J. Physiol. (1981), **319**, *pp.* 555–567
With 5 text-figures
Printed in Great Britain

SELECTIVE SYNAPSE FORMATION DURING SPROUTING AFTER PARTIAL DENERVATION OF THE GUINEA-PIG SUPERIOR CERVICAL GANGLION

BY J. MÆHLEN AND A. NJÅ

From the Institutes of Physiology and Neurophysiology, University of Oslo, Karl Johansgt. 47, Oslo 1, Norway

(*Received 26 January 1981*)

SUMMARY

1. The synaptic connexions established by sprouting of intact preganglionic sympathetic axons were examined by intracellular recording *in vitro* and by observing the sympathetic end organ responses to ventral root stimulation *in vivo*.

2. The superior cervical ganglion of the guinea-pig was partially denervated (70–85 %) by crushing the cervical sympathetic trunk at the level of the subclavian artery, leaving the *ansa subclavia* intact. The intact nerve carried some preganglionic axons arising from each of the eight spinal cord segments (C8–T7) contributing innervation to the ganglion.

3. During the first 4 weeks after the operation, there was a two- to threefold increase in the number of steps in the synaptic response elicited in individual ganglion cells by graded stimulation of the *ansa subclavia*. There was also an increase in the amplitude of the synaptic potential elicited by each preganglionic axon.

4. This increase in the synaptic contribution of the intact nerve to neurones in the superior cervical ganglion after partial denervation was attributed to sprouting of residual preganglionic axons. A major contribution from collateral connexions between ganglion cells was ruled out by intracellular recording from neurones during antidromic stimulation of their axons in the inferior post-ganglionic nerve.

5. After sprouting, the specificity of the sympathetic end organ responses elicited by stimulation of the ventral roots of spinal segments T1 and T4 *in vivo* was indistinguishable from normal, although the strength of these responses increased from just perceptible acutely after partial denervation to near normal 3–6 weeks after the operation, when sprouting was largely complete.

6. These results show that intact preganglionic axons arising from different spinal levels established selective connexions with different classes of ganglion cells during sprouting.

INTRODUCTION

In the mammalian superior cervical ganglion, neurones supplying different peripheral regions are selectively innervated by preganglionic axons arising from overlapping but different levels of the spinal cord (Langley, 1892; Njå & Purves, 1977*a*;

Lichtman, Purves & Yip, 1979). This pattern of ganglionic connexions is faithfully re-established after section and regeneration of the preganglionic nerve in adult animals (Langley, 1895, 1897; Guth & Bernstein, 1961; Njå & Purves, 1977*b*, 1978; see also Purves & Thompson, 1979). However, if the ganglion is partially denervated by interrupting the sympathetic rami T1–T3, the residual preganglionic axons, arising from T4–T7, will sprout and establish new synaptic connexions with ganglion cells that are obviously inappropriate to them. Thus several weeks after partial denervation, stimulation of the residual preganglionic axons elicits an abnormally wide range of peripheral sympathetic effects, including dilatation of the pupil, a response which is normally elicited only by preganglionic axons arising from T1–T3 (Murray & Thompson, 1957; Guth & Bailey, 1961; Guth & Bernstein, 1961). This loss of specificity might be a consequence of the complete removal of preganglionic axons arising from particular spinal levels. Alternatively, the result of sprouting in these experiments might not be due to the way of partial denervation, but rather reflect a general inability of the preganglionic axons to form selective synaptic connexions during sprouting. Thus, for example, during re-innervation each regenerating axon may to some extent re-establish its original contacts with ganglion cells, whereas during sprouting intact axons generate new synapses *in addition* to the ones that they maintain in normal ganglia. We have now studied the course of sprouting after a different type of partial denervation. Thus in the present work we removed most, but not all, the preganglionic axons arising from each of the spinal cord segments contributing innervation to the guinea-pig superior cervical ganglion. The results show that after sprouting, the specificity of the sympathetic end organ responses to stimulation of the ventral roots T1 and T4 was indistinguishable from normal. This implies that selective synapse formation is possible during sprouting, and that the outcome of sprouting depends on the type of partial denervation.

Some of these results have been presented in preliminary form (Mæhlen & Njå, 1979).

METHODS

Partial denervation

The connexion between the stellate ganglion and the inferior cervical ganglion of the guinea-pig sympathetic chain consists of two separate nerves, which run on each side of the subclavian artery. These are the cervical sympathetic trunk and the *ansa subclavia*, running on the dorsal and the ventral side of the subclavian artery, respectively (Fig. 1). As will be shown in the Results section, each of these two nerves carries preganglionic axons that arise from spinal cord segments C8–T7, and innervate neurones in the superior cervical ganglion.

