabsorption was seen in neurites projecting towards the anode, 67 % (32/52), and there was a greater net loss in neurite length compared with controls, 23 ± 3 μm (Pl. 1 *A–D*). By contrast the

incidence of reabsorption in cathodal-projecting neurites, 16% (13/61), was much lower than for either controls or for anodally directed neurites and the mean length of neurite reabsorbed was less than for anodally directed neurites (12 ± 3 μm: $P < 0.05$). These events showed no clear relationship to the size of the electric field used.

TABLE 2. Incidence and duration of periods of arrested neurite growth

	Number of neurites	Percentage showing no growth period	Duration of arrested growth (min)
Controls	85	30	22 ± 2
To anode	53	23	45 ± 7
To cathode	61	21	60 ± 17

$P < 0.001$ (controls vs. to cathode)

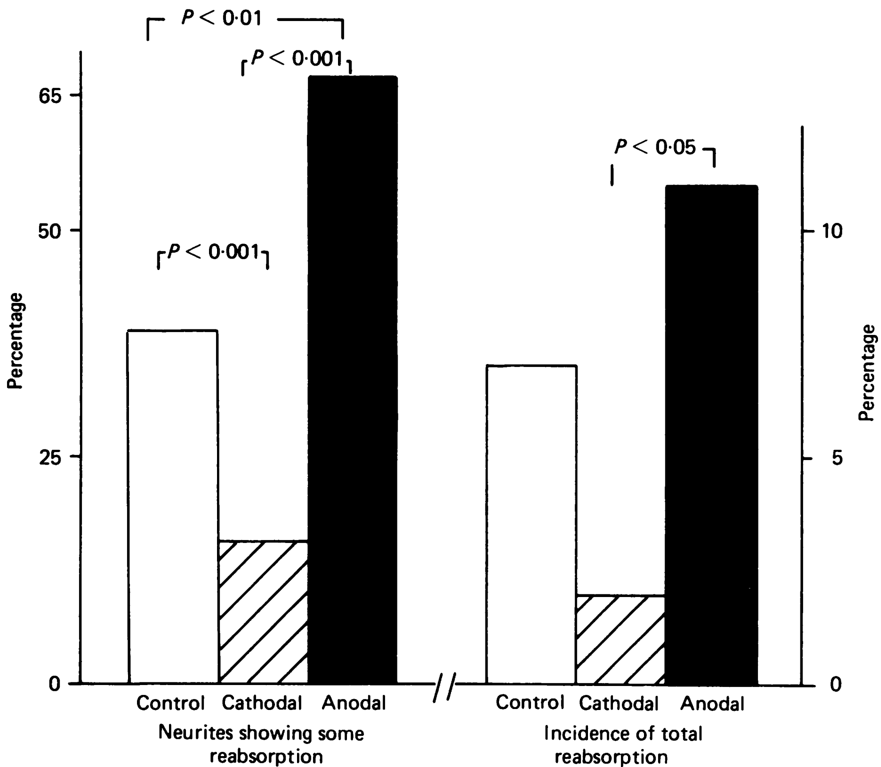

Fig. 4. Incidence of neurite reabsorption *in vitro*. Control (total $n = 85$ neurites). Anodal directed (52 neurites). Cathodal directed (61 neurites).

Some neurites were reabsorbed completely and were included in the above analysis. The data for these alone appears also in Fig. 4 and shows a similar trend with reabsorption being much less common in cathodal-directed neurites than in anodal-directed neurites. In four cells, immediately following the total reabsorption of an anodal-directed neurite, the polarity of the electric field was reversed. In two of the cells a neurite reappeared from the same site and grew for 10 μm and 90 μm

respectively during the subsequent 1 h. No regrowth was seen in the other two cells. A resprouting of neurites was never seen in the six (out of eighty-five) control cells which showed total reabsorption of a neurite.

Periods of reabsorption in general began about 1 h after exposure to the electric field (60 ± 6 min: $n = 35$) and lasted for $\frac{1}{2}$ h (97 ± 8 min) although in the six anodal-facing

TABLE 3. Incidence of complete neurite reabsorption

	Number reabsorbed / Total number	Mean angle (deg)	Length reabsorbed (μm)	Control rate (μm/h)	Mean rate of reabsorption (μm/h)
Control	6/85	—	36 ± 5	-19 ± 4	-41 ± 6
Anode directed	6/53	$+78 \pm 5$	52 ± 4	$+42 \pm 8$	-57 ± 12

