

## THE EFFECTS OF POST-GANGLIONIC AXOTOMY ON SELECTIVE SYNAPTIC CONNEXIONS IN THE SUPERIOR CERVICAL GANGLION OF THE GUINEA-PIG

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### SUMMARY

Stimulation of preganglionic axons arising from different levels of the thoracic spinal cord causes different effects on end-organs supplied by the superior cervical ganglion (Langley, 1892; Njå & Purves, 1977*a*; Lichtman, Purves & Yip, 1979). For example, stimulation of the first thoracic ventral root (T1) causes pupillary dilatation and widening of the palpebral fissure; stimulation of T4, on the other hand, has little effect on the eye, even though axons arising from this level innervate about as many superior cervical ganglion cells as those from T1. Thus ganglion cell innervation is selective.

(1) Three months after crushing the major post-ganglionic branches of the superior cervical ganglion this differential effectiveness is lost: T1 and T4 stimulation have approximately equal effects on the end-organs of the eye.

(2) In normal animals, the cellular counterpart of selective end-organ effects is the innervation of each ganglion cell by a contiguous subset of the spinal segments that innervate the ganglion as a whole. One of these segments is usually dominant, the strength of innervation from adjacent segments falling off as a function of distance from the dominant one (Njå & Purves, 1977*a*). Intracellular recordings from ganglion cells 3 months after post-ganglionic axotomy showed that this selective pattern is re-established.

(3) Since the innervation of ganglion cells appears normal, the abnormal end-organ responses after post-ganglionic axotomy suggest that ganglion cell axons are not limited to their original targets during peripheral re-innervation. This suggestion is supported by the finding that ganglion cells sending axons to different peripheral destinations via the second and third cervical spinal nerves were no longer distinguishable on the basis of their segmental inputs 3 months after post-ganglionic axotomy.

(4) Similar results were obtained when the preganglionic cervical trunk was cut at the same time as the post-ganglionic axons were crushed: the pattern of end-organ responses was abnormal, whereas individual ganglion cells were re-innervated according to the rules of contiguity and segmental dominance.

(5) These results indicate that ganglion cells do not undergo a compensatory change in the segmental innervation they receive when their axons regenerate to

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targets different from, or in addition to, those they originally innervated, even when an entirely new set of ganglionic connexions is formed. This suggests that ganglion cells, or some aspect of their immediate environment, possess a permanent label that determines the segmental innervation they receive.

#### INTRODUCTION

The innervation of the guinea-pig superior cervical ganglion is selective in that each neurone is contacted by a restricted subset of the spinal segments innervating the ganglion as a whole (Njå & Purves, 1977*a*; see also Langley, 1892). Moreover, regenerating preganglionic sympathetic axons are capable of selectively re-innervating ganglion cells in adult animals. This conclusion follows from two observations. First, regeneration of cut preganglionic axons restores the normal pattern of sympathetic end-organ responses observed upon stimulating each of the ventral roots that supply the ganglion (Langley, 1895, 1897; Njå & Purves, 1977*b*). Secondly, preganglionic regeneration largely restores the normal, ordered pattern of synaptic inputs from thoracic ventral roots to individual neurones in the ganglion (Njå & Purves, 1977*b*, 1978*b*).

The basis of this remarkable selectivity in synapse formation is not known. One possibility is that a retrograde influence from peripheral targets dictates the segmental innervation of ganglion cells. This view is attractive in principle because the formation and maintenance of ganglionic synapses does depend, at least to some degree, on a retrograde trophic effect from the periphery (Black, Hendry & Iversen, 1972; Purves, 1975, 1976; Njå & Purves, 1978*a*; see also Landmesser & Pilar, 1978). The purpose of the present work was to examine the effect of post-ganglionic targets on selectivity by asking whether the segmental innervation of ganglion cells is altered when they innervate novel targets. The approach we took was suggested by an observation on a single cat reported by Langley (1897). The major post-ganglionic nerve of the superior cervical ganglion in this animal had been cut several months previously; when Langley examined the effects of stimulating the thoracic ventral roots, he found that the normal pattern of end-organ responses was altered, and suggested that the regeneration of post-ganglionic axons might be non-specific. In the studies reported here we confirm Langley's result, and use the imprecision of post-ganglionic re-innervation to assess the influence of the periphery on the segmental innervation of adult ganglion cells.

#### METHODS

The pre- and post-ganglionic innervation of the superior cervical ganglion of adult guinea-pigs was studied *in vivo* and *in vitro* by techniques described in previous papers (Purves, 1975; Njå & Purves, 1977*a*; Lichtman *et al.* 1979). In brief, end-organ responses to stimulation of each of the first five thoracic ventral roots (T1–T5) were observed *in vivo* after removal of the spinal cord in animals anaesthetized with pentobarbitone. These roots supply most of the innervation to the superior cervical ganglion, although some axons also arise from C8, T6 and T7 (Njå & Purves, 1977*a*). Dilatation of the pupil and widening of the palpebral fissure were estimated on a semi-quantitative, 0 to + + + + scale. We also observed vasoconstriction of the ear and piloerection, but these responses were more difficult to interpret. The posterior part of the ear is normally supplied by post-ganglionic axons from both the stellate and the superior

cervical ganglion; thus the contribution of the superior cervical ganglion is not easy to dissociate (Njá & Purves, 1977*b*; Lichtman *et al.* 1979). Furthermore, since many ganglion cells die during recovery from axotomy (Purves, 1975), some sprouting of stellate axons in border regions is expected. The responses of hairs after axotomy were also difficult to evaluate because pilomotor effects were very weak.

