Spinal neurite reabsorption and regrowth in vitro depend on the polarity of an applied electric field

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Summary

Retraction and regrowth of frog neural tube neurites have been studied in vitro in control cultures and in the presence of a small, continuously applied electrical field. In control cultures, some degree of retraction was seen in 39% of neurites while 7% were reabsorbed completely. Reabsorption of anodal-facing neurites was at least twice as common, with 67% showing some retraction and 17% almost totally reabsorbed. Cathodal-facing neurites were spared from retraction. Following extreme reabsorption of anodal-facing neurites, reversal of the electric field promoted regeneration in 47% (9/19) of cases studied.

Growth cone morphology also was determined by the polarity of the applied field. Anodal-facing growth cones had fewer filopodia than cathodal-facing growth cones sharing the same cell body. Field reversal induced a polarity-specific change in filopodia number on individual growth cones: a shift from anodal to cathodal increased filopodia numbers and vice versa. Some possible mechanisms involved and the significance of these results are discussed.

Key words: electric field, nerve reabsorption, nerve regeneration, spinal neurite, polarity, Xenopus laevis.

Introduction

Lesions to the nervous system generally induce nerve degeneration distally and die-back of nerve proximally, followed by some regenerative effort. In the central nervous system the extent of the latter is limited, perhaps more by unfavourable environmental conditions than by intrinsic inadequacies of the neurones. CNS axons, which show little regeneration in situ, can be induced to grow through the microenvironment offered by a grafted peripheral nerve (e.g. David & Aguayo, 1985). A simple in vitro model in which the growth of future spinal neurites is studied individually in response to a controlled alteration in their environment has indicated one potential therapeutic way of approaching the problem of CNS regeneration in vivo: by the application of an electric field. Small DC electric fields direct nerves towards the cathode (Ingvar, 1920; Marsh & Beams, 1946; Jaffe & Poo, 1979; Hinkle, McCaig & Robinson, 1981; Patel & Poo, 1982; McCaig, 1986). Pulsed and focal electrical fields also direct growth to a cathode (Patel & Poo, 1984). Nerves growing towards an anode in a DC field are slowed down (Jaffe & Poo, 1979; Patel & Poo, 1982; McCaig, 1986), sometimes to the extent of becoming partially or totally reabsorbed (McCaig, 1986). This field-induced degeneration is characterized further here.

Interest has developed in using applied electric fields to stimulate nerve regeneration across lesions in both the peripheral and central nervous systems (Ziegenbein, Westerman, Silberstein, Krantz, Cassell, Finkelstein & Bettess, 1983; Borgens, Roederer & Cohen, 1981; Roederer, Goldberg & Cohen, 1983). Recently, Borgens, Blight, Murphy & Stewart (1986) have shown that electric field stimulated guinea-pig dorsal column axons regenerate and grow around and through the glial scar formed after a spinal cord lesion. To aid this type of effort, detailed information on all aspects of the behaviour of nerves in the presence of electric fields is essential.

In this in vitro system, an electric field promotes nerve growth in one direction, while simultaneously inhibiting it in the opposite direction. Application of
an electric field to a neural lesion in vivo, as above, also would face this problem. However, reversing the polarity of the electric field might reverse the degenerative process and initiate regeneration. I have found that 47% of cells that showed extreme degeneration when facing an anode regenerated substantially soon after reversal of the field.

Methods

Cultures of the earliest developing spinal neurites from Xenopus laevis embryos were used for all experiments (stage 19/20; Nieuwkoop & Faber, 1956). The culture method was adapted from Jones & Elsdale (1963) and has been described previously (Hinkle et al. 1981). Briefly, the dorsal third of an embryo was excised and soaked in collagenase for 10 min, 1 mg ml⁻¹ in Steinberg’s solution (composition mm⁻¹: NaCl, 58; KCl, 0.67; Ca(NO₃)₂, 0.44; MgSO₄, 1.3; Tris, 4.6; pH 7.9). The neural tube was dissected out and pipetted into a dissociating medium for 15–30 min (divalent ion-free Steinberg’s plus 0.4 mM EDTA). Cells were picked up using a fine flame-drawn Pasteur pipette and dispersed into culture medium in the centre of a chamber constructed on the base of a tissue culture dish (Falcon type 3003F). Culture medium was Steinberg’s solution supplemented with 20% Liebowitz L15 solution, 1% fetal bovine serum and 2% penicillin (5000 i.u. ml⁻¹)/streptomycin (5000 µg ml⁻¹) [all from Flow Laboratories, Irvine] and used at pH 7.9. Total ionic strength was 81 mm⁻¹. The culture medium lay in a very shallow trough formed by two strips of No. 1 cover glass (64x10 mm) glued parallel to each other with silicone rubber, 1 cm apart. Cells were allowed 15–30 min to settle and attach to the dish before 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 64x10x0.5 mm.