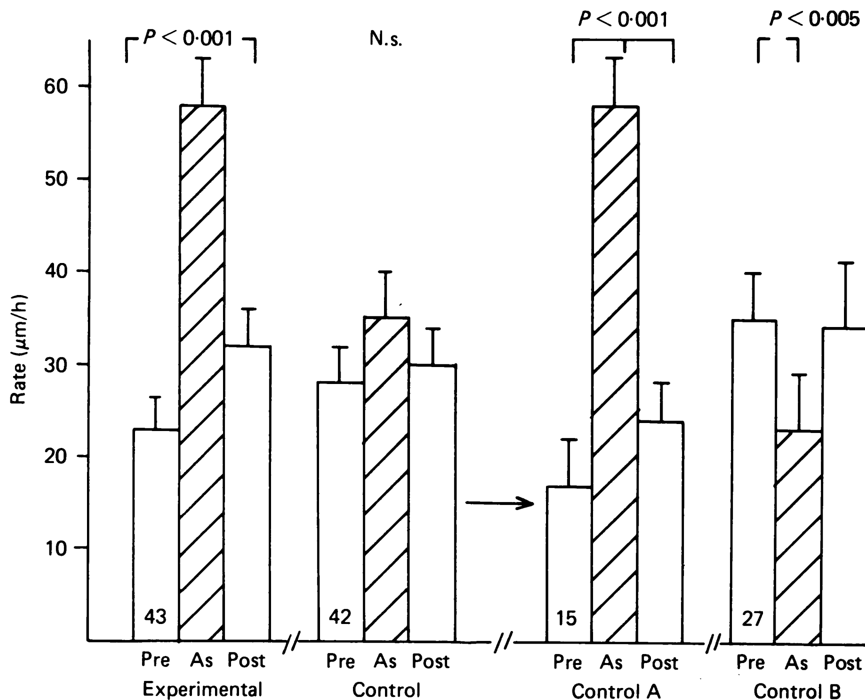

Fig. 5. Rate of growth of neurites 10–20 min before a turn, for 10–30 min during a turn and during 20–40 min after a turn in controls or towards the cathode in an applied electric field. Towards the cathode (experimental): $n = 43$ neurite turns. Control: $n = 42$ turns.

neurites showing total reabsorption the mean onset time was earlier; 26 ± 9 min. The mean rate of neurite reabsorption was around -25 μm/h for all control, or cathodal, or anodal-facing neurites. In both controls and anodally directed cases of total reabsorption, mean rates of reabsorption were considerably higher (Table 3). This seemed to result from increasingly high rates of reabsorption as neurites became smaller and smaller. Table 3 also shows that the six neurites that were reabsorbed totally in controls were reabsorbing at the onset of observations. The anodal-facing

neurites, however, were growing at normal rates before the field was switched on and represent field-induced reabsorption.

(*c*) *Rate of growth on turning.* Large numbers of neurites (33–46 %) turned to grow towards the cathode in an electric field. As this occurred, most showed a transient increase in rate of extension. Fig. 5 shows a 2·5-fold increase in growth rate of neurites turning through 55 ± 5 deg over 36 ± 4 min (i.e. at 1·5 deg/min). This pattern of a transient increase in growth rate was observed in 86 % of cathode-directed turns (38/44 neurite turns: 33 neurites).

Turning generally began very soon after exposure to an electric field, in 75 % of the cases at a mean time of 13 ± 3 min. The other neurites apparently grew unaffected by the electric field for 1–1½ h before beginning to respond by turning towards the cathode.

In control cultures, neurites which changed direction tended to turn through smaller angles than those responding to an electric field 38 ± 2 deg in 24 ± 2 min ($n = 42$), although the rate of angular displacement was the same. Considered as a single homogeneous population of cells, no net increase in growth rate was seen during these turns (Fig. 5). However, two populations of neurones appear to exist in controls: those which do and those which do not grow faster as they turn. One-third of neurites showed a transient increased rate of growth as they turned (15/42: Fig. 5: control A), while the other two-thirds if anything slowed down on turning (Fig. 5: control B).

The growth cone

I have made a light microscopic study of the distribution of filopodia at the neurite growth cone over time in the presence and in the absence of an electric field (30–233 mV/mm). Multiple photographs of the same growth cone (usually six or seven) were taken over a period of 1½–2 h and the number of filopodia counted from prints at a magnification of 545 times, viewed under a dissecting microscope. The number of filopodia per growth cone varied considerably. Nevertheless, neurites exposed to an electric field possessed 30–50 % more filopodia than were found on control neurites. This was true irrespective of whether the neurite projected towards the cathode or to the anode (Fig. 6). Comparing the number of filopodia on the same growth cone before and after switching on the electric field, revealed that 85 % of cathodally directed neurites showed a field-induced increase (28/33), with 63 % of anodally directed neurites (27/43) similarly increasing their number of filopodia (Fig. 6). This was not the result of the length of time in culture, nor was there a clear relationship to field strength.

