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SPECIFIC INNERVATION OF GUINEA-PIG SUPERIOR CERVICAL GANGLION CELLS BY PREGANGLIONIC FIBRES ARISING FROM DIFFERENT LEVELS OF THE SPINAL CORD

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SUMMARY

1. The synaptic contribution of preganglionic nerve fibres arising from the last cervical (C8) and the first seven thoracic spinal cord segments (T1–T7) to neurones of the guinea-pig superior cervical ganglion has been studied by means of intracellular recording during ventral root stimulation *in vitro*.

2. The majority of neurones received innervation from the middle segments (T2 and T3) of the length of spinal cord from which preganglionic fibres derive; an intermediate number of ganglion cells were innervated by fibres from the segments adjacent to these (T1, T4, and T5), and relatively few neurones by fibres from the most rostral and caudal segments supplying innervation to the ganglion (C8, T6 and T7).

3. Each neurone received preganglionic terminals from multiple thoracic segments (range 1–7, mean = 4·0). The estimated minimum number of preganglionic fibres contacting each neurone was 10, on average.

4. As a rule, the spinal segments innervating a neurone were contiguous. Thus we rarely encountered neurones innervated by segments located both rostrally and caudally to a segment which failed to provide innervation.

5. Neurones tended to be innervated predominantly by axons arising from a single spinal segment, with adjacent segments contributing a synaptic influence that diminished as a function of their distance from the dominant segment. All segments provided dominant innervation to at least some neurones.

6. Stimulating the ventral roots of C8–T7 *in vivo* showed that the axons arising from each segment produced a characteristic pattern of peripheral

effects. Thus different populations of neurones in the superior cervical ganglion of the guinea-pig are innervated by preganglionic axons from different levels of the spinal cord, as originally suggested by Langley (1892) for the cat, dog, and rabbit.

7. On the basis of our *in vitro* studies we conclude that underlying the specificity of innervation of neurones of the superior cervical ganglion that can be inferred from *in vivo* experiments is a tendency for individual neurones to be innervated in a systematically graded fashion by a contiguous subset of the eight spinal segments which provide innervation to the ganglion.

INTRODUCTION

The way in which neurones establish connexions with particular sources of innervation during development, and maintain these connexions throughout life, is largely unknown. An attractive preparation for the study of this problem at the cellular level is the mammalian autonomic nervous system where individual peripheral neurones can be routinely impaled with micro-electrodes, and where the presynaptic inputs are well defined anatomically. The advantages of the cervical sympathetic system in studying the specificity of neuronal connexions were realized many years ago by Langley (1892, 1895, 1897) whose classical experiments on the superior cervical ganglion of the cat, dog, and rabbit provided evidence that mammalian neurones can in some way select between competing preganglionic fibres to achieve a specific pattern of innervation. In these experiments groups of nerve cells mediating distinct functions (such as dilatation of the pupil or constriction of the vessels of the ear) were found to be preferentially innervated (and re-innervated) by fibres arising from different segments of the thoracic spinal cord. Thus, for example, pupillary dilatation was elicited by stimulation of the outflow of the first thoracic segment (T1) but not by the fourth thoracic segment (T4); conversely, ear vessel constriction was caused by stimulation of T4 but not T1. These results were subsequently confirmed using essentially the same methods (Murray & Thompson, 1957; Guth & Bernstein, 1961). From his findings Langley (1895) inferred a 'chemiotactic' mechanism which would allow neurones to recognize innervation appropriate to them both during development and during re-innervation in adult animals.

The importance of the mechanism underlying these results prompted us to study the innervation of ganglion cells in a more direct way. In this report we describe the organization of preganglionic inputs to neurones of the adult guinea-pig superior cervical ganglion, based on intracellular recordings from ganglion cells while stimulating the lowest cervical (C8)

and the upper thoracic (T1–T7) ventral roots *in vitro*. The results show that, in general, each nerve cell is dominated by innervation from a single spinal cord segment with adjacent segments contributing a synaptic influence that decreases as a function of the segment's distance from the dominant segment. These findings provide a cellular basis for the original *in vivo* observations of Langley, which we confirm in the guinea-pig. Moreover, they suggest a mechanism which allows particular preganglionic neurones to establish synaptic connexions in a graded fashion with a distinct population of ganglion cells.

METHODS

Stimulation of the ventral roots in vivo

Five young adult albino guinea-pigs (200–300 g) were anaesthetized with pentobarbitone (30–40 mg/kg I.P.) and maintained on a positive pressure respirator. The spinal cord from the level of C8–T7 was exposed by laminectomy and removed. The ventral roots were drawn one at a time into a close fitting suction electrode for stimulation (1·0 msec, 100 V, 20/sec). The effect of stimulating the outflow of each segment was determined by observing the diameter of the pupil, the width of the palpebral fissure, the appearance of the vessels of the ear, and the degree of piloerection on the head and neck. Vasoconstriction of the ear was observed by a stereomicroscope using transillumination at × 6 magnification. All effects were graded subjectively on a 0 to + + + + scale. At the end of each experiment the level of the ventral roots which had been stimulated was confirmed by removing the thoracic cage and counting caudally from the first rib. We assume throughout these experiments that the axons of the preganglionic neurones of a spinal cord segment emerge in that segment's ventral root.

Intracellular recording from ganglion cells during ventral root stimulation in vitro

An additional fifteen animals were anaesthetized with pentobarbitone and perfused through the heart with oxygenated mammalian Ringer fluid (Liley, 1956). The right superior cervical ganglion was removed from the animal in continuity with the cervical sympathetic trunk and a part of the thoracic cage including the sympathetic chain and spinal column to about the level of T10. Further dissection was carried out in Ringer fluid. The spinal canal was opened and the cord excised leaving the ventral roots as long as possible. We dissected away the soft tissues and bone covering the dorsal root ganglia, spinal nerves, rami communicantes and sympathetic chain so that all of this portion of the peripheral sympathetic system was exposed to the bathing fluid. The preparation was then placed in a perfusion chamber at room temperature and the first seven thoracic ventral roots were drawn into close fitting suction electrodes, as were the superior and inferior post-ganglionic nerves of the superior cervical ganglion (Fig. 1). In about half the experiments the eighth cervical ventral root was also stimulated. The bath volume was 60 ml. and the rate of perfusion with oxygenated Ringer fluid was about 2 ml./min. An isolated stimulus (40–100 V, 0·5–1·0 msec) was led to a switching box so that each of the ventral roots could be activated in turn; the stimulus could also be applied to the two major post-ganglionic nerves of the superior cervical ganglion. The compound action

potentials elicited in the post-ganglionic nerves by ventral root stimulation were recorded by means of an AC-coupled differential amplifier with a 0·1 Hz low frequency cut-off; these recordings allowed us to compare the relative effect of stimulating different ventral roots and to maintain a supramaximal stimulus strength throughout the experiment.

