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SPECIFICITY OF INITIAL SYNAPTIC CONTACTS MADE ON GUINEA-PIG SUPERIOR CERVICAL GANGLION CELLS DURING REGENERATION OF THE CERVICAL SYMPATHETIC TRUNK

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SUMMARY

1. Largely appropriate synaptic connexions are formed with neurones in the superior cervical ganglion at long intervals after interruption of the preganglionic nerve. In the present study we have assessed the accuracy of connexions during the early stages of re-innervation by observing end-organ responses to ventral root stimulation *in vivo*, and by recording intracellularly from ganglion cells during ventral root stimulation in isolated preparations.

2. Appropriate, but weak, end-organ responses were elicited by stimulation of the first and fourth thoracic ventral roots (T1 and T4) 15–30 days after freezing the cervical sympathetic trunk.

3. Intracellular recordings from ganglion cells during stimulation of the ventral roots C8–T7 *in vitro* showed that synaptic contacts are first re-established 8–11 days after freezing the preganglionic nerve. The proportion of re-innervated cells, and the strength of innervation of individual neurones, increased rapidly for up to about 3 months after nerve injury, but showed little change thereafter. Innervation remained weaker than normal even after 6 months.

4. Patterns of segmental innervation recorded intracellularly during the early stages of regeneration were similar to, but more restricted than normal. Even 13–19 days after interruption of the preganglionic nerve, neurones re-innervated by more than one spinal cord segment tended to be innervated by a *contiguous* subset of the spinal segments which contribute innervation to the ganglion. The incidence of neurones receiving innervation from a discontinuous segmental subset was about the same at early and late stages of re-innervation.

5. Throughout the course of nerve regeneration, re-innervated neurones tended to receive dominant synaptic input from axons arising at a particular spinal level, as do normal cells, with adjacent segments contributing a synaptic influence that diminished as a function of distance from the dominant segment.

6. The results of these experiments argue against the initial formation of imprecise connexions with subsequent retention of appropriate contacts and a loss of

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inappropriate ones. Rather our findings suggest that the re-innervation of ganglion cells proceeds by a gradual accumulation of synaptic connexions which are, from the outset, appropriate.

INTRODUCTION

Preganglionic fibres arising from neurones in the lower cervical and the upper thoracic spinal cord innervate nerve cells in the mammalian superior cervical ganglion in a characteristic way. *In vivo* stimulation of each of the ventral roots which supply the ganglion elicits distinct patterns of end-organ responses (Langley, 1892; Murray & Thompson, 1957; Guth & Bernstein, 1961; Njå & Purves, 1977*a*, *b*). Stimulation of the uppermost thoracic ventral root (T1), for example, excites the pupillary and palpebral musculature, but has relatively little effect on the blood vessels of the ear, while stimulation of a lower thoracic root such as T4 has little or no effect on the eye, but causes vasoconstriction of the ear and piloerection on the face and neck. Thus preganglionic axons arising from different levels of the spinal cord make selective contacts with neurones in the superior cervical ganglion.

Intracellular recording from individual neurones *in vitro* suggests that this selectivity of ganglionic innervation is only broadly determined: although individual neurones prefer innervation from particular segments, synaptic contacts from other segments are not excluded, but only less likely to occur. Thus the innervation of ganglion cells is to some extent stochastic. Typically, ganglionic neurones are innervated by at least ten to twelve preganglionic axons which arise from a contiguous subset of four of the eight ventral roots which supply the ganglion. The input from a single segment usually dominates each cell, with the strength of the synaptic response to stimulation of adjacent segments falling off as a function of distance from the dominant level (Njå & Purves, 1977*a*, *b*). This arrangement, in which neurones innervated by rostral segments are less likely to receive synaptic contacts from more caudal segments, and vice versa, is presumably the cellular basis for the specificity of connexions inferred from ventral root stimulation *in vivo*.

The characteristic pattern of both end-organ responses and the innervation of individual neurones in normal animals has allowed experiments which test the ability of the sympathetic nervous system to re-establish appropriate connexions during re-innervation. The outcome is that end-organ responses, and the pattern of innervation of individual cells, are restored with considerable fidelity when tested several months after section of the cervical sympathetic trunk (Langley, 1895, 1897; Njå & Purves, 1977*b*; see also Guth & Bernstein, 1961; Landmesser & Pilar, 1970).

In the present work we have examined the early course of re-innervation of the guinea-pig superior cervical ganglion to see whether appropriate connexions are established from the outset, or whether the final result is achieved by a re-arrangement of connexions which are initially less precise. Our results show that selective connexions are formed during the earliest stages of regeneration.

METHODS

The methods used are, for the most part, similar to those we have previously described (Purves, 1975; Njå & Purves, 1977*a*, *b*). However, in order that axons regenerate with a minimum of misdirection and interference from scarring, the preganglionic nerve was interrupted by

freezing (Raisman, Field, Ostberg, Iversen & Zigmond, 1974; Ostberg, Raisman, Field, Iversen & Zigmond, 1975), rather than by transection. In fact, the method of interruption made no difference in the outcome (see Results section). As normal controls we have used results obtained in unoperated guinea-pigs of similar size and age in an earlier study (Njå & Purves, 1977 *b*).

Interruption of the cervical sympathetic trunk by freezing

Fifty-seven young adult guinea-pigs (200–300 g) were anaesthetized with pentobarbitone (30–40 mg/kg, I.P.), and the right cervical sympathetic trunk exposed under aseptic conditions (Fig. 1). The preganglionic nerve was touched lightly with a small piece of dry ice about 5–6 mm below the caudal pole of the ganglion. This procedure, which was repeated 3–4 times, caused freezing of the nerve and surrounding tissues for a distance of 2–3 mm. On the basis of electron microscopical examination, freezing by dry ice, as nerve section, has been reported to produce complete degeneration of the preganglionic fibres within several days in the rat (Raisman *et al.* 1974; Ostberg *et al.* 1975). To ensure the efficacy of the method in our hands, we impaled seventy-one neurones in three ganglia 4–5 days after freezing the sympathetic trunk, and stimulated the preganglionic nerve distal to the lesion with strong current pulses (1·0 msec, 100 V). Although weaker stimulation causes suprathreshold synaptic responses in every neurone in normal ganglia (Purves, 1975), no synaptic potentials could be detected in the neurones impaled after freezing the sympathetic trunk.

Stimulation of the ventral roots in vivo

Fifteen to thirty days after freezing the preganglionic nerve, the end-organ responses to ventral root stimulation were studied in twenty animals anaesthetized with pentobarbitone, and maintained on a respirator. As in 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 were graded subjectively on a 0 to + + + + scale (Njå & Purves, 1977 *a*, *b*). In most experiments we exposed and stimulated only the ventral roots of T1 or T4 on both sides (1·0 msec pulses at 20/sec for several sec; 50–100 V). This limited operation resulted in animals that were in better condition than after more extensive ventral root exposures (Njå & Purves, 1977 *a*). The ventral roots of T1 and T4 were chosen because fibres from each of these normally innervate 60–80% of the neurones in the superior cervical ganglion, and yet the pattern of end-organ responses elicited by the two roots is obviously different (Njå & Purves, 1977 *a*; see also Langley, 1892).