Young adult albino guinea-pigs of either sex (200–400 g) were anaesthetized with Na pentobarbitone (30–40 mg/kg I.P.) and the right inferior cervical ganglion exposed through a ventral midline incision. The *ansa subclavia* was identified and the cervical sympathetic trunk was crushed twice 1–2 mm caudal to the inferior cervical ganglion (for about 3 sec with forceps that closed precisely over an area about as wide as the transverse dimension of the nerve). Since partial denervation invariably caused moderate ptosis and miosis, occasional animals showing no effects of the operation, or signs of full denervation, were not kept for further use. Partial denervation also caused vasodilatation of the ear, which lasted only several hours. The effects on the eye after partial denervation disappeared gradually over a period of 1–2 weeks.

Intracellular recording from ganglion cells in vitro

The animals were killed and the right superior cervical ganglion was removed in continuity with the cervical and part of the thoracic sympathetic chain, including a piece of the thoracic cage, as

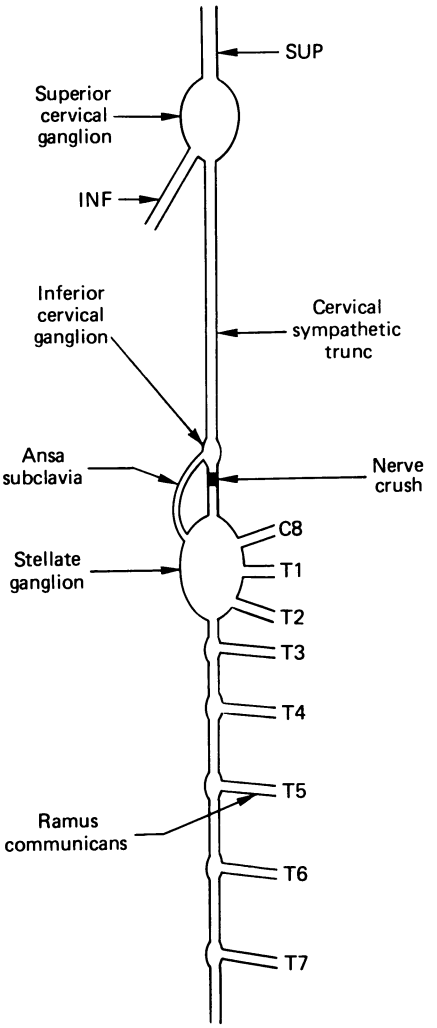

Fig. 1. Diagram of the peripheral sympathetic nervous system in the neck and upper thorax. The superior cervical ganglion, which is innervated by preganglionic axons arising from the last cervical and the first seven thoracic segments of the spinal cord (C8–T7), was partially denervated by crushing the cervical sympathetic trunk as shown, leaving the *ansa subclavia* intact. The two major post-ganglionic branches of the superior cervical ganglion are the superior (SUP) and inferior (INF) nerves.

previously described (Njå & Purves, 1977*a*). The *ansa subclavia* and the rest of the cervical sympathetic trunk were both cut at their exits from the rostral pole of the stellate ganglion, and the dissected preparation was placed in a chamber perfused with oxygenated mammalian Ringer fluid. The two preganglionic nerves were stimulated while recording from individual ganglion cells with an intracellular microelectrode (80–150 MΩ). The methods of impalements of neurones, estimation of the number of preganglionic axons innervating each neurone by graded nerve stimulation and measuring the amplitude of the excitatory post-synaptic potential (e.p.s.p.) in the refractory period of a directly elicited action potential have been described (Purves, 1975; Njå & Purves, 1977*a*, 1978).

Stimulation of the ventral roots in vivo

The pattern of peripheral sympathetic effects elicited by ventral root stimulation in anaesthetized animals (Njå & Purves, 1977*a*) was studied in five guinea-pigs after partial denervation and in another five animals 3–6 weeks after the operation. By this time, sprouting of the remaining preganglionic axons was near the end-stage, as judged by intracellular recording from ganglion cells *in vitro*. As in the earlier work, dilatation of the pupil, widening of the palpebral fissure, vasoconstriction of the ear and piloerection on the face and neck in response to stimulation of individual ventral roots (at 20 Hz) were graded subjectively on a 0 to + + + + scale. Although we frequently stimulated several different ventral roots, our attention was focused on the responses to stimulation of T1 and T4. In normal animals, each of these innervates about 60 % of the neurones in the superior cervical ganglion, and yet their peripheral sympathetic effects are obviously different (Njå & Purves, 1977*a*).

Histology

The *ansa subclavia* was removed in continuity with the cervical sympathetic trunk and fixed overnight in 2 % paraformaldehyde and 2·5 % glutaraldehyde in 0·1 M-Na cacodylate (pH 7·4), post-fixed in 1 % osmium tetroxide in 0·1 M-Na cacodylate for 1 hr, dehydrated in acetone and embedded in TAAB embedding resin. Transverse sections (2–3 μm) were cut from the *ansa subclavia* and the whole cervical sympathetic trunk, about 2 mm from the inferior cervical ganglion (Fig. 1). Sections were stained with 1 % *p*-phenylenediamine in ethanol/isopropanol (Holländer & Vaaland, 1968) and 0·5 % toluidine blue in phosphate buffer.