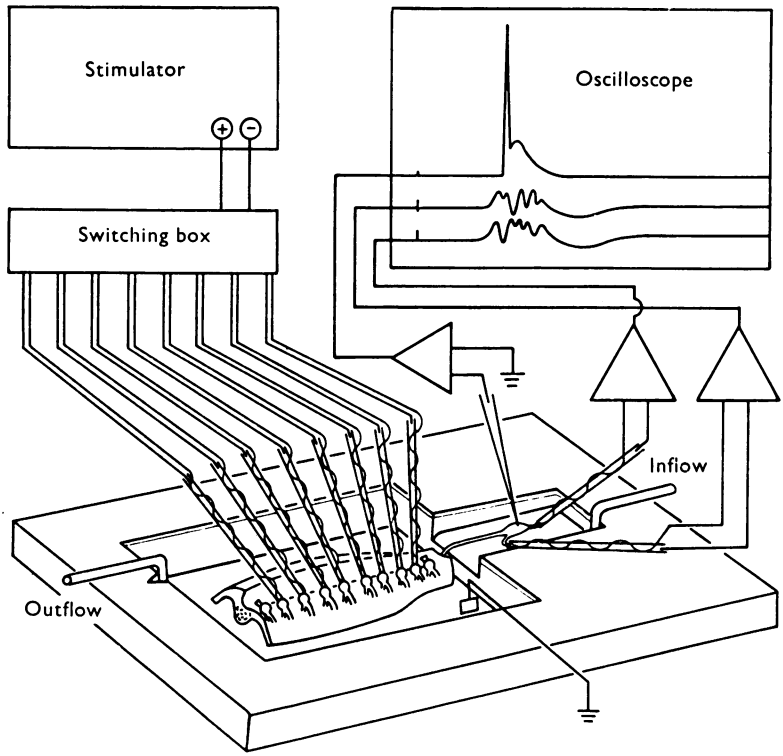

Fig. 1. Apparatus used to record intracellularly from neurones in the superior cervical ganglion while stimulating the last cervical and the first seven thoracic ventral roots with suction electrodes. Compound action potentials in response to ventral root stimulation were recorded from the superior and inferior post-ganglionic nerves. Additional features not shown are the ability to inject current through the recording micro-electrode, and the ability to switch the suction electrodes on the two major post-ganglionic nerves to a stimulator.

Intracellular recordings were made from ganglion cells with glass micro-electrodes filled with 0·5 M potassium citrate (60–100 MΩ); the methods of impalement and other details have been described (Purves, 1975*a*, 1976*a*). As in previous studies, only neurones giving an action potential of 60 mV or more in response to depolarizing current injected through the recording micro-electrode were examined.

In general, we followed a standard procedure. The integrity of the preparation was first tested by measuring the relative amplitude of the compound action potentials recorded in the post-ganglionic nerves in response to ventral root stimulation. If compound action potentials of what in preliminary experiments we came to

recognize as usual size (see Results) were recorded in response to stimulation of each of the first six thoracic ventral roots, we proceeded with impalements of ganglion cells. The compound action potentials arising from stimulation of C8 and T7 were too small and variable to be of use in this respect. If no compound action potential could be recorded in response to stimulation of any one of the first six thoracic ventral roots (or if a response was clearly less than expected) the preparation was abandoned. Following impalement of a neurone, the presence or absence of an excitatory post-synaptic potential (e.p.s.p.) in response to supramaximal stimulation of each ventral root was noted. A neurone was considered uninnervated by a particular segment if no e.p.s.p. could be observed on five successive trials of ventral root stimulation using both stimulus polarities. For those segments which gave rise to a synaptic response following supramaximal stimulation of the ventral root, the amplitude of the e.p.s.p. was measured during the refractory period of an action potential initiated in the cell soma by depolarizing current injection (Purves, 1975*a*, and Fig. 6 below). The interval between the peak of the directly initiated action potential and the e.p.s.p. (10–30 msec) was adjusted in each case to give the maximum e.p.s.p. amplitude without a superimposed regenerative response. This interval range is somewhat greater than that used previously because the slow time base (50 msec/cm) required by the long preganglionic conduction time limited the accuracy of the adjustment. As in earlier experiments, measurement of e.p.s.p.s in this way enabled us to compare synaptic responses which were above and below the threshold of the post-synaptic cell. In cases where two or more distinct e.p.s.p.s were elicited by supramaximal stimulation of a ventral root, only the largest one was measured. The number of axons from each segment innervating the impaled neurone was then estimated by gradually increasing the strength of ventral root stimulation from zero to a supramaximal value while observing the number of stable incremental amplitude steps and latency differences in the synaptic response. The measured e.p.s.p. amplitudes and the estimated number of preganglionic fibres were used to determine which segment provided the dominant innervation to a neurone. Finally, each of the two major post-ganglionic nerves was stimulated in turn to determine (by initiation of an antidromic action potential) whether the neurone being recorded from sent its axon into the superior or inferior post-ganglionic branch. Neurones which could not be held without deterioration while this series of observations was made were not included in the results. Occasionally neurones were impaled which, although innervated by one or a few segments, could not be brought to threshold by stimulation of any of the ventral roots (seven of 210 impalements). Since all neurones in the superior cervical ganglion are brought to threshold with a large safety factor when the entire cervical sympathetic trunk is stimulated (Purves, 1975*a*), it seemed likely that some of the preganglionic fibres contacting these cells had been injured (presumably due to damage during the dissection), and these seven neurones were omitted from the results.