In presenting these results, we have relied primarily on the effects of T1 stimulation, since the interpretation of T4 responses is complicated by the following considerations. In normal guinea-pigs, sympathetic end-organ responses of the head and neck are largely mediated by neurones in the superior cervical ganglion. This has been shown by eliciting the end-organ effects under study here by post-ganglionic nerve stimulation 2–4 days after cervical trunk section (Njå & Purves, 1977 *a*), and by showing that the sympathetic effects of ventral root stimulation are grossly abolished by acute section of the cervical trunk (Langley & Dickinson, 1889; Langley, 1897; A. Njå & D. Purves, unpublished). However, because the end-organ responses elicited during early regeneration were usually weak, we repeated our observations on ventral root stimulation after acute interruption of the cervical trunk to make sure that the responses disappeared completely. Thus in six animals in this series the cervical trunk on both the normal and operated side was avulsed with fine ligatures which had been placed around the nerve at the time of tracheal cannulation, and the ventral roots again stimulated. In confirmation of previous results, end-organ responses were completely abolished by preganglionic nerve avulsion on the normal side, although blood vessels continued to react to T4 stimulation along the caudal margin if the ear in some animals. This presumably represents the border between the region innervated by the superior cervical ganglion and post-ganglionic fibres arising from the stellate ganglion. Avulsion of the regenerating cervical trunk also abolished the reaction of the eye to stimulation of T1. However, vasoconstriction of the entire ear could still be elicited by T4 stimulation on the operated side in some animals. In only two of the six animals was the response abolished by avulsion of the regenerating cervical trunk; in one animal the vascular response was sharply reduced by avulsion but still apparent over the whole pinna, and in three remaining animals little or no reduction was observed. Thus the initial operation interrupting the cervical trunk apparently caused blood vessel supersensitivity or sprouting of the thoracic post-ganglionic

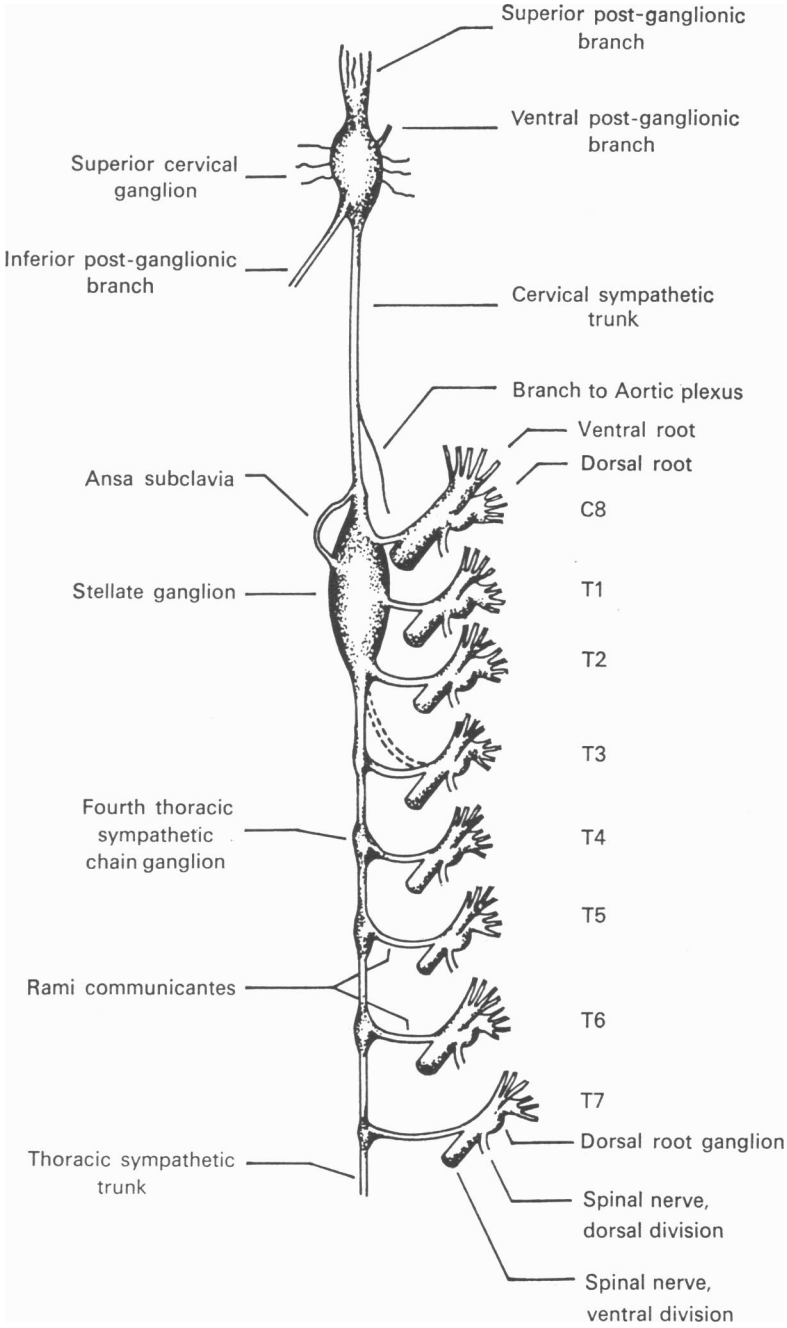

Fig. 1. Diagram of the portion of the guinea-pig peripheral sympathetic system studied in these experiments. Right side, ventral view. Dashed line indicates that the rami of T3 run directly to the stellate ganglion in some animals.

axons which supply the caudal margin of the pinna. The basis for these effects may be partial denervation arising from the interruption of some post-ganglionic fibres ascending in the cervical trunk. Such axons probably arise from small groups of neurones found within the cervical trunk in some guinea-pigs (Njå & Purves, 1977*b*). Alternatively, inactivity of innervated but decentralized peripheral smooth muscle might also lead to supersensitivity and sprouting (see, for example, Lømo & Rosenthal, 1972; Brown & Ironton, 1977).

Intracellular recording from re-innervated ganglion cells during ventral root stimulation

Thirty-seven animals were sacrificed at various intervals after freezing the cervical sympathetic trunk, and the right superior cervical ganglion removed in continuity with the preganglionic nerve and a portion of the thoracic cage including the sympathetic chain and spinal column (Njå & Purves, 1977*a*, *b*). Following dissection and complete exposure of the ventral roots, spinal nerves, rami communicantes and the sympathetic trunk (Fig. 1), the preparation was placed in a bath perfused with oxygenated mammalian Ringer fluid (Liley, 1956) at room temperature. The right ventral roots of C8 through T7 were drawn into suction electrodes for stimulation (0·5 msec pulses at 0·5/sec, 50–100 V); the compound action potentials in response to ventral root stimulation were recorded with suction electrodes applied to the superior and inferior post-ganglionic nerves (see Fig. 1). Intracellular recordings from about 200 neurones in the superior cervical ganglion were made at each of four stages of re-innervation following cervical trunk interruption: after 13–19 days (0·5 months), 30–38 days (1 month), 86–107 days (3 months), and after 167–206 days (6 months). A smaller number of neurones were studied 8–11 days after preganglionic nerve section. For purposes of comparison we have assumed that the pooled results at each interval are homogeneous. In fact there was some variability in the degree of re-innervation amongst different animals at each stage, although the differences were generally minor.