Statistical procedures

Two-tailed significance levels were computed by the Wilcoxon test. Some minor violations of the assumptions in this test (such as the dependence between a few of the observations) were ignored. The resulting errors were negligible.

RESULTS

Normal contribution of the ansa subclavia to the innervation of the superior cervical ganglion

In the present experiments, partial denervation of the superior cervical ganglion was achieved by interrupting the cervical sympathetic trunk at the level of the subclavian artery, leaving the *ansa subclavia* intact. In order to assess the partial denervation thus obtained, we examined the normal contribution of the *ansa subclavia* to the innervation of the superior cervical ganglion.

Post-ganglionic compound action potentials elicited by stimulation of individual ventral roots

The superior cervical ganglion of the guinea-pig is innervated by preganglionic axons arising from the last cervical (C8) and the first seven thoracic (T1–T7) segments of the spinal cord (Njå & Purves, 1977*a*). Thus a post-ganglionic compound action potential could be elicited by stimulation of the sympathetic outflow from each of these spinal cord segments *in vitro* (Fig. 2*A*). The cervical sympathetic trunk was then cut at the level of the subclavian artery, and each ventral root was stimulated again in the same preparation (Fig. 2*B*). It was clear that each ventral root from C8–T7 still elicited a compound action potential after partial denervation, although the magnitude of the responses became smaller. In fact, the *relative* reduction in the size of each post-ganglionic compound action potential appeared to be equal for most of the ventral roots contributing innervation to the ganglion. Similar results were

obtained in the two other preparations examined in this way. These results could be explained if ascending preganglionic sympathetic axons mixed extensively before they part to run on either side of the subclavian artery. In any case, we conclude that the present method of partial denervation tended to preserve the numerical balance between preganglionic axons arising from different spinal levels. In contrast, the partial denervation performed in the earlier studies on sprouting in the superior cervical ganglion of the cat involved complete interruption of the sympathetic outflow

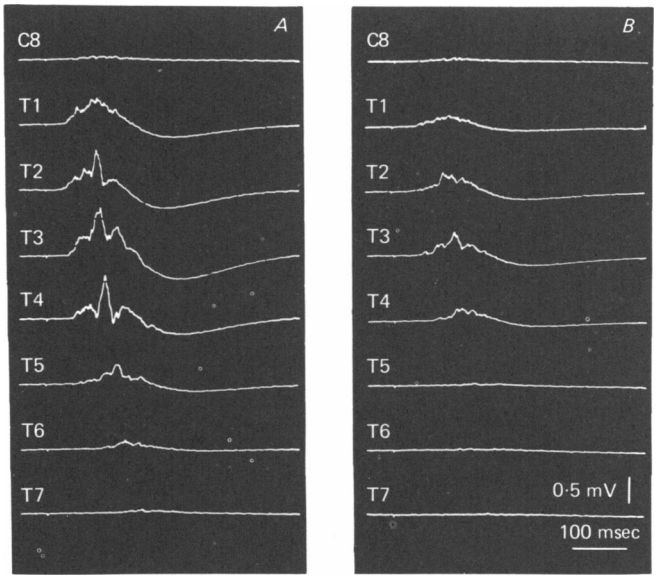

Fig. 2. Spinal level of origin of the preganglionic axons remaining after partial denervation. *A*, compound post-ganglionic action potentials recorded from the superior nerve in response to stimulation of the ventral roots of C8–T7 in a normal preparation and *B*, in the same preparation immediately after cutting the cervical sympathetic trunk caudal to the inferior cervical ganglion (see Fig. 1). The responses that remain were due to preganglionic axons running in the *ansa subclavia*.

of selected spinal cord segments (Murray & Thompson, 1957; Guth & Bailey, 1961; Guth & Bernstein, 1961).

The possibility that the *ansa subclavia* might preferentially supply a particular subregion of the superior cervical ganglion was examined by comparing the responses obtained from the superior and inferior post-ganglionic nerves of one ganglion. Partial denervation had indistinguishable effects on the compound action potentials recorded from these two nerves, whose cells of origin are located in largely different regions of the ganglion (Purves, 1975).