To study the innervation of individual ganglion cells *in vitro*, the superior cervical ganglion was dissected in continuity with the cervical sympathetic trunk, the sympathetic chain, communicating rami, and ventral roots C8–T7. Neurones in the superior cervical ganglion were then

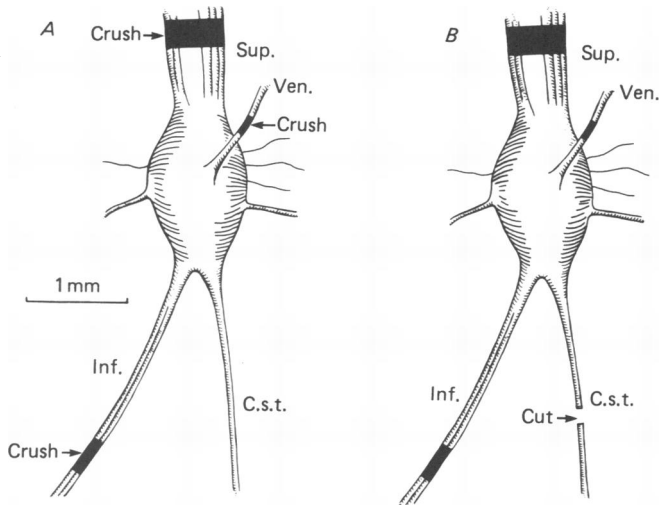


Fig. 1. Diagram of the superior cervical ganglion, its branches, and the operations performed. *A*: in one series of experiments all three major post-ganglionic branches were crushed (superior branch = Sup.; inferior branch = Inf.; ventral branch = Ven.; cervical sympathetic trunk = C.s.t.). *B*: in another series the preganglionic trunk (C.s.t.) was cut at the same time the post-ganglionic branches were crushed.

impaled with a micro-electrode and each of these ventral roots was stimulated in turn (Njá & Purves, 1977*a*). We determined the number of segments contributing innervation to each cell and the amplitude of the compound excitatory post-synaptic potential (e.p.s.p.) elicited by stimulating each ventral root; we also estimated the number of axons contacting a cell from each segment by counting the steps in the synaptic response upon gradually increasing the strength of ventral root stimulation.

In some animals we examined the segmental innervation of neurones whose axons ran to different peripheral destinations in the cervical spinal nerves C2 and C3 (Lichtman *et al.* 1979). In order to do this, the inferior post-ganglionic branch was further dissected in continuity with the ventral division of C2 and C3, and suction electrodes were applied to each of these spinal nerves medially and laterally to the juncture of the ramus. Impaled neurones could then be identified by antidromic stimulation as running either dorsomedially or ventrolaterally in these spinal nerves (see Fig. 3*A* in Lichtman *et al.* 1979).

Both *in vivo* and *in vitro* observations were made 4 days to 20 months after an initial operation. The surgery was performed on the right side of young adult guinea-pigs (250–400 g) and was of two sorts (Fig. 1):

(1) In thirty-eight animals all three major post-ganglionic nerves of the superior cervical ganglion (the inferior, superior and ventral branches) were crushed within 1–3 mm of the ganglion (Fig. 1*A*). This procedure interrupts the axons of more than 80% of the ganglion cells (Purves, 1975).

(2) In addition to crushing the major post-ganglionic branches, in fifty-eight animals the

cervical sympathetic trunk was also cut 1–3 mm from the caudal pole of the ganglion (Fig. 1*B*). As controls for these latter experiments the cervical sympathetic trunk was cut while leaving the post-ganglionic nerves intact in twenty-three other animals.

In all *in vitro* experiments neurones were identified as running in either the inferior or superior post-ganglionic branches by antidromic stimulation (Purves, 1975). Thus neurones running in smaller, uninjured branches were excluded from the study.

Mean values are given throughout as  $\pm$  the standard error.

## RESULTS

### *The effects of post-ganglionic axotomy on the segmental innervation of the superior cervical ganglion*

#### *End-organ responses in vivo*

In normal guinea-pigs stimulation of progressively more caudal thoracic ventral roots has a progressively different effect on the end-organs supplied by the post-ganglionic axons of the superior cervical ganglion (Njå & Purves, 1977*a*; Lichtman *et al.* 1979). Stimulation of the first thoracic ventral root, for example, causes dilatation of the pupil and widening of the palpebral fissure; stimulation of T4, however, has relatively little effect on the eye, but causes pronounced effects on other end-organs, as has been observed in other mammals (see Purves & Lichtman, 1978, for a review).

These differences in end-organ effects of the eye in response to stimulating the preganglionic outflow from different spinal levels are markedly altered after regeneration of the post-ganglionic nerves (Fig. 2). In each of ten animals in which the ventral roots T1–T5 were stimulated *in vivo* 3 months after axotomy, stimulation of each root no longer elicited selective responses but rather affected end-organs in rough proportion to its over-all contribution to the superior cervical ganglion. For example, T1 and T4, which innervate about the same number of ganglion cells (see Fig. 3 below), caused approximately equal eye responses after recovery from post-ganglionic axotomy. These *in vivo* results confirm Langley's observation on a cat studied in the same general way (Langley, 1897).

