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# THE EFFECTS OF ELECTRICAL STIMULATION ON SPROUTING AFTER PARTIAL DENERVATION OF GUINEA-PIG SYMPATHETIC GANGLION CELLS

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## SUMMARY

1. The effects of electrical stimulation of presynaptic and post-synaptic cells on sprouting after partial denervation were examined in the guinea-pig superior cervical ganglion with intracellular recording. The partial denervation reduced the mean number of preganglionic axons innervating each ganglion cell from about eleven to about two (Mæhlen & Njå, 1981).

2. One week after partial denervation alone, the number of *residual* preganglionic axons innervating each superior cervical ganglion cell was increased by about 30 %. This means that the number of ganglion cells contacted by each residual preganglionic axon was increased by the same amount, which represents an early stage of sprouting.

3. Preganglionic stimulation for 1 hr immediately after the partial denervation (with 100 pulses at 20 Hz every 25 sec) increased the rate of sprouting. Thus 1 week after partial denervation and preganglionic stimulation the number of residual preganglionic axons innervating each ganglion cell was increased by about 70 %. Preganglionic stimulation had no similar effect on the innervation of normal ganglia.

4. The acceleration of sprouting caused by preganglionic stimulation was abolished by blocking ganglionic transmission with hexamethonium (30–60 mg $kg^{-1}$ $hr^{-1}$ I.V.) during the stimulation.

5. Furthermore, the rate of sprouting of residual preganglionic axons was increased by electrical stimulation of the ganglion cells alone.

6. These results show that after partial denervation of the superior cervical ganglion, a period of impulse activity in ganglion cells enhances their subsequent ability to receive innervation from sprouts arising from residual preganglionic axons.

## INTRODUCTION

New branches grow out from intact axons not only during the normal development of the nervous system, but also after a variety of manipulations in the adult animal. This suggests that the extent of axonal arborization is controlled throughout life, and provides a favourable assay by which to study how axonal sprouting and synapse formation are regulated. The way in which sprouting is normally controlled in adult animals is not fully known, but there is evidence that sprouting occurs in response

to: (1) removal of neighbouring nerve terminals (Hoffman, 1950; Edds, 1953), (2) inactivity of post-synaptic cells (Betz, Caldwell & Ribchester, 1980; Brown, Holland & Ironton, 1980), (3) blockage of axonal transport in neighbouring axons (Aguilar, Bisby, Cooper & Diamond, 1973), (4) products of nerve degeneration (Brown, Holland & Ironton, 1978), (5) exposure of sensitive neurones to exogenous nerve growth factor (see Thoenen & Barde, 1980 for a review) or denervated target tissue (Olson & Malmfors, 1970; see also Ebendal, Olson, Seiger & Hedlund, 1980).

An additional factor affecting sprouting, for which there is some anatomical evidence in mammalian skeletal muscle, is that a brief period of excessive activity of presynaptic cells immediately after partial denervation can accelerate the production of sprouts (Hoffman, 1952). The aim of the present work was to confirm this aspect of the regulation of axon terminal sprouting with electrophysiological techniques, and to extend such a study to a system where the target cells are neurones rather than muscle fibres. The guinea-pig superior cervical ganglion is well suited for this purpose, since ganglion cells can be routinely impaled with micro-electrodes, and the pre- and post-ganglionic axons are readily accessible for manipulation. Our results show that electrical stimulation of the preganglionic axons immediately after partial denervation of the ganglion causes an acceleration of sprouting. Further experiments demonstrate the relative importance of pre- and post-synaptic activity in producing this effect.

Some of these results have been briefly reported (Mæhlen & Njå, 1979, 1980*a*, *b*).

## METHODS

The experiments were carried out on albino guinea-pigs of either sex weighing 200–400 g.

### *Partial denervation*

The animals were anaesthetized with sodium pentobarbitone (30–40 mg $kg^{-1}$ I.P.) and the right thoraco-cervical sympathetic trunk was cut at the level of the subclavian artery, leaving the ansa subclavia intact (Fig. 1). This procedure interrupts 70–85 % of the preganglionic axons innervating the superior cervical ganglion (Mæhlen & Njå, 1981).

### *Electrical stimulation*

Immediately after partial denervation (and in the same session) the cervical sympathetic trunk was stimulated electrically for 1 hr. During this period, one or more additional doses of the anaesthetic were given (10–50 mg $kg^{-1}$ $hr^{-1}$ I.P.). Using a dissecting microscope at 25 × magnification, a 2–3 mm long segment of the cervical sympathetic trunk together with a coat of surrounding loose connective tissue was lifted onto the stimulating electrode (Fig. 2) in a pool of sterile mammalian Ringer solution (Liley, 1956). A region of the nerve was chosen where major blood vessels would not be interrupted, and care was taken not to interfere with the circulation in the longitudinal vessels seen along the surface of the nerve.