Although we have previously described the methods of impalement, measurement of the excitatory post-synaptic potential (e.p.s.p.) amplitude within the refractory period of a directly evoked action potential, and estimation by graded nerve stimulation of the number of preganglionic fibres innervating each cell (Purves, 1975; Njå & Purves, 1977*a*), the limitations of these methods should be re-emphasized. Measurement of e.p.s.p. amplitude by timing transmitter release during the refractory period of a preceding action potential is less accurate when the ventral roots are stimulated than following stimulation of the cervical trunk. The reason for this is that while transmitter release from different axons occurs almost simultaneously when stimulation is carried out close to the superior cervical ganglion, a complex e.p.s.p. often results when axons with somewhat different conduction velocities are stimulated at the level of the ventral root (see, for example, Fig. 4, Njå & Purves, 1977*b*, and Fig. 5 below). As a result, the response which we measured was often smaller than it would have been if each axon in a ventral root released its transmitter at the same time. Similarly, counts of the number of preganglionic fibres innervating each neurone based on the number of step-like increments in the complex e.p.s.p. upon increasing the strength of stimulation are subject to considerable error. If the number of axons from a particular root contacting a cell is large, one or more incremental steps may be lost because of their small size, or the increased chance of two or more fibres having approximately the same threshold. This is the probable cause of the lower estimates of the number of axons innervating ganglionic neurones when the entire cervical trunk is stimulated (5–7 axons, Purves, 1975, 1976*a*), than when the input to each cell is fractionated by stimulating individual ventral roots (10–12 axons, Njå & Purves, 1977*a*, *b*). Therefore, measurements of e.p.s.p. amplitude and the number of preganglionic fibres innervating neurones represent minimum estimates which are, however, suitable for comparisons of the relative strength of innervation to individual neurones from different roots.

In presenting our results we have ignored the possibility that some synaptic responses elicited by ventral root stimulation might be indirectly mediated by collateral branches of ganglion cells, rather than by preganglionic terminals (Purves, 1976*b*). It seems unlikely that the presence of these relatively rare and generally weak connexions would have much influence on our observations.

RESULTS

In vivo stimulation of ventral roots during early regeneration of the cervical sympathetic trunk

A generally normal pattern of peripheral sympathetic effects is observed in cats and guinea-pigs in response to stimulation of individual ventral roots several months after section of the cervical sympathetic trunk (Langley, 1895, 1897; Njå & Purves, 1977*b*; see also Guth & Bernstein, 1961). In order to examine the specificity of re-innervation during the initial stages of recovery, we studied the pattern of end-organ responses elicited 15–30 days after preganglionic nerve injury. Intracellular recordings showed that a few neurones received a detectable synaptic input as early as 8–11 days after nerve interruption, and that by 13–19 days small to moderate sized e.p.s.p.s could be evoked in about half the neurones (see below). After 1 month, most cells received innervation from at least one preganglionic fibre (see below and Purves, 1976*b*). In accord with these results, most animals showed detectable peripheral responses to ventral root stimulation within 15–30 days after preganglionic nerve injury.

TABLE 1. End-organ responses to *in vivo* stimulation of T1 20–30 days after freezing the cervical trunk. Dilatation of the pupil (P), widening of the palpebral fissure (F), piloerection on the face and neck (H), and vasoconstriction of the ear (E) were graded subjectively on a 0 to + + + + scale

Time after nerve injury (days)	Normal side				Re-innervated side			
	P	F	H	E	P	F	H	E
20	+ + +	+ + +	0	0	+	+	0	0
21	+ + +	+ + +	0	0	0	0	0	0
23	+ + +	+ + +	+ +	0	+	+ +	0	0
23	+ +	+ + +	+	0	0	+	0	0
24	+ + +	+ + +	+	0	+	+	0	0
24	+ + +	+ + +	+	0	+ +	+ +	+	0
26	+ +	+ +	0	0	+ +	+ +	0	0
27	+ + +	+ + +	+ + +	0	+ +	+	0	0
28	+ + +	+ +	+	0	+	+	0	+
30	+ + +	+ + +	+ +	0	+	+ +	0	0

The results of stimulating T1 in ten animals on the normal and operated side 20–30 days after nerve injury are presented in Table 1. In normal guinea-pigs stimulation of T1 causes pupillary dilatation and widening of the palpebral fissure, but relatively little piloerection on the face and neck, or vasoconstriction of the ear, responses which are produced by stimulation of more caudal ventral roots (Njå & Purves, 1977*a*). Although most of the responses to ventral root stimulation during this early phase of re-innervation were weak (and sometimes absent), the great majority of end-organ effects were appropriate. In only one of the ten experiments did we observe very weak vasoconstriction of the ear during stimulation of T1 on the re-innervated side. In a previous study, however, a similar weak response was occasionally seen in normal animals (Njå & Purves, 1977*a*).

In ten other animals the response to stimulation of T4 was tested. Stimulation of T4 normally causes vasoconstriction of the ear and piloerection on the face and neck,

but usually has no effect on the pupil or palpebral fissure. For the reasons discussed in the Methods section, the *in vivo* responses of ear vessels to stimulation of T4 during the early course of re-innervation are difficult to evaluate. With only one exception, however, T4 stimulation did not activate the pupillary or palpebral musculature, but caused only varying degrees of piloerection and vasoconstriction of the ear. Since the results of intracellular recording show that many neurones are re-innervated by T4 fibres at this stage (see next section), and since the synaptic responses are often suprathreshold, dilatation of the pupil and widening of the palpebral fissure should

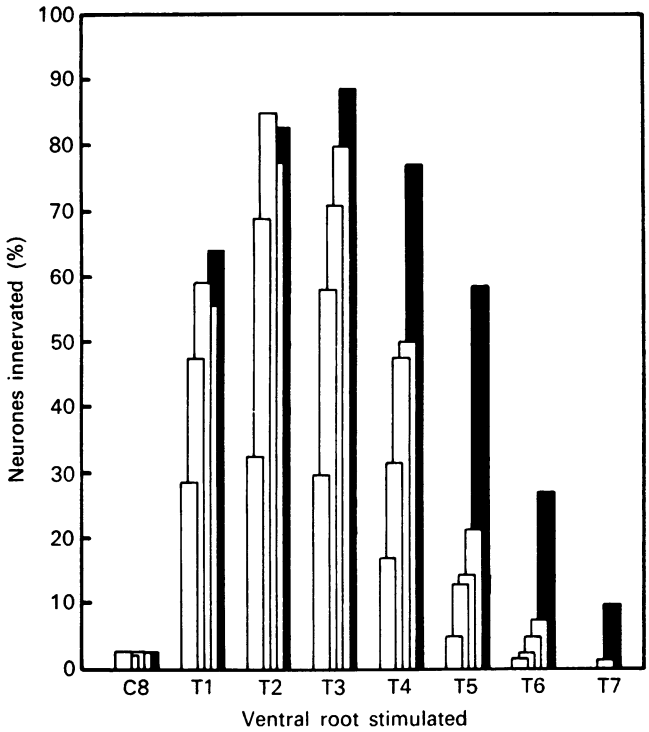

Fig. 2. Proportion of superior cervical ganglion neurones receiving innervation from different spinal segments 0·5, 1, 3 and 6 months after preganglionic nerve interruption (open bars; earliest stage of re-innervation is in the foreground). The filled bars are normal values (replotted from Njå & Purves, 1977*b*). About 200 neurones were impaled at each interval.

have occurred if neurones subserving these functions had been widely re-innervated by T4 axons. Thus, in spite of the difficulty in interpreting the response of ear vessels to T4 stimulation, axons running in this ventral root also appear to re-establish synapses with the neurones they normally contact early during the course of nerve regeneration.