#### *Innervation of ganglion cells*

The loss after axotomy of the normal polarity of end-organ responses to stimulation of different ventral roots might have several explanations: (1) as suggested by Langley (1897), regenerating post-ganglionic axons might innervate sympathetic targets more or less randomly; (2) since post-ganglionic axotomy causes an acute, if transient, loss of about two-thirds of the ganglionic synapses (Mathews & Nelson, 1975; Purves, 1975), post-ganglionic neurones might re-innervate their original targets but recover a largely random set of preganglionic connexions; or (3) both post-ganglionic re-innervation and recovery of ganglion cell synapses after axotomy might be selective, but the normal polarity lost because of a disproportionate loss or recovery of synapses after axotomy. For example, if the initial loss of synapses arising from T1 were much greater than T4, or their recovery less, then some loss of differential end-organ effects might be seen.

To explore these possibilities, we examined the segmental innervation of individual ganglion cells 3 months after post-ganglionic axotomy, when the recovery of the ganglionic synapses initially lost is complete (Purves, 1975).

(a) Pattern of segmental innervation after recovery from post-ganglionic axotomy

In general, the segmental innervation of ganglion cells appeared to be normal 3 months after post-ganglionic axotomy. As in unoperated animals, neurones after recovery from axotomy were innervated by about twelve axons (mean = 12.2,  $n = 200$ ) arising from about four segments (4.3,  $n = 200$ ) (see Njå & Purves, 1977a, b).

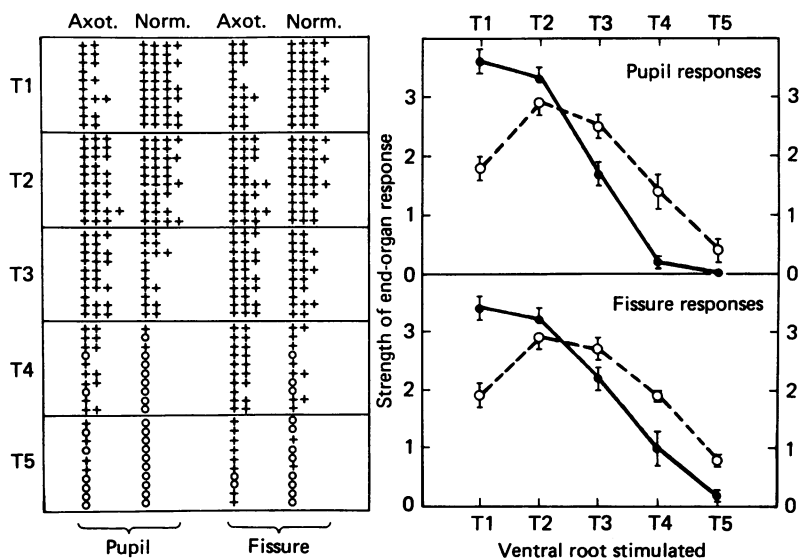


Fig. 2. Change in the normal differential effectiveness of ventral root stimulation on sympathetic end-organ responses after post-ganglionic nerve regeneration. Left: the effects of *in vivo* stimulation of thoracic ventral roots T1-T5 on the pupil and palpebral fissure in ten guinea-pigs 3 months (81-114 days) after post-ganglionic axotomy (axot.). Operated right side is compared to normal (norm.) end-organ responses on the animal's left side. The scale is subjective; 0 = no detectable response, + + + = maximum response observed. Both sides were judged according to the maximum normal response. Right: graphic summary of the tabulated results. Average end-organ responses of the eye on the normal side are represented by filled circles, and on the operated side by open circles. The change observed is consistent with a loss of specificity, since the activation of the end-organs of the eye after post-ganglionic axotomy is roughly proportional to the over-all innervation of the superior cervical ganglion by the different spinal segments (see Fig. 3).

The over-all distribution of the segmental innervation to ganglion cells, however, was slightly abnormal: axons arising from rostral segments innervated (and dominated) a somewhat greater proportion of ganglion cells than caudal segments, compared to normal animals (Fig. 3). A similar shift in segmental innervation occurs after re-innervation of ganglion cells (Njå & Purves, 1977b, 1978b). Whatever the cause of this slight difference in the recovery of synapses from rostral *vs.* caudal segments after axotomy, its effect presumably would be to preserve the normally greater influence of rostral segments on the end-organs of the eye. Since the influence of rostral segments on the eye was reduced, the abnormality of end-organ responses

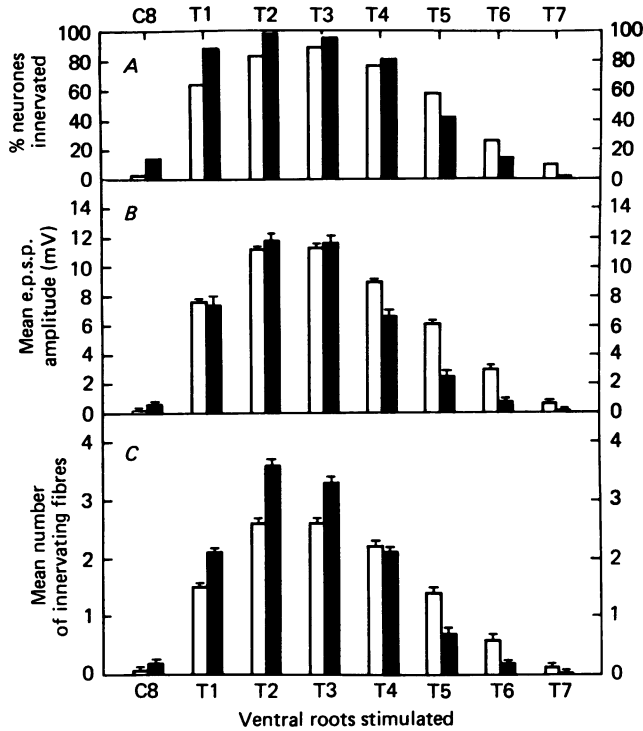


Fig. 3. Segmental innervation of ganglion cells by spinal segments C8-T7 3 months (84-98 days) after post-ganglionic axotomy (filled bars) compared to the segmental innervation of normal neurones (open bars). Normal histograms are redrawn from Njå & Purves, 1977*b*. *A*, percentage of neurones innervated; *B*, mean e.p.s.p. amplitudes; *C*, mean number of innervating axons.

cannot be due to these small changes in the relative segmental contributions to the superior cervical ganglion after recovery from axotomy.