The peripheral sympathetic effects most easily observed during stimulation were dilatation of the pupil, widening of the palpebral fissure, vasoconstriction of the ear, piloerection of the face and neck, and salivation. The voltage and current configurations of the biphasic rectangular stimulus pulses were monitored continually on an oscilloscope screen (±5–15 V, ±0·2–0·4 mA, 0·5 msec of each polarity); the net current was adjusted to zero. The stimulus pulses were delivered in trains of 100 pulses at 20 Hz, repeated every 25 sec. The current strength was adjusted in order to produce slightly submaximal sympathetic end organ responses. In this way, the magnitude of the response to each pulse train was maintained at the initial current setting throughout the 1 hr period of stimulation. However, we can not exclude that some axons were damaged by the stimulation. Such nerve injury, if present, would cause an underestimate of the effect of impulse activity on sprouting. In sham-stimulated control animals we followed the same procedures except

that the current was turned off after one or two test trains of stimulus pulses. In other experiments the inferior post-ganglionic nerve of the superior cervical ganglion was prepared and stimulated in the same way (Fig. 1). Ganglionic transmission was blocked during preganglionic stimulation in some experiments with hexamethonium (Sigma, St Louis, MO, U.S.A.) supplied through a catheter in the left internal jugular vein (30–60 mg $kg^{-1}$ $hr^{-1}$).

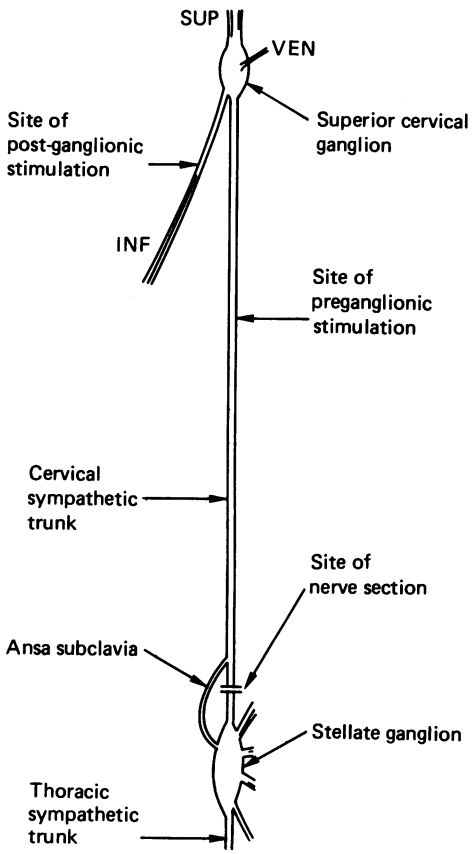


Fig. 1. The peripheral sympathetic nervous system of the guinea-pig on the right side of the neck and upper thorax (ventral aspect). About 20 % of the preganglionic axons to the superior cervical ganglion run isolated from the rest of the cervical sympathetic trunk in the ansa subclavia. The three major post-ganglionic branches of the superior cervical ganglion are the superior (SUP), inferior (INF) and ventral (VEN) post-ganglionic nerves.

*Intracellular recording in vitro*

Seven days after the operation the animals were killed and the right superior cervical ganglion with appropriate lengths of the pre- and post-ganglionic nerves was removed and maintained in a flow of oxygenated mammalian Ringer fluid at room temperature (Mæhlen & Njå, 1981). The methods of impaling neurones, estimating the number of preganglionic axons innervating each neurone by graded nerve stimulation, and measuring the amplitude of the excitatory postsynaptic potential (e.p.s.p.) in the refractory period of a directly elicited action potential have been described (Purves, 1975; Njå & Purves, 1977, 1978*b*; Lichtman & Purves, 1980).

*Statistical procedures*

Since the tendency to underestimate the number of steps in the synaptic potential increases with increasing numbers of innervating axons (Lichtman & Purves, 1980), the distribution of the number of synaptic steps is probably artificially truncated. Therefore, we used the median rather than the mean as a measure of location (using linear interpolation). The difference between the observed mean and interpolated median was small (in the order of 0·1 synaptic steps).

In each experimental (and control) group 150 neurones were impaled in ten to twenty ganglia. We used the interpolated median from each ganglion as the observations in the Wilcoxon two-sample test. The Wilcoxon–van Eltern test (Lehmann, 1975) was used to compare data from stimulated and unstimulated neurones in the same ganglion.

## RESULTS

### *Responses of the sympathetic end-organs during preganglionic stimulation*

In the present experiments, peripheral sympathetic nerves were stimulated electrically in order to examine the effect of impulse activity on sprouting. When selecting a current strength that excited the majority of axons but did not cause nerve injury, we relied mainly on visual observation of the peripheral sympathetic effects produced by the stimulation. With the appropriate current strength, each pulse train (5 sec at 20 Hz, repeated every 25 sec) elicited powerful and reproducible end-organ responses throughout the 1 hr period of stimulation. Thus the pupil dilated strongly, the palpebral fissure opened widely and the pinna showed marked vasoconstriction. The effects on the eye and the ear of each 5 sec pulse train subsided before the onset of the next train, facilitating the comparison of successive responses. Piloerection on the face and neck was also evident. Successive pilomotor responses appeared equal if the fur was flattened manually between trains. Finally, saliva accumulated in the oral cavity within several minutes and dripped slowly from the mouth during the remaining period of stimulation.