Intracellular recordings from ganglion cells during the early stages of re-innervation

The earliest time at which e.p.s.p.s could be recorded in a few ganglion cells after freezing the cervical sympathetic trunk was 8–11 days, when four of ninety-five

neurones impaled (4 %) gave small subthreshold responses. After 13–19 days 56 % of the neurones impaled received some innervation (n = 200); 91 % were innervated after 1 month (n = 198), and 98 % after 3 months (n = 200) and 6 months (n = 200).

Innervation by ventral roots at different stages of re-innervation

In normal animals, the preganglionic fibres running in each ventral root innervate a characteristic fraction of the neurones in the superior cervical ganglion (Njå & Purves, 1977*a*). At long times after preganglionic nerve section (4–9 months), the proportion of cells contacted by each root is nearly normal for the rostral segments (C8–T3), but falls progressively short of normal for more caudal segments (T4–T7) (Njå & Purves, 1977*b*). This general relation among the different ventral roots was evident from the earliest stages of re-innervation (Fig. 2): 13–19 days after nerve

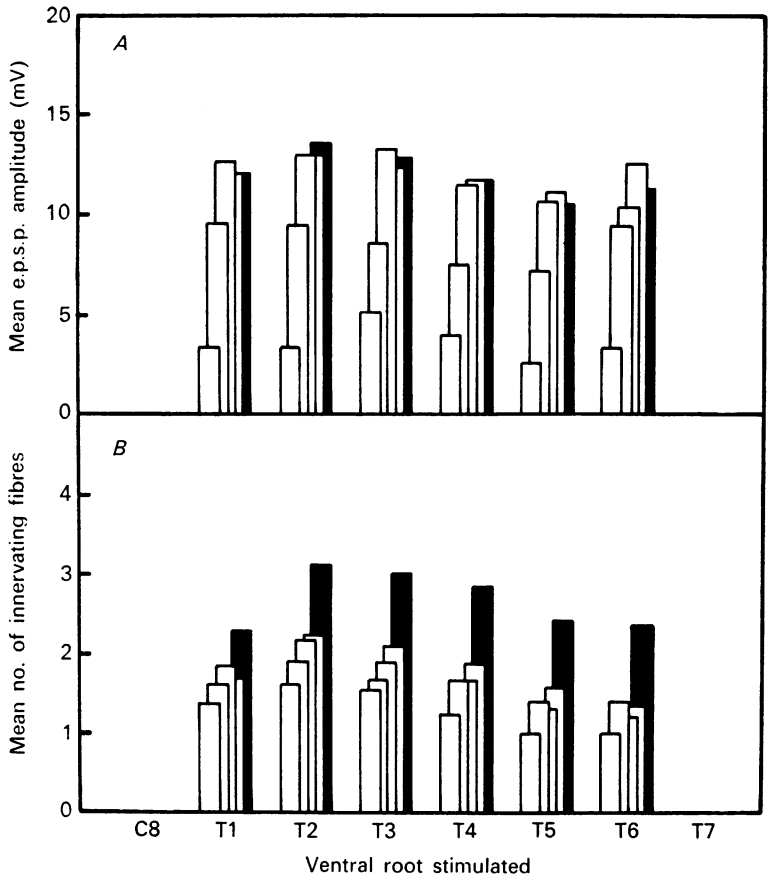

Fig. 3. Course of re-innervation of the superior cervical ganglion as measured by the mean e.p.s.p. amplitude in response to stimulation of each ventral root (*A*), and the mean number of axons contributed to particular neurones by each ventral root (*B*). Only neurones receiving *some* innervation from the root under consideration are included. Open bars represent results at 0·5 and 1, 3 and 6 months, as in Fig. 2. Filled bars are normal values (replotted from Njå & Purves, 1977*b*). C8 and T7 are not included in the results because of the small number of cells innervated by these roots throughout regeneration (see Fig. 4).

injury, each ventral root innervated a fraction of the total number of neurones impaled that was roughly proportional to the percentage of neurones innervated by each segment at later stages of regeneration. Thus there was no obvious tendency for the innervation arising from some segments to give way in favour of another segment during the course of regeneration. Rather the population of neurones re-innervated by each root appeared to gradually increase, with the proviso that the caudal roots were, on average, less efficient in this task than rostral ones.

To further test the strength of innervation from various ventral roots at different stages of regeneration, we determined the average e.p.s.p. amplitude, and the average estimated number of preganglionic fibres supplied to neurones by each root. Neurones in which no synaptic responses could be elicited by stimulation of a particular ventral root were excluded in calculating the average value for that root. Both the number of preganglionic fibres supplied to ganglion cells by each root, and the amplitude of the synaptic response, increased progressively as a function of time after preganglionic nerve injury (Fig. 3). While this is in accord with the results presented in Fig. 2, there is no apparent difference in the performance of rostral and caudal roots when their effectiveness is measured by the amplitude of the synaptic response, or the number of fibres contributed to each neurone innervated by the various ventral roots. Although caudal segments re-innervate a smaller fraction of their normal share of ganglion cells than rostral segments, the average synaptic contribution to cells which *are* innervated appears to reach roughly the same relative final level at approximately the same rate for caudal and rostral segments (Fig. 3). This suggests that those caudal axons which establish some synaptic contacts with ganglion cells are as effective in synapse formation as axons from the more rostral segments. Thus, the over-all inefficiency of re-innervation by caudal segments may lie in the number of axons from these roots which reach the ganglion, rather than the synaptogenic efficacy of those fibres which are able to establish contacts with ganglion cells. Since, on average, fewer axons contact each neurone after regeneration, but elicit synaptic potentials of approximately normal amplitude (Fig. 3 and below; see also Purves, 1976*b*; Njå & Purves, 1977*b*), many axons from the rostral segments, at least, must make a larger number of synaptic contacts after re-innervation than they do normally.

Pattern of synaptic contacts with individual neurones: specificity during early re-innervation

The characteristic pattern of innervation of individual ganglion cells is largely restored when tested several months after preganglionic nerve section (Njå & Purves, 1977*b*). Each re-innervated neurone is generally contacted by a contiguous subset (three on average) of the eight spinal segments which contribute innervation to the ganglion, the strength of segmental input declining as a function of distance from the dominant segment.