The approximately normal segmental innervation of ganglion cells shown in Fig. 3 implies that the acute loss of synapses which follows post-ganglionic axotomy (Matthews & Nelson, 1975; Purves, 1975) is evenly distributed amongst preganglionic axons arising from different spinal segments. To verify this point we compared the e.p.s.p. amplitudes elicited by ventral root stimulation in 205 neurones 4-7 days after axotomy of one post-ganglionic branch (inferior) to the e.p.s.p. distribution in 206 neurones in the same ganglia whose post-ganglionic axons (running in the superior branch) were intact. The decline in average e.p.s.p. amplitude, and presumably the loss of synapses from injured neurones, was apparent for each of the segments T1-T5 and was roughly proportional for each (the number of cells innervated by C8, and T6 and T7, was too small to allow comparison). It appears, therefore, that synapse loss (and other causes of synaptic depression after post-ganglionic axotomy - see Purves, 1975; Brenner & Martin, 1976) affects axons arising from different thoracic segments about equally.

The cellular counterpart of selective end-organ effects is the innervation of ganglion cells by a contiguous subset of the spinal segments that innervate the ganglion as a whole; one of these segments is usually dominant, the strength of innervation from adjacent segments falling off as a function of distance from the dominant one (Njå & Purves, 1977*a*). Following recovery from axotomy, the segments

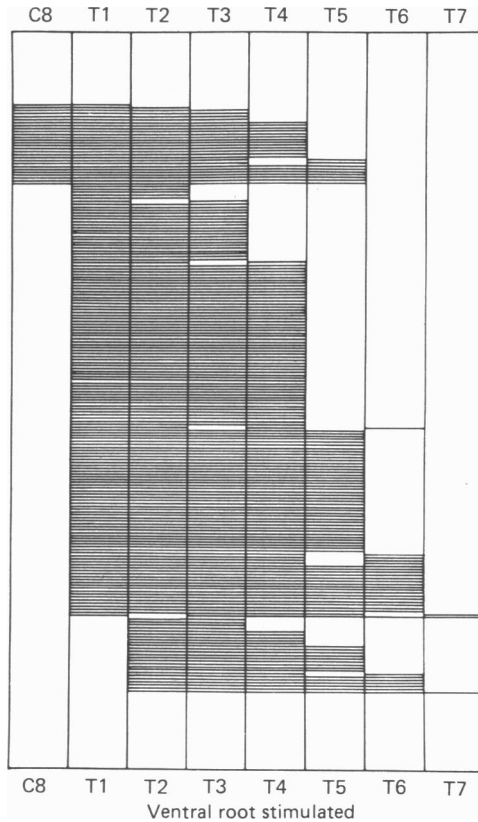


Fig. 4. Contiguity of segmental innervation of ganglion cells 3 months after post-ganglionic axotomy. Each horizontal line represents an individual neurone; the length and continuity of the line reflect segmental innervation. The line is filled in for a cell if any innervation is detected in response to supramaximal stimulation of the corresponding ventral root. The strong tendency for neurones to be innervated by a contiguous subset of spinal segments is similar to that observed in normal animals (see Njå & Purves, 1977a, b).

innervating particular neurones were also generally contiguous (Fig. 4); furthermore, the strength of the segmental innervation of a cell tended to decrease with increasing distance from the dominant segment (Fig. 5). Thus there is a discrepancy between the *in vivo* results presented in the preceding section and the results of intracellular recording from ganglion cells: while there is little or no evidence of selectivity in the end-organ responses, the neuronal counterpart of selectivity within the superior cervical ganglion is still apparent. This suggests that the recovery of ganglionic synapses is selective, but that post-ganglionic regeneration is not.

(b) Segmental innervation of neurones running to different destinations after recovery from post-ganglionic axotomy

To test the idea that post-ganglionic axons regenerate in a relatively imprecise way with respect to their original peripheral course, we studied the segmental innervation of neurones running to different destinations in the second and third cervical

spinal nerves. In normal animals, sympathetic post-ganglionic axons running ventrolaterally in the spinal nerves of C2 and C3 receive relatively more rostral segmental inputs than neurones whose axons run dorsomedially (Lichtman *et al.* 1979). In similar experiments on fifteen animals 3 months after post-ganglionic axotomy this difference was no longer apparent. Thus the average e.p.s.p. amplitude in response to stimulating each ventral root in neurones whose axons ran ventro-