The superior cervical ganglion of the guinea-pig contains a small number (less than 3%) of intrinsic synapses probably deriving from post-ganglionic axon collaterals and interneurones (Purves, 1976*b*). In presenting the results we have ignored the possibility that ventral root stimulation might occasionally elicit a polysynaptic response mediated by these connexions.

Figs. 5 and 6 have been retouched for clarity.

RESULTS

In vivo stimulation of the last cervical and the first seven thoracic ventral roots

In his original experiments on the superior cervical ganglion of the cat, dog, and rabbit Langley (1892; see also Murray & Thompson, 1957; Guth & Bernstein, 1961) inferred that different populations of neurones receive preganglionic fibres from different levels of the spinal cord. This conclusion was based on the observation that stimulation of each of the spinal nerves innervating the superior cervical ganglion in these animals produced a different pattern of peripheral sympathetic effects. As a first step, we repeated these experiments in the guinea-pig.

In five guinea-pigs we exposed and removed the upper part of the spinal cord and stimulated in turn the last cervical and the first seven thoracic ventral roots with a suction electrode while observing the diameter of the pupil, the width of the palpebral fissure, piloerection on the head and neck, and the vasculature of the ear. These functions are known to be mediated by the superior cervical ganglion in other species (Langley & Dickinson, 1889; Langley, 1897) and this was confirmed in the guinea-pig by stimulation of the major post-ganglionic nerves with suction electrodes 2–4 days after section of the cervical sympathetic trunk. The results of ventral root stimulation were similar from animal to animal, and are presented in Table 1. Stimulation of each root produced a characteristic pattern of peripheral effects. The most apparent aspect of the pattern was that the more rostral roots (C8, T1) had a greater effect on pupil diameter and palpebral fissure width than on piloerection or vasoconstriction, while stimulation of the more caudal roots (T4–7) produced the opposite effect. Stimulation of T2 and T3 activated all the peripheral functions observed, although T2 usually had a stronger effect on the eye than T3. These results are similar to Langley's observations with the exception of the response to stimulation of C8 which was not described in the animals he studied. Thus we confirm in the guinea-pig that preganglionic fibres arising from different levels of the spinal cord innervate functionally distinct populations of neurones in the superior cervical ganglion.

In vitro determination of the contribution of spinal segments to neurones of the superior cervical ganglion

In order to study the synaptic organization responsible for these effects at the cellular level, intracellular recordings were made from individual ganglion cells during ventral root stimulation. E.p.s.p.s could be recorded in at least some neurones in response to stimulation of the last cervical

TABLE 1. The effects of *in vivo* stimulation of the last cervical and the first seven thoracic ventral roots in five guinea-pigs. Stimulation of T6 and T7 also caused piloerection on the shoulder and upper thorax, but these effects were not mediated by the superior cervical ganglion

Segment stimulated	Dilatation of the pupil	Widening of the palpebral fissure	Piloerection on face and neck	Vasoconstriction of the ear
C8	0	0	0	0
	+	0	0	0
	+	++	0	0
	+	+++	0	0
	0	0	0	0
T1	+++	++	0	0
	+++	+++	++	+
	+++	+++	0	+
	++++	+++	0	0
	++	++	+	++
T2	+++	++	++	++
	++++	++++	++	+++
	+++	++	+	+++
	+++	++	+++	+++
	++	+++	+++	+++
T3	++	++	+++	+++
	+++	++	++	+++
	+	+	++	+++
	++	+	++++	++++
	0	+	++	++++
T4	0	0	++	++++
	0	+	++	++++
	0	0	+++	++++
	0	0	++++	++++
	0	0	++	++++
T5	0	0	++	+++
	0	0	+++	+++
	0	0	+	++++
	0	0	++	+++
	0	0	++	+++
T6	0	0	0	++
	0	0	0	+
	0	0	0	+++
	0	0	0	0
	0	0	0	0
T7	0	0	0	0
	0	0	0	+
	0	0	0	+
	0	0	0	0
	0	0	0	0

and each of the first seven thoracic ventral roots. However, the fraction of neurones innervated by fibres arising from each of these segments was different (Fig. 2*A*): about nine out of ten neurones impaled responded to stimulation of T2 and T3, while only one or two in ten responded to

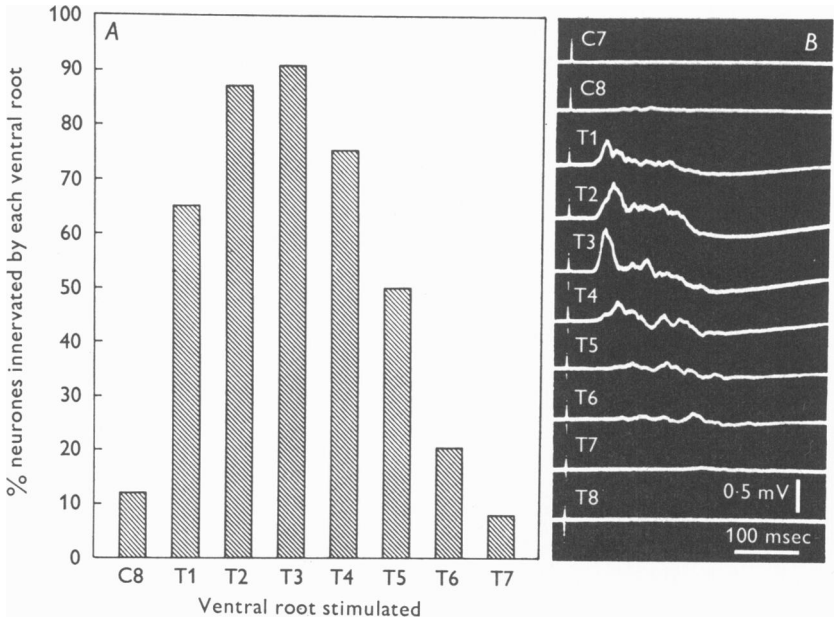

Fig. 2. Fraction of superior cervical ganglion cells innervated by different spinal segments. *A*, % neurones impaled (n = 203 for thoracic segments, 74 for C8) receiving detectable innervation in response to stimulation of a particular ventral root. *B*, compound action potentials recorded from the superior post-ganglionic nerve. This less accurate but more general measure of the degree of innervation provided by each segment is in agreement with the intracellular sample (see also Fig. 5). No response was elicited by stimulation of C7 or T8.