The sympathetic effects that we observed were limited to the head and neck on the side of stimulation (not checked for salivation). Therefore, it is unlikely that sympathetic reflexes or catecholamines from the adrenal medulla contributed to these effects. We could see no obvious signs of sympathetic activity in animals that were similarly treated but not stimulated.

Extracellular recording of the post-ganglionic compound action potential was done during stimulation in a few animals, and the results confirmed that ganglionic transmission was well maintained during each pulse train (Fig. 2), and throughout the period of stimulation. The base line was almost quiet between trains, showing that the activity produced by the stimulation was substantial compared to the level of spontaneous sympathetic activity in anaesthetized animals. Intracellular recording from ganglion cells during light pentobarbitone anaesthesia confirms that under these conditions the majority of neurones are either silent or firing at low frequencies (J. W. Yip, personal communication). Single unit recording from preganglionic nerve filaments in the cat suggests that this may be the rule in awake animals, as well (Passatore, 1976; Passatore & Pettorossi, 1976).

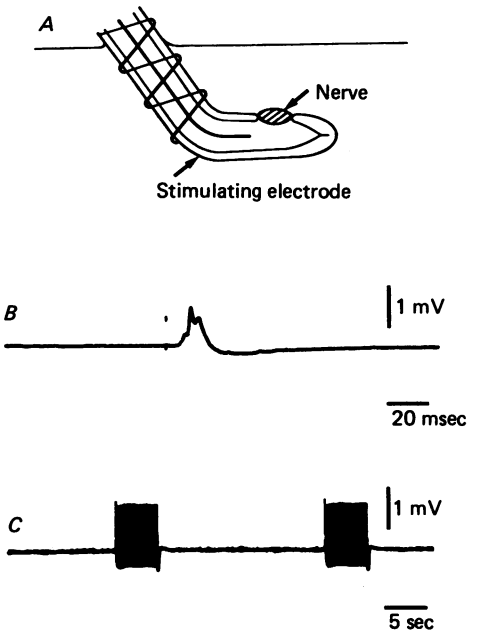


Fig. 2. Extracellular recording from the cut superior post-ganglionic nerve during preganglionic stimulation in a normal guinea-pig anaesthetized with pentobarbitone. *A*, glass capillary electrode for *en passant* stimulation. The nerve covered the opening between the two Ringer compartments in which the internal and external leads were located. *B*, response to a single stimulus pulse. *C*, response to trains of 100 pulses at 20 Hz, repeated every 25 sec. At this sweep speed (250 times slower than in *B*) the responses to individual pulses fused on the oscilloscope screen during each train. The ripples on the base line show spontaneous sympathetic activity, which could be abolished by cutting the preganglionic nerve.

*Normal ganglia examined one week after preganglionic stimulation*

Repetitive stimulation of the cervical sympathetic trunk, comparable to that used in the present experiments, has been shown to cause long-term biochemical changes in the normal superior cervical ganglion of the rat. Thus the activity of the enzyme tyrosine hydroxylase (the rate-limiting enzyme in the synthesis of noradrenaline) increases by 30–40 % a few days after electrical stimulation of the preganglionic nerve at 10 Hz for 30 min (Zigmond & Ben-Ari, 1977). Therefore, as a first step, we examined whether preganglionic stimulation caused any changes in the innervation of normal ganglia.

One week after electrical stimulation of the cervical sympathetic trunk for 1 hr, the number of preganglionic axons in the ansa subclavia innervating each neurone in the superior cervical ganglion was estimated by intracellular recording *in vitro* (Fig. 3). A group of sham-stimulated animals was studied in parallel as a control. The distribution of the number of steps in the synaptic response was similar after sham stimulation and after electrical stimulation (Fig. 4). In both series of experiments, the interpolated median (2·2 and 2·1, respectively) was close to that observed in normal ganglia taken from unoperated animals (2·3, see Fig. 5*A*). Moreover, the amplitude of the synaptic potential recorded in neurones innervated by a given