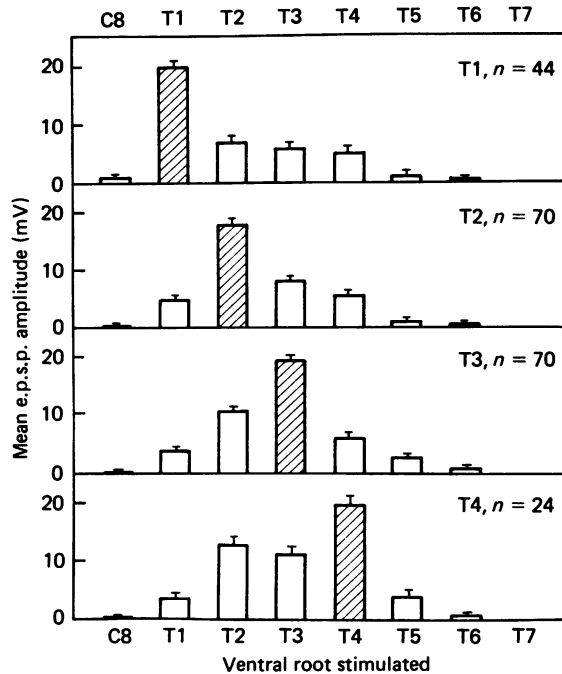


Fig. 5. Distribution of e.p.s.p. amplitudes in neurones dominated by different thoracic segments 3 months after post-ganglionic axotomy. Histograms show mean amplitudes in neurones dominated by innervation from the spinal segment indicated to the right. Cross-hatched bars are responses to the dominant segment. The gradual shift in average segmental innervation with the dominant segment is similar to that seen in normal animals (see Njå & Purves, 1977 *a, b*). The sum of the neurones in these classes exceeds 200 because stimulation of more than one ventral root elicited an equally large e.p.s.p. in some cells. Neurones dominated by T5 are not shown because  $n$  was too small to construct a valid histogram.

laterally ( $n = 16$ ) was, on average, the same as in neurones whose axons ran dorsomedially ( $n = 50$ ). Of the ninety-three cells antidromically driven by C2 or C3 in these experiments twenty-seven had axons which divided to send branches both ventrolaterally and dorsomedially; this fraction (29%) is about the same as that in normal animals (30%; Lichtman *et al.* 1979). The percentages of neurones running either dorsomedially or ventrolaterally were also similar to values in normal animals. The simplest explanation of these results is that post-ganglionic axons do not regenerate selectively to their original peripheral targets.



*The effects of post-ganglionic axotomy on the re-innervation of ganglion cells after section of the cervical sympathetic trunk*

The findings described in the preceding sections suggest that post-ganglionic axons do not regenerate with any precision to their original destination, and that this abnormality causes no obvious compensatory change in the segmental innervation of individual ganglion cells. This implies that adult ganglion cells are not readily re-specified when they re-innervate targets in positions different from their original ones.

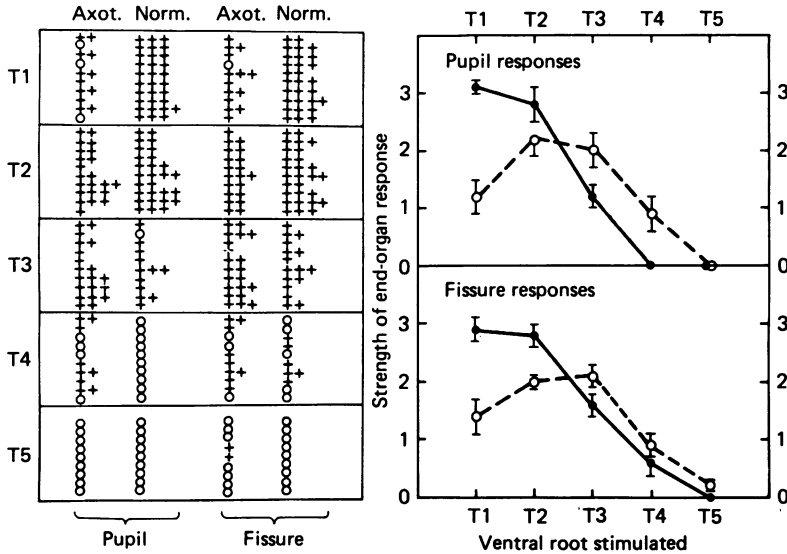


Fig. 6. Change in the normal differential effectiveness of ventral root stimulation on end-organ responses after regeneration of both pre- and post-ganglionic nerves. Left: the effects of *in vivo* stimulation of thoracic ventral roots T1-T5 on the pupil and palebral fissure in ten guinea-pigs 3 months (79-89 days) after post-ganglionic axotomy combined with section of the cervical trunk. Operated right side is compared to normal responses elicited on the animal's left side. Scale and abbreviation as in Fig. 2. Right: graphic summary of the tabulated results. Average end-organ responses of the eye on the normal side are represented by filled circles and on the operated side by open circles.

This conclusion, however, is weakened by the fact that some synapses remain on ganglion cells after axotomy and that little is known about the degree of degeneration of those preganglionic terminals which are lost within a few days of the post-ganglionic injury (Purves, 1975). For example, if the detached preganglionic terminals remained nearby during the regeneration of post-ganglionic axons, the apparent recovery of contacts from original segments might be due simply to the proximity of the original axons. On the other hand, if preganglionic axons had an unbiased choice of ganglion cells, perhaps some re-specification would be apparent. We therefore tested the ability of *regenerating* preganglionic axons to make synapses which might restore the normal pattern of end-organ effects after post-ganglionic axotomy.