Cells were left to develop unstimulated for 4–6 h (first outgrowths appear after 3–4 h) before one dish was selected for continual observation either with an electric field applied or as a control. The electric field was applied through a pair of agar–Steinberg’s salt bridges, 15 cm long. Chamber dimensions were 64x10x0.5 mm. Cells were left to develop unstimulated for 4–6 h (first outgrowths appear after 3–4 h) before one dish was selected for continual observation either with an electric field applied or as a control. The electric field was applied through a pair of agar–Steinberg’s salt bridges, 15 cm long. This is more than sufficient to prevent diffusion of electrode products into the culture chamber. A constant field of 50–155 mV mm⁻¹ extreme reabsorption was 2.5 times more common in neurites facing an anode than in control cultures (P < 0.01). In total 25 out of 149 anodal-facing neurites were reabsorbed (to a length less than 10 µm): an incidence of 17% (Figs 5–8). Only 1/61 cathodally projecting neurites was reabsorbed fully i.e. 2%. Table 1 compares the characteristics of anodal-induced reabsorption with reabsorption in control neurites. With the exception of the incidence of the events, there is no statistical difference between any of the parameters. In general, neurites lost about 6–10 times under a dissecting microscope. Values are expressed throughout as means ± standard errors of the mean.

Results

Incidence of reabsorption

Previous work has outlined the incidence of reabsorption in control neurites and in neurites facing either an anode or a cathode in a field of 30–233 mV mm⁻¹ (McCaig, 1986). Briefly, 39% of neurites in control cultures (33/85) show some degree of reabsorption within 2–4 h of beginning growth. A significantly greater incidence of reabsorption was seen in neurites projecting towards the anode 67% (32/48; P < 0.001), while much less reabsorption was seen for cathodal-projecting neurites, 16% (13/61) than for either controls or anodal-directed neurites (P < 0.01 and P < 0.001 respectively; see Bailey, 1981, p. 39). Reabsorption therefore is a common event in neural tissue culture and one that can be regulated by the polarity of an applied electric field.

Extreme cases of reabsorption occurred in some neurites. In control cultures 6/85 neurites became completely reabsorbed over a mean period of 82 ± 11 min: an incidence of 7% (Figs 1–4). At field strengths between 50–155 mV mm⁻¹ extreme reabsorption was 2.5 times more common in neurites facing an anode than in control cultures (P < 0.05). In total 25 out of 149 anodal-facing neurites were reabsorbed (to a length less than 10 µm): an incidence of 17% (Figs 5–8). Only 1/61 cathodally projecting neurites was reabsorbed fully i.e. 2%.

Incidence of reabsorption

The morphology of neurite reabsorption

Reabsorption in vitro, both in controls and in field-stimulated neurites, was characterized by the withdrawal of laterally directed filopodia from the growth cones and the assumption of a lanceolate or club-like shape by the nerve ending (Figs 6, 7). In most cases,
Nerve die-back and regrowth in an electric field

Figs 1-4. Complete reabsorption of a neurite in a control culture. Bar, 50 μm.
Fig. 1. 0 min.
Fig. 2. 28 min.
Fig. 3. 58 min.
Fig. 4. 76 min – a fine filament remains in place of the reabsorbed neurite.