stimulation of C8, T6 and T7. An intermediate proportion gave e.p.s.p.s in response to stimulation of T1, T4 and T5. The relative contribution of different spinal segments could also be assessed by observing the size of the post-ganglionic compound action potentials recorded in the superior and inferior nerves (Fig. 2*B*; see also Fig. 5). The largest compound action potentials were invariably recorded in response to stimulation of T2 and T3, the smallest in response to C8, T6 and T7, and potentials of intermediate size in response to stimulation of T1, T4 and T5. Apart from differences in size, post-ganglionic compound action potentials were roughly similar in configuration, having multiple peaks with latencies between 40 and 400 msec. No compound action potentials were elicited by stimulation

of C7 and T8 (Fig. 2*B*); however, intracellular recordings were not made while stimulating these ventral roots, and it is possible that occasional fibres from C7 and T8 also innervate a few ganglion cells. These findings are generally consistent with the proportion of fibres remaining in the cervical sympathetic trunk after severing different combinations of thoracic rami communicantes in the cat (Murray & Thompson, 1957).

The amplitude distributions of the measured e.p.s.p.s elicited by stimulation of each of the ventral roots contributing fibres to the superior cervical ganglion (see below) were generally similar. Although we did not study the e.p.s.p.s in response to stimulation of single preganglionic axons in detail, it was clear that single fibres could evoke e.p.s.p.s in ganglion cells which ranged from our limit of detection (1 mV) to more than 40 mV in amplitude. The average number of preganglionic axons innervating individual neurones, estimated by the sum of the incremental steps in the synaptic response for each root, was ten.

Pattern of innervation of individual neurones by spinal segments

Most neurones received innervation from several spinal segments, and all but two of 203 neurones impaled in 15 preparations were innervated by more than one segment (Figs. 3 and 5). The average number of segments contributing innervation to single neurones was 4·0.

A striking feature of the innervation of individual cells was the contiguity of the contributing segments (Fig. 3). Almost invariably, the segments which gave rise to e.p.s.p.s in a cell were adjacent to one another; thus 'holes' in the pattern of innervation were rare. Furthermore, when innervation by non-contiguous segments occurred, the discontinuity was always limited to the absence of a single spinal segment from an otherwise contiguous set. The degree to which the data presented in Fig. 3 differ from the pattern of innervation expected if segmental contributions innervated neurones independently of each other can readily be calculated. For example, the probability of a given neurone being innervated by T3 is 0·91, by T4 0·75 and by T5 0·49 (Fig. 2*A*). Thus the number of neurones in this series of 203 impalements expected to be innervated by T3 and T5, but *not* by T4 is given by $(0{\cdot}91 \times 0{\cdot}25 \times 0{\cdot}49) \times (203)$ or about 23. Repeating this calculation for each of the first six thoracic segments, a total of seventy-five neurones would be predicted to have a single break in the continguity of innervation. Since only four single discontinuities (and no multiple discontinuities) were observed (Fig. 3), we conclude that the innervation of neurones by preganglionic fibres from different spinal segments is not independent. Rather the innervation must have been established according to a rule or rules involving an increased probability

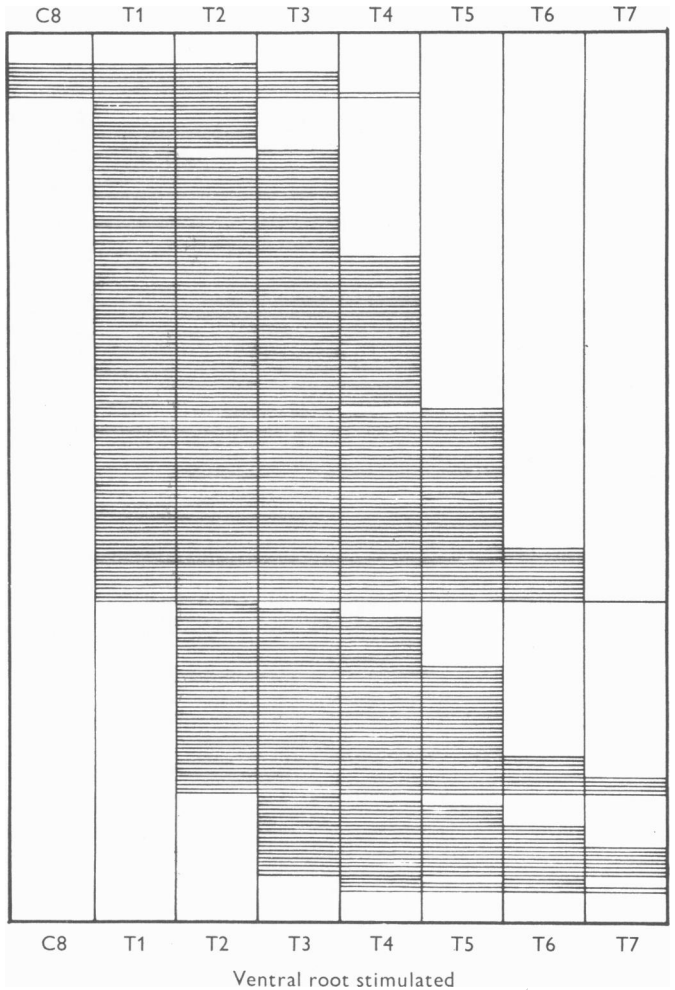

Fig. 3. Segmental innervation of individual ganglion cells. Each horizontal line represents a different neurone; length of the line corresponds to the number of segments contributing innervation to the neurone. The results have been ordered to show that neurones innervated by more rostral segments have a low probability of being innervated by the caudal segments, and conversely. 'Holes' in the pattern of innervation (neurones innervated by the segments rostral and caudal to a segment which fails to contribute innervation) are rare. Innervation by C8 is under-represented since this segment was tested in only seventy-four neurones, while the other segments were stimulated for all impalements (n = 203). In arriving at a mean value for the number of segments innervating single neurones, we assume that the proportion of neurones innervated by C8 in the sample in which this root was stimulated would be similar if C8 had been tested for all neurones impaled.