*End-organ responses in vivo three months after interruption of both pre- and post-ganglionic axons*

Ten animals were studied *in vivo* 3 months after cervical trunk section and post-ganglionic axotomy by stimulating the thoracic ventral roots of T1–T5 while observing the end-organ responses in the territory supplied by the superior cervical ganglion. The same change in the differential effectiveness of ventral root stimulation on end-organ responses of the eye was observed as after axotomy alone (Fig. 6; cf. Fig. 2): the selective responses seen in normal animals were no longer apparent.

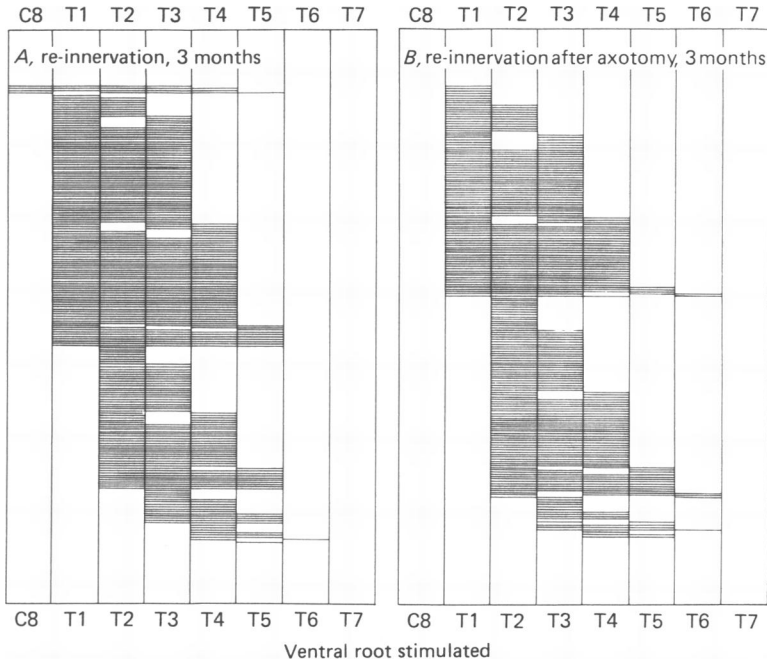


Fig. 7. Contiguity of the segmental innervation of ganglion cells 3 months (87–95 days) after cervical trunk section (A) compared to the segmental innervation of neurones 3 months (85–98) days after cervical trunk section and post-ganglionic axotomy (B). Each horizontal line represents an individual neurone, as in Fig. 4.

*Innervation of ganglion cells three months after interruption of both pre- and post-ganglionic axons*

Re-innervation of *normal* ganglion cells, while selective, is quantitatively different from the usual pattern of innervation (Njä & Purves, 1977*b*; 1978*b*). Thus when re-innervation is complete (3 months), ganglion cells are contacted by about three instead of four segments, and by about six instead of twelve axons; moreover, relatively caudal segments show some deficiency during re-innervation (see above). Consequently, the results of re-innervation after axotomy are compared to control ganglia examined after re-innervation alone.

Neurones re-innervated after post-ganglionic axotomy were contacted by about the same number of segments and axons as neurones re-innervated in intact ganglia

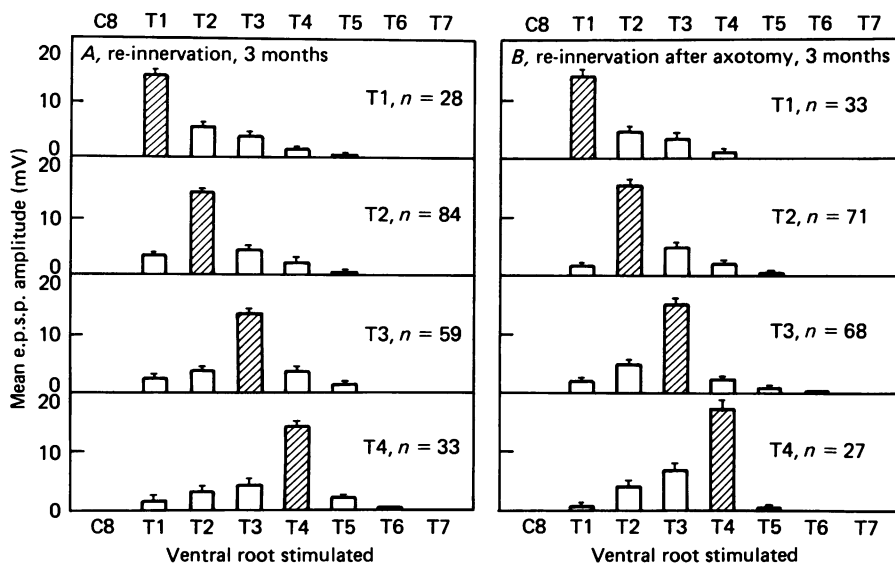


Fig. 8. Distribution of e.p.s.p. amplitudes in neurones dominated by different thoracic segments 3 months after cervical trunk section (A) compared to re-innervation 3 months after cervical trunk section and post-ganglionic axotomy (B). Histograms show mean amplitudes in neurones dominated by innervation from spinal segment indicated to the right; cross-hatched bars are responses to the dominant segment.

TABLE 1.