Number of myelinated axons in the ansa subclavia and the whole cervical sympathetic trunk

About 25 % of the preganglionic axons in the guinea-pig cervical sympathetic trunk are myelinated (Purves, 1976). The actual proportion of myelinated axons may differ

among preganglionic axons contributed to the cervical trunk from different levels of the spinal cord (Njå & Purves, 1977*b*; see also Murray & Thompson, 1957). However, since the relative contributions from individual ventral roots to the *ansa subclavia* and the whole cervical sympathetic trunk were similar (Fig. 2), we assumed that the proportion of myelinated preganglionic axons in the *ansa subclavia* was comparable to that in the whole cervical sympathetic trunk. Thus an estimate of the relative size of the *ansa subclavia* could be obtained by counting the number of myelinated axonal

TABLE 1. The number of myelinated axonal profiles in cross-sections of the *ansa subclavia* and the whole cervical sympathetic trunk in five normal guinea-pigs

Animal no.	*Ansa subclavia*	Cervical sympathetic trunk	*Ansa*/cervical trunk (%)
1	162	731	22
2	108	813	13
3	120	731	16
4	91	415	22
5	97	685	14
Mean	116	675	17

profiles in cross-sections of these two nerves. (We assume here that myelinated sensory axons were distributed like myelinated preganglionic axons in the two nerves.) The results obtained in five guinea-pigs showed that the number of myelinated axons in the *ansa subclavia* was about 17% (range 13–22%) of that in the whole cervical sympathetic trunk (Table 1). The variation between animals probably represents real differences in the relative size of the *ansa subclavia* in different guinea-pigs (see also Fig. 5).

Innervation of single neurones by preganglionic axons running in the ansa subclavia

The innervation of individual neurones was examined by intracellular recording *in vitro*, as shown in Fig. 3. The total number of preganglionic axons contacting a neurone in the guinea-pig superior cervical ganglion is about eleven, on average, as estimated by counting the number of steps in the synaptic response to graded stimulation of the individual ventral roots (Njå & Purves, 1977*b*). By graded stimulation of the *ansa subclavia*, we found that the mean number of preganglionic axons contacting each neurone from this source was about two (2·2, range 0–5, $n = 150$, see Fig. 4), or about 20% of the total. Thus, the degree of partial denervation predicted by the mean number of preganglionic axons contacting each neurone was in good agreement with the estimate obtained from the number of myelinated axons counted in nerve cross-sections (Table 1).

Most neurones (130/150) received some innervation from the *ansa subclavia* (Fig. 4), whereas all neurones were innervated by the remainder of the cervical sympathetic trunk. These results indicate that after partial denervation of the ganglion, all neurones will lose at least some of their innervating axons. About 13% of the cells (20/150) will be fully denervated, while the rest will all be *partially* denervated, retaining a variable proportion of their normal complement of preganglionic axons. On average, the estimated number of innervating axons lost by each neurone will

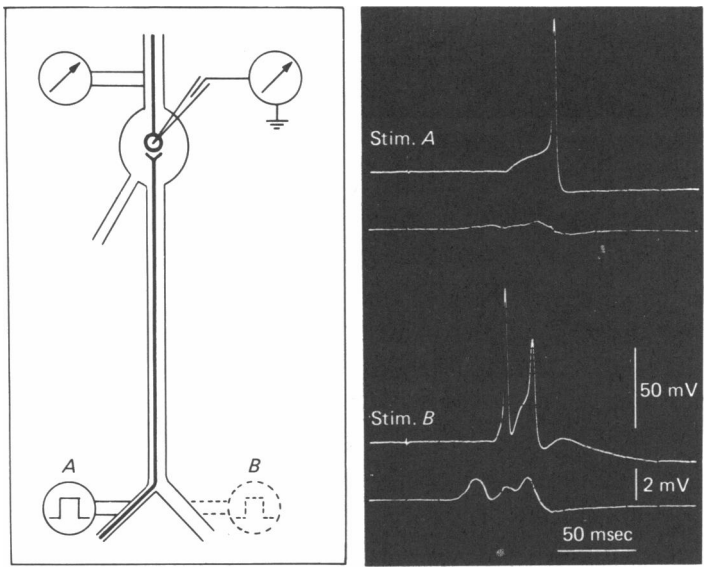

Fig. 3. Contribution of the *ansa subclavia* (*A*) and the rest of the cervical sympathetic trunk (*B*) to a neurone in the normal superior cervical ganglion as shown by intracellular recording (diagram of the experimental arrangement on the left). The estimated number of innervating axons running in the smaller and larger nerve was one and nine, respectively. The lower trace in each pair of recordings shows the post-ganglionic compound action potential.

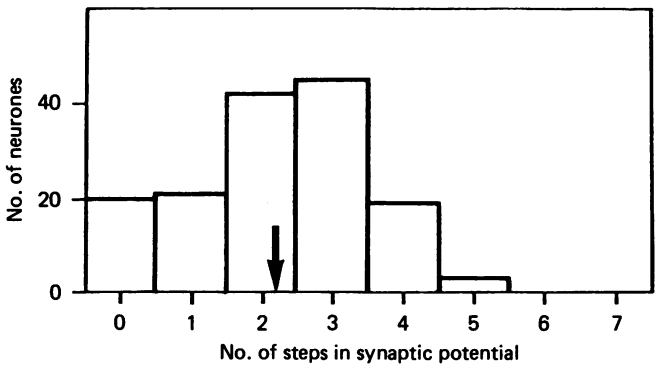

Fig. 4. The distribution of the number of steps in the synaptic response recorded in 150 ganglion cells impaled in nineteen normal ganglia upon graded stimulation of the *ansa subclavia*. The mean was 2·2 (arrow).