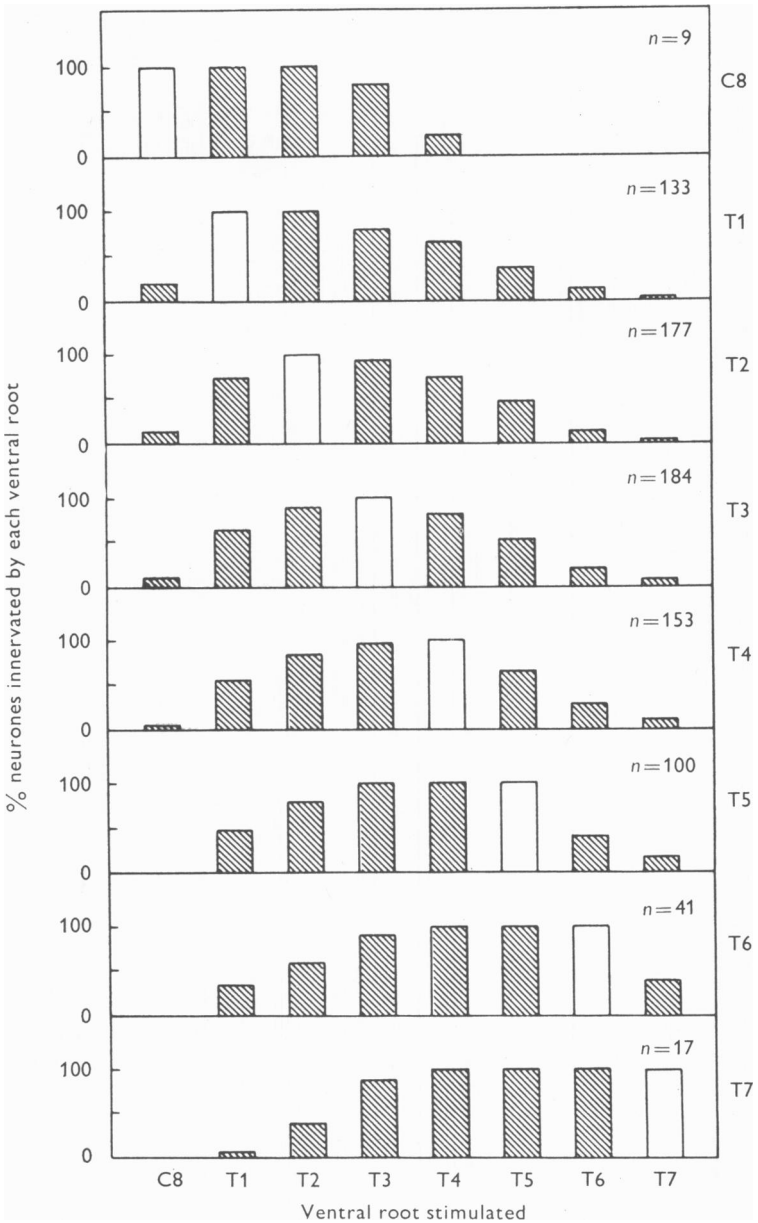

Fig. 4. Likelihood of other segments innervating neurones receiving synapses from particular spinal levels. Each histogram is based on all the neurones in the total sample (Fig. 3) innervated by a particular ventral root (indicated to right). The open bars by definition equal 100 %.