Distribution of filopodia

To assess the distribution of filopodia, each control growth cone was bisected by a line continuing along the direction of growth (Fig. 1) and the number of filopodia projecting to the left and to the right of this line was counted. Filopodia were distributed symmetrically on growth cones from all control neurites (Fig. 7). Sixteen neurites were photographed while growing perpendicular (0 deg), to the lines of force of the electric field and the numbers of filopodia on the anodal and cathodal sides of a vertical line bisecting the growth cone were counted. Twice as many filopodia

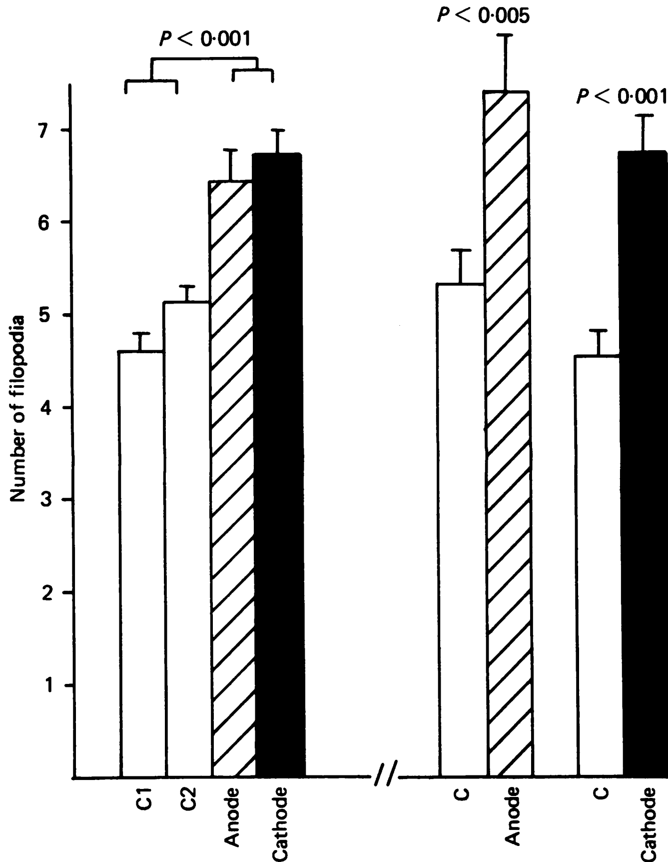

Fig. 6. Number of filopodia per growth cone on control neurites (C) and on neurites projecting towards the anode or cathode. Controls C1 (n = 68 neurites: 32 neurones). C2 pre-field controls (n = 76 neurites: 42 neurones). To cathode: (n = 38 neurites: 34 neurones). To anode: (n = 43 neurites: 40 neurones). The field-induced increase in number of filopodia shown by 63 % of anodally directed neurites and by 81 % of cathodally directed neurites also is shown at right.

projected towards the cathode than towards the anode (Fig. 7; Pl. 1 *F–H*). In thirteen of these cases, turning had not begun, i.e. initial growth was perpendicular to the long axis. An asymmetry of filopodia generally was an early response to the electric field occurring in 16/18 cases within 14 ± 3 min. In two other cases no asymmetry was seen until $1\frac{1}{2}$ h and $3\frac{1}{2}$ h in the field. In all cases the appearance of growth cone asymmetry was coincident with the onset of turning.

Neurites turning in control cultures also possessed about twice as many filopodia on the side to which they were turning (Fig. 7). This also was evident as turning was beginning in 75 % of cases (12/16).