be about $11-2=9$ (see above). Counting the number of steps in the synaptic response to graded nerve stimulation is likely to produce an underestimate. Probably, counts that are as low as one, two or three (as are often obtained by stimulating the *ansa subclavia*, or an individual ventral root) are fairly reliable. However, the error grows progressively with the number of steps to be counted. Thus, for example, when estimated by stimulation of nerve *B* in Fig. 3 the mean number of innervating axons expected to be lost by each neurone after partial denervation was only 5·5 ($n = 150$).

Preganglionic sympathetic axons can branch to innervate neurones in several ganglia (Lichtman, Purves & Yip, 1980; see also Langley, 1899; Perri, Sacchi & Casella, 1970; Purves, 1975). Thus preganglionic axons ascending in the *ansa subclavia* might possibly give off collateral branches descending for some distance in the cervical sympathetic trunk and vice versa, in which case the same preganglionic axon might sometimes be activated by stimulation of either nerve. However, intracellular recording from thirty-two neurones in the rostral pole of the *stellate ganglion* (Fig. 1) showed that none of these cells responded with a synaptic potential to stimulation of the rostral cut end of the *ansa subclavia*, which communicated with the stellate ganglion via the cervical sympathetic trunk. In contrast, synaptic potentials were elicited in all but three of these neurones by stimulation of the cervical sympathetic trunk above the inferior cervical ganglion (Fig. 1). Corresponding results were obtained in preparations in which the *ansa subclavia* was left intact while the corresponding segment of the cervical sympathetic trunk was cut and stimulated. Thus, although preganglionic axons in each nerve presumably give off collateral branches in several ganglia, few if any of the collaterals ramifying in the inferior cervical ganglion descend far towards the stellate ganglion in the other nerve.

Preganglionic axons might also branch in the stellate ganglion and run in both the *ansa subclavia* and the cervical sympathetic trunk. However, this pattern of branching is uncommon since only one of thirty-three neurones impaled in the *inferior cervical ganglion* (Fig. 1) showed a synaptic potential in response to stimulation of the caudal cut end of the *ansa subclavia*, leaving the other nerve intact.

Intracellular recording from ganglion cells after partial denervation

During the first 4 weeks after partial denervation there was a progressive increase in the number of steps in the synaptic response elicited in individual ganglion cells by graded stimulation of the *ansa subclavia* (Fig. 5). On average, the number of steps in the synaptic response increased from about two to about five. However, since this number approached that obtained in normal ganglia by graded stimulation of nerve *B* in Fig. 3 (5·5), and since the number of innervating axons per cell from this source was probably about $11-2=9$ (see above) each neurone may be innervated by as many as seven or eight axons, on average, four weeks after partial denervation. Collateral branching of ganglion cell axons (see Sargent & Dennis, 1977) could be excluded as a source of new synaptic contacts, since stimulation of the inferior post-ganglionic nerve caused only an antidromic action potential, and no synaptic potential, in the majority of ganglion cells projecting through this nerve. In fact, a synaptic potential was observed in only two of eighty neurones (2·5 %) tested in this way 2–16 weeks after partial denervation. This is similar to the incidence obtained in normal ganglia (two of sixty cells, or 3 %; see also Perri *et al.* 1970; Purves, 1975). Ganglionic interneurones might also mediate synaptic potentials recorded in principal cells. The function of the small intensely fluorescent cells in the superior cervical ganglion is unknown, but probably they do not cause fast excitatory synaptic potentials in ganglion cells (Burnstock & Costa, 1975).

In ganglia examined 4–16 weeks after partial denervation, occasional ganglion cells were re-innervated by the crushed nerve. Early re-innervation was characterized by small synaptic potentials having a longer than normal latency. The earliest interval at which some ganglion cells were re-innervated by the crushed nerve was 4 weeks, but re-innervation was not the rule even 10 and 16 weeks after the operation. All ganglion cells that were re-innervated by the crushed nerve (seventy-nine of 410 neurones impaled 4–16 weeks after the operation) were excluded from the results presented here. However, if the injured preganglionic axons grew back via the *ansa subclavia*, re-innervation might be faster, and the result might imitate sprouting of

intact preganglionic axons. This possibility was almost certainly ruled out by observations made on neurones in the stellate ganglion. It is known that about 90 % of these neurones receive innervation from preganglionic axons that ascend to the superior cervical ganglion (Lichtman *et al.* 1980). By stimulating the *ansa subclavia*, we observed a synaptic response in about half the stellate ganglion cells impaled in normal preparations. However, the proportion of stellate ganglion cells responding with a synaptic potential to stimulation of the *ansa subclavia* was reduced at long intervals after the operation, contrary to expectation if the crushed preganglionic axons were to regrow via the intact *ansa subclavia* (J. Mæhlen & A. Njå, to be published).