A similar, although more restricted, pattern was observed during the early stages of re-innervation. Thus, 13–19 days after nerve injury, those neurones which received innervation from more than a single segment were usually contacted by fibres arising from contiguous ventral roots (Figs. 4*A*, 5). During the course of re-innervation, the average number of segments innervating all the cells impaled gradually increased

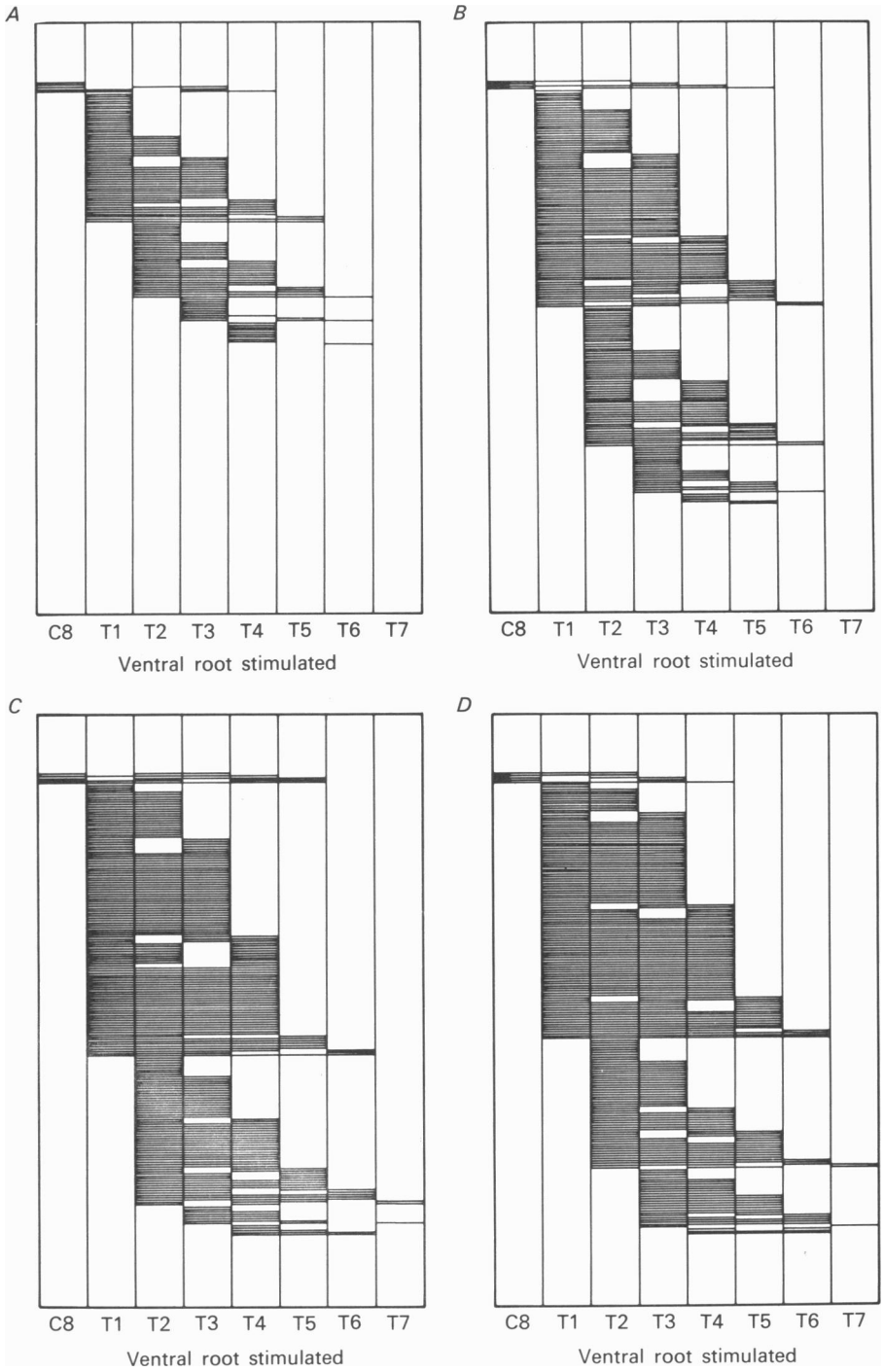

Fig. 4. Contiguity of segmental innervation to particular ganglion cells. Each horizontal line represents the pattern of segmental innervation to a single neurone impaled after 5·0 months (*A*), 1 month (*B*), 3 months (*C*) and 6 months (*D*).

from 1·2 after 13–19 days to 3·0 after 6 months (average in normal animals = four segments).

The relative proportion of continuous and discontinuous segmental patterns of innervation appeared to be about the same throughout the course of re-innervation (Fig. 4, Table 2). In order to assess the prevalence of contiguous innervation at each stage of regeneration, we calculated the number of neurones expected to show discontinuous patterns if the contributions from each ventral root to particular cells are established independently. By comparing the calculated values to those observed, we obtained a measure of the deviation from random at each interval. Assuming segmental independence, the probability of a particular pattern of segmental innervation is given by the product of the probabilities of innervation (or the lack of it) for each of the eight contributing ventral roots. The probability of innervation from each segment was estimated from Fig. 4 by dividing the number of neurones receiving innervation from each root by the total number of neurones impaled. By calculating the sum of the probabilities for each of the thirty-six possible continuous combinations (including innervation by single segments) and the probability of no innervation, we obtained the complementary probability of discontinuous patterns of innervation (Table 2; see also Njå & Purves, 1977*a*, *b*).

The presence of a large fraction of uninnervated neurones 13–19 days after preganglionic nerve injury (see Fig. 4) raises the question of whether this approach remains valid during the initial stages of re-innervation. This was checked by calculating the conditional probability of discontinuous patterns for neurones innervated by a particular number of segments. The expected number of neurones showing discontinuous patterns of innervation at each stage is given by the sum of the predicted discontinuities for neurones innervated by 2, 3, ..., 7 segments (innervation by one, or all eight segments, cannot be discontinuous). To obtain the expected incidence of discontinuous patterns in neurones innervated by two to seven segments, all possible patterns of segmental input were listed, and the discontinuous combinations determined. For neurones innervated by a particular number of segments, the probability of a discontinuous pattern is then given by the sum of the probability of all the possible *discontinuous* patterns, divided by the sum of the probability of all possible patterns. These determinations were calculated using the data shown in Fig. 4. The predicted number of cells with discontinuous patterns of innervation calculated in this way was the same as that obtained by the simpler method described above.

At all stages of re-innervation, the number of neurones showing discontinuous patterns of segmental innervation was much smaller than expected if re-innervation by fibres arising from each spinal segment occurred independently; conversely, the number of cells receiving continuous input was much greater than expected by chance (Table 2). Although the ratio of observed to expected patterns varied, there was no evidence of a systematic change during the course of re-innervation: the ratios were about the same after 13–19 days as after 6 months. This suggests that the mechanism producing the characteristic pattern of innervation to individual cells operates effectively from the earliest stages of re-innervation.

The process of re-innervation was further analysed by measuring the synaptic response to supramaximal stimulation of each ventral root as a function of proximity to the spinal segment providing the dominant input to each neurone. Re-innervated ganglion cells, like normal neurones, tended to be dominated by axons from a particular ventral root, with other roots contributing a synaptic influence which decreased with increasing distance from that segment (Figs. 6, 7). This tendency was also

apparent from the earlist stages of re-innervation, and did not appear to be stronger after 6 months than at 13–19 days. Thus, throughout re-innervation, the average pattern of synaptic responses tended to shift in a continuous way according to the level of the dominant segment, whether e.p.s.p. amplitude (Fig. 6) or preganglionic fibre number (Fig. 7) was used as the criterion of segmental dominance. Re-innervated neurones appeared to follow this general rule of segmental dominance somewhat less well than normal neurones (see also Njå & Purves, 1977*b*). This deviation from normal presumably reflects the moderate increase in discontinuous patterns of innervation after preganglionic nerve regeneration, as well as the relative deficiency of re-innervation from the more caudal segments. These discrepancies are equally evident at early, intermediate, and late stages of re-innervation.