	No. cells	Innervated (%)	Mean no. of segments innervating cells	Mean no. of axons innervating cells	Sum of segmental e.p.s.p.s (mV)
<i>A. Comparison of the innervation of superior cervical ganglion cells 3 months after cervical trunk section and 3 months after trunk section combined with post-ganglionic axotomy.</i>					
Cervical trunk section only	199	100.0	2.9 ± 0.1	5.8 ± 0.2	29.0 ± 1.1
Cervical trunk section and post-ganglionic axotomy	200	98.5	2.6 ± 0.1	5.2 ± 0.2	31.8 ± 1.3
<i>B. Comparison of the innervation of superior cervical ganglion cells 1 month after cervical trunk section and 1 month after trunk section combined with post-ganglionic axotomy.</i>					
Cervical trunk section only	200	81.5	2.0 ± 0.1	3.5 ± 0.2	13.5 ± 1.0
Cervical trunk section and post-ganglionic axotomy	200	55.0	1.2 ± 0.1	2.0 ± 0.2	6.6 ± 0.8

after 3 months (Table 1A); moreover both groups showed about the same tendency towards innervation by contiguous segments (Fig. 7), and the same decrease in e.p.s.p. amplitude as a function of distance from the dominant segment (Fig. 8). Therefore, it appears that the re-innervation of ganglion cells whose axons have been interrupted obeys the rules of contiguity and segmental dominance as strictly as the re-innervation of intact ganglion cells. Since, however, the end-organ responses mediated by ganglion cells whose axons have regenerated are abnormal (see preceding

section), the newly formed ganglionic connexions are not appropriate to the targets innervated during post-ganglionic regeneration.

*Innervation of ganglion cells one month after interruption of both pre- and post-ganglionic axons*

In contrast to the re-innervation of axotomized ganglion cells at 3 months, at one month after post-ganglionic axotomy there was an obvious quantitative deficiency of re-innervation compared to experiments in which only the cervical trunk had been

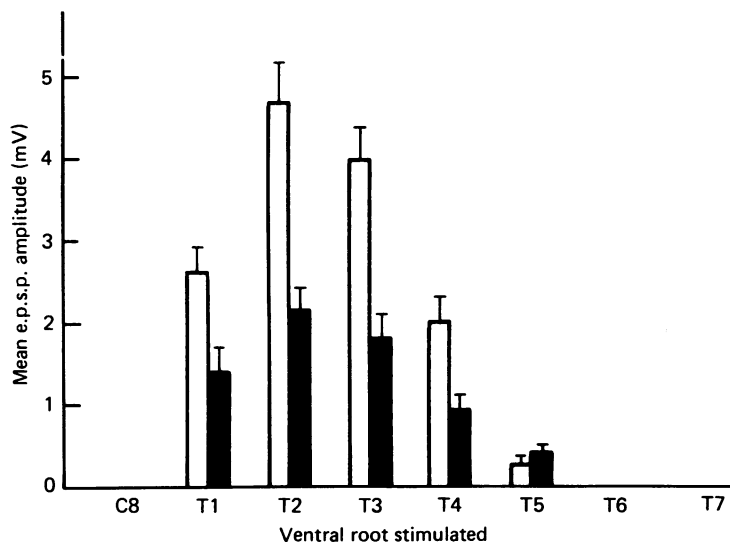


Fig. 9. Distribution of mean e.p.s.p. amplitudes in ganglion cells re-innervated 1 month (27–35 days) after cervical trunk section and post-ganglionic axotomy (filled bars;  $n = 200$ ), compared to re-innervation of neurones in intact ganglia after 1 month (26–35 days; open bars;  $n = 200$ ).

cut: the average e.p.s.p. amplitude recorded in ganglion cells was smaller, the number of innervating segments and axons per cell was less, and the percentage of innervated cells was reduced (Fig. 9 and Table 1*B*). Since there are no significant differences in these parameters at 3 months or later (see Table 1*A* and below), axotomy appears to delay synaptogenesis without altering the final outcome of re-innervation.

It is possible, however, that an axotomized ganglion might somehow decrease the *rate* of preganglionic regeneration, without affecting synaptogenesis *per se*. We therefore studied the re-innervation of ganglia in which only one of the major post-ganglionic branches was crushed (either the inferior or superior branch; see Fig. 1). Neurones regenerating their axons could be identified by antidromic stimulation of the crushed branch, and their state of re-innervation compared to that of neurones in the same ganglion antidromically driven from the intact post-ganglionic branch. Recordings from eighty-five cells with intact axons and seventy-three injured neurones showed that the regenerating cells were much less well re-innervated after one month. In fact, more injured cells were uninnervated after one month in these experiments

(78%) than neurones one month after crushing all three of the major post-ganglionic branches (45% uninervated; see Table 1*B*), suggesting that injured neurones are even less attractive when they are juxtaposed to substantial numbers of normal ganglion cells. In sum, axotomy appears to reduce sharply the attractiveness of ganglion cells to regenerating preganglionic axons for several weeks, without affecting the capacity of the preganglionic cells to regenerate.

*Innervation of ganglia and peripheral targets at long times after  
post-ganglionic axotomy*

Compensatory changes in the pattern of ganglion cell or peripheral innervation might occur at longer times after recovery from post-ganglionic axotomy. To rule out this possibility we stimulated the ventral roots T1–T5 *in vivo* in eight animals 6–20 months after axotomy of the major post-ganglionic branches while observing the end-organ responses mediated by the superior cervical ganglion. The change in the differential effectiveness of ventral root stimulation was approximately the same as in animals studied 3 months after this operation (see Fig. 2). We also impaled 200 neurones in twelve animals 9–12 months after sectioning the cervical sympathetic trunk and crushing the major post-ganglionic branches. The average number of innervating segments and axons, the contiguity, and the distribution of e.p.s.p. amplitudes according to the dominant segment were not significantly different from the results obtained in neurones studied 3 months after the same operation (see Figs. 7 and 8). Thus the patterns of pre- and post-ganglionic innervation established at 3 months are probably permanent.