Figs 5-8. Reabsorption of a neurite facing the anode in an electric field of 143 mV mm⁻¹. Bar, 50 μm.
Fig. 5. 0 min – control.
Fig. 6. 43 min in electric field – anode at right.
Fig. 7. 76 min in electric field – anode at right.
Fig. 8. 116 min in electric field – anode at right. A fine filament remains in place of the reabsorbed neurite. 1 h later the remaining stump of neurite also had disappeared.
Table 1. Characteristics of the reabsorption of neurites in control cultures (n = 6) and of neurites facing an anode in an applied electric field (n = 25)

<table>
<thead>
<tr>
<th></th>
<th>Initial length (µm)</th>
<th>Final length (µm)</th>
<th>Mean rate of reabsorption (µm h⁻¹)</th>
<th>Maximum rate of reabsorption (µm h⁻¹)</th>
<th>Duration of maximal rate of reabsorption (min)</th>
<th>Change in length during reabsorption (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>37 ± 4</td>
<td>0</td>
<td>-35 ± 6</td>
<td>-73 ± 12</td>
<td>60 to 77</td>
<td>23 to 4</td>
</tr>
<tr>
<td>Anodal (n = 25)</td>
<td>49 ± 4</td>
<td>5 ± 1</td>
<td>-49 ± 4</td>
<td>-86 ± 9</td>
<td>40 to 62</td>
<td>36 to 11</td>
</tr>
</tbody>
</table>

Mean rate of reabsorption is the collated average rates obtained from repeated photographs of individual neurites during the period of reabsorption, which was 91 ± 10 min for anodal-facing neurites and 82 ± 11 min for control neurites. Maximum rate of reabsorption: repeated photographs taken of each neurite during reabsorption give rise to measures of the rate of reabsorption at different time intervals throughout the entire process. The mean of the largest of these rates is the maximum rate of reabsorption.

When neurite reabsorption was complete, there remained a long slender filopodial-like thread of membrane attached to the culture substratum along the path previously occupied by the neurite. This was seen in five of the six control cases where neurites were reabsorbed (Fig. 4). At its maximum, this extension was 47 ± 3 µm long, longer than the neurites were originally (Table 1), however their measurement did not include the length of any filopodia. This remnant of the neurite could persist for at least 1 h in the absence of any regeneration.

Of the ten anodally directed neurites thus analysed, five had fine membranous remnants of their original neurites left after reabsorption. The maximal length of these was 57 ± 14 µm; the longest being 100 µm (e.g. Figs 8, 10). This mean value corresponds to the original length of this group of neurites before field-induced reabsorption. In two cases regeneration took place along the path of these filaments, which appeared to be absorbed into the newly expanding neurite (Figs 9–13).

Growth cone filopodia

Nerve growth occurs by addition of new membrane at the growth cone. Since cathodal growth is promoted while simultaneously anodal-facing processes on the same cell may be reabsorbed, I have studied the growth cones on apposed processes that share a common cell soma.

In control neurones, oppositely projecting neurites had the same number of filopodia on their growth cones (Fig. 19). After 1 h of growth in an applied electric field (50–155 mV mm⁻¹), cathodal-facing neurites had 66% more filopodia on their growth cones than were seen on anodal-facing neurites from the same cell body (P < 0.001; Figs 19, 22, 23). In agreement with a previous report, cathodal-facing neurites had significantly more filopodia on their growth cones than were seen on growth cones of control neurites (McCaig, 1986). Anodal-facing neurites, however, in contrast to previous work, were no different to control growth cones in terms of numbers of filopodia.

The effect of reversing the electric field on the neurite

1. **Anodal to cathodal**

   Table 2 shows that reversal of the field polarity from anodal to cathodal caused a marked shift in mean rate of neurite growth from negative to positive values. In 19 of the 25 cells in which anodal-induced reabsorption was studied the polarity of the field then was reversed to see if this might promote regeneration. In control cultures no regrowth was seen over a 12 h period from any of the six neurites that reabsorbed completely. Reversal of the electric field however prompted regrowth in 47% of neurites (9/19) [Figs 9–13 & 14–18]. Fig. 20 shows that neurites that had grown to more than 50 µm before the field was applied, reabsorbed to less than 10 µm after 65 ± 12 min facing the anode, then on reversal of the field had regenerated to their original length after 75 ± 5 min facing a cathode (n = 8). Table 3 describes the reabsorption that occurred both in the group that regenerated after revering the field and for the group that did not regenerate but remained reabsorbed. Essentially this presents the same picture as that seen for all the experimental neurites in Table 1, however it serves to point out that there were no major differences between the characteristics of the reabsorption that preceded either unsuccessful or successful efforts to regrow. As before, peak rates of reabsorption occurred after about 1 h, once neurites were shortening and were roughly twice the mean rate.