TABLE 2. Differences between the observed segmental patterns of re-innervation, and the patterns expected if the innervation contributed by each ventral root were established independently. The significance levels (one-sided) were obtained from the normal approximation of a binomial distribution with a mean, np, equal to the expected number of cells receiving discontinuous innervation, and a standard deviation = $\sqrt{(npq)}$ (Noether, 1976)

Time after freezing the preganglionic nerve (months)	No. of neurones impaled	No. of neurones inner-vated	Ratio of discont./ cont. patterns	No. of neurones receiving discontinuous segmental innervation			
				Obs.	Exp.	Ratio of obs./exp.	Level of significance
0·5	200	112	0·16	18	32·5	0·55	0·0003
1	198	180	0·21	37	58·7	0·63	0·0005
3	200	196	0·19	37	62·9	0·59	< 0·0001
6	200	196	0·16	32	70·4	0·45	< 0·0001
4–9 after nerve section (from Njå & Purves, 1977*b*)	200	200	0·16	32	78·6	0·41	< 0·0001
Normal (from Njå & Purves, 1977*b*)	202	202	0·02	5	90·6	0·06	< 0·0001

Fig. 5. Intracellular recordings from neurones 13–19 days after freezing the cervical sympathetic trunk. *A*, most neurones received innervation from a contiguous subset of spinal cord segments during the initial stage of reinnervation (see Fig. 4 *A*). Typically, innervation derived from a particular ventral root was dominant (in this case T3; see Methods), with progressively weaker innervation from segments increasingly distant from the dominant level. Eighteen days after cervical trunk injury; resting potential − 59 mV. *B*, even when innervation to a neurone was too weak to fire an action potential, if more than one ventral root contributed innervation, the roots tended to be contiguous. Fourteen days after cervical trunk injury; resting potential − 80 mV. An action potential 130 mV in amplitude could be elicited by direct stimulation. *C*, neurone showing a discontinuous pattern of segmental innervation. The incidence of such abnormal patterns was similar throughout the couse of re-innervation (see Fig. 4 and Table 2). Fourteen days after cervical trunk injury; resting potential − 62 mV. An action potential 100 mV in amplitude could be elicited by direct stimulation.

Re-innervation after freezing compared to re-innervation following nerve section

The degree of re-innervation of the superior cervical ganglion 6 months after freezing the preganglionic nerve was indistinguishable from results obtained 4–9 months after nerve section (Njå & Purves, 1977*b*). The final number of neurones

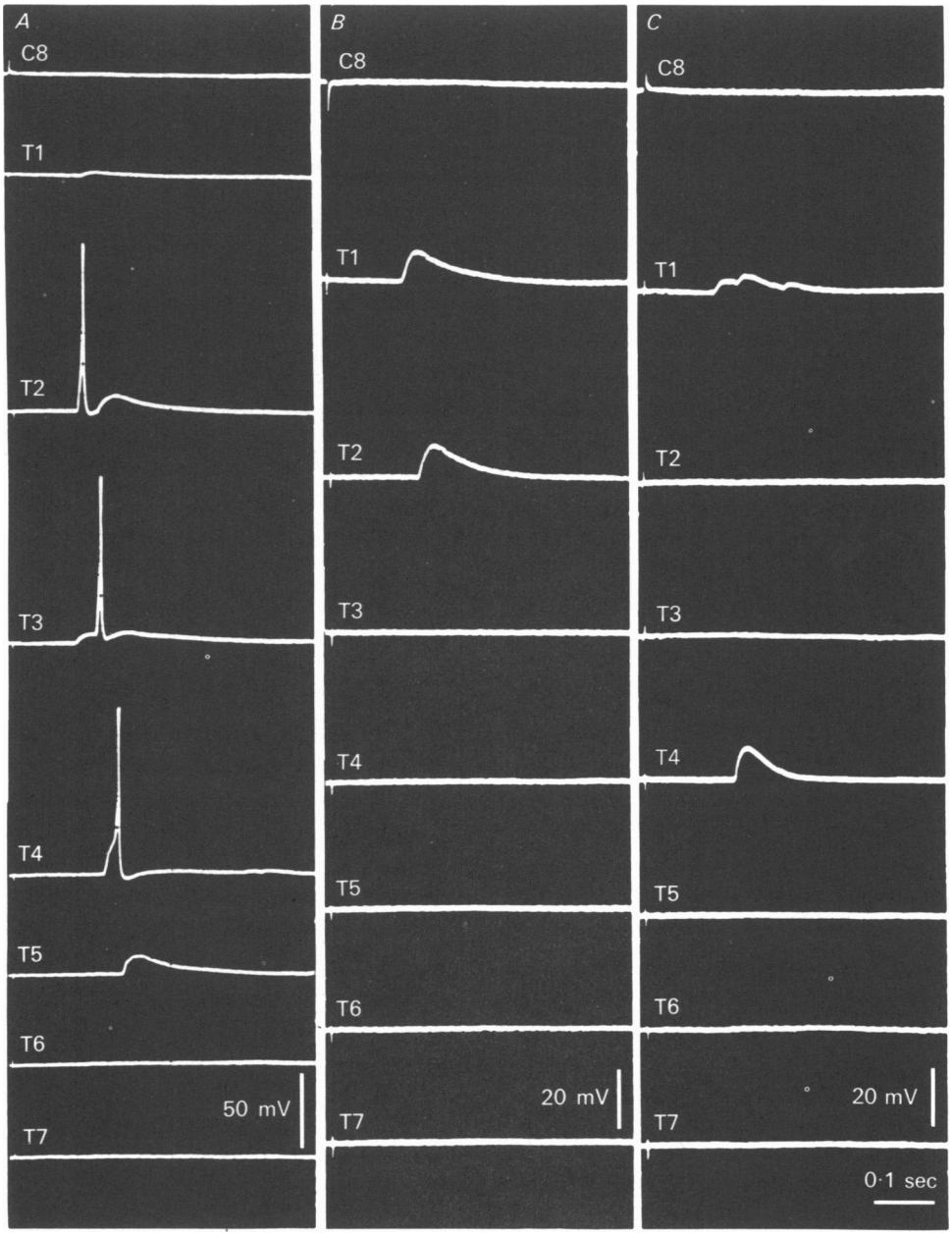

Fig. 5. For legend see facing page.

innervated (98 % after freezing, 100 % after section), the mean number of axons innervating each neurone (5·9 ± 0·2 after freezing, 6·1 ± 0·2 after section (± S.E. of mean)), the mean number of segments innervating each neurone (3·0 after freezing,