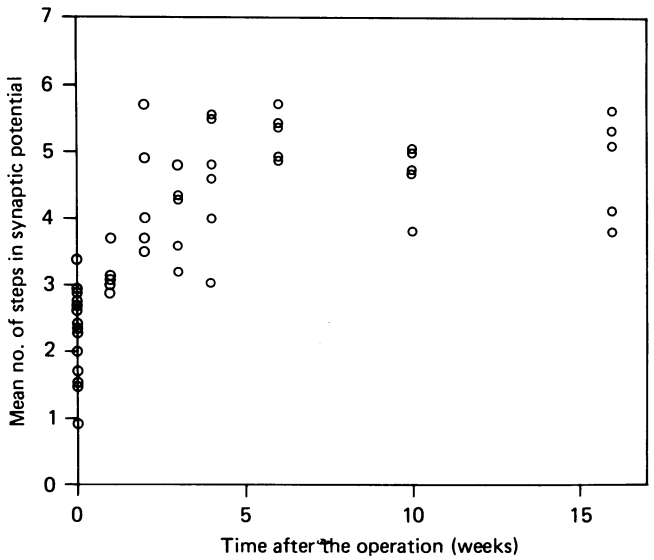

Fig. 5. Increased contribution of the *ansa subclavia* to neurones in the superior cervical ganglion after partial denervation. The number of steps in the synaptic response to graded nerve stimulation is shown as a function of time after the operation. Each symbol represents the mean for five to twenty neurones impaled in one ganglion.

Therefore, each ganglion cell appeared to become innervated by a larger number of the intact (or, uninjured) preganglionic axons after partial denervation. If other sources of innervation are disregarded, this increase in the number of intact preganglionic axons converging on each ganglion cell is equivalent to an increase in the number of ganglion cells contacted by each intact preganglionic axon. Thus our results demonstrate a substantial sprouting of residual preganglionic axons after partial denervation.

We also measured the amplitude of the compound e.p.s.p. (see Njå & Purves, 1978) elicited in each ganglion cell by supramaximal stimulation of the *ansa subclavia*. These measurements were ordered according to the number of steps in the synaptic response to graded nerve stimulation (for neurones showing $\leqslant$ four steps) in order to see whether there were any changes in the amplitude of the unitary e.p.s.p. during sprouting (Table 2). The median e.p.s.p. amplitude in neurones thought to be

innervated by a single preganglionic axon from the *ansa subclavia* was greatly increased after sprouting (by about 200%), and the increase was also large for neurones innervated by two and three preganglionic axons. Thus, the data shown in Table 2 suggest that the synaptic influence on each ganglion cell by each innervating preganglionic axon increases during sprouting.

TABLE 2. The median amplitude of the composite e.p.s.p. recorded in ganglion cells thought to be innervated by one, two, three and four intact preganglionic axons acutely after partial denervation and 3–16 weeks after the operation. The numbers of neurones are shown in parentheses

	E.p.s.p. amplitude (mV)			
No. of steps in synaptic potential	Acutely after partial denervation	3–16 weeks after partial denervation	% increase	Level of significance
0	— ($n = 20$)	— ($n = 5$)	—	—
1	3 ($n = 21$)	10 ($n = 7$)	233	< 0.050
2	6 ($n = 41$)	18 ($n = 17$)	200	< 0.001
3	9 ($n = 44$)	22 ($n = 25$)	144	< 0.001
4	12 ($n = 19$)	22 ($n = 65$)	83	< 0.001
$\geqslant 5$	— ($n = 2$)	— ($n = 204$)	—	—

Stimulation of the ventral roots in vivo

The selectivity of the synaptic connexions formed by sprouting was examined by observing the peripheral sympathetic effects elicited by stimulation of the ventral roots T1 and T4 in anaesthetized animals. In five acutely operated guinea-pigs, which served as controls, the pattern of sympathetic effect on the eye and the ear in response to stimulation of these ventral roots on the partially denervated side was similar to that observed on the normal side, except that the responses were greatly reduced in strength (Table 3). Thus, on both sides, dilatation of the pupil was elicited by stimulation of T1 but not T4, whereas vasoconstriction of the ear occurred in response to stimulation of T4 but not T1. The only exception was a weak vasoconstriction elicited by stimulation of T1 on the normal side in one animal (Table 3). This pattern is the same as that previously described in guinea-pigs (Njå & Purves, 1977*a*) and other mammals (Langley, 1892).