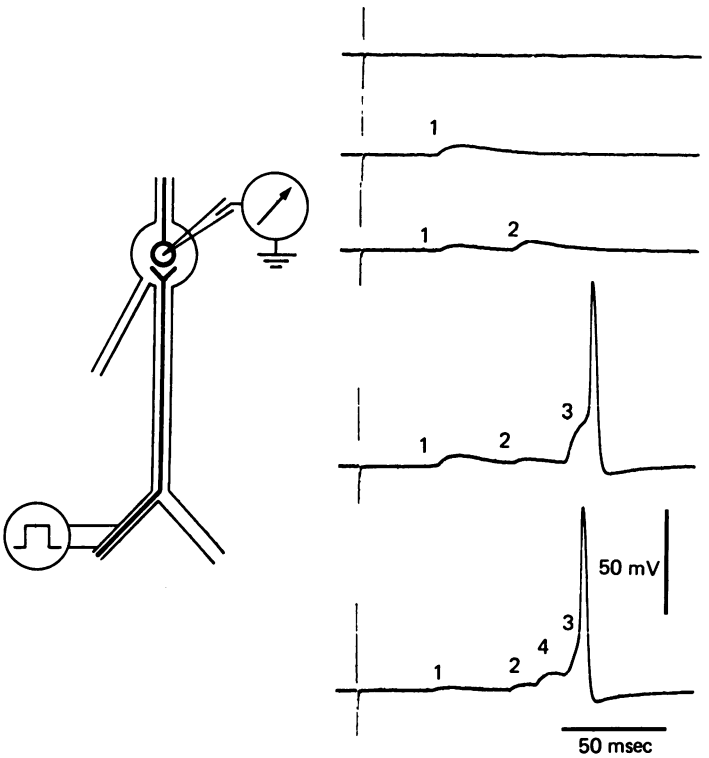


Fig. 3. Demonstration of the method of estimation of the number of preganglionic axons in the ansa subclavia innervating each neurone. The nerve was stimulated with single pulses, the intensity of which was adjusted from zero and upwards in small steps, while recording intracellularly from a neurone in the superior cervical ganglion and counting each stable incremental step in the synaptic potential (diagram of the experimental arrangement on the left). In this cell we counted four steps (numbered according to their order of recruitment), suggesting that the cell was innervated by four different preganglionic axons running in the ansa subclavia.

TABLE 1. The median amplitude of the compound e.p.s.p. recorded in ganglion cells thought to be innervated by one, two and three preganglionic axons from the ansa subclavia (number of samples in parentheses). Notice that only the ansa subclavia was examined in both intact and partially denervated ganglia (see Fig. 3). There were no obvious effects of either partial denervation or electrical stimulation on the e.p.s.p. amplitudes measured 1 week after the operation (compare with Table 2 in Mæhlen & Njå, 1981)

| | E.p.s.p. amplitude (mV) | | | |
|---|---|---|---|---|
| | Intact ganglia | | Partially denervated ganglia | |
| No. of steps in synaptic potential | Control | Stimulated | Control | Stimulated |
| 1 | 4 (51) | 3 (30) | 4 (26) | 4 (22) |
| 2 | 7 (74) | 6 (49) | 10 (70) | 7 (35) |
| 3 | 9 (71) | 12 (23) | 13 (81) | 13 (65) |

number of preganglionic axons was within normal range (Table 1). Therefore, preganglionic stimulation had no detectable effect on the innervation of normal ganglia.

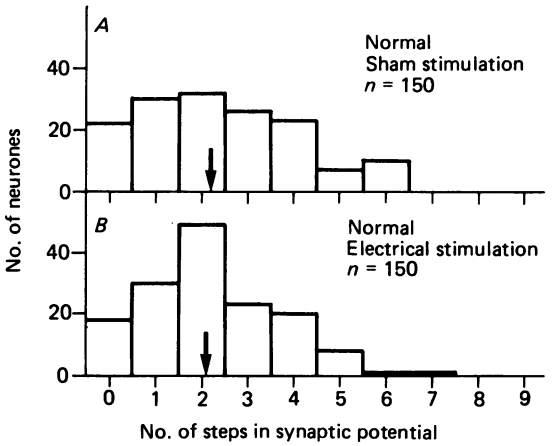


Fig. 4. Distributions of the number of steps in the synaptic response to graded stimulation of the ansa subclavia. *A*, normal neurones examined 1 week after sham stimulation of the preganglionic nerve. *B*, normal neurones examined 1 week after electrical stimulation of the preganglionic nerve. In both cases the nerve was placed on a stimulating electrode for 1 hr during an initial operation and the ganglion removed after 7 days for intracellular recording *in vitro*. Each histogram shows the results from ten ganglia. Arrows show interpolated medians.

*Effect of preganglionic stimulation on sprouting after partial denervation*

During the first month after partial denervation of the mammalian superior cervical ganglion, there is an extensive sprouting of the remaining intact preganglionic axons (Murray & Thompson, 1957; Mæhlen & Njå, 1981). In the guinea-pig, there is a 2–3-fold increase in the number of steps in the synaptic potential elicited in ganglion cells by graded stimulation of the intact ansa subclavia. There is also a large increase in the amplitude of the synaptic potential elicited in ganglion cells innervated by the same number of preganglionic axons (and hence in the synaptic contribution to a neurone from each individual axon). These changes represent sprouting of intact preganglionic axons, since there is little or no contribution from ganglion cell collaterals or ganglionic interneurones. Thus an increase in the number of preganglionic axons innervating each ganglion cell is equivalent to an increase in the number of ganglion cells contacted by each preganglionic axon (Mæhlen & Njå, 1981).

In the present experiments, the effect of electrical stimulation was examined 1 week after partial denervation, which represents an early stage of sprouting. Obviously, the distribution of the number of steps in the synaptic potential elicited in normal ganglion cells by preganglionic axons in the ansa subclavia (Figs. 4 and 5) is broad compared to the shift in this distribution expected one week after partial denervation. This broad distribution is largely caused by biological variation rather than by our experimental techniques. First, the total number of preganglionic axons innervating each neurone in the normal superior cervical ganglion of the guinea-pig varies considerably, the estimates in different neurones ranging from three to

twenty-two (A. Njå and D. Purves, unpublished). Secondly, the proportion of these axons running in the ansa subclavia ranges from zero and upwards for different cells (Mæhlen & Njå, 1981). Thirdly, the size of the ansa subclavia relative to the whole cervical sympathetic trunk may vary by a factor of two in different animals (Mæhlen & Njå, 1981). Nevertheless, we could demonstrate a small but significant amount of sprouting 1 week after partial denervation (Fig. 5*A*, *B*). Thus compared with neurones impaled in ganglia from normal unoperated animals (Fig. 5*A*), there were fewer cells receiving no innervation and more cells with multiple inputs (Fig. 5*B*). The interpolated median was 2·9, or 26% above normal.