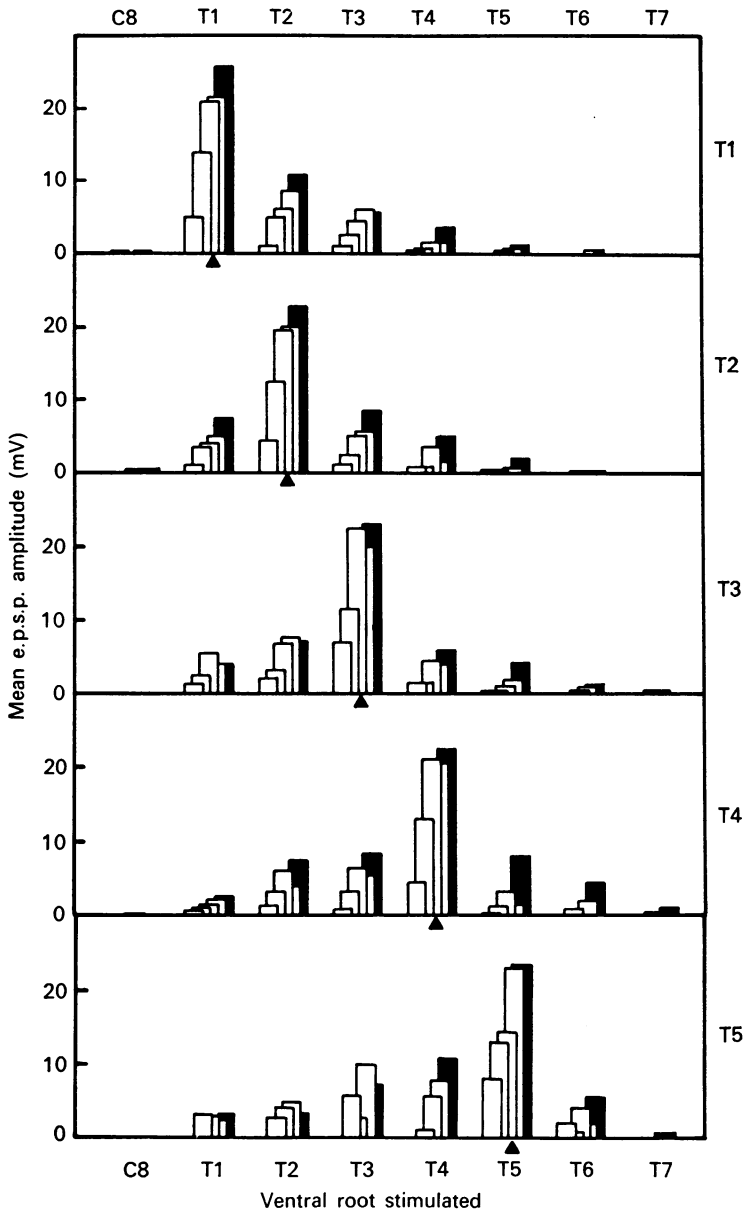

Fig. 6. Distribution of the average synaptic responses recorded in neurones dominated by different spinal segments 0·5, 1, 3 and 6 months after nerve injury (open bars, from foreground to background) and in normal neurones (filled bars, replotted from Njå & Purves, 1977*b*). Each histogram indicates all the neurones in which the largest amplitude e.p.s.p. was elicited by stimulating the ventral root indicated to the right (arrowheads show responses to the dominant segment). About 200 neurones were impaled at each interval. Too few neurones were dominated by C8, T6 and T7 to be included.

3·0 after section), and the mean e.p.s.p. amplitude measured in response to stimulating each ventral root (Fig. 6; compare with Fig. 5, Njå & Purves, 1977*b*) were not significantly different for the two procedures (see also Table 2).

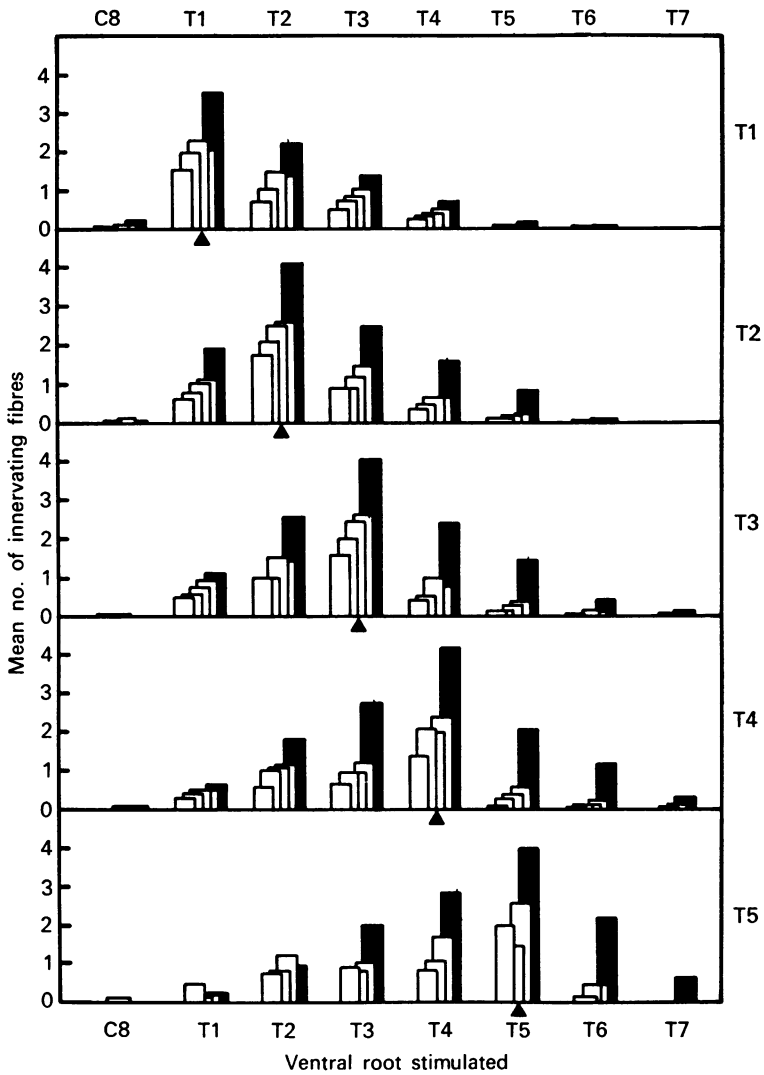

Fig. 7. Distribution of innervation to neurones using the estimated number of preganglionic fibres contacting a neurone as the criterion of segmental dominance. Open bars represent re-innervated neurones after 0·5, 1, 3 and 6 months as in Fig. 6; filled bars are the results in normal neurones (replotted from Njå & Purves, 1977*b*). Dominant segments indicated to the right of the histograms; arrowheads show responses to the dominant segment. Too few neurones were dominated by C8, T6, and T7 to be included.

DISCUSSION

Both *in vivo* and *in vitro* examination of re-innervated superior cervical ganglia suggest that, from the outset, different classes of pre- and post-synaptic cells make connexions with each other which are selective. At the earliest stage at which

a significant number of ganglion cells received innervation from regenerating preganglionic fibres, stimulation of the first and fourth thoracic ventral roots elicited distinct patterns of end-organ responses which were weaker but otherwise similar to the patterns elicited by stimulation of these roots in normal animals. Intracellular recordings were in agreement with these findings: as early as 13–19 days after nerve injury, when cells had been re-innervated for at most a few days, innervation of individual cells tended to be contiguous, with neurones showing a progressive reduction in the strength of innervation from segments increasingly removed from those providing the dominant input.

Throughout the course of re-innervation, rostral segments were relatively more successful than caudal ones in establishing connexions with the number of neurones normally innervated by each root. The reason for this difference is uncertain. Since the preganglionic axons probably branch extensively to innervate neurones in several ganglia (Langley, 1899; Perri, Sacchi & Casella, 1970; Purves, 1975), a possible cause of this result is the presumably greater number of intact connexions remaining for more caudally located preganglionic neurones after cervical trunk injury. If the vigor with which re-innervation proceeds depends on the degree to which the injured neurones are deprived of their normal complement of target cells, then axons originating from more caudal spinal levels might regenerate less effectively after interruption of the cervical trunk.