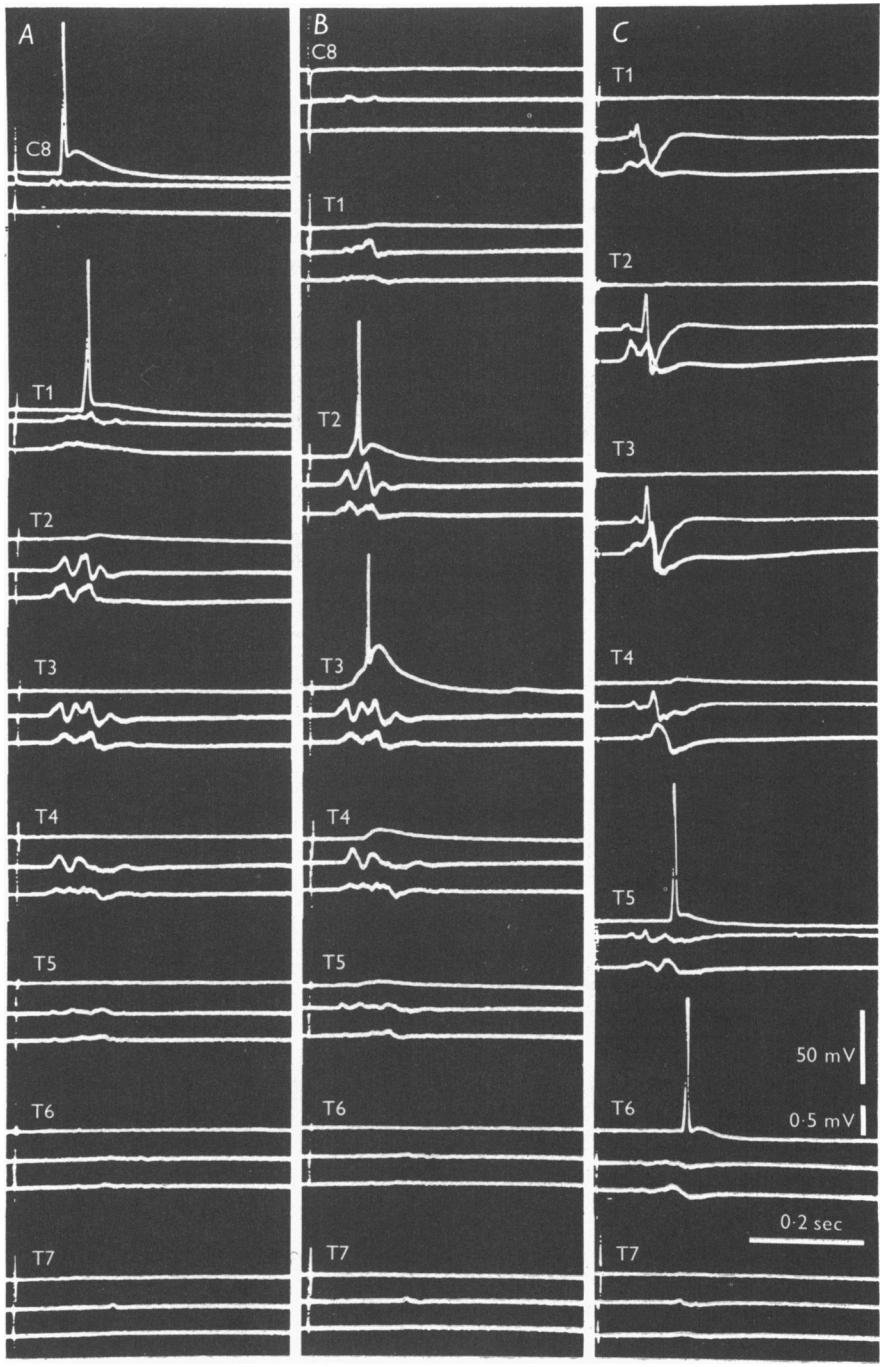

Fig. 5. For legend see facing page.

of innervation from adjacent segments. A simple statement of this rule might be that individual neurones prefer a contiguous subset (four on average) of the eight segments which provide innervation to the ganglion.

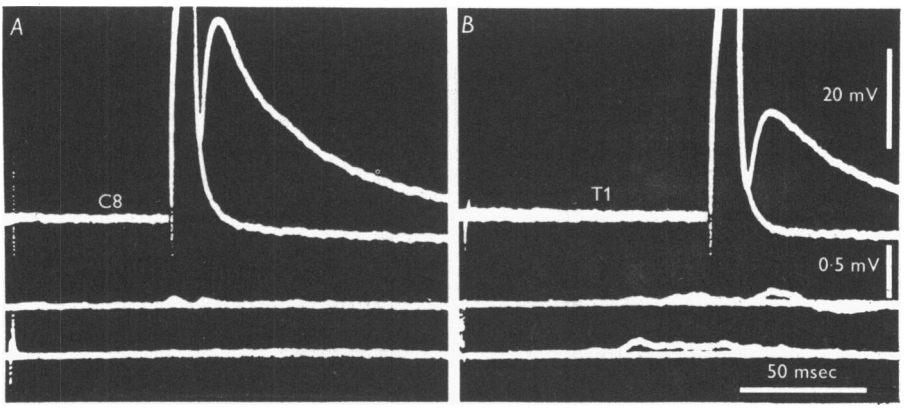

Fig. 6. Determination of the dominant segment by measurement of the e.p.s.p. amplitude. Same neurone as in Fig. 5*A*. *A*, stimulation of C8 was timed so that the peak of the e.p.s.p. occurred 10–30 msec after an action potential directly elicited by current injection into the neuronal soma. *B*, stimulation of T1 resulted in a smaller e.p.s.p.; thus, although both C8 and T1 caused a suprathreshold response, the neurone received a stronger synaptic input from C8. Two traces are superimposed; the action potential is not fully seen at this gain.

It is apparent from Fig. 3 (see also Fig. 5) that neurones innervated by rostral segments tend to be innervated less often by caudal segments and vice versa. To examine this tendency we grouped all of the neurones innervated by a particular segment and, for each subgroup, determined the fraction innervated by each of the remaining segments (Fig. 4). Reordering the information presented in Fig. 3 in this way shows that there

Fig. 5. Tendency of neurones to receive graded innervation from contiguous spinal segments. Upper traces are intracellular recordings, middle and lower traces are recordings of the compound action potential elicited in the inferior and superior post-ganglionic branches respectively. *A*, neurone receiving dominant innervation (see Fig. 6) from C8 receives a progressively smaller contribution from the adjacent rostral segments, and no innervation from the more caudal segments. *B*, most neurones received their dominant innervation from T2 to T4, and showed a progressive fall in the intensity of synaptic influence from increasingly distant rostral and caudal segments. When e.p.s.p.s were measured during the refractory period of an action potential, T3 was found to provide the dominant innervation to this neurone. *C*, neurone receiving dominant innervation from T5 is innervated by adjacent caudal segments but not by rostral segments. Neurones shown in *A* and *B* are from the same preparation.