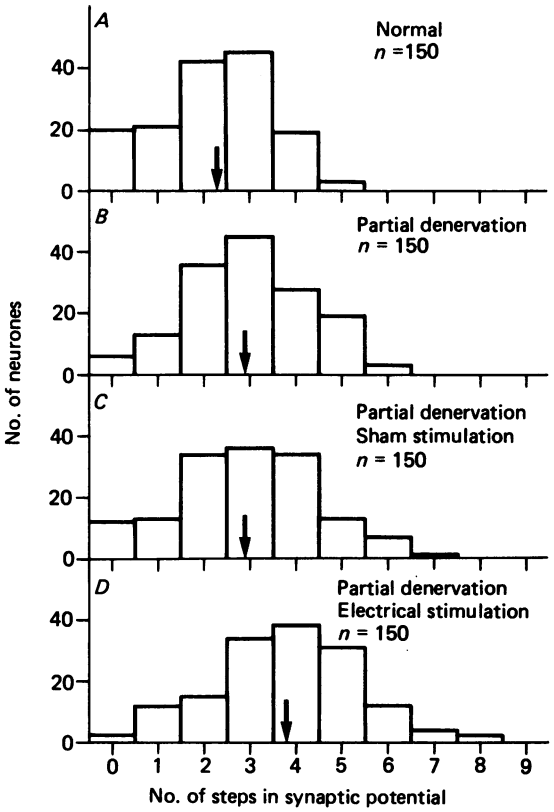


Fig. 5. Distributions of the number of steps in the synaptic response to graded stimulation of the ansa subclavia, showing the effect of preganglionic stimulation on sprouting after partial denervation. *A*, neurones impaled in ganglia from normal unoperated animals (reproduced from Mæhlen & Njå, (1981). *B*, neurones impaled in partially denervated ganglia 1 week after the operation. *C*, neurones impaled in ganglia 1 week after partial denervation and sham stimulation of the preganglionic nerve for 1 hr. *D*, neurones impaled in ganglia 1 week after partial denervation and electrical stimulation. Arrows show interpolated medians. The shifts from *A* to *B* ($\alpha = 0{\cdot}03$) and from *C* to *D* ($\alpha = 0{\cdot}001$) are statistically significant.

The effect of preganglionic stimulation on sprouting was examined in two series of animals in which one group was stimulated immediately after the partial denervation and the other was treated identically except that the current was not turned on (Fig. 5*C*, *D*). One week after partial denervation and sham stimulation, the results were indistinguishable from those obtained after partial denervation alone (Fig. 5*B*),

showing that the electrode caused no mechanical injury to the nerve (compare also Fig. 4*A* with Fig. 5*A*). The results obtained 1 week after partial denervation and electrical stimulation, however, (Fig. 5*D*) showed a substantial increase in the number of steps in the synaptic potential elicited in ganglion cells. Thus the interpolated median increased to 3·8, or 65% above the value obtained in normal ganglia upon activation of the preganglionic axons running in the ansa subclavia. Collateral connexions between ganglion cells were excluded as a source of this increase by examining the responses of ganglion cells to antidromic activation (Table 2; see also Mæhlen & Njå, 1981). These results show that preganglionic stimulation for 1 hr immediately after the partial denervation increased the amount of sprouting of residual preganglionic axons during the first week after the operation.

Several weeks after partial denervation, sprouting causes an increase in the amplitude of the synaptic potential recorded in ganglion cells innervated by a given number of preganglionic axons (Mæhlen & Njå, 1981). However, one week after the operation no such increase (or decrease) was found, whether the preganglionic nerve had been stimulated or not (Table 1). Thus at this early stage of sprouting, there was no obvious change in the average synaptic contribution to each ganglion cell from each innervating axon.

*Ganglionic blockage during preganglionic stimulation*

Preganglionic stimulation caused excitation of both preganglionic neurones and ganglion cells. Therefore, it was of interest to examine whether the activity in ganglion cells, evidenced by the occurrence of sympathetic end-organ responses, was necessary in order to cause acceleration of sprouting. This question was approached by administering the ganglionic blocking agent hexamethonium intravenously during preganglionic stimulation, at a rate sufficient to block the sympathetic end-organ responses almost completely (30–60 mg $kg^{-1}$ $hr^{-1}$). The blocking effect of hexamethonium disappeared before the animal recovered from the anaesthesia, when judged by the appearance of the eye and the ear on the normal side. One week after partial denervation, hexamethonium and sham stimulation, we observed the usual amount of sprouting (Fig. 6*A*; the interpolated median was 2·9), showing that a few hours of ganglionic blockage had no effect on the subsequent rate of sprouting. However, after partial denervation, hexamethonium and electrical stimulation, the enhancement of sprouting was absent or at least greatly reduced (Fig. 6*B*; the interpolated median was 3·1). In contrast, the expected effect on sprouting was observed 1 week after partial denervation, saline infusion and electrical stimulation (Fig. 6*C*; the interpolated median was 3·9). From these results, it is clear that the acceleration of sprouting produced by preganglionic stimulation depends on the binding of the transmittor substance acetylcholine to nicotinic receptors, or on some later step(s) in the sequence of events initiated by the transmitter–receptor complex.