Neuritic spines

At several points along the shaft of a neurite filopodial-like projections are seen and may help in anchoring the neurite to its substrate. More of these are present (64 %)

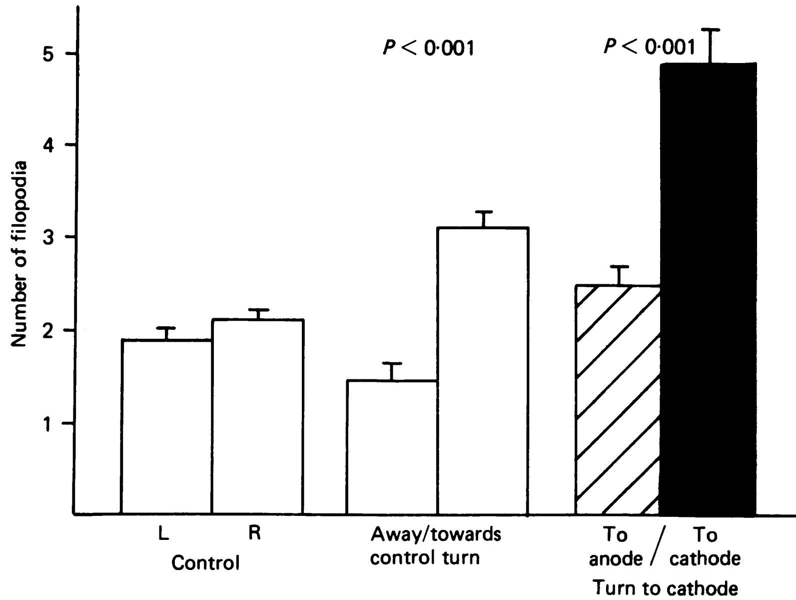

Fig. 7. Spatial distribution of growth cone filopodia on all control neurites; on control neurites as they turn, and on neurites turning towards the cathode. Control (number of growth cones = 35). L = left; R = right. Control turns (n = 12). Turns to cathode (n = 16).

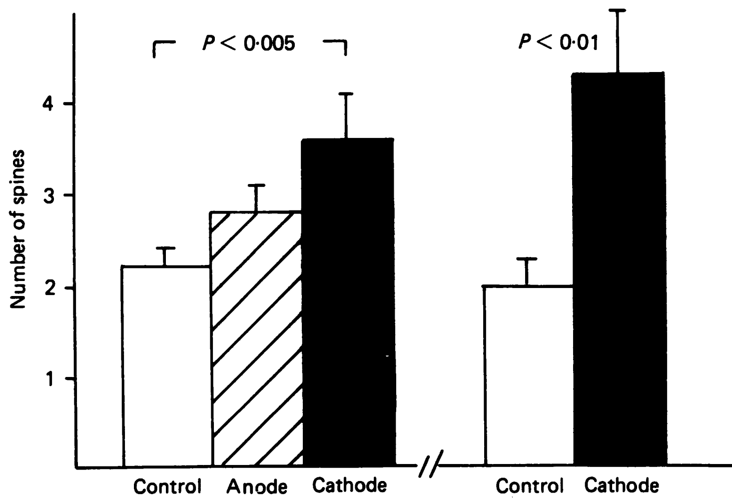

Fig. 8. Number of neurite spines (cytoplasmic projections) on control neurites and on anodal and cathodal-projecting neurites. Control (75 neurites: 42 neurones). Neurites projecting to anode (44 neurites: 40 neurones). Neurites projecting to cathode (35 neurites: 34 neurones). The field-induced increase occurring in 61 % of cathodal-directed neurites also is shown at right.

on neurites projecting towards the cathode than on control neurites (Fig. 8; Pl. 1*I* and *J*). Comparing the number of spines on the same neurite before and after switching on an electric field shows that 61 % (19/31) show an increase over their own control value (Fig. 8). Analysis of the distribution of these projections along the

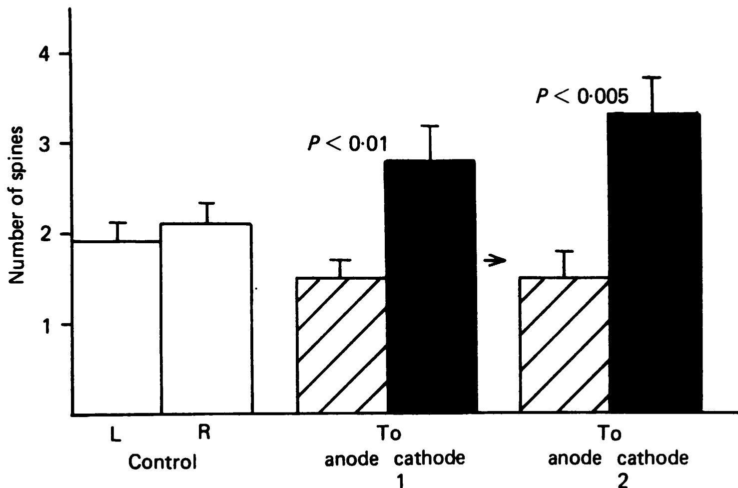

Fig. 9. Distribution of neurite spines on control neurites and on neurites exposed to an electric field. Controls, to left/to right of shaft (n = 38 neurites: 21 neurones). To anode/to cathode 1 (n = 21 neurites: 16 neurones). To anode/to cathode 2 (75 % show increase over own control) (n = 16 neurites).