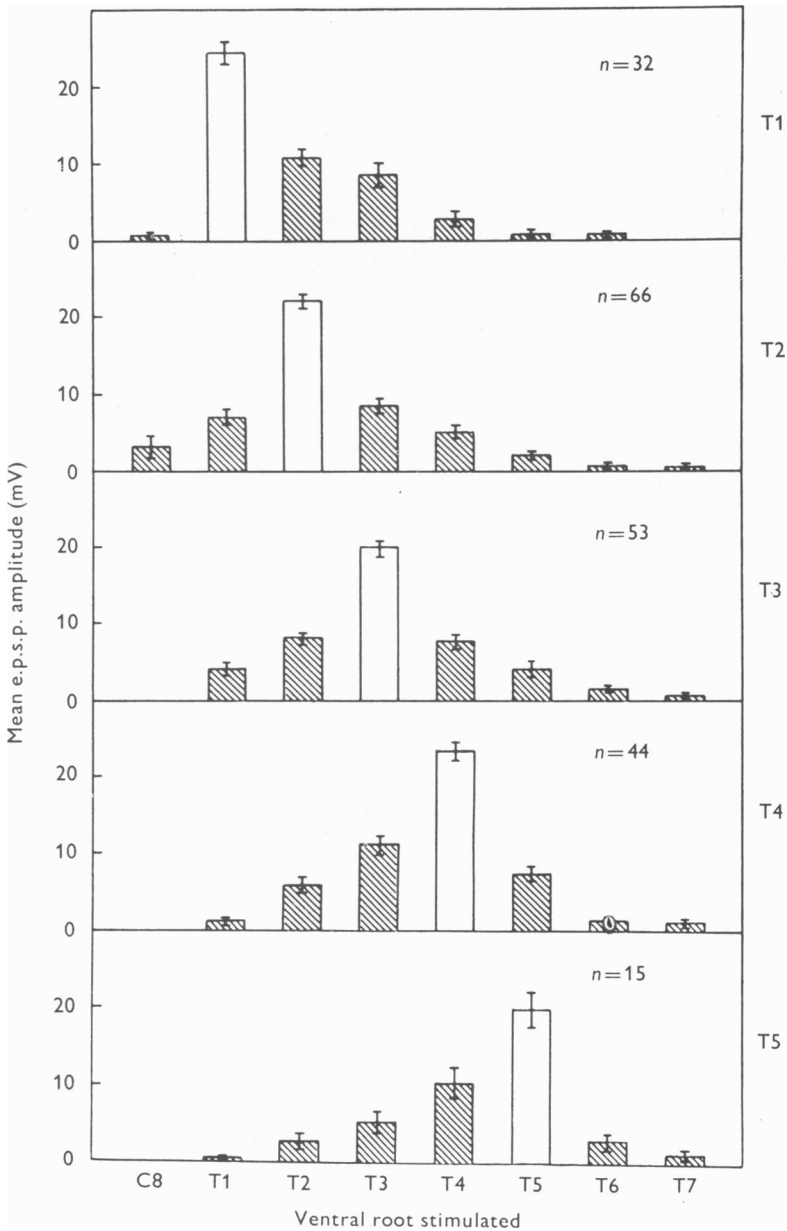

Fig. 7. Distribution of innervation to individual neurones using e.p.s.p. amplitude as the criterion of segmental dominance. Histograms show mean e.p.s.p. amplitudes recorded in neurones dominated by innervation from different spinal segments (± S.E.); open bars show the response to stimulation of the dominant segment (indicated to the right). E.p.s.p. amplitude distributions shift continuously with the level of the dominant spinal cord

is an apparently smooth transition from one subgroup to the next of the level at which preganglionic innervation tends to arise.

The innervation of individual neurones was studied in more detail by measuring the amplitude of the e.p.s.p.s elicited by stimulation of each of the ventral roots (Figs. 5, 6 and 7). The method of measuring the synaptic response during the refractory period of a directly initiated action potential is shown in Fig. 6. In general, neurones were most effectively innervated by a single segment with adjacent segments contributing a synaptic influence that diminished as a function of their distance from the dominant segment. Thus a neurone dominated by one of the rostral segments (Fig. 5*A*) was usually well innervated by the adjacent rostral segments but innervated poorly or not at all by the more caudal segments; the opposite was true for neurones dominated by one of the caudal segments (Fig. 5*C*). Neurones whose dominant innervation derived from one of the middle segments (T2 or T3) were usually innervated to an increasingly weak degree by both more caudal and more rostral segments (Fig. 5*B*).

Although some neurones disobeyed this rule in being weakly innervated by preganglionic fibres from a segment between two segments providing stronger innervation, the prevailing pattern of segmental innervation was graded in a unimodal way (Figs. 7 and 8). Each of the histograms in Fig. 7 represents the average synaptic responses of all the neurones dominated by a particular segment to stimulation of the remaining ventral roots. The average measured e.p.s.p. was generally larger for segments adjacent to the dominant one, although there was some skewing. This skewing, and the smaller number of neurones receiving dominant innervation from the most rostral and caudal segments, probably occur because the most rostral and caudal segments contribute fewer fibres to the superior cervical ganglion than the middle segments (see Fig. 2). However, Fig. 7 shows that the average e.p.s.p. amplitudes elicited by stimulation of different ventral roots are unique for each group of neurones, changing systematically with the level of the dominant spinal cord segment.

When the estimated number of preganglionic fibres instead of e.p.s.p. amplitude was used as the criterion of segmental dominance of a ganglion cell a similar pattern was observed: the number of fibres contributed by a segment tended to fall with increasing distance from the dominant segment

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level. In twelve impalements the maximum e.p.s.p. measured was the same for stimulation of two ventral roots; these cells were thus counted twice. None of the neurones impaled in this series of experiments were dominated by innervation from T7, one neurone was dominated by C8, and four by T6. In later experiments occasional neurones have been found to receive dominant innervation from T7 as well.