Another five animals were examined 3–6 weeks after partial denervation, when sprouting was near the end stage, as judged by intracellular recording (Fig. 5). In these animals, the end organ responses on the normal and operated side were almost equally strong. However, there was no loss of specificity. Only in one case did we observe a response on the operated side that was not present on the normal side in the same animal (a weak vasoconstriction of the ear in response to stimulation of T1), but as already described, a similar weak response occurred on the normal side in one of the animals examined acutely after partial denervation (Table 3). Thus after sprouting, as in normal guinea-pigs (see Njå & Purves, 1977*a*), stimulation of T1 caused near maximal dilatation of the pupil, but just threshold effects on the blood vessels of the ear. Such differential effects on end organs elicited by stimulation of individual ventral roots show that sprouting preganglionic axons established selective connexions with ganglion cells.

TABLE 3. Responses of the pupil and of the blood vessels of the ear to stimulation of the ventral roots T1 and T4 in five animals studied acutely after partial denervation and in another five animals 3–6 weeks after the operation. The responses were graded subjectively on a 0 to + + + + scale

		Normal side		Operated side	
		Dilatation of the pupil	Vasoconstriction of the ear	Dilatation of the pupil	Vasoconstriction of the ear
Acutely	T1	+ + +	0	+	0
		+ + +	+	+	0
		+ + +	0	+	0
		+ + +	0	+	0
		+ + +	0	+	0
	T4	0	+ + + +	0	+ +
		0	+ + +	0	+
		0	+ + +	0	+
		0	+ + + +	0	+ +
		0	+ + +	0	+
After 3–6 weeks	T1	+ + + +	0	+ + +	+
		+ + +	0	+ + +	0
		+ + +	0	+ + +	0
		+ + +	0	+ +	0
		+ + +	0	+ + +	0
	T4	0	+ + + +	0	+ + + +
		0	+ + + +	0	+ + + +
		0	+ + + +	0	+ + +
		0	+ + + +	0	+ + +
		0	+ + +	0	+ + +

DISCUSSION

Restoration of normal sympathetic end organ responses

The present results show that selective synaptic connexions are established during sprouting of intact preganglionic sympathetic axons. Thus 3–6 weeks after partial denervation of the superior cervical ganglion, the sympathetic end organ responses to ventral root stimulation, which were very weak acutely after the operation, had grown to almost normal strength without losing specificity: just as on the normal side of the same animals, stimulation of T1 affected the eye and stimulation of T4 affected the ear, with little or no overlap (Table 1). It is highly unlikely that regeneration of the injured axons contributed to this result, since intracellular recording *in vitro* showed that re-innervation was infrequent, and always weak when present, until after the time when the end organ responses were examined. In contrast, the strength of innervation of individual ganglion cells by residual preganglionic axons, and the magnitude of the sympathetic end organ responses to ventral root stimulation, were both several times greater 3–6 weeks after partial denervation than they were acutely after the operation.

It is well known that the normal pattern of end organ responses is re-established by re-innervation of the superior cervical ganglion after complete denervation (Langley, 1895, 1897; Guth & Bernstein, 1961; Njå & Purves, 1977*b*, 1978). Re-innervation also restores, with considerable fidelity, the characteristic pattern of

innervation of individual ganglion cells by ventral roots. Thus re-innervated neurones, like normal ones, tend to be strongly innervated by a single spinal cord segment, while the synaptic influence of adjacent segments diminishes as a function of distance from the dominant segment (see the review by Purves & Lichtman, 1978). It is likely that a similar pattern can be established by sprouting after partial denervation, but this remains to be experimentally confirmed.

Connexions established between preganglionic axons and ganglion cells not contacting each other in normal ganglia

By the present way of partial denervation, most (perhaps all) ganglion cells lost a substantial part of their normal preganglionic innervation. Thus at the outset of sprouting, each intact ganglionic axon had the possibility to form new synapses on almost any neurone in the ganglion (there is no obvious somatotopical organization within the superior cervical ganglion, see Lichtman *et al.* 1979). One way in which the specificity of the sympathetic end organ responses might be preserved during sprouting would be that each intact preganglionic axon formed new synapses exclusively on ganglion cells with which it already maintained some synaptic connexions. However, this was ruled out by intracellular recording, which showed that during the course of sprouting, each intact preganglionic axon established synaptic connexions with a substantial number of ganglion cells that it did not innervate normally. On the other hand, the restoration of normal sympathetic end organ responses to ventral root stimulation shows that sprouting did not occur at random. From these results, it appears that the mechanisms governing the formation of selective synaptic connexions in the superior cervical ganglion operated efficiently between preganglionic axons and ganglion cells that did not form synaptic connexions with each other in the normal ganglion.

Effect of the type of partial denervation

Within several weeks after interruption of the sympathetic rami T1–T3 in the cat, stimulation of the preganglionic axons arising from T4–T7 causes strong activation of end organs that were previously activated only by T1–T3 (Murray & Thompson, 1957). By contrast, we find in the guinea-pig that the normal end organ responses to stimulation of T1 and T4 are restored after removing some, but not all, the preganglionic axons arising from each spinal segment innervating the ganglion. Since the normal pattern of sympathetic end organ responses in cats and guinea-pigs are similar (see Langley, 1892 and Njå & Purves, 1977*a*), we conclude that the type of partial denervation determines the range of peripheral sympathetic effects elicited by an individual spinal cord segment after sprouting. Thus the presence of some preganglionic axons arising from each of the spinal segments involved appears to be necessary for selective synapse formation during sprouting. This type of behaviour suggests a competitive interaction between the preganglionic axons.