*Effect of post-ganglionic stimulation on sprouting after partial denervation*

The results described above show that a 1 hr period of excessive activity of the preganglionic neurones *alone* is unable to influence the rate of sprouting of intact preganglionic nerve terminals after partial denervation. Therefore, the effect of preganglionic stimulation might depend on the simultaneous activity of pre- and post-synaptic neurones. Alternatively, the important factor might be the activity

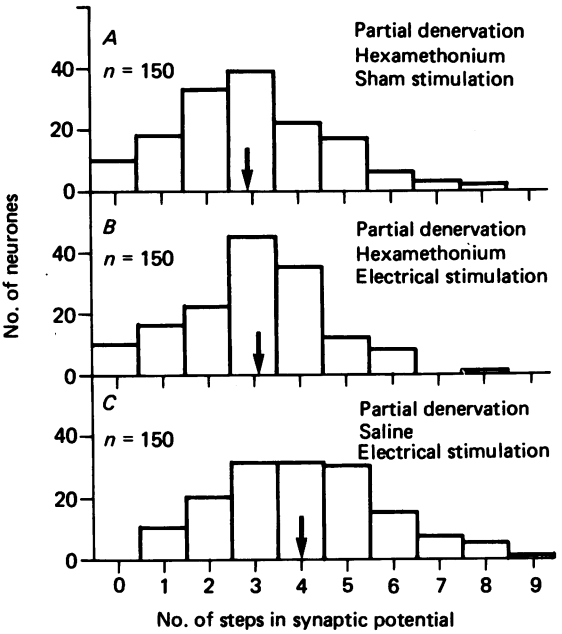


Fig. 6. Distributions of the number of steps in the synaptic response to graded stimulation of the ansa subclavia, showing the effect of ganglionic blockage during preganglionic stimulation. *A*, *B* and *C*, show the results from neurones impaled in partially denervated ganglia 1 week after blockage and sham stimulation, blockage and electrical stimulation, and electrical stimulation without the blockage, respectively. Arrows show interpolated medians. The shift from *B* to *C* is statistically significant ($\alpha = 0{\cdot}003$).

TABLE 2. The occurrence of synaptic potentials in neurones located in the lower third of the superior cervical ganglion in response to *in vitro* stimulation of the inferior post-ganglionic nerve. The majority of cells in this region responded with an antidromic action potential, which could be blocked by collision with an action potential initiated in the soma (Fig. 7). Only a few cells showed synaptic potentials, which were resistant to block by collision (see Fig. 8 in Purves, 1976). These synaptic potentials were often subthreshold and seemed to be no more frequent after partial denervation or electrical stimulation than in normal ganglia

| Experiment | No. of ganglia examined | No. of neurones | | |
|---|---|---|---|---|
| | | Total | Antidr. action potential | Synaptic potential |
| Normal, unoperated | 6 | 67 | 65 | 2 |
| Normal, sham stim. (pre) | 6 | 78 | 61 | 6 |
| Normal, electrical stim. (pre) | 2 | 22 | 21 | 1 |
| Part. denervation, only | 2 | 27 | 13 | 0 |
| Part. denervation, sham stim. (pre) | 3 | 32 | 18 | 1 |
| Part. denervation, electrical stim. (pre) | 3 | 31 | 24 | 1 |
| Part. denervation, sham stim. (post) | 12 | 150 | 150 | 0 |
| Part. denervation, electrical stim. (post) | 17 | 150 | 150 | 5 |

induced in the ganglion cells and/or sympathetic effector cells. A final possibility is that the acceleration of sprouting requires binding of the released acetylcholine to nicotinic receptors, but not the impulse activity produced in ganglion cells. In order to distinguish between these hypotheses we stimulated the ganglion cells after partial denervation to see whether the activity thus produced (confined to the ganglion cells and the sympathetic effector cells that they control) affected the rate of sprouting

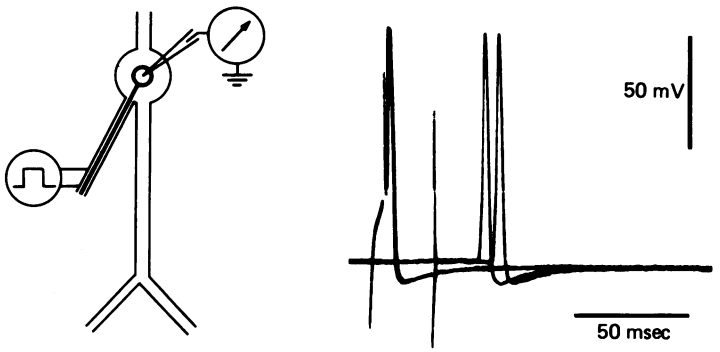


Fig. 7. Intracellular recording from a neurone whose axon emerged in the inferior post-ganglionic nerve, as shown by antidromic stimulation. Each stimulus polarity gave rise to an antidromic action potential (with different latencies), but no synaptic potential (see Table 2). The antidromic action potential was identified by collision with an orthodromic action potential set up by a depolarizing current pulse through the intracellular micro-electrode. Two sweeps were superimposed for each stimulus polarity, the second one with an orthodromic action potential added. The nerve was stimulated distal to the site of contact with the stimulating electrode used in the initial operation 1 week earlier.