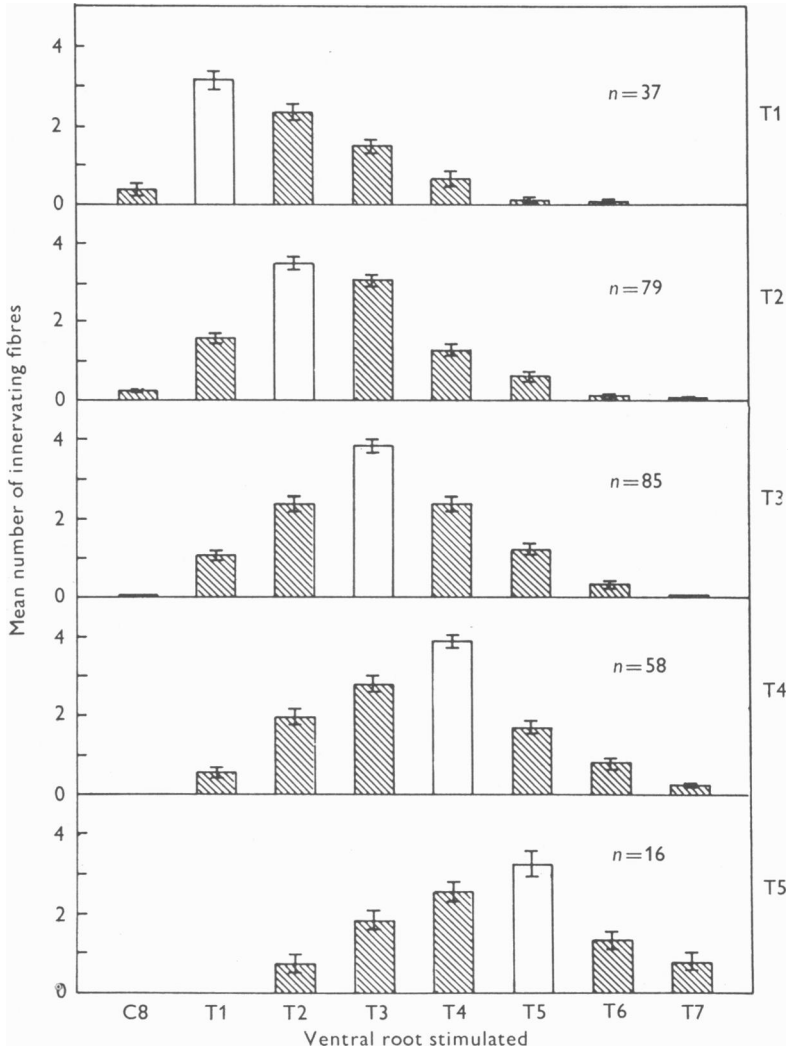

Fig. 8. Distribution of innervation of neurones using the estimated number of preganglionic fibres contacting a neurone as the criterion of segmental dominance. Histograms show the mean number of preganglionic fibres determined in neurones dominated by innervation from different spinal segments (±S.E.); open bars show the response to stimulation of the dominant segment (indicated to the right). The distributions of the number of innervating fibres shift continuously with the level of the dominant spinal cord segment. This result is complementary to that shown in Fig. 7. In 71% of the neurones impaled the dominant segments were the same whether determined by e.p.s.p. amplitude or fibre number. In some neurones the maximum number of innervating fibres was identical in response to stimulation of two (or sometimes three) ventral roots; these cells were thus counted more than once.

(Fig. 8). This finding further supports the systematic grading of ganglion cell innervation.

During the course of these observations we noted that the probability of innervation by each spinal segment was roughly similar for neurones sending their axons into the superior and inferior post-ganglionic nerves. This finding was in accord with the result of stimulating these post-ganglionic nerves *in vivo*. For example, stimulation of the superior nerve caused piloerection on the face (a function mediated mainly by the second to the fifth thoracic segment – see Table 1), while stimulation of the inferior post-ganglionic nerve caused marked vasoconstriction of the ear (also mediated by T2–T5). The functions elicited by stimulation of the post-ganglionic nerves, however, showed little or no overlap. Although we often encountered neighbouring neurones sharing a similar pattern of innervation, we noticed no clear-cut grouping of neurones dominated by particular segments. Some independent evidence consistent with our failure to detect an anatomical grouping of neurones with similar patterns of innervation is the apparent dispersion of labelled neurones in the superior cervical ganglion of the rat after injection of a radioactive marker into the eye (Hendry, Stöckel, Thoenen & Iversen, 1974, their Fig. 4).

DISCUSSION

It has been known for many years that functionally distinct populations of neurones in the mammalian superior cervical ganglion are innervated by preganglionic fibres arising from different levels of the spinal cord (Langley, 1892, 1895, 1897; Murray & Thompson, 1957; Guth & Bernstein, 1961). The finding that stimulation of different ventral roots *in vivo* produces different patterns of end-organ responses in guinea-pigs, as well as other mammals in which this has been tested, could have several explanations at the level of the nerve cells which comprise the superior cervical ganglion. The present experiments show that individual neurones are innervated by a contiguous subset of spinal segments (about four on average), and are generally dominated by the innervation arising from a single segment with adjacent segments contributing a synaptic effect that decreases with increasing distance from the dominant segment.

A major question arising from these results is what factors underlie the tendency of neurones to be innervated in a systematic way by a particular subset of preganglionic fibres. An orderly arrangement of multiple synaptic inputs on individual nerve cells is presumably commonplace in the nervous system, but little information is available to indicate how specific patterns of synaptic contacts are established. In general, the matching of pre- and post-synaptic elements might come about in two different ways, either as a result of a developmental sequence, or because of unique 'labels' associated

with each neurone or class of neurones. The present findings might arise from a developmental sequence if, for example, different classes of nerve cells matured sequentially together with fibres from different spinal levels. Although there is no direct evidence against this possibility, Langley (1895, 1897), and subsequently Guth & Bernstein (1961), found that a similar pattern of end-organ responses was re-established after re-innervation of the superior cervical ganglion in the adult cat, suggesting that the ability of ganglion cells to establish specific connexions is not limited to the period of development, but rather is present throughout life (see also Landmesser & Pilar, 1970; Jansen & Nicholls, 1972). In concluding his original experiments, Langley (1895) suggested that 'there is some special chemical relation between each class of nerve fibre and each class of nerve cell, which induces each fibre to grow towards a cell of its own class and there to form its terminal branches' (p. 284). This view remains a plausible explanation for the present results. However, the finding that neurones are innervated by several segments in a generally graded way (Figs. 5, 7 and 8) suggests that the matching of pre- and post-ganglionic elements is statistical rather than unique. Another example of a failure to distinguish between alternate sources of innervation in an absolute way occurs when these ganglion cells are simultaneously re-innervated with native and foreign (vagal) fibres (Purves, 1975*b*, 1976*b*); although foreign axons are less successful in making synapses on ganglion cells than the native fibres, many neurones are contacted by both classes of synapses and remain dually innervated indefinitely. This is in contrast to the situation at some lower vertebrate nerve-muscle junctions where inappropriate foreign contacts are recognized as such and apparently are completely repressed or rejected (Cass, Sutton & Mark, 1973; Yip & Dennis, 1976).

There is as yet little basis for speculating about the nature of a 'chemotactic' mechanism that could promote or inhibit the formation of particular synaptic contacts on sympathetic ganglion cells. It is worth noting, however, that the integrity of the post-ganglionic axons is necessary for the normal maintenance of preganglionic endings (Purves, 1975*a*; see also Matthews & Nelson, 1975). Several lines of evidence suggest that the role of the axon in this regard may be the retrograde transport of a trophic agent, possibly nerve growth factor (Hendry *et al.* 1974; Hendry, 1975; Purves, 1975*a*, 1976*a*; Purves & Njå, 1976). The results we report here might be explained by a retrograde trophic signal which acted differentially on preganglionic neurones from different levels of the spinal cord.

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