#### DISCUSSION

The major question we address in these experiments is whether changing an adult neurone's target leads to compensatory changes in the innervation it receives. Our ability to evaluate the problem of re-specification in the superior cervical ganglion depends on the fact that its normal connexions appear to match positional attributes of the pre- and post-synaptic neurones (Njå & Purves, 1977*a*; Lichtman *et al.* 1979). Thus all sympathetic end-organs which occupy a particular position within the territory served by the superior cervical ganglion tend to be activated by the same spinal segments, whereas end-organs at increasingly different locations within the territory are activated by overlapping but increasingly different segmental subsets (Lichtman *et al.* 1979). Because different post-ganglionic nerves of the guinea-pig superior cervical ganglion innervate widely overlapping positions, they are comprised of neurones which, on average, receive the same segmental innervation (Lichtman *et al.* 1979); therefore, the strategy of testing re-specification by cross re-innervation was not feasible. An alternative approach was suggested by Langley's evidence that the regeneration of post-ganglionic axons might be relatively imprecise (Langley, 1897).

To confirm Langley's suggestion we tested the ability of regenerating ganglion cells to re-innervate their original peripheral targets. Following recovery from post-ganglionic axotomy the effect of stimulating each thoracic ventral root was no longer limited to the region normally activated by that segment: although T1 normally has

much stronger effects on the end-organs of the eye than T4, after post-ganglionic regeneration each of these segments affected the eye about equally. The simplest explanation of this result would be that interrupted post-ganglionic axons grow out with little or no preference for their original peripheral targets. This view is supported by the finding that regenerated sympathetic fibres travelling to different destinations in the cervical spinal nerves no longer arise from ganglion cells receiving predictably different segmental innervation. The relatively non-specific outgrowth of post-ganglionic axons is probably similar to the outgrowth of mammalian skeletal motor neurones after nerve injury: in this case as well, axons appear to contact targets of opportunity along whatever path they happen to follow (Sperry, 1945; Bernstein & Guth, 1961).

The loss of the specificity inferred from *in vivo* experiments implies that the innervation of a new portion of the peripheral territory supplied by the ganglion does not cause a compensatory change in the innervation of the ganglion cells themselves. If re-specification *had* occurred, the pattern of end-organ responses observed *in vivo* should have been normal, even though the responses would be mediated by a novel set of ganglion cells. Moreover, there should have been no change in the segmental innervation of neurones whose axons travel to different destinations in the cervical spinal nerves. The finding that end-organ responses are also abnormal after re-innervation of axotomized neurones by surgically interrupted preganglionic axons strengthens the conclusion that adult ganglion cells have little ability to alter their identity with respect to the segmental innervation they receive. The apparent failure of spinal motor neurones to be re-specified by innervation of novel skeletal muscles (Sperry, 1945) may be an analogous result.

Since the segmental innervation of ganglion cells after recovery from post-ganglionic axotomy does not change in response to novel peripheral innervation, we have assumed that the continued expression of selectivity at the level of ganglion cells reflects each cell's *original* segmental preferences (as after re-innervation alone; Njä & Purves, 1977*b*, 1978*b*). However, since in these experiments we have no means of knowing an individual cell's original preference, we cannot rule out the possibility that contiguity and segmental dominance after recovery from axotomy represent neither the original preference, nor a novel preference retrogradely imposed from the periphery. One should also be cautious in extending our interpretation of these experiments to developmental events. It does not necessarily follow, for instance, that the innervation of peripheral targets in ontogeny plays no role in specifying segmental innervation. Indeed, whether the identity of ganglion cells with respect to synaptogenesis derives from targets they innervate, from the preganglionic innervation they receive, from the positions they occupy at an early embryonic stage, or from intrinsic genetic information, remains an open question. Our experiments suggest, however, that whatever the source of a ganglion cell's identity, this information is a permanent feature of the neurone.

#### *Comparison of the specificity of pre- and post-ganglionic regeneration*

Regeneration of interrupted preganglionic axons in adult mammals results in the accurate re-establishment of segmental connexions in the superior cervical ganglion (Langley, 1895, 1897; Njä & Purves, 1977*b*; 1978*b*). Taken together with the present

results, this finding raises an important question: why is post-ganglionic regeneration less precise than preganglionic regeneration?

The answer may lie in the different tasks presented to the interrupted pre- and post-ganglionic axons. Pre-ganglionic axons need to grow only one or a few millimetres over an unbranched course to reach the ganglion where they presumably respond differentially to local cues which promote the formation of appropriate synapses. Post-ganglionic axons, on the other hand, must follow a longer and repeatedly branching course to reach peripheral targets. Since our experiments do not assess the accuracy of post-ganglionic re-innervation with respect to modality, but only position, we do not know whether post-ganglionic axons form appropriate connexions according to this or some other criterion (see Purves & Lichtman, 1978, for further discussion). Our results, however, support the view that post-ganglionic axons have little ability to follow their original path. In consequence, the difference in the specificity of pre- and post-ganglionic re-innervation may reflect a deficiency in axon guidance, which is important after post-ganglionic axotomy, but is presumably less important after cervical trunk section. Implicit in this distinction is the idea that specific neural connectivity has at least two components: the guidance of growing axons to a generally appropriate destination, and the subsequent response to local cues which promote selective synaptogenesis.

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