Table 4 describes the regeneration that took place following field reversal (anodal to cathodal facing). The mean rate of regrowth was 48 µm h⁻¹ although this varied considerably with time. 5/8 neurites continued to reabsorb for the first 20 min in the reversed field. By the end of the first hour these neurites were regenerating at a rate of 35 ± 11 µm h⁻¹. 3/8 neurites began to regenerate within the first 10 min with very
Fig. 9. 49 min in electric field – anode at right.
Fig. 10. 101 min in electric field – anode at right.
Fig. 11. 13 min after reversal of field – cathode at right.
Fig. 12. 51 min after reversal of field – cathode at right.
Fig. 13. 72 min after reversal of field – cathode at right.
(Polarity of electric field reversed after 118 min.)

Figs 14–18. Reabsorption of a neurite facing the anode in a field of 120 mV mm$^{-1}$ (Figs 14–16). The polarity of the field was reversed and the neurite regenerated towards the new cathode (Figs 17, 18). Bar, 50 μm.
Fig. 14. 0 min.
Fig. 15. 27 min with anode at the right.
Fig. 16. 0 min after reversal of field – cathode at right.
Fig. 17. 35 min after reversal of field – cathode at right.
Fig. 18. 93 min after reversal of field – cathode at right.
(Polarity of field reversed after 47 min.)
high rates of growth i.e. $91 \pm 18 \mu m h^{-1}$, maintained throughout the first 20 min of growth. In some cases therefore, reabsorption could be replaced by substantial rates of regrowth within 10 min of field reversal. In all cases maximal rates of regeneration were seen between 13 to 36 min after field reversal.

(2) Cathodal to anodal
While the growth of anodal-directed neurites was promoted by reversal of the field polarity, the growth of initially cathode-directed neurites in general was halted or reversed by a switch of polarity (Table 2). The mean growth rate fell from $42 \mu m h^{-1}$ to only $2 \mu m h^{-1}$ and in four out of seven neurites some degree of active reabsorption had occurred within an hour of reversing the field. During this period these cells were reabsorbing at a rate of $-11 \pm 7 \mu m h^{-1}$. Thus following a polarity shift, cathodal to anodal, neurites were slow to reabsorb by comparison with the speed at which some regrow when the polarity switch is made in the opposite direction (anodal to cathodal, see above).

The effect of reversing field polarity on the growth cone

(1) Anodal to cathodal
The number of filopodia on growth cones was counted immediately before reversing the polarity of the electric field and during a 40 min period after this was done. Fig. 21 shows that anodal-facing neurites had simple growth cones with only about two filopodia immediately before field reversal (i.e. after about 50 min in the field). [This number is markedly less than that found in growth cone filopodia above. The difference lies in the fact that the larger figure is a mean value derived from repeated observations over the 1h period in the field ($62 \pm 6 \text{ min}$). The smaller value in Fig. 21 was taken immediately before field reversal i.e. at the end of a $47 \pm 5 \text{ min}$ spell facing the anode. The difference therefore indicates the progressive nature of the decline in growth cone filopodia over time on anodal-facing neurites.] After reversing the polarity of the field, the same neurites had almost double the number of filopodia on what now were cathodal-facing growth cones ($P < 0.001$; Figs 24–27).

(2) Cathodal to anodal
It could be argued that this increase in number of growth cone filopodia was a nonspecific effect of polarity reversal and not necessarily the direct result of a shift in the anode-to-cathode direction. However, Fig. 21 also shows the effect of polarity reversal on the growth cones of cathodal-facing neurites. After 1h in the field, counts made immediately before field reversal showed about seven filopodia per growth cone. This number had been pruned by 40% to about four per growth cone after $43 \pm 9 \text{ min}$ facing the new anode ($P < 0.05$; Figs 28–30). As shown already, apposed neurites in control cultures have similar numbers of growth cone filopodia. Thus the polarity of the electric field has specific and reversible effects on growth cone form.