neurite shaft reveals that in control cultures as many are found on the left of the shaft as are found on the right of the shaft (Fig. 9). In an electric field the number of spines on the cathodal-facing side and on the anodal-facing side of neurites growing within 30 deg to the perpendicular were counted. Fig. 9 shows that roughly twice as many spines were present on the cathodal side of the shaft than were present on anodal-facing sides. This was true in 75 % of cases (16/21; Fig. 9).

DISCUSSION

In a series of control experiments, it was shown that the orienting responses of neurites in an electric field for 16–20 h were primary responses to the electric field and not secondary responses to physical or chemical changes induced in the substrate by the electric field (Hinkle *et al.* 1981). These controls were not repeated here.

Both control and cathodally directed neurites grew at the same rate of around 30 μm/h. This agrees with previous estimates of nerve growth in development and regeneration: *in vivo* and *in vitro* (Harrison, 1910; Speidel, 1941; Hughes, 1953). A 30 % increase over control values in growth rate of amphibian neurites towards the cathode was reported using field strengths of 250 mV/mm (Patel & Poo, 1982). No significant increase was apparent in this work at 233 mV/mm. Growth of anodal-facing neurites was suppressed severely, an effect seen as soon as the field was switched on. Also, reabsorption of anodal-facing neurites was more common and more severe than

in control or cathode-directed neurites. Possible causes of these events include (1) the electric field directly inhibiting fast axonal transport of axolemmal components, (2) a block on the incorporation of these into new membrane, (3) the electric field inhibiting cytoskeletal assembly/promoting disassembly. There is as yet no direct evidence for any of these proposals. However, an applied electric field could influence these events in one of three ways. (a) It can be shown that approximately two-thirds of an external electric field will be transmitted across the membrane and exist as an internal voltage drop within a long, slender cytoplasmic tube ($100 \times 0{\cdot}3\ \mu m$), a reasonable estimate for a filopodium (see Jaffe & Nuccitelli, 1977). Thus a direct effect of the electric field on the distribution and function of growth cone organelles could occur. (b) The redistribution by electrophoresis of charged integral membrane components proximally towards the cell body and hence the cathode (Poo & Robinson, 1977; McLaughlin & Poo, 1981; Patel & Poo, 1982) could inhibit microfilament extension and filopodia protrusion. (c) Field-induced changes in membrane potential, specifically hyperpolarization of the anodal-facing membrane. This is thought likely to raise intracellular [Ca^{2+}] and to cause contraction of the cytoskeleton and retraction of anodal-facing protrusions in neural crest cells (Cooper & Keller, 1984). Similar events may bring about retraction of anodal-facing neurites. Extreme increases in Ca^{2+} influx also cause depolymerization of microtubules and breakdown of neurofilaments (Wilson, Bryan, Ruby & Mazia, 1970; Gilbert, Newby & Anderton, 1975) and are thought to be responsible predominantly for Wallerian degeneration in rat peripheral nerve (Schlaepfer, 1974). These events also may occur in neurites facing an anode for long periods.

Most neurites display brief periods of arrested growth lasting 10–20 min (e.g. Argiro, Bunge & Johnson, 1984). However, an applied electric field prolonged these periods 2–3-fold in both anodally and cathodally directed spinal neurites. The mechanism(s) controlling interruptions in growth, normally, are not known, but may involve transient disruption of some aspect of new membrane transport, incorporation or cytoskeletal assembly. This finding provides further indirect evidence to add to that offered by the slowing and retraction of anodal-directed neurites, that an electric field can influence profoundly one or more of these same events, even in neurites projecting towards the cathode.

The electric field also caused most cells to show a transient increase in growth rate as they turned towards the cathode. A much smaller proportion of control neurites also exhibit this behaviour. This implies addition of new membrane at a much greater rate than normal as the neurite turns to lie more and more along the lines of force of the electric field. Since the phenomenon was transient, lasting only 20 or 30 min, it is not known whether it could have occurred without an increase in fast axonal transport. There is probably a pool of membrane components at the growing tip, but the time needed to exhaust this is unknown.