of intact preganglionic axons. In these experiments, we used the inferior post-ganglionic nerve, which contains very few preganglionic axons (Table 2; see also Perri, Sacchi & Casella, 1970), and placed the stimulus electrode some distance away from the ganglion in order to reduce the chance of activating preganglionic axons by current spread (see Fig. 1). Using the same time pattern of stimulation as was used for preganglionic stimulation, we observed similar end-organ responses, except that they were now confined to the region supplied by the inferior post-ganglionic nerve. For example, there was no effect on the eye during the stimulation.

In the final experiments, neurones that sent their axons into the inferior post-ganglionic nerve were identified by antidromic activation (Fig. 7). In ganglion cells examined 1 week after partial denervation and sham stimulation, the distribution of the number of steps in the synaptic potential elicited by the residual preganglionic axons indicated a normal amount of sprouting (Fig. 8*A*; the interpolated median was 3·1). However, the results obtained 1 week after partial denervation and electrical stimulation showed that sprouting was enhanced (Fig. 8*B*; the interpolated median was 4·0). Since pre- and post-ganglionic stimulation was about equally effective in causing acceleration of sprouting, we conclude that ganglion cell activity was instrumental in mediating the effect of preganglionic stimulation on sprouting. In some of these experiments, the innervation of ganglion cells whose axons had been stimulated was compared with that of neurones whose axons ran in the untreated

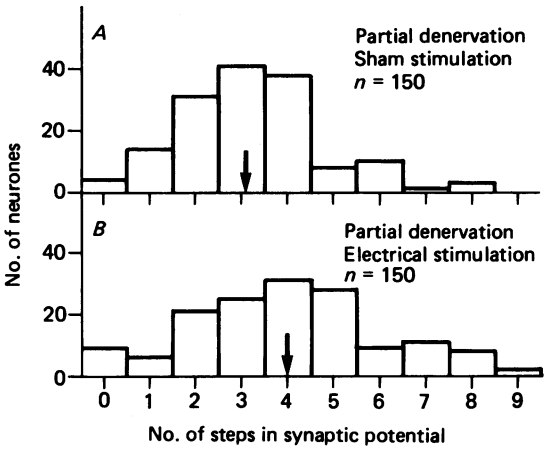


Fig. 8. Distributions of the number of steps in the synaptic response to graded stimulation of the ansa subclavia, showing the effect of post-ganglionic stimulation on sprouting after partial denervation. *A*, neurones impaled in partially denervated ganglia 1 week after sham stimulation of the inferior post-ganglionic nerve for 1 hr. *B*, neurones impaled in ganglia 1 week after partial denervation and electrical stimulation. In both *A* and *B*, only neurones whose axons were shown to run in the inferior post-ganglionic nerve by antidromic stimulation were included (Fig. 7). Arrows show interpolated medians. The shift from *A* to *B* is statistically significant ($\alpha = 0{\cdot}01$).

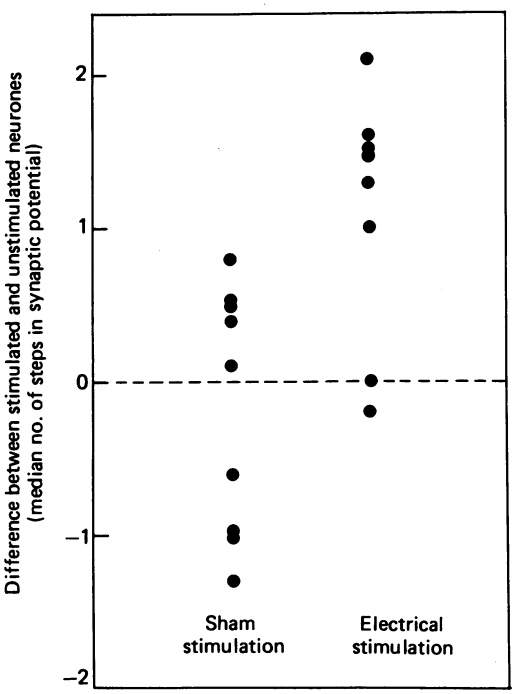


Fig. 9. Difference between the innervation of stimulated and unstimulated neurones in the same ganglion. The inferior post-ganglionic nerve was either sham-stimulated or electrically stimulated after partial denervation, and each symbol shows the difference between the interpolated median number of steps in the synaptic response in about ten stimulated and about ten unstimulated neurones in one ganglion (the unstimulated neurones were cells whose axons ran in the superior post-ganglionic nerve). The tendency of the electrically stimulated ganglion cells to receive innervation from a greater number of residual preganglionic axons than their unstimulated counterparts in the same ganglia is statistically significant ($\alpha = 0{\cdot}001$).

superior post-ganglionic nerve of the same ganglion. The difference between the (interpolated) median number of steps in the synaptic potential elicited in stimulated and unstimulated neurones in the same ganglion (Fig. 9) was about the same as that observed between stimulated and sham-stimulated neurones impaled in different ganglia (see Fig. 8). These results show that a period of electrical stimulation of a group of ganglion cells immediately after the partial denervation enhances the ability of these neurones to receive innervation from sprouts arising from residual preganglionic axons.