### Table 2. Effect of reversing the polarity of an applied electric field on mean neurite length and rate of growth

<table>
<thead>
<tr>
<th></th>
<th>Mean rate of growth</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Anodal : Cathodal</td>
</tr>
<tr>
<td></td>
<td>($\mu m h^{-1}$)</td>
</tr>
<tr>
<td></td>
<td>($\mu m h^{-1}$)</td>
</tr>
<tr>
<td>Anodal to cathodal</td>
<td>$-24 \pm 6 \rightarrow +36 \pm 6$</td>
</tr>
<tr>
<td>($n = 19$)</td>
<td></td>
</tr>
<tr>
<td>Cathodal to anodal</td>
<td>$+2 \pm 6 \rightarrow +42 \pm 11$</td>
</tr>
<tr>
<td>($n = 7$)</td>
<td></td>
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</table>
Table 3. Characteristics of the field induced reabsorption of neurites which did regenerate and of those which did not regenerate following reversal of the polarity of the applied electric field

<table>
<thead>
<tr>
<th>Rate of reabsorption (μm h⁻¹)</th>
<th>Maximal rate of reabsorption</th>
<th>Duration (min)</th>
<th>Change in length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial length (μm)</td>
<td>Final length (μm)</td>
<td>Mean</td>
<td>Maximal</td>
</tr>
<tr>
<td>Reabsorbed (10/19)</td>
<td>45 ± 5</td>
<td>3 ± 2</td>
<td>-44 ± 6</td>
</tr>
<tr>
<td>Regrew (9/19)</td>
<td>50 ± 7</td>
<td>7 ± 1</td>
<td>-57 ± 8</td>
</tr>
</tbody>
</table>

See legend to Table 1 for explanation of mean and maximum rate of reabsorption.

Fig. 20. Mean neurite length before application of the electric field, after facing the anode for roughly one hour and following reversal of the electric field (n = 8). Neurites underwent extreme reabsorption when facing the anode, then regenerated when facing the cathode.

Table 4. Characteristics of the field-induced regeneration of reabsorbed neurites following reversal of the polarity of the applied electric field such that anodal-facing neurites become cathodal-facing neurites

<table>
<thead>
<tr>
<th>Rate of regeneration (μm h⁻¹)</th>
<th>Time of maximal rate of regeneration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Maximal</td>
</tr>
<tr>
<td>+48 ± 10</td>
<td>+73 ± 18</td>
</tr>
</tbody>
</table>

Fig. 21. Numbers of filopodia on the same growth cone immediately before and during a 40 min period after changing the polarity of an applied electric field. Anodal to cathodal, n = 17: cathodal to anodal, n = 5. Numbers of filopodia immediately before field reversal were counted from photographs of individual growth cones after 1 h exposure to an electric field. Numbers of filopodia on individual growth cones after field reversal were obtained as means from repeated photographs taken over a 40 min period.

Discussion

Reabsorption occurred frequently in this mixed population of neural tube neurites. Two out of every three anodal-facing neurites underwent some degree of reabsorption, while one in five could disappear altogether. However, depending on the polarity of the electric field, neurites either were induced to reabsorb or were spared from reabsorption. There was no obvious relationship between the severity of reabsorption and field strength, although a fairly narrow range of small field strengths were used (50–155 mV mm⁻¹). While higher field strengths might reveal such a relationship, the values used here already lie at the upper end of the field strengths likely to exist in embryos. (Endogenous currents up to 100 μA/cm² have been measured in chick and quail embryos [Jaffe & Stern, 1979; Erickson & Nuccitelli, 1984]. The internal resistivity of small confined tissue spaces in any embryo is not known, but could be close to that of muscle i.e. 2000 Ω cm. This would give an internal field strength of the order of 20 mV mm⁻¹. Of course in vivo, other factors may potentiate the effectiveness of a 20 mV mm⁻¹ voltage drop and these...
Fig. 22. Oppositely directed neurites in a control culture have similar numbers of growth cone filopodia. Bar, 50 µm.

Fig. 23. Cathodal-directed neurites (to left) have more filopodia than anodal-directed neurites. E, 120 mV mm⁻¹ for 26 min. Bar, 50 µm.

Factors may not be present in tissue culture. Thus effective field strengths an order of magnitude greater than the estimated in vivo values nevertheless may lie within the physiological range.