Most neurites turning towards the cathode began to do so within about 10 min. Filopodial asymmetry at the growth cone also was a very early response to the electric field and was coincident with turning. Filopodia attached to a substrate generate tension in the neurite and the distribution of tension at the growth cone determines the direction of neurite growth (Bray, 1979). More tension is likely to be produced on the cathodal side of the growth cone by the greater numbers of filopodia than on

the anodal side. The same mechanism of turning appears to occur in controls although the cause of growth cone asymmetry is unknown. A likely explanation for field-induced growth cone asymmetry and hence turning may involve lateral electrophoresis towards the cathode of integral membrane proteins embedded in the filopodia of the growth cone. These appear to be tethered to the cytoplasmic microfilament lattice (Rees & Reese, 1981) which may be pulled over towards the cathode by the movements of membrane proteins. This would favour cathodal-directed orientation of filopodia. Concanavalin A receptors accumulate on the cathodal-facing membranes of neurones, while pre-incubation with concanavalin A, which immobilizes its membrane receptors, before applying an electric field, prevents oriented neurite outgrowth (Patel & Poo, 1982). The rapid onset of turning and filopodial asymmetry fit this hypothesis since a steady-state redistribution of integral membrane proteins is achieved after 10 min in a field of 100 mV/mm (Poo, 1981).

Twice as many filopodia were present on growth cones in an electric field than on controls. If this occurs *in vivo*, then a localized electric field present at a decision point on a neurite pathway could enhance the ability of the growth cone to sense and respond to electrical, chemical or physical cues present in the micro-environment, by increasing the number of filopodia at the growth cone.

Other field-induced morphological changes may have important implications for the ultimate structural form adopted by a neurite. The increased number of neuritic spines and their predominant distribution on the cathodal side suggest that an electric field can disrupt the cytoskeleton locally at points along the length of a neurite and direct the formation of cytoplasmic protrusions. Spines often form the basis of a lateral branch to a neurite (Pl. 1 *K* and *L*). Growth cones can be induced pharmacologically to form along the length of a neurite (Bray, Thomas & Shaw, 1978). A focally applied electric field may therefore induce branching from any point along a neurite. This could be of importance in the light of observations of changes in dendritic branching occurring in adult neurones *in vivo* (Purves & Hadley, 1985).

Clearly a d.c. electric field can guide neurite growth but may in addition influence neuronal architecture by determining levels of reabsorption of selected neurites and possibly by determining the foundations for branch points along a neurite.

The time course of neurite retraction is relevant to experiments which use electric field to improve peripheral nerve or spinal cord regeneration. Larval lampreys spontaneously regenerate a severed spinal cord (Selzer, 1978). D.c. stimulation in one direction for 5 days improved regeneration across a lesion after 2 months and reduced the extent of die-back of axons normally present after 5 days (Borgens *et al.* 1981; Roederer *et al.* 1983). Since axons course in both directions, constant stimulation in one direction may cause retraction or reabsorption of many anodal-facing axons. The spinal neurites of *X. laevis* begin to die back within $1-1\frac{1}{2}$ h of growing towards the anode. A schedule of stimulation across a lesion in which the direction of current flow was alternated every $\frac{1}{2}-1$ h would be expected to minimize reabsorption of anodally directed neurites, while promoting and directing growth towards the cathode.

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REFERENCES

Argiro, V., Bunge, M. B. & Johnson, M. I. (1984). Correlation between growth cone form and movement and their dependence on neuronal age. *Journal of Neuroscience* **4**, 3051–3062.

Blackshaw, S. & Warner, A. (1976). Onset of acetylcholine sensitivity and end-plate activity in developing myotome muscles of *Xenopus*. *Nature* **262**, 217–218.

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

Bray, D. (1979). Mechanical tension produced by nerve cells in tissue culture. *Journal of Cell Science* **37**, 391–410.

Bray, D., Thomas, C. & Shaw, G. (1978). Growth cone formation in cultures of sensory neurones. *Proceedings of the National Academy of Sciences of the U.S.A.* **75**, 5226–5229.

Cooper, M. S. & Keller, R. E. (1984). Perpendicular orientation and directional migration of amphibian neural crest cells in d.c. electrical fields. *Proceedings of the National Academy of Sciences of the U.S.A.* **81**, 160–164.

Erickson, Carol E. & Nuccitelli, R. (1984). Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. *Journal of Cell Biology* **98**, 296–307.

Gilbert, D. S., Newby, B. J. & Anderton, B. H. (1975). Neurofilament disguise, destruction and discipline. *Nature* **256**, 586–589.

Harrison, R. G. (1910). The outgrowth of the nerve fiber as a mode of protoplasmic movement. *Journal of Experimental Zoology* **9**, 787–846.