The outcome of the present experiments, as well as previous studies of the segmental innervation of normal and re-innervated ganglia (Njå & Purves, 1977*a*, *b*; see also Langley, 1892, 1895, 1897; and Guth & Bernstein, 1961), suggests that the basis of selective synapse formation in the superior cervical ganglion involves differences amongst the population of both the pre- and the post-synaptic neurones. It seems likely that a differentiating feature amongst preganglionic neurones is their rostro-caudal position in the spinal cord. The distinguishing features of postsynaptic cells, on the other hand, are less clear. Neurones innervating the same target are not grouped within the ganglion in any obvious way, and are similar to other classes of ganglion cells by electrophysiological criteria (Purves, 1975; Njå & Purves, 1977*a*, *b*; D. Purves and J. W. Lichtman, unpublished). The apparent absence of a topographic arrangement of segmental connexions within the ganglion makes attractive the view that the distinctiveness of ganglion cells has something to do with their targets. One possibility is that the segmental level of the inputs a ganglion cell receives is dictated by the target it contacts. Some basis for this supposition is provided by the dependence of ganglionic synapses on the integrity of the post-ganglionic axons (Purves, 1975, 1976*a*; Njå & Purves, 1978; see also Matthews & Nelson, 1975). If the post-ganglionic targets are indeed important in identifying ganglion cells, it would be of interest to know whether ganglion cells are made distinctive by virtue of the *nature* of their targets (e.g. arteriolar smooth muscle versus the smooth muscle of the iris) or the *position* of their target. Alternatively, both the targets a cell contacts, and the inputs it receives, might be the result of an inherent property of the nerve cell.

How might selective synaptogenesis between distinctive classes of pre- and postsynaptic neurones occur? Among the explanations which have been considered are (*a*) the formation of more or less random connexions between classes with later

elimination of functionally incorrect contacts, and (*b*) the initial formation of correct connexions by virtue of (i) extraneuronal guidance mechanisms, (ii) surface interactions between the pre- and post-synaptic neurones, (iii) responses to trophic agents secreted by the pre- or the post-synaptic neurones (or both). The present results argue against random synapse formation and later correction as a necessary feature of specific connectivity in the adult superior cervical ganglion. However, synapse elimination does occur in the normal development of some mammalian autonomic ganglia (Lichtman, 1977), and may occur during the initial innervation of the superior cervical ganglion as well. It will be of interest to see whether synapse elimination during development, if it occurs in this system, promotes selectivity or only operates in parallel with it. The outcome of our experiments does not distinguish among mechanisms which might lead to correct connexions from the outset. As in other circumstances, appropriate guidance of axons (see, for example, Attardi & Sperry, 1963; Landmesser & Morris, 1975; Van Essen & Jansen, 1977) must play a role in establishing innervation of the superior cervical ganglion: if foreign axons are re-routed to a denervated adult ganglion, they form permanent contacts with ganglion cells, even in the face of native competition (Purves, 1976*b*). Once native axons have reached the ganglion, however, extraneuronal guidance of groups of preganglionic fibres to particular regions of the ganglion is probably not the cause of selective synoptogenesis. Preganglionic fibres arising from different spinal segments appear to provide innervation more or less uniformly throughout the ganglion (D. Purves, A. Njå & J. W. Lichtman, unpublished). Moreover, the final result of re-innervation is no different following section of the preganglionic nerve, when mechanical guidance mechanisms might be expected to be comprised to some extent, than after freezing the preganglionic nerve. While extraneuronal guidance of axons to particular regions cannot be ruled out, it seems likely that the mechanism of synaptic selectivity in sympathetic ganglia resides, at least in large part, at the level of synapse formation itself.

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REFERENCES

ATTARDI, D. G. & SPERRY, R. W. (1963). Preferential selection of central pathways by regenerating optic fibres. *Expl Neurol.* **7**, 46–64.

BROWN, M. C. & IRONTON, R. (1977). Motor neurone sprouting induced by prolonged tetrodotoxin block of nerve action potentials. *Nature, Lond.* **265**, 459–461.

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.

LANDMESSER, L. & MORRIS, D. G. (1975). The development of functional innervation in the hind limb of the chick embryo. *J. Physiol.* **249**, 301–326.

LANDMESSER, L. & PILAR, G. (1970). Selective re-innervation of two cell populations in the adult pigeon ciliary ganglia. *J. Physiol.* **211**, 203–216.

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 pre-ganglionic fibres of the sympathetic. *J. Physiol.* **18**, 280–284.

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

LANGLEY, J. N. (1899). On axon reflexes in the pre-ganglionic fibres of the sympathetic system. *J. Physiol.* **25**, 365–398.

LANGLEY, J. N. & DICKINSON, W. L. (1889). On the local paralysis of peripheral ganglia, and on the connexion of different classes of nerve fibres between them. *Proc. R. Soc.* **46**, 423–431.

LICHTMAN, J. W. (1977). The reorganization of synaptic connexions in the rat submandibular ganglion during post-natal development. *J. Physiol.* **273**, 155–177.

LILEY, A. W. (1956). An investigation of spontaneous activity at the neuromuscular junction of the rat. *J. Physiol.* **132**, 650–666.

LØMO, T. & ROSENTHAL, J. (1972). Control of ACh-sensitivity by muscle activity in the rat. *J. Physiol.* **221**, 493–513.

MATHEWS, M. R. & NELSON, V. (1975). Detachment of structurally intact nerve endings from chromatolytic neurones in the rat superior cervical ganglion during depression of synaptic transmission induced by postganglionic axotomy. *J. Physiol.* **245**, 91–135.

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

NOETHER, G. (1976). *Introduction to Statistics: a Nonparametric Approach*, chap. 5, pp. 44–60. Boston: Houghton Mifflin.

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). The effects of nerve growth factor and its antiserum on synapses in the superior cervical ganglion of the guinea-pig. *J. Physiol.* **277**, 53–75.

OSTBERG, A. J. C., RAISMAN, G., FIELD, P. M., IVERSEN, L. L. & ZIGMOND, R. E. (1975). A quantitative comparison of the formation of synapses in the rat superior cervical sympathetic ganglion by its own and foreign nerve fibres. *Brain Res.* **107**, 445–470.

PERRI, V., SACCHI, O. & CASELLA, C. (1970). Synaptically mediated potentials elecited 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*a*). Functional and structural changes in mammalian sympathetic neurones following colchicine application to post-ganglionic nerves. *J. Physiol.* **259**, 159–175.

PURVES, D. (1976*b*). Competitive and non-competitive re-innervation of mammalian sympathetic neurones by native and foreign fibres. *J. Physiol.* **261**, 453–475.

RAISMAN, G., FIELD, P. M., OSTBERG, A. J. C., IVERSEN, L. L. & ZIGMOND, R. E. (1974). A quantitative ultrastructural and biochemical analysis of the process of reinnervation of the superior cervical ganglion of the adult rat. *Brain Res.* **71**, 1–17.

VAN ESSEN, D. C. & JANSEN, J. K. S. (1977). The specificity of re-innervation by identified sensory and motor neurones in the leech. *J. comp. Neurol.* **171**, 533–454.