Not every anodal-facing neurite was reabsorbed. This could indicate a differential sensitivity of the different neuronal types present in these mixed cultures to anodal-induced reabsorption, an hypothesis worth testing with homogeneous cultures of specific

Figs 24–27. Effect of a change of polarity, anodal to cathodal, on growth cone morphology. Bar, 50 µm.

Fig. 24. 4 min in 150 mV mm⁻¹. Cathodal- and anodal-facing processes have similar numbers of filopodia. Anode at right.

Fig. 25. 34 min in 150 mV mm⁻¹. Filopodia are more numerous on the cathodal-facing neurite and have been lost from the anodal-facing neurite. Anode at right.

Fig. 26. 40 min in E reversed; 104 min total E, 150 mV mm⁻¹. Increasing number of filopodia on NEW cathodal-facing neurite. Other neurite is turning away from NEW anode. Cathode at right.

Fig. 27. 85 min in E reversed; 150 min total E, 150 mV mm⁻¹. Further increase in number of filopodia on NEW cathodal-facing neurite. Cathode at right.
neuronal types. Also, individual neurones responded to differing extents, so that even within a homogeneous neuronal population graded responses to an electric field might be expected. The reasons for this variability are not known.

The mean rate of the field-induced reabsorption was similar to reported rates for both degeneration and for the rate of elongation in vivo and in vitro (Harrison, 1910; Speidel, 1941; Hughes, 1953; McCaig, 1986). One feature of both natural and field-induced degeneration was the progressive nature of the increasing rate of reabsorption: as neurites became smaller, reabsorption progressed more rapidly. In the last stages of extreme reabsorption the average rate of neurite loss was more than twice the overall mean rate (Table 1). These changes occurred within about 1 h of the start of degeneration or of switching on the electric field. It could be that something critical and irreversible occurs in terms of a structural breakdown in the neurite during this high-speed loss. In the two anodal-facing neurites with by far the highest rates of neurite loss, no regeneration occurred on reversing the field polarity. This may indicate that the best time to use an electric field in any effort to reverse the die-back of nerve would be before this has progressed to the later/faster stages, that is within the first hour after a lesion occurs.

The time course of neurite loss (and regeneration) in an electric field may have implications for possible underlying mechanisms. In 17 of the 25 cases observed reabsorption was occurring inside 20 min. Thus the electric field must act on the neurite by a mechanism capable of producing a noticeable loss of neurite inside 20 min, and in some cases within a 10 min period. There are at least three types of possible mechanism.

(1) The electric field might directly block fast axonal transport and the supply of axolemmal components to the growth cone or their incorporation into new membrane. The extent to which an externally applied electric field is ‘felt’ as a voltage drop within a neurite depends in particular on specific membrane resistance, axoplasmic resistivity, neurite length and cross-sectional area. In a long slender neurite the internal axoplasmic resistivity becomes significant and may exceed membrane resistance. Since the applied voltage drop becomes distributed across membrane and axoplasm in proportion to their relative resistances, a substantial proportion of an external field will be seen as a voltage drop along the axoplasm of a slender neurite or filopodium (see Jaffe & Nuccitelli, 1977; equation 9). Half of the applied voltage drop (100 mV mm⁻¹) therefore might exist within a 50 µm long 0.5 µm wide filopodium. This would give a voltage drop of 2.5 mV along the length of the filopodial cytoplasm which may be sufficient to influence motility and distribution of cytoplasmic components. Thus direct effects of an applied electric field on cytoplasmic organelles and their function may result.

A second possibility involves an action of the electric field at the cell surface. Charged integral membrane proteins protrude from the outer surface of the axolemma and are translocated physically.
facing an anode is unknown. The extent to which these events may occur in neurites within 0–20 min of reversing the field. Within lh of facing an anode began to grow and to grow faster (Newby & Anderton, 1975; Schlaepfer, 1974). The nerve (Wilson, Bryan, Ruby & Mazia, 1970; Gilbert, 1979) neurites. Extreme increases in Ca$^{2+}$ influx also cause neural crest cells causes contraction of the cytoskeleton and may be responsible predominantly for Wallerian degeneration in rat peripheral nerve (Rees & Reese, 1981). In neurites facing an anode, integral membrane proteins would tend to be electrophoresed towards the cell body (cathodal side) and this could impair severely the ability to extend the microfilament lattice of the cytoskeleton away from the cell body, towards the anode. Under these circumstances, filopodia may be forced back on to the neurite shaft reducing the extent of their lateral spread (as observed) and promoting neurite reabsorption. The observations of relatively rapid onset and completion of reabsorption fit such a hypothesis. In a field of 100 mV mm$^{-1}$ a steady state redistribution of integral membrane proteins is reached after 10 min (Poo, 1981). This is about the time taken to observe a noticeable loss in length of anodally directed neurites and thus electrophoresis of integral membrane proteins is a feasible basis for the mechanism of reabsorption.