Hinkle, Laura, McCaig, 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.

Hughes, A. (1953). The growth of embryonic neurites. A study on cultures of chick neural tissue. *Journal of Anatomy* **87**, 150–162.

Ingvar, S. (1920). Reaction of cells to the galvanic current in tissue cultures. *Proceedings of the Society of Experimental Biology and Medicine* **17**, 198–199.

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.

Jones, K. W. & Elsdale, T. R. (1963). The culture of small aggregates of amphibian embryonic cells in vitro. *Journal of Embryology and Experimental Morphology* **11**, 135–154.

Kullberg, R. W., Lentz, T. L. & Cohen, M. W. (1977). Development of the myotomal neuromuscular junction in *Xenopus laevis*: An electrophysiological and fine-structural study. *Developmental Biology* **60**, 101–129.

McLaughlin, S. & Poo, M-m. (1981). The role of electro-osmosis in the electric field-induced movement of charged macromolecules on the surfaces of cells. *Biophysical Journal* **34**, 85–93.

Nieuwkoop, P. D. & Faber, J. (1956). *Normal Table of Xenopus Laevis (Daudin)*. Amsterdam: North-Holland.

Patel, N. & Poo, M-m. (1982). Orientation of neurite growth by extracellular electric fields. *Journal of Neuroscience* **2**, 483–496.

Patel, N. & Poo, M-m. (1984). Perturbation of the direction of neurite growth by pulsed and focal electric fields. *Journal of Neuroscience* **4**, 2939–2947.

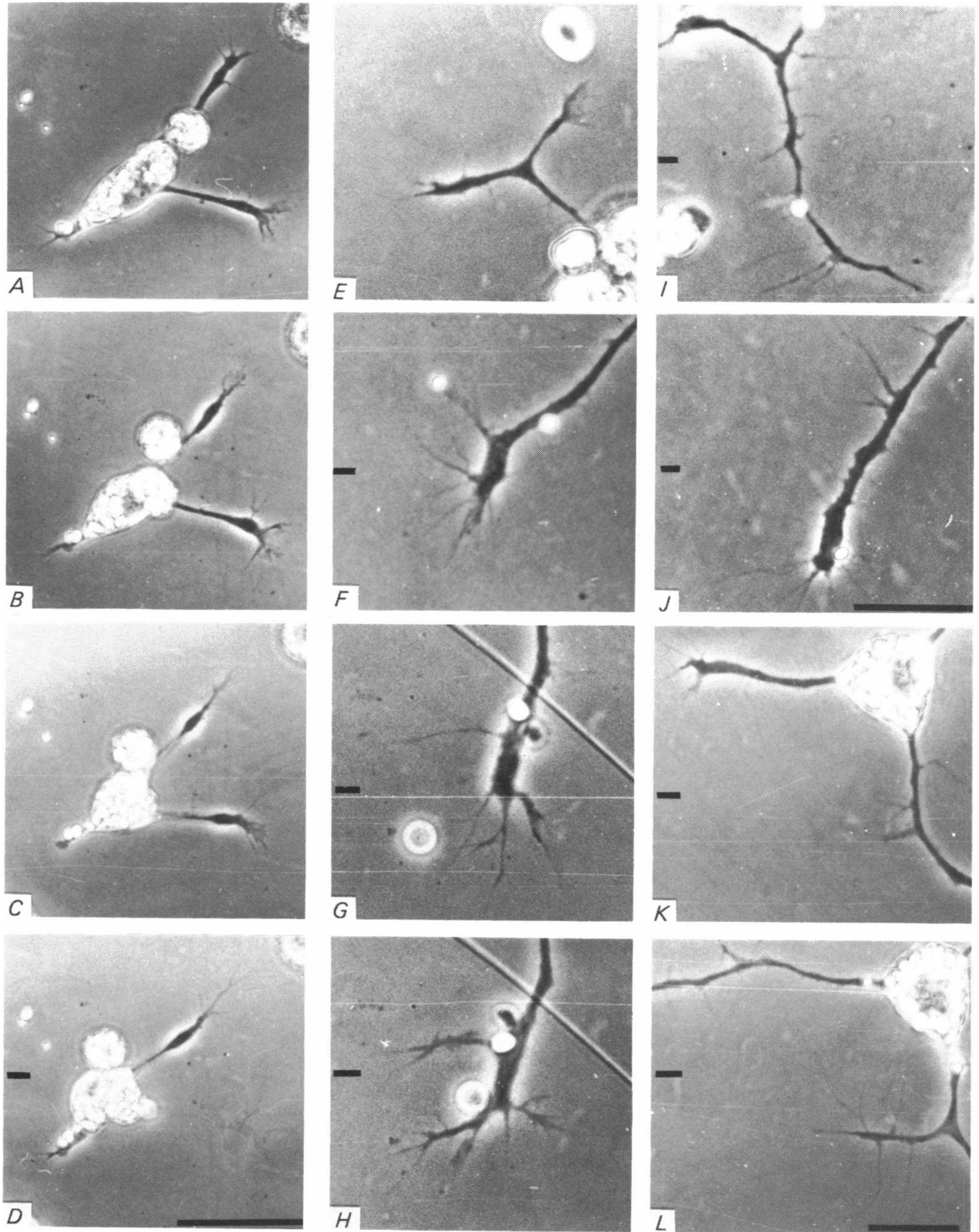
Poo, M-m. (1981). In situ electrophoresis of membrane components. *Annual Review of Biophysics and Bioengineering* **10**, 245–276.