## DISCUSSION

The present results show that a single 1 hr period of excessive activity of the post-synaptic cells enhances the sprouting of residual presynaptic axons after partial denervation. The three lines of evidence supporting this conclusion are: (1) preganglionic stimulation for 1 hr immediately after partial denervation of the guinea-pig superior cervical ganglion increased the number of residual preganglionic axons innervating each ganglion cell one week after the operation, (2) this effect of preganglionic stimulation was abolished by blocking ganglionic transmission with hexamethonium during the stimulation, (3) direct electrical stimulation of the ganglion cells after partial denervation also increased the rate of sprouting of residual preganglionic axons. These findings suggest a mechanism by which the magnitude of a retrograde trans-synaptic trophic effect can be modulated by impulse activity.

In mammalian skeletal muscle, changes in the impulse activity of the muscle fibres have marked effects on the production of sprouts from motor nerve terminals. For example, continuous electrical stimulation of a partially denervated or impulse blocked skeletal muscle can prevent terminal (but not collateral) sprouting from intact motor end-plates (Brown & Ironton, 1977*b*; Brown *et al.* 1980). Conversely, chronic inactivity of the muscle causes terminal sprouting (although not formation of functional synapses) in the absence of partial denervation (Brown & Ironton, 1977*a*; Betz *et al.* 1980; Brown *et al.* 1980). The prevention of terminal sprouting is caused by chronic stimulation lasting for several days, with a pattern of stimulation which effectively inhibits the changes occurring in denervated muscle fibres (Brown *et al.* 1980; see also Lømo & Westgaard, 1976). In contrast, the electrical stimulation performed in the present experiments lasted only 1 hr, followed by several days of subnormal activity, caused by the partial denervation. Although we can not distinguish collateral and terminal sprouting in the superior cervical ganglion, both the present results and the work on muscle inactivity are consistent with the view that part of the stimulus for sprouting after partial denervation derives from post-synaptic cells deprived of their normal impulse activity. In addition, the present experiments indicate that such an influence of ganglion cells on preganglionic nerve terminals is enhanced by a brief episode of hyperactivity preceding the period of subnormal activity in ganglion cells (for further discussion of trophic interactions occurring in sympathetic ganglia, see Njå & Purves, 1978*a* and the reviews by Purves & Lichtman, 1978 and Black, 1978).

An acceleration of sprouting caused by a brief period of electrical nerve stimulation was also reported by Hoffman (1952) in partially denervated skeletal muscles of the

rat. Unfortunately, the amounts of pre- and post-synaptic hyperactivity resulting from the stimulation in his study were poorly defined, so that a direct comparison with the present experiments is not possible. A further difficulty arises because histological sprouting does not always imply the formation of functional synapses, at least not in intact impulse-blocked muscles (Brown & Ironton, 1977*a*; Betz *et al.* 1980). Nevertheless, it appears likely that the phenomenon which we describe in the sympathetic system is related to that observed in skeletal muscle.

Although the present results are consistent with the view that neuronal activity affects specific biological signals which serve to maintain or regulate synaptic connexions, alternative explanations are possible. For example, the preganglionic stimulation might change the circulation in the region of the ganglion, or cause a more rapid degeneration of the injured axons (see Brown *et al.* 1978). Nevertheless, the activation of a relatively small population of ganglion cells during post-ganglionic stimulation, with cell bodies spread out over the lower one third of the ganglion and axon terminals located mostly in distal end-organs, also increased the number of residual preganglionic axons innervating each ganglion cell. Moreover, post-ganglionic stimulation had little, if any, effect on the innervation of unstimulated neurones in the same ganglion. These results argue against the possibility that sprouting is enhanced by some non-specific effects of the stimulation and support the idea that activity can alter the degree of sprouting to individual neurones.

The dose–response relationship for the observed effect is unknown. The amount and pattern of activity are important factors determining the properties of mammalian skeletal muscle fibres (Lømo & Westgaard, 1976; Lømo, Westgaard & Engebretsen, 1980), and may also be crucial in the regulation of nerve terminal sprouting in muscle (Brown *et al.* 1980) and ganglia. It seems likely that the exact time of the stimulation in the present experiments could have been changed a few hours in either direction relative to the time of partial denervation without seriously affecting the results, but we have not examined this aspect, or the effect of *repeated* periods of stimulation. For example, it would be of interest to examine whether the activity of the presynaptic cells might play a role in the control of sprouting during a different pattern of stimulation, since it has been suggested (Hebb, 1949; Rauschecker & Singer, 1981) that simultaneous activity of the pre- and post-synaptic cells can cause a strengthening of synaptic connexions in the central nervous system.

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