The third possibility is that nerve retraction could arise because of the field-induced hyperpolarization that will occur at the anodal-facing membrane. For a spherical neurone of 30 μm diameter in an electric field of 100 mV mm$^{-1}$ this hyperpolarization would amount to about 2 mV (see Jaffe & Nuccitelli, 1977). Cooper & Keller (1984) suggested the following attractive hypothesis to explain the retraction of anodal-facing membranes of neural crest cells. Since hyperpolarization increases the driving force for Ca$^{2+}$ entry into cells, this is likely to cause a rise in cytoplasmic [Ca$^{2+}$]. Raising intracellular [Ca$^{2+}$] in neural crest cells causes contraction of the cytoskeleton and retraction of cell protrusions. Similar events may contribute to the retraction of anodal-facing neurites. Extreme increases in Ca$^{2+}$ influx also cause depolymerization of microtubules and breakdown of neurofilaments and may be responsible predominantly for Wallerian degeneration in rat peripheral nerve (Wilson, Bryan, Ruby & Mazia, 1970; Gilbert, Newby & Anderson, 1975; Schlaepfer, 1974). The extent to which these events may occur in neurites facing an anode is unknown.

Reversing the polarity of the electric field, reversed the reabsorption process. Neurites that had undergone some reabsorption or had been slowed down by facing an anode began to grow and to grow faster within 0–20 min of reversing the field. Within 1 h of reversal the mean growth rate had gone from $-24 \mu m h^{-1}$ to $+36 \mu m h^{-1}$. Similarly, neurites that had been growing towards the cathode at normal rates, quickly slowed down or began to reabsorb when a polarity switch left them facing the anode.

About half of the anodal-facing neurites that had almost disappeared regrew to their original length within an hour or so. Control neurites that became reabsorbed have never been seen to regenerate spontaneously. Field reversal, anodal to cathodal, therefore can induce regrowth of reabsorbed neurites.

The polarity of the applied electric field also determined growth cone morphology. Neurites facing a cathode had 66% more filopodia (mean over a 1 h period) than neurites from the same cell body that faced the anode. Reversing the electric field from anode to cathode doubled the number of filopodia at the growth cone, while a polarity shift from cathode to anode roughly halved the number of growth cone filopodia. In both cases the effects could be apparent within 10–15 min. Thus growth cone form is modulated by an electrical field and correlated with the response of the neurite. Previously it was shown that the distribution of filopodia at the growth cone is determined by an electric field and in turn determines the direction of neurite growth; more filopodia are found on the cathodal side of growth cones and neurites grow towards the cathode (McCaig, 1986). Here it is seen that cathodal-facing growth cones tend to have more filopodia and to grow faster than anodal-facing growth cones. This implies that an electric field has a direct influence on one of several processes; axonal transport of materials to form new filopodia (cytoskeleton and plasmalemma) or the mechanism of assembly of these structures. In anodal-facing neurites, some or all of these events must be inhibited, while being enhanced in cathodal-facing neurites. Interestingly, the growth cones of motor but not sensory neurones increase in complexity at critical decision regions during chick limb innervation (Tosney & Landmesser, 1985). The local environmental influence that causes this is not known.

In conclusion, reabsorption induced by an electric field was most severe within 1–1.5 h, while regeneration was promoted optimally within 20 min of reversing the field. If these data reflect what might happen in vivo then an optimal regime for electrical stimulation across a lesion might be to alternate the polarity of the field every 0.5–1 h, while ensuring that the electric field was applied early on, before excessive die-back of axons had occurred. In this way, growth may be promoted in one direction during a period of limited die-back of axons projecting in the opposite direction.
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References