We would like to thank Ingerid Mürer Knutzen for preparing the histological sections, Karl Buday for help with the illustrations and Håvard Tønnesen for technical assistance. We are also grateful to Jan Jansen, David Johnson, Knut Liestøl, Dale Purves, Richard Ribchester, Eric Rubin and Donald Wigston for helpful comments. This work was supported by The Norwegian Research Council for Science and the Humanities (projects C.10.10.-31. and C.10.10.-44).

REFERENCES

BURNSTOCK, E. & COSTA, M. (1975). *Adrenergic Neurones*. London: Chapman & Hall.

GUTH, L. & BAILEY, C. J. (1961). Pupillary function after alteration of the preganglionic sympathetic innervation. *Expl Neurol.* **3**, 325–332.

GUTH, L. & BERNSTEIN, J. J. (1961). Selectivity in the re-establishment of synapses in the superior cervical sympathetic ganglion of the cat. *Expl Neurol.* **4**, 59–69.

HOLLÄNDER, H. & VAALAND, J. L. (1968). A reliable staining method for semi-thin sections in experimental neuroanatomy. *Brain Res.* **10**, 120–126.

LANGLEY, J. N. (1892). On the origin from the spinal cord of the cervical and upper thoracic sympathetic fibres, with some observations on white and grey rami communicantes. *Phil. Trans. R. Soc.* B **183**, 85–124.

LANGLEY, J. N. (1895). Note on regeneration of preganglionic fibres of the sympathetic. *J. Physiol.* **18**, 280–284.

LANGLEY, J. N. (1897). On the regeneration of preganglionic and of post-ganglionic visceral nerve fibres. *J. Physiol.* **22**, 215–230.

LANGLEY, J. N. (1899). On axon-reflexes in the preganglionic fibres of the sympathetic system. *J. Physiol.* **25**, 364–398.

LICHTMAN, J. W., PURVES, D. & YIP, J. W. (1979). On the purpose of selective innervation of guinea-pig superior cervical ganglion cells. *J. Physiol.* **292**, 69–84.

LICHTMAN, J. W., PURVES, D. & YIP, J. W. (1980). Innervation of sympathetic neurones in the guinea-pig thoracic chain. *J. Physiol.* **298**, 285–299.

MÆHLEN, J. & NJÅ, A. (1979). Formation and regression of synapses in the guinea-pig superior cervical ganglion after partial denervation. *Acta physiol. scand.* Suppl. **473**, 56.

MURRAY, J. G. & THOMPSON, J. W. (1957). The occurrence of collateral sprouting in the sympathetic nervous system of the cat. *J. Physiol.* **135**, 133–162.

NJÅ, A. & PURVES, D. (1977*a*). Specific innervation of guinea-pig superior cervical ganglion cells by preganglionic fibres arising from different levels of the spinal cord. *J. Physiol.* **264**, 565–583.

NJÅ, A. & PURVES, D. (1977*b*). Re-innervation of guinea-pig superior cervical ganglion cells by preganglionic fibres arising from different levels of the spinal cord. *J. Physiol.* **272**, 633–651.

NJÅ, A. & PURVES, D. (1978). Specificity of initial synaptic contacts made on guinea-pig superior cervical ganglion cells during regeneration of the cervical sympathetic trunk. *J. Physiol.* **281**, 45–62.

PERRI, V., SACCHI, O. & CASELLA, C. (1970). Synaptically mediated potentials elicited by the stimulation of post-ganglionic trunks in the guinea-pig superior cervical ganglion. *Pflügers Arch.* **314**, 55–67.

PURVES, D. (1975). Functional and structural changes of mammalian sympathetic neurones following interruption of their axons. *J. Physiol.* **252**, 429–463.

PURVES, D. (1976). Functional and structural changes in mammalian sympathetic neurones following colchicine application to post-ganglionic nerves. *J. Physiol.* **259**, 157–175.

PURVES, D. & LICHTMAN, J. W. (1978). Formation and maintenance of synaptic connexions in autonomic ganglia. *Physiol. Rev.* **58**, 821–862.

PURVES, D. & THOMPSON, W. (1979). The effects of post-ganglionic axotomy on selective synaptic connexions in the superior cervical ganglion of the guinea-pig. *J. Physiol.* **297**, 95–110.

SARGENT, P. B. & DENNIS, M. J. (1977). Formation of synapses between parasympathetic neurones deprived of preganglionic innervation. *Nature, Lond.* **268**, 456–458.