Poo, M-m. & Robinson, K. R. (1977). Electrophoresis of concanavalin A receptors along embryonic muscle cell membrane. *Nature* **265**, 602–605.

Purves, D. & Hadley, R. (1985). Changes in the dendritic branching of adult mammalian neurones revealed by repeated imaging *in situ*. *Nature* **315**, 404–406.

Rees, R. P. & Reese, T. S. (1981). New structural features of freeze-substituted neuritic growth cones. *Neuroscience* **6**, 247–254.

Roberts, A. & Taylor, J. S. H. (1982). A scanning electron microscopy study of the development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L

of a peripheral sensory neurite network. *Journal of Embryology and Experimental Morphology* **69**, 237–250.

ROBINSON, K. R. & STUMP, R. F. (1984). Self-generated electrical currents through *Xenopus* neurulae. *Journal of Physiology* **352**, 339–352.

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.

SCHLAEPFER, W. W. (1974). Calcium-induced degeneration of axoplasm in isolated segments of rat peripheral nerve. *Brain Research* **69**, 203–215.

SELZER, M. E. (1978). Mechanisms of functional recovery and regeneration after spinal cord transection in larval sea lamprey. *Journal of Physiology* **277**, 395–408.

SPEIDEL, C. C. (1941). Adjustments of nerve endings. *Harvey Lectures* **36**, 126–158.

TAYLOR, J. S. H. & ROBERTS, A. (1983). The early development of the primary sensory neurones in an amphibian embryo: a scanning electron microscopy study. *Journal of Embryology and Experimental Morphology* **75**, 49–66.

WEISS, P. (1934). In vitro experiments on the factors determining the course of the outgoing nerve fibre. *Journal of Experimental Zoology* **68**, 393–448.

WILSON, L., BRYAN, J., RUBY, A. & MAZIA, D. (1970). Precipitation of proteins by vinblastine and calcium ions. *Proceedings of the National Academy of Sciences of the U.S.A.* **66**, 807–814.

ZIEGENBEIN, R. W., WESTERMAN, R. A., SILBERSTEIN, R., KRANTZ, H., CASSELL, J., FINKELSTEIN, D. I. & BETTESS, D. (1983). Effect of electric fields on nerve regeneration and functional recovery in the cat hindlimb. In *Molecular Pathology of Nerve and Muscle, ed.* KIDMAN, A. D., TOMKINS, J. K., MORRIS, C. A. & COOPER, N. A. New Jersey: Humana Press.

EXPLANATION OF PLATE

A–D, two neurites growing towards the anode in an electric field of 130 mV/mm. One is completely reabsorbed, the other partially reabsorbed within 2 h. Time in field is shown in each frame. Scale bar = 50 μm. *E*, growth cones from neurites in control cultures; few filopodia are seen. *F–H*, asymmetric distribution of filopodia on growth cones exposed to 100 mV/mm, *F*, after 33 min; *G*, after 4 min; *H*, same as in *G*, 30 min later. Note the predominance of filopodia projecting to the cathode (left). *I* and *J*, asymmetric distribution of neuritic spines. More spines project towards the cathode (left) than towards the anode. *I*, 230 min in 90 mV/mm; *J*, 85 min in 60 mV/mm. *K* and *L*, a cathode-directed (left) neurite spine (*K*) forms the basis for a lateral branch which grows towards the cathode (*L*). *K*, 0 min in 90 mV/min; *L*, +99 min in 90 mV/mm. Scale bars *E–L* = 